



PERSPECTIVE

The future of genomics in polar and alpine cyanobacteria

Nathan A. M. Christmas^{1,2,*}, Alexandre M. Anesio¹ and Patricia Sánchez-Baracaldo¹

¹Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, University Road, Bristol, BS8 1SS, UK and ²Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK

*Corresponding author: Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill Plymouth, Plymouth, PL1 2PB, UK. Tel: +44-1752-426-493; E-mail: natchr@mba.ac.uk

One sentence summary: This review discusses how the use of genomics has the potential to greatly expand our understanding of cyanobacteria in cold environments.

Editor: Marcus Horn

¹Nathan A. M. Christmas, <http://orcid.org/0000-0002-2165-3102>

ABSTRACT

In recent years, genomic analyses have arisen as an exciting way of investigating the functional capacity and environmental adaptations of numerous micro-organisms of global relevance, including cyanobacteria. In the extreme cold of Arctic, Antarctic and alpine environments, cyanobacteria are of fundamental ecological importance as primary producers and ecosystem engineers. While their role in biogeochemical cycles is well appreciated, little is known about the genomic makeup of polar and alpine cyanobacteria. In this article, we present ways that genomic techniques might be used to further our understanding of cyanobacteria in cold environments in terms of their evolution and ecology. Existing examples from other environments (e.g. marine/hot springs) are used to discuss how methods developed there might be used to investigate specific questions in the cryosphere. Phylogenomics, comparative genomics and population genomics are identified as methods for understanding the evolution and biogeography of polar and alpine cyanobacteria. Transcriptomics will allow us to investigate gene expression under extreme environmental conditions, and metagenomics can be used to complement tradition amplicon-based methods of community profiling. Finally, new techniques such as single cell genomics and metagenome assembled genomes will also help to expand our understanding of polar and alpine cyanobacteria that cannot readily be cultured.

Keywords: cyanobacteria; cryosphere; polar; alpine; genomics

INTRODUCTION

The application of genomic technologies has emerged as a powerful tool in helping to understand the diversity, function, adaptation and evolution of microbes and microbial communities in diverse global environments. In habitats where light and liquid water are readily available, cyanobacteria can make up an

important component of these communities, contributing to both carbon and nitrogen fixation and often acting as ecosystem engineers (see Whitton 2012 and chapters therein). Cyanobacteria have had billions of years of evolution (Schirrmeister, Sánchez-Baracaldo and Wacey 2016) and have persisted through

Received: 25 October 2017; Accepted: 23 February 2018

© FEMS 2018. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

profound global environmental including extreme climatic fluctuations and global-scale glaciation (e.g. Neoproterozoic Snowball Earth) (Fairchild and Kennedy 2007). As such, they are highly resilient organisms and have evolved strategies to survive in many extreme environments, with evolutionary radiations into the cryosphere occurring multiple times (Christmas, Anesio and Sánchez-Baracaldo 2015). In the polar regions, the biomass of vascular plants is reduced with increasing latitude (Walker et al. 2016). As such, the relative importance of other photoautotrophs such as cyanobacteria is enhanced, and on glaciers and ice sheets cyanobacteria are responsible for considerable carbon sequestration and driving of the microbial food chain (Anesio et al. 2009; Stibal, Šabacká and Žárský 2012). With the advent of -omic technologies (e.g. genomics, metagenomics, transcriptomics and proteomics), the limits of our appreciation of these cyanobacteria mediated processes have expanded from observations and measurements of nutrient fluxes to a much deeper understanding of the molecular mechanisms that allow these processes to take place, while simultaneously shedding light on how cyanobacteria have evolved and adapted to a variety of different ecosystems. For example, the use of genomics (in particular where studies have involved the sequencing of complete or near complete genomes) has expanded our knowledge of cyanobacteria in marine ecosystems by allowing considerable insight into niche differentiation, functional adaptation and biogeography in globally distributed lineages such as *Trichodesmium erythraeum* (Walworth et al. 2015), *Crocospaera watsonii* (Shi et al. 2010; Bench et al. 2011), *Synechococcus* spp. (Palenik et al. 2003; Palenik et al. 2006; Six et al. 2007; Scanlan et al. 2009) and *Prochlorococcus* spp. (Dufresne et al. 2003; Scanlan et al. 2009; Coleman and Chisholm 2010; Biller et al. 2014; Kashtan et al. 2014; Sun and Blanchard 2014; Kent et al. 2016). Similarly, whole genome sequences of cyanobacteria from hot springs have helped to elucidate the mechanisms by which they survive in such extreme environments (Bhaya et al. 2007; Klatt et al. 2011). Genomics studies based on organisms kept in culture collections such as the Pasteur Culture Collection of Cyanobacteria have yielded information about the production of cyanobacterial secondary metabolites (Pancrace et al. 2017) and allowed for broad reaching studies covering the entire cyanobacterial phylum (Shih et al. 2013). Yet cyanobacteria in the cryosphere have received much less attention at a genomic level, despite having high levels of local ecological importance (Anesio et al. 2009; Anesio and Laybourn-Parry 2012).

Until recently, microbial genomics in the cryosphere has been limited to a few studies using metagenomics as a means of evaluating overall community composition or bioprospecting for cold active genes (e.g. Arctic (Choudhari et al. 2013), Antarctic (Berlemont et al. 2013; Lopatina et al. 2016), alpine (Edwards et al. 2013)). Much more widespread use has been made of amplicon sequencing. Cyanobacteria specific studies have mainly revolved around the use of SSU rRNA, ITS and LSU rRNA sequences to examine the extent of cyanobacterial diversity in a variety of polar environments including Antarctica (Taton et al. 2003; Wood et al. 2008; Namsaraev et al. 2010) and Svalbard (Strunecký, Komárek and Elster 2012; Pushkareva et al. 2015; Palinska, Schneider and Surosz 2017) as well as possible biogeographic links between them (Casamatta et al. 2005; Jungblut, Lovejoy and Vincent 2010; Strunecký, Elster and Komárek 2010; Christmas, Anesio and Sánchez-Baracaldo 2015; Segawa et al. 2017). Although these studies are both useful and of considerable interest, they do not address the full extent of functional diversity that only full genome sequences can reveal.

Only now are we truly beginning to look at cyanobacteria from the cryosphere from a genomic perspective (Christmas et al. 2016; Christmas 2017). *Phormidesmis priestleyi* BC1401 (Accession number: LXYSR01000000), *Leptolyngbya* sp. BC1307 (Accession number: NRTA01000000) and *Pseudanabaena* sp. BC1403 (Accession number: PDDM01000000) (Fig. 1) are among the first cyanobacteria from polar environments to have their genomes sequenced and are yielding new information about how cyanobacteria might be adapted to these environments. No genomic indications of true psychrophily were found in these genomes, but genes for other important adaptations such as EPS production, which is implicated in freezing tolerance (Christmas et al. 2016), and mechanisms for tolerating light conditions in Antarctica (Christmas 2017) were revealed. This work represents the first steps in this area. There are many ways in which the genomics of polar and alpine cyanobacteria might move forward our understanding in a variety of currently underexplored areas including evolutionary biology, functional adaptation to cold environments, regulation and activation of cold associated genes, interactions with viruses and microbial community ecology.

With each year setting a new low point in global glacier coverage (Zemp et al. 2015), it is imperative that we explain how key organisms in these environments, such as cyanobacteria, might respond and evolve with anthropogenic climate change. This article serves to outline the prospects for future research into the genomics of cyanobacteria in the cryosphere. We introduce a variety of ways in which genomics can be used to answer biological questions and give examples of how these applications have been previously used. Most of these examples are taken from studies that have used genomics to investigate cyanobacteria in marine ecosystems, although some from more diverse environments such as hot springs are also given. We discuss how these methods can be used to answer specific questions about cyanobacteria in polar and alpine environments.

