Supplementary material

Using Captain Scott's Discovery specimens to unlock the past: Has Antarctic cyanobacterial diversity changed over the last 100 years?

Anne D. Jungblut, Ian Hawes

Supplementary material and methods

Study sites and samples

Cyanobacterial mat samples were studied from the Ross Island and McMurdo Ice Shelf in Southern Victoria Land Antarctica. Six samples were from the McMurdo Ice Shelf and one from close to winter quarters on Ross Island near Hut Point, currently the location of the historic Discovery Hut and the current US Antarctic McMurdo Station (Supplementary Table S1). McMurdo Ice Shelf samples were from an area approximately half way between Brown and Black Peninsulas (12). One sample was collected by Dr Wilson and the other likely by Dr Koettlitz (12). The dried, still green-pigmented cyanobacterial mat samples (Figure 1) are held in the Natural History Museum Botanical collections, London, UK. The mm to cm thick dried cyanobacterial mats were found preserved dry on paper herbarium sheets, in the dark, at room temperature. No records are available how the specimens were dried and kept during the first half of the century. However, based on expedition records (14), herbarium specimens were likely prepared by drying and pressing of material in the field directly after collection, by Dr R. Koettlitz.

The Scott's Discovery samples were compared with present-day cyanobacteria from microbial mats collected from freshwater meltwater ponds on the McMurdo Ice Self, Cape Royds and Cape Evans on Ross Island in January 2011, and Hut Point, Ross

Island near the Discovery Hut in January 2012 (Supplementary Figure S1 and Table S2). Samples from the McMurdo Ice Shelf were from near Bratina Island and half way between Brown Peninsula and Black Island. Sample sites were chosen to be the same geographic regions as described from the Discovery Expedition and listed as sample locations in the Terra Nova and Nimrod expedition, as well as to cover a range of water chemistries (Supplementary Table S2). Modern samples were collected in sterile plastic containers and frozen within 24h. Frozen samples were transported to the NHM and stored at -80°C until further use.

PCR, PCR-product purification, and pyrosequencing

Cyanobacterial 16S rRNA genes were amplified using Platinum High Fidelity Taq Polymerase (Invitrogen) in triplicate using cyanobacterial specific primers 16S378F (5' GGGGAATYTTCCGCAATGGG T ‘3) and 16S781R (5'GAC TAC WGG GGT ATC TAA TCC C W T T ‘3) modified from Taton et al (18) containing the linker A (Primer A: 5' – CGT ATC GCC TCC CTC GCG CCA TCAG-MID– template specific sequence – 3') and B (Primer B: 5' – CTA TGC GCC TTG CCA GCC CGC TCAGC – template specific sequence – 3') using the recommended Roche barcodes (MID1-8). These primers provide a broad coverage across the phylum cyanobacteria (18). Barcodes were designed to be on Primer A followed by the 16S781R. PCR-products were pooled per sample and purified using Qiagen gel-purification (Qiagen, Hilden, Germany) according to the manufacturer’s instructions, and quantified in duplicate by Qubit. Eight samples were multiplexed per Junior 454-pyrosequencing run (Roche) to generate approximately 450 bp long reads and was performed at the Biochemistry DNA Sequencing Facility, Department of Biochemistry, University of Cambridge, UK. We used cyanobacteria-specific protocols that allowed us to exclude

any other bacteria and microbial eukaryote contaminants from the century-long storage at the Natural History Museum.

Sequencing and statistical analysis

Sequences from the three runs were combined and operational taxonomic units (OTUs) generated using the standard operation protocol for 454 data sequencing by Qiime (19) Sequences were clustered in OTUs based on 99% sequence similarity using uclust for highest taxonomic resolution. OTUs were then classified to identify taxonomic annotation using the Greengenes taxonomic database (20), and chimeras removed using Chimera Slayer. Subsequently, sequences of unassigned, eukaryotic origin, non-cyanobacterial bacteria taxa, and Cyanobacteria sequences with assignment to only phyla level ("Cyanobacteria" phyla level) were removed from further analysis. The remaining OTUs were at least assigned to class level (Synechococcophycideae, Oscillatoriophycideae, Nostocophycideae, Gloeobacterphycideae). OTUs with abundance of less than 0.01% were filtered and removed from further analysis and samples were subsequently rarefied using 1267 sequences.

