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Research Article

RESEARCH ARTICLE

Community structure and distribution of benthic cyanobacteria in Antarctic lacustrine microbial mats

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One sentence summary: Benthic cyanobacterial communities in Antarctic lakes are mostly structured by lake conductivity.

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ABSTRACT

The terrestrial Antarctic Realm has recently been divided into 16 Antarctic Conservation Biogeographic Regions (ACBRs) based on environmental properties and the distribution of biota. Despite their prominent role in the primary production and nutrient cycling in Antarctic lakes, cyanobacteria were only poorly represented in the biological dataset used to delineate these ACBRs. Here, we provide a first high-throughput sequencing insight into the spatial distribution of benthic cyanobacterial communities in Antarctic lakes located in four distinct, geographically distant ACBRs and covering a range of limnological conditions. Cyanobacterial community structure differed between saline and freshwater lakes. No clear bioregionalization was observed, as clusters of community similarity encompassed lakes from distinct ACBRs. Most phylotypes (77.0%) were related to cyanobacterial lineages (defined at ≥99.0% 16S rRNA gene sequence similarity) restricted to the cold biosphere, including lineages potentially endemic to Antarctica (55.4%). The latter were generally rare and restricted to a small number of lakes, while more ubiquitous phylotypes were generally abundant and present in different ACBRs. These results point to a widespread distribution of some cosmopolitan cyanobacterial phylotypes across the different Antarctic ice-free regions, but also suggest the existence of dispersal barriers both within and between Antarctica and the other continents.

Keywords: Antarctica; bioregionalization; cosmopolitanism; endemism; microbial community ecology; cyanobacteria; high-throughput sequencing; lakes

INTRODUCTION

Terrestrial life in Antarctica is largely restricted to sparse ice-free areas such as coastal oases, inland nunataks and mountain ranges, which make up only *ca.* 0.34% of the continent's surface area (Convey *et al.* 2008). Recent studies have revealed a relatively high incidence of endemism and clear bioregionalization patterns in various taxonomic groups in these terrestrial ecosystems, with some taxa being restricted to specific ice-free regions (Convey *et al.* 2008; De Wever *et al.* 2009; Vyverman *et al.* 2010). These patterns have been attributed to the long-term isolation of the continent from other landmasses, dispersal limitation between isolated ice-free regions and the survival of a number of well-adapted organisms in ice-free refugia during past glaciations (Fraser *et al.* 2014). These findings have led to an increased awareness regarding the need to protect Antarctic ecosystems against environmental perturbations, the introduction of alien

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species and the transportation of taxa between the isolated ice-free regions (Cowan *et al.* 2011; Chown *et al.* 2015). The most up-to-date framework for protecting Antarctic terrestrial life is represented by the Antarctic Conservation Biogeographic Regions (ACBRs; Terauds *et al.* 2012; Terauds and Lee 2016). These biogeographic provinces were identified based on environmental domains in combination with expert consultation and multivariate analyses of biological datasets. In total, 16 ACBRs have been recognized (Terauds and Lee 2016).

Lakes are found in the vast majority of these ACBRs and are of crucial importance for conservational purposes for several reasons. First, Antarctic lakes are highly sensitive to climate-induced environmental change (Quayle *et al.* 2002), as deglaciation of snow banks in the catchment area and reductions in snow and lake ice cover have a profound effect on nutrient concentration, moisture balance, water temperature and light climate, which in turn affect the lake biota (Verleyen *et al.* 2012). Moreover, the amplitude of climate change appears variable across Antarctic regions, with the Antarctic Peninsula and, to a lesser extent, West Antarctica showing a clear warming trend, while temperature variability during the past decades has been modest on the remainder of the continent (Steig *et al.* 2009; Turner *et al.* 2014; Lee *et al.* 2017). Second, lakes are hotspots of biodiversity in the cold desert biomes of the Antarctic continent (Verleyen *et al.* 2012). This is mainly related to the influence of lake water and ice cover, which provide a buffer against climatic extremes and thus result in favorable habitats for a diverse range of organisms (Vincent 2004; Laybourn-Parry and Wadham 2014). Moreover, Antarctic lakes encompass a high diversity of limnological conditions ranging from freshwater to hypersaline, from small and ephemeral water bodies to large deep and perennial systems, and from seasonally ice-free to permanently ice-covered (Laybourn-Parry and Wadham 2014). This in turn leads to the availability of a wide range of ecological niches that are occupied by relatively diverse food webs in comparison to terrestrial ecosystems (Laybourn-Parry and Pearce 2007; Verleyen *et al.* 2010, 2012). Third, Antarctic lakes are among the most productive ecosystems in terrestrial Antarctica, and, as such, are an important source of organic carbon and nutrients for the ultra-oligotrophic surrounding soils (Priscu *et al.* 1999; Hopkins *et al.* 2006).

Most terrestrial life in Antarctica, including that in lacustrine ecosystems, is microbial (Wynn-Williams 1996; Laybourn-Parry and Pearce 2007). Cyanobacteria are ubiquitous members of Antarctic lake ecosystems, where they characteristically form diverse and conspicuous microbial mat communities in the lake benthos (Vincent and Quesada 2012). Cyanobacteria appear adapted to the environmental conditions found in Antarctic lakes and are the main drivers of organic matter accumulation in these ecosystems (Singh and Elster 2007; Vincent and Quesada 2012). Despite their major role as the main primary producers, the diversity and biogeographic distribution of cyanobacteria in Antarctic lakes are relatively poorly understood since most studies have been confined to one or few Antarctic regions (Gibson *et al.* 2006). Moreover, the current census of cyanobacterial diversity in Antarctica remains considerably incomplete due to technical limitations. The large majority of investigations carried out so far have applied microscopy, culturing and/or traditional molecular ecology approaches such as cloning and denaturing gradient gel electrophoresis. These studies have shown that Antarctic lacustrine microbial mats are dominated by filamentous cyanobacteria from the genera *Leptolyngbya*, *Phormidium*, *Microcoleus* and *Oscillatoria* (Hodgson *et al.* 2004; Taton *et al.* 2006a,b; Taton, Hoffman and Wilmotte 2008; Fernandez-Carazo *et al.* 2011; Michaud, Šabacká and Priscu 2012). Moreover, studies have shown that salinity, pH, lake water depth and related factors such as the light climate (PAR and UVR) and physical stresses imposed by lake ice cover are important drivers of cyanobacterial community structure in Antarctic lakes (Hodgson *et al.* 2004; Sabbe *et al.* 2004; Jungblut *et al.* 2005; Verleyen *et al.* 2005, 2010). Nonetheless, while microscopy-based investigations are limited due to the relatively simple morphology of many cyanobacterial taxa which hides a large genetic diversity (Wilmotte and Golubić 1991), traditional molecular methods only give information on the dominant members of the communities (Shokralla *et al.* 2012). High-throughput sequencing (HTS) of 16S rRNA gene amplicons using cyanobacteria-specific primers has been recently shown as a valuable tool for studying Antarctic cyanobacterial communities as it is also able to detect less dominant taxa (Kleinteich *et al.* 2014; Pushkareva *et al.* 2015; Pessi *et al.* 2016; Pushkareva *et al.* 2018). In addition, in studies dealing with the investigation of a given microbial group rather than the whole microbial community, the use of specific primers is preferred over universal ones, as the latter can lead to the underrepresentation of the group of interest (Klindworth *et al.* 2013).