GENOMICS OF POLAR AND ALPINE CYANOBACTERIA

Evolution and adaptation

Recently, phylogenomics (phylogenetic analysis using multiple conserved genes) has emerged as an important tool for understanding the evolution of diverse groups of cyanobacteria over broad timescales and may help us to understand the mechanisms by which cyanobacteria radiated into cold environments. Expanding phylogenies to include more than just the rRNA genes has been shown to give much improved resolution of clades of cyanobacteria such as the *Synechococcus/Prochlorococcus* group (Cabello-Yeves et al. 2017), unicellular marine diazotrophs such as *Cyanothece*, *Crocospaera*, and UCYNA (Bombar et al. 2014; Cornejo-Castillo et al. 2016) and the Nostocales (Dagan et al. 2013; Warshan et al. 2017). Furthermore, phylogenomic analyses have allowed for links between cyanobacterial diversification and global scale changes in the environment to be inferred (Larsson, Nylander and Bergman 2011; Schirrmeyer et al. 2011; Schirrmeyer et al. 2013; Shih et al. 2013; Sánchez-Baracaldo, Ridgwell and Raven 2014; Sánchez-Baracaldo 2015; Schirrmeyer, Sánchez-Baracaldo and Wacey 2016). The current lack of genomes from cold environments has prevented true phylogenomic studies of polar and alpine cyanobacteria from being carried out. However, the use of a phylogenomic tree using cyanobacterial genomes from many environments to constrain phylogenies constructed from SSU rRNA sequences of polar and

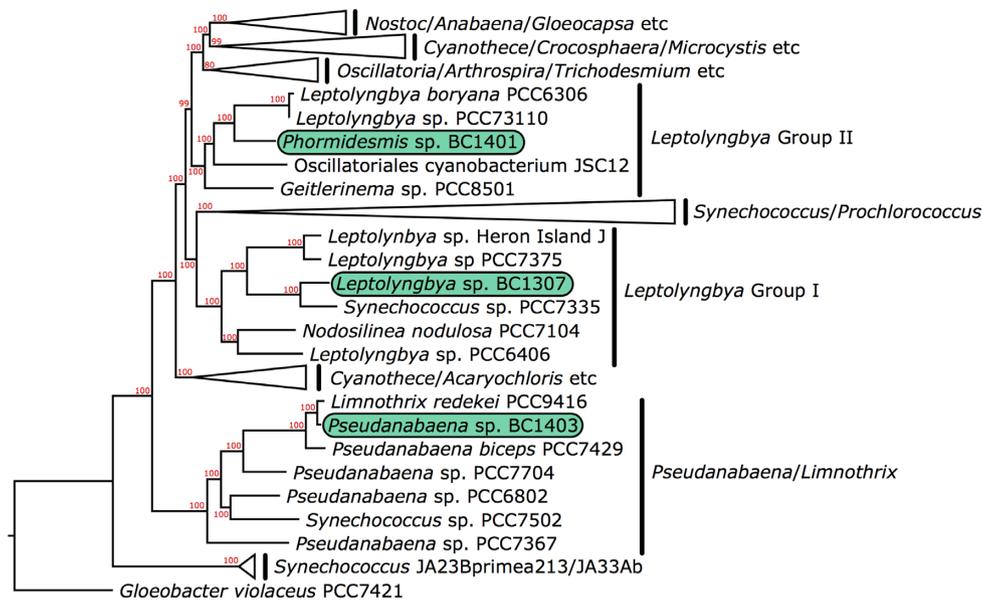


Figure 1. Phylogenomic tree using 136 proteins genes (Blank and Sánchez-Baracaldo 2010) of 95 cyanobacterial taxa indicating the positions of the polar strains *Phormidesmis priestleyi* BC1401, *Pseudanabaena* sp. BC1403 (Greenland, Arctic) and *Leptolyngbya* sp. BC1307 (McMurdo Dry Valleys, Antarctica).

alpine lineages has helped to begin to explain the complexities of cyanobacterial diversity in the cryosphere (Chrismas Anesio and Sánchez-Baracaldo 2015). By using this approach, Chrismas, Anesio and Sánchez-Baracaldo (2015) suggested a mixture of mechanisms for different lineages, varying between (i) ancient cold tolerant ancestors to entire groups of cyanobacteria, and (ii) recent radiations of temperate strains into cryo-ecosystems. Once more genomes from polar and alpine cyanobacteria are available, robust phylogenomic trees can be used to perform more in-depth evolutionary studies and molecular clock analyses. These will help to determine the time that such radiations occurred, and inferring the ecological conditions that prevailed at the time will help us explain how different cold tolerant cyanobacteria originated, while highlighting any links that may exist between the appearance of cold tolerant cyanobacteria and global environmental change.

Arguably one of the most important reasons for generating complete genome sequences of cyanobacteria from cold environments is to better understand how those cyanobacteria are adapted to the variety of environmental pressures of the cryosphere (e.g. freezing, desiccation, high light in summer and low light in winter) (Laybourn-Parry et al. 2012), and the implications that such adaptations have for overall ecosystem function (e.g. ecosystem engineering) (Cook, Edwards and Hubbard 2015). While it is becoming increasingly clear from both growth experiments and genomic analysis that cyanobacteria in the cryosphere are not true psychrophiles (Tang and Vincent 1999; Chrismas et al. 2016; Chrismas 2017), there is still speculation as to what other mechanisms exist to protect cyanobacteria from the harsh polar and alpine environments, and how those mechanisms first evolved.

Cyanobacteria genomes can be divided into two parts, a ‘stable core’ and a ‘variable shell’ (Shi and Falkowski 2008). The stable core consists of conserved genes needed for essential cellular components such as ribosomes and parts of the photosynthetic apparatus. The variable shell includes metabolically non-essential genes that may confer important environmental adaptations, and may be subject to evolution and periodic loss/acquisition of genes via horizontal gene transfer (HGT)

(Mulikidjanian et al. 2006; Shi and Falkowski 2008). This flexible shell (also referred to as the pan-genome (Vernikos et al. 2015)) also includes strain-specific genes and it is here that we might expect to find the genes responsible for allowing the survival of cyanobacteria in a variety of polar and alpine environments.

Variation of genome content within the cyanobacterial pan-genome that includes ecologically relevant genes has already been demonstrated in marine cyanobacteria. For example, changes in levels of nutrient availability have led to quantifiable differences in the genomes of the picocyanobacteria *Prochlorococcus*, where different ecotypes can be found crossing environmental gradients (Johnson et al. 2006; Kashtan et al. 2014; Kashtan et al. 2017). Differences in phosphorous acquisition genes in *Prochlorococcus* can be seen between the phosphorous rich Pacific and phosphorous deplete North Atlantic (Coleman and Chisholm 2010). A further example of environmentally driven changes in physiological capabilities can be seen in *Synechococcus* isolated from alkaline siliceous hot springs, which contained ferrous iron transport related genes not present in a related reference genome (Klatt et al. 2011). Conversely, considerable variation in genomic amino acid identity can also occur between closely related organisms with the same functional and ecological role such as in the symbiotic diazotroph UCYN-A (Bombar et al. 2014). Changes in gene complement such as the examples above highlight the plasticity of cyanobacterial genomes, and how knowledge of genomic variation within both lineages and populations is fundamental to our understanding of how cyanobacteria interact with, and contribute to, the environment.