Phylogenetic analysis was performed with a representative sequence for each genotype (99% clustered) that was either one of the most abundant genotypes, or only present either in the old or new samples. In addition, at least one of the closest cultured and uncultured match based using a BLASTn search (5 March 2017, 22) to GenBank was included in the analysis. For comparison, a range of cultured and environmental cyanobacterial sequences were also included from the Antarctic, Arctic and other climate zones. Sequences were aligned using Clustal X (version 2.0.9) and

manual edited with Mesquite (3.04). Raw sequences are available at the National Centre for Biotechnology Information Sequence Read Archive under the accession number PRJNA323585.

Table S1. Location, description, date and accession numbers of cyanobacteria-based microbial mat samples collected during Captain Scott's Discovery expedition to Antarctica' * Ponds half-way between Black Island and Brown Peninsula as reported by Fritsch (*5*).

Sample name	Descriptions accompanying herbarium specimens	Location	Date	Catalogues number	Number of OTUs	Chao1 richness
SCT1	Portion of enormous freshwater algae found growing in through ice in pond among the eskers which border the shore of West Mainland*	McMurdo Ice Shelf	2 Dec. 1902	BM001062584	85	108.9
SCT 2	Fragment of large algae (freshwater) west eskers*	McMurdo Ice Shelf	2 Dec. 1902	BM001062585	111	167.4
SCT 3	Portion of enormous fresh water fungus found penetrating ice (by thaw) in pond anchoring esker of West mainland shore*	McMurdo Ice Shelf	2 Dec. 1902	BM001062586	105	153.2
SCT 4	Portion of enormous fresh water*	McMurdo Ice Shelf	2 Dec. 1902	BM001062587	84	107.9
SCT 5	Dried fragment of alga driven by wind upon lea-shore of freshwater pond on islet in "old ice" McMurdo strait, collected by S Wilson*	McMurdo Ice Shelf	Dec. 1903	BM001062588	93	147.7
SCT 6	From dry pond winter quarter	Ross Island	Feb. 1902	BM001062589	100	147.7
SCT 7	Esker Pond, Shore Western land*	McMurdo Ice Shelf	14 Dec. 1902	BM001062590	54	64.5

Table S2.

Location, date of collection, latitude-longitude and properties of cyanobacteria-base microbial mats and properties such as conductivity, temperature and pH. Legend: *unofficial names

Sample name	Site*	Location	Latitude longitude	Date	Conductivity ($\mu S\ cm^{1}$)	pH	Number of OTUs	Chao 1 richness
SLT	Salt Pond	MIS	78°00'0.96" 164°00'32.7"	14 Jan. 2011	51400	9.8	59	175.25
FRE	Fresh Pond	MIS	78°00'0.93" 164°00'32.6"	14 Jan. 2011	595	9.5	54	90.1
ORG	Orange Pond	MIS	78°00'0.82" 165°00'33.40"	14 Jan. 2011	2102	10.1	69	115.5
DIS	Pond half way between Brown Peninsula and Black Island	MIS	78º07'38.7" 165º48'50.0"	14 Jan. 2011	2657	9.4	57	74.8
HTP2	Hut Point Pond 2	Hut Point, RI	77º 50'41.8" 166º 38'36.8"	21 Jan. 2012	2763	9.5	48	61.3
HTP3	Hut Point Pond 3	Hut Point, RI	77º 50'41.4" 166º 38'35.5"	21 Jan. 2012	1779	9.7	35	38.0
OBH	Observation Hill Pond	Cape Armitage, RI	77º 51'20.9" 166º 41'25.7"	21 Jan. 2012	541	9.9	50	92.9
SKU	Skua Pond	Cape Evans, RI	77º 38'07 166º25 38.4	16 Jan. 2011	1026	9.5	101	128.8
COA	Coast Pond	Cape Royds, RI	77º 32'35.8" 166º 08'55.1"	6 Jan.2011	789	10.0	84	151.6

