

# 1 Novel diversity of polar Cyanobacteria revealed by 2 genome-resolved metagenomics

3 Igor S. Pessi<sup>1,2,\*</sup>, Rafael Vicentini Popin<sup>1</sup>, Benoit Durieu<sup>3</sup>, Yannick Lara<sup>4</sup>,  
4 Valentina Savaglia<sup>3,5</sup>, Beatriz Roncero-Ramos<sup>3,6</sup>, Jenni Hultman<sup>1,2,7</sup>, Elie Verleyen<sup>5</sup>,  
5 Wim Vyverman<sup>5</sup>, and Annick Wilmotte<sup>3</sup>

6 <sup>1</sup>Department of Microbiology, University of Helsinki, Helsinki, Finland

7 <sup>2</sup>Helsinki Institute of Sustainability Science (HELSUS), Helsinki, Finland

8 <sup>3</sup>InBioS – Centre for Protein Engineering, University of Liège, Liège, Belgium

9 <sup>4</sup>Early Life Traces & Evolution-Astrobiology, UR-Astrobiology, University of Liège, Liège, Belgium

10 <sup>5</sup>Laboratory of Protistology & Aquatic Ecology, Ghent University, Ghent, Belgium

11 <sup>6</sup>Department of Plant Biology and Ecology, University of Sevilla, Sevilla, Spain

12 <sup>7</sup>Natural Resources Institute Finland (LUKE), Helsinki, Finland

13 \*Corresponding author: [igor.pessi@helsinki.fi](mailto:igor.pessi@helsinki.fi); [igor.pessi@gmail.com](mailto:igor.pessi@gmail.com)

## 14 Abstract

15 Benthic microbial mats dominated by Cyanobacteria are important features of polar lakes.  
16 Although culture-independent studies have provided important insights into their diversity,  
17 only a handful of genomes of polar Cyanobacteria have been sequenced to date. Here, we applied  
18 a genome-resolved metagenomics approach to data obtained from Arctic, sub-Antarctic, and  
19 Antarctic microbial mats. We recovered 22 unique metagenome-assembled genomes (MAGs) of  
20 Cyanobacteria, most of which are only distantly related to genomes that have been sequenced  
21 so far. These include i) lineages that are common in polar microbial mats such as the  
22 filamentous taxa *Pseudanabaena*, *Leptolyngbya*, *Microcoleus/Tychonema*, and *Phormidium*; ii)  
23 the less common taxa *Crinalium* and *Chamaesiphon*; iii) an enigmatic Chroococcales lineage  
24 only distantly related to *Microcystis*; and iv) an early branching lineage in the order  
25 Gloeobacterales that is almost exclusively restricted to the cold biosphere, for which we propose  
26 the name *Candidatus Sivonemia alaskensis*. Our results show that genome-resolved  
27 metagenomics is a powerful tool for expanding our understanding of the diversity of  
28 Cyanobacteria, especially in understudied remote and extreme environments.

## 29 **Data summary**

30 The sequencing data generated in this study have been submitted to the European Nucleotide  
31 Archive (ENA) under the BioProject PRJEB59431. Individual accession numbers for raw reads  
32 and genomic bins are listed in **Table S1** and **Table S3**, respectively. Genomic bins can also be  
33 downloaded from [doi.org/10.6084/m9.figshare.22003967](https://doi.org/10.6084/m9.figshare.22003967). The commands used throughout this  
34 study are available in [github.com/igorspp/polar-cyanobacteria-MAGs](https://github.com/igorspp/polar-cyanobacteria-MAGs).

## 35 **Impact statement**

36 Cyanobacteria are photosynthetic microorganisms that play important roles in polar lacustrine  
37 ecosystems. Many Cyanobacteria are difficult to grow in the laboratory, particularly in isolation  
38 from other organisms, which makes it challenging to sequence their genomes. As such,  
39 considerably fewer genomes of Cyanobacteria have been sequenced so far compared to other  
40 bacteria. In this study, we used a metagenomics approach to recover novel genomes of  
41 Cyanobacteria from Arctic and Antarctic microbial mats without the need to isolate the  
42 organisms. The community DNA was extracted and sequenced, and the genomes of individual  
43 populations were separated using bioinformatics tools. We recovered 22 different genomes of  
44 Cyanobacteria, many of which have not been sequenced before. We describe in more detail an  
45 interesting lineage of ancestral Cyanobacteria in the order Gloeobacterales, for which we  
46 propose the name *Candidatus* Sivonenia alaskensis. Our study shows that genome-resolved  
47 metagenomics is a valuable approach for obtaining novel genomes of Cyanobacteria, which are  
48 needed to improve our understanding of life in the polar regions and the planet at large.

## 49 **Introduction**

50 Microbial mats are highly successful and productive ecosystems found in a wide range of  
51 environments since the dawn of life on Earth [1, 2]. Microbial mats commonly comprise a vast  
52 diversity of microorganisms such as auto- and heterotrophic bacteria, fungi, microalgae, and  
53 heterotrophic protists embedded in an exopolysaccharide matrix [3]. Benthic microbial mats  
54 represent an important survival strategy against the harsh environmental conditions in polar  
55 and alpine lakes, and have Cyanobacteria as their primary source of organic carbon and  
56 nitrogen [4, 5]. In addition to aquatic microbial mats, Cyanobacteria are also important  
57 members of terrestrial and epi- and supraglacial communities in polar environments [6, 7].

58 Despite their importance, knowledge on the diversity and ecology of Cyanobacteria in polar  
59 environments is fragmentary [8]. Studies on the diversity of polar Cyanobacteria have mostly  
60 focused on microscopic identification and strain isolation [9–15], analysis of environmental 16S  
61 rRNA gene sequences [16–21], or a combination of these methods [22–24]. On one hand, the

62 microscopic identification of Cyanobacteria is hindered by the high plasticity of taxonomic  
63 markers such as cell dimensions and division patterns and the relative paucity of morphological  
64 characters [25]. In addition, morphology-based assessments underestimate the diversity of  
65 Cyanobacteria in the environment compared to molecular approaches based on environmental  
66 DNA [19]. Molecular approaches, in turn, are hampered by the scarcity of cyanobacterial  
67 genomes stored in public databases, which are largely underrepresented compared to other  
68 microbial phyla and heavily biased towards the *Prochlorococcus/Synechococcus* clade [26, 27].

69 The genomic catalogue of polar Cyanobacteria is currently limited to a handful of strains,  
70 including *Pseudanabaena* sp. BC1403 and *Phormidesmis priestleyi* BC1401 from Greenland  
71 [28], *Leptolyngbya* sp. Cla-17 from the Canadian High Arctic [29], and the Antarctic strains *P.*  
72 *priestleyi* ULC007 [30], *Leptolyngbya* sp. BC1307 [31], *Synechococcus* sp. SynAce01 [32], and  
73 *Nostoc* sp. SO-36 [33]. Twelve other low-quality genomes obtained by a metagenome-like  
74 assembling approach of non-axenic strains are also available [34]. Genome-resolved  
75 metagenomics has been established in recent years as a powerful approach to obtain microbial  
76 genomes, as it circumvents the difficulties associated with culturing microorganisms by  
77 reconstructing microbial genomes directly from environmental DNA [35–38]. Several genomes  
78 of uncultured polar Cyanobacteria have been obtained recently using this approach, including  
79 several novel lineages of early branching Cyanobacteria in the order Gloeobacterales [39–41].

80 In this study, we aimed to expand the genomic catalogue of polar Cyanobacteria. To achieve  
81 this, we applied a genome-resolved metagenomics approach to data obtained from microbial  
82 mats from Arctic, sub-Antarctic, and Antarctic lakes spanning a wide geographic and  
83 limnological range. Our results include the recovery of novel genomes of polar Cyanobacteria  
84 and the description of an early branching lineage that is distributed across polar and alpine  
85 environments.

## 86 **Methods**

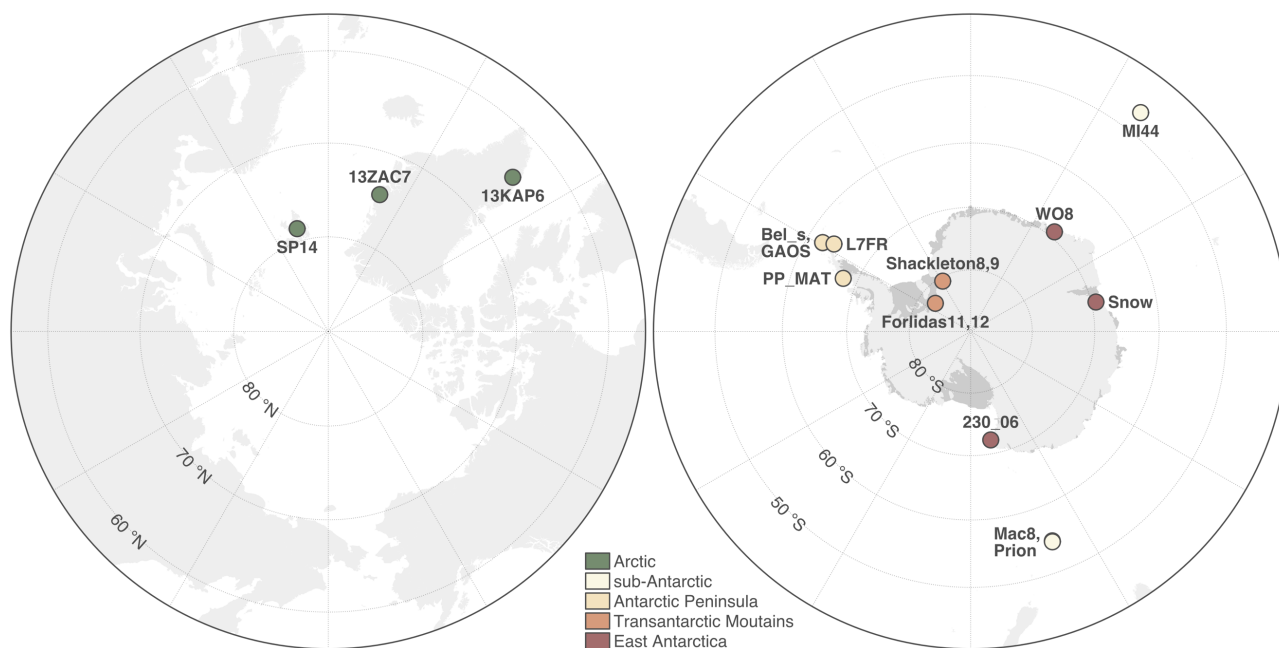
### 87 **Sample description**

88 We analysed 17 microbial mat samples obtained from 15 Arctic, sub-Antarctic, and Antarctic  
89 lakes (**Fig. 1, Table S1**). The Arctic lakes are located in Svalbard and Greenland. The sub-  
90 Antarctic samples come from lakes in Macquarie Island (South Pacific Ocean) and Marion  
91 Island (South Indian Ocean), and sampling in Antarctica covered several locations in the  
92 Antarctic Peninsula, Transantarctic Mountains, and East Antarctica. The Antarctic lakes are  
93 distributed across five Antarctic Conservation Biological Regions (ACBRs) [42]. We analysed  
94 one microbial mat sample taken from the shallow region of each lake (*ca.* 0.2 m depth). In lakes

95 Lundström and Forlidas, we analysed one additional sample taken from a deeper saline and  
96 hypersaline layer, respectively.