Exactly what these adaptations are in polar and alpine cyanobacteria requires further investigation. The production of EPS is known to confer freezing and desiccation tolerance in cyanobacteria (Tamaru et al. 2005; Knowles and Castenholz 2008) and the genes for EPS production have already been identified in *Phormidesmis priestleyi* BC1401 (Chrismas et al. 2016), but how they are regulated or vary between cold tolerant lineages across the cyanobacterial phylum is as yet unknown. Ice binding proteins (IBPs) are another key adaptation in ice dwelling organisms. IBPs are a diverse group of proteins (Davies 2014; Bar Dolev, Braslavsky and Davies 2016) that prevent ice nucleation and

have been found in bacteria from polar environments such as sea ice (Raymond, Fritsen and Shen 2007) and cryoconite (Singh et al. 2014). Antarctic cyanobacterial mats have also been shown to inhibit ice crystal formation, a property not seen in temperate cyanobacterial mats (Raymond and Fritsen 2001), and combined proteomic and genomic approaches will help to understand the importance of IPBs in cold adapted cyanobacteria. Fatty acid desaturation represents another mechanism of cold tolerance in cyanobacteria, helping to maintain membrane fluidity at low temperatures (Murata and Wada 1995; Chintalapati, Kiran and Shivaji 2004). Fatty acid desaturase gene complement varies across the cyanobacterial phylum (Chi et al. 2008) and, by expanding upon the number of available genomes of polar cyanobacteria, it will be possible to see the full extent that desaturase genes vary in cold tolerant lineages. Other adaptations unrelated to cold but needed for withstanding other environmental stressors such as light are also important. Antarctic eukaryotic algae are adapted to survival under polar light regimes (Morgan-Kiss et al. 2006; Morgan-Kiss et al. 2015), and there is evidence for adaptation of the light harvesting complex in the Antarctic *Leptolyngbya* sp. BC1307 (Christmas 2017); combining genome interrogation with photophysiology experiments will therefore be key to explaining light adaptation in these organisms. There are doubtless other adaptations yet to be discovered, and investigating the presence of these ecologically important genes in polar and alpine cyanobacteria is essential to understanding how they have evolved to survive in such extreme environments.

Biogeography and population genomics

There is considerable scope to investigate genomic differentiation within lineages that are only found in cold habitats that can tell us about both adaptation and biogeography of these organisms. For example, *Phormidesmis priestleyi* is an ecologically important cyanobacterium that can be found both in the Arctic and Antarctica (Komárek et al. 2009; Christmas, Anesio and Sánchez-Baracaldo 2015) with highly similar SSU rRNA sequences between populations from either side of the globe. Investigating genomic variability between organisms isolated from the Arctic and Antarctica such as *Phormidesmis priestleyi* BC1401 (Christmas et al. 2016) and *Phormidesmis priestleyi* ULC007 (Lara et al. 2017) may tell us a great deal about similarity between these geographically distant but ecologically related environments (Fig. 2). Additionally, many lineages of cyanobacteria found in the cryosphere (e.g. *Nostoc*, *Leptolyngbya*, *Chroococcidiopsis*) are thought to be cosmopolitan with some members of a lineage being found in cold habitats, while others may exist in temperate, tropical or arid environments (Bahl et al. 2011; Christmas, Anesio and Sánchez-Baracaldo 2015). Comparing between the genomes of organisms from these distinct populations can yield information into subtle adaptive changes between them depending on the prevailing ecological conditions. For example, variation in the photosynthetic genes in *Leptolyngbya* sp. BC1307 compared to closely related lineages suggests the ability to account for light conditions in Antarctic terrestrial environments (Christmas 2017). Many other such adaptations are likely to exist and by expanding the number of sequenced genomes of cyanobacteria from the cryosphere and further identifying genomic components likely to be under selection in cold environments, we may begin to observe ecological differentiation within lineages found both in and out of cold environments that is masked by closely related SSU rRNA sequences.

Environmental gradients within an ecosystem can also be big drivers for both changes in cyanobacterial community structure (Bolhuis, Fillinger and Stal 2013) and genomic diversification (Koza et al. 2011; Hahn et al. 2016). Many types of environmental gradient can be seen in the cryosphere, and they have been shown to influence microbial community composition and drive interspecific, or between species, variation in cyanobacteria. For example, on the Tibetan Plateau over an elevation gradient of 5300 m–5900 m, the relative abundance of cyanobacteria has been shown to shift in response to an increase in elevation and a decrease in phosphorous and nitrogen availability (Janatková et al. 2013). Similar changes in microbial community structure caused by local environmental conditions such as nutrient availability (Logares et al. 2013; Borghini et al. 2016) and oxygen gradients (Jungblut et al. 2016) have been demonstrated in Antarctic lakes, in the Arctic along developing soils in pro-glacial moraines (Hodkinson, Coulson and Webb 2003; Kwon et al. 2015) and in the Alps in recently isolated proglacial lakes (Peter and Sommaruga 2016). While interspecific differences in polar cyanobacterial communities in these situations are well documented, much less is understood about intraspecific or within species variation. In many of the situations described above, certain key cyanobacteria (e.g. *Leptolyngbya* and *Phormidesmis*) can be found in varying abundance across the entire environmental gradients, yet the extent to which these organisms vary at a genomic level is unknown.

Population genomics, which deals with genome wide variation within lineages to understand biogeographical links and the extent of dispersal between populations, will be of great importance in understanding how distinct populations of polar and alpine cyanobacteria interconnect. Evidence for population structuring within the SSU rRNA and ITS sequences of several lineages of cryoconite cyanobacteria has already been shown (Segawa et al. 2017), and by expanding this to look at differences across the genome, a much clearer resolution of cyanobacterial biogeography will become apparent. Single cell sequencing is now emerging as the optimal way of doing population genomics (Kashtan et al. 2014) as it has the benefit of producing an entire genome and single nucleotide polymorphisms (SNPs) for a single cell. Methods of cell isolation are more complex than culture-based techniques, with cells being captured by a variety of methods including flow cytometry, microfluidics and dilution to extinction. However, alternative approaches to cell isolation may be applicable to cyanobacteria. Hayes et al. (2002) successfully carried out PCR of specific loci on single filaments of *Nodularia* derived from natural populations in the Baltic sea allowing for SNP analysis in single genes. This might be a suitable approach for cyanobacteria from the cryosphere since many cold environments are dominated by filamentous lineages; when combined with whole genome amplification and sequencing this could represent an efficient way of investigating population level diversity in filamentous lineages of polar and alpine cyanobacteria without the need to resort to cell isolation methods requiring expensive specialized equipment. Alternatively, new bioinformatics approaches are improving on our ability to generate metagenome assembled genomes (MAGs). This allows draft genomes to be obtained directly from environmental samples (Hugerth et al. 2015; Parks et al. 2017; Tully et al. 2017) and has the potential to greatly increase the number of available cyanobacterial genomes directly linked with specific geographical locations. Together, these methods will make it possible to

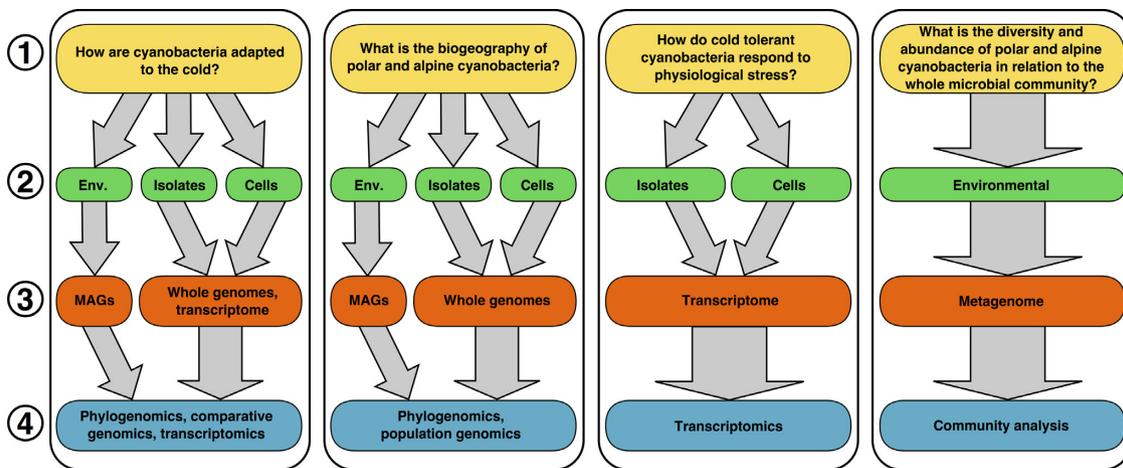


Figure 2. Potential methods to be used for investigating (1) important questions regarding polar and alpine cyanobacteria, including (2) sampling type (e.g. environmental samples, isolated strains or single cells/filaments), (3) data generated (e.g. metagenomes, metagenome assembled genomes (MAGs), whole genomes or transcriptomes) and (4) analytical methods required (e.g. analysis of community composition, phylogenomics, comparative genomics or transcriptomics).

use cyanobacteria in the cryosphere as model systems for investigating mechanisms of microbial evolution, including environmental adaptation, rates of genomic evolution and the extent of gene flow and recombination within and between populations.

