RESEARCH ARTICLE

Low cyanobacterial diversity in biotopes of the Transantarctic Mountains and Shackleton Range (80–82°S), Antarctica

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Abstract

The evolutionary history and geographical isolation of the Antarctic continent have produced a unique environment rich in endemic organisms. In many regions of Antarctica, cyanobacteria are the dominant phototrophs in both aquatic and terrestrial ecosystems. We have used microscopic and molecular approaches to examine the cyanobacterial diversity of biotopes at two inland continental Antarctic sites (80–82°S). These are among the most southerly locations where freshwater-related ecosystems are present. The results showed a low cyanobacterial diversity, with only 3–7 operational taxonomic units (OTUs) per sample obtained by a combination of strain isolations, clone libraries and denaturing gradient gel electrophoresis based on 16S rRNA genes. One OTU was potentially endemic to Antarctica and is present in several regions of the continent. Four OTUs were shared by the samples from Forlidas Pond and the surrounding terrestrial mats. Only one OTU, but no internal transcribed spacer (ITS) sequences, was common to Forlidas Pond and Lundström Lake. The ITS sequences were shown to further discriminate different genotypes within the OTUs. ITS sequences from Antarctic locations appear to be more closely related to each other than to non-Antarctic sequences. Future research in inland continental Antarctica will shed more light on the geographical distribution and evolutionary isolation of cyanobacteria in these extreme habitats.

Introduction

Cyanobacteria are the dominant phototrophs in both aquatic and terrestrial ecosystems in many regions of Antarctica. They develop considerable biomass in microbial mats (laminated biofilms) that typically form on the benthos and littoral zones of lakes and ponds, and on the surface of areas of soil where seasonal snow patches accumulate or summer water availability is reliable (Vincent, 1988).

Despite the prominent role of cyanobacteria in Antarctica, it is still unclear whether geographic isolation is a structuring factor in the biogeography of microorganisms in this region. Several studies elsewhere have shown that biogeographical distribution patterns do occur in free-living microorganisms, although separating the respective roles of contemporary environmental conditions and historical contingencies is often difficult (see Martiny *et al.*, 2006 for a review). Recently, Bahl *et al.* (2010) found that the global distribution of desert cyanobacteria is the result of an ancient evolutionary legacy, rather than contemporary dispersal, highlighting the importance of temporal scales in biogeographical studies. In Antarctica, endemism has been reported in several taxa, for example in terrestrial metazoa and lichens (see Convey & Stevens 2007; Convey *et al.*, 2008 for a review). Similarly, the chironomid midges (*Diptera*) appear to be restricted to tectonically distinct parts of the Antarctic Peninsula and the Scotia Arc (Allegrucci *et al.*, 2006), and possibly all nematode species present in Antarctica are endemic to the continent (and to smaller regions within it) (Andrássy, 1998; Maslen & Convey, 2006). Finally, the review of Vyverman *et al.* (2010) identified a high degree of endemism in the diatom flora. However, although endemism has been commonly recognized in higher eukaryotic taxa, prokaryotes are commonly assumed to be widely dispersed by effective mechanisms that should reduce the occurrence of endemicity (Baas Becking, 1934; Finlay, 2002).

One of the challenges in determining the presence of endemic organisms is applying an appropriate level of taxonomic resolution. This is because different resolution molecular markers can influence the interpretation of

the biogeographic distribution of particular taxa. For example, Cho & Tiedje (2000) demonstrated that the use of different markers [16S rRNA gene, internal transcribed spacer (ITS) and genomic fingerprints] on fluorescent strains of *Pseudomonas* showed no endemicity on the basis of 16S rRNA gene, but a high level of endemicity was revealed by the genomic fingerprints.

Previous molecular studies of Antarctic cyanobacteria have shown that they include both cosmopolitan and potentially endemic operational taxonomic units (OTUs) (Taton *et al.*, 2003, 2006a, b). The OTUs were defined as groups of 16S rRNA gene sequences that shared more than 97.5% similarity (Taton *et al.*, 2006a). The observed divergence of Antarctic endemic OTUs implies an isolation of at least 125 My, as the mutation rate of 16S rRNA genes has been estimated as 1% every 50 million years (Ma) (Ochman & Prager, 1987). The most comprehensive studies on cyanobacterial endemism in Antarctica, to date, have worked on samples from coastal regions of east Antarctica (Taton *et al.*, 2006a, b) and the McMurdo Dry Valleys (Taton *et al.*, 2003). These studies led to two conclusions: (1) the molecular approach suggested that potential Antarctic endemic OTUs (57%), including a previously undiscovered diversity (39%), were more abundant than previously estimated using morphological approaches (35%) and (2) cosmopolitan OTUs were more widespread over the continent than potential endemics. However, since 2006, the databases have grown and the proportion of these potential Antarctic endemic OTUs has declined to 28.5%.

As previous studies have mostly focused on near-coastal locations, little is known about the relative abundances of cosmopolitan vs. endemic cyanobacteria in the more southerly, inland, extreme and isolated freshwater-related ecosystems of Antarctica. To address this, we examined the morphological and molecular diversity of cyanobacteria inhabiting Forlidas Pond in the Transantarctic Mountains (TAM) and Lundström Lake in the Shackleton Range (80–82°S). This involved a polyphasic study of the diversity and distribution of the cyanobacteria in four samples, including an analysis of the ITS region to improve the precision of groupings based on 16S rRNA genes (Taton *et al.*, 2006a). The results were compared with sequence databases, in order to better understand the distribution patterns of the cyanobacterial genotypes identified. This work is the first report of the cyanobacterial diversity, studied using a polyphasic approach, in the TAM and the Shackleton Range.

Materials and methods

Sampling and site description

The TAM are one of the world's longest continental rift flank uplifts. They divide the continent into two unequal (West and East) elements. The mountains rise from the sea level to elevations of *c.* 4000 m. They constitute one of the harshest ecological areas of Antarctica (Turner *et al.*, 2009; Hodgson *et al.*, 2010).

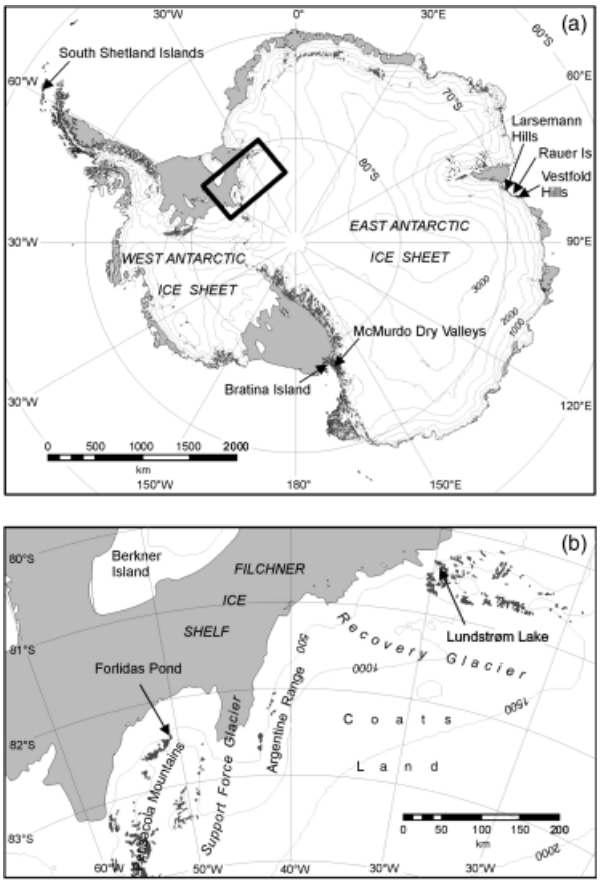

Fig. 1. Location map of the sampled water bodies. (a) The sampled area is marked with a black rectangle. (b) Lakes are indicated by arrows.