## 97 DNA extraction and metagenome sequencing

98 We used the DNeasy PowerBiofilm DNA Isolation kit (QIAGEN, Hilden, Germany) to extract  
99 DNA from *ca.* 0.5 g of each microbial mat sample and checked the concentration and quality of  
100 the DNA extracts using the Qubit dsDNA BR Assay kit (Thermo Fisher Scientific, Waltham,  
101 MA, USA). We used the Nextera XT kit (Illumina, San Diego, CA, USA) to prepare the  
102 metagenomic libraries, which were then sent to Eurofins Genomics (Ebersberg, Germany) for  
103 sequencing using the Illumina HiSeq 2500 platform (2x100 bp). We checked the quality of the  
104 raw sequencing data with fastQC v0.11.9 ([bioinformatics.babraham.ac.uk/projects/fastqc](http://bioinformatics.babraham.ac.uk/projects/fastqc)) and  
105 multiQC v1.8 [43], and used Cutadapt v1.16 [44] to trim adapters, low-quality base calls (Phred  
106 score <20), and discard short reads (<50 bp). Finally, we used METAXA v2.2 [45] to extract  
107 reads matching the 16S rRNA gene, which were then classified with mothur v1.44.3 [46] using  
108 the SILVA database release 138.1 [47] and the Naïve Bayesian Classifier with a confidence cut-  
109 off of 80% [48].



110 **Fig. 1.** Location of the Arctic and Antarctic lakes where microbial mats were sampled. Maps were created  
111 with public data from the Norwegian Polar Institute (Tromsø, Norway). More information about the  
112 samples can be found in **Table S1**.

## 113 **Metagenome assembling**

114 We assembled and binned each metagenome individually and as two co-assemblies. One co-  
115 assembly was done by grouping samples from the Arctic (n=3) and sub-Antarctic (n=3). The  
116 second co-assembly comprised the remaining samples from the Antarctic Peninsula,  
117 Transantarctic Mountains, and East Antarctica (n=11). We assembled the metagenomes with  
118 MEGAHIT v1.1.1.2 [49] and obtained 176,097 and 72,514 contigs  $\geq 1000$  bp for the Antarctic and  
119 Arctic/sub-Antarctic co-assemblies, respectively. The total assembled length was 447.6 and  
120 182.2 Mb, respectively. The output of the individual assemblies ranged from 218 contigs/0.3 Mb  
121 (sample ‘13ZAC7’) to 96722 contigs/262.4 Mb (sample ‘PP\_MAT’). The assembly of sample  
122 ‘Forlidas11’ did not yield any contig due to the very low sequencing depth achieved for this  
123 sample (**Table S1**).

## 124 **Metagenome binning**

125 For each individual and co-assembly, we used *anvi'o* v7.0 [50] to bin contigs  $\geq 2500$  bp into  
126 metagenome-assembled genomes (MAGs) as previously described [37, 38]. In brief, we used  
127 *Prodigal* v2.6.3 [51] to find gene calls, *HMMER* v.3.3 [52] to identify a set of 71 bacterial and 76  
128 archaeal single-copy genes [53], and *DIAMOND* v0.9.14 [54] to assign taxonomy to the single-  
129 copy genes according to the Genome Taxonomy Database (GTDB) release 04-RS89 [55]. We used  
130 *bowtie* v2.4.2 [56] to map the quality-filtered reads from all samples to the contigs and *SAMtools*  
131 v1.1 [57] to sort and index the mapping output. We then used the *anvi-interactive* interface of  
132 *anvi'o* to manually sort the contigs into genomic bins based on differential coverage and  
133 tetranucleotide frequency. Bins that were  $\geq 50\%$  complete according to the presence of 71 single-  
134 copy genes [53] were manually curated using the *anvi-refine* interface of *anvi'o*. We refined the  
135 bins by removing outlying contigs according to coverage, tetranucleotide frequency, and  
136 taxonomic signal. We assigned taxonomy to the refined bins based on 122 archaeal and 120  
137 bacterial single-copy genes with *GTDB-Tk* v1.3.0 [58] and the GTDB release 05-RS95 [55]. Bins  
138 assigned to the phylum Cyanobacteria that were  $\geq 50\%$  complete and  $\leq 10\%$  redundant –  
139 hereafter referred as MAGs – were kept for downstream analyses.

## 140 **Phylogenetic analysis**

141 We used a concatenated alignment of 38 ribosomal proteins to place the MAGs in a phylogenetic  
142 tree alongside all genomes assigned to the Cyanobacteria/Melainabacteria group in GenBank  
143 (NCBI:txid1798711, accessed on 17 November 2022). We used *ncbi-genome-download* v0.3.1  
144 ([github.com/kbclin/ncbi-genome-download](https://github.com/kbclin/ncbi-genome-download)) to recover the genomes from GenBank. In *anvi'o* v7.0  
145 [50], we retrieved the translated amino acid sequence of each ribosomal protein with *HMMER*  
146 v.3.3 [52] and aligned them with *MUSCLE* v3.8.1551 [59]. We concatenated the alignments of

147 the 38 ribosomal proteins and built a maximum-likelihood tree with *IQ-TREE* [60] using the  
148 automatic model selection and 1000 ultrafast bootstrap approximation replicates. We also used  
149 *fastANI* v1.32 [61] to calculate the genome-wide average nucleotide identity (ANI) between  
150 MAGs and GenBank genomes. For better visualization, we computed a more compact  
151 maximum-likelihood tree including only the MAGs, their closest neighbours in GenBank,  
152 strains from the Pasteur Culture Collection of Cyanobacteria (PCC), and other selected  
153 genomes. We classified the MAGs based on their phylogenetic placement following the  
154 taxonomic system of Komárek *et al.* [62].

## 155 **Gene annotation**

156 In *anvi'o* v7.0 [50], we annotated the gene calls identified by *Prodigal* v2.6.3 [51] against the  
157 KOfam [63] and the Pfam [64] databases with *HMMER* v.3.3 [52] and the COG [65] database  
158 with *DIAMOND* v0.9.14 [54]. We also used *tblastn* (web interface, available at  
159 [blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE\\_TYPE=BlastSearch](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=tblastn&PAGE_TYPE=BlastSearch)) to search for  
160 additional genes involved in mechanisms of resistance to stress. Only hits with e-value  $<10^{-5}$   
161 and bitscore  $>50$  were considered, according to Pearson [66].

## 162 **Distribution analyses**

163 We used metagenomic read recruitment to compute the relative abundance of the MAGs across  
164 the 17 microbial mat samples. Prior to this, we used *dRep* v3.2.2 [67] to dereplicate the MAGs  
165 based on a 99% ANI threshold. We then used *CoverM* v0.6.1 ([github.com/wwood/CoverM](https://github.com/wwood/CoverM)) to  
166 map the quality-filtered reads to the MAGs with *minimap* v2.17 [68] and compute relative  
167 abundances based on the proportion of reads recruited by the MAGs. For this, we considered  
168 only matches with  $\geq 95\%$  identity and  $\geq 75\%$  coverage. We also used *sourmash branchwater* [69,  
169 70] and *IMNGS* [71] to search the two Gloeobacterales MAGs against metagenomic and  
170 amplicon sequencing datasets in the Sequence Read Archive (SRA), respectively. For the first,  
171 we used the *mastiff* implementation of *sourmash branchwater* ([github.com/sourmash-bio/2022-  
172 search-sra-with-mastiff](https://github.com/sourmash-bio/2022-search-sra-with-mastiff)). The datasets where significant matches were found (containment  
173  $\geq 20\%$ ) were downloaded from SRA with *fasterq-dump* v3.0.1 ([github.com/nbci/sra-tools](https://github.com/nbci/sra-tools)) and  
174 mapped back to the two Gloeobacterales MAGs with *CoverM* v0.6.1 as described above. For the  
175 analysis of amplicon sequencing datasets, we used the web interface of *IMNGS* ([imngs.org](https://imngs.org)) and  
176 only considered datasets where significant matches ( $\geq 99\%$  similarity) accounted for  $\geq 0.1\%$  of the  
177 sequences.

## 178 **Results and discussion**

179 We obtained around 500 million paired-end metagenomic reads (99.3 Gb) from 17 Arctic, sub-  
180 Antarctic, and Antarctic microbial mat samples (**Fig. 1, Table S1**). Taxonomic profiling based  
181 on reads matching the 16S rRNA gene revealed Cyanobacteria as the second most abundant  
182 microbial phylum after Proteobacteria (mean relative abundance of 20.8 and 24.0%,  
183 respectively) (**Table S2**). The dominance of these two phyla is commonly observed in polar  
184 microbial mats [72–74]. After assembling the reads with *MEGAHIT* [49], we used *anvi'o* [50] to  
185 manually bin and curate MAGs. Taxonomic classification based on the GTDB release 05-RS95  
186 [55] assigned 37 MAGs to the phylum Cyanobacteria (**Fig. 2, Table 1, Table S3**). These include  
187 two MAGs ('PMM\_0025' and 'PMM\_0089') belonging to the order Obscuribacterales of the  
188 Melainabacteria, a sister lineage to the Cyanobacteria *stricto sensu* (clade Oxyphotobacteria)  
189 that lacks the photosynthetic machinery [75]. Indeed, annotation of protein-coding genes  
190 revealed that the two Obscuribacterales MAGs do not encode proteins of the Calvin cycle (Rbc),  
191 photosystems I and II (Psa and Psb), cytochrome *b<sub>cf</sub>* complex (Pet), and phycobilisomes (Apc,  
192 Cpc, Cpe, and Pec) (**Fig. 3**). Interestingly, the presence of genes for the small and large subunits  
193 of the nitric oxide reductase (NorC and NorB, respectively) indicates a potential role of this  
194 lineage in the production of the greenhouse gas nitrous oxide [37].

## 195 **Genome-resolved metagenomics is a reliable tool for the investigation of** 196 **cyanobacterial diversity**

197 Phylogenetic analysis based on a concatenated alignment of 38 ribosomal proteins assigned the  
198 35 Oxyphotobacteria MAGs to five orders according to the taxonomic system of Komárek *et al.*  
199 [62]: Gloeobacterales (n=2), Synechococcales (n=19), Oscillatoriales (n=8), Chroococcales (n=2),  
200 and Nostocales (n=4) (**Fig. 2**). Most MAGs originated from the individual (n=20) and Antarctic  
201 (n=15) (co-)assemblies (**Table S3**). We did not recover any MAG from the individual assemblies  
202 of Arctic samples despite the high abundance of Cyanobacteria in these samples (5.9–28.6% of  
203 the reads matching the 16S rRNA gene) (**Table S2**) and the high sequencing depth (3.6–7.5 Gb)  
204 (**Table S1**). MAG dereplication based on a 99% ANI threshold grouped the 37 Cyanobacteria  
205 MAGs into 22 unique clusters (**Table S3**). In general, we observed a good correspondence  
206 between individual and co-assembly MAGs, *i.e.* closely related genomic bins with  $\geq 99\%$  ANI  
207 were recovered from the two assembly types.