Here, we used 454 pyrosequencing of partial 16S rRNA gene sequences to investigate the composition of benthic cyanobacterial communities in 13 Antarctic lakes with different limnological properties and located in four distinct and geographically distant ACBRs. We aimed to assess (i) the spatial patterns of cyanobacterial community structure across Antarctic lakes; (ii) the correlation between environmental factors and cyanobacterial community structure; and (iii) the geographic span at a global scale of the cyanobacterial lineages observed. The latter was carried out via comparison with sequences available in public databases.

MATERIALS AND METHODS

Study area and sampling

The studied lakes are distributed across eight Antarctic regions belonging to four distinct ACBRs (Fig. 1 and Table 1). Col Lake is located in Pourquoi-Pas Island, an island off the west coast of Palmer Land (Antarctic Peninsula) and belongs to ACBR1 'NW Antarctic Peninsula' (AP). Lakes Higashi and Nishi (West Ongul), Taratine (East Ongul), Akebi (Langhovde) and Mago (Skarvsnes) are located in Lützow-Holm Bay, a 100-km ice-free coastline in ACBR5 'Enderby Land' (EL) (Kimura *et al.* 2010; Verleyen *et al.* 2017). Lakes L59b (Larsemann Hills), Rauer2 (Rauer Islands), Highway and Waterfall (Vestfold Hills) are located in Prydz Bay, Princess Elizabeth Land and belong to ACBR7 'East Antarctica' (EA) (Roberts and McMinn 1996; Hodgson, Vyverman and Sabbe 2001a; Hodgson, Noon and Vyverman 2001b). Lake M5, also in ACBR7 (EA), is located in the Bunger Hills (Queen Mary Land) (Kaup, Haendel and Vaikmäe 1993). Forlidas Pond and Lundström Lake are located in the Dufek Massif and the Shackleton Range, respectively (Hodgson *et al.* 2010; Fernandez-Carazo *et al.* 2011). Both lakes are located within ACBR10 'Transantarctic Mountains' (TM).

Benthic microbial mats were sampled during the period 1997–2007 (Table 1). While we are aware that this is a relatively long time window, we are confident that potential environmental changes in the studied lakes were relatively small and have not profoundly affected the cyanobacterial communities therein. Indeed, except for Col Lake, all water bodies are situated in the East Antarctic and Transantarctic Mountains

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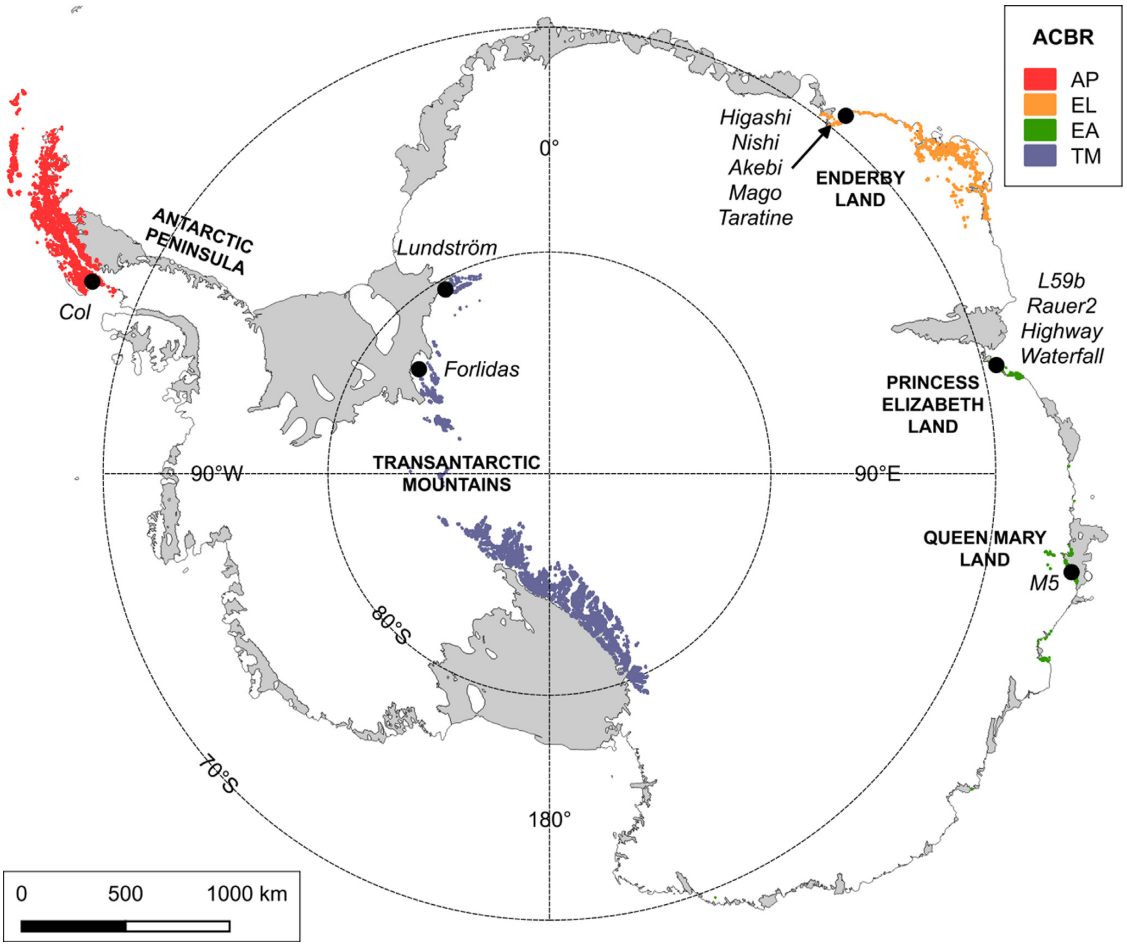

Figure 1. Map of Antarctica showing the studied lakes and their location within each Antarctic Conservation Biogeographic Region (ACBR). Map generated using the Quantarctica QGIS package (Norwegian Polar Institute, Tromsø, Norway) and ACBR data from the Australian Antarctic Data Centre (Kingston, Australia).

Table 1. Geographic information on the studied Antarctic lakes and their location within each Antarctic Conservation Biogeographic Region (ACBR).

Lake	Region	ACBR	Latitude	Longitude	Sampling year
Col	Pourquoi-Pas Island, Palmer Land	AP	67°50′ S	67°14′ W	2002
Higashi	West Ongul, Enderby Land	EL	69°01′ S	39°34′ E	2007
Nishi	West Ongul, Enderby Land	EL	69°01′ S	39°31′ E	2007
Taratine	East Ongul, Enderby Land	EL	69°01′ S	39°35′ E	2007
Akebi	Langhovde, Enderby Land	EL	69°12′ S	39°39′ E	2007
Mago	Skarvsnes, Enderby Land	EL	69°28′ S	39°38′ E	2007
L59b	Larsemann Hills, Princess Elizabeth Land	EA	69°24′ S	76°21′ E	1997
Rauer2	Rauer Islands, Princess Elizabeth Land	EA	68°48′ S	77°51′ E	1997
Highway	Vestfold Hills, Princess Elizabeth Land	EA	68°28′ S	78°11′ E	1997
Waterfall	Vestfold Hills, Princess Elizabeth Land	EA	68°33′ S	78°20′ E	1997
M5	Bunger Hills, Queen Mary Land	EA	66°17′ S	100°41′ E	1997
Forlidas	Dufek Massif, Pensacola Mountains	TM	82°27′ S	51°21′ W	2003
Lundström	Shackleton Range, Coats Land	TM	80°27′ S	29°29′ W	2003

AP: ACBR1 'NW Antarctic Peninsula'.
EL: ACBR5 'Enderby Land'.
EA: ACBR7 'East Antarctica'.
TM: ACBR10 'Transantarctic Mountains'.

regions, where temperature and environmental changes in the past decades have been relatively modest compared with the Antarctic Peninsula (Steig *et al.* 2009; Turner *et al.* 2014; Lee *et al.* 2017).