Our first study site, 'Forlidas Pond' (51°16′48″W, 82°27′28″S, within the Antarctic Specially Protected Area 119), is a 90 m diameter frozen freshwater lake overlying a 20 cm thick layer of hypersaline brine located in an ice-free 'dry' valley situated at the north-eastern end of the Dufek Massif (see Hodgson *et al.*, 2010 for details). Our second study site is Lundström Lake (29°26′56″W, 80°26′30″S) (Fig. 1 and Supporting Information, Fig. S1a and b), located in the Haskard Highlands of the Shackleton Range, 420 km north-east of Forlidas Pond. Lundström Lake is a shallow, perennially ice-covered lake, 400 m long and 300 m wide and, like Forlidas Pond, is the remnant of a much larger proglacial lake that last filled the valley in the mid-Holocene (Höfle & Buggisch, 1995). The lake, at 640 m above sea level, is frozen down to the bed at 3.14 m ice depth. The conductivity in the interstitial water in the ice at the bed was higher (2.9 mS cm^{-1}) than the littoral surface water

($0.227\ mS\ cm^{-1}$), suggesting a limited accumulation of catchment-derived salts in the lake basin (Table S1).

Four samples were collected during the 2003/2004 Antarctic summer at the two study sites. Three samples were analyzed from Forlidas Pond (TM1 and TM2, from the hypersaline brine and the freshwater littoral zone, respectively) and its catchment (TM3) (see Hodgson *et al.*, 2010 for details). The TM3 sample was selected as an example of the cyanobacterial mats that are frequently observed in the dry valleys of the Dufek Massif (Hodgson *et al.*, 2010). A fourth microbial mat sample (TM4) was taken from the littoral zone of Lundström Lake.

Samples of cyanobacteria in the benthic brine layer of Forlidas Pond were obtained using a UWITEC water sampler (Hodgson *et al.*, 2010) and those from the littoral and terrestrial zones of both lakes were sampled manually into sterile bags using tweezers. All samples were stored in sterile Whirlpak bags, frozen in the field and transferred frozen to the UK via the British Antarctic Survey station at Rothera Point. Subsamples were then sent to the University of Liège under dry ice.

Morphological characterization and cultures

The major cyanobacterial taxa were examined in both the environmental samples and cultures using a Leica DM LB2 microscope (Leica Microsystems GmbH, Wetzlar, Germany). The taxonomic work of Komárek & Anagnostidis (2005) was used as a reference, and the diacritical morphological traits used for botanical species identification followed Taton *et al.* (2006b). Cells were measured using the AXIOVISION software (Carl Zeiss MicroImaging GmbH, Germany), and the results were expressed as the mean measurements of at least five cells per morphotype.

Environmental samples were grown in several media to isolate strains. In addition to BG11 (Rippka *et al.*, 1981), we used media with different salinities ($0.068–33\ g\ L^{-1}$ NaCl) because the TM1 sample was collected from a hypersaline brine. Thus, media $BG11_0$, 2, 3 (nitrogen-free media) and 2NP, 3NP, ASNIII/2 and ASNIII media (nitrogen-containing media) were used (Rippka *et al.*, 1979; Taton *et al.*, 2006b). Cycloheximide ($50\ mg\ L^{-1}$) was used when inoculating the environmental samples on culture plates for the first time, to avoid eukaryotic contaminants (Taton *et al.*, 2006b). The temperatures of incubation used were 12 and 22 °C, to allow the growth of psychrophilic and/or psychrotolerant cyanobacteria, respectively (Morita, 1975).

DNA extraction and purification

The DNA extraction method was modified from Taton *et al.* (2003). A 0.5-g mat sample was thawed and then frozen overnight and thawed again, and then rinsed two times with 1 mL of phosphate-buffered saline (0.08 M Na_2HPO_4, 0.03 M NaH_2PO_4, 1.50 M NaCl, pH 7.2). Glass beads (0.25 g) (diameter 0.17–0.18 mm; Braun Biotech) were added and pressed with a pestle (Eppendorf, Hamburg, Germany) several times in a 2-mL Eppendorf tube. Next, 250 μL of SNT solution was added (500 mM Tris-HCl pH 8, 100 mM NaCl, 25% saccharose) supplemented with the addition of 130 μL of fresh lysozyme ($50\ mg\ mL^{-1}$). Cells were further disrupted using the pestle, followed by the addition of 250 μL of SNT solution. The suspension was then incubated for 1 h at 37 °C with mixing every 15 min. After this incubation, 0.5 mL of solution II (Tris base 500 mM, EDTA 500 mM, SDS 1%, phenol 6%) was added and the suspension was vortexed continuously for 30 min. The suspension was then placed on ice for 1 h and shaken briefly by vortex every 10 min. After this incubation, the suspension was centrifuged for 10 min at 720 ***g*** (Centrifuge 5424, Eppendorf) and 1 mL of the aqueous phase was mixed with an equal volume of phenol, after which it was centrifuged for 5 min at 13 600 ***g***. The supernatant was then transferred into new tubes, extracted with equal volumes of phenol–chloroform–isoamyl alcohol (25 : 24 : 1) and re-extracted with equal volumes of chloroform–isoamyl alcohol (24 : 1). Then, a Na acetate–ethanol precipitation was performed and the dried pellet was resuspended in 200 μL of TE-4 buffer (10 mM Tris-HCl pH 8, 0.1 mM Na_2EDTA pH 8). The environmental DNA was purified using the Wizard DNA clean-up system (Promega, Madison). The DNA was eluted by adding 100 μL of TE-4 buffer.

Cloning and screening of clone libraries

PCR amplification of the cyanobacterial 16S rRNA gene plus the ITS was performed as described by Taton *et al.* (2003), with the minor modifications that 1 μL of DNA was added and the PCR cycles were altered: amplification was carried out using an Icycler thermocycler (Bio-Rad), with one cycle of 5 min at 95 °C; 35 cycles of 45 s at 95 °C, 45 s at 54 °C and 3 min at 68 °C; and a final elongation step of 7 min at 68 °C. Three PCRs were carried out and the products were pooled. Negative controls (PCR mix with no DNA) were always included. This mix of amplicons was purified two times using Quantum Prep PCR Kleen Spin columns (Bio-Rad). Poly(A) extension was performed using the Qiagen A-addition Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Cloning of the PCR products (*c.* 1600 bp) utilized the TOPO TA cloning kit (Invitrogen BV, Breda, the Netherlands) as described by Taton *et al.* (2003), except that the screening PCR was performed with the 16S378F and 16S784R primers. The amplification conditions described above were used, except that 0.8 U of Super *Taq* polymerase (HT Biotechnology, Cambridge, UK) were used and amplification was carried out as follows: 10 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 60 °C

and 1 min at 72 °C; and a final elongation step of 7 min at 72 °C. Plasmid DNAs were extracted using a Quantum Prep Plasmid Miniprep kit (Bio-Rad) following the manufacturer's instructions. All the primers used have been described by Taton *et al.* (2003, 2006a).

Amplified ribosomal DNA analysis (ARDRA)

The direct sequencing of a number of clones with primer 16S1092R was complemented by a screening of the remaining clones with ARDRA to avoid replicates. The clones of each clone library were digested together, with at least one sequence belonging to the different OTUs detected by direct sequencing. The inserted 16S rRNA gene plus ITS was reamplified with primers 16S378F and 23S30R as described above. All steps were performed as described previously (Scheldeman *et al.*, 1999; Taton *et al.*, 2003), except for the following changes: MboI and HpaII (MBI Fermentas, Vilnius, Lithuania) were used as restriction enzymes and electrophoresis was performed at a constant voltage of $3\,V\,cm^{-1}$ for 235 min in NuSieve (3 : 1) agarose gels (Cambrex, NJ). The numbers of clones analyzed by ARDRA were 40, 25, 55 and 44 for TM1, TM2, TM3 and TM4, respectively (Table S2). Clones with different band patterns were sequenced.