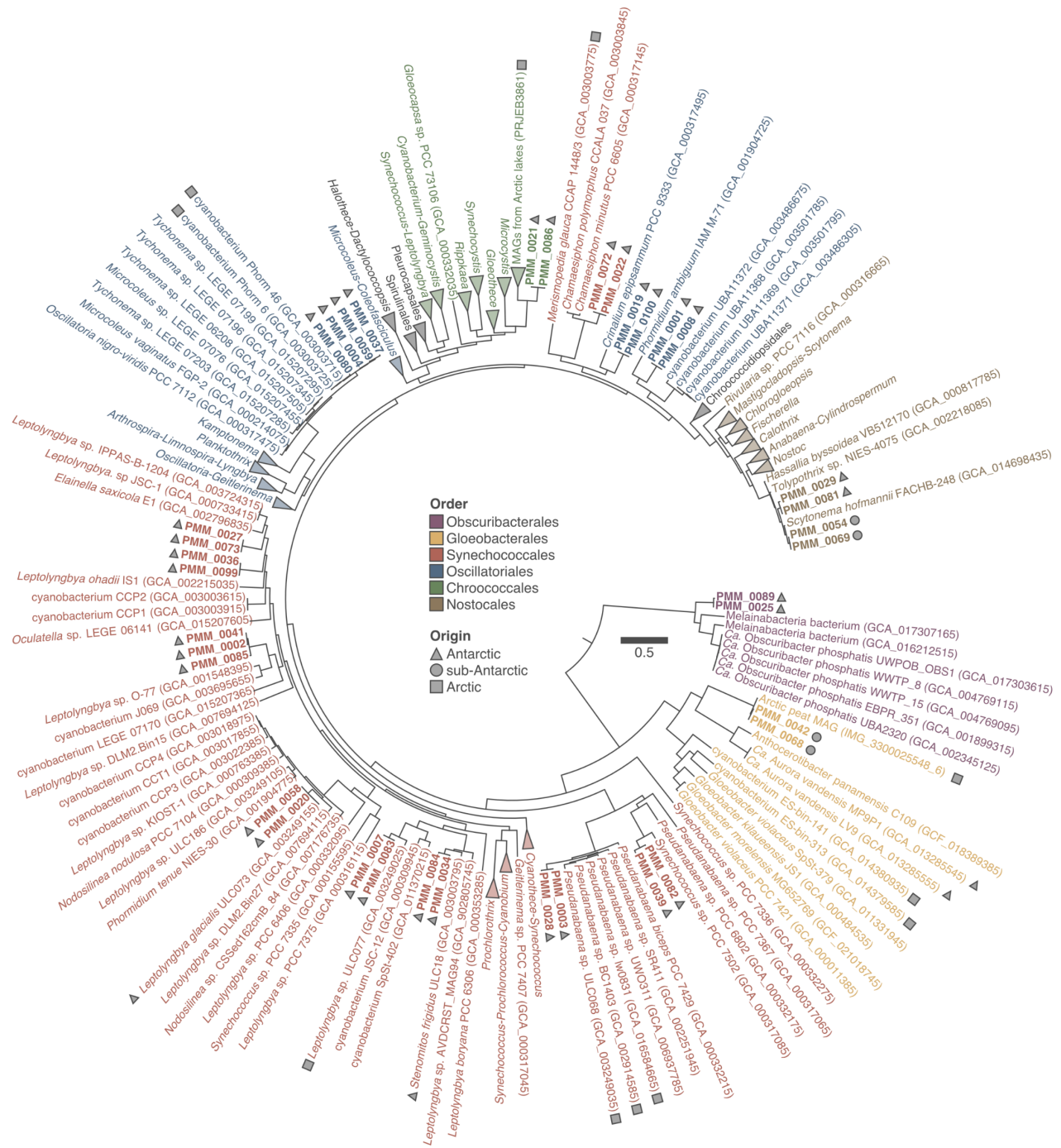
208 **Table 1.** Information on 37 metagenome-assembled genomes (MAGs) of Cyanobacteria *stricto sensu*  
 209 (clade Oxyphotobacteria) and Melainabacteria recovered from polar microbial mats.

210	<b>Group</b>	<b>MAG</b>	<b>Size (Mb)</b>	<b>Compl-</b>	<b>Redund-</b>	<b>GC (%)</b>
211				<b>tion (%)</b>	<b>ancy (%)</b>	
212	<b>Melainabacteria</b>					
213	Obscuribacterales	PMM_0025	6.1	90.1	8.5	47.7
214		PMM_0089	4.7	77.5	5.6	47.9
215	<b>Oxyphotobacteria</b>					
216	Gloeobacterales	PMM_0042	2.9	97.2	0.0	49.3
217		PMM_0068	2.8	95.8	0.0	48.8
218	Synechococcales	PMM_0039	1.5	57.7	0.0	41.5
219	( <i>Pseudanabaena</i> )	PMM_0082	2.7	85.9	0.0	40.3
220		PMM_0003	3.1	78.9	5.6	42.9
221		PMM_0028	3.7	84.5	8.5	42.6
222	Synechococcales	PMM_0034	3.3	66.2	0.0	51.3
223	( <i>Leptolyngbya</i> )	PMM_0084	4.8	81.7	4.2	51.8
224		PMM_0007	5.2	94.4	1.4	49.0
225		PMM_0083	4.6	94.4	1.4	48.4
226		PMM_0020	3.2	90.1	2.8	57.2
227		PMM_0058	2.8	84.5	1.4	56.9
228		PMM_0002	2.9	90.1	1.4	52.5
229		PMM_0041	2.5	50.7	1.4	52.4
230		PMM_0085	3.7	81.7	4.2	52.5
231		PMM_0036	4.6	66.2	4.2	49.4
232		PMM_0099	4.7	67.6	5.6	49.3
233		PMM_0027	2.2	73.2	1.4	55.0
234		PMM_0073	3.6	85.9	5.6	55.3
235	Synechococcales	PMM_0022	2.5	80.3	2.8	44.9
236	( <i>Chamaesiphon</i> )	PMM_0072	2.9	83.1	1.4	44.4
237	Oscillatoriales	PMM_0004	4.4	60.6	7.0	45.6
238	( <i>Tychonema/Microcoleus</i> )	PMM_0037	5.2	67.6	8.5	45.3
239		PMM_0059	4.9	90.1	5.6	45.6
240		PMM_0080	6.4	94.4	2.8	45.1
241	Oscillatoriales	PMM_0019	5.7	95.8	2.8	45.2
242	( <i>Crinalium</i> )	PMM_0100	3.8	80.3	2.8	45.4
243	Oscillatoriales	PMM_0001	5.8	94.4	2.8	45.4
244	( <i>P. ambiguum</i> )	PMM_0008	5.8	94.4	2.8	45.4
245	Chroococcales	PMM_0021	4.0	77.5	2.8	39.0
246		PMM_0086	3.8	80.3	2.8	38.9
247	Nostocales	PMM_0029	2.7	73.2	7.0	42.0
248		PMM_0081	4.4	91.5	2.8	42.1
249		PMM_0054	5.1	90.1	2.8	42.2
250		PMM_0069	3.0	76.1	0.0	41.6

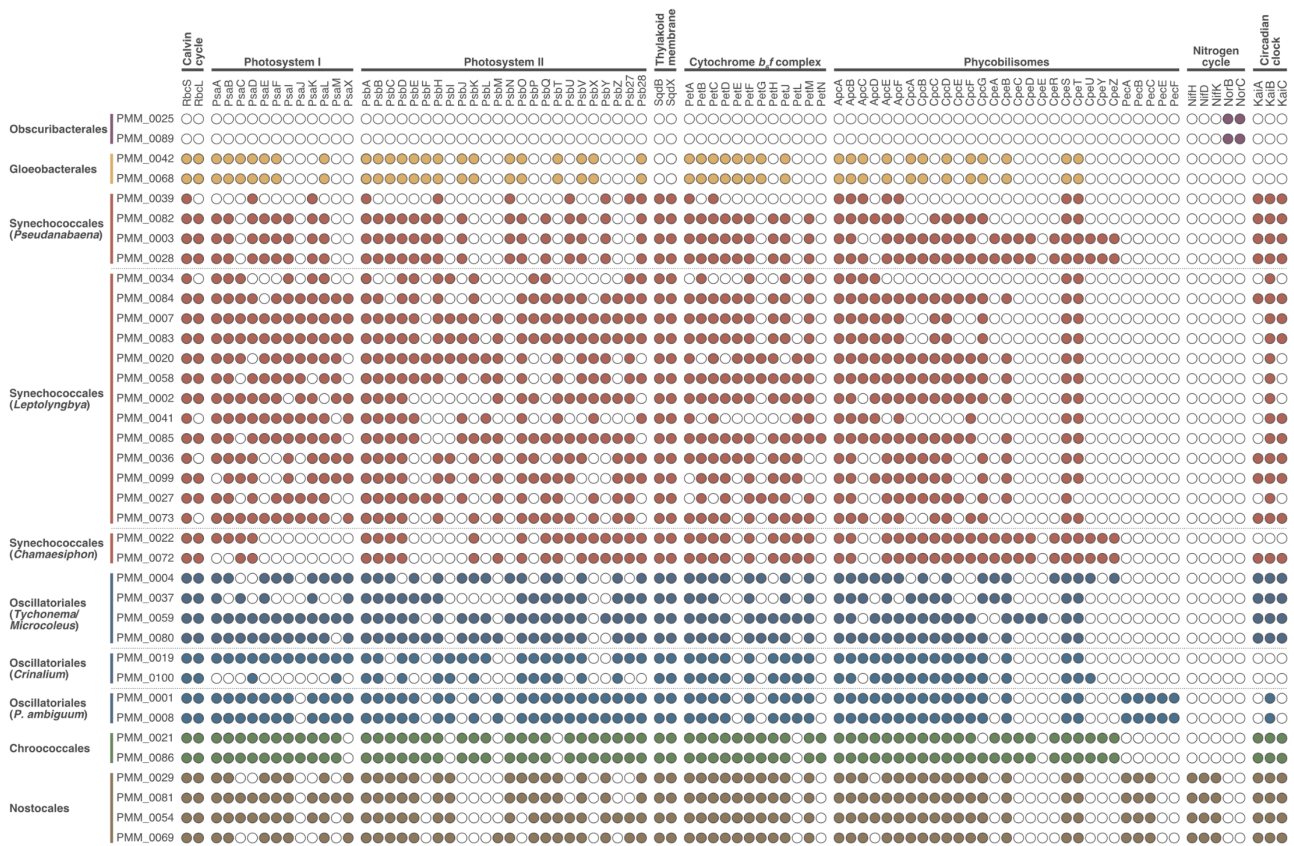
251 Completion and redundancy were estimated based on the presence of 71 single-copy genes [53].

252 More information about the MAGs can be found in **Table S3**.





253 **Fig. 2.** Phylogenetic analysis of 37 metagenome-assembled genomes (MAGs) assigned to the phylum  
 254 Cyanobacteria, including both Cyanobacteria *stricto sensu* (clade Oxyphotobacteria) and the  
 255 Melainabacteria. Maximum-likelihood tree (LG+R8 model) based on a concatenated alignment of 38  
 256 ribosomal proteins from the MAGs (in bold), their closest neighbours in GenBank, PCC strains, and other  
 257 selected genomes. The geographic origin of polar MAGs and strains are indicated. Order-level  
 258 classification is shown according to the taxonomic system of Komárek *et al.* [62].



