DOI: 10.1111/jbi.13089

ORIGINAL ARTICLE

WILEY Journal of Biogeography

Biogeography of cryoconite forming cyanobacteria on polar and Asian glaciers

Takahiro Segawa[1,2,3*] | Takahiro Yonezawa[4*] | Arwyn Edwards[5,6] | Ayumi Akiyoshi[2,3] | Sota Tanaka[7] | Jun Uetake[2,3] | Tristram Irvine-Fynn[6,8] | Kotaro Fukui[9] | Zhongqin Li[10] | Nozomu Takeuchi[7]

[1]Center for Life Science Research, University of Yamanashi, Yamanashi, Japan

[2]Transdisciplinary Research Integration Center, Tokyo, Japan

[3]National Institute of Polar Research, Tokyo, Japan

[4]School of Life Sciences, Fudan University, Shanghai, China

[5]Institute of Biological, Environmental & Rural Sciences (IBERS), Aberystwyth University, Aberystwyth, UK

[6]Interdisciplinary Centre for Environmental Microbiology, Aberystwyth University, Aberystwyth, UK

[7]Department of Earth Sciences, Graduate School of Science, Chiba University, Chiba, Japan

[8]Institute of Geography and Earth Sciences, Aberystwyth University, Aberystwyth, UK

[9]Tateyama Caldera Sabo Museum, Toyama, Japan

[10]Tienshan Glaciological Station, Chinese Academy of Science, Lanzhou, China

Correspondence
Takahiro Segawa, Center for Life Science Research, University of Yamanashi, Yamanashi, Japan.
Email: tsegawa@yamanashi.ac.jp

Funding information
Grants-in-Aid for Scientific Research, Grant/Award Number: 17H01854, 26281017, 16H01772, 26247078, 26241020; CAS, Grant/Award Number: KJZD-EW-G03-01; Aberystwyth University Research Fund; Great Britain Sasakawa Foundation

Editor: Peter Linder

Abstract

Aim: Cryoconite, a microbe-mineral aggregate found on glaciers worldwide, is formed by microbial phototrophs, principally cyanobacteria. Despite their ecological importance in supraglacial environments, the phylogeographical distributions of supraglacial cyanobacteria are poorly understood. Here, we investigate the biogeographical distribution of cyanobacteria on glaciers in the Antarctic, Arctic and Asia.

Location: Glaciers in the Antarctic, Arctic and Asia.

Methods: We analysed contiguous sequences of 16S rRNA genes and 16S–23S internal transcribed spacer (ITS) regions, determined by a long read strategy and single-filament PCR analysis in 38 glacial samples. We analysed cyanobacterial distribution patterns and genetic differentiation.

Results: The cyanobacterial 16S rRNA gene sequences were grouped into 20 operational taxonomic units (OTUs), and the six major OTUs that accounted for 88% of sequences were distributed broadly from polar to Asian glaciers, suggesting that they are cosmopolitan at the species level. However, analysis of the more variable ITS region revealed geographical differentiation at the strain level. Nineteen OTUs, including the six major OTUs, showed considerable genetic differentiation among geographical regions; at the population level, they are, thus, geographically restricted. Only one of the phylotype exhibits a population structure which does not show a relationship with geographical distribution, suggesting that is cosmopolitan, even at the strain level.

Main conclusions: Our 16S rRNA gene analyses suggest a global distribution of species of cyanobacteria colonizing glacier surfaces; however, the 16S–23S ITS regions revealed that most of the phylotypes are fundamentally endemic to particular areas at the population level and indicate limited migration among regions. Our result suggests that selection pressures among geographical regions are strong driving forces shaping genetic structure in cyanobacteria.

KEYWORDS
cryoconite, cryosphere, cyanobacteria, dispersal, glacier, phylogeography

*These authors contributed equally to this study.

1 | INTRODUCTION

Cyanobacteria are prominent photoautotrophs growing in various extreme environments. Glaciers, which are characterized by low temperature and poor nutrient status, are commonly inhabited by cyanobacteria capable of surviving in cold environments (Edwards et al., 2014; Langford, Hodson, Banwart, & Boggild, 2010). They are usually found in cryoconite holes, which are small meltwater ponds on ablating ice surfaces in which dark-coloured spherical aggregates (cryoconite granules) form. The presence of cryoconite results in the accumulation of organic matter; therefore, cryoconite holes sustain diverse heterotrophic organisms on glaciers. Cyanobacteria are found on glaciers worldwide in geographically widely separated regions, such as the Arctic, Antarctic and high-altitude mountains at lower latitudes (Edwards et al., 2014; Foreman, Sattler, Mikucki, Porazinska, & Priscu, 2007; Takeuchi & Li, 2008). However, it is unclear how cyanobacteria expand their distribution, whether they can disperse to other glaciers locally or globally. Furthermore, their phylogeographical distribution patterns are not known. Understanding the distribution pattern and dispersal process of cyanobacteria is important to interpret recent changes in glacier ecosystems in Arctic and other regions under the conditions of global warming and potential de-glaciation (Wientjes, Van de Wal, Reichart, Sluijs, & Oerlemans, 2011).

Several studies based on 16S rRNA gene analysis have concluded that cryospheric cyanobacteria are cosmopolitan (Harding, Jungblut, Lovejoy, & Vincent, 2011; Jungblut, Lovejoy, & Vincent, 2010). The results of a recent study based on 16S rRNA gene sequences suggested that several clades of cold-dwelling cyanobacteria are common to polar and alpine regions, and showed that 20 clades independently evolved from the ancestors with high probabilities of being able to survive in cold environments (Chrismas, Anesio, & Sanchez-Baracaldo, 2015). The global distribution of glacier cyanobacteria implies that their dispersion and evolution are associated with climate variations, rather than geographical distance (Pointing et al., 2015). Cryosphere-specialist cyanobacteria may expand their ranges during cold climatic periods, but become increasingly fragmented in warmer climates (Pointing et al., 2015; Vincent, 2000). However, little is known about the evolutionary history of glacier cyanobacteria, especially microevolutionary processes such as regional differentiation. While conventional 16S rRNA gene analyses have broad utility in resolving taxonomic affiliations to the genus and species levels (Liguori et al., 2011), they have limited ability to resolve population-level genetic structure within species due to the low evolutionary rates of the gene. The 16S–23S internal transcribed spacer (16S–23S ITS) region has a faster evolutionary rate, and therefore, it is more appropriate for resolving population structure (Dvořák, Hašler, & Poulíčková, 2012). The differential resolution provided by the 16S–23S ITS region and the 16S rRNA gene in genetic analyses may improve our insights in population genetics and geographical patterns.

In this study, we investigated the biogeographical distribution patterns of cyanobacteria among glaciers in the Antarctic, Arctic and Asia, the three largest glacierized regions of the world. Given the physical distance between each region, we hypothesized that there would be a corresponding influence of geographical region upon the cyanobacterial community. Consequently, we hypothesized that the extent of dispersal among the major regions could be revealed, and ultimately whether the dispersal limitation is prevalent at species or strain level. On the basis of the sequences from cyanobacteria collected from glaciers in these regions, we estimated cyanobacterial population structure on global and regional scales (e.g. Arctic, Asian, or each glacial scale). The analyses based on genetic markers for regions with different evolution rates (16S rRNA gene and 16S–23S ITS region) provided evidence for the endemic distribution of major cyanobacterial phylotypes. Using this experimental strategy, we identified species (or genera) by inter-species comparison and analysed population genetic structure by intra-species comparison.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We used cryoconite samples collected from 38 sites on 15 glaciers in the Arctic, Antarctic and Asian mountains (Figure 1 and Table S1 in Appendix S1). The glaciers sampled in the Arctic include sites from Greenland, Svalbard and Alaska, those of the Antarctic glaciers include King George and James Ross islands, and those of the Asian mountains include Himalayas, Pamir, Tienshan and Qilian mountains. At each of site, we collected cryoconite in cryoconite holes from five randomly selected points, which were representative surfaces in terms of slope, roughness and meltwater, and away from the glacier margins. Cryoconite samples were collected using a pipette or stainless steel scoop which had previously been washed by Milli-Q water (Millipore, USA) within a clean room and sterilized by UV treatment. Samples were stored in sterile 5- or 50-ml plastic conical tubes (Assist, Japan). The samples were frozen on dry ice during transportation to Japan and kept frozen at −80°C before use. PCR amplification and sequencing were performed no later than 1 year after collection.

