



## MICROBIOLOGY

# Morphological diversity of benthic cyanobacterial assemblages in meltwater ponds along environmental gradients in the McMurdo Sound region, Antarctica

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**Abstract:** Benthic cyanobacterial assemblages from ponds distributed along inland-coastal gradients in the McMurdo Sound region were studied during the 2011/12 Antarctic summer season. Twenty-five ponds were sampled in four distinct geographic locations, including the Lower and Upper Wright Valleys, Ross Island and the McMurdo Ice Shelf. For morphological identification, benthic mat samples were thawed and a subsample was directly observed by light microscopy. Remaining sample material was stored in 50 ml sterile polycarbonate bottles containing the mineral nutrient medium MLA for future studies, maintained at a temperature of 21°C. Ten morphological criteria were used to describe the morphotypes (trichome shape, number of trichomes in sheath, presence or absence of terminal attenuation of trichome, calyptra on mature apical cell, shape of apical cell, presence or absence of constrictions at transverse walls, granules, branching, range in width of trichomes and range of cell length) with reference to available identification literature. All morphospecies were documented using photomicrography. In total, 29 morphospecies were described, four assigned to the order Chroococcales, three to Nostocales and 22 to Oscillatoriales. The four geographic locations had similar taxonomic richness, sharing many morphospecies. However, each also contained distinct floristic elements that were rare or absent from the others.

**Key words:** cyanobacteria, microbial mats, meltwater, ponds, Antarctica.

## INTRODUCTION

The Cyanobacteria (Cyanophyceae, blue-green algae) are an ancient group of microorganisms that comprises unicellular to multicellular species which possess chlorophyll *a* and perform oxygenic photosynthesis (Schopf 2000, Frey 2012). They are found in almost all terrestrial and aquatic biotopes (Whitton & Potts 2000) and are well known for their ability to dominate habitats in temperate and extreme environments (Pearl & Huisman 2008, Wood et al. 2010). In Antarctica, cyanobacteria are widespread in lakes, ponds, streams, soils and on the surfaces of and within rocks, where they can form macroscopic visible

crusts and thin biofilms (Friedmann 1982, Vincent 2000, Andersen et al. 2011, Hawes et al. 2011). They often dominate the biomass and biological productivity of Antarctic regions due to their multiple adaptations to polar environmental extremes (Vincent 2000, Jungblut et al. 2009).

Antarctic cyanobacteria tolerate a wide range of climatic conditions and harsh physicochemical parameters (e.g. high salinity, UV radiation, extended periods of darkness, freezing and desiccation) (Zakhia et al. 2009). They have evolved various stress response mechanisms and are highly responsive to the sudden availability of moisture after periods

of desiccation and freezing (Hawes et al. 1992, Vincent 2007), giving them an impressive ability to colonise and dominate different habitats in Antarctica.

Many studies have addressed the diversity of Antarctic cyanobacteria using morphological and molecular methods (e.g. Broady & Kibblewhite 1991, Komárek 1999, Taton et al. 2003, 2006a, b, Jungblut et al. 2005, 2016, Comte et al. 2007, Zhang et al. 2015) and both cosmopolitan and endemic taxa have been described. Taxonomic understanding of cyanobacteria has developed substantially over the last two decades with the application of molecular phylogenetics. However, the identities of many cyanobacteria remain unclear, as does the number of taxa recorded in Antarctica (Garcia-Pichel 2009, Frey 2012), while many Antarctic locations have yet to be investigated. Unlike molecular methods, classifications based entirely on morphological features, such as whether a cyanobacterium is filamentous, colonial or unicellular, and whether or not it possesses heterocytes, do not reflect evolutionary relationships between the identified species (Nadeau et al. 2001) but rather the possession of consistent features and common characters which can be used to identify and sort them into recognisably distinct groups. Additionally, morphological features of cyanobacteria are often directly related with the habitat characteristics and ecological dynamics of these microorganisms (Sánchez-Baracaldo et al. 2005, Uyeda et al. 2016).