259 **Fig. 3.** Presence of genes involved in carbon fixation, photosynthesis, nitrogen cycle, and circadian clock  
 260 in 37 metagenome-assembled genomes (MAGs) of Cyanobacteria *stricto sensu* (clade Oxycyphotobacteria)  
 261 and Melainabacteria.

262 The robustness of our metagenomic approach is further illustrated by the high similarity that  
 263 some of the MAGs share with genomes available in GenBank. In particular, two MAGs obtained  
 264 from different assemblies ('PMM\_0058' and 'PMM\_0020') are almost identical (99.7–99.8% ANI)  
 265 to the genome of the strain *Leptolyngbya glacialis* ULC073 (**Fig. 2, Table S3**). This is not  
 266 surprising given that the three genomes originate from the same hypersaline brine layer in the  
 267 benthos of Forlidas Pond, Transantarctic Mountains [24]. *L. glacialis* ULC073 and *L. antarctica*  
 268 ULC047 (Ace Lake, Princess Elizabeth Land) [12], which share identical 16S rRNA gene  
 269 sequences, are representative strains of an ubiquitous morphotype in Antarctic lakes belonging  
 270 to the *Leptolyngbya-Nodosilinea* clade [12, 20, 23, 24]. Despite the importance of this lineage,  
 271 the genome of *L. glacialis* ULC073 currently available in GenBank (accession  
 272 GCA\_003249155.1), which was obtained from a non-axenic unialgal culture using a  
 273 metagenome-like approach [34], is very fragmented (650 contigs,  $N_{50}$ =10.7 Kbp) and somewhat  
 274 redundant (7.0% according to our analysis of 71 single-copy genes). Based on these parameters,  
 275 the MAGs 'PMM\_0058' and 'PMM\_0020', which are 84.5–90.1% complete, 1.4–2.8% redundant,

276 comprise 290–339 contigs, and have an N<sub>50</sub> of 12.1–13.0 Kbp (**Table 1, Table S3**), can be  
277 considered better representatives of this important lineage of Antarctic Cyanobacteria.

278 Other MAGs that are closely related to strains are the Nostocales MAGs ‘PMM\_0054’ and  
279 ‘PMM\_0069’. Genome-wide analysis revealed that they share 93.8–94.5% ANI with their closest  
280 genome on GenBank, *Scytonema hofmannii* FACHB-248 (**Fig. 2, Table S3**). However, their  
281 16S rRNA gene is 99.4% similar to the sequence of *Dactylothamnos antarcticus* CENA433  
282 isolated from a freshwater biofilm in the Antarctic Peninsula [76], for which genomic  
283 information is currently lacking. The other two Nostocales MAGs (‘PMM\_0029’ and  
284 ‘PMM\_0081’) are also likely related to *D. antarcticus* given their close phylogenetic relationship  
285 with ‘PMM\_0054’ and ‘PMM\_0069’ (**Fig. 2**). Finally, the Gloeobacterales MAGs ‘PMM\_0042’  
286 and ‘PMM\_0068’ share 97.2% ANI with the MAG ‘IMG\_3300025548\_6’ recovered from peat soil  
287 in Alaska [39] (**Fig. 2, Table S3**).

## 288 **Metagenomics reveals novel genomic diversity of polar Cyanobacteria**

289 Phylogenetic placement and genome-wide comparison with sequences from GenBank revealed  
290 that most MAGs differ from genomes that have been sequenced so far (**Fig. 2, Table S3**). In  
291 particular, 19 of the 37 MAGs have <80% ANI with genomes currently available in GenBank  
292 and 12 are only distantly related to existing genomes (80.1–93.2% ANI). Interestingly,  
293 phylogenetic placement clustered 16 and eight MAGs alongside polar and alpine strains,  
294 respectively (**Fig. 2**). This is in agreement with previous studies showing that many lineages of  
295 Cyanobacteria are distributed across the cold biosphere [20, 77, 78]. Most MAGs are affiliated  
296 with filamentous taxa in the orders Synechococcales (n=17), Oscillatoriales (n=8), and  
297 Nostocales (n=4), highlighting the importance of filamentous Cyanobacteria as the ecosystem  
298 builders of polar microbial mats [4, 5, 79, 80]. Moreover, Cyanobacteria belonging to the order  
299 Nostocales often dominate the microbial communities in oligotrophic polar environments due to  
300 their ability to fix atmospheric nitrogen [4–7]. As observed previously (*e.g.* Olson *et al.* [81]),  
301 genes encoding the different subunits of the nitrogenase enzyme (NifHDK) involved in nitrogen  
302 fixation were exclusive to the four Nostocales MAGs (**Fig. 3**).

303 Most Synechococcales MAGs (n=13) are phylogenetically related to strains that have been  
304 traditionally classified as *Leptolyngbya*, which is a morphological group comprising  
305 Cyanobacteria with a thin, simple filamentous morphotype that includes many different genera  
306 according to molecular data [27, 62]. Our *Leptolyngbya* MAGs can be broadly categorized into  
307 four major lineages (**Fig. 2**): i) *Leptolyngbya stricto sensu* (‘PMM\_0007’ and ‘PMM\_0083’), ii)  
308 *Leptolyngbya-Stenomitos* (‘PMM\_0034’ and ‘PMM\_0084’), iii) *Leptolyngbya-Nodosilinea*  
309 (‘PMM\_0020’ and ‘PMM\_0058’), and iv) *Leptolyngbya-Oculatella-Elainella* (‘PMM\_0085’,

310 ‘PMM\_0002’, ‘PMM\_0041’, ‘PMM\_0099’, ‘PMM\_0036’, ‘PMM\_0073’, and ‘PMM\_0027’). The other  
311 four MAGs of filamentous Synechococcales are affiliated with the early branching  
312 *Pseudanabaena* (**Fig. 2**). Two of these (‘PMM\_0003’ and ‘PMM\_0028’) are most closely related  
313 (80.9–81.0% ANI) to the strain *Pseudanabaena* sp. ULC068 isolated from a lake in the Canadian  
314 sub-Arctic (W. Vincent, unpublished) (**Table S3**), and also clustered alongside the strains  
315 BC1403 from Greenland [28] and lw0831 from Svalbard [82] (**Fig. 2**). The other two  
316 *Pseudanabaena* MAGs (‘PMM\_0039’ and ‘PMM\_0082’) are distantly related (<80% ANI) to  
317 *Synechococcus* sp. PCC 7502, a unicellular strain isolated from an alpine *Sphagnum* bog that  
318 clusters with the early branching *Pseudanabaena* [26].

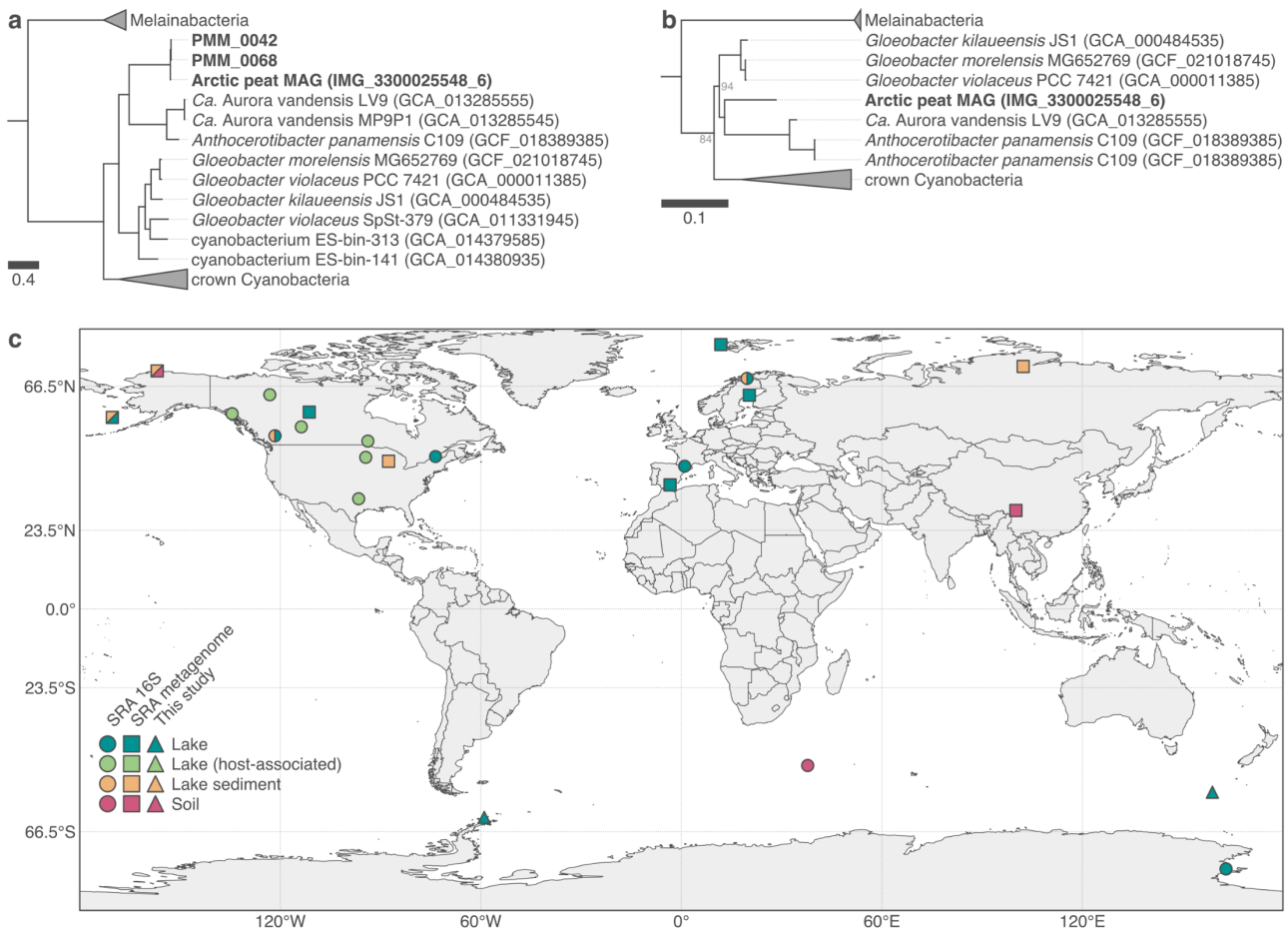
319 Other MAGs of filamentous Cyanobacteria are affiliated with the order Oscillatoriales (n=8)  
320 (**Fig. 2**). Four of these (‘PMM\_0004’, ‘PMM\_0037’, ‘PMM\_0059’, and ‘PMM\_0080’) are most  
321 closely related (90.1–93.2% ANI) to the strain Phorm 46 isolated from a lake in the Canadian  
322 High-Arctic [83] (**Table S3**), and also clustered alongside strains of *Tychonema* and *Microcoleus*  
323 *vaginatus* (**Fig. 2**). The other two MAGs of filamentous Oscillatoriales (‘PMM\_0001’ and  
324 ‘PMM\_0008’) are distantly related (<80% ANI) to the strain *Phormidium ambiguum* IAM M-71,  
325 which has an uncertain phylogenetic placement. Phylogenetic analysis of the amplified 16S  
326 rRNA gene sequence (accession AB003167) originally placed *P. ambiguum* IAM M-71 alongside  
327 other Oscillatoriales such as *Oscillatoria* and *Lyngbya* [84, 85]. However, a later 16S rRNA  
328 phylogeny [86] and a phylogenomic tree based on 834 single-copy genes [87] both placed the  
329 strain IAM M-71 in a similar phylogenetic position as the one inferred here, *i.e.* basal to the  
330 Nostocales (**Fig. 2**). A BLAST analysis suggests that the AB003167 sequence is chimeric with  
331 *Phormidium muscicola* IAM M-221, but a phylogenetic artefact based on long branch attraction  
332 is also possible given the lack of related genomes. Interestingly, the *P. ambiguum* MAGs were  
333 the most widespread MAGs in our dataset, being detected in five samples in the Antarctic  
334 Peninsula, Transantarctic Mountains, and East Antarctica (**Table S4**). Finally, the two  
335 remaining Oscillatoriales MAGs (‘PMM\_0019’ and ‘PMM\_0100’) clustered alongside *Crinalium*  
336 *epipsammum* PCC 9333 (**Fig. 2**). *Crinalium* is a filamentous genus of Cyanobacteria with  
337 unusual elliptical trichomes [88]. Sequences related to *Crinalium* have been recovered from  
338 different alpine habitats [89] and a new species, *C. glaciale*, has been described from cryoconite  
339 pools in Antarctica on the basis of morphological identification [90].