Response to physiological stress

As discussed in the previous section, evidence is growing to support the fact that cyanobacteria from the cryosphere are not true psychrophiles. The overwhelming majority of cultured cold cyanobacteria have thermal optima well above their ambient environments (Tang, Tremblay and Vincent 1997), and there are no clear genomic signatures of cold adaptation in the genomes of the Arctic *Phormidesmis priestleyi* BC1401 and the Antarctic *Lepolyngbya* sp. BC1307 (Chrismas et al. 2016; Chrismas 2017). Yet these and other cyanobacteria are still capable of withstanding the intense environmental pressures of the polar regions. The mechanisms for doing so may, therefore, lie in the regulation of existing mechanisms that are common throughout the group (Chrismas et al. 2016; Sinetova and Los 2016a).

Levels of gene expression are known to vary dramatically under different environmental conditions with upregulation of stress response genes being a prime example of this. In cyanobacteria, iron limitation (Ludwig and Bryant 2012; Kopf et al. 2014), light stress (Billis et al. 2014; Kopf et al. 2014; Kopf et al. 2015), salt (Billis et al. 2014; Al-Hosani et al. 2015), and nitrogen limitation (Kopf et al. 2014; Choi et al. 2016) among others all initiate expression of groups of specific genes in order to alleviate the cellular stress that these environmental pressures incur. Cold stress is no different. In microarray expression experiments, over 100 genes in *Synechocystis* were upregulated by a factor of at least two under cold stress (although only 38 of these being exclusively implicated in cold stress response) (Sinetova and Los 2016b). Since most cyanobacteria from the cryosphere do not grow preferentially at low temperatures (Tang, Tremblay and Vincent 1997) (except for Ant-Orange (Nadeau and Castenholz 2000)), they are likely to be experiencing constant stress in their environment. However, constant expression of cold shock genes is likely to be metabolically expensive and as such cells may be acclimated to growth at low temperatures rather than exhibiting a persistent cold shock response. Determining the time and rate at which cold response genes in cold-adapted cyanobacteria are expressed is therefore essential if we are to understand

how these organisms survive. Where the mechanisms allowing cold tolerance are common throughout the cyanobacterial phylum (e.g. the production of EPS, Pereira et al. 2015; fatty acid desaturase genes, Chi et al. 2008), differences may exist in the way these shared characteristics are regulated to account for the increased levels of expression required in cold environments. Indeed, in Antarctic *Nostoc* sp., constitutive expression of desaturase genes has been observed rather than being upregulated upon temperature reduction, as is seen in temperate lineages (Chintalapati et al. 2007). Identifying how these processes are regulated is therefore key to explaining mechanisms of long term cold tolerance.

Investigation into the cyanobacterial transcriptome can take one of two forms. Either a global transcriptome can be generated under stress conditions (e.g. Ludwig and Bryant 2012; Harke and Gobler 2013; Teikari et al. 2015) to show all genes transcribed at any given time, or RNA-sequencing can be targeted at key transcripts or regulatory RNAs of interest. The availability of the genomes of key organisms is essential for this; by identifying key genes of interest within the genome (e.g. the genes responsible for the production of EPS in *Phormidesmis priestleyi* BC1401, Chrismas et al. 2016, or genes involved in cold shock response), transcriptomic studies can be targeted towards these genes and their putative regulatory networks to establish how cold tolerant cyanobacteria might be reacting to their environment at a molecular level.

Community ecology and interactions

While cyanobacteria often dominate the habitats that they inhabit in the cryosphere, they do not exist in isolation within their environment. Instead, they are members of complex communities containing multiple types of cyanobacteria, heterotrophic bacteria and eukaryotes (e.g. Gordon et al. 2000; Paerl, Pinckney and Steppe 2000; Torre et al. 2003; Jungblut et al. 2012; Edwards et al. 2013; Ambrosini et al. 2017). Determining the structure of these communities is fundamental to understanding the overall function of microbially dominated cryo-environments. Typically, SSU rRNA gene amplicon-based approaches have been used to determine abundances of different cyanobacteria within cryospheric environments. Such techniques have been used to show that the relative abundance of the cyanobacterial component of snow communities varies from site to site in alpine

snow (Wunderlin, Ferrari and Power 2016), and revealed regional scale variation in cryoconite communities from glaciers on the Tibetan Plateau (Liu *et al.* 2017). Community profiling such as this has revealed many insights into how cyanobacteria interact with the environment. These include how cyanobacterial abundance varies in response to soil development and abiotic factors like pH (Pushkareva *et al.* 2015), succession in glacial forefields (Knelman *et al.* 2012; Rime *et al.* 2015), and changes in altitude and nutrient composition as discussed earlier (Janatková *et al.* 2013; Logares *et al.* 2013; Borghini *et al.* 2016; Jungblut *et al.* 2016). However, PCR bias is known to influence the resolution and sensitivity of operational taxonomic unit (OTU) recovery, and metagenomics can recover 1.5 to $\sim 10 \times$ more phyla than amplicon-based approaches (Poretsky *et al.* 2014). The extent of cyanobacterial diversity (indeed, all microbial diversity) in the cryosphere is therefore likely to be much greater than is presently known, which has considerable implications for how we interpret microbial ecology in the cryosphere and associated biogeochemical processes. Expanding on existing studies with metagenomics is therefore essential if we are to know the true diversity of both polar cyanobacteria and their associated microbial communities, and the ability to obtain cyanobacterial MAGs from these metagenomes will allow for deeper investigation of cyanobacteria that cannot be isolated or cultured using traditional methods (Hugerth *et al.* 2015; Parks *et al.* 2017; Tully *et al.* 2017).

Understanding how members of these communities interact with each other is also of great importance. For example, laminated cyanobacterial mats (such as those common in polar and alpine environments) include layers of methanogens and sulfur-reducing bacteria (Stal 1995; Bolhuis, Fillinger and Stal 2013) that interact to form a network of metabolic interdependencies. In many cases, such interactions are essential for survival, and when cyanobacteria are removed from their community, growth can sometimes be impaired or inhibited altogether (Xie *et al.* 2016). Multi-omic techniques can help us understand these community interactions (Franzosa *et al.* 2015). Differences in transcriptional regulation were observed in different strains of *Prochlorococcus* when they were grown in co-culture with marine *Alteromonas* (Aharonovich and Sher 2016), and metagenomics has revealed that *Microcystis* is dependent on associated microbiota for Vitamin B12 synthesis (Xie *et al.* 2016). The extent to which cyanobacteria are reliant upon the community and vice versa is therefore a key question in microbial ecology, and cyanobacteria-dominated communities in the cryosphere represent excellent systems for investigating these kinds of community interactions. In particular, cryoconite holes act as a semi-closed system of cyanobacteria-dominated microbial communities that may be investigated in the field or reproduced in the lab.