2.2 | 16SrRNA–ITS long read strategy by PCR amplification and sequencing

Within 1 year of sample collection, genomic DNA was extracted from supraglacial samples using a FastDNA spin kit for soil (Qbiogene, USA) with a Multi-beads shocker at 2,500 rpm for 30 s (Yasui Kikai, Japan). Between 1 and 2 ml of each sample was centrifuged at 10,000 ***g*** for 10 min and the pellets from five replicate samples collected at each site pooled prior to the extraction. All DNA extractions were conducted in a class 100 clean bench (MHE-130AB3; Sanyo, Japan), and the subsequent procedures were carried out in another class 100 clean bench.

While high-throughput sequencing is commonly applied to 16S rRNA gene amplicon analyses, the read lengths of currently used sequencing platforms could not provide contiguous 16S–ITS rRNA sequences from the same template molecules. Since this was a pre-

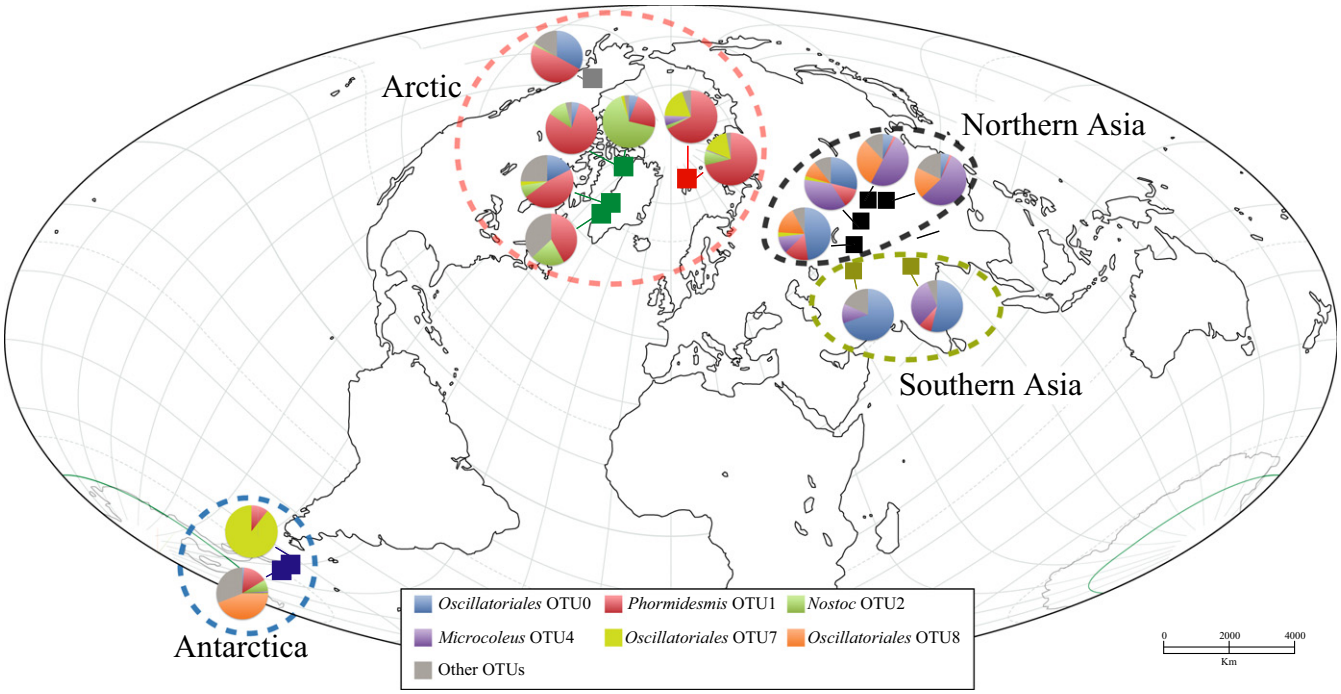

FIGURE 1 Map showing locations of snow and ice sampling sites. Cryoconite samples were collected from 38 sites on 15 glaciers in the Arctic, Antarctic and Asian mountains. Pie charts depict distribution and abundance of cyanobacterial OTUs obtained from each glacier. Six major 16S OTUs and low-abundance OTUs (grouped into "Other OTUs") are represented in pie charts. Colour points on map represent regions: blue: Antarctica, green: Greenland, red: Svalbard, grey: Alaska, black: Northern Asia and yellow: Southern Asia [Colour figure can be viewed at wileyonlinelibrary.com]

requisite to perform parallel analyses of broad-scale taxonomic (16S rRNA gene) or fine-scaled (16S–23S ITS) on the same individuals, we accordingly selected Sanger based sequencing of clone libraries as the study's strategy, accepting the limitation of shallower sequencing depth.

PCR amplification of cyanobacterial 16S rRNA gene to 23S rRNA ITS regions was performed using high-fidelity PrimeStar polymerase (Takara, Japan) and primers 27FHT (Isenbarger, Finney, Rios-Velazquez, Handelsman, & Ruvkun, 2008) and 23S30R (Lepère, Wilmotte, & Meyer, 2000). To reduce PCR artefacts, the number of PCR cycles was kept to a minimum. The PCR conditions were as follows: 3 min of initial denaturation at 94°C, then 12–20 cycles at 98°C for 10 s, 57°C for 15 s and 72°C for 4 min, and a final extension at 72°C for 7 min. To minimize PCR drift, 3–5 independent reactions were conducted. The pooled PCR products were purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany) and cloned using the pCR4Blunt-TOPO with a Zero Blunt TOPO PCR Cloning Kit for Sequencing (Life technologies, USA). *Escherichia coli* HST08 Premium Competent Cells (Takara) were transformed with the cloning vector. We sequenced approximately 3,000 clones in total. Sequencing was achieved using Big Dye Terminator 3.1 and an ABI 3130xl automatic sequencer.

2.3 | Single-filament PCR analysis and microscopy

To directly identify the phylotypes forming cryoconite granules, single-filament PCR analysis was conducted. Cryoconite samples collected from the two glaciers in Asian mountains were used for dissection, and three taxa with different morphological characteristics were selected according to Takeuchi and Li (2008).

Cryoconite samples collected from the Urumqi Glacier No. 1, China, and Gregoriev Glacier, Kyrgyzstan, were used for dissection. The samples were placed on a PPS membrane slide (Leica, Germany) and allowed to dry. Single cyanobacterial filaments were isolated using a Laser Microdissection System (model LMD 7000; Leica, Germany). The dissected cyanobacterial filament was directly transferred to PCR tubes containing a sterilized lysis buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA, and 0.1% Tween 20 in Milli-Q water). Tubes containing cyanobacterial filaments were subjected to three consecutive freeze–thaw cycles to lyse the filaments and then incubated at 55°C for 2 h. PCR amplification of the 16S rRNA-ITS region genes was performed using Ex Taq Hot Start Version (Takara, Japan) and primers 359F (Nübel, Garcia-Pichel, & Muyzer, 1997) and 23S30R under the following conditions: 3 min initial denaturation at 94°C; 45 cycles of 94°C for 45 s, 52°C for 45 s and 72°C for 3 min 30 s; final extension at 72°C for 7 min. Sequencing procedures were the same as described in the above.

2.4 | Sequencing and phylogenetic analysis

Sequencing data were analysed using MOTHUR 1.33.3 (Schloss et al., 2009). Chimeric sequences were identified and removed using chimera.uchime (Edgar, Haas, Clemente, Quince, & Knight, 2011). The sequences were clustered into operational taxonomic units (OTUs)

with a criterion of 98% identity of 16S rRNA gene using the furthest neighbour algorithm. BLASTN searches were performed to identify the closest sequences in the NCBI-nt database. MOTHUR data were used to calculate a coverage (the formula [1−*n*/*N*], where *n* is the number of singletons and *N* is the total number of sequences).

Operational taxonomic units were initially clustered at the 98% nucleotide identity level based only on the 16S rRNA gene sequences (defined as "species"). These 16S rRNA-based OTUs were then subclustered using ITS sequences with a criterion of 100% identity. These unique ITS sequences were defined as "strains." We searched for tRNA sequences using tRNADB-CE (Abe et al., 2014) and removed tRNA sequences for subsequent analysis. Representative sequences of 16S rRNA gene and ITS sequences in each haplotype were aligned using the program MAFFT (Katoh & Toh, 2010), respectively. These alignments were carefully checked by eye and all ambiguous sites, and sequences were manually deleted. Maximum likelihood trees were reconstructed using RAxML v.7.2.8 (Stamatakis, 2006) with the GTR+Γ models for 16S and ITS sequences. Weighted UniFrac analyses were performed to calculate pairwise distances between cyanobacterial communities, and permutation analysis was used to test whether they differ significantly (Lozupone & Knight, 2005). Weighted UniFrac analyses were performed in MOTHUR. Similarity of cyanobacterial community structures was calculated using the Bray–Curtis similarity index implemented in the QIIME v1.9.1 package (Caporaso et al., 2010). Differences among clusters were statistically tested by analysis of similarity (ANOSIM) with 999 permutations.