In Mago Lake, surface sediments were sampled at a depth of 5.8 m using a UWITEC gravity corer (UWITEC, Mondsee, Austria). In the remaining lakes, samples were taken in the littoral zone

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at *ca*. 20 cm depth and *ca*. 40 cm from the lake edge, by manually collecting microbial mats attached to rocks and gravel. All samples were kept frozen (−20°C) until analysis. Conductivity and pH were measured in the field using a YSI 600 water quality meter (Xylem Analytics, Beverly, MA, USA). Water samples for chemical analysis were collected in sterile Nalgene bottles and kept frozen (−20°C) until analysis immediately upon return to the laboratory. A detailed description of the field measurements, sampling procedures and analytical techniques used for the chemical characterization of the lake water can be found in Verleyen *et al*. (2012) and Tavernier *et al*. (2014).

DNA extraction, PCR and sequencing

Pyrosequencing analyses were carried out according to the study of Pessi *et al*. (2016), from which data for lakes L59b, Rauer2, Highway, Forlidas and Lundström were retrieved. The remaining samples were processed using the same experimental procedures. Briefly, DNA was extracted using the PowerSoil DNA Isolation Kit (MOBIO, Carlsbad, CA, USA) and partial (V3–V4 region) 16S rRNA gene sequences were obtained by PCR using the cyanobacteria-specific primers CYA359F and CYA781Ra/CYA781Rb (Nübel, Garcia-Pichel and Muyzer 1997). Six independent PCR reactions were pooled for each sample (three for each variation of the reverse primer), purified using the GeneJet PCR Purification Kit (ThermoScientific, Waltham, MA, USA) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). Libraries were pooled in equimolar concentrations and sent to Beckman Coulter Genomics (Danvers, MA, USA), where sequencing adapters were ligated to the amplicons and sequencing was performed using the 454 GS FLX + Titanium platform (454 Life Sciences, Branford, CT, USA).

Bioinformatics procedures

Quality control of reads, removal of chimeric sequences and operational taxonomic unit (OTU) clustering were performed using UPARSE (Edgar 2013) following the method validated by Pessi *et al*. (2016) using data from artificial cyanobacterial communities. Briefly, two and zero mismatches were allowed to the primer and barcode sequences, respectively, and reads were required to have a maximum expected error of 0.5 and a length of 370 bp. Quality-filtered sequences were clustered into OTUs at 95.0%, 97.5% and 99.0% similarity thresholds, corresponding roughly to the genera, species and strain levels, respectively (Yarza *et al*. 2014). Analyses were carried out primarily with OTUs clustered at 97.5% similarity, unless mentioned otherwise. The most abundant unique sequence in each OTU cluster was selected as the representative sequence of that OTU. These were classified using CREST (Lanzén *et al*. 2012) based on the Greengenes database (2011 Release; McDonald *et al*. 2012) and non-cyanobacterial OTUs were removed from the datasets.

Beta diversity analyses

Beta diversity analyses were performed in PRIMER 7 (Primer-E, Plymouth, UK). To investigate differences in community structure between the lakes, OTU abundance data were square root-transformed to down-weight the importance of highly abundant phylotypes, and pairwise Bray–Curtis similarities were computed. Unweighted pair group method with arithmetic mean (UPGMA) analyses were performed for OTUs clustered at 95.0%, 97.5% and 99.0% similarity thresholds, after resampling the dataset to 1480, 1225 and 1064 sequences per sample, respectively. In order to further compare the bioregionalization patterns of the cyanobacterial communities with the ones used to delineate the ACBRs, an additional UPGMA analysis based on Jaccard distances computed from OTU presence-absence data was performed. The robustness of the UPGMA groups observed in both the abundance and presence–absence analyses was assessed using a similarity profile (SIMPROF) test with 1000 permutations.

Distance-based linear model (distLM) was applied to assess the correlation between cyanobacterial community structure and lake water chemical composition and depth. Explanatory variables were log(x + 1) transformed (except pH) and standardized by subtracting the mean and dividing by the standard deviation of the variable, in order to attenuate skewness and compensate for differences in measuring units. Normality and correlation between variables were assessed using Draftsman plots. SO_4, Mg, Ca, K, Na and Cl concentrations were strongly correlated with conductivity (see results) and thus only the former was included in the model. The distLM procedure was performed using 10 000 permutations and did not include lakes M5, Highway and Waterfall, which were removed due to missing limnological data.

BLAST and phylogenetic analyses

For the representative sequence of each OTU, all closely related (≥99.0% similarity) isolate and environmental sequences were retrieved from GenBank using BLAST, and information regarding the geographic origin of each hit was obtained using in-house bash scripts. BLAST searches were carried out on 27 October 2016 using the partially non-redundant nucleotide sequences database (nt) and resulted in 2339 hits for all 112 OTUs. Phylotypes were classified as 'Novel' (if no related sequences were found in GenBank at this similarity threshold), 'Endemic' (when including only hits from Antarctica), 'Polar' (when hits were distributed across Arctic and Antarctic biotopes), 'Polar/Alpine' (when included hits from polar and high altitude biotopes) and 'Cosmopolitan' (when at least one hit originated from non-polar and non-alpine regions).

Multilevel pattern analysis was carried out using the 'indicspecies' package in R (De Cáceres and Legendre 2009) in order to investigate the association between individual phylotypes and the UPGMA groups. Phylotypes significantly associated with one UPGMA group ($P < 0.05$) were considered as indicator phylotypes for that particular group and were the subject of further phylogenetic analysis. Representative sequences were aligned using MUSCLE (Edgar 2004) along with all closely related isolate sequences retrieved from GenBank (≥99.0% similarity). A maximum likelihood tree based on the Kimura 2-parameter model (Kimura 1980) with 1000 bootstraps was computed using MEGA 7 (Kumar, Stecher and Tamura 2016). The Kimura 2-parameter substitution model was chosen based on a best-fit model selection carried out in MEGA prior to the phylogenetic analysis. The final alignment contained 401 sites (*Escherichia coli* positions 380–780). Indels were not taken into account.

RESULTS

Limnological conditions of the studied lakes

Specific conductivity ranged from freshwater (0.1–0.7 mS cm^{-1}) to brackish (1.8–4.5 mS cm^{-1}) and hypersaline (131.5 mS cm^{-1}; Table S1, Supporting Information). pH was neutral to

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slightly alkaline in all lakes (7.5–9.0). Lakes were generally (ultra)oligotrophic, with dissolved organic carbon (DOC) ranging from 1.0 mg L^{-1} in the two TM lakes (Forlidas and Lundström) to 3.8 mg L^{-1} in Mago Lake. Nutrient levels were extremely low, with NH_4 and PO_4 concentrations below the detection limit in all lakes and NO_3 in all but one lake (Forlidas Pond). The concentrations of the main ions (SO_4, Mg, Ca, K, Na and Cl) were highly correlated with specific conductivity ($r \geq 0.8$; data not shown), and thus only the latter was used in the statistical analyses, as it can be used as a proxy for the total ion sum.

Taxonomic composition of Antarctic lacustrine cyanobacterial communities

A total of 249 191 pyrosequencing reads with an average length of 410 bp were obtained. This included 101 788 reads that had been generated previously for five of the studied lakes (Pessi *et al.* 2016). After removal of low-quality and chimeric sequences, 177 482 sequences (71.2%) remained. From these, 578 sequences (0.3%) were assigned to plastid sequences of eukaryotic microalgae and 788 sequences (0.4%) to other bacterial phyla such as Acidobacteria, Chloroflexi, Planctomycetes, TM7 and Verrucomicrobia.