Another important ecological interaction in polar environments is the cyanobacteria-fungus symbiosis in cyanolichens. Cyanobacteria are the main photobiont in several Arctic lichens such as *Peltigera*, *Solorina* and *Nephroma* spp. (Rikkinen 2015) and *Peltigaria* spp. have also been shown to exhibit considerable diversity in maritime Antarctica (Zúñiga *et al.* 2015). Lichen cyanobionts are primarily diazotrophs such as *Nostoc* and *Stigonema*, which can also exist as nitrogen fixing symbionts alongside green algal photobionts in tripartite lichens (Rozema, Aerts and Cornelissen 2007). The importance of cyanolichens for nitrogen fixation in Arctic environments is clear; Weiss, Hobbie and Gettel (2005) showed that abundance of *Peltigaria aphthosa* in tundra was diminished when an eternal source of nitrogen was added, and cyanolichen-mediated nitrogen cycling is likely to have widespread ecosystem implications in the Arctic (Wooley

et al. 2009). However, the molecular biology of the cyanobacteria-fungus symbiosis is still developing (Rikkinen 2013) and genomic approaches have great potential to improve our understanding of polar cyanolichens in terms of their evolution and ecology. Metagenomics can be used to investigate the entire lichen consortium (Grube *et al.* 2013), which can shed light on the genomic composition of not only the main symbiotes but also the associated microbiome (Bates *et al.* 2011; Sigurbjörnsdóttir, Andrésón and Vilhelmsson 2015). Investigating the cyanobiont alone may also provide interesting evolutionary insights. Genome reduction is common in symbiotic prokaryotes (McCutcheon and Moran 2012) including cyanobacteria (Bombar *et al.* 2014), and by sequencing cyanobiont isolates from cyanolichens we may better understand the extent of the symbiotic relationship. However, culturing the cyanobiont from lichens is not trivial. Isolates obtained from cyanolichens are often found not to be the main photobiont (Summerfield, Galloway and Eaton-Rye 2002), and using new single cell genomics approaches may be of help here.

It is well understood that interactions with viruses are an important part of the processes that drive the evolution of microbial genomes (Weinbauer and Rassoulzadegan 2004), and the same is likely to hold true for cyanobacteria in the cryosphere. It has been proposed that viruses are one of many factors that contribute to the evolution of cyanobacteria (Shestakov and Karbysheva 2015) and direct interactions of viruses with cyanobacterial genomes are fundamental to this. Genes native to cyanobacteria (e.g. genes involved in the cyanobacterial photosynthetic apparatus) are regularly found within the genomes of cyanophage (Sullivan *et al.* 2005) and the genomes of some cyanobacterial T4-like myophages have been found to be significantly shaped by their host organism (Ignacio-Espinoza and Sullivan 2012). Likewise, cyanophage help to mold the genomes of their cyanobacterial hosts (Coleman *et al.* 2006; Lindell *et al.* 2007) and virus-mediated HGT is thought to be responsible for the acquisition of novel genes and may be involved in the rearrangement of genome structures (Kuno, Sako and Yoshida 2014). There is considerable potential for these processes to be acting on cyanobacteria from the cryosphere (Anesio and Bellas 2011; Rassner *et al.* 2016). Viruses are found at relatively high numbers in cryoconite holes. In cryoconite from Greenland and Svalbard, abundance of viruses was between 5.62×10^8 (Midtre Lovénbreen, Svalbard) and 24.5×10^8 (Greenland Ice Sheet, 11 km) virus-like particles per gram of dry weight sediment (Bellas *et al.* 2013). The assembly of potential virus genomes from the virus size fraction of these cryoconites resulted in four scaffolds of viruses that had putative cyanobacterial hosts (Bellas, Anesio and Barker 2015) and the genomes of eight viruses were recovered from cyanobacterial mats on the McMurdo Ice Shelf (Zawar-Reza *et al.* 2014). A high proportion of cyanobacterial cells in various Antarctic lakes were observed to contain prophage (Säwström *et al.* 2007). In some cases, these viruses may be specific to polar cyanobacteria and a novel lineage of cyanophage, S-EIV1, was found to infect Arctic *Synechococcus* (Chénard *et al.* 2015).

Modern genomics techniques can be used to investigate the influence of these abundant viruses on cyanobacteria in the cryosphere. Metagenomes can be used to link virus-host interactions in cyanobacterial mats (Voorhies *et al.* 2016) and once a cyanobacterial genome has been sequenced, detecting past virus-host interactions is possible due to viruses leaving distinct signatures on microbial genomes. Insertion elements can be evidence of HGT, while clustered regularly interspaced short palindromic repeats (CRISPRs) are evidence of previous exposure to

viruses. More direct evidence might be found in the form of in situ prophage integrated into a cyanobacterial genome (Chénard, Wirth and Suttle 2016). With the availability of new genomes of cyanobacteria from the cryosphere, our knowledge of the effect of viruses in cyanobacterial dominated ecosystems will increase correspondingly.

CONCLUSION

Genomics is no longer next-generation science; it is both contemporary and essential. The advances that large-scale genomics projects have had on our understanding of marine cyanobacteria have been substantial, leading to new discoveries in terms of their global biodiversity and biogeochemistry. The earth's polar and alpine regions are ripe for expanding these techniques into extreme environments that have been hitherto underexplored in respect to cyanobacterial genomic diversity. By sequencing many genomes of single cyanobacterial lineages from diverse polar and alpine habitats, we may better understand their population structure and begin to answer questions about biogeography, dispersal and functional adaptation. Such genomes will help to complement metagenomic and transcriptomic approaches allowing us to better understand their role in polar microbial communities and how they might react to environmental pressures. In a changing climate where the extent of glaciers is in widespread decline, efforts should be made how these organisms might respond to these changes from both an evolutionary and ecological perspective.

ACKNOWLEDGEMENTS

The authors wish to thank Martyn Tranter and Birgit Sattler for interesting discussions relating to this work and three anonymous reviewers for providing thorough and constructive reviews.

FUNDING

This work was supported by NERC GW4+(NAMC), NERC grant NE/J02399X/1 (AMA) and a Royal Society University Research Fellowship (PSB).

Conflict of interest. None declared.

REFERENCES

- Aharonovich D, Sher D. Transcriptional response of *Prochlorococcus* to co-culture with a marine *Alteromonas*: differences between strains and the involvement of putative infochemicals. *ISME J* 2016;10:2892–906.
- Al-Hosani S, Oudah MM, Henschel A et al. Global transcriptome analysis of salt acclimated *Prochlorococcus* AS9601. *Microbiol Res* 2015;176:21–8.
- Ambrosini R, Musitelli F, Navarra F et al. Diversity and assembling processes of bacterial communities in cryoconite holes of a Karakoram glacier. *Microb Ecol* 2017;73:827–37.
- Anesio AM, Bellas CM. Are low temperature habitats hot spots of microbial evolution driven by viruses? *Trends Microbiol* 2011;19:52–7.
- Anesio AM, Hodson AJ, Fritz A et al. High microbial activity on glaciers: importance to the global carbon cycle. *Global Change Biol* 2009;15:955–60.
- Anesio AM, Laybourn-Parry J. Glaciers and ice sheets as a biome. *Trends Ecology Evolut* 2012;27:219–25.
- Bahl J, Lau MCY, Smith GJD et al. Ancient origins determine global biogeography of hot and cold desert cyanobacteria. *Nat Commun* 2011;2:163.
- Bar Dolev M, Braslavsky I, Davies PL. Ice-Binding proteins and their function. *Annu Rev Biochem* 2016;85:515–42.
- Bates ST, Cropsey GWG, Caporaso JG et al. Bacterial communities associated with the lichen symbiosis. *Appl Environ Microbiol* 2011;77:1309–14.
- Bellas CM, Anesio AM, Barker G. Analysis of virus genomes from glacial environments reveals novel virus groups with unusual host interactions. *Front Microbiol* 2015;6:656.
- Bellas CM, Anesio AM, Telling J et al. Viral impacts on bacterial communities in Arctic cryoconite. *Environ Res Lett* 2013;8:045021.
- Bench SR, Ilikchyan IN, Tripp HJ et al. Two strains of *Crocospaera watsonii* with highly conserved genomes are distinguished by strain-specific features. *Front Microbiol* 2011;2:261.
- Berlemont R, Jacquin O, Delsaute M et al. Novel cold-adapted esterase MhIip from an Antarctic soil metagenome. *Biology* 2013;2:177–88.
- Bhaya D, Grossman AR, Steunou A-S et al. Population level functional diversity in a microbial community revealed by comparative genomic and metagenomic analyses. *ISME J* 2007;1:703–13.
- Billler SJ, Berube PM, Berta-Thompson JW et al. Genomes of diverse isolates of the marine cyanobacterium *Prochlorococcus*. *Sci Data* 2014;1:140034.
- Billis K, Billini M, Tripp HJ et al. Comparative transcriptomics between *Synechococcus* PCC 7942 and *Synechocystis* PCC 6803 provide insights into mechanisms of stress acclimation. *PLOS One* 2014;9:e109738.
- Blank CE, Sánchez-Baracaldo P. Timing of morphological and ecological innovations in the cyanobacteria—a key to understanding the rise in atmospheric oxygen. *Geobiology* 2010;8:1–23.
- Bolhuis H, Fillinger L, Stal LJ. Coastal microbial mat diversity along a natural salinity gradient. *PLOS One* 2013;8:e63166.
- Bombar D, Heller P, Sánchez-Baracaldo P et al. Comparative genomics reveals surprising divergence of two closely related strains of uncultivated UCYN-A cyanobacteria. *ISME J* 2014;8:2530–42.
- Borghini F, Colacevich A, Caruso T et al. Algal biomass and pigments along a latitudinal gradient in Victoria Land lakes, East Antarctica. *Polar Res* 2016;35:20703.
- Cabello-Yeves PJ, Haro-Moreno JM, Martín-Cuadrado A-B et al. Novel *Synechococcus* genomes reconstructed from freshwater reservoirs. *Front Microbiol* 2017;8:1151.
- Casamatta DA, Johansen JR, Vis ML et al. Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (cyanobacteria). *J Phycol* 2005;41:421–38.
- Chi X, Yang Q, Zhao F et al. Comparative analysis of fatty acid desaturases in cyanobacterial Genomes. *Compar Funct Genom* 2008; 2008:284508.
- Chintalapati S, Kiran MD, Shivaji S. Role of membrane lipid fatty acids in cold adaptation. *Cell Mol Biol* 2004;50:631–42.
- Chintalapati S, Prakash JSS, Singh AK et al. Desaturase genes in a psychrotolerant *Nostoc* sp. are constitutively expressed at low temperature. *Biochem Biophys Res Commun* 2007;362:81–7.
- Choi SY, Park B, Choi I-G et al. Transcriptome landscape of *Synechococcus elongatus* PCC 7942 for nitrogen starvation responses using RNA-seq. *Sci Rep* 2016;6:srep30584.