This study presents morphological descriptions of cyanobacterial strains obtained from 25 ponds sampled along inland to coastal gradients in the McMurdo Sound region, Victoria Land, Antarctica, including locations which have not previously been sampled.

## MATERIALS AND METHODS

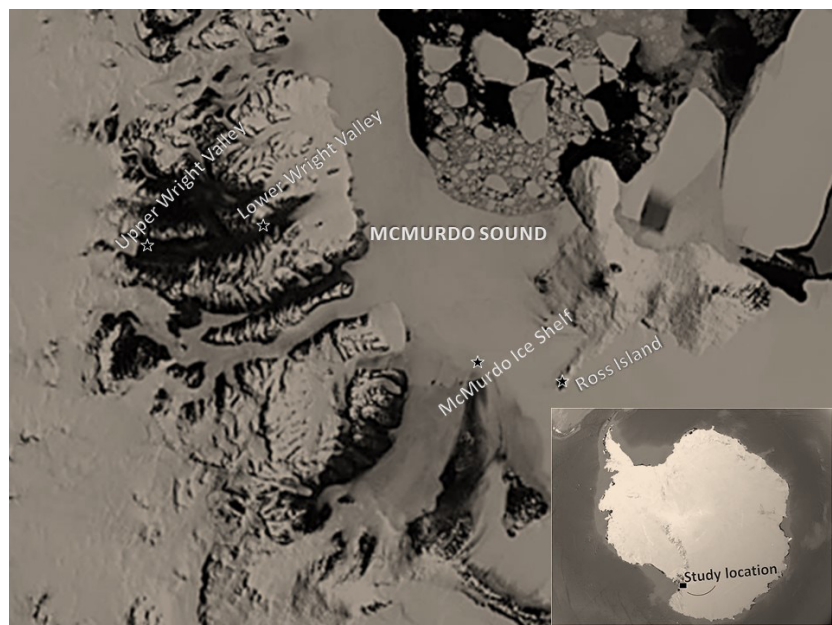
### Sampling sites

Samples of benthic microbial mats from 25 ponds distributed along inland-coastal gradients in the McMurdo Sound region were collected during the austral summer between December 2011 and January 2012. Four separate locations within this region were sampled: (i) Ross Island - RI, (ii) McMurdo Ice Shelf - MIS, (iii) Lower Wright Valley - LWV, and (iv) Upper Wright Valley - UWV (Fig. 1). The sampled ponds ranged in electrical conductivity from 1.92 to 42.6 mS/cm and in pH from 7.93 to 10.27. Most of the ponds sampled (RI, LWV and UWV) were located on terrestrial substrates, with only ponds from the MIS area being situated on ice surfaces. The location and brief description of each pond are given in Table I. Samples were collected from the pond margins using a cut-off 20 mL syringe. Cores were taken with care in order to collect only microbial mat material and no underlying sediment. The resulting mat core was carefully lifted from the sediment and transferred to a plastic container, kept chilled while in the field and subsequently frozen (-20°C) for return to New Zealand.

### Sample analysis

For morphological identification, benthic mat samples were thawed and a subsample was directly observed under light microscopy. Remaining sample material was placed in 50 mL sterile polycarbonate bottles (Biolab, NIWA) containing mineral nutrient medium MLA (Bolch & Blackburn 1996) for future observation, and maintained at a laboratory temperature of about 21°C. No attempt was made to isolate cyanobacterial morphospecies into unialgal cultures.

Cyanobacterial morphospecies were identified using an Olympus light microscope



**Figure 1.** McMurdo Sound region and the location of the four field areas. Satellite images from Google earth pro, Image © 2020 DigitalGlobe, US Geological Survey.