340 In addition to filamentous taxa, we also recovered MAGs related to unicellular Cyanobacteria  
341 in the orders Gloeobacterales (n=2), Synechococcales (n=2), and Chroococcales (n=2) (**Fig. 2**).  
342 All except the two Gloeobacterales MAGs were distantly related (<80% ANI) to genomes  
343 currently available in GenBank (**Table S3**). The two Synechococcales MAGs (‘PMM\_0022’ and  
344 ‘PMM\_0072’) clustered alongside *Chamaesiphon minutus* PCC 6605 and *Chamaesiphon*

345 *polymorphus* CCALA 037 (**Fig. 2**). *Chamaesiphon* is a cosmopolitan genus that is often reported  
346 in polar and alpine terrestrial and aquatic environments, and includes two species potentially  
347 endemic to Antarctica (*C. arctowskii* and *C. austro-polonicus*) [15, 19, 20, 79, 80, 91]. Finally,  
348 the two Chroococcales MAGs ('PMM\_0021' and 'PMM\_0086') formed a distinct lineage related  
349 to *Microcystis* and several MAGs recovered from Arctic lakes (BioProject PRJEB38681) (**Fig.**  
350 **2**).

### 351 **Description of *Candidatus Sivonenia alaskensis***

352 We investigated in more detail the two Gloeobacterales MAGs 'PMM\_0042' and 'PMM\_0068'  
353 given the importance of this group as the most basal lineage of extant Cyanobacteria [92–94].  
354 Phylogenetic analysis based on a concatenated alignment of 38 ribosomal proteins placed both  
355 MAGs alongside the Arctic Peat MAG 'IMG\_3300025548\_6' [39], with which they share 97.2%  
356 ANI (**Fig. 4a**). This is above the threshold of 95–96% ANI commonly used for delineating  
357 microbial species [95, 96], which thus suggests that the three MAGs ('PMM\_0042', 'PMM\_0068',  
358 and 'IMG\_3300025548\_6') belong to the same species. Furthermore, their phylogenetic  
359 placement and low ANI (<80%) with other Gloeobacterales indicate that they constitute a  
360 distinct genus in this order. Separation from the other Gloeobacterales is also supported by  
361 analysis of the 16S rRNA gene of the MAG 'IMG\_3300025548\_6', which is 91.8–92.0%, 90.0%,  
362 and 89.3% similar to the sequences of *Gloeobacter* spp., *Candidatus Aurora vandensis*, and  
363 *Anthocerotibacter panamensis*, respectively (**Fig. 4b**). We consider that the MAGs 'PMM\_0042',  
364 'PMM\_0068', and 'IMG\_3300025548\_6' represent a novel lineage in the order Gloeobacterales  
365 and propose the name *Candidatus Sivonenia alaskensis* (*Sivonenia*: in honour of our colleague  
366 and Cyanobacteria expert Dr. Kaarina Sivonen, *professor emerita* of the University of Helsinki;  
367 *alaskensis*: relative to the geographic origin of the MAG 'IMG\_3300025548\_6', which is proposed  
368 here as the nomenclatural type for this species according to the SeqCode initiative [97]).



369 **Fig. 4. *Candidatus Sivonienia alaskensis*, a lineage of early branching Cyanobacteria in the**  
370 **order Gloeobacterales. a)** Maximum-likelihood tree (LG+R8 model) based on a concatenated alignment  
371 of 38 ribosomal proteins from the three *Ca. Sivonienia alaskensis* MAGs (in bold) and other  
372 Gloeobacterales and selected genomes from GenBank. All nodes have bootstrap support  $\geq 95\%$ . **b)**  
373 Maximum-likelihood tree (GTR+F+R8 model) of the 16S rRNA gene of *Ca. Sivonienia alaskensis*. Nodes  
374 have bootstrap support  $\geq 95\%$  unless shown otherwise. **c)** Geographic distribution of *Ca. Sivonienia*  
375 *alaskensis* based on significant matches with metagenomic and 16S rRNA gene amplicon sequencing  
376 datasets in SRA ( $\geq 20\%$  containment and  $\geq 0.1\%$  relative abundance, respectively).

377 ***In silico* analysis indicates that *Ca. Sivonienia alaskensis* is a thylakoid-less**  
378 **cyanobacterium**

379 Analysis of the protein-coding genes of the *Ca. Sivonienia alaskensis* MAGs revealed many  
380 similarities with other Gloeobacterales, thus supporting their phylogenetic placement within  
381 this order of early branching Cyanobacteria (Fig. 4ab). For instance, strains of *Gloeobacter* spp.  
382 and *A. panamensis* differ notoriously from other Cyanobacteria by the lack of thylakoid  
383 membranes and the presence of a reduced photosynthetic apparatus [98–101]. These traits are  
384 considered ancestral features of oxygenic photosynthesis given the basal position of  
385 Gloeobacterales in the evolution of Cyanobacteria and plastids [92–94]. Like the genomes of  
386 other Gloeobacterales [39, 101–104], the *Ca. Sivonienia alaskensis* MAGs lack the genes for

387 several subunits of the photosystems I (PsaI, PsaJ, PsaK, and PsaX) and II (PsbY, PsbZ, and  
388 Psb27), the circadian clock (KaiA, KaiB, and KaiC), and the thylakoid membrane (SqdB and  
389 SqdX) (**Fig. 3, Table S5**). Moreover, similarly to *A. panamensis* and *Ca. Aurora vandensis* but  
390 unlike *Gloeobacter* spp. [101], *Ca. Sivonienia alaskensis* lacks two subunits of the photosystem  
391 II (PsbM and PsbU) and the cytochrome *b<sub>6</sub>f* complex (PetM and PetN), and does not contain any  
392 gene involved in the synthesis of phycoerythrin (Pec). Overall, the *in silico* analysis of the  
393 proteome of *Ca. Sivonienia alaskensis* suggests that this lineage comprises organisms without  
394 thylakoid membranes and with a reduced photosynthetic machinery, both of which are the  
395 defining characteristics of the order Gloeobacterales [62]. Moreover, the predicted structure of  
396 the photosystem II, cytochrome *b<sub>6</sub>f*, and phycobilisome machineries of *Ca. Sivonienia alaskensis*  
397 holds more similarities with *A. panamensis* and *Ca. Aurora vandensis* than with *Gloeobacter*  
398 spp., supporting the evolutionary relationship inferred from the analysis of ribosomal proteins  
399 and the 16S rRNA gene (**Fig. 4ab**). Whether the unique characteristics of *Gloeobacter* spp.  
400 reflect the ancestral state of the phylum Cyanobacteria has been an open question in the study  
401 of the early evolution of this group for many decades. The discovery of *Ca. Sivonienia alaskensis*,  
402 *A. panamensis* [101], and *Ca. Aurora vandensis* [40] suggests that characteristics such as the  
403 lack of thylakoid membranes and a reduced photosynthetic apparatus are indeed a defining  
404 characteristic of the early branching Gloeobacterales.

#### 405 ***Ca. Sivonienia alaskensis* is predominantly distributed in the cold biosphere**

406 Read recruitment analysis revealed that the two *Ca. Sivonienia alaskensis* MAGs are found in  
407 four microbial mat samples from the sub-Antarctic and Antarctic Peninsula, where they  
408 constitute up to 1.0% of the metagenomes (**Table S4**). To gain further insights into the ecology  
409 of *Ca. Sivonienia alaskensis*, we used *sourmash branchwater* [69, 70] to search metagenomic  
410 datasets in SRA for sequences matching the *Ca. Sivonienia alaskensis* MAGs. We also searched  
411 its 16S rRNA gene in amplicon sequencing datasets in SRA using IMNGS [71]. This extensive  
412 search, which included collectively *ca.* 1.3 million public datasets from around the globe,  
413 revealed sequences related to *Ca. Sivonienia alaskensis* in lakes, sediments, and soils mainly in  
414 polar, sub-polar, and alpine environments (**Fig. 4c**). Sequences matching the *Ca. Sivonienia*  
415 *alaskensis* MAGs were particularly abundant (0.4–8.0%) in amplicon sequencing datasets of  
416 active communities (*i.e.* derived from RNA molecules) in the sediment of thermokarst lakes near  
417 Barrow (Alaska) [105], as well as in metagenomic datasets from the sediment of Lake Hill (St.  
418 Paul Island, Alaska) [106] (0.8–3.1% of the reads). Interestingly, sequences matching the 16S  
419 rRNA gene of *Ca. Sivonienia alaskensis* were found in several datasets obtained from the gut  
420 microbiome of stickleback fishes (Actinopterygii: Gasterosteidae) and mayflies (Insecta:  
421 Ephemeroptera) [107–109].

422 Despite their importance for the study of the evolution of oxygenic photosynthesis, little is  
423 known about the ecology of the early branching Gloeobacterales compared to the other  
424 Cyanobacteria [83, 92, 101, 110]. *Gloeobacter*, which was for many decades the only described  
425 genus in this order, is typically found in low light, wet rock habitats [98, 111, 112]. Amplicon  
426 sequencing studies have also reported 16S rRNA gene sequences loosely related to *Gloeobacter*  
427 spp. in Arctic [17, 21] and temperate [113] soil crusts, and in Arctic [78, 114] and Antarctic [115]  
428 microbial mats. The phylogenetic and ecological range of the order Gloeobacterales has  
429 expanded recently with the discovery of *Ca. Aurora vandensis* from Antarctic lakes [40], *A.*  
430 *panamensis* associated with a tropical bryophyte [101], and five MAGs from different  
431 ecosystems including the *Ca. Sivonienia alaskensis* MAG ‘IMG\_3300025548\_6’ recovered from  
432 Arctic peat soil [39]. Apart from *A. panamensis*, Gloeobacterales appear to show a preference  
433 for low light and cold environments. This has been linked to their slow growth which, in turn,  
434 appears to be a consequence of their reduced photosynthetic apparatus [101]. In agreement with  
435 this, our results suggest that *Ca. Sivonienia alaskensis* is predominant in cold regions, especially  
436 polar and alpine lakes and sediments (**Fig. 4c**). Its high abundance in an RNA-derived amplicon  
437 sequencing dataset of lake sediments in Alaska [105] suggests that *Ca. Sivonienia alaskensis*  
438 forms active populations in this habitat. By contrast, the detection of sequences matching the  
439 16S rRNA gene of *Ca. Sivonienia alaskensis* in the microbiome of stickleback fishes does not  
440 entail that they are active members of gut communities. These sequences likely represent cells  
441 that were ingested either incidentally or collaterally via zooplankton that is consumed by the  
442 fish (Daniel Bolnick, personal communication).