Remaining cyanobacterial sequences (176 116 reads, 99.3%) were grouped into 112 OTUs at 97.5% similarity. Pseudanabaenales comprised the majority of the phylotypes (61 OTUs, 54.5%), followed by Oscillatoriales, Synechococcales (11 OTUs each, 9.8%), Nostocales (10 OTUs, 8.9%) and Chroococcales (8 OTUs, 7.2%). Eleven OTUs (9.8%) could not be unambiguously assigned to a specific cyanobacterial order. Filamentous cyanobacteria were also dominant in terms of read abundance (Fig. 2). Pseudanabaenales was the dominant cyanobacterial order in all lakes, ranging from 52.3% of the quality-filtered reads in Col Lake to 100.0% in Taratine Lake. Oscillatoriales was also an important fraction of the cyanobacterial community in some lakes, accounting for up to 35.7% and 42.6% of the reads in Forlidas Pond and Col Lake, respectively. Unicellular cyanobacteria (orders Synechococcales and Chroococcales) were found at minor abundances in most lakes. Heterocystous cyanobacteria (order Nostocales) were only found in EA lakes, most notably in lakes L59b and Waterfall.

Variability in cyanobacterial community structure across Antarctic lakes

The UPGMA/SIMPROF analysis of OTU abundance data discriminated between five community groups, two of which were composed of one single lake, namely lakes Rauer2 and Mago (Fig. 3A). However, we only considered the second level of clustering and grouped the individual clusters with their respective higher order cluster, which resulted in three distinct community clusters. Each cluster comprised lakes from at least two different ACBRs. It follows that the lakes did not cluster according to the ACBR to which they belong. A similar grouping pattern was observed when OTUs were clustered at the 95.0% and 99.0% similarity thresholds but, as expected, SIMPROF groups were wider and narrower, respectively (Fig. S1, Supporting Information).

Cluster I comprised the brackish lakes Forlidas, Akebi and Highway, which were part of a significant SIMPROF group, and Rauer2, a hypersaline lake with a significantly different cyanobacterial community structure (Fig.3A and B). Multilevel pattern analysis identified four phylotypes that were consistently more abundant in cluster I lakes but were absent or rare in the others (Fig. 3C). Phylogenetic analysis (Fig. 4) revealed that OTU1 (clade VI), the dominant phylotype in all cluster I lakes, and OTU10 (clade V) were identical to *Leptolyngbya antarctica* strains isolated from Antarctic saline lakes and ponds in ACBR7 (EA), ACBR9 'South Victoria Land' (SV) and ACBR10 (TM) (Taton *et al.* 2006b; Fernandez-Carazo *et al.* 2011; Martineau *et al.* 2013). OTU4 (clade XVII) was identical to several *Phormidium/Microcoleus* strains isolated from Antarctic terrestrial and aquatic habitats in ACBR9 (SV) and ACBR10 (TM) (Nadeau, Milbrandt and Castenholz 2001; Fernandez-Carazo *et al.* 2011).

Cluster II comprised the freshwater lakes Taratine, M5, Higashi, and Nishi, which formed a significant SIMPROF group, and Mago, which was separated from the remaining lakes (Fig. 3A and B). Five phylotypes were identified as characteristic of cluster II lakes (Fig. 3C). OTU3 and OTU7 were the dominant phylotypes in all cluster II lakes except Mago Lake, in which OTU9 was the most dominant phylotype. OTU3 (clade IX) was phylogenetically affiliated with a *Leptolyngbya* lineage with no cultured relatives in GenBank (≥99.0% similarity) alongside OTU156. OTU7 (clade I) was identical to several *L. antarctica* strains isolated from a range of Antarctic freshwater lakes in ACBR7 (EA) and ACBR9 (SV) (Taton *et al.* 2006b). OTU9 (clade III) was identical to a *Phormidium* sp. strain isolated from an Antarctic pond in ACBR9 (SV) (Martineau *et al.* 2013).

Cluster III comprised the remaining freshwater lakes, namely Lundström, Col, L59b and Waterfall (Fig. 3A and B), for which a high number of indicator phylotypes was found (27 OTUs; Fig. 3C). Among them is OTU2 (clade IV), the dominant phylotype in Lundström Lake, which belonged to a cluster of phylotypes with no cultured relatives in GenBank alongside OTU123 and OTU44. OTU15 (clade VIII), the dominant phylotype in Col Lake, belonged to a cluster of 'Polar/Alpine' phylotypes (see below) comprising also OTUs 11, 26, 99, 125 and 162. These were related (99.2%–100.0% similarity) to several *Phormidesmis priestleyi* strains isolated from a range of Antarctic lakes in ACBR7 (EA) and ACBR10 (TM) (Taton *et al.* 2006b; Fernandez-Carazo *et al.* 2011; Lara *et al.* 2017). Four phylotypes (OTUs, 18, 93, 109 and 148; clade X) formed a cluster of 'Polar' phylotypes (see below) without any cultured relatives in GenBank. Finally, five phylotypes (OTUs 28, 36, 55 and 96; clades XIII, XIV and XV) were related (99.7%–100.0% similarity) to heterocystous cyanobacteria from the order Nostocales, including *Nostoc*, *Coleodesmium* and *Calothrix* strains isolated from Antarctic lakes in ACBR7 (EA) (Taton *et al.* 2006b).

The separation between cyanobacterial communities from saline versus freshwater lakes irrespectively of the ACBR to which they belong (Fig. 3) suggests an association between the cyanobacterial community structure and the chemical composition of the lake water. This was confirmed by the distLM procedure, which revealed that the variation in community structure between lakes was correlated with conductivity and DOC, although the latter was only marginally significant (Table 2). The other chemical parameters (as well as lake depth) showed no correlation with cyanobacterial community structure ($P > 0.1$) or were not included in the model because they were below the detection limit.

Bioregionalization patterns based on presence–absence data

Following the methodology used by Terauds *et al.* (2012) to describe the ACBRs, an additional UPGMA/SIMPROF analysis based on presence-absence data was performed to further

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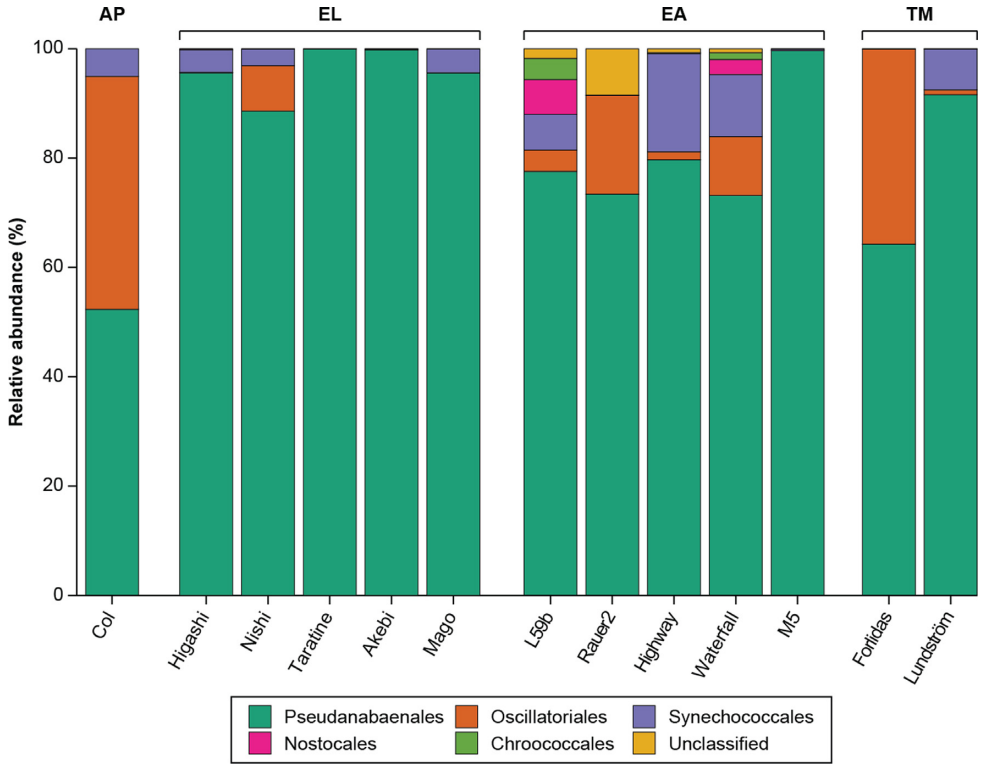

Figure 2. Cyanobacterial community structure summarized at the order level. Relative abundances were computed after resampling the dataset to 1225 sequences per sample.