2.5 | Haplotype network and population genetic diversity analysis of ITS regions

Haplotype networks were reconstructed on the basis of the ITS sequences with the median joining (MJ) method (Bandelt, Forster, & Röhl, 1999) using NETWORK 4.6.11 (http://www.fluxus-engineering.com/sharenet.htm). To estimate genetic diversities within each population, nucleotide diversity was calculated using p-distances calculated in MEGA 5 (Tamura et al., 2011). Standard deviations were calculated using 1,000 bootstrap replicates. To clarify phylogenetic relationships among populations, the net distances between the populations were also calculated based on p-distances. The neighbour joining (NJ) method was used to reconstruct phylogenetic relationships. Geographical distances between sampling points were calculated with the Perpendicular Distance Calculator v1.2.2 (American Museum of Natural History, http://biodiversityinformatics.amnh.org/open_source/pdc). Mantel tests of geographical distances versus pairwise raw net genetic distances were performed with PAST 3.1 (Hammer, Harper, & Ryan, 2001), using 100,000 permutations and the Pearson's correlation coefficient.

The presence or absence of geographical structures within each OTU defined by 16S rRNA gene was investigated by measuring G_{ST} and N_{ST} using PERMUT (Pons & Petit, 1996; Burban, Petit, Carcreff, & Jactel, 1999; https://www6.bordeaux-aquitaine.inra.fr/biogeco/Production-scientifique/Logiciels/Contrib-Permut/Permut), F_{ST} and analysis of molecular variance (AMOVA) using the ARLEQUIN 3.5 program (Excoffier & Lischer, 2010). For the AMOVA analysis, two kinds of hierarchical structures were adopted. The first specified regions and subregions as higher and lower hierarchical structures, respectively. Each region (Antarctica, Asia and Arctic) consists of more than one subregion (Antarctica, Northern Asia, Southern Asia, Alaska, Greenland and Svalbard). The second hierarchical structure adopted subregions and glaciers as higher and lower structures.

3 | RESULTS

3.1 | Cyanobacterial phylotypes as inferred from 16S rRNA analysis

The 16S–ITS sequence dataset comprised a total of 2,938 reads with an average of 78 ± 32 sequences per glacier sample and an average sequence length of 1,843 bp. The coverage value per 16S rRNA gene clone library was 94%–100%. We assume that the sequencing effort was sufficient to detect most, if not all, cyanobacterial diversity present at each site. Most 16S rRNA genes were affiliated with Oscillatoriales (87% of the total number of OTUs), Nostocales (9%) and Chroococcales (3%; Table 1). The most frequently detected genera were *Phormidesmis* (29%) and *Microcoleus* (16%) in Oscillatoriales; unclassified Oscillatoriales accounted for 22% of OTUs. The 16S rRNA gene sequences were grouped into 20 cyanobacterial OTUs (Table 1). The phylogenetic composition of the cyanobacterial communities differed significantly among regions (UniFrac $p < .001$) and among individual glaciers (ANOSIM: global $R = .68$, $p < .001$, $n = 38$, number of groups = 15 in 16S rRNA gene, $R = .64$, $p < .001$, $n = 38$, number of groups = 15 in ITS region).

A heatmap analysis 16S rRNA gene OTUs showed that the phylotype composition was related to the geographical region (Figure 2). Of the 20 OTUs, 11 were found in multiple regions. These 11 OTUs accounted for 97% of the sequences in the clone libraries, suggesting that the most abundant phylotypes were present in multiple regions. Of the 20 OTUs detected, only six OTUs were present at abundances >5% in the clone libraries (OTU 0, 1, 2, 4, 7 and 8; Table 1). Of these, five were assigned to Oscillatoriales, while one (OTU2) was assigned to Nostocales. Together, these six most abundant phylotypes represented 45%–100% of each clone library, averaging 88% ± 13% of the clones sequenced across the total library.

3.2 | Morphological classification and single-filament PCR

Clone libraries from cryoconite sampled from Urumqi Glacier No. 1, China, and Gregoriev Glacier, Kyrgyzstan, contained 11 of the 20 OTUs (Figure 2). Together, these 11 OTUs accounted for 90% of the clone library. The cryoconite granules from these two glaciers contained abundant filamentous cyanobacteria, and at least three morphotypes could be identified with optical or fluorescence microscopy. The three morphotypes from both glaciers were classified as Oscillatoriaceae cyanobacterium 1, Oscillatoriaceae cyanobacterium

TABLE 1 The list of cyanobacterial 16S rRNA gene sequences (OTU0–OTU19) detected on the glacier samples in the Arctic, Antarctic and Asian mountains

OTU numbers	Highest match/accession number Highest cultured match/accession number	% similarity	Order	ITS length (bp) (removed tRNA)	Single-filament PCR	% in the total
OTU0	Uncultured Oscillatoriales cyanobacterium clone LI06st1/JF733399	99	Oscillatoriales	354–356	+	21.9
	Cyanobacterium enrichment culture clone CAWBG90/KC818279	99				
	Alkalinema pantanalense CENA531/KF246497	94				
OTU1	Uncultured cyanobacterium clone H-B07/DQ181687	99	Oscillatoriales	358–465		28.9
	Phormidesmis priestleyi ANT.L66.1/AY493581	99				
OTU2	Uncultured *Nostoc* sp. clone K_J52/JQ007764	99	Nostocales	324–387		6.7
	Nostoc sp. "*Nephroma arcticum* UK103 cyanobiont"/KF359688	99				
OTU3	Uncultured *Chamaesiphon* sp. clone 5f-17/JF832304	99	Chroococcales	460–494		3.0
	Chamaesiphon polonicus SAG 32.87/KM019983	98				
OTU4	–		Oscillatoriales	420–442	+	16.1
	Microcoleus vaginatus SRS1-KK2/EF654078	99				
OTU5	*Pseudanabaena* sp. lw0831/KF208379	99	Oscillatoriales	380–394	+	2.3
	Pseudanabaena catenata SAG 254.80/KM020004	98				
OTU6	Uncultured bacterium clone Bas-7-20/GQ495376	95	Unclassified	446–447		0.8
	Cyanobacterium TDX16/KJ599678	80				
OTU7	Uncultured cyanobacterium clone 10.1.11-006_Isolat_1/KF029609	100	Oscillatoriales	345–346		6.3
	Cyanobacterium enrichment culture clone CAWBG81/KC818277	98				
	Alkalinema pantanalense CENA531/KF246497	93				
OTU8	Uncultured bacterium clone: MPB1-5/AB630387	99	Oscillatoriales	432–435	+	7.6
	Leptolyngbya frigida ANT.L53B.2/AY493576	93				
OTU9	Uncultured cyanobacterium clone A206/DQ181671	98	Oscillatoriales	337	+	1.9
	Geitlerinema sp. Sai004/GU935348	98				
OTU10	Uncultured bacterium clone: Qiyi-cya-OTU 5/AB569630	100	Chroococcidiopsidales	334		0.03
	Aliterella antarctica CENA408/KU291459	96				
OTU11	Uncultured cyanobacterium clone RJ094/DQ181682	98	Oscillatoriales	390–416		1.2
	Leptolyngbya frigida ANT.L52.2/AY493575	95				
OTU12	–		Oscillatoriales	538–539		0.1
	Phormidesmis priestleyi ANT.LPR2.6 1/AY493585	100				
OTU13	Uncultured bacterium clone TP-Snow-9/HQ327233	99	Oscillatoriales	416		0.1
	Phormidesmis priestleyi ANT.L52.6/AY493579	99				
OTU14	Uncultured bacterium clone EpiUMA24/FJ849205	98	Chroococcales	522		0.1
	Chamaesiphon polonicus SAG 32.87/KM019983	97				

(Continues)

TABLE 1 (Continued)