(BX51, Olympus) at up to 1000× magnification. Ten morphological criteria (Supplementary Material - Table SI) were used to describe the morphotypes (trichome shape, number of trichomes in sheath, presence or absence of terminal attenuation of trichome, calyptra on mature apical cell, shape of apical cell, presence or absence of constrictions at transverse walls, granules, branching, range in width of trichomes and range of cell length) with reference to the identification keys of Komárek & Anagnostidis (1989, 2000, 2005) and other specialized literature (Broady & Kibblewhite 1991, Taton et al. 2011, Strunecký et al. 2011). The cyanobacterial morphotypes were documented by photomicrography.

The percentage of frequency of occurrence of each morphospecies in each studied area was calculated by:  $FO = (N_{pi} \times 100) / N_{tp}$ ; where FO = Frequency of occurrence,  $N_{pi}$  = number of ponds in which the morphospecies was found, and  $N_{tp}$  = total number of studied ponds.

## RESULTS AND DISCUSSION

Twenty-nine cyanobacterial morphospecies were distinguished in the samples examined. Four were assigned to the order Chroococcales, 22 to Oscillatoriales and three to Nostocales. The morphotypes (Mph) are designated 1-29 and summary morphological and morphometric descriptions and data on site of occurrence of each morphotype are given in Table SI. Photomicrographs are provided in Figures 2-4.

### Morphological diversity and descriptions

#### Oscillatoriales

##### Morphotype 1 (Fig. 2a1-a2)

**Description:** Trichome generally forms short filaments (4 to 16 celled), rarely solitary. Cells cylindrical or up to barrel-shaped, ± isodiametric by fragmentation of trichome, sheaths lacking, immotile, constricted at the cross-walls.

**Remarks:** This morphotype was assigned to the genus *Borzia* and was present in 11 of the studied samples. *Borzia* has previously reported in continental Antarctic systems by

**Table I. Locations of study sites and mat description.**

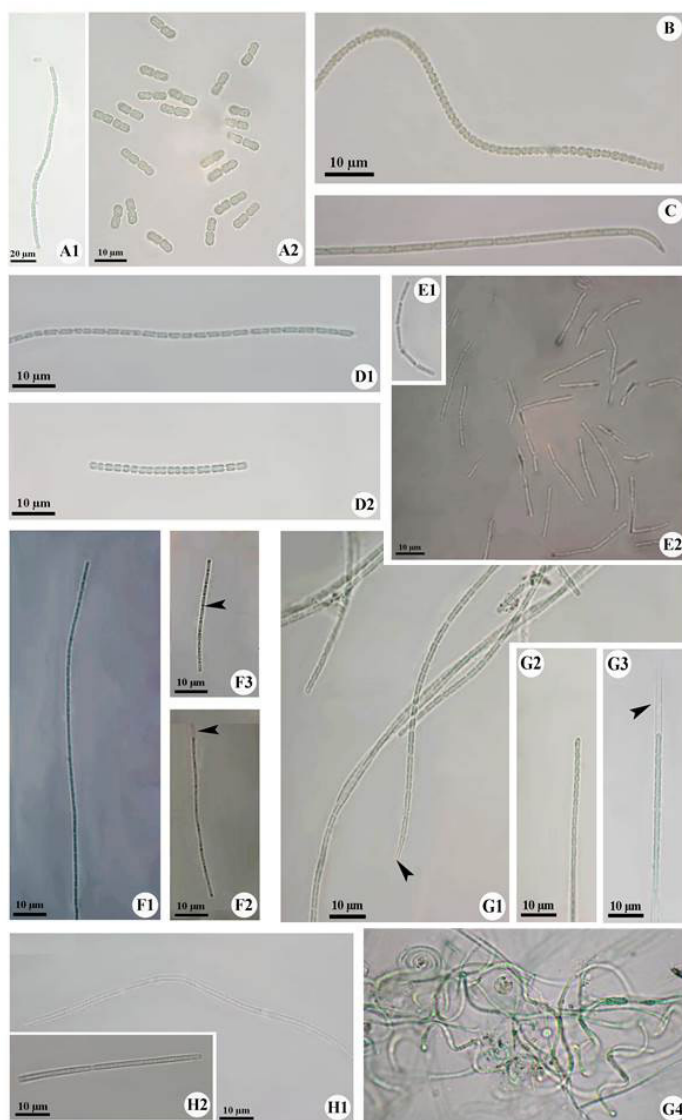
Area	Location			Mat description			
	Pond	Lat.	Long.	Colour	bilayer	cohesive	thickness
Upper Wright Valley	L26	77 33 03.2	160 43 24.0	1	1	2	1
	L09	77 33 02.4	160 44 26.2	2	2	3	2
	L15	77 33 13.1	160 43 01.2	3	1	3	2
	L16	77 32 29.0	160 45 20.0	1	1	1	2
	E9	77 31 35.1	160 46 16.7	2	1	1	1
	E4	77 31 21.3	160 44 17.9	2	1	1	1
Lower Wright Valley	LW2	77 26 43.9	162 41 30.3	1	1	3	2
	LW12	77 27 00.1	162 39 00.5	1	1	3	2
	LW13	77 27 02.0	162 38 52.5	1	2	3	2
	LW14	77 27 02.8	162 38 44.7	1	2	3	2
	LW15	77 27 03.1	162 38 35.3	1	2	3	2
	LW16	77 27 03.0	162 38 28.6	1	2	3	2
	LW18	77 27 02.7	162 38 19.3	1	1	2	2
Ross Island	OHP	77 51 20.9	166 41 25.7	1	1	2	2
	HP1	77 50 36.6	166 38 41.6	1	1	2	1
	HP2	77 50 41.8	166 38 36.8	1	2	3	2
	HP3	77 50 41.4	166 38 35.5	1	2	3	2
McMurdo IceShelf	FH	78 00.962	165 33.070	2	2	2	1
	Nostoc	78 00.832	165 33.288	2	1	2	1
	P70	78 00.892	165 33.136	1	2	3	2
	Skua	78 00.798	165 33.113	2	2	3	2
	Orange	78 00.838	165 33.339	1	2	3	2
	P70E	78 00.949	165 33.082	2	1	2	1
	Brack	78 00.947	165 32.723	1	2	3	2
	Salt	78 00.963	165 32.734	4	1	2	2