Denaturing gradient gel electrophoresis (DGGE) analysis

Two successive PCR reactions were run to produce 422-bp-long fragments, as described in Taton *et al.* (2003), except that electrophoresis was performed for 999 min at 75 V and 60 °C. Two DGGE (a and b) were performed for each sample. The PCR primers used to perform the DGGE (a) and (b) targeted filamentous and unicellular cyanobacteria, respectively (Nübel *et al.*, 1997). A marker was provided by S. Cousin (U. Ghent, pers. commun.), which provided nine bands uniformly distributed along the gel. The excised DGGE bands were incubated in TE-4 buffer for 12 h at 4 °C. Each DNA solution was used as a template for PCR reamplification (primers 16S378R and 16S784R), followed by purification (Ilustra DNA and band gel purification Kit, GE Healthcare, Belgium) and sequencing. When several bands with identical or quasi-identical sequences were obtained for one sample, only one representative is shown in the tree.

Sequencing was carried out by GIGA (http://www.giga.ulg.ac.be/) (Liège, Belgium) using an ABI 3730xls DNA analyser (Applied Biosystems, Foster City). DGGE bands were sequenced using the 16S784R and/or 16S359F primers to obtain sequences of about 350 bp. Almost complete 16S rRNA gene (*Escherichia coli* positions 364–1526) plus ITS sequences were determined for clones from the clone libraries using the sequencing primers 16S1092R (Taton *et al.*, 2006a), 16S1494R (Wilmotte *et al.*, 2002) and 23S30R (Taton *et al.*, 2003). Thirty sequences were submitted to GenBank and assigned the following accession numbers for 16S rRNA genes from sample TM4 (EU852501–EU852506, HQ219056–HQ219059, HQ219060–HQ219062) and for ITS from samples TM1 to TM4 (EU852516–EU852519, EU852521–EU852532 and HQ336421).

Analysis of sequence data

Partial sequences of 16S rRNA genes from clones (*E. coli* positions 379–1542) and DGGE bands (*E. coli* positions 359–800) were used for the BLAST analyses. The 16S rRNA gene sequences from the DGGE and clone libraries from samples TM1, TM2 and TM3 were reported by Hodgson *et al.* (2010) and a BLAST analysis including these data and those obtained here from TM4 was carried out (July 17, 2010). Similarly, complete ITS sequences from the four clone libraries (TM1 to TM4 samples) were used for BLAST analysis, with the exception of some sequences that were slightly shorter. Chimeras were detected using CHIMERA CHECK in the Ribosomal Database Project (Maidak *et al.*, 2001), PINTAIL (Ashelford *et al.*, 2005) and BELLEROPHON (Huber *et al.*, 2004) and then excluded from the analysis. Aligned partial 16S rRNA gene sequences corresponding to *E. coli* sequence positions 379–806 were used to select the three most similar strain sequences and three uncultured sequences using the option SEQMATCH in RDPII (http://rdp.cme.msu.edu) (Cole *et al.*, 2009). A distance tree was constructed using the software package TREECON for Windows 1.3b (Van de Peer & De Wachter, 1997) not taking into account indels and ambiguous bases. The dissimilarity values were corrected for multiple substitutions using the method of Jukes & Cantor (1969) and were used to calculate a distance matrix. The tree was constructed using the neighbor-joining method (Saitou & Nei, 1987). A bootstrap analysis was performed that involved the construction of 1000 resampled trees.

The sequences were grouped into OTUs, following the numeration proposed by Taton (2005). A distance matrix with the Jukes and Cantor correction was calculated using the DNADIST software from the package PHYLIP 3.67 (Felsenstein, 1989). Clustering into OTUs was carried out with the DOTUR software (Schloss & Handelsman, 2005) using the average neighbor method (*E. coli* positions 405–780).

The ITS sequences of several clones were determined to assess the homogeneity of ITS within the same OTU. The ITS sequences were analyzed by a similarity search using the BLAST program and then aligned on the basis of conserved domains (Iteman *et al.*, 2000). The ITS sequences were grouped into 'ITS types', which are groups of sequences that can be meaningfully aligned (Wilmotte *et al.*, 1994), sharing at least 75% pairwise identity.

The clone library accumulation curves, coverage index and Berger–Parker index were analyzed using the software 'SPECIES DIVERSITY AND RICHNESS' (Pisces Conservation Ltd, New Milton, UK).

Results

Microscopic diversity

Six morphotypes were observed in the four samples, all belonging to the order *Oscillatoriales* (Table 1 and Table S3). Four of these were isolated in a pure culture using BG11 media. Three isolated strains, in unicyanobacterial cultures, were added to the BCCM/ULC Belgian collection of (sub)-polar cyanobacteria (http://bccm.belspo.be/about/ulc.php). The diacritical characters used for botanical species identification and their corresponding values are listed in Table 1.

No heterocystous or unicellular cyanobacteria were detected by microscopy in this study, although they have been observed in lakes located at lower latitudes (Taton *et al.*, 2006a; Verleyen *et al.*, 2010).

The isolated strains were characterized at the species level on the basis of their morphology. The two TM4 morphotypes (Fig. 2c and e) that could not be cultured, possibly because of their rareness, were detected only by microscopy in the environmental samples. The first was identified as *Phormidium crassior* (Fig. 2c). The small size of the second morphotype (Fig. 2e) only allowed assignment at the genus level as *Leptolyngbya* sp., because of its cell shape, thin sheaths and usually regularly twisted trichomes.

Molecular diversity

The complete 16S rRNA gene sequences plus ITS were obtained from the four strains isolated in this study (Tables 2 and 3). The 16S rRNA gene sequences from TM1, TM2 and TM3 analyzed in Discussion are reported from Hodgson *et al.* (2010) to allow for the comparison of the diversity found in Forlidas Pond and Lundström Lake. The analysis of the four clone libraries (TM1, TM2, TM3, TM4) characterized 264 clones (16S plus ITS) with an insert of the correct size (*c.* 1700 bp). To assign clones to taxonomic clusters, 100 clones chosen at random were partially sequenced (*E. coli* positions 364–1044) and corresponded to 1, 3, 2 and 1 OTUs for TM1, TM2, TM3 and TM4, respectively. The remaining 164 clones were studied by ARDRA (16S plus ITS) and belonged to seven different clusters based on the band patterns. At least one complete sequence from the clone libraries was determined for each OTU. In total, 12 different almost complete sequences were obtained for seven OTUs.