- Choudhari S, Smith S, Owens S *et al.* Metagenome sequencing of prokaryotic microbiota collected from Byron Glacier, Alaska. *Genome Announc* 2013;1:e00099–13.
- Christmas NAM, Anesio AM, Sánchez-Baracaldo P. Multiple adaptations to polar and alpine environments within cyanobacteria: a phylogenomic and Bayesian approach. *Front Microbiol* 2015;6:1070.
- Christmas NAM, Barker G, Anesio AM *et al.* Genomic mechanisms for cold tolerance and production of exopolysaccharides in the Arctic cyanobacterium *Phormidesmis priestleyi* BC1401. *BMC Genomics* 2016;17:533.
- Christmas NAM. Exploring the diversity and evolution of Cyanobacteria in the cryosphere, through phylogenetics and comparative genomics. Ph.D. Thesis. University of Bristol 2017.
- Chénard C, Chan AM, Vincent WF *et al.* Polar freshwater cyanophage S-EIV1 represents a new widespread evolutionary lineage of phages. *ISME J* 2015; 9:2046–58.
- Chénard C, Wirth JF, Suttle CA. Viruses infecting a freshwater filamentous cyanobacterium (*Nostoc* sp.) encode a functional CRISPR array and a proteobacterial DNA polymerase B. *mBio* 2016;7:e00667–16.
- Coleman ML, Chisholm SW. Ecosystem-specific selection pressures revealed through comparative population genomics. *Proc Natl Acad Sci* 2010;107:18634–9.
- Coleman ML, Sullivan MB, Martiny AC *et al.* Genomic islands and the ecology and evolution of *Prochlorococcus*. *Science* 2006;311:1768–70.
- Cook J, Edwards A, Hubbard A. Biocryomorphology: integrating microbial processes with ice surface hydrology, topography, and roughness. *Front Earth Sci* 2015;3:78.
- Cornejo-Castillo FM, Cabello AM, Salazar G *et al.* Cyanobacterial symbionts diverged in the late Cretaceous towards lineage-specific nitrogen fixation factories in single-celled phytoplankton. *Nat Commun* 2016;7:11071.
- Dagan T, Roettger M, Stucken K *et al.* Genomes of Stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. *Genome Biol Evol* 2013;5:31–44.
- Davies PL. Ice-binding proteins: a remarkable diversity of structures for stopping and starting ice growth. *Trends Biochem Sci* 2014;39:548–55.
- de la Torre JR, Goebel BM, Friedmann EI *et al.* Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys, Antarctica. *Appl Environ Microbiol* 2003;69:3858–67.
- Dufresne A, Salanoubat M, Partensky F *et al.* Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. *Proc Natl Acad Sci* 2003;100:10020–5.
- Edwards A, Pachebat JA, Swain M *et al.* A metagenomic snapshot of taxonomic and functional diversity in an alpine glacier cryoconite ecosystem. *Environ Res Lett* 2013;8:035003.
- Fairchild IJ, Kennedy MJ. Neoproterozoic glaciation in the Earth System. *J Geol Soc* 2007;164:895–921.
- Franzosa EA, Hsu T, Sirota-Madi A *et al.* Sequencing and beyond: integrating molecular “omics” for microbial community profiling. *Nat Rev Microbiol* 2015;13:360–72.
- Gordon DA, Priscu J, Giovannoni S. Origin and phylogeny of microbes living in permanent Antarctic lake ice. *Microb Ecol* 2000;39:197–202.
- Grube M, Berg G, S. Andrésón Ó *et al.* Lichen genomics. In: Martin F (ed). *The Ecological Genomics of Fungi*, Hoboken, NJ: John Wiley & Sons, Inc 2013 pp. 191–212.
- Hahn MW, Jezberová J, Koll U *et al.* Complete ecological isolation and cryptic diversity in *Polynucleobacter* bacteria not resolved by 16S rRNA gene sequences. *ISME J* 2016;10:1642–55.
- Harke MJ, Gobler CJ. Global transcriptional responses of the toxic cyanobacterium, *Microcystis aeruginosa*, to nitrogen stress, phosphorus stress, and growth on organic matter. *PLoS One* 2013;8:e69834.
- Hayes PK, Barker GLA, Batley J *et al.* Genetic diversity within populations of cyanobacteria assessed by analysis of single filaments. *Antonie Leeuwenhoek* 2002;81:197–202.
- Hodkinson ID, Coulson SJ, Webb NR. Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. *J Ecol* 2003;91:651–63.
- Hugerth LW, Larsson J, Alneberg J *et al.* Metagenome-assembled genomes uncover a global brackish microbiome. *Genome Biol* 2015;16:279.
- Ignacio-Espinoza JC, Sullivan MB. Phylogenomics of T4 cyanophages: lateral gene transfer in the “core” and origins of host genes. *Environ Microbiol* 2012;14:2113–26.
- Janatková K, Reháková K, Doležal J *et al.* Community structure of soil phototrophs along environmental gradients in arid Himalaya. *Environ Microbiol* 2013;15:2505–16.
- Johnson ZI, Zinser ER, Coe A *et al.* Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. *Science* 2006;311:1737–40.
- Jungblut AD, Hawes I, Mackey TJ *et al.* Microbial mat communities along an oxygen gradient in a perennially ice-covered Antarctic lake. *Appl Environ Microbiol* 2016;82:620–30.
- Jungblut AD, Lovejoy C, Vincent WF. Global distribution of cyanobacterial ecotypes in the cold biosphere. *ISME J* 2010;4:191–202.
- Jungblut AD, Wood SA, Hawes I *et al.* The Pyramid Trough Wetland: environmental and biological diversity in a newly created Antarctic protected area. *FEMS Microbiol Ecol* 2012;82:356–66.
- Kashtan N, Roggensack SE, Berta-Thompson JW *et al.* Fundamental differences in diversity and genomic population structure between Atlantic and Pacific *Prochlorococcus*. *ISME J* 2017;11:1997–2011.
- Kashtan N, Roggensack SE, Rodrigue S *et al.* Single-cell genomics reveals hundreds of coexisting subpopulations in wild *Prochlorococcus*. *Science* 2014;344:416–20.
- Kent AG, Dupont CL, Yooseph S *et al.* Global biogeography of *Prochlorococcus* genome diversity in the surface ocean. *ISME J* 2016;10:1856–65.
- Klatt CG, Wood JM, Rusch DB *et al.* Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. *ISME J* 2011;5:1262–78.
- Knelman JE, Legg TM, O'Neill SP *et al.* Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. *Soil Biol Biochem* 2012;46:172–80.
- Knowles EJ, Castenholz RW. Effect of exogenous extracellular polysaccharides on the desiccation and freezing tolerance of rock-inhabiting phototrophic microorganisms. *FEMS Microbiol Ecol* 2008;66:261–70.
- Komárek J, Kaštovský J, Ventura S *et al.* The cyanobacterial genus *Phormidesmis*. *Algol Stud* 2009;129:41–59.
- Kopf M, Klähn S, Scholz I *et al.* Comparative analysis of the primary transcriptome of *Synechocystis* sp. PCC 6803. *DNA Res* 2014;21:527–39.