**Colour:** 1 = orange; 2=brown; 3=black; 4=green. **Bilayer:** 1= no; 2= yes. **Degree of cohesion:** 1=flake; 2=sheet; 3= mat. **Thickness:** 1= thin; 2=thick.

Hodgson et al. (2001) and Broady (2005). The genus has not been found in Antarctic Peninsula habitats, suggesting a restricted distribution in continental areas.

### Morphotype 2 (Fig. 2b)

**Description:** Trichome forming fine filament, a little flexuous, elongated, broadly constricted at the cross-walls, immotile, without firm sheaths, simple. Necridic cells, akinetes, aerotopes and heterocytes were not observed. Cells barred-shaped or sometimes almost isodiametric.



**Figure 2.** Benthic cyanobacterial morphospecies in meltwater ponds along environmental gradients in the McMurdo Sound region. (a-g) morphospecies belonging to the Oscillatoriales order: (a1-a2) *Borzia* sp. (Mph 1); (a1) formation of filament; (a2) short trichome; (b) cf. *Kamvophoron* sp. (Mph 2); (c) *Geitlerinema* cf. *ionicum* (Mph 3); (d1-d2) *Pseudanabaena* sp. (Mph 4); (d1) long filament; (d2) short filament; (e1-e2) cf. *Pseudanabaena* (Mph 5); (e1) detail of trichome; (f1-f3) *Pseudanabaena* sp. (Mph 6); (f1) formation of long filament; (f2) ends of trichome with sheath (arrow); (f3) ends of cell with granule (arrow); (g1-g4) *Leptolyngbya* sp. (Mph 7); (g1) ends of trichome with slightly attenuation (arrow); (g2) filament without sheath; (g3) end of trichome with sheath (arrow); (g4) coiled filaments; (h1-h2) *Leptolyngbya* sp. (Mph 8). Mph= morphotype.