Table 2. Distance-based linear model (distLM) results showing the proportion of the variation in cyanobacterial community structure explained by lake water chemical parameters (Table S1, Supporting Information).

Parameter	Pseudo-F	P-value	Explained variation (%)
Conductivity	2.54	0.001	24.1
pH	0.67	0.760	7.7
DOC	1.68	0.090	17.4
NO_3	nd	nd	nd
NH_4	nd	nd	nd
PO_4	nd	nd	nd
SiO_4	1.41	0.209	15.0

DOC: dissolved organic carbon.
nd: not determined.

assess the bioregionalization patterns of the cyanobacterial communities. In general, this analysis confirmed the findings obtained based on the abundance data, namely that a clear bioregionalization in cyanobacterial community structure is lacking (Fig. S2, Supporting Information). A separation between saline and freshwater lakes irrespectively of the ACBR to which they belong was observed. A similar conclusion can be drawn from a UPGMA/SIMPROF analysis considering only 'Novel' and 'Endemic' OTUs (see below). In this analysis, all lakes from the four ACBRs formed a single significant SIMPROF group (Fig. S3, Supporting Information).

The absence of clear bioregionalization patterns is likely related to the fact that a large fraction of the phylotypes (44 OTUs, 39.3%) occurred exclusively in one lake, and only 17 OTUs (15.2%) were present in at least half of the studied lakes (Fig. 5A). Furthermore, a positive relationship between phylotype abundance and site occupancy (i.e. the number of samples in which a phylotype was found) was observed (Fig. 5B). In other words, phylotypes which were more abundant were also generally those with the broadest geographic distribution.

Global geographic distribution of phylotypes

All phylotypes were compared to closely related (≥99.0% similarity) isolate and environmental sequences available in GenBank in order to verify their global geographic distribution. Thirty-seven OTUs (33.0%) were related to sequences with a wide global distribution and were thus classified as 'Cosmopolitan'. The remaining phylotypes (79 OTUs, 77.0%) appeared to be restricted to the cold biosphere, i.e. polar and alpine environments. More specifically, 42 OTUs (37.5%) had no related sequences in GenBank at this similarity threshold ('Novel' OTUs) and are thus considered as potentially endemic; 20 OTUs (17.9%) were exclusively related to sequences from Antarctic biotopes ('Endemic'); 6 OTUs (5.4%) included hits from both the Arctic and the Antarctic ('Polar'); and 7 OTUs (6.3%) also included hits from high

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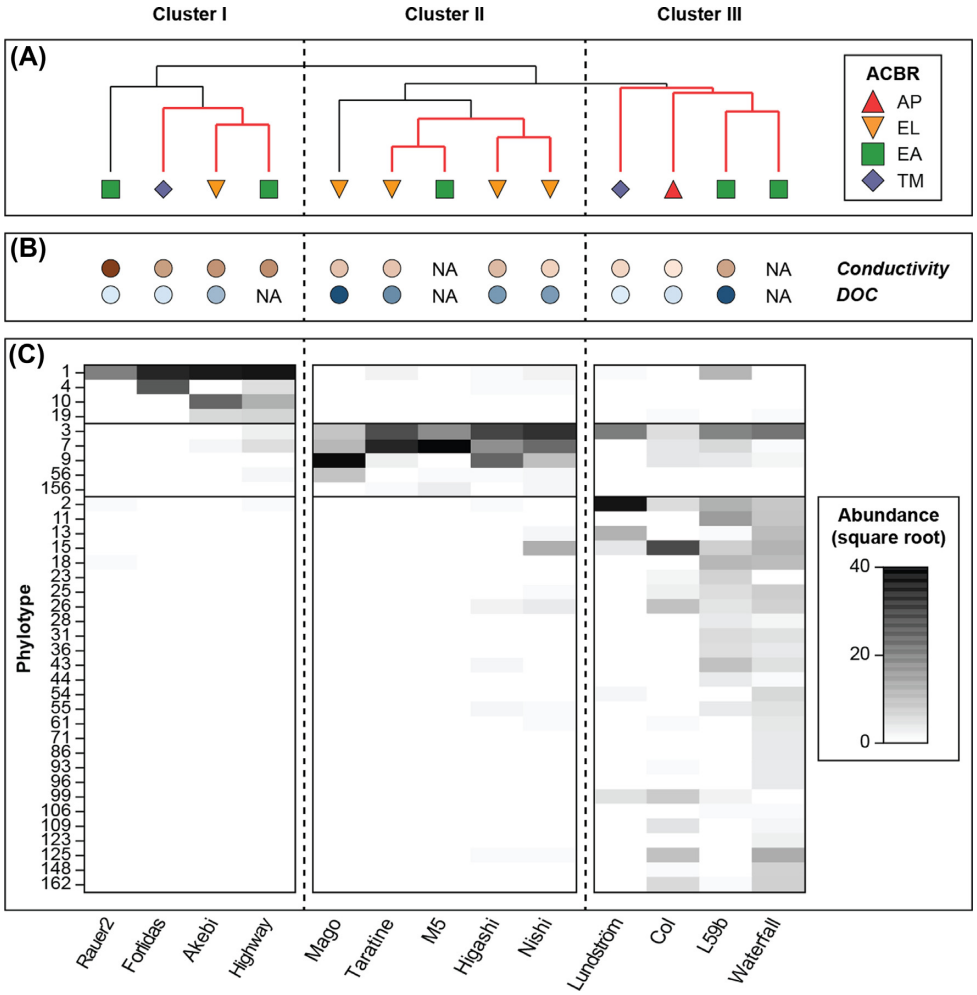

Figure 3. (**A**) Unweighted pair group method with arithmetic mean (UPGMA) analysis of OTU abundance data (Bray–Curtis similarities) between cyanobacterial assemblages (OTUs defined at 97.5% similarity). Red lines represent significant SIMPROF groups ($P < 0.05$). (**B**) Schematic representation of conductivity and dissolved organic carbon (DOC) content in each lake. For absolute values see Table S1 (Supporting Information). (**C**) Heatmap analysis of indicator phylotypes (multilevel pattern analysis; $P < 0.05$). The gray scale represents the abundance of a phylotype and shows their association with one of the UPGMA groups.

altitude regions such as the Alps, the Andes or the Himalayas ('Polar/Alpine').