- Kopf M, Möke F, Bauwe H et al. Expression profiling of the bloom-forming cyanobacterium *Nodularia* CCY9414 under light and oxidative stress conditions. *ISME J* 2015;9:2139–52.
- Koza A, Moshynets O, Otten W et al. Environmental modification and niche construction: developing O₂ gradients drive the evolution of the Wrinkly Spreader. *ISME J* 2011;5:665–73.
- Kuno S, Sako Y, Yoshida T. Diversification of CRISPR within coexisting genotypes in a natural population of the bloom-forming cyanobacterium *Microcystis aeruginosa*. *Microbiology* 2014;160:903–16.
- Kwon HY, Jung JY, Kim O-S et al. Soil development and bacterial community shifts along the chronosequence of the Midtre Lovénbreen glacier foreland in Svalbard. *J Ecol Environ* 2015;38:461–76.
- Lara Y, Durieu B, Cornet L et al. Draft genome sequence of the axenic strain *Phormidesmis priestleyi* ULC007, a cyanobacterium isolated from Lake Bruehwiler (Larsemann Hills, Antarctica). *Genome Announc* 2017;5:e01546–16.
- Larsson J, Nylander JA, Bergman B. Genome fluctuations in cyanobacteria reflect evolutionary, developmental and adaptive traits. *BMC Evol Biol* 2011;11:187.
- Laybourn-Parry J, Hodson A, Tranter M. *The Ecology of Snow and Ice Environments*. Oxford, NY: Oxford University Press 2012.
- Lindell D, Jaffe JD, Coleman ML et al. Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. *Nature* 2007;449:83–6.
- Liu Y, Vick-Majors TJ, Priscu JC et al. Biogeography of cryoconite bacterial communities on glaciers of the Tibetan Plateau. *FEMS Microbiol Ecol* 2017;93:fix072.
- Logares R, Lindström ES, Langenheder S et al. Biogeography of bacterial communities exposed to progressive long-term environmental change. *ISME J* 2013;7:937–48.
- Lopatina A, Medvedeva S, Shmakov S et al. Metagenomic analysis of bacterial communities of Antarctic surface snow. *Front Microbiol* 2016;7:398.
- Ludwig M, Bryant DA. Acclimation of the global transcriptome of the cyanobacterium *Synechococcus* sp. Strain PCC 7002 to nutrient limitations and different nitrogen sources. *Front Microbiol* 2012;3:145.
- McCutcheon JP, Moran NA. Extreme genome reduction in symbiotic bacteria. *Nat Rev Microbiol* 2012;10:13.
- Morgan-Kiss RM, Lizotte MP, Kong W et al. Photoadaptation to the polar night by phytoplankton in a permanently ice-covered Antarctic lake. *Limnol Oceanogr* 2015;61:3–13.
- Morgan-Kiss RM, Priscu JC, Pockock T et al. Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiol Mol Biol Rev* 2006;70:222–52.
- Mulkidjanian AY, Koonin EV, Makarova KS et al. The cyanobacterial genome core and the origin of photosynthesis. *Proc Natl Acad Sci* 2006;103:13126–31.
- Murata N, Wada H. Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochem J* 1995;308:1–8.
- Nadeau TL, Castenholz RW. Characterization of psychrophilic oscillatorians (cyanobacteria) from Antarctic meltwater ponds. *J Phycol* 2000;36:914–23.
- Namsaraev Z, Mano M-J, Fernandez Carazo R et al. Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas. *Ann Glaciol* 2010;51:171–7.
- Paerl HW, Pinckney JL, Stegge TF. Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. *Environ Microbiol* 2000;2:11–26.
- Palenik B, Brahamsha B, Larimer FW et al. The genome of a motile marine *Synechococcus*. *Nature* 2003;424:1037–42.
- Palenik B, Ren Q, Dupont CL et al. Genome sequence of *Synechococcus* CC9311: insights into adaptation to a coastal environment. *Proc Natl Acad Sci* 2006;103:13555–9.
- Palinska KA, Schneider T, Surosz W. Phenotypic and phylogenetic studies of benthic mat-forming cyanobacteria on the NW Svalbard. *Polar Biol* 2017;40:1607–16.
- Pancrace C, Barny M-A, Ueoka R et al. Insights into the *Planktothrix* genus: genomic and metabolic comparison of benthic and planktic strains. *Sci Rep* 2017;7:41181.
- Parks DH, Rinke C, Chuvochina M et al. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nat Microbiol* 2017;2:1533.
- Pereira SB, Mota R, Vieira CP et al. Phylum-wide analysis of genes/proteins related to the last steps of assembly and export of extracellular polymeric substances (EPS) in cyanobacteria. *Sci Rep* 2015;5:14835.
- Peter H, Sommaruga R. Shifts in diversity and function of lake bacterial communities upon glacier retreat. *ISME J* 2016;10:1545–54.
- Poretsky R, Rodriguez-R LM, Luo C et al. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. *PLoS One* 2014;9:e0093827.
- Pushkareva E, Pessi IS, Wilmette A et al. Cyanobacterial community composition in Arctic soil crusts at different stages of development. *FEMS Microbiol Ecol* 2015;91:fv143.
- Rassner SME, Anesio AM, Girdwood SE et al. Can the bacterial community of a high Arctic glacier surface escape viral control? *Front Microbiol* 2016;7:956.
- Raymond JA, Fritsen C, Shen K. An ice-binding protein from an Antarctic sea ice bacterium. *FEMS Microbiol Ecol* 2007;61:214–21.
- Raymond JA, Fritsen CH. Semipurification and ice recrystallization inhibition activity of ice-active substances associated with Antarctic photosynthetic organisms. *Cryobiology* 2001;43:63–70.
- Rikkinen J. Cyanolichens. *Biodivers Conserv* 2015;24:973–93.
- Rikkinen J. Molecular studies on cyanobacterial diversity in lichen symbioses. *MycKeys* 2013;6:3–32.
- Rime T, Hartmann M, Brunner I et al. Vertical distribution of the soil microbiota along a successional gradient in a glacier forefield. *Mol Ecol* 2015;24:1091–108.
- Rozema J, Aerts R, Cornelissen H. *Plants and Climate Change*. Dordrecht, the Netherlands: Springer 2007.
- Scanlan DJ, Ostrowski M, Mazard S et al. Ecological genomics of marine picocyanobacteria. *Microbiol Mol Biol Rev* 2009;73:249–99.
- Schirrmeyer BE, Anisimova M, Antonelli A et al. Evolution of cyanobacterial morphotypes: taxa required for improved phylogenomic approaches. *Commun Integr Biol* 2011;4:424–7.
- Schirrmeyer BE, Sánchez-Baracaldo P, Wacey D. Cyanobacterial evolution during the Precambrian. *Int J Astrobiology* 2016;15:187–204.
- Schirrmeyer BE, de Vos JM, Antonelli A et al. Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event. *Proc Natl Acad Sci* 2013;110:1791–6.
- Segawa T, Yonezawa T, Edwards A et al. Biogeography of cryoconite forming cyanobacteria on polar and Asian glaciers. *J Biogeogr* 2017;44:1–13.
- Shestakov SV, Karbysheva EA. The role of viruses in the evolution of cyanobacteria. *Biol Bull Rev* 2015;5:527–37.