All TM1 sequences and ARDRA patterns were identical. It can be assumed that all the clones were identical, especially as the PCR product included the variable ITS (Taton *et al.*, 2003). For the TM2 sample, 12 different restriction patterns

Table 1. Diacritical characteristics of the cyanobacterial morphotypes in the samples, presence and correspondence with the OTUs

Morphospecies	Strain name	Isolation medium	Sheath	False branching	Cross-wall constriction	Necridic cell	Cell shape	Apical cell shape	Cell width (minimum–maximum) (μm)	Cell length (minimum–maximum) (μm)	Presence in samples*	OTU
A – *Leptolyngbya glacialis*	TM1ULC73†	BG11	+	–	+	–	c	r	1.2 (0.9–1.3)	1.7 (1.4–2.4)	TM1, TM2	16ST63
B – *Phormidium murrayi*	TM2ULC130†	BG11	–	+	–	+	c	c	4.9 (4.2–5.8)	9.5 (6.9–14.1)	TM2	16ST49
C – *Leptolyngbya* cf. *foveolarum*	TM2ULC129†	BG11	–	–	+	+	b	r	1.2 (1.1–1.3)	0.8 (0.7–1.0)	TM2, TM3	16ST80
	TM3FOS129											
D – *Phormidium crassior*	¶	–	+	–	–	+	b	r	8.3 (8.0–9.1)	NA	TM4	NA
E – *Phormidium priestleyi*	TM4LUS131	BG11	+	–	+	–	a	r	2.6 (2.2–3.0)	1.8 (1.2–2.3)	TM4	16ST10
F – *Leptolyngbya* sp.	¶	–	+	–	–	+	a, b	r	1.2	0.9	TM4	NA

*Observation of the morphotypes in the samples.
†Strains added to the BCCM/ULC Belgian collection of (sub)polar cyanobacteria.
¶, Morphospecies was detected in the environmental sample, but not isolated in culture; a, isodiametric; b, wider than long; c, longer than wide; r, rounded; c, calyptrate; NA, data were not available.

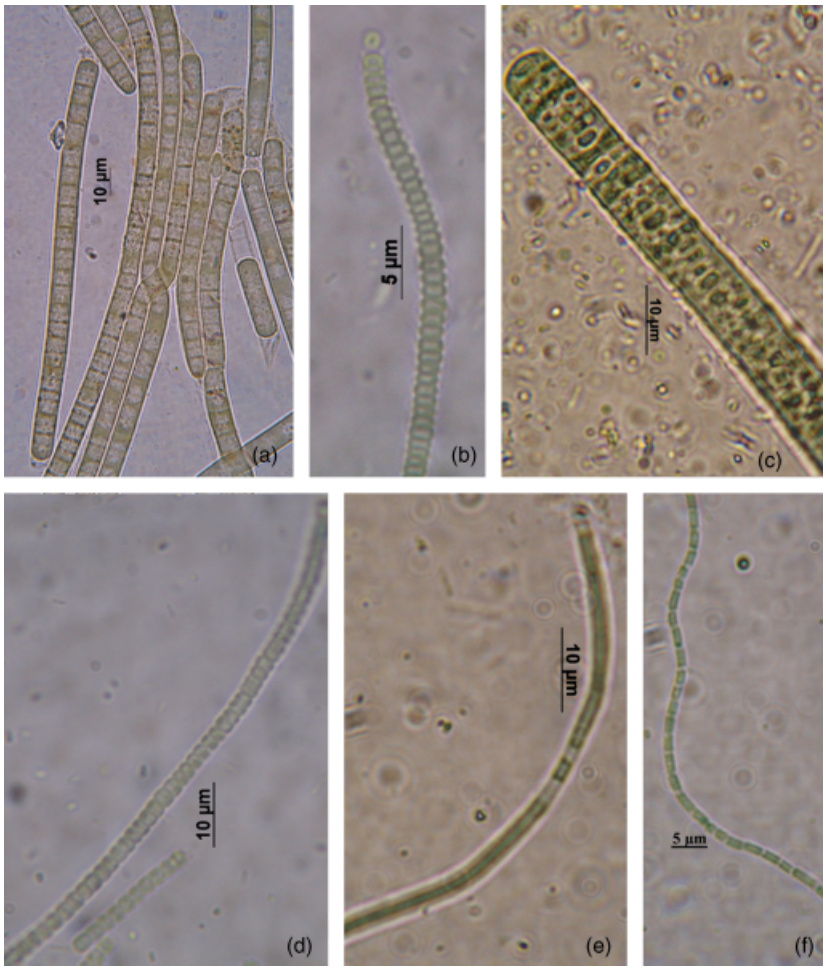

Fig. 2. Diversity of cyanobacterial morphotypes identified in environmental samples and cultures from Forlidas Pond and Lundström Lake. (a) *Phormidium murrayi* (TM2ULC130); (b) *Leptolyngbya* cf. *foveolarum* (TM2ULC129); (c) *Phormidium crassior*, TM4 environmental sample; (d) *Phormidium priestleyi* (TM4LUS131); and (e) *Leptolyngbya* sp., TM4 environmental sample; (f) *Leptolyngbya glacialis* (TM1ULC73).

were found by ARDRA. Eight were chimeras and only the clone TM2FOD1 belonged to a new genotype by comparison with the OTUs obtained by direct sequencing. For TM3, seven different ARDRA restriction patterns were obtained, but they were all chimeric. For TM4, the ARDRA analysis showed four different restriction patterns, but two of them were chimeric. Chimera analysis showed that chimerical sequences were absent from the TM1 sample, but accounted for 22% of the clones for TM2, 10% for TM3 and 3% for TM4. One of the TM4's chimeras contained a fragment derived from the OTU 16ST16, but this sequence is not shown in the distance tree (Fig. 3) as the fragment location (*E. coli* positions 811–1534) did not correspond to the positions used for the phylogenetic analysis.

The DGGE analysis of the sample TM4 revealed four OTUs (16ST02, 16ST44, 16ST53, 16ST92). In total, seven OTUs were retrieved from TM4, including clones, DGGE sequences and an isolated strain. The presence of OTUs from clone libraries, DGGE gels and isolated strains from all the samples is given in Table 4.

The clone library accumulation curves approached an asymptote (data not shown), indicating that it is unlikely that the diversity would be increased significantly if more clones were sequenced. In addition, the coverage index (Good, 1953) was 100% for TM1 and TM3 and $> 97.5\%$ for TM2 and TM4 (Table S2). The Berger–Parker index corroborated the low biodiversity, as it was > 0.94 for all the samples, except for TM2, where it was 0.43.

Distribution analysis

The 16S rRNA gene sequences from the cultures, the clone libraries and the DGGE were grouped into 12 OTUs, including the sequences from Forlidas Pond (Hodgson *et al.*, 2010) (Fig. 3). Five OTUs were only found in Forlidas Pond and its catchment (16ST07, 16ST14, 16ST49, 16ST63 and 16ST80), six were only present in Lundström Lake (16ST02, 16ST11, 16ST16, 16ST53, 16ST57 and 16ST92), while only one OTU (16ST44) was observed in both lakes.

Table 2. Analysis of the 16S rRNA gene sequences from Forlidas Pond (Hodgson *et al*., 2010) and Lundström Lake and the geographical location of OTUs