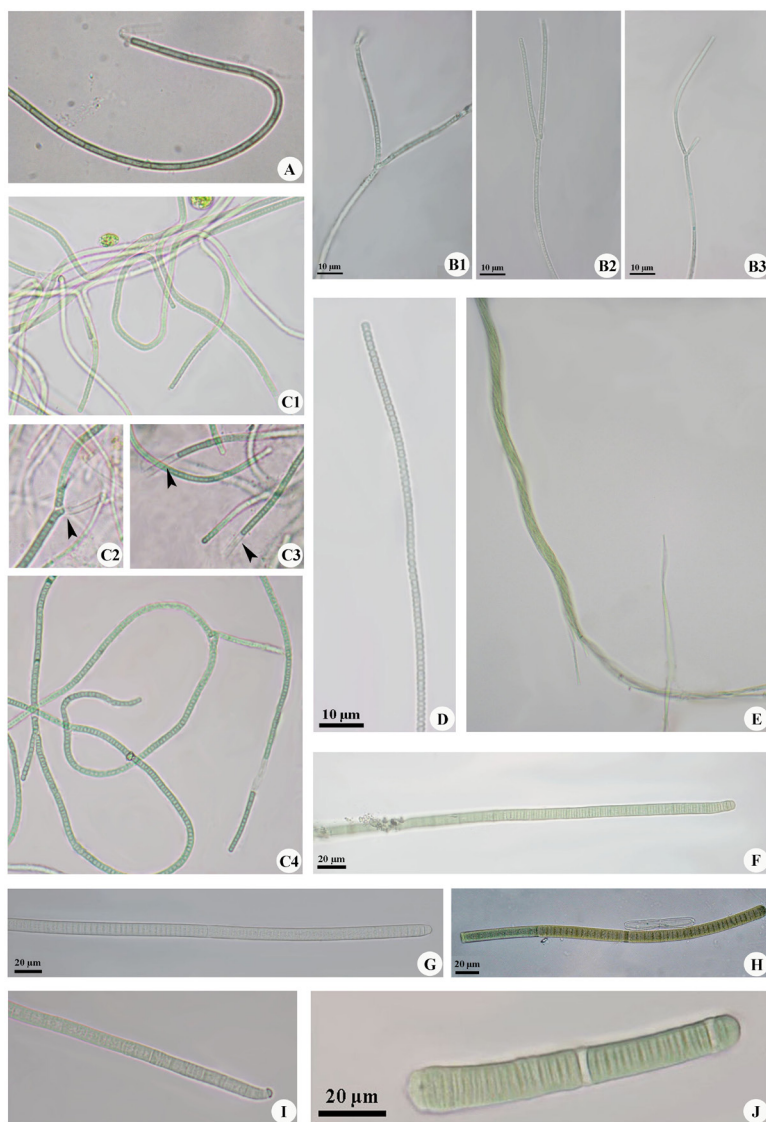
**Remarks:** Mph 2 was present in only one sample and, based on the classical literature, resembles the genus *Komphovoron*, although the width range given by Komárek & Anagnostidis (2005) is somewhat wider than that observed in the present study.

### Morphotype 3 (Fig. 2c)

**Description:** Filamentous trichome, thin, cylindrical, straight or slightly flexuous, without sheaths, not constricted at the cross-walls,  $\pm$  gradually attenuated and bent or coiled at the ends. Trichome strongly motile, with intense

gliding in the direction of the longitudinal axis, both forwards and backwards or waving (oscillation). Cells longer than wide, sometimes with large prominent granules. Apical cells usually conical, hooked or bent.

**Remarks:** The consistent characters observed allowed assignment to *Geitlerinema* cf. *ionicum*. This morphotype was observed in nine samples. Studies from the Antarctic Peninsula (Komárek 1999) and Antarctic continent (Taton et al. 2006a) have documented the presence of *Geitlerinema* spp., indicating the genus is widely distributed across Antarctica.