Cold biosphere phylotypes ('Novel', 'Endemic', 'Polar' and 'Polar/Alpine) were not restricted to a particular cyanobacterial lineage or taxon (Fig. 4). Two notable exceptions were a group of six phylotypes related to several *P. priestleyi* strains which appear currently restricted to the cold biosphere (clade VIII), and four phylotypes which formed a cluster of polar lineages without any cultured relatives in GenBank (clade X). Geographic distribution was, on the other hand, related to site occupancy and read abundance. 'Novel' and 'Endemic' phylotypes were found in a maximum of six lakes, while all phylotypes found in more than six lakes were 'Polar', 'Polar/Alpine' or 'Cosmopolitan' (Fig.5A). A similar trend was observed in respect to phylotype abundance; 'Polar', 'Polar/Alpine' and 'Cosmopolitan' phylotypes were in general more abundant than 'Novel' and 'Endemic' phylotypes (Fig. 5B).

DISCUSSION

Our HTS analysis revealed benthic cyanobacterial mat communities dominated by filamentous cyanobacteria from the orders Pseudanabaenales and Oscillatoriales (Fig.2), which is in line with earlier studies of cyanobacterial diversity in Antarctic lakes (e.g. Hodgson *et al.* 2004; Taton *et al.* 2006a,b; Fernandez-Carazo *et al.* 2011). Filamentous cyanobacteria form the fabric of the three-dimensional structure of microbial mats and, as such, play a pivotal role in these ecosystems. They provide substrate and nutrients for a relatively diverse community consisting of other bacteria, unicellular eukaryotes such as diatoms and green algae, as well as microinvertebrates (Singh and Elster 2007; Verleyen *et al.* 2010,2012; Vincent and Quesada 2012). Nitrogen-fixing cyanobacteria from the order Nostocales are usually important members of microbial mat communities in Antarctic lakes but made up only a small portion of the communities investigated here. This is, however, in agreement with previous studies which could not detect the presence of heterocystous morphotypes in Antarctic mat communities by molecular methods (Taton *et al.* 2006a,b; Fernandez-Carazo *et al.* 2011). Indeed, high amounts of EPS produced by heterocystous cyanobacteria can affect negatively the efficiency of cell lysis procedures, thus hindering their detection in molecular investigations of cyanobacterial communities (Gaget *et al.* 2016). Notwithstanding, the low abundance of Nostocales observed here might be due to the low phosphorus availability in the studied lakes (Table S1, Supporting Information), which is a known

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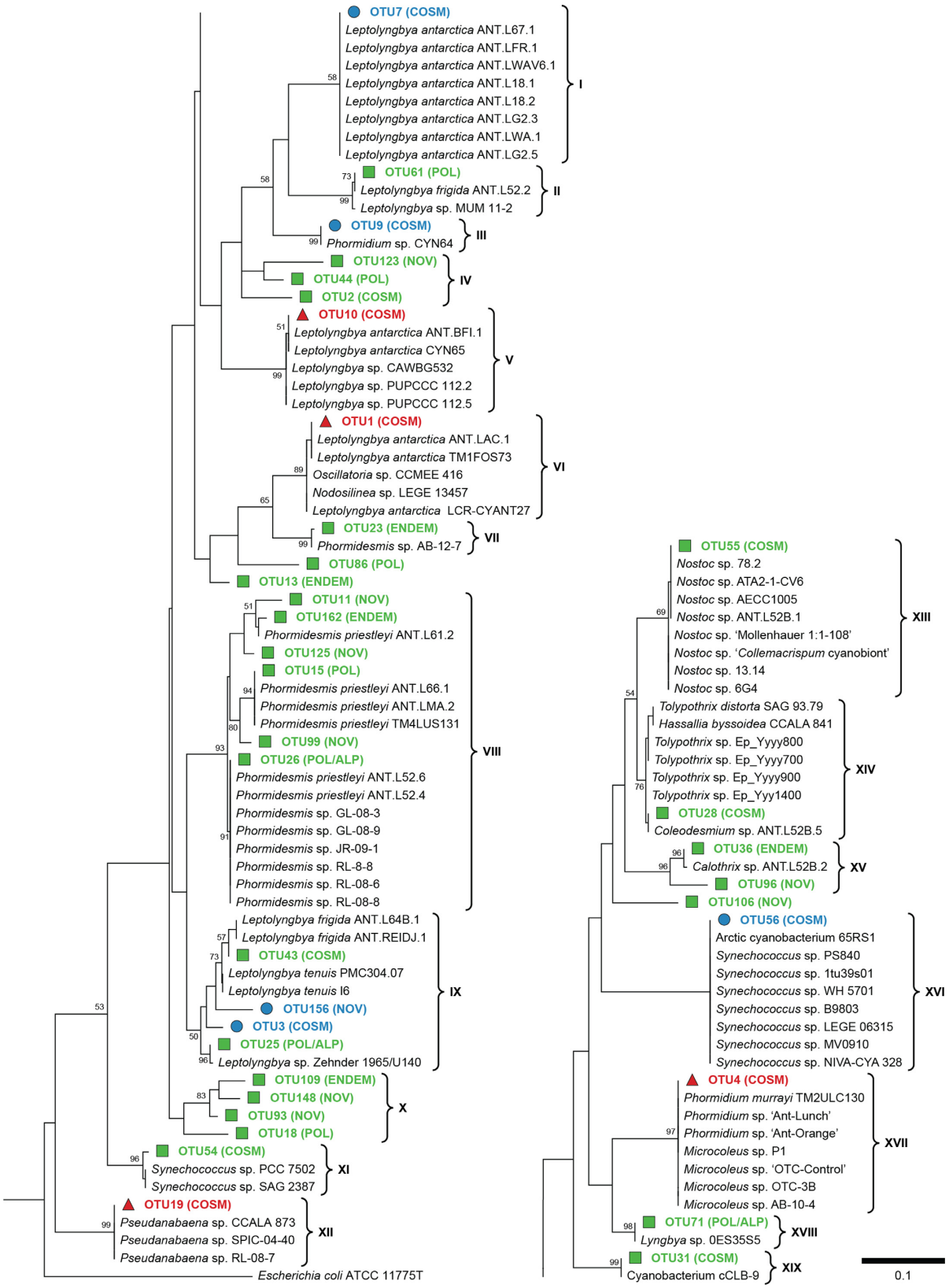

Figure 4. Phylogenetic analysis of indicator phylotypes (Fig. 3C) of cluster I (red), cluster II (blue) and cluster III (green) lakes and all closely related (≥99.0% similarity) isolate sequences retrieved from GenBank. Maximum likelihood tree (log likelihood: 5311.8582) computed using the Kimura 2-parameter model and 1000 bootstraps. Bootstrap values <50% are omitted. Roman numbers denote clades discussed in the text.