Table S2. continued

Location, date of collection, latitude-longitude and properties of cyanobacteria-base microbial mats and properties such as conductivity, temperature and pH. Legend: *unofficial names

Sample name	Site*	Location	Latitude longitude	Date	Conductivity ($\mu S\ cm^{1}$)	pH	Number of OTUs	Chao 1 richness
BLU	Blue Lake	Cape Royds, RI	77º 32'41.6" 166º 10'55.7"	7 Jan.2011	129.03	10.2	59	109
CLR	Clear Pond	Cape Royds, RI	77º 32'28.5" 166º 09'23.5"	7 Jan.2011	546.33	10.1	102	151.3
BRT	Bart Pond	Cape Royds, RI	77º 32'57.5" 166º 69'52.3"	6 Jan.2011	56633.3	9.2	32	36.7
SHL	Shacketon Pond	Cape Royds RI	77º 32'20.5" 166º 10'06.9"	5 Jan.2011	1117.67	9.7	80	117
ERNST	Ernest Pond	Cape Royds, RI	77º 32'16.9" 166º 10'01.8"	5 Jan.2011	2423.33	9.6	100	125.1

Table S3: Highest uncultured and cultured BLAST similarity match of the 10 most abundant cyanobacterial 16S rRNA genotypes in the historic and modern Antarctic cyanobacterial mat communities.

16S rRNA genotype	%	accession number	highest uncultured BLAST match	%	accession number	highest BLAST match
OTU1	100	KU230335	Uncultured Antarctic cyanobacterium clone HL820 16S ribosomal RNA gene	100	AY493590.	Leptolyngbya antarctica ANT.BFI.1
OTU2	100	KM112130	Uncultured cyanobacterium clone LJ6_294	100	KU219691	Microcoleus sp. P1 16S ribosomal
OTU3	100	AY541567	Uncultured Antarctic cyanobacterium clone FreP17	99	AY493607	Leptolyngbya antarctica ANT.L18.1
OTU4	100	KM112108	Uncultured cyanobacterium clone LH12_01	100	DQ493873	Phormidium autumnale Arct-Ph5
OTU5	99	HG932422	Uncultured cyanobacterium partial 16S rRNA gene, isolate BI02, clone 97BI02D01b	99	AY493588	Leptolyngbya antarctica ANT.LAC.1
OTU6	100	KP190129	Uncultured cyanobacterium clone BTRA-39	99	KU219729.1	Phormidesmis sp. HOR_11_6
OTU7	99	KF029608	Uncultured cyanobacterium clone 20.1.11-009_Isolat_1	99	KT347094	Phormidium pseudopriestleyi FRX01 1
OTU8	100	KU230341	Uncultured Antarctic cyanobacterium clone HL172 1	100	LT600732.1	Pseudanabaena sp. RL-08-7
OTU9	100	JX948577	Uncultured bacterium clone 4I.2.31	98	KU161657	Leptolyngbyaceae cyanobacterium EY08-AM1 clone 10B
OTU10	100	KC895987	Uncultured cyanobacterium clone OABF_3 1	99	AY493587	Pseudophormidium sp. ANT.LPE.3

Table S4. Cyanobacterial 16S rRNA genotypes that contribute up to 25% of cumulative dissimilarity based on SIMPER analysis; OTUs only present in modern samples are marked in bold. Any OTUS present in historic samples only contributed < 25%.