**Figure 3.** Benthic cyanobacterial morphospecies in meltwater ponds along environmental gradients in the McMurdo Sound region. (a-j) morphospecies belonging to the Oscillatoriales order: (a) cf. *Leptolyngbya* (Mph 9); (b1-b3) *Plectolynbya* cf. *hodgsonii* (Mph 10); (c1-c4) cf. *Plectolynbya* (Mph 11); (c2) detail of false branching; (c3) ends of filament with sheath (arrow); (d) *Leptolyngbya* with resemblance to *Phormidium priestleyi* (Mph 12); (e) cf. *Schizothrix* (Mph 13); (f) *Oscillatoria* sp. (Mph 14); (g) *Oscillatoria* sp. (Mph 15); (h) cf. *Oscillatoria* (Mph 16); (i) *Oscillatoria* sp. (Mph 17); (j) *Crinalium* cf. *glaciale* (Mph 18). Mph = morphotype.

#### Morphotype 4 (Fig. 2d1-d2)

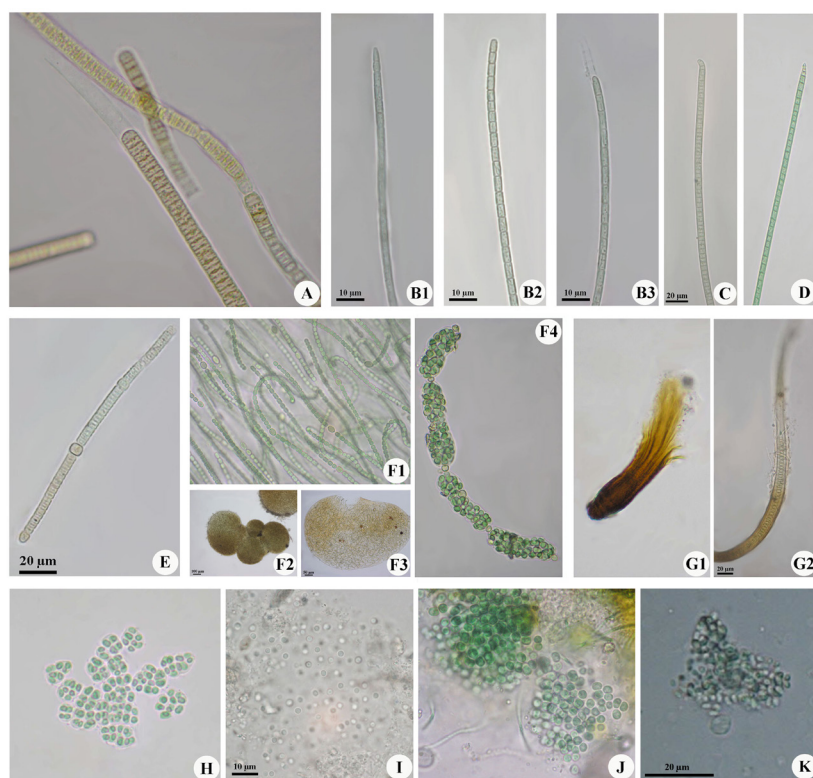
**Description:** Trichomes usually straight or a little waved, consisting of few to several cells, with broad constrictions at cross-walls. Trichomes without sheaths. Motility lacking. Cells usually cylindrical with rounded ends, sometimes almost barred-shaped, apical cells not differentiated.

**Remarks:** This morphotype might be further separable into two morphospecies based on the length of the trichome, in some cases possessing a long filament (Fig. 2d1) and occasionally short

(Fig. 2d2). However, no consistent characters could be used to separate these forms. Mph 4 was recorded in 14 samples and was assigned to the genus *Pseudanabaena*.

#### Morphotype 5 (Fig. 2e1-e2)

**Description:** Filament very short, few-celled (3 - 6 celled), thin, straight or slightly curved. Cell cylindrical, constantly longer than wide, sometimes granules were present at the ends. Trichome constricted, not attenuated, with trembling-like motility.