OTU numbers	Highest match/accession number Highest cultured match/accession number	% similarity	Order	ITS length (bp) (removed tRNA)	Single-filament PCR	% in the total
OTU15	–		Oscillatoriales	329		0.2
	Phormidium murrayii ANT.LPE.2/AY493598	98				
OTU16	Uncultured cyanobacterium clone FQSS103/EF522323	99	Nostocales	486–494		2.2
	Cylindrospermum stagnale PCC 7417/CP003642	94				
OTU17	Uncultured bacterium clone EpiUMB54/FJ849308	98	Chroococcales	475		0.1
	Chamaesiphon polonicus SAG 32.87/KM019983	96				
OTU18	Uncultured cyanobacterium clone RJ102/DQ181684	99	Oscillatoriales	468		0.1
	Leptolyngbya antarctica ANT.L67.1/AY493572	99				
OTU19	Uncultured bacterium clone: G2CLN39/AB696223	97	Unclassified	522–526		0.1
	Gloeobacter kilaueensis JS1/CP003587	85				

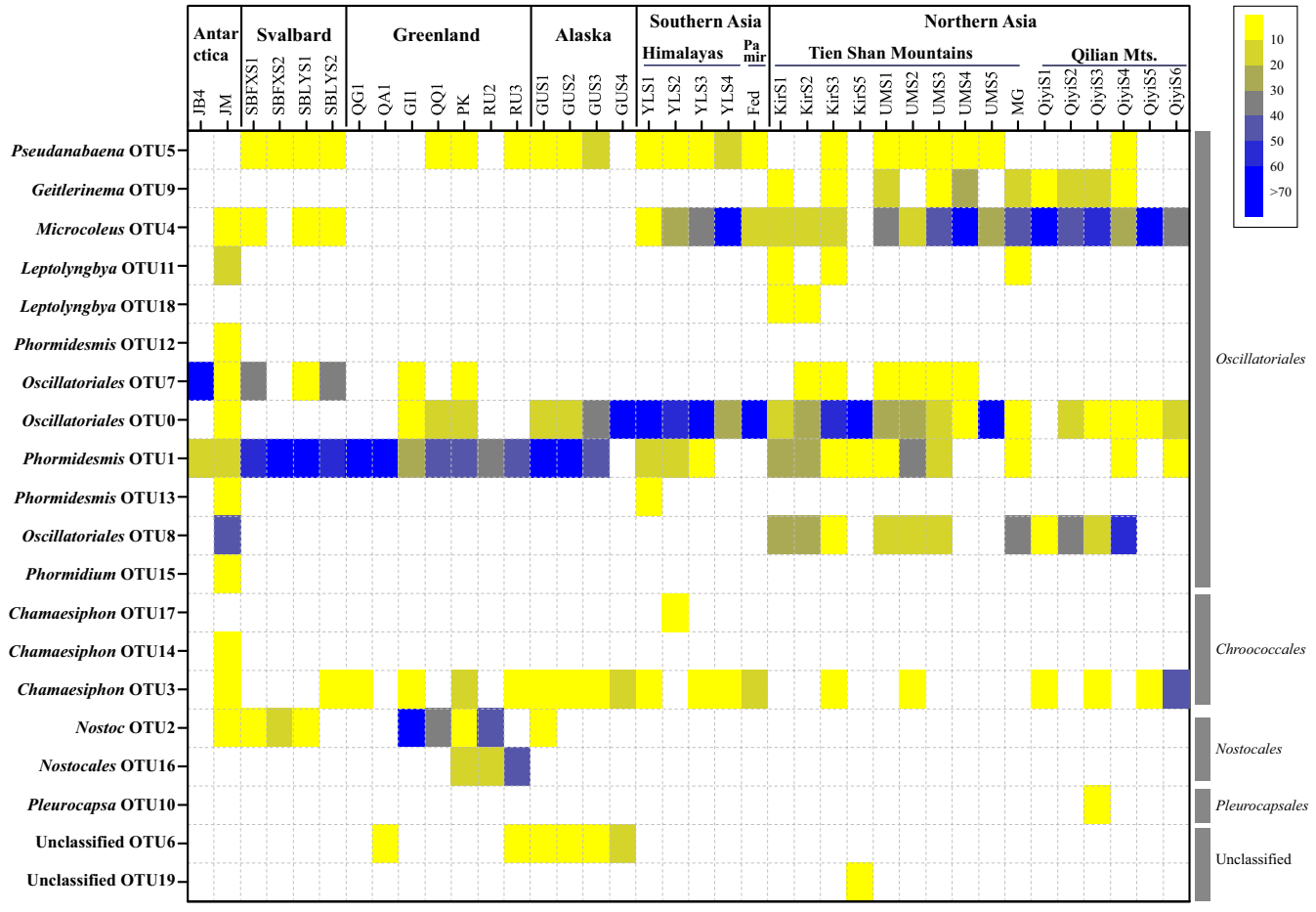

FIGURE 2 Geographical distribution of cyanobacteria on glacier samples as determined by 16S rRNA gene analysis. Representative heat map of clone percentage in samples is shown. Blue and yellow heat map indicate high and low expression, respectively, of each OTU at sample sites. White, no detectable signal [Colour figure can be viewed at wileyonlinelibrary.com]

2 and Oscillatoriaceae cyanobacterium 3. Oscillatoriaceae cyanobacterium 1 lacked a sheath and its mean cell diameter (±*SD*) was 1.6 ± 0.20 μm (Urumqi Glacier No. 1) and 1.3 ± 0.19 μm (Gregoriev Glacier). Oscillatoriaceae cyanobacterium 2 had a sheath and its mean cell diameter (±*SD*) was 3.1 ± 0.50 (Urumqi Glacier No. 1) and 3.4 ± 0.73 μm (Gregoriev Glacier). Oscillatoriaceae cyanobacterium 3 also had a sheath and its mean cell diameter (±*SD*) was 1.5 ± 0 .22 μm (Urumqi Glacier No. 1) and 1.4 ± 0.29 μm (Gregoriev Glacier) (Figure S1 in Appendix S2).

The 16S–ITS sequences of these three morphotypes were amplified by single-filament PCR. Five OTUs (*Oscillatoriales* OTU0, *Microcoleus* OTU4, *Pseudanabaena* OTU5, *Oscillatoriales* OTU8 and *Geitlerinema* OTU9; Figure S2 in Appendix S2) were identified with 99.5%–100% similarity to clone library sequences of the16S rRNA gene. These five OTUs represented 50% of the sequences in the clone libraries. Single-filament PCR analyses of the microscopic morphotypes showed that the filamentous Oscillatoriales cyanobacteria were abundant in the Asian glacier samples, consistent with the clone library analysis.

3.3 | Cyanobacterial analysis based on 16S–23S ITS region

To examine phylogenetic variation on a finer scale, we independently examined the 16S–23S ITS region contiguously sequenced within the clone libraries. The 16S–23S ITS region sequences of nine of the 20 OTUs analysed showed 96%–100% similarity with Antarctic and glacier cyanobacterial sequences in the NCBI-nt database (>96.0% similarity; Table S2 in Appendix S2). The nucleotide diversity ($\Theta\pi$) within the ITS varied among OTUs with values ranging from 0.0030 to 0.097 (0.035 ± 0.005). The 16S–23S ITS intra-OTU nucleotide diversities were not correlated with the abundance of 16S-defined OTUs within the clone library; for example, the greatest nucleotide diversities were in OTU1 (*Phormidesmis*, 28.9% of clone library), OTU3 (*Chamaesiphon*, 3.0% of clone library) and OTU11 (*Leptolyngbya*, 1.2% of clone library) (Figure S3 in Appendix S2). The nucleotide diversities were higher in *Phormidesmis* OTU1 and *Chamaesiphon* OTU3 than in the other OTUs. *Oscillatoriales* OTU0, the second-most dominant OTU, had low nucleotide diversity (0.0077 ± 0.0023; see Figure S3 in Appendix S2). Thus, nucleotide diversities were not related to the dominance of OTUs or to their phylogenetic relationships.

While the 16S rRNA-based OTUs were often widely distributed, a geographical network analysis of 16S–23S ITS showed that almost all phylotypes had regional distribution patterns. The haplotype networks detected in this study indicated that several geographical regions shared ancestral haplotypes with endemic haplotypes independently derived from them multiple times (Figure 3 and Figure S4–S8 in Appendix S2). The results of the 16S–23S ITS analyses showed that only *Nostoc* OTU2 was cosmopolitan with a global distribution at the strain level; the other OTUs, together accounting for 93% of the library clones, were regionally endemic at the strain level.