- Shi T, Falkowski PG. Genome evolution in cyanobacteria: the stable core and the variable shell. *Proc Natl Acad Sci* 2008;**105**:2510–5.
- Shi T, Ilikchyan I, Rabouille S et al. Genome-wide analysis of diel gene expression in the unicellular N₂-fixing cyanobacterium *Crocospaera watsonii* WH 8501. *ISME J* 2010;**4**:621–32.
- Shih PM, Wu D, Latifi A et al. Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. *Proc Natl Acad Sci* 2013;**110**:1053–8.
- Sigurbjörnsdóttir MA, Andrésón ÓS, Vilhelmsson O. Analysis of the *Peltigera membranacea* metagenome indicates that lichen-associated bacteria are involved in phosphate solubilization. *Microbiology* 2015;**161**:989–96.
- Sinetova MA, Los DA. Lessons from cyanobacterial transcriptomics: universal genes and triggers of stress responses. *Mol Biol* 2016a;**50**:606–14.
- Sinetova MA, Los DA. New insights in cyanobacterial cold stress responses: genes, sensors, and molecular triggers. *Biochim Biophys Acta* 2016b;**1860**:2391–403.
- Singh P, Hanada Y, Singh SM et al. Antifreeze protein activity in Arctic cryoconite bacteria. *FEMS Microbiol Lett* 2014;**351**:14–22.
- Six C, Thomas J-C, Garczarek L et al. Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study. *Genome Biol* 2007;**8**:R259.
- Stal LJ. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytol* 1995;**131**:1–32.
- Stibal M, Šabacká M, Žárský J. Biological processes on glacier and ice sheet surfaces. *Nat Geosci* 2012;**5**:771.
- Strunecký O, Elster J, Komárek J. Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? *Polar Biol* 2010;**33**:1419–28.
- Strunecký O, Komárek J, Elster J. Biogeography of *Phormidium autumnale* (Oscillatoriales, Cyanobacteria) in western and central Spitsbergen. *Pol Polar Res* 2012;**33**:369–82.
- Sullivan MB, Coleman ML, Weigle P et al. Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. *PLoS Biol* 2005;**3**:e144.
- Summerfield TC, Galloway DJ, Eaton-Rye JJ. Species of cyanolichen from *Pseudocyphellaria* with indistinguishable ITS sequences have different photobionts. *New Phytol* 2002;**155**:121–9.
- Sun Z, Blanchard JL. Strong genome-wide selection early in the evolution of *Prochlorococcus* resulted in a reduced genome through the loss of a large number of small effect genes. *PLoS One* 2014;**9**:e88837.
- Sánchez-Baracaldo P, Ridgwell A, Raven JA. A Neoproterozoic transition in the marine nitrogen cycle. *Curr Biol* 2014;**24**:652–7.
- Sánchez-Baracaldo P. Origin of marine planktonic cyanobacteria. *Sci Rep* 2015;**5**:17418.
- Sävström C, Granéli W, Laybourn-Parry J et al. High viral infection rates in Antarctic and Arctic bacterioplankton. *Environ Microbiol* 2007;**9**:250–5.
- Tamaru Y, Takani Y, Yoshida T et al. Crucial role of extracellular polysaccharides in desiccation and freezing tolerance in the terrestrial cyanobacterium *Nostoc commune*. *Appl Environ Microbiol* 2005;**71**:7327–33.
- Tang EPY, Tremblay R, Vincent WF. Cyanobacterial dominance of polar freshwater ecosystems: are high-latitude mat-formers adapted to low temperature? *J Phycol* 1997;**33**:171–81.
- Tang EPY, Vincent WF. Strategies of thermal adaptation by high-latitude cyanobacteria. *New Phytol* 1999;**142**:315–23.
- Taton A, Grubisic S, Brambilla E et al. Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo dry valleys, Antarctica): a morphological and molecular approach. *Appl Environ Microbiol* 2003;**69**:5157–69.
- Teikari J, Österholm J, Kopf M et al. Transcriptomics and proteomics profiling of *Anabaena* sp. Strain 90 under inorganic phosphorus stress. *Appl Environ Microbiol* 2015;**81**:5212–22.
- Tully BJ, Sachdeva R, Graham ED et al. 290 metagenome-assembled genomes from the Mediterranean Sea: a resource for marine microbiology. *PeerJ* 2017;**5**:e3558.
- Vernikos G, Medini D, Riley DR et al. Ten years of pan-genome analyses. *Curr Opin Microbiol* 2015;**23**:148–54.
- Voorhies AA, Eisenlord SD, Marcus DN et al. Ecological and genetic interactions between cyanobacteria and viruses in a low-oxygen mat community inferred through metagenomics and metatranscriptomics. *Environ Microbiol* 2016;**18**:358–71.
- Walker DA, Daniëls FJA, Alsos I et al. Circumpolar Arctic vegetation: a hierarchic review and roadmap toward an internationally consistent approach to survey, archive and classify tundra plot data. *Environ Res Lett* 2016;**11**:055005.
- Walworth N, Pfreundt U, Nelson WC et al. *Trichodesmium* genome maintains abundant, widespread noncoding DNA in situ, despite oligotrophic lifestyle. *Proc Natl Acad Sci* 2015;**112**:4251–6.
- Warshan D, Espinoza JL, Stuart RK et al. Feathermoss and epiphytic *Nostoc* cooperate differently: expanding the spectrum of plant-cyanobacteria symbiosis. *ISME J* 2017;**134**:2821–33.
- Weinbauer MG, Rassoulzadegan F. Are viruses driving microbial diversification and diversity? *Environ Microbiol* 2004;**6**:1–11.
- Weiss M, Hobbie S, Gettel G. Contrasting responses of nitrogen fixation in Arctic lichens to experimental and ambient nitrogen and phosphorous availability, Arctic, Antarctic, and Alpine Research 2005;**37**:396–401.
- Whitton BA, (ed). *Ecology of Cyanobacteria II*. Dordrecht, the Netherlands: Springer 2012.
- Wood SA, Mountfort D, Selwood AI et al. Widespread distribution and identification of eight novel microcystins in Antarctic cyanobacterial mats. *Appl Environ Microbiol* 2008;**74**:7243–51.
- Wookey PA, Aerts R, Bardgett RD et al. Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. *Global Change Biol* 2009;**15**:1153–72.
- Wunderlin T, Ferrari B, Power M. Global and local-scale variation in bacterial community structure of snow from the Swiss and Australian Alps. *FEMS Microbiol Ecol* 2016;**92**:fiw132.
- Xie M, Ren M, Yang C et al. Metagenomic analysis reveals symbiotic relationship among bacteria in *Microcystis*-dominated community. *Front Microbiol* 2016;**7**:56.
- Zawar-Reza P, Argüello-Astorga GR, Kraberger S et al. Diverse small circular single-stranded DNA viruses identified in a freshwater pond on the McMurdo Ice Shelf (Antarctica). *Infect Genet Evol* 2014;**26**:132–8.
- Zemp M, Frey H, Gärtner-Roer I et al. Historically unprecedented global glacier decline in the early 21st century. *J Glaciol* 2015;**61**:745–62.
- Zúñiga C, Leiva D, Ramírez-Fernández L et al. Phylogenetic diversity of *Peltigera* cyanolichens and their photobionts in Southern Chile and Antarctica. *Microbes Environ* 2015;**30**:172–9.