Selected sequences*	Accession number	Closest BLAST uncultured hit†	Closest BLAST strain hit†	OTUs	Origin of related sequences in Antarctica‡	Origin of related sequences outside Antarctica‡
TM4LUD1a3	HQ219059	Clone H-C30 (DQ181727) (99%)	*Synechococcus* sp. PCC 7502 (AF44808) (96%)	16ST02[A]	Fresh Pond, McMurdo Ice Shelf (AY541565); Lake Rauer7, Rauer Islands, (EU009706); Heart Lake, Larsemann Hills (DQ181726); Pup Lagoon (EU009721); and Lake Reid (DQ181712, EU009717), Larsemann Hills	–
TM3FODb1¶	EU852512	Clone ANTLV1_H06 (DQ521499) (100%)	*Leptolyngbya appalachiana* GSM-SFF-MF60 (EF429286) (93%)	16ST07[C]	Lakes Vida (DQ521499) and Fryxell (AY151728), McMurdo Dry Valleys; Lake Reid (DQ181673)	High Arctic microbial mat (FJ977124); Artic soil from ML-RS1 site (AM940881)
TM4LUS131‖	EU852504	Clone H-B07 (DQ181687) (98%)	*Phormidium priestleyi* ANT.LH66.1 (AY493581) (100%)	16ST11[C]	Lake in Larsemann Hills (AY493581); red snow, Langhovde (AB519661)	Soil sample in Svalbard, Norway (AM940913)
TM2FOCD1	EU852493	Clone LMM1-4 (EU032358) (97%)	*Phormidium priestleyi* ANT. L61.2 (AY493582) (92%)	16ST14[C]	Lake Miers, McMurdo Dry Valleys (EU032358)	Lake Tuusulanjarvi, Filand (AM259242); Bogotá river (Colombia) (EF111085); Shiga river biofilm, Japan (AB275532); Sedge Bay, Yellowstone Lake (United States) (EU340161); high Arctic microbial mat (FJ977133)
TM1FOD1b1	EU852508	Clone MIS92 (FJ977133) (99%)	cf. *Leptolyngbya* sp. Greenland (DQ431004) (89%)	16ST14[C]		
TM4LUCC30	EU852502	Clone LMM1-5 (EU032359) (98%)	*Phormidium* sp. SAG 37.90 (AM398795) (97%)	16ST16[C]	Lake Reid (DQ181681); Lake Miers (EU032359); Larsemann Hills (AY493576)	*Pseudanabaena tremula* (AF218371) isolated in Canada
TM2FOCH1	EU852489	Clone UMAB-cl-34 (FN811218) (98%)	*Microcoleus vaginatus* UBI-KK2 (EF654079) (99%)	16ST44[C]	Lake Fryxell (AY151749); Orange and Lunch Ponds, Bratina Island (AF263336, AF263335)	Douglas River, Australia (F438215); river Garonne (France) (AY456650); Canyonlands National Park (United States) (AF428550); Kansas (United States) (EU300529)
TM3FOCB5	EU852490	Clone UMAB-cl-34 (FN811218) (98%)	*Microcoleus vaginatus* UBI-KK2 (EF654079) (99%)	16ST44[C]		
TM4LUDa1¶	EU852514	DGGE gel band OTU_23 (FJ796382) (99%)	*Phormidium autumnale* CYN52 (GQ451424) (99%)	16ST44[C]		
TM2ULC130‖	EU852498	Clone AK4DE1 (GQ397048) (98%)	*Microcoleus vaginatus* UBI-KK2 (EF654079) (99%)	16ST44[C]		
TM3FODa4	HQ219058	Clone KuyT-ice-36 (EU263778) (100%)	*Phormidium* sp. Ant-Orange (AF263336) (100%)	16ST44[C]		
TM3FOCA2	EU852492	Clone A206 (DQ181671) (99%)	*Geitlerinema* sp. Sai004 (GU935348) (99%)	16ST49[C]	Lake Fryxell (AY151723, AY151768); Ace Lake,Vestfold Hills (DQ181671); Lake Firelight, Bølingen Islands (EU009679); Lake Manning, Larsemann Hills (EU009719)	Loa river, Chile (AF317510); freshwater bloom (Sweden) (AY874006); Andes Mountains (Bolivia) (EU728890); Great Sulphur Spring (United States) (FJ967937); Thermal spring (Australia) (EU106086)
TM2FOCA9	EU852500	Clone A206 (DQ181671) (99%)	*Geitlerinema* sp. Sai004 (GU935348) (99%)	16ST49[C]		
TM2FOCA2	EU852491	Clone A206 (DQ181671) (99%)	*Geitlerinema* sp. Sai004 (GU935348) (99%)	16ST49[C]		
TM4LUD2a3¶	HQ219060	Clone H0w-51 (EF632992) (98%)	*Lyngbya* sp. 0ES35S5 (DQ264199) (98%)	16ST53[C]	Lake Fryxell (AY151731, AY151724); benthic communities in King George Island (DQ533827)	Salar de Huasco (Chile) (AY151731); soil crusts of the Colorado Plateau (F428510); planktonic european cyanobacteria (DQ264199)
TM4LUCG9	EU852503	Clone RD017 (DQ181674) (100%)	*Leptolyngbya badia* CRS-1 (EF429297) (94%)	16ST57[c]	Lake Reid (DQ181675)	High mountain lake epilithic biofilm. Pyrenees (Spain) (FR667367)
TM1FOCA5	EU852494	Clone R8-R56 (DQ181691) (99%)		16ST63[C]	Ace Lake (AY493589); Lake Rauer 8 (DQ181691) and Salt Pond, McMurdo Ice Shelf (AY541528)	Salar de Huasco (EF633019); Shark Bay (Australia) (AY430152); agricultural soil (Spain) (AM503974);

Table 2. Continued.

Selected sequences*	Accession number	Closest BLAST uncultured hit†	Closest BLAST strain hit†	OTUs	Origin of related sequences in Antarctica‡	Origin of related sequences outside Antarctica‡
			Leptolyngbya antarctica ANT.ACEV6.1 (AY493589) (100%)			Bubano Basin (Italy) (AJ639892); Chaerhan Lake (Tibet) (HM127136); Lake Ferto (Hungary) (EU914882); Yellowstone National Park (United States) (AY790846)
TM1ULC73‖	EU852495	Clone R8-R56 (DQ181691) (99%)	*Leptolyngbya antarctica* ANT.ACEV6.1 (AY493589) (100%)	16ST63^{C}		
TM1FODa1	EU852507	Clone R8-R56 (DQ181691) (99%)	*Leptolyngbya antarctica* ANT.ACEV6.1 (AY493589) (100%)	16ST63^{C}		
TM2FOCH9	EU852499	Clone R8-R56 (DQ181691) (99%)	*Leptolyngbya antarctica* ANT.ACEV6.1 (AY493589) (99%)	16ST63^{C}		
TM2FOCF1	EU852517	ND	ND	16ST63^{C}		
TM2ULC129‖	EU852496	Clone GBII-52 (GQ441323) (98%)	*Leptolyngbya* sp. OU-6 (GQ162217) (98%)	16ST80^{C}	–	Intertidal beach (the Netherlands) (GQ441323)
TM3FOS129‖	EU852497	Clone GBII-52 (GQ441323) (98%)	*Leptolyngbya* sp. OU-6 (GQ162217) (98%)	16ST80^{C}		
TM4LUCF12	EU852505	Clone D1G12 16S (EU753634) (98%)	*Leptolyngbya badia CRS-1* (EF429297) (95%)	16ST92^{C}	–	Dry stromatolites, Ruidera Pools Natural Park (Spain) (EU753629); Rocky Mountain sandstone (United States) (EF522259)
TM4LUCE5	EU852501	Clone D1G12 16S (EU753634) (98%)	*Leptolyngbya badia CRS-1* (EF429297) (95%)	16ST92^{C}		
TM4LUCF10	EU852506	Clone D1G12 16S (EU753634) (98%)	*Leptolyngbya badia CRS-1* (EF429297) (95%)	16ST92^{C}		
TM4LUDb1	HQ219061	Clone D1F08 (EU753629) (99%)	*Pseudanabaena tremula* UTCC 471 (AF218371) (92%)	16ST92^{C}		
TM4LUDb3	HQ219062	Clone D1F08 (EU753629) (99%)	*Leptolyngbya* sp. CNP1-B1-4 (AY239603) (91%)	16ST92^{C}		
TM4LUDb5	HQ219057	Clone D1F08 (EU753629) (99%)	*Leptolyngbya* sp. CNP1-B1-4 (AY239603) (91%)	16ST92^{C}		
TM4LUD1b3	HQ219056	Clone D1F08 (EU753629) (99%)	*Leptolyngbya* sp. CNP1-B1-4 (AY239603) (91%)	16ST92^{C}		

*We selected more than one sequence per OTU if they came from different samples or using different molecular methods.