#### 443 **Analysis of resistance mechanisms to environmental stress in *Ca. Sivonienia*** 444 ***alaskensis***

445 To obtain insights regarding the distribution of *Ca. Sivonienia alaskensis* across the cold  
446 biosphere, we searched the MAGs for genes involved in resistance mechanisms to  
447 environmental stress. We found 75 genes related to mechanisms to cope with desiccation, cold,  
448 and ultraviolet radiation (UVR) stresses in at least one of the *Ca. Sivonienia alaskensis* MAGs  
449 (**Table S6**). Among these are genes involved in the Wzy- and ABC transporter-dependent  
450 pathways for the assembly and export of extracellular polymeric substances (EPS). The  
451 production of an EPS matrix is a mechanism that is commonly employed by Cyanobacteria to  
452 cope with desiccation and freezing [116]. Genes involved in the synthase-dependent pathway of  
453 EPS production were not found. Scytonemin and mycosporine-like amino acids (MAAs) are often  
454 produced by Cyanobacteria as UVR-screening compounds [117]. Despite having several of the  
455 genes involved in the production of scytonemin and MAAs, the genes encoding the key proteins  
456 ScyC, ScyD, EboA, EboB, EboC, and MysC were not found. As such, the production of these



457 compounds by *Ca. Sivonenia alaskensis* is unlikely. We identified several mechanisms of  
458 resistance to cold in the *Ca. Sivonenia alaskensis* MAGs, including proteins involved in the  
459 regulation of the cell membrane fluidity, regulation of replication and translation, and RNA  
460 metabolism (**Table S6**). Finally, mechanisms of DNA repair include the base excision repair  
461 pathway for several glycosylases, the homologous recombination pathway for single-stranded  
462 breaks, and one of the subtypes of the nuclear excision repair pathway.

## 463 **Conclusion**

464 We investigated 17 polar microbial mat metagenomes and recovered 37 MAGs of Cyanobacteria  
465 representing different levels of phylogenetic novelty: around half of the MAGs are very distant  
466 (<80% ANI) to genomes currently available in GenBank; the other half are related to polar and  
467 alpine strains with varying levels of genome similarity (80.1–99.8% ANI). Among the latter, we  
468 describe the phylogenetic, metabolic potential, and ecological characteristics of a lineage in the  
469 early branching Gloeobacterales. *In silico* analyses indicate that this lineage – which we name  
470 *Ca. Sivonenia alaskensis* – is a thylakoid-less cyanobacterium that is mostly found in cold  
471 environments and harbours common mechanisms of resistance to environmental stress. Our  
472 study shows that genome-resolved metagenomics is a reliable and straightforward way of  
473 recovering novel genomes of Cyanobacteria without the need for strain isolation. However,  
474 strain isolation is still useful for many purposes and may in fact benefit from genomic  
475 information obtained from MAGs to design protocols for targeted isolation. Based on the ANI  
476 thresholds commonly used for delineating microbial species [95, 96], most of the MAGs obtained  
477 represent different species or even genera from the ones currently represented by genomes in  
478 GenBank. Comparison with strains without genome data was not possible as only one of the 37  
479 MAGs included the 16S rRNA gene, which is the most widely used molecular marker for the  
480 taxonomy of Cyanobacteria [27]. The use of long read technologies (*e.g.* Oxford Nanopore and  
481 PacBio SMRT sequencing) could help alleviate this issue. Altogether, our study highlights the  
482 uniqueness of polar microbiomes and their specialized communities of Cyanobacteria, of which  
483 a large fraction is yet to be characterized.

## 484 **Author statements**

### 485 **Author contributions**

486 ISP, YL, EV, and AW conceived the experiments. ISP performed most of the analysis, and RVP,  
487 VS, and BRR contributed with minor parts. ISP and RVP wrote the manuscript. All authors  
488 provided important feedback, helped shape the study, and contributed to the writing of the  
489 manuscript.

## 490 **Conflicts of interest**

491 The authors declare that there are no conflicts of interest.

## 492 **Funding information**

493 This work was supported by the Belgian Federal Science Policy Office (BELSPO) (projects  
494 AMBIO – SD/BA/01A and CCAMBIO – SD/BA/03A), the Belgian National Fund for Scientific  
495 Research (FRS-FNRS) (grants 2.4570.09 and CR.CH.10-11-1.5139.11), and the EU-Interact  
496 project MiBiPol. ISP and JH were supported by the Academy of Finland grant 1314114, RVP  
497 by the Doctoral Program in Microbiology and Biotechnology (University of Helsinki), BD and  
498 VS by the FRS-FNRS, BRR by the Special Funds for Research (University of Liège), the IPD-  
499 STEMA Programme, and the Junta de Andalucía (PAIDI-DOCTOR 21\_00571), and AW is  
500 Senior Research Associate of the FRS-FNRS.

## 501 **Acknowledgments**

502 The authors would like to acknowledge Sofie D’Hondt (UGent) and Bjorn Tytgat for help with  
503 DNA extraction and library preparation, the IT Centre for Science – CSC (Finland) for providing  
504 the computational resources used in the study, and Kaarina Sivonen, Daniel Bolnick, Danillo  
505 Alvarenga, and Tânia Shishido for comments. We also thank Dominic A. Hodgson, Steve J.  
506 Roberts, Wim Van Nieuwenhuyze, Koen Sabbe, Dagmar Obbels, Otakar Strunecký, Kate  
507 Kopalová, Jan Kavan, Josef Elster, Pieter Vanormelingen, and Eveline Pinseel for help during  
508 sampling campaigns or/and sharing samples.

## 509 **References**

- 510 1. **Golubic S.** Modern stromatolites: a review. In: Riding R (editor). *Calcareous Algae and*  
511 *Stromatolites*. Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 541–561.
- 512 2. **Stal LJ.** Cyanobacterial mats and stromatolites. In: Whitton BA (editor). *Ecology of*  
513 *Cyanobacteria II*. Dordrecht: Springer Netherlands. pp. 65–125.
- 514 3. **Bolhuis H, Cretoiu MS, Stal LJ.** Molecular ecology of microbial mats. *FEMS Microbiol*  
515 *Ecol* 2014;90:335–350.
- 516 4. **Singh SM, Elster J.** Cyanobacteria in Antarctic lake environments: a mini-review. In:  
517 Seckbach J (editor). *Algae and Cyanobacteria in Extreme Environments*. Dordrecht:  
518 Springer Netherlands. pp. 303–320.
- 519 5. **Vincent WF, Quesada A.** Cyanobacteria in high latitude lakes, rivers and seas. In:  
520 Whitton BA (editor). *Ecology of Cyanobacteria II*. Dordrecht: Springer Netherlands. pp.  
521 371–385.

- 522 6. **Quesada A, Vincent WF**. Cyanobacteria in the cryosphere: snow, ice and extreme cold.  
523 In: Whitton BA (editor). *Ecology of Cyanobacteria II*. Dordrecht: Springer Netherlands. pp.  
524 387–399.
- 525 7. **Van Goethem MW, Cowan DA**. Role of Cyanobacteria in the ecology of polar  
526 environments. In: Castro-Sowinski S (editor). *The Ecological Role of Micro-organisms in*  
527 *the Antarctic Environment*. Cham: Springer International Publishing. pp. 3–23.
- 528 8. **Christmas NAM, Anesio AM, Sánchez-Baracaldo P**. The future of genomics in polar  
529 and alpine cyanobacteria. *FEMS Microbiol Ecol* 2018;94:fiy032.
- 530 9. **Vincent WF, Downes MT, Castenholz RW, Howard-Williams C**. Community  
531 structure and pigment organisation of cyanobacteria-dominated microbial mats in  
532 Antarctica. *Eur J Phycol* 1993;28:213–221.
- 533 10. **Elster J, Komarek O**. Ecology of periphyton in a meltwater stream ecosystem in the  
534 maritime Antarctic. *Antarct Sci* 2003;15:189–201.
- 535 11. **Elster J, Lukesová A, Svoboda J, Kopecky J, Kanda H**. Diversity and abundance of  
536 soil algae in the polar desert, Sverdrup Pass, central Ellesmere Island. *Polar Rec*  
537 1999;35:231–254.
- 538 12. **Taton A, Grubisic S, Ertz D, Hodgson DA, Piccardi R, et al**. Polyphasic study of  
539 Antarctic cyanobacterial strains. *J Phycol* 2006;42:1257–1270.
- 540 13. **Palinska KA, Schneider T, Surosz W**. Phenotypic and phylogenetic studies of benthic  
541 mat-forming cyanobacteria on the NW Svalbard. *Polar Biol* 2017;40:1607–1616.
- 542 14. **Strunecky O, Raabova L, Bernardova A, Ivanova AP, Semanova A, et al**. Diversity  
543 of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella*  
544 and *Shackletoniella*. *FEMS Microbiol Ecol* 2020;96:fiz189.
- 545 15. **Taton A, Hoffmann L, Wilmotte A**. Cyanobacteria in microbial mats of Antarctic lakes  
546 (East Antarctica): a microscopical approach. *Algol Stud* 2008;126:173–208.
- 547 16. **Namsaraev Z, Mano M-J, Fernandez R, Wilmotte A**. Biogeography of terrestrial  
548 cyanobacteria from Antarctic ice-free areas. *Ann Glaciol* 2010;51:171–177.
- 549 17. **Pushkareva E, Pessi IS, Wilmotte A, Elster J**. Cyanobacterial community composition  
550 in Arctic soil crusts at different stages of development. *FEMS Microbiol Ecol*  
551 2015;91:fiv143.