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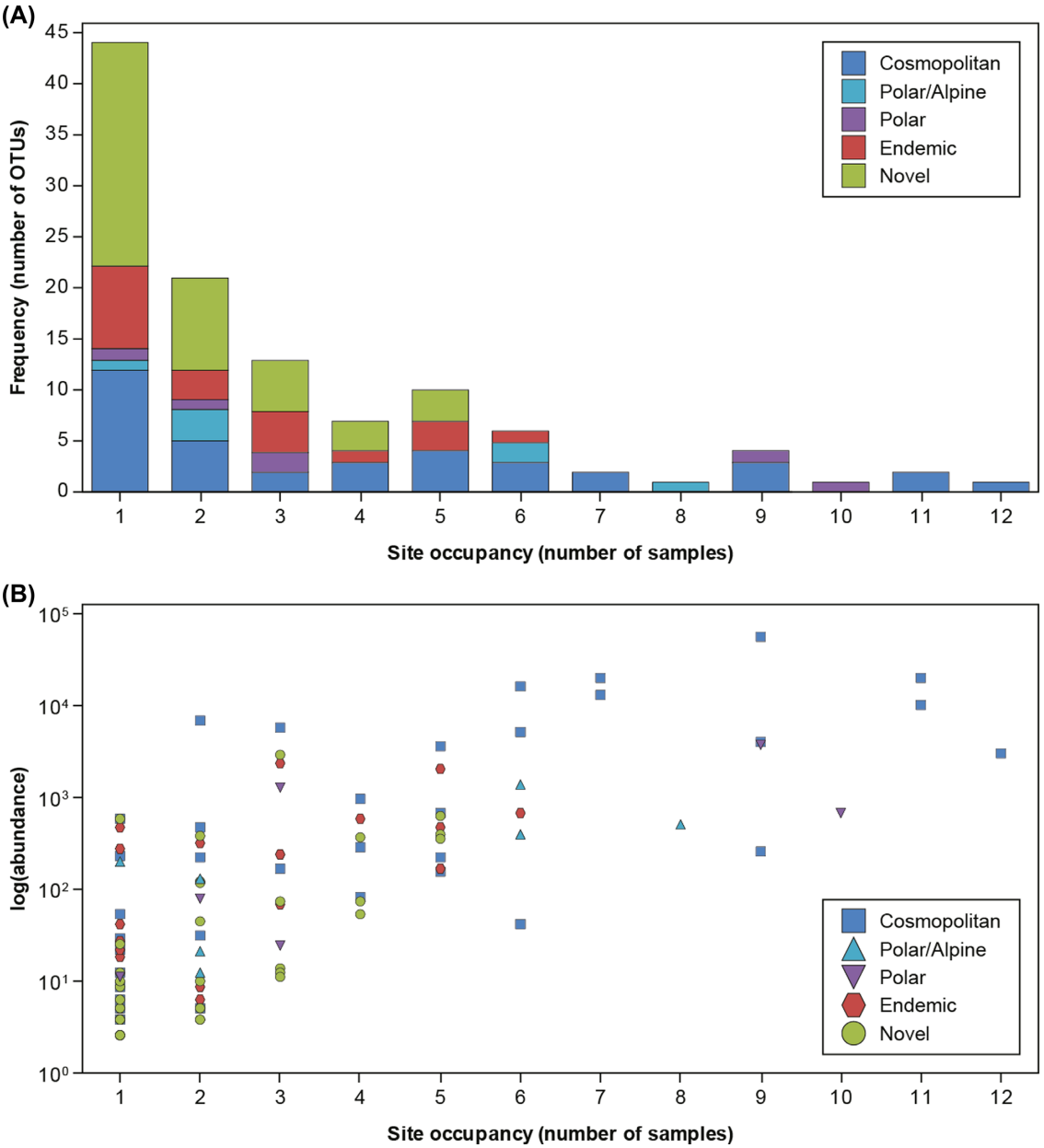

Figure 5. (**A**) Occupancy-frequency histogram of phylotype distribution showing that most phylotypes (specially 'Novel' and 'Endemic') were restricted to a small number of samples. (**B**) Relationship between site occupancy rate and phylotype abundance showing that phylotypes found in more samples (specially 'Cosmopolitan' and 'Polar/Alpine') had generally higher abundances. In both figures, phylotypes are discriminated based on their global geographic distribution.

limiting factor for nitrogen fixation activity (Wynn-Williams 1996). Interestingly, our HTS analysis revealed the presence of two phylotypes (OTU62 and OTU129) related to the Melainabacteria, a lineage of non-photosynthetic bacteria, which are a sister group to the cyanobacteria (Soo *et al.* 2014). These phylotypes were found at very low abundances (<0.01% of the reads) and were only present in two lakes (L59b and Highway; data not shown). A few reports of Melainabacteria in polar lakes exist (e.g. Jungblut *et al.* 2016; Schütte *et al.* 2016), but their ecological role remains unknown.

Spatial patterns of cyanobacterial community structure were mainly associated with lake water chemistry and appeared to diverge from the recently proposed ACBR partitioning of the Antarctic continent (Fig. 3). These ACBRs have been identified based on the distribution of a variety of taxa belonging to different phyla, but mainly Ascomycota (43.9% of the records) and Bryophyta (35.9%). Cyanobacteria, on the other hand, represented only 2.2% of the records and identifications were based on microscopic observations only (Terauds *et al.* 2012; Terauds and Lee 2016). The dataset used for the delineation of the ACBRs was developed by combining existing taxonomic inventories collected over several years, which included literature reviews . This dataset is thus well suited as a baseline data against which our study comprising samples collected over a 10-year period could be compared. In our study, lakes with similar conductivity and located in different, geographically distant ACBRs harbored comparable communities (Fig.3). Cluster I, for example, comprised saline lakes from three different ACBRs located up to

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3000 km apart (Fig. 1). These results suggest a widespread distribution of the more abundant cyanobacterial phylotypes across the different Antarctic ice-free regions studied. Cyanobacterial distribution within Antarctica thus appears mostly constrained by the availability of suitable habitats and, to a lesser extent, geographic barriers to dispersal. Although Antarctic lakes are largely isolated and have little or no physical connection, dispersal of microbial propagules has been proposed to be readily accomplished via wind currents and animal vectors such as migratory birds (Wynn-Williams 1991; Pearce *et al*. 2009). In the case of microbial mat communities, small mat fragments can become detached, float to the surface of the lake and are then transferred to nearby habitats by the wind (Vincent 2004; Gibson *et al*. 2006). For example, Hodgson *et al*. (2010) showed that in permanently ice-covered lakes such as those in the Dufek Massif (Transantarctic Mountains) cyanobacteria can become detached from the lake benthos and migrate up through the ice profile. These lift-off mats eventually break out at the ice surface and can be dispersed by wind onto the shoreline or even further away. The reverse dispersal, from terrestrial habitats to ice inclusions, has also been demonstrated by molecular probes (Gordon, Priscu and Giovannoni 2000). Aerial transfer of propagules between coastal habitats is favored by strong katabatic winds blowing away from the continent, which then follow a circumpolar course along the Antarctic coast (Wynn-Williams 1991; Hughes *et al*. 2006). Moreover, microbial mats appear to be well adapted to withstand cold and dry conditions. In particular, a cyanobacterial mat in the McMurdo Dry Valleys became active within a week after the stream, which was dry for decades, was experimentally reactivated (McKnight *et al*. 2007). This suggests that the mats had been preserved in a cryptobiotic state, which is expected to be a good adaptation for surviving the conditions experienced during dispersal.

Although the observed lack of bioregionalization suggests a widespread distribution of cyanobacterial phylotypes across the different Antarctic regions, this seems to be the case for only a relatively small number of abundant phylotypes. Indeed, the vast majority of the phylotypes were found at low abundances and had a very limited distribution (Fig. 5). A similar trend was observed at the global level; abundant phylotypes with a wide distribution were also globally distributed, while endemic and novel phylotypes were generally found at low abundances and were constrained to only a few Antarctic lakes. This is in agreement with the study of Taton *et al*. (2006a) on Antarctic lacustrine cyanobacteria, which suggested that cosmopolitan cyanobacteria are better adapted to long-range transportation by the wind—or potentially via migrating birds both between polar regions and within Antarctica (Schlichting, Speziale and Zink 1978)—than potentially endemic ones and are thus more likely to disperse between the different Antarctic regions (Taton *et al*. 2006a). Furthermore, our results are in agreement with the study of Nemergut *et al*. (2011), who found that 65%–85% of the phylotypes in a range of habitats worldwide were confined to a single microbial assemblage. They also reported a positive relationship between phylotype abundance and distribution as observed here (Fig. 5). This can be attributed to a link between demography and dispersal potential, with populations of abundant taxa having a higher probability to disperse in comparison to rare taxa (Fierer *et al*. 2010; Nemergut *et al*. 2011).