†BLAST analysis based on 16S rRNA gene sequences.

‡Selection of locations of related sequences from the same OTU. OTUs superscripts are A for potentially endemic to Antarctica and C for cosmopolitan.

¶Only sequenced in one direction.

‖The sequence belongs to a strain.

Table 3. Summary of ITS sequences obtained by clone library

ITS sequences	Accession numbers	ITS types	Closest BLAST hit (similarity %)
TM1FOCA5	EU852516	ITS02	Clone R8-R56 (DQ181761) (98%)
TM1ULC73*	EU852528	ITS02	
TM2FOCH9	EU852532	ITS02	
TM2FOCF1	EU852517	ITS02	
TM3FOCA2	EU852521	ITS23	Cyanobacterium clone A206 (DQ181751) (99%)
TM2FOCA9	EU852522	ITS23	
TM2ULC129*	EU852531	ITS26	*Oscillatoriales* cyanobacterium OU4 (GQ162321) (79%)
TM3FOS129*	EU852530	ITS26	
TM2FOCD1	EU852523	ITS27	Clone Qiyi-cya-OTU 0 (AB569623) (89%)
TM2FOCH1	EU852518	ITS27	
TM3FOCB5	EU852519	ITS27	
TM2ULC130*	EU852529	ITS27	
TM4LUCG9	EU852526	ITS28	Clone LMM1-21 (EU032366) (99%)
TM4LUCC30	HQ336421	ITS29	*Leptolyngbya* sp. LLi18 (DQ786166) (77%)
TM4LUCF12	EU852524	ITS29	Cyanobacterium clone LMM1-11 (EU032362) (79%)
TM4LUCF10	EU852525	ITS29	Cyanobacterium clone LMM1-11 (EU032362) (99%)
TM4LUS131*	EU852527	ITS30	Cyanobacterium clone LMM1-18 (EU032363) (89%)

*The sequence belongs to a strain.

The sequences exhibited 97–100% sequence identity with their highest matches found by BLAST analysis. Eleven out of 12 OTUs showed a cosmopolitan distribution while the remaining OTU, 16ST02 from Lundström Lake, is potentially endemic to Antarctica (Table 2).

ITS analysis

Seventeen ITS sequences were obtained from the eight OTUs found by clone library or in the isolated strains (Table 3). The 17 ITS sequences were distributed into seven ITS types (Table 5). Only ITS sequences from related organisms can be meaningfully aligned because this spacer is highly variable (Wilmotte *et al.*, 1994). In each OTU based on 16S rRNA gene, one or two ITS types were detected. The $tRNA^{Ala}$ and $tRNA^{Ile}$ were present in all the ITS types. The aligned ITS types showed 75–100% similarity (indels were taken into account). Ten ITS sequences, from OTUs 16ST44, 16ST49 and 16ST63, corresponded to ITS types (ITS02, ITS23 and 27) already reported by Taton *et al.* (2006a). The ITS types ITS02, ITS23, ITS27, ITS29 and ITS30 were divided into subgroups when they contained sequences that were $< 96\%$ similar and formed clear subgroups (Table 5 and Fig. S2).

Discussion

Methodological considerations

The analysis of the OTUs recorded in the three samples from Forlidas Pond (Hodgson *et al.*, 2010) and one sample from Lundström Lake identified 12 OTUs. Three OTUs were only detected by DGGE, two only by clone library and two only by sequencing the isolates obtained (Table 4). The sequences from the strains *Phormidium priestleyi* (TM2ULC129) and *Leptolyngbya* cf. *foveolarum* (TM4LUS131) form two OTUs not retrieved by clone libraries or DGGE (Table 4). The high sensitivity level of the molecular detection could imply that these two morphotypes were rare in the environmental samples. It is a common observation that 16S rRNA genes retrieved directly from environmental samples rarely match those of cultivated strains or are distantly related (Nübel *et al.*, 2000). This emphasizes the complementary nature and advantage of applying both approaches.

In TM1, only one of the three OTUs was detected by clone library. In TM3, two OTUs were not detected by clone library, but by the DGGE method. Similarly, a higher number of OTUs were detected by DGGE in TM4 (Table 4). Although clone libraries are generally expected to reveal a higher diversity than DGGE, this was not observed here. This could be due to the number of sequenced clones and the use of two PCRs (semi-nested PCR) with two different primers specific for cyanobacteria [16S781R(GC) (a) and (b)] in the DGGE, whereas only one PCR was used with the primers 16S378F–23S30R for the clone library. In addition, the ARDRA analyses used to screen the clone libraries could underestimate the diversity (Cho & Tiedje, 2000).

Phylogenetic resolution and biogeographical patterns

Comparison of our sequences with the ITS sequences available in GenBank revealed that, within the same OTU, different ITS sequences could be found that were too different to be aligned. For instance, the 16S rRNA gene sequence of the clone TM4LUCG9 belonged to the OTU 16ST57 and its corresponding ITS to the type ITS28. In GenBank, only three ITS sequences corresponding to the