- 552 18. **Pushkareva E, Pessi IS, Namsaraev Z, Mano M-J, Elster J, et al.** Cyanobacteria  
553 inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica. *Syst*  
554 *Appl Microbiol* 2018;41:363–373.
- 555 19. **Pessi IS, Maalouf PDC, Laughinghouse HD, Baurain D, Wilmotte A.** On the use of  
556 high-throughput sequencing for the study of cyanobacterial diversity in Antarctic aquatic  
557 mats. *J Phycol* 2016;52:356–368.
- 558 20. **Pessi IS, Lara Y, Durieu B, Maalouf P de C, Verleyen E, et al.** Community structure  
559 and distribution of benthic cyanobacteria in Antarctic lacustrine microbial mats. *FEMS*  
560 *Microbiol Ecol* 2018;94:fiy042.
- 561 21. **Pessi IS, Pushkareva E, Lara Y, Borderie F, Wilmotte A, et al.** Marked succession of  
562 cyanobacterial communities following glacier retreat in the High Arctic. *Microb Ecol*  
563 2019;77:136–147.
- 564 22. **Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A.** Cyanobacterial diversity in  
565 natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica):  
566 a morphological and molecular approach. *Appl Environ Microbiol* 2003;69:5157–5169.
- 567 23. **Taton A, Grubisic S, Balthasart P, Hodgson DA, Laybourn-Parry J, et al.**  
568 Biogeographical distribution and ecological ranges of benthic cyanobacteria in East  
569 Antarctic lakes. *FEMS Microbiol Ecol* 2006;57:272–289.
- 570 24. **Fernandez-Carazo R, Hodgson DA, Convey P, Wilmotte A.** Low cyanobacterial  
571 diversity in biotopes of the Transantarctic Mountains and Shackleton Range (80–82°S),  
572 Antarctica. *FEMS Microbiol Ecol* 2011;77:503–517.
- 573 25. **Wilmotte A, Golubić S.** Morphological and genetic criteria in the taxonomy of  
574 Cyanophyta/Cyanobacteria. *Algol Stud* 1991;64:1–24.
- 575 26. **Shih PM, Wu D, Latifi A, Axen SD, Fewer DP, et al.** Improving the coverage of the  
576 cyanobacterial phylum using diversity-driven genome sequencing. *Proc Natl Acad Sci*  
577 2013;110:1053–1058.
- 578 27. **Mareš J.** Multilocus and SSU rRNA gene phylogenetic analyses of available  
579 cyanobacterial genomes, and their relation to the current taxonomic system. *Hydrobiologia*  
580 2018;811:19–34.
- 581 28. **Christmas NAM, Barker G, Anesio AM, Sánchez-Baracaldo P.** Genomic mechanisms  
582 for cold tolerance and production of exopolysaccharides in the Arctic cyanobacterium  
583 *Phormidismis priestleyi* BC1401. *BMC Genomics* 2016;17:533.

- 584 29. **Péquin B, Tremblay J, Maynard C, Wasserscheid J, Greer CW.** Draft whole-genome  
585 sequences of the polar cyanobacterium *Leptolyngbya* sp. strain Cla-17 and its associated  
586 flavobacterium. *Microbiol Resour Announc* 2022;11:e00059-22.
- 587 30. **Lara Y, Durieu B, Cornet L, Verlaine O, Rippka R, et al.** Draft genome sequence of  
588 the axenic strain *Phormidesmis priestleyi* ULC007, a cyanobacterium isolated from Lake  
589 Bruehwiler (Larsemann Hills, Antarctica). *Genome Announc* 2017;5:e01546-16.
- 590 31. **Christmas NAM, Williamson CJ, Yallop ML, Anesio AM, Sánchez-Baracaldo P.**  
591 Photoecology of the Antarctic cyanobacterium *Leptolyngbya* sp. BC1307 brought to light  
592 through community analysis, comparative genomics and in vitro photophysiology. *Mol Ecol*  
593 2018;27:5279–5293.
- 594 32. **Tang J, Du L-M, Liang Y-M, Daroch M.** Complete genome sequence and comparative  
595 analysis of *Synechococcus* sp. CS-601 (SynAce01), a cold-adapted cyanobacterium from an  
596 oligotrophic Antarctic habitat. *Int J Mol Sci* 2019;20:152.
- 597 33. **Effendi DB, Sakamoto T, Ohtani S, Awai K, Kanasaki Y.** Possible involvement of  
598 extracellular polymeric substrates of Antarctic cyanobacterium *Nostoc* sp. strain SO-36 in  
599 adaptation to harsh environments. *J Plant Res* 2022;135:771–784.
- 600 34. **Cornet L, Bertrand AR, Hanikenne M, Javaux EJ, Wilmotte A, et al.** Metagenomic  
601 assembly of new (sub)polar Cyanobacteria and their associated microbiome from non-  
602 axenic cultures. *Microb Genomics* 2018;4:212.
- 603 35. **Chen L-X, Anantharaman K, Shaiber A, Eren AM, Banfield JF.** Accurate and  
604 complete genomes from metagenomes. *Genome Res* 2020;30:315–333.
- 605 36. **Delmont TO.** Discovery of nondiazotrophic *Trichodesmium* species abundant and  
606 widespread in the open ocean. *Proc Natl Acad Sci* 2021;118:e2112355118.
- 607 37. **Pessi IS, Viitamäki S, Virkkala A-M, Eronen-Rasimus E, Delmont TO, et al.** In-  
608 depth characterization of denitrifier communities across different soil ecosystems in the  
609 tundra. *Environ Microbiome* 2022;17:30.
- 610 38. **Pessi IS, Rutanen A, Hultman J.** *Candidatus Nitrosopolaris*, a genus of putative  
611 ammonia-oxidizing archaea with a polar/alpine distribution. *FEMS Microbes*  
612 2022;3:xtac019.
- 613 39. **Grettenberger CL.** Novel *Gloeobacterales* spp. from diverse environments across the  
614 globe. *mSphere* 2021;6:e00061-21.

- 615 40. **Grettenberger CL, Sumner DY, Wall K, Brown CT, Eisen JA, et al.** A  
616 phylogenetically novel cyanobacterium most closely related to *Gloeobacter*. *ISME J*  
617 2020;14:2142–2152.
- 618 41. **Lumian JE, Jungblut AD, Dillion ML, Hawes I, Doran PT, et al.** Metabolic capacity  
619 of the Antarctic cyanobacterium *Phormidium pseudopriestleyi* that sustains oxygenic  
620 photosynthesis in the presence of hydrogen sulfide. *Genes* 2021;12:426.
- 621 42. **Terauds A, Lee JR.** Antarctic biogeography revisited: updating the Antarctic  
622 Conservation Biogeographic Regions. *Divers Distrib* 2016;22:836–840.
- 623 43. **Ewels P, Magnusson M, Lundin S, Källner M.** MultiQC: summarize analysis results for  
624 multiple tools and samples in a single report. *Bioinformatics* 2016;32:3047–3048.
- 625 44. **Martin M.** Cutadapt removes adapter sequences from high-throughput sequencing reads.  
626 *EMBnet.journal* 2011;17:10.
- 627 45. **Bengtsson-Palme J, Hartmann M, Eriksson KM, Pal C, Thorell K, et al.** METAXA2:  
628 improved identification and taxonomic classification of small and large subunit rRNA in  
629 metagenomic data. *Mol Ecol Resour* 2015;15:1403–1414.
- 630 46. **Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, et al.** Introducing mothur:  
631 open-source, platform-independent, community-supported software for describing and  
632 comparing microbial communities. *Appl Environ Microbiol* 2009;75:7537–7541.
- 633 47. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, et al.** The SILVA ribosomal  
634 RNA gene database project: improved data processing and web-based tools. *Nucleic Acids*  
635 *Res* 2013;41:D590–D596.
- 636 48. **Wang Q, Garrity GM, Tiedje JM, Cole JR.** Naïve Bayesian classifier for rapid  
637 assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*  
638 2007;73:5261–5267.
- 639 49. **Li D, Liu C-M, Luo R, Sadakane K, Lam T-W.** MEGAHIT: an ultra-fast single-node  
640 solution for large and complex metagenomics assembly via succinct de Bruijn graph.  
641 *Bioinformatics* 2015;31:1674–1676.
- 642 50. **Eren AM, Kiefl E, Shaiber A, Veseli I, Miller SE, et al.** Community-led, integrated,  
643 reproducible multi-omics with anvi'o. *Nat Microbiol* 2021;6:3–6.
- 644 51. **Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, et al.** Prodigal: prokaryotic  
645 gene recognition and translation initiation site identification. *BMC Bioinformatics*  
646 2010;11:119.

- 647 52. **Eddy SR.** Accelerated profile HMM searches. *PLoS Comput Biol* 2011;7:e1002195.
- 648 53. **Lee MD.** GToTree: a user-friendly workflow for phylogenomics. *Bioinformatics*  
649 2019;35:4162–4164.
- 650 54. **Buchfink B, Xie C, Huson DH.** Fast and sensitive protein alignment using DIAMOND.  
651 *Nat Methods* 2015;12:59–60.
- 652 55. **Parks DH, Chuvochina M, Rinke C, Mussig AJ, Chaumeil P-A, et al.** GTDB: an  
653 ongoing census of bacterial and archaeal diversity through a phylogenetically consistent,  
654 rank normalized and complete genome-based taxonomy. *Nucleic Acids Res* 2022;50:D785–  
655 D794.
- 656 56. **Langmead B, Salzberg SL.** Fast gapped-read alignment with Bowtie 2. *Nat Methods*  
657 2012;9:357–359.
- 658 57. **Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, et al.** The Sequence  
659 Alignment/Map format and SAMtools. *Bioinformatics* 2009;25:2078–2079.
- 660 58. **Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH.** GTDB-Tk: a toolkit to classify  
661 genomes with the Genome Taxonomy Database. *Bioinformatics* 2020;36:1925–1927.
- 662 59. **Edgar RC.** MUSCLE: multiple sequence alignment with high accuracy and high  
663 throughput. *Nucleic Acids Res* 2004;32:1792–1797.
- 664 60. **Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ.** IQ-TREE: a fast and effective  
665 stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*  
666 2015;32:268–274.
- 667 61. **Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S.** High  
668 throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat*  
669 *Commun* 2018;9:5114.
- 670 62. **Komárek J, Kaštovský J, Mareš J, Johansen JR.** Taxonomic classification of  
671 cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. *Preslia*  
672 2014;86:295–335.
- 673 63. **Aramaki T, Blanc-Mathieu R, Endo H, Ohkubo K, Kanehisa M, et al.** KofamKOALA:  
674 KEGG ortholog assignment based on profile HMM and adaptive score threshold.  
675 *Bioinformatics* 2020;36:2251–2252.
- 676 64. **Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, et al.** Pfam: the  
677 protein families database in 2021. *Nucleic Acids Res* 2021;49:D412–D419.

- 678 65. **Galperin MY, Wolf YI, Makarova KS, Vera Alvarez R, Landsman D, et al.** COG  
679 database update: focus on microbial diversity, model organisms, and widespread  
680 pathogens. *Nucleic Acids Res* 2021;49:D274–D281.
- 681 66. **Pearson WR.** An introduction to sequence similarity (“homology”) searching. *Curr Protoc*  
682 *Bioinforma* 2013;42:3.1.1-3.1.8.
- 683 67. **Olm MR, Brown CT, Brooks B, Banfield JF.** dRep: a tool for fast and accurate genomic  
684 comparisons that enables improved genome recovery from metagenomes through de-  
685 replication. *ISME J* 2017;11:2864–2868.
- 686 68. **Li H.** Minimap and miniasm: fast mapping and de novo assembly for noisy long sequences.  
687 *Bioinformatics* 2016;32:2103–2110.
- 688 69. **Irber L, Pierce-Ward NT, Brown CT.** *Sourmash branchwater enables lightweight*  
689 *petabyte-scale sequence search.* Preprint (BioRxiv). Epub ahead of print 3 November 2022.  
690 DOI: 10.1101/2022.11.02.514947.
- 691 70. **Lumian J, Sumner D, Grettenberger C, Jungblut AD, Irber L, et al.** *Biogeographic*  
692 *distribution of five Antarctic cyanobacteria using large-scale k-mer searching with*  
693 *sourmash branchwater.* Preprint (BioRxiv). Epub ahead of print 30 October 2022. DOI:  
694 10.1101/2022.10.27.514113.
- 695 71. **Lagkouvardos I, Joseph D, Kapfhammer M, Giritli S, Horn M, et al.** IMNGS: a  
696 comprehensive open resource of processed 16S rRNA microbial profiles for ecology and  
697 diversity studies. *Sci Rep* 2016;6:33721.
- 698 72. **Zaikova E, Goerlitz DS, Tighe SW, Wagner NY, Bai Y, et al.** Antarctic relic microbial  
699 mat community revealed by metagenomics and metatranscriptomics. *Front Ecol Evol*  
700 2019;7:1.
- 701 73. **Slattery M, Lesser MP.** Allelopathy-mediated competition in microbial mats from  
702 Antarctic lakes. *FEMS Microbiol Ecol* 2017;93:fix019.
- 703 74. **Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J.** Metagenomic analysis of  
704 stress genes in microbial mat communities from Antarctica and the High Arctic. *Appl*  
705 *Environ Microbiol* 2012;78:549–559.
- 706 75. **Soo RM, Skennerton CT, Sekiguchi Y, Imelfort M, Paech SJ, et al.** An expanded  
707 genomic representation of the phylum Cyanobacteria. *Genome Biol Evol* 2014;6:1031–  
708 1045.