3.4 | Population genetic analysis

The six major globally distributed 16S rRNA-based OTUs were then analysed based on their 16S–23S ITS sequences. The N_{ST} values were larger than the G_{ST} values for each OTU (Table 2), indicating that there was genetic differentiation among geographical regions for all six OTUs.

Mantel tests showed that genetic distance was correlated with geographical distance for *Oscillatoriales* OTU0, 4 and 8 (Table 2). Almost all F_{ST} values of the *Oscillatoriales* (OTU0, 1, 4, 7 and 8) were statistically significant ($p < .05$). In contrast, differences in F_{ST} values for *Nostoc* OTU2 were mostly non-significant among regions, except for Alaska–Greenland (Table S3 in Appendix S2). In the AMOVA analysis, most of the variance (>60%) was "within populations" for the major OTUs (Table 2). The proportion of variance among regions (Antarctica, Asia, Arctic) and among subregions (Antarctica, Northern Asia, Southern Asia, Alaska, Greenland and Svalbard) differed among the OTUs. The proportion of variance among subregions was smaller than that among glaciers within subregions for OTU0, OTU1 and OTU2. The proportion of variance among subregions was larger than the proportion of variance among glaciers for OTU4, OTU7 and OTU8.

4 | DISCUSSION

4.1 | Cryoconite cyanobacterial phylotypes on glaciers

Based on their 16S rRNA sequences, most of the cyanobacteria detected in this study were affiliated with Oscillatoriales, which have been previously described from glacier environments (Michaud, Šabacká, & Priscu, 2012; Segawa & Takeuchi, 2010; Segawa et al., 2014). Specifically, these cyanobacteria detected in this study belonged to the genera *Phormidesmis* and *Microcoleus* in Oscillatoriales, which appear to be both ubiquitous and dominant in glacier environments and are capable of growing in cold conditions (Strunecký, Komárek, Johansen, Lukešová, & Elster, 2013; Taton et al., 2006; Webster-Brown, Hawes, Jungblut, Wood, & Christenson, 2015). *Phormidesmis* was the dominant genus, accounting for 29% of the total sequences (*Phormidesmis* OTU1, *Phormidesmis* OTU12 and *Phormidesmis* OTU13). In a previous report, an analysis of the origins of cold-tolerant clades of cyanobacteria indicated that *Phormidesmis* is highly likely to be derived from a cold-tolerant ancestor clade (Chrismas et al., 2015).

The results of this study indicate that *Phormidesmis* is a prominent cyanobacterial taxon in cryoconite in the Arctic region. However, since *Phormidesmis* is not included in the Silva or Greengenes databases for QIIME, it has not been described in some previous high-throughput sequencing studies (Cameron et al., 2016; Stibal et al., 2015). However, a *Phormidesmis* OTU was found to be dominant in a dataset produced from cryoconite samples collected from an ice cap in Greenland (based on a manual annotation of 16S amplicon semiconductor sequencing data) (Uetake et al., 2016), and

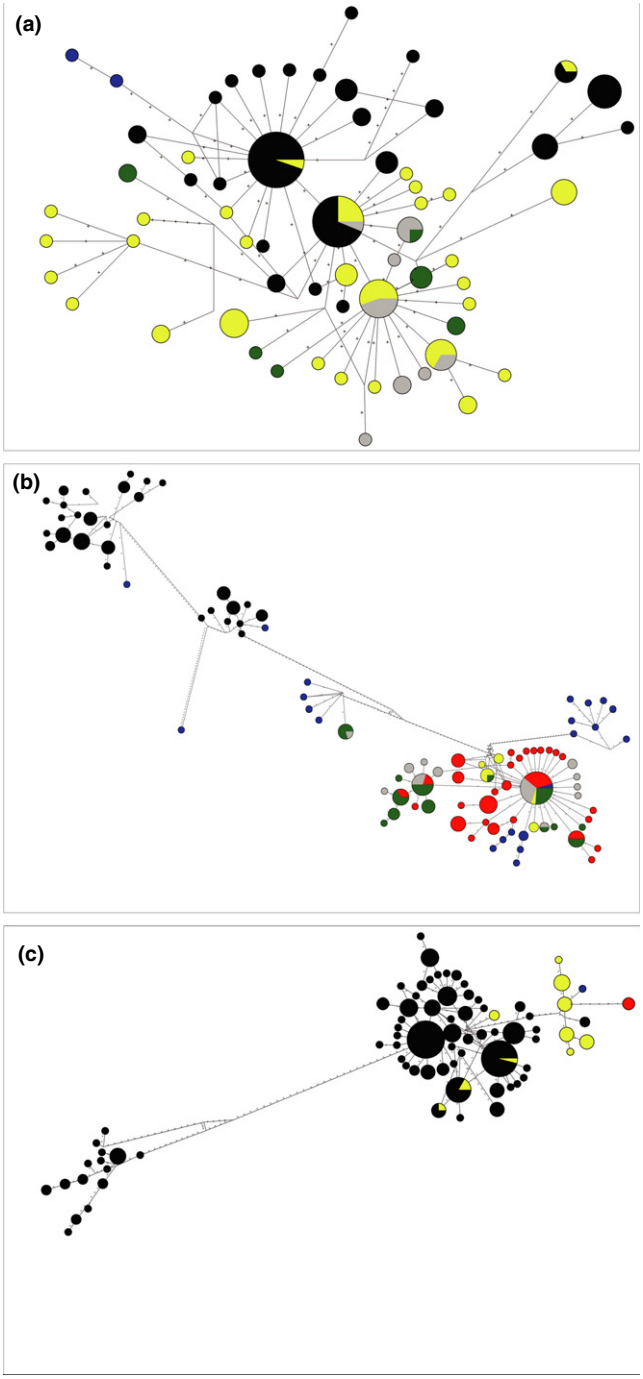

FIGURE 3 Haplotype networks of 16S–23S ITS regions on OTU0 (a), OTU1 (b) and OTU4 (c), the three most abundant OTUs in this study. Median joining method was used for this analysis. Circles indicate haplotypes; size of each haplotype is proportional to clone number of unique sequence. Haplotypes are coloured according to geographical region of the glacier: blue: Antarctica, green: Greenland, red: Svalbard, grey: Alaska, black: Northern Asia and yellow: Southern Asia. Most closely related haplotypes are directly connected by lines ("links"). + indicates mutation events [Colour figure can be viewed at wileyonlinelibrary.com]

Phormidesmis also dominated cyanobacterial OTUs in Sanger-sequenced clone libraries generated from Svalbard cryoconite (Edwards et al., 2011).

Microcoleus OTU4 has been recorded from soils in cold environments (DNA database with similarities >99%, e.g. EF654078 and KF494564); furthermore, *Microcoleus* was found to be dominant in reverse-transcribed cDNA 16S rRNA profiles from Asian cryoconites, suggesting it is commonly distributed on glaciers (Segawa et al., 2014). Therefore, it is likely that *Phormidesmis* and *Microcoleus* are the major groups in the Arctic and Asia, respectively. The existence of sequences in the NCBI-nt database with similarities >98% suggests that these cyanobacterial taxa are widely distributed among different habitats such as soil (e.g. EF654078), fresh water (e.g. DQ181687), and snow and ice environments (e.g. HQ595191). According to Jungblut et al. (2010), the major cyanobacterial 16S rRNA gene phylotypes within the cryosphere matched those of perennially cold terrestrial ecosystems. The results of the 16S rRNA gene analyses in this study imply that cyanobacteria on glaciers are unlikely to be specialist taxa restricted solely to snow and ice. Indeed, most of the cyanobacterial species detected in cryoconite have also been found in soil samples (Kaštovská, Elster, Stibal, & Šantrůčková, 2005). Moraine sediments were shown to be a source of cryoconite microbes (Segawa et al., 2014). Consistent with this potential source of cyanobacteria inoculating glaciers, we found that many of the OTUs detected in this study have also been detected in soil environments.

A global phylogenetic analysis focusing on the evolution of cold-environment cyanobacteria showed that several clades of cold-dwelling cyanobacteria are common among cold regions (Chrismas et al., 2015). Ten OTUs in this study, including four of the major six OTUs (*Phormidesmis* OTU1, *Nostoc* OTU2, *Microcoleus* OTU4 and *Oscillatoriales* OTU8), were grouped into putative ancestral cold-tolerant clusters defined by Chrismas et al. (2015). However, *Oscillatoriales* OTU0, the second-most dominant OTU in this study, was not recognized in their study. On the basis of 16s rRNA gene analyses, Chrismas et al. (2015) suggested that there are biogeographical links between the Arctic and Northern Hemisphere alpine regions. *Pseudanabaena* OTU5, which was included in the Northern Hemisphere cluster described by Chrismas et al. (2015), was detected in glaciers across a wide geographical range from the Arctic to Asia, but not in Antarctica.