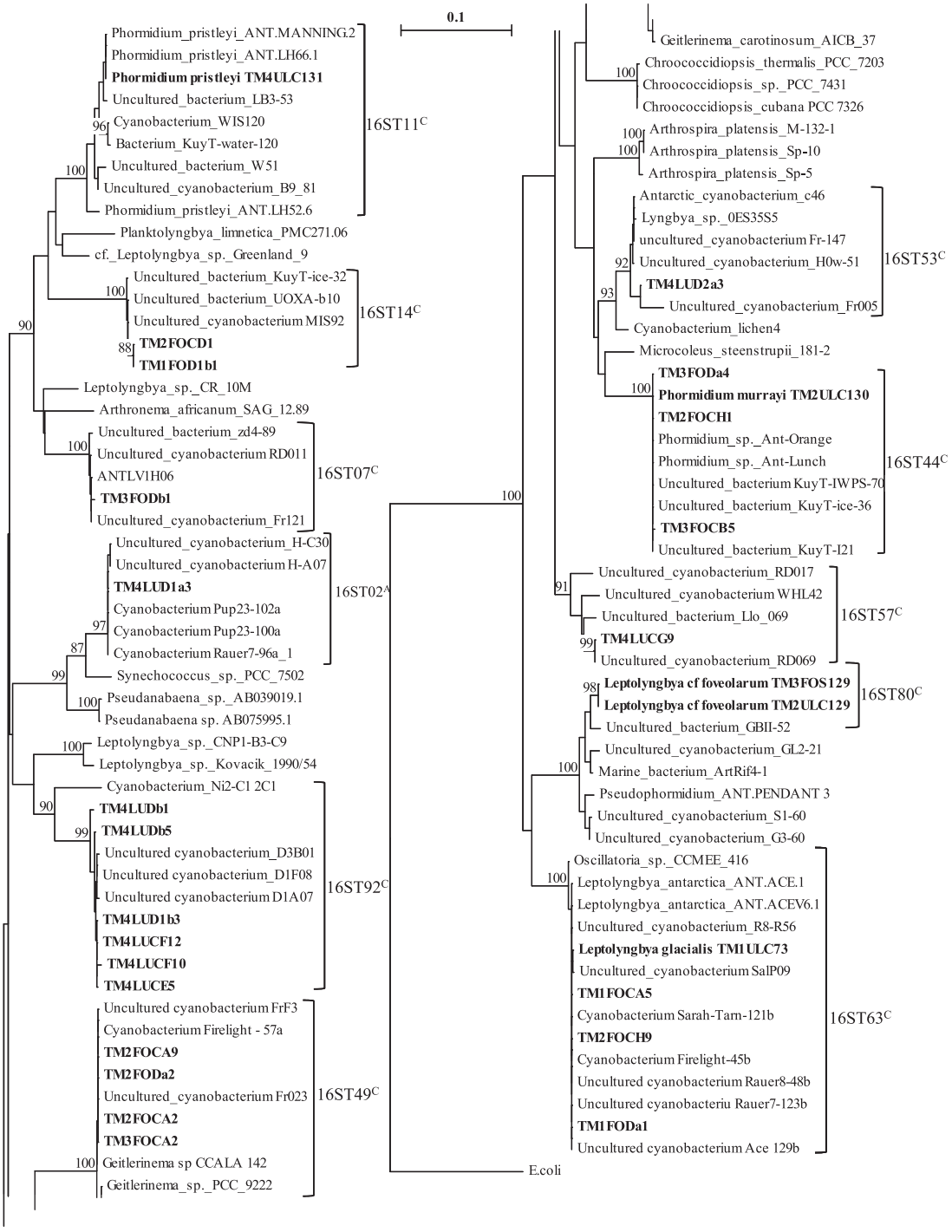

Fig. 3. Distance tree based on cyanobacterial partial 16S rRNA gene sequences (*Escherichia coli* positions 379–806) constructed using the neighbor-joining method (Saitou & Nei, 1987). A bootstrap analysis was performed that involved the construction of 1000 resampled trees (values indicated at the node). The tree comprised the sequences of 10 DGGE bands, 12 clones and five strains from the Forlidas Pond samples (from Hodgson *et al*., 2010) and Lundström Lake (both in bold) and their three most similar strain sequences and three uncultured sequences from RDPII (http://rdp.cme.msu.edu). *Escherichia coli* sequence is used as an outgroup. The OTU numbers are indicated on the right. OTU 16ST16 composed by TM4LUCC30 is not shown in the tree because the fragment of this sequence did not include the positions used for the construction of the tree. The evolutionary distance between two sequences is obtained by adding the lengths of the horizontal branches connecting them and using the scale bar (0.1 mutation per position). The superscripts in the OTUs numbering mean: 'C' for cosmopolitan distribution and 'A' for potentially endemic to Antarctica.

Table 4. Presence/absence of OTUs from clone libraries, DGGE gels and isolated strains

	16ST02	16ST07	16ST11	16ST14	16ST16	16ST44	16ST49	16ST53	16ST57	16ST63	16ST80	16ST92
TM1*				D			D			C+S+D		
TM2*				C		C+S+D	C+D			C+D	S	
TM3*		D				C+D	C+D			D	S	
TM4	D		S		C	D		D	C			C+D

*The 16S rRNA gene sequences from these samples have been obtained from Hodgson *et al*. (2010).

The presence of OTUs in the samples is indicated by dark shading. The origins of the sequences from an OTU in a sample are: D, DGGE; C, clone library; and S, isolated strain.

16S rRNA gene sequences from OTU 16ST57 were available and only one sequence (EU032366) belonged to the ITS28. The other two ITS sequences could not be aligned with this ITS type and were isolated from Lake Reid (Larsemann Hills, East Antarctica).

The study of the ITS clearly increases the phylogenetic resolution provided by the 16S rRNA genes. The sequences of TM2ULC129 and TM3FOS129 showed 97.3% 16S rRNA gene similarity to *Leptolyngbya* sp. ANT.LH52 (AY493584), but they were included in a new OTU (16ST80). Indeed, the ITS sequences from both strains were identical, but clearly not related to the *Leptolyngbya* sp. ANT.LH52 sequence. This emphasizes that, although they were at the limit of the OTU definition, they were distinct and, therefore, the ITS supported the creation of the new OTU 16ST80. The ITS analysis also confirmed that the same *Phormidium autumnale* population was present both in the terrestrial and in the aquatic samples. Indeed, two sequences from the Forlidas Pond shoreline water sample (TM2FOCH1 and TM2ULC130) and one sequence from the terrestrial mat (TM3FOCB5) shared identical 16S rRNA gene and ITS sequences (Fig. 3 and alignment in Fig. S2).

The distribution pattern of the ITS could be linked to the different physicochemical parameters or the distinct geographical origin of the samples. For instance, ITS02, which was divided into four subgroups, contained sequences from TM1 and TM2 (Forlidas Pond) and Lakes Ace (DQ181748, AY493632, AY493633) and Rauer 8 (DQ181759, DQ181761, DQ181762) (Vestfold Hills and Rauer Islands, respectively). These lakes are quite different, Lakes Rauer 8 and Ace being hyposaline (6.26 and 25.9 mS cm^{-1}, respectively) while the TM1 and TM2 sequences were isolated from hypersaline and oligosaline samples (142.37 and 0.227 mS cm^{-1}, respectively) (Table S1). The ITS27 is divided into two subgroups. The first contains sequences from Forlidas Pond and its catchment while the second is composed by sequences from Lake Fryxell (AF547634, AF547628), a brackish meromitic lake sampled in its freshwater moat (Taton *et al*., 2003). The physicochemical parameters differ considerably between these lakes and appear to coincide with the ITS subgroups. A positive correlation with local factors can be observed for ITS28, which contains two sequences (99.6% pairwise identity) originating from distant lakes sharing similar environmental parameters. The sequence EU032366 was isolated from Lake Miers (Miers Valley, Southern Victoria Land) while TM4LUCG9 comes from Lundström Lake. Both samples were taken at the shoreline and their physicochemical parameters were similar (Bell, 1967).

The ITS sequences recorded in Antarctica appear to be more similar among themselves than to non-Antarctic ITS. The type ITS23 contained two sequences from samples TM2 (TM2FOCH1) and TM3 (TM3FOCA9), which were related to five sequences from two Antarctic lakes (Ace Lake and Lake Fryxell, in the Vestfold Hills and McMurdo Dry Valleys, respectively) and to a strain (*Geitlerinema carotinosum* AICB 37; AY423710) isolated in Banloc (Romania). The Antarctic ITS sequences from ITS23 were 98.7% similar between themselves, but only 93% similar to the non-Antarctic ITS sequences. Similarly, only two Antarctic ITS from Lake Fryxell could be aligned within ITS27. They correspond to the OTU 16ST44, for which non-Antarctic sequences are available (16S and ITS), although these ITS were too dissimilar to be aligned into ITS27 (e.g. AM778715, EF18274). However, the low number of ITS sequences in the databases impedes a deeper analysis and further studies are required.

Impoverished diversity

The autotrophs recorded in the Dufek Massif appear limited to cyanobacteria and a few green algae and lichens. In addition, this area has been reported to harbor the most reduced metazoan terrestrial and freshwater ecosystems known from Antarctica (Hodgson *et al*., 2010). The only previous description of the ecology in this area was given by Neuburg *et al*. (1959). During this first visit to the region in the International Geophysical Year (1957/1958), some vegetation was collected from Forlidas Pond for microscopic observation and identified as '*Phormidium incrustatum* and possibly *Phormidium retzii*' (G. Llano & G. Prescott, pers. commun.). The fact that we did not find these species probably results from the different approach rather than a change in the biodiversity (Hodgson *et al*., 2010). The description of *P. retzii* could correspond to the morphotype illustrated in

Table 5. ITS types and subgroups present in the clone libraries and isolated strains

ITS type	ITS02		ITS23		ITS26	ITS27	ITS28	ITS29		ITS30
ITS sequences*	Subgroups	TM1ULC73†	Subgroups	TM3FOCA2	TM3FOS129†	TM2FOCH1	TM4LUCG9	Subgroups	TM4LUCC30	TM4ULC131
		TM1FOCA5		TM2FOCA9	TM2ULC129†	TM2FOCD1	EU032366			
		TM2FOCF1		DQ181751		TM3FOCB5				
		TM2FOCH9		AF547633		TM2ULC130†				
				AF547632						
				AF547630						
				AF547626					TM4LUCF10	EU032363
		DQ181759								EU032367
		DQ181761								
		AY493632		AY423710		AF547634			TM4LUCF12	DQ181755
		AY493633				AF547628				
		DQ181762								
		DQ181748								
Corresponding OTU‡	16ST63		16ST49		16ST80	16ST44	16ST57	16ST16 and 16ST92		16ST11

*The ITS sequences from Forlidas Pond, Lundström Lake and GenBank sequences that could be meaningfully aligned.
†Strains.
‡The OTU corresponding to the ITS sequences obtained in this study.
Sequences in the same types shared at least 75% pairwise identity. The OTUs based on 16S rRNA gene and corresponding to the ITS types are indicated.