- 709 76. **Komárek J, Genuário DB, Fiore MF, Elster J.** Heterocytous cyanobacteria of the Ulu  
710 Peninsula, James Ross Island, Antarctica. *Polar Biol* 2015;38:475–492.
- 711 77. **Christmas NAM, Anesio AM, Sánchez-Baracaldo P.** Multiple adaptations to polar and  
712 alpine environments within cyanobacteria: a phylogenomic and Bayesian approach. *Front*  
713 *Microbiol* 2019;10:1070.
- 714 78. **Jungblut AD, Lovejoy C, Vincent WF.** Global distribution of cyanobacterial ecotypes in  
715 the cold biosphere. *ISME J* 2010;4:191–202.
- 716 79. **Velichko N, Smirnova S, Averina S, Pinevich A.** A survey of Antarctic cyanobacteria.  
717 *Hydrobiologia* 2021;848:2627–2652.
- 718 80. **Davydov D.** Cyanobacterial diversity of Svalbard Archipelago. *Polar Biol* 2021;44:1967–  
719 1978.
- 720 81. **Olson JB, Steppe TF, Litaker RW, Paerl HW.** N<sub>2</sub>-fixing microbial consortia associated  
721 with the ice cover of Lake Bonney, Antarctica. *Microb Ecol* 1998;36:231–238.
- 722 82. **Su H-N, Wang Q-M, Li C-Y, Li K, Luo W, et al.** Structural insights into the cold  
723 adaptation of the photosynthetic pigment-protein C-phycoyanin from an Arctic  
724 cyanobacterium. *Biochim Biophys Acta BBA - Bioenerg* 2017;1858:325–335.
- 725 83. **Moore KR, Magnabosco C, Momper L, Gold DA, Bosak T, et al.** An expanded  
726 ribosomal phylogeny of Cyanobacteria supports a deep placement of plastids. *Front*  
727 *Microbiol* 2019;10:1612.
- 728 84. **Ishida T, Yokota A, Sugiyama J.** Phylogenetic relationships of filamentous  
729 cyanobacterial taxa inferred from 16S rRNA sequence divergence. *J Gen Appl Microbiol*  
730 1997;43:237–241.
- 731 85. **Honda D, Yokota A, Sugiyama J.** Detection of seven major evolutionary lineages in  
732 cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five  
733 marine *Synechococcus* strains. *J Mol Evol* 1999;48:723–739.
- 734 86. **Ishida T, Watanabe MM, Sugiyama J, Yokota A.** Evidence for polyphyletic origin of  
735 the members of the orders of Oscillatoriales and Pleurocapsales as determined by 16S  
736 rDNA analysis. *FEMS Microbiol Lett* 2001;201:79–82.
- 737 87. **Chen M-Y, Teng W-K, Zhao L, Hu C-X, Zhou Y-K, et al.** Comparative genomics reveals  
738 insights into cyanobacterial evolution and habitat adaptation. *ISME J* 2021;15:211–227.

- 739 88. **de Winder B, Stal LJ, Mur LR.** *Crinalium epipsammum* sp. nov.: a filamentous  
740 cyanobacterium with trichomes composed of elliptical cells and containing poly- $\beta$ -(1,4)  
741 glucar (cellulose). *J Gen Microbiol* 1990;136:1645–1653.
- 742 89. **Bohunická M, Mareš J, Hrouzek P, Urajová P, Lukeš M, et al.** A combined  
743 morphological, ultrastructural, molecular, and biochemical study of the peculiar family  
744 Gomontiellaceae (Oscillatoriales) reveals a new cylindrospermopsin-producing clade of  
745 cyanobacteria. *J Phycol* 2015;51:1040–1054.
- 746 90. **Broady PA, Kibblewhite AL.** Morphological characterization of Oscillatoriales  
747 (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. *Antarct Sci*  
748 1991;3:35–45.
- 749 91. **Komárek J.** Phenotypic and ecological diversity of freshwater coccoid cyanobacteria from  
750 maritime Antarctica and Islands of NW Weddell Sea. II. *Czech Polar Rep* 2014;4:17–39.
- 751 92. **Demoulin CF, Lara YJ, Cornet L, François C, Baurain D, et al.** Cyanobacteria  
752 evolution: insight from the fossil record. *Free Radic Biol Med* 2019;140:206–223.
- 753 93. **Seo P-S, Yokota A.** The phylogenetic relationships of cyanobacteria inferred from 16S  
754 rRNA, *gyrB*, *rpoC1* and *rpoD1* gene sequences. *J Gen Appl Microbiol* 2003;49:191–203.
- 755 94. **Gupta RS, Mathews DW.** Signature proteins for the major clades of Cyanobacteria. *BMC*  
756 *Evol Biol* 2010;10:24.
- 757 95. **Konstantinidis KT, Rosselló-Móra R, Amann R.** Uncultivated microbes in need of  
758 their own taxonomy. *ISME J* 2017;11:2399–2406.
- 759 96. **Ciufo S, Kannan S, Sharma S, Badretdin A, Clark K, et al.** Using average nucleotide  
760 identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. *Int J Syst*  
761 *Evol Microbiol* 2018;68:2386–2392.
- 762 97. **Hedlund BP, Chuvochina M, Hugenholtz P, Konstantinidis KT, Murray AE, et al.**  
763 SeqCode: a nomenclatural code for prokaryotes described from sequence data. *Nat*  
764 *Microbiol* 2022;7:1702–1708.
- 765 98. **Rippka R, Waterbury J, Cohen-Bazire G.** A cyanobacterium which lacks thylakoids.  
766 *Arch Microbiol* 1974;100:419–436.
- 767 99. **Guglielmi G, Cohen-Bazire G, Bryant DA.** The structure of *Gloeobacter violaceus* and  
768 its phycobilisomes. *Arch Microbiol* 1981;129:181–189.
- 769 100. **Bryant DA, Cohen-Bazire G, Glazer AN.** Characterization of the biliproteins of  
770 *Gloeobacter violaceus*. *Arch Microbiol* 1981;129:190–198.

- 771 101. **Rahmatpour N, Hauser DA, Nelson JM, Chen PY, Villarreal A. JC, et al.** A novel  
772 thylakoid-less isolate fills a billion-year gap in the evolution of Cyanobacteria. *Curr Biol*  
773 2021;31:2857–2867.
- 774 102. **Nakamura Y.** Complete genome structure of *Gloeobacter violaceus* PCC 7421, a  
775 cyanobacterium that lacks thylakoids. *DNA Res* 2003;10:137–145.
- 776 103. **Saw JH, Cardona T, Montejano G.** Complete genome sequencing of a novel *Gloeobacter*  
777 species from a waterfall cave in Mexico. *Genome Biol Evol* 2021;13:evab264.
- 778 104. **Saw JHW, Schatz M, Brown MV, Kunkel DD, Foster JS, et al.** Cultivation and  
779 complete genome sequencing of *Gloeobacter kilaueensis* sp. nov., from a lava cave in  
780 Kīlauea Caldera, Hawai‘i. *PLoS ONE* 2013;8:e76376.
- 781 105. **Matheus Carnevali PB, Herbold CW, Hand KP, Priscu JC, Murray AE.** Distinct  
782 microbial assemblage structure and archaeal diversity in sediments of Arctic thermokarst  
783 lakes differing in methane sources. *Front Microbiol* 2018;9:1192.
- 784 106. **Graham RW, Belmecheri S, Choy K, Culleton BJ, Davies LJ, et al.** Timing and  
785 causes of mid-Holocene mammoth extinction on St. Paul Island, Alaska. *Proc Natl Acad*  
786 *Sci* 2016;113:9310–9314.
- 787 107. **Bolnick DI, Snowberg LK, Caporaso JG, Lauber C, Knight R, et al.** Major  
788 Histocompatibility Complex class IIb polymorphism influences gut microbiota composition  
789 and diversity. *Mol Ecol* 2014;23:4831–4845.
- 790 108. **Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Knight R, et al.** Individuals’ diet  
791 diversity influences gut microbial diversity in two freshwater fish (threespine stickleback  
792 and Eurasian perch). *Ecol Lett* 2014;17:979–987.
- 793 109. **Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, et al.** Individual diet has  
794 sex-dependent effects on vertebrate gut microbiota. *Nat Commun* 2014;5:4500.
- 795 110. **Delwiche CF.** Microbial biodiversity: a newly isolated cyanobacterium sheds light on the  
796 evolution of photosynthesis. *Curr Biol* 2021;31:R843–R845.
- 797 111. **Mareš J, Hrouzek P, Kaňa R, Ventura S, Strunecký O, et al.** The primitive thylakoid-  
798 less cyanobacterium *Gloeobacter* is a common rock-dwelling organism. *PLoS ONE*  
799 2013;8:e66323.
- 800 112. **Golubic S, Campbell SE.** Analogous microbial forms in recent subaerial habitats and in  
801 Precambrian cherts: *Gloethece coerulea* Geitler and *Eosynechococcus moorei* Hofmann.  
802 *Precambrian Res* 1979;8:201–217.

- 803 113. **Williams L, Loewen-Schneider K, Maier S, Büdel B.** Cyanobacterial diversity of  
804 western European biological soil crusts along a latitudinal gradient. *FEMS Microbiol Ecol*  
805 2016;92:fiw157.
- 806 114. **Lionard M, Péquin B, Lovejoy C, Vincent WF.** Benthic cyanobacterial mats in the  
807 High Arctic: multi-layer structure and fluorescence responses to osmotic stress. *Front*  
808 *Microbiol* 2012;3:140.
- 809 115. **Nakai R, Abe T, Baba T, Imura S, Kagoshima H, et al.** Microflorae of aquatic moss  
810 pillars in a freshwater lake, East Antarctica, based on fatty acid and 16S rRNA gene  
811 analyses. *Polar Biol* 2012;35:425–433.
- 812 116. **Pereira SB, Mota R, Vieira CP, Vieira J, Tamagnini P.** Phylum-wide analysis of  
813 genes/proteins related to the last steps of assembly and export of extracellular polymeric  
814 substances (EPS) in cyanobacteria. *Sci Rep* 2015;5:14835.
- 815 117. **Gao Q, Garcia-Pichel F.** Microbial ultraviolet sunscreens. *Nat Rev Microbiol* 2011;9:791–  
816 802.
- 817