4.2 | Geographical variations in cryoconite cyanobacterial communities

Although OTUs were detected across multiple regions, the 16S rRNA gene analysis suggested that the distribution of cyanobacteria in various regions had a geographical structure. Based on their detection rate in geographical regions, 16S phylotypes were categorized as either cosmopolitan (OTU0, 1, 2, 3, 4, 5, 7, 8, 11 and 13), an Arctic type dominant at Greenland, Spitsbergen and Alaska sites (OTU6 and 16), an Antarctic type (OTU12, 14 and 15), and an Asian type dominant at northern and southern Asia sites (OTU9, 10, 17, 18 and 19). Six major phylotypes representing 88% of the total clone library were cosmopolitan taxa.

TABLE 2 Analysis of molecular variance (AMOVA), genetic differentiation and Mantel test of the six major OTUs (OTU0, 1, 2, 4, 7 and 8) on the glaciers in the Antarctic, Arctic and Asian mountains

		Variance components (subregions—glaciers)				Variance components (regions—subregions)				Genetic differentiation		Mantel test	
OTUs		Among subregions (Va)	Among glaciers within subregions (Vb)	Within glaciers (Vc)	Total variance	Among regions (Va)	Among subregions within regions (Vb)	Within subregions (Vc)	Total variance	G_{ST}	N_{ST}	Correlation *R*	*p*-value
OUT0	σ^2	0.28 (15.6%)	0.64 (35.5%)**	0.88 (48.9%)**	1.8	0.07 (3.9%)	0.64 (34.4%)**	1.14 (61.8%)**	1.85	0.29 ± 0.059	0.59 ± 0.16	.172	.044
	Φ	0.16	0.043	0.19		0.002	0.27	0.28					
OTU1	σ^2	0.03 (6.0%)	0.05 (13.0%)**	0.34 (81.0%)**	0.42	0.05 (11.7%)*	0.04 (8.3%)**	0.36 (80.1%)**	0.45	0.19 ± 0.058	0.66 ± 0.12	.100	.158
	Φ	0.060	0.14	0.19		0.117	0.093	0.20					
OTU2	σ^2	−0.0041 (−1.2%)	0.03 (9.7%)**	0.31 (91.5%)**	0.33	−0.0050 (−1.5%)	0.02 (4.7%)	0.32 (96.8%)	0.34	0.14 ± 0.19	0.15 ± 0.23	−.164	.815
	Φ	−0.0058	0.067	0.061		−0.015	0.046	0.032					
OTU4	σ^2	0.09 (16.9%)**	0.02 (4.4%)**	0.42 (78.8%)**	0.53	0.17 (24.8%)	0.08 (11.6%)**	0.43 (63.6%)**	0.68	0.59 ± 0.25	0.66	.389	.032
	Φ	0.17	0.053	0.21		0.25	0.15	0.36					
OTU7	σ^2	0.05 (13.2%)*	0.02 (4.6%)	0.31 (82.3%)**	0.38	−0.03 (−6.8%)	0.09 (23.2%)	0.32 (83.6%)**	0.38	0.19	0.29 ± 0.074	−.177	.780
	Φ	0.13	0.053	0.18		−0.068	0.22	0.16					
OTU8	σ^2	0.21 (37.1%)	−0.0082 (1.4%)**	0.35 (61.5%)**	0.57	–	–	–	–	0.44	0.58	.572	.008
	Φ	0.37	0.023	0.39									

σ2: variance components (percentages of variations) and total variances.

Φ: Φ statistics (fixation indices) corresponds to Φct (among groups), Φsc (among populations within groups) and Φst (among populations among groups).

"Regions" are defined as Antarctica, Asia and Arctic; "subregions" are defined as Antarctica, Northern Asia, Southern Asia, Alaska, Greenland and Svalbard.

OTU8 was collected from only two regions (Antarctica and Northern Asia). OTU8 was excluded from the AMOVA analysis.

N_{ST} and G_{ST} values among subregions (Antarctica, Northern Asia, Southern Asia, Alaska, Greenland and Svalbard) are shown for each OTU.

* and **, statistically significant at $p < .05$ and $p < .01$, respectively (Va and Φ_{CT}, Vb and Φ_{SC}, Vc and Φ_{ST}).

Single-filament PCR conducted on microscopic morphotypes showed that filamentous cyanobacteria were abundant in the Asian glacier samples, consistent with the clone library analysis. These results were compatible with our previous findings, since these filamentous cyanobacteria were detected at a high density in cryoconite granules (Takeuchi, Kohshima, & Seko, 2001). These findings also indicate that the phylotypes detected using the clone library strategy correspond to those of cyanobacteria forming cryoconite granules on the ice surface. However, these categories are based on a limited number of glaciers investigated in this study, especially in Antarctica. To confirm these categories, further investigations in different regions are necessary.

Most of the major 16S rRNA gene phylotypes were detected on both polar and Asian glaciers. However, the dominant type in the cyanobacterial community varied among the regions. *Phormidesmis* OTU1, *Nostoc* OTU2 and *Oscillatoriales* OTU7 were relatively abundant in polar regions; *Oscillatoriales* OTU0, *Microcoleus* OTU4 and *Oscillatoriales* OTU8 were abundant in Asia; *Oscillatoriales* OTU0 was dominant in Southern Asia; and *Microcoleus* OTU4 and *Oscillatoriales* OTU8 were dominant in Northern Asia. Since these dominant phylotypes appeared to be common within each region, the community structure may have been determined by the physical or chemical characteristics of glaciers in these regions. There are a number of differences in chemical and physical conditions between Asian and polar glaciers. For example, solar radiation is more intense at Asian glaciers than at those in polar regions because Asian glaciers are located at lower latitudes. Such differences in local environmental conditions may affect cyanobacterial communities, especially since cyanobacteria are more abundant on Asian glaciers than on polar glaciers (e.g. Takeuchi & Li, 2008). Although the life cycles and favourable growth conditions for these dominant cyanobacterial species are still unknown, they are the key phylotypes for understanding the expansion of cryoconite coverage on glaciers.

4.3 | Biogeography and dispersal processes of cyanobacterial phylotypes

In this study, we addressed the question of whether cyanobacterial species on glaciers are cosmopolitan or restricted in their distribution. We analysed genetic markers with different evolution rates to investigate large- and fine-scale genetic structures. The 16S–23S ITS haplotype networks showed that most OTUs were restricted in their regional distribution. Several ancestral haplotypes were detected in multiple regions, while the derived haplotypes showed restricted distributions (Figure 3). This result indicates that the ranges of most of the cyanobacterial OTUs were predominantly local, within glacier regions rather than intercontinental or inter-island.

For all OTUs, the N_{ST} was larger than the G_{ST} (Table 2), indicating that all the OTUs showed genetic differentiation among the geographical regions. The F_{ST} values of the most of the *Oscillatoriales* (OTU0, 1, 4, 7 and 8) were statistically significant ($p < .05$). In contrast, the F_{ST} values of the *Nostoc* OTU2 were mostly non-significant among regions except for Alaska–Greenland (Table S3). Figure 4 illustrates the types of cyanobacterial geographical distributions. The F_{ST} values suggested that five of the six major OTUs (*Oscillatoriales* OTU0, *Phormidesmis* OTU1, *Microcoleus* OTU4, *Oscillatoriales* OTU7 and *Oscillatoriales* OTU8) showed restricted distribution at the strain level. In contrast, *Nostoc* OTU2 lacked a geographical structure among subregions and was, therefore, characterized as cosmopolitan. *Oscillatoriales* OTU0 and *Phormidesmis* OTU1 showed strong signals of geographical structure in the N_{ST}–G_{ST} analysis. *Microcoleus* OTU4, *Oscillatoriales* OTU7 and *Oscillatoriales* OTU8 showed moderate or weak geographical structures. The G_{ST} and N_{ST} values were almost equal for *Nostoc* OTU2, also supporting its lack of geographical structure among subregions.