Taxon	Average dissimilarity	Contribution %	Cumulative %	Mean 1	Mean 2
denovo4878	0.7887	1.13	1.13	2.1	3.14
denovo4756	0.7871	1.128	2.258	1.39	1.78
denovo1261	**0.7731**	**1.108**	**3.366**	**0.188**	**1.75**
denovo10	0.7021	1.006	4.372	2.46	3.07
denovo5012	0.6852	0.9818	5.353	2.77	2.36
denovo4290	0.6764	0.9692	6.323	0.837	1.78
denovo3486	0.6655	0.9536	7.276	3.28	2.65
denovo131	0.6599	0.9456	8.222	2.84	2.55
denovo1370	0.6476	0.928	9.15	1.56	1.46
denovo1987	0.5952	0.8528	10	1.38	0.637
denovo13	0.5883	0.843	10.85	1.04	1.34
denovo619	0.5321	0.7624	11.61	3.07	2.19
denovo4212	0.5312	0.7612	12.37	2.95	2.78
denovo3348	0.4941	0.7079	13.08	1.28	1.56
denovo1895	0.4669	0.6691	13.75	1.06	0.112
denovo1042	0.465	0.6664	14.41	0.143	0.975
denovo698	0.4542	0.6508	15.06	0.946	0.0714
denovo4337	0.4508	0.646	15.71	0.955	0.0714
denovo5213	0.4484	0.6426	16.35	0	0.983
denovo2516	0.4448	0.6373	16.99	1.15	0.398
denovo4343	0.4405	0.6312	17.62	1.36	1.2
denovo766	0.4357	0.6243	18.24	0.989	0.179
denovo4682	0.4314	0.6181	18.86	1.53	0.901
denovo4163	0.4219	0.6046	19.47	0.17	0.755
denovo1938	0.4097	0.5871	20.05	0.313	0.91
denovo5038	0.4056	0.5811	20.64	0.912	0.237
denovo778	0.3871	0.5547	21.19	0.903	0.874
denovo667	0.3851	0.5518	21.74	0.849	0.143
denovo1989	0.3813	0.5464	22.29	0.873	0.351
denovo3244	0.3713	0.532	22.82	0.419	0.703
denovo957	0.3705	0.5309	23.35	0.843	0.53
denovo1569	0.3607	0.5168	23.87	0.716	0.927
denovo2101	0.36	0.5158	24.38	0.786	0.13
denovo4573	0.3558	0.5098	24.89	0.815	0.525
denovo2803	0.353	0.5058	25.4	0.809	0.241

Figure S1: A) Geographic location of Ross Island (RI) and McMurdo Ice Shelf (MIS) in Antarctica. B) Locations of the herbarium cyanobacteria specimens collected during Captain Scott's "Discovery" National Antarctic Expedition at their winter quarters on Ross Island and eskers on the McMurdo Ice Shelf halfway between Black Island (BI) and Brown Peninsula (BP) in 1902-03. Modern Antarctic cyanobacterial communities were collected on Ross Island (Cape Royds and Evans as well as Armitage and Hut Point adjacent to location of former winter quarters) and on the McMurdo Ice Shelf including halfway between Black Island and Brown Peninsula and near Brown Peninsula in 2011-2012.