The importance of lake conductivity and hence salinity as a driver of cyanobacterial community composition in Antarctic lakes is well known (e.g. Hodgson *et al*. 2004; Jungblut *et al*. 2005; Verleyen *et al*. 2010). Cyanobacteria differ widely in respect to salinity tolerance, with several taxa having developed various mechanisms that allow them to withstand the osmotic stresses associated with high salt concentrations (Oren 2012). In addition to the direct effect of osmotic stress, salinity also affects the physical properties of the water column in Antarctic lakes. More specifically, hypersaline lakes can be permanently stratified and remain (partially) ice-free during winter in contrast to freshwater systems (Verleyen *et al*. 2012). Lake ice and water column stability, in turn, have an effect on the light climate and nutrient dynamics of lake systems and, as such, play an important role on the structuring of the microbial mat communities (Vincent 2004; Laybourn-Parry and Wadham 2014). In our study, hypersaline and slightly brackish lakes shared four abundant phylotypes related to *L. antarctica* and *P. murrayi* strains isolated from a range of Antarctic saline lakes (Figs 3 and 4). The fact that these phylotypes were mainly absent in the freshwater lakes emphasizes that, for conservational purposes, different types of lake environments should be targeted within each ACBR. This is important given that a wide variety of lake types can be found in many Antarctic regions, ranging from highly diluted glacial systems to hypersaline isolation lakes (Verleyen *et al*. 2012). Despite this, little consideration has been given to include cyanobacteria and other microorganisms in the development of conservation plans in Antarctica (Hughes, Cowan and Wilmotte 2015). This is surprising given that Antarctic ecosystems are essentially microbial realms (Wynn-Williams 1996; Laybourn-Parry and Pearce 2007).

Although 16S rRNA gene sequences do not provide direct information about functional and ecophysiological traits, phylogenetic reconstructions can offer some insights into the distribution of particular taxa. For example, two phylotypes associated with saline lakes had sequences related (99.5%–100.0%) to *L. antarctica* strains isolated from a wide range of Antarctic saline lakes (clades VI and V; Fig. 4). *Leptolyngbya antarctica* morphotypes are common in Antarctic lakes (Komárek 2007) and recent polyphasic studies have shown that, behind their simple morphology, a large genotypic diversity can be found (Taton *et al*. 2006b). One of these genotypes, represented by the *L. antarctica/Nodosilinea* lineage (clade VI; Fig. 4), was the most abundant group in the saline lakes investigated here (Fig. 3C). *Nodosilinea* characteristically forms nodules of tightly coiled trichomes embedded in a polysaccharide sheath, a morphological feature that has been associated with several traits such as nitrogen fixation, buoyancy and anti-herbivory (Li and Brand 2007; Perkerson III *et al*. 2011). Here, we speculate that these polysaccharide-rich nodules might contribute to maintain a balanced osmolarity within the microenvironment, which would explain the ecological success of the *L. antarctica/Nodosilinea* lineage in saline lakes. However, confirmation of this hypothesis requires further investigations of the morphology and ecophysiology of *L. antarctica* strains isolated from different habitats, including freshwater and saline lakes. Although the specific physiological and/or structural traits underlying their ecological range remain unknown, our results give further support to the heterogeneous nature of *L. antarctica* morphotypes, both from phylogenetic and ecological perspectives (Taton *et al*. 2006b; Komárek 2007).

At the global level, more widely distributed lineages comprised an important fraction of the cyanobacterial communities investigated here ('Cosmopolitan' OTUs; 33.0%). Even though the Antarctic circumpolar current and the Southern Ocean act as a strong barrier for both marine and terrestrial flora and fauna (Convey *et al*. 2008), high altitude aeolian processes likely allow the dispersal of microorganisms both to and from Antarctica (Wynn-Williams 1991; Pearce *et al*. 2009,2016). Many cyanobacterial taxa seem well adapted to long-range aerial transport

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due to their resistance to desiccation, freezing and UV radiation (Singh and Elster 2007; Vincent and Quesada 2012). Our results agree with the findings of Jungblut, Lovejoy and Vincent (2010), who reported a ubiquitous distribution of cyanobacterial phylotypes throughout the cold biosphere. Altogether, these findings suggest contemporary or relatively modern genetic exchanges between Antarctica and similar habitats in other polar and alpine regions. On the other hand, the majority of the cyanobacterial diversity observed in the present study comprised potentially endemic lineages, including phylotypes with no closely related (≥99.0% similarity) sequences in GenBank ('Novel' OTUs; 37.5%) and phylotypes associated with cyanobacterial lineages currently restricted to Antarctica ('Endemic' OTUs; 17.9%). The relatively high level of endemism reported here as well as in other studies of Antarctic microbial communities (e.g. Taton *et al.* 2006a,b; De Wever *et al.* 2009; Peeters *et al.* 2012), though with different criteria and thresholds, suggests an ancient, pre-Holocene origin for an important fraction of the contemporary Antarctic microbiota (Convey and Stevens 2007; Convey *et al.* 2008,2009). It has become evident that Antarctic organisms from various taxonomic groups have likely survived past glaciations in ice-free refugia such as inland nunataks and coastal oases (Fraser *et al.* 2014). For example, the presence of endemic arthropods in isolated nunataks cannot be explained by recent colonization events and is probably the result of an ancient origin on the continent and hence persistence and survival in refugia during glacial maxima (Wise and Gressitt 1965; Gressitt 1967; also see Convey *et al.* 2008 for a detailed discussion). Recent studies have also suggested that some cyanobacterial taxa were able to survive in glacial refugia during previous ice ages. More specifically, some *Chroococcidiopsis* and *Phormidium autumnale* lineages were shown to have an ancient evolutionary history in Antarctica and were suggested to have diverged from non-polar lineages since before the breakup of Gondwana (Bahl *et al.* 2011; Strunecký, Elster and Komárek 2012). Altogether, these observations suggest that a fraction of the extant Antarctic cyanobacterial diversity is likely endemic and thus may have persisted *in situ* for several glacial cycles (Convey *et al.* 2008,2009).

In conclusion, our results based on 13 lakes from four ACBRs suggest that the spatial patterns of Antarctic lacustrine cyanobacteria are not congruent with those observed in a recent study based on a comprehensive dataset of terrestrial biota (Terauds *et al.* 2012). More specifically, no apparent bioregionalization was observed, and the cyanobacterial community structure was associated with salinity and salinity-associated factors such as lake ice cover and water column stability. Conservation measures for the protection of Antarctic biota should thus consider the variation in community composition between limnologically distinct lakes within the same biogeographic province. Our results also point to the presence of a few highly abundant, widely distributed and cosmopolitan phylotypes, while the majority of the cyanobacterial diversity observed consists of rare, regionally constrained and (potentially) endemic phylotypes. While the former points to more recent dispersal events, the latter might represent lineages which have evolved *in situ* over larger temporal scales. The rareness of endemic taxa further underlines the vulnerability of Antarctic lake biota in response to environmental disturbances. As the climate continues to warm, endemic lineages could be displaced or eliminated by invasive generalists from lower, warmer latitudes (Gibson *et al.* 2006). Furthermore, changes in the precipitation-evaporation balance due to increasing temperatures and/or changing wind patterns is expected to have a direct influence on lake salinity (Verleyen *et al.* 2003,2010; Hodgson *et al.* 2006). This, in turn, can be expected to have an impact on the structure and composition of cyanobacterial mat communities in Antarctic lakes.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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Conflict of interest. None declared.

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