To understand the formation of these biogeographical patterns, it is necessary to distinguish between the migration ability of the groups and their sensitivity to environmental conditions. The AMOVA analysis allowed us to compare the variance among subregions (Va) with the variance among the glaciers within subregions (Vb). The OTUs with high variance among subregions (Va%) (e.g. OTU8: 37.1%, OTU4: 16.9%, OTU0: 15.6%) showed a significant correlation between geographical and genetic distances in the Mantel test (Table 2). In contrast, those with lower Va% (OTU2: −1.2%, OTU1: 6.0%, OTU7: 13.2%) showed little variance among subregions, and no significant correlation between geographical and genetic distances. If the migration ability is high, geographical structuring will be diluted and the correlation between geographical and genetic distances will be weaker. Therefore, Va% is a good indicator of limited geographical migration ability. The Va% of the OTU2 was negative; a negative value can occur without genetic structure at a given level, confirming that there was no genetic structure in OTU2 among subregions.

If biogeographical structure has resulted from limited migration ability, then genetic differentiation will be determined by geographical distance. If this is the case, then Va% > Vb% would be expected, since the geographical distances among subregions were larger than those among glaciers within subregions. However, if the distribution of cyanobacterial OTUs has been determined mainly by environmental conditions (e.g. pH, irradiance and nutrients), then Vb% > Va% would be expected, because such conditions vary among glaciers within subregions. For this reason, the Vb%/Va% ratio may distinguish between differentiation caused by geographical distance and that resulting from ecological filtering in assembling communities.

The genetic differentiation among glaciers within a subregion seemed to be related to the adaptabilities of genotypes to various glacial environments, rather than their migration ability. Five of the six major phylotypes showed significant genetic differentiation among glaciers, the only exception being *Oscillatoriales* OTU7. The variance among glaciers within subregions was larger than the variance among subregions for *Oscillatoriales* OTU0, *Phormidesmis* OTU1 and *Nostoc* OTU2. This result suggests that their genetic differentiation was mainly caused by environmental differences among glaciers, rather than geographical isolation. In terms of geographical structure, OTU0 and OTU2 were the extreme cases in our samples. *Oscillatoriales* OTU0 populations were typically geographically restricted,

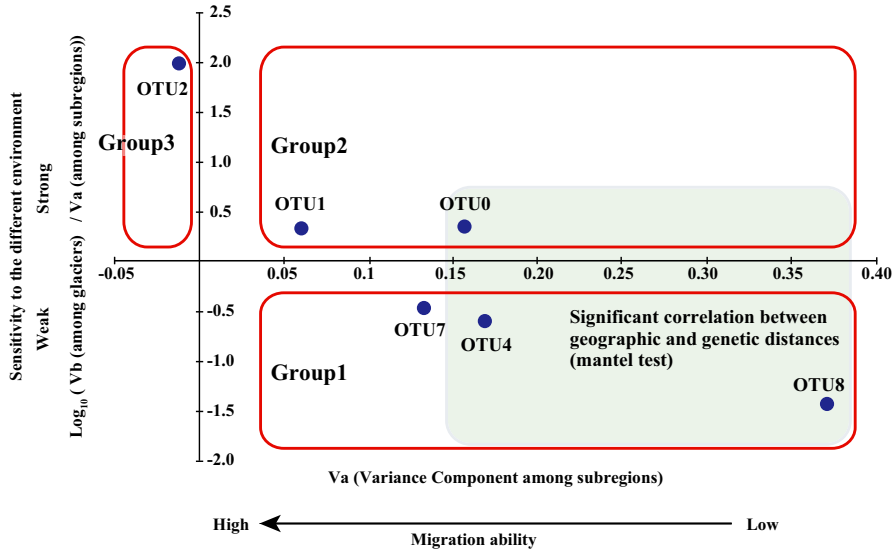

FIGURE 4 Types of geographical distribution of major cyanobacterial OTUs based on mobility and adaptability to glacial environments. Geographical mobility (*X* axis) was measured by percentage of variance components among subregions (Va%); adaptability to glacial environments (*Y* axis) was measured by $\log_{10}$ (Vb%/Va%), where Vb% is percentage of the variance among glaciers within subregions. Negative values of variance components indicate absence of genetic structure at a given level. Negative value of Va% indicates no genetic structure among subregions (absolute value of Va% has no meaning in this case). Va% was assumed to have a small positive value (0.1%) in calculation of $\log_{10}$ (Vb%/Va%) in this case [Colour figure can be viewed at wileyonlinelibrary.com]

whereas *Nostoc* OTU2 showed a cosmopolitan population structure. The OTUs showing a large proportion of variance among glaciers were highly sensitive to environmental factors, and therefore, have been under strong selective pressure at each site.

Nostoc OTU2 was not detected from Asian glaciers in this study. However, the 16S rRNA gene of *Nostoc* OTU2 showed 98%–99% similarity with Asian lichens, for example, *Peltigera degenii* in China (JQ007760) and *Peltigera praetextata* in Japan (JQ007787) and with lichen-symbiotic *Nostoc* in Finland and the USA (KF359688, KF359683). This 16S rRNA gene phylotype can, therefore, be classified as cosmopolitan, which is supported by the results of our 16S-23S ITS haplotype network analysis. Domaschke, Fernández-Mendoza, Garcia, Martín, and Printzen (2012) reported the long-distance dispersal of *Cetraria aculeate*, the algal partner of this lichen. In this study, *Nostoc* OTU2 had almost no geographical structure, as indicated in the Mantel test of the correlation between the geographical and genetic distances (p-value = .815, R = −.164). The results suggest that this OTU has a high migration rate among regions, weakening its geographical structure.

The proportion of variance among glaciers within subregions was smaller than that among subregions for *Microcoleus* OTU4, *Oscillatoriales* OTU7 and *Oscillatoriales* OTU8. This result suggests that their genetic differentiation was mainly caused by geographical isolation rather than by environmental differences among glaciers.

The geographical structures detected in this study could be categorized into three groups (Figure 4): the first group had a geographically restricted but ubiquitous distribution within different glacial environments (*Microcoleus* OTU4, *Oscillatoriales* OTU7, and *Oscillatoriales* OTU8). These OTUs probably have lower migration ability among regions and low sensitivity to the environment. The second group showed a geographically restricted and specific distribution in different environments (*Oscillatoriales* OTU0 and *Phormidesmis* OTU1). This group may have also low migration ability among regions (a strong signal of the geographical structure by long history), but high sensitivity to different environmental conditions. This group showed the strongest biogeographical structure in the G_{ST}–N_{ST} analysis, probably because their geographical distribution has been affected by both geographical distance and environmental conditions. The geographical distribution of these two types can be explained by the regional characteristics in glaciers or the change of glacial extent due to climatic changes from glacial to interglacial periods. The third group showed a cosmopolitan distribution across different environments (*Nostoc* OTU2). This was the only group with a global distribution, indicating that it readily migrates among regions.

In conclusion, the results of the 16S rRNA gene analyses suggest that there is a global distribution of cyanobacterial species colonizing glacier surfaces. However, the results of the 16S–23S ITS analyses, which identified most of the phylotypes to the strain level, revealed regional adaptations indicative of dispersal limitation. Only one phylotype showed no genetic structure among the geographical regions, suggesting that it is cosmopolitan even at the strain level. The geographical distributions of the OTUs revealed by this rapidly evolving genetic marker could not be explained by the hypothesis of "Everything is everywhere, but the environment selects" suggested by Baas Becking (De Wit and Bouvier, 2006). Only the distribution of the cosmopolitan OTU2 supported this hypothesis. Most of the phylotypes showed restricted distributions at the strain level, indicating

that there is limited migration among regions. We detected significant levels of genetic differentiation, which may indicate the different adaptabilities of genotypes to various glacial environments. This novel perspective on the restricted distribution of glacier cyanobacteria highlights the potential specialization of biodiversity and the differences in glacier ecosystems within and among regions.

4.4 | Nucleotide sequences

The nucleotide sequences obtained in this study have been deposited in DDBJ/EMBL/GenBank under the accession numbers LC103274–LC104281.

ACKNOWLEDGEMENTS

We thank the editors, Dr Mark Carine and Dr. Peter Linder, as well as three anonymous reviewers, whose comments greatly improved our manuscript. This study was supported by the Grants-in-Aid for Scientific Research (no. 17H01854, 26281017, 23221004, 26247078 and 26241020) from the Japan Society for Promotion of Science and CAS (KJZD-EW-G03-01) from China. Svalbard sampling was supported by a grant from the Aberystwyth University Research Fund to A.E. and by grants from the Great Britain Sasakawa Foundation to A.E., N.T. and T.I.-F. The University Centre in Svalbard (UNIS) is gratefully acknowledged for logistical support.