Fig. 2b, but we identify this morphotype as *P. crassior* because of its ecology and the absence of truncated end cells, a very characteristic feature in the literature. Similarly, *P. incrustatum* shares several characteristics with the morphotype illustrated in Fig. 2b, but it has a narrower cell width.

Our data show a quite impoverished cyanobacterial diversity in Forlidas Pond (5 OTUs) and Lundström Lake (7 OTUs) compared with lakes in the coastal regions of Antarctica. Indeed, using the same methodology, we calculated an average of 8.5 OTUs in lakes of the McMurdo Dry Valleys and other sites in East Antarctica, ranging from 4 OTUs for Lake Rauer 8 (Rauer Islands, East Antarctica) to 15 OTUs in Lake Fryxell (McMurdo Dry Valleys) (Taton *et al.*, 2003, 2006a). Sample TM1 comes from a hypersaline brine, with a salinity four times that of seawater. Hypersaline conditions, in addition to the other extreme physical and chemical conditions present at these latitudes, are likely to account for the particularly low biodiversity (3 OTUs) in this sample.

Forlidas Pond and Lundström Lake shared only one OTU (16ST44). The high salinity of Forlidas Pond suggests a long history of evaporation in this lake. In contrast, Lundström Lake has a low salinity, with only a slight increase at the base of the water column (Table S1). Because of their different evaporation history and geomorphology, we can infer that these lakes have also been subject to different ecological processes, and this could be one reason for the differences in diversity. Moreover, geomorphological features that separate the two lakes, such as the Support Force Glacier, Argentina Range and Recovery Glacier, and the 420 km distance between them, could also play a role.

Spatial distribution

Four OTUs found in Forlidas Pond were also present in terrestrial habitats in its catchment (Fig. S3), showing that these taxa could inhabit both aquatic and terrestrial biotopes (Gordon *et al.*, 2000). Although some terrestrial mats near melting snow were damp, most terrestrial mats were completely dry at the time of sampling. It seems likely that the terrestrial mats were once growing on the bottom of the lake before its size began to shrink, and that they now survive due to water sourced from seasonally melting snow patches (Hodgson *et al.*, 2010). The ITS distribution shows a similar spatial distribution as observed by analysis of the 16S rRNA gene sequences (Fig. S4). Lundström Lake's ITS sequences are not shared with any sample from the Forlidas Valley while one ITS type is shared between the TM1 and the TM2 samples (ITS02) and three ITS types are shared between the TM2 and the TM3 samples (ITS23, ITS26, ITS27). In addition, when our ITS sequences were compared with the ITS sequences from GenBank, we observed that the intra-Antarctic variability was smaller than the differences between the Antarctic ITS and those of other continents.

Numerous terrestrial mats were present around Forlidas Pond and in the adjacent Davis Valley. We only found *Leptolyngbya*, *Geitlerinema* and *Phormidium* sp. in the sample taken from one of these terrestrial mats (TM3). Because of the large size of the mat, we would also expect to find ensheathed filamentous cyanobacteria producing large amounts of exopolysaccharides (i.e. *Coleodesmium* or *Stigonema*). However, the main cyanobacteria responsible for the production of these macroscopic structures remain uncertain, as *Leptolyngbya* are too small and rare in the samples, *Geitlerinema* does not possess sheaths and *Phormidium* produce only thin sheaths. Nevertheless, it is known that meshes of filaments of *P. autumnale* can provide physical support and protection against desiccation for other algae, mainly associated cyanobacterial filaments and diatoms (Wynn-Williams, 1996). Moreover, the importance of its soil-binding role for the physical structure of the community has been shown before (Mataloni *et al.*, 2000).

Endemism

The results of this study support the hypothesis that, in the more inhospitable regions of continental Antarctica, cyanobacterial communities are characterized by a low biodiversity, taxa tolerant of extreme cold and dry conditions, and marginal endemism (Hodgson *et al.*, 2010). Specifically, samples from Forlidas Pond contained only cosmopolitan OTUs while Lundström Lake included one potential Antarctic endemic OTU (16ST02). However, the 16S rRNA gene marker is highly conserved and might underestimate the phenomenon of endemism, as shown by Cho & Tiedje (2000). Cosmopolitan OTUs must possess features enabling the processes of dispersal, colonization and establishment between different continents and, thus, possess resistance capacities that could explain their presence in the two locations studied here. The higher colonization rates (Hodgson *et al.*, 2010 and references therein) attributed to cosmopolitan OTUs could also explain their abundance. However, Antarctic endemic OTUs will also have well-developed resistance adaptations, being recorded across distant locations within the Antarctic continent.

The harsh environmental conditions, together with geographical isolation, could explain the marginal endemism found in Lundström Lake (Vyverman *et al.*, 2007; Souza *et al.*, 2008; Hodgson *et al.*, 2010). The unique OTU from this study, which was exclusively recorded in Antarctica (OTU 16ST02), has been recorded in two very distant locations (*c.* 2600 km apart): in the Larsemann Hills (DQ181726) (East Antarctica) (Taton *et al.*, 2006a) and on the McMurdo Ice Shelf (AY541565) (Jungblut *et al.*, 2005). It is possible that OTU 16ST02 present in Lundström Lake originated in one of these Antarctic locations (or *vice versa*). However, we cannot definitively state that this OTU is not present in other (including temperate) regions, but is as yet unrecorded (Martiny *et al.*, 2006). Therefore, its description as endemic should be considered with caution. Other cyanobacterial sequences previously considered as endemic in 2006 are now known to be cosmopolitan, as new sequences have been added to the databases (e.g. OTUs 16ST07, 16ST11, 16ST14, 16ST16, 16ST53 in this study). We therefore use the term 'potentially endemic'. The ITS types defined in this study, except ITS23, are all potentially endemic to Antarctica, in contrast to the corresponding OTUs (based on 16S rRNA gene), which were all cosmopolitan. This is in agreement with the conclusion of Cho & Tiedje (2000), who showed higher endemicity levels for ITS than for 16S rRNA gene sequences. However, the ITS database remains fragmentary. As these databases expand, future research in inland continental Antarctica will shed more light on the geographical distribution and evolutionary isolation of cyanobacteria in these extreme habitats.

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Supporting Information

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

Fig. S1. Photographs of the sampling locations, Forlidas Pond (a) and Lundström Lake (b).
Fig. S2. Alignment of the ITS sequences from the Forlidas Pond and Lundström Lake environmental samples and their closest relatives found by BLAST analysis.
Fig. S3. Spatial distribution of cyanobacterial OTUs in Forlidas Valley.
Fig. S4. Spatial distribution of ITS groups observed with the clone libraries of samples from the Forlidas Valley and Lundström Lake.
Table S1. Water chemistry of Forlidas Pond and Lundström Lake.
Table S2. Molecular diversity summary and richness indices.
Table S3. Definition of morphospecies described in this study.

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