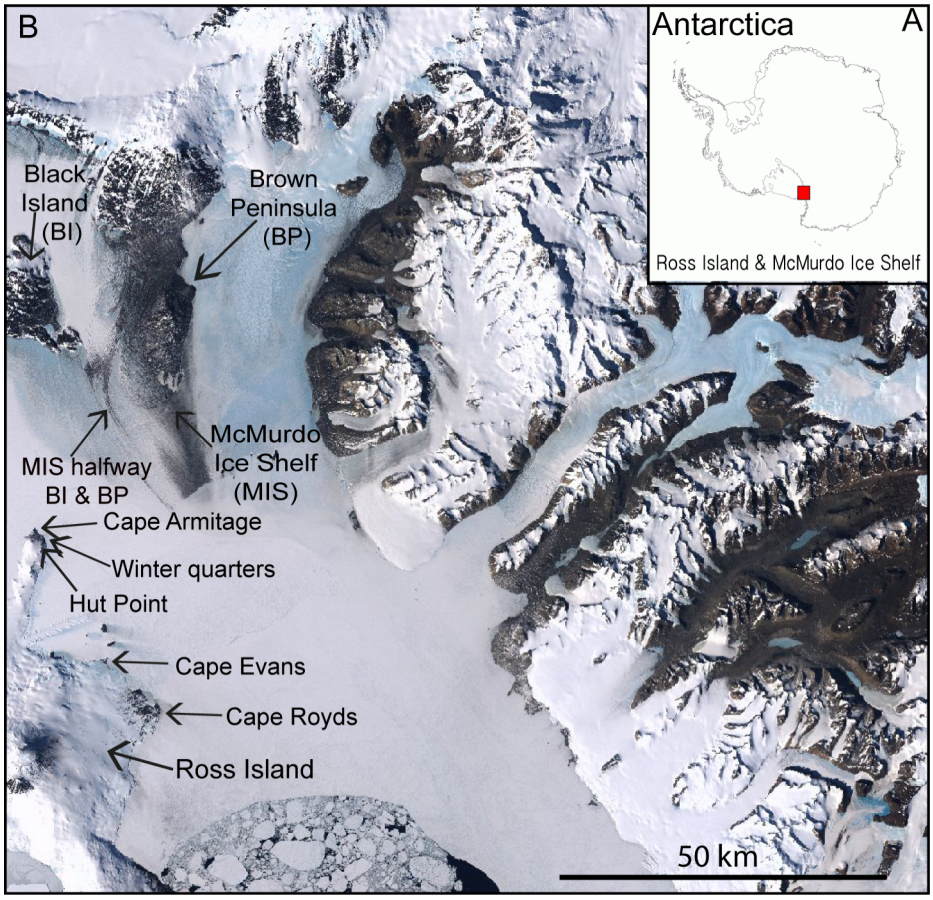


Figure S2.
Number of sequences of the 10 most abundant OTUs (99% clustering) in the historic and modern Antarctic cyanobacteria mat community samples.