ORCID

Takahiro Segawa http://orcid.org/0000-0002-3111-708X

REFERENCES

Abe, T., Inokuchi, H., Yamada, Y., Muto, A., Iwasaki, Y., & Ikemura, T. (2014). tRNADB-CE: tRNA gene database well-timed in the era of big sequence data. *Frontiers in Genetics*, *5*, 114.

Bandelt, H. J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, *16*, 37–48.

Burban, C., Petit, R. J., Carcreff, E., & Jactel, H. (1999). Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. *Molecular Ecology*, *8*, 1593–1602.

Cameron, K. A., Stibal, M., Zarsky, J. D., Gözdereliler, E., Schostag, M., & Jacobsen, C. S. (2016). Supraglacial bacterial community structures vary across the Greenland ice sheet. *FEMS Microbiology Ecology*, fiv164.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K. … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336.

Chrismas, N. A. M., Anesio, A., & Sanchez-Baracaldo, P. (2015). Multiple adaptations to polar and alpine environments within cyanobacteria: A phylogenomic and Bayesian approach. *Frontiers in Microbiology*, *6*, 1070.

De Wit, R. & Bouvier, T. (2006). 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say?. *Environmental Microbiology*, *8*, 755–758.

Domaschke, S., Fernández-Mendoza, F., Garcia, M. A., Martín, M. P., & Printzen, C. (2012). Low genetic diversity in Antarctic populations of the lichenforming ascomycete *Cetraria aculeata* and its photobiont. *Polar Research*, *31*, 17353.

Dvořák, P., Hašler, P., & Poulíčková, A. (2012). Phylogeography of the *Microcoleus vaginatus* (Cyanobacteria) from three continents – a spatial and temporal characterization. *PLoS ONE*, *7*, e40153.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*, 2194–2200.

Edwards, A., Anesio, A. M., Rassner, S. M., Sattler, B., Hubbard, B. P., & Perkins, W. T. (2011). Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard. *ISME Journal*, *5*, 150–160.

Edwards, A., Mur, L. A. J., Girdwood, S. E., Anesio, A. M., Stibal, M., Rassner, S. M. E., … Sattler, B. (2014). Coupled cryoconite ecosystem structure–function relationships are revealed by comparing bacterial communities in alpine and Arctic glaciers. *FEMS Microbiology Ecology*, *89*, 222–237.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

Foreman, C. M., Sattler, B., Mikucki, J. A., Porazinska, D. L., & Priscu, J. C. (2007) Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica. *Journal of Geophysical Research*, *112*, G04S32.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*, 1–9.

Harding, T., Jungblut, A. D., Lovejoy, C., & Vincent, W. F. (2011). Microbes in high arctic snow and implications for the cold biosphere. *Applied and Environment Microbiology*, *77*, 3234–3243.

Isenbarger, T. A., Finney, M., Rios-Velazquez, C., Handelsman, J., & Ruvkun, G. (2008). Miniprimer PCR, a new lens for viewing the microbial world. *Applied and Environmental Microbiology*, *74*, 840–849.

Jungblut, A. D., Lovejoy, C., & Vincent, W. F. (2010). Global distribution of cyanobacterial ecotypes in the cold biosphere. *ISME Journal*, *4*, 191–202.

Kaštovská, K., Elster, J., Stibal, M., & Šantrůčková, H. (2005). Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). *Microbial Ecology*, *50*, 396–407.

Katoh, K., & Toh, H. (2010). Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics*, *26*, 1899–1900.

Langford, H., Hodson, A., Banwart, S., & Boggild, C. (2010). The microstructure and biogeochemistry of Arctic cryoconite granules. *Annals of Glaciology*, *51*, 87–94.

Lepère, C., Wilmotte, A., & Meyer, B. (2000). Molecular diversity of microcystis strains (Cyanophyceae, Chroococcales) based on 16S rDNA sequences. *Systematics and Geography of Plants*, *70*, 275–283.

Liguori, A. P., Warrington, S. D., Ginther, J. L., Pearson, T., Bowers, J., Glass, M. B., … Tuanyok, A. (2011). Diversity of 16S-23S rDNA internal transcribed spacer (ITS) reveals phylogenetic relationships in *Burkholderia pseudomallei* and its near-neighbors. *PLoS ONE*, *6*, e29323.

Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*, 8228–8235.

Michaud, A. B., Šabacká, M., & Priscu, J. C. (2012). Cyanobacterial diversity across landscape units in a polar desert: Taylor Valley, Antarctica. *FEMS Microbiology Ecology*, *82*, 268–278.

Nübel, U., Garcia-Pichel, F., & Muyzer, G. (1997). PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and Environmental Microbiology*, *63*, 3327–3332.

Pointing, S., Buedel, B., Convey, P., Gillman, L., Koerner, C., Leuzinger, S., & Vincent, W. (2015). Biogeography of photoautotrophs in the high polar biome. *Frontiers in Plant Science*, *6*, 692.

Pons, O., & Petit, R. J. (1996). Measuring and testing genetic differentiation with ordered *Versus* unordered alleles. *Genetics*, *144*, 1237–1245.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*, 7537–7541.

Segawa, T., Ishii, S., Ohte, N., Akiyoshi, A., Yamada, A., Maruyama, F., … Takeuchi, N. (2014). The nitrogen cycle in cryoconites: Naturally occurring nitrification-denitrification granules on a glacier. *Environmental Microbiology*, *16*, 3250–3262.

Segawa, T., & Takeuchi, N. (2010). Cyanobacterial communities on Qiyi glacier, Qilian Shan China. *Annals of Glaciology*, *51*, 135–144.

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690.

Stibal, M., Schostag, M., Cameron, K. A., Hansen, L. H., Chandler, D. M., Wadham, J. L., & Jacobsen, C. S. (2015). Different bulk and active bacterial communities in cryoconite from the margin and interior of the Greenland ice sheet. *Environmental Microbiology Reports*, *7*, 293–300.

Strunecký, O., Komárek, J., Johansen, J., Lukešová, A., & Elster, J. (2013). Molecular and morphological criteria for revision of the genus *Microcoleus* (Oscillatoriales, Cyanobacteria). *Journal of Phycology*, *49*, 1167–1180.

Takeuchi, N., Kohshima, S., & Seko, K. (2001). Structure, formation, darkening process of albedo reducing material (cryoconite) on a Himalayan glacier: A granular algal mat growing on the glacier. *Arctic, Antarctic, and Alpine Research*, *33*, 115–122.

Takeuchi, N., & Li, Z. (2008). Characteristics of surface dust on Ürümqi glacier no. 1 in the Tien Shan Mountains, China. *Arctic, Antarctic, and Alpine Research*, *40*, 744–750.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739.

Taton, A., Grubisic, S., Balthasart, P., Hodgson, D. A., Laybourn-Parry, J., & Wilmotte, A. (2006). Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. *FEMS Microbiology Ecology*, *57*, 272–289.

Uetake, J., Tanaka, S., Segawa, T., Takeuchi, N., Nagatsuka, N., Motoyama, H., & Aoki, T. (2016). Microbial community variation in cryoconite granules on Qaanaaq Glacier, NW Greenland. *FEMS Microbiology Ecology*, *92*, fiw127

Vincent, W. F. (2000). Evolutionary origins of Antarctic microbiota: Invasion, selection and endemism. *Antarctic Science*, *12*, 374–385.

Webster-Brown, J. G., Hawes, I., Jungblut, A. D., Wood, S. A., & Christenson, H. K. (2015) The effects of entombment on water chemistry and bacterial assemblages in closed cryoconite holes on Antarctic glaciers. *FEMS Microbiology Ecology*, *91*, fiv144.

Wientjes, I. G. M., Van de Wal, R. S. W., Reichart, G. J., Sluijs, A., & Oerlemans, J. (2011). Dust from the dark region in the western ablation zone of the Greenland ice sheet. *Cryosphere*, *5*, 589–601.

BIOSKETCH

Takahiro Segawa is interested in the phylogeography, adaptive evolutions and evolution of microorganisms in glacier ecosystems. Our group seeks improved understanding of ecology of glacial biology and relationship between the organisms and glacial environment.

Author contributions: T.S., T.Y. and N.T. conceived the ideas; T.S., A.E., S.T., J.U., T.I.F., K.F. and Z.L. collected glacial samples; A.A. supplied the sequences; T.S. and T.Y. analysed the data; T.S., T.Y., A.E. and N.T. wrote the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Segawa T, Yonezawa T, Edwards A, et al. Biogeography of cryoconite forming cyanobacteria on polar and Asian glaciers. *J Biogeogr*. 2017;44:2849–2861. https://doi.org/10.1111/jbi.13089