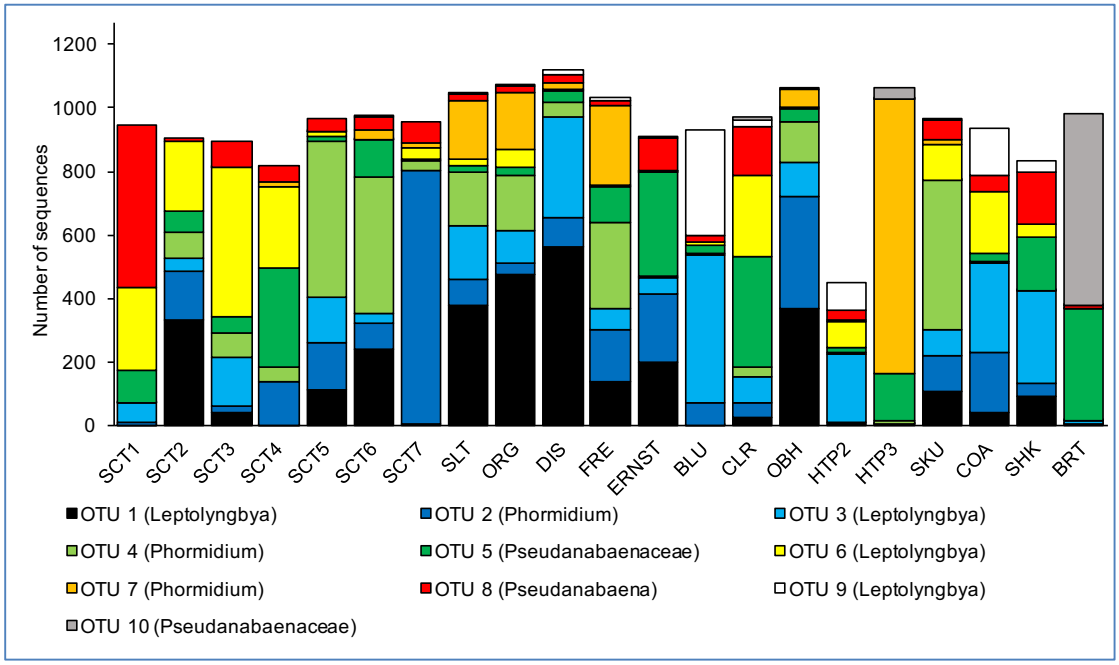
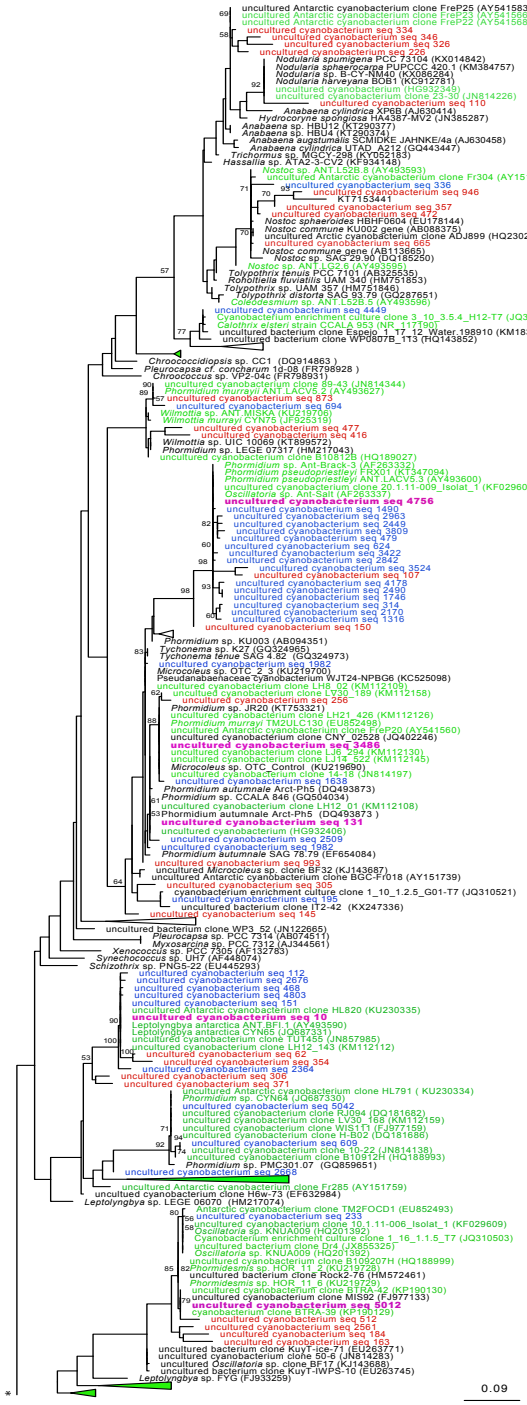
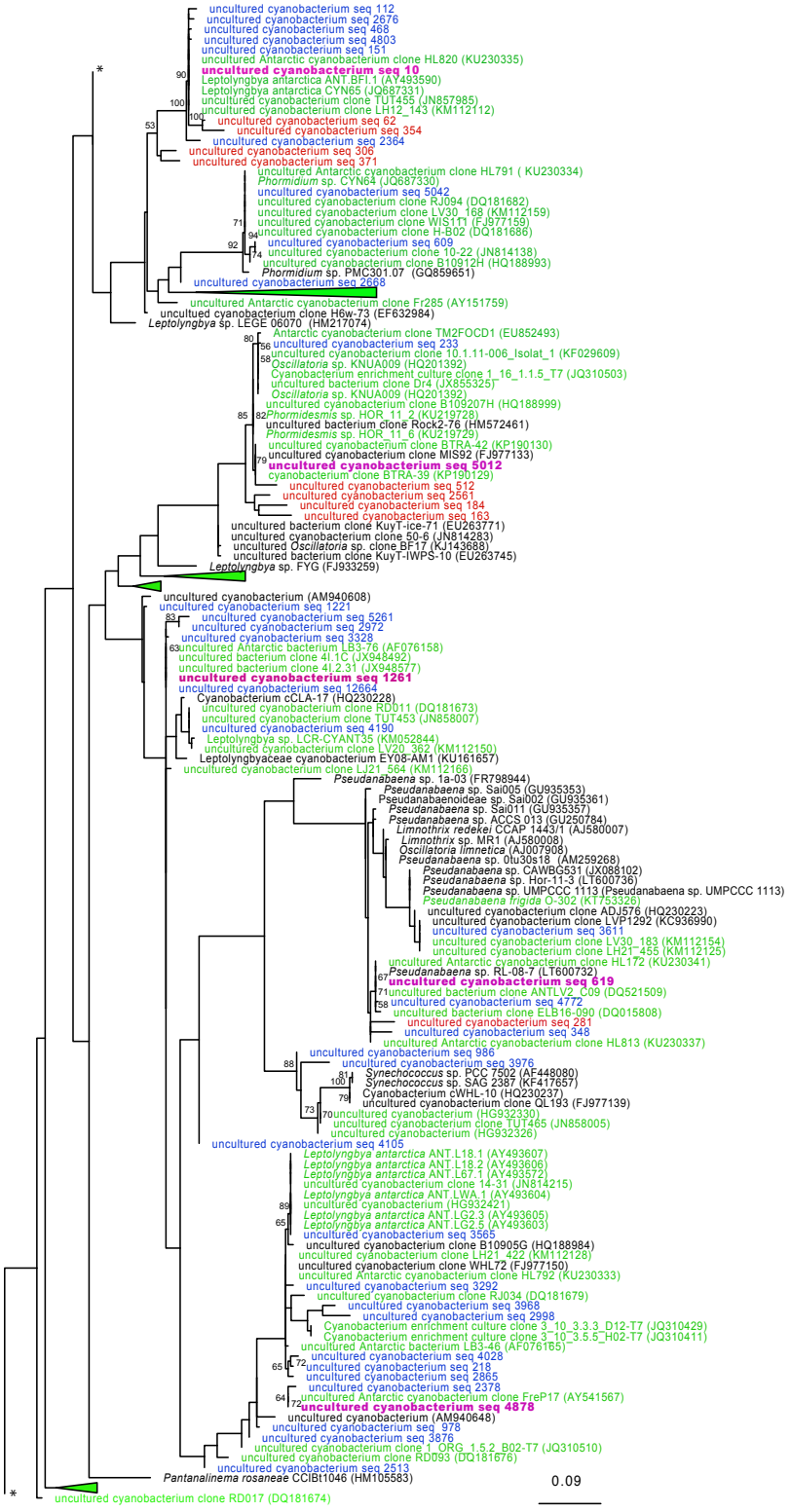


Figure S3. Phylogentic analysis of representative sequences from the 10 most abundant cyanobacterial 16S rRNA genotypes (pink) as well as representative sequences from the OTUS that were identified either only in the historic (red) or modern cyanobacteria communities (blue) from Antarctic microbial mat samples. Bootstrap support for nodes is shown for values greater than 50%. The 16S rRNA gene sequences of *Gloeobacter violaceus* PCC 7421 (BA000045) was used as an outgroup.



Supplementary Figure S2 continued



Supplementary Figure S2 continued