**Figure 4.** Benthic cyanobacterial morphospecies in meltwater ponds along environmental gradients in the McMurdo Sound region. (a-d) morphospecies belonging to the Oscillatoriales order: (a) cf. *Lyngbya* (Mph 19); (b1-b3) *Phormidium* cf. *murrayi* (Mph 20); (b1) terminal cells conical-shaped; (b2) terminal cells rounded-shaped (b3) presence of sheath; (c) *Phormidium* cf. *autumnale* (Mph 21); (c) *Phormidium* cf. *setchellianum* (Mph 22). (e-g) morphospecies belonging to the Nostocales order: (e) *Nodularia* sp. (Mph 23); (f1-f4) *Nostoc* sp. (Mph 24). (g1-g2) *Calothrix* sp. (Mph 25). (h-k) morphospecies belonging to the Chroococcales order: (h) *Chroococcus* sp. (Mph 26); (i) cf. *Aphanocapsa* (Mph 27); (j) cf. *Aphanocapsa* (Mph 28); (k) cf. *Aphanocapsa* (Mph 29). Mph=morphotype.

**Remarks:** This morphotype was similar to the genus *Pseudanabaena* and present in eight samples.

#### Morphotype 6 (Fig. 2f1-f3)

**Description:** Trichome very thin, not attenuated at the ends, with firm, thin sheaths (Fig. 2f2) or sheath not present (Fig. 2f1), prominent granules constantly present at the ends of the cells (Fig. 2f3), with indistinct trembling.

**Remarks:** Filaments assigned to Mph 6 were thinner than those of Mph 4 but similar to width of those Mph 5. However, Mph 6 forms long filament while the possession of Mph 5 is characterized by consistently short filaments. The features of Mph 6 are also consistent with the genus *Pseudanabaena* and was present in 14 samples.

#### Morphotype 7 (Fig. 2g1-g4)

**Description:** Filamentous trichome, straight or flexuous, finely waved, long, with sheath (Fig. 2g3) or sheath not present (Fig. 2g2), most filaments not attenuated at the ends although occasionally so (Fig. 2g1). Trichomes non-motile or with indistinct trembling. Cells mostly cylindrical, although some isodiametric or a little longer than wide.

**Remarks:** Mph 7 was assigned to the genus *Leptolyngbya*. This morphotype may also possibly be subdivided through the possession in some instances of very densely spirally coiled trichomes (Fig. 2g4). Komárek (2007) separated and classified the spirally coiled trichome form as *L. borchgrevinkii*. Mph 7 was present in all samples.

#### Morphotype 8 (Fig. 2h1-h2)

**Description:** Filaments nearly straight, sometimes slightly curved, pale blue-green,

not constricted at cross-walls. Cells of various lengths, sometimes longer than wide but also wider than long. Apical cell somewhat elongated, sometimes rounded.

**Remarks:** This morphotype was assigned to the genus *Leptolyngbya*. The main characteristic separating Mph 7 from Mph 8 was the cell shape, which was nearly square in Mph 8 and slightly cylindrical or isodiametric in Mph 7. Mph 8 was present in 22 samples.

### Morphotype 9 (Fig. 3a)

**Description:** Trichome long, curved, with sheath, thin, straight at the ends, not attenuated, not constricted.

**Remarks:** This morphotype was distinguished from Mph 6, Mph 7 and Mph 8 by cell length. Mph 9 had cells consistently longer than wide (approximately 5× longer than wide). This morphotype may also be a representative of the genus *Leptolyngbya* and was present in only one sample.

### Morphotype 10 (Fig. 3b1-b3)

**Description:** Filaments solitary or in small free clusters, thin, slightly curved, long with false branching with very thin sheath. Trichomes cylindrical, slightly constricted at cross-walls, not attenuated towards the ends. Cells cylindrical, ± isodiametric or slightly longer or shorter than wide, end cells rounded.

**Remarks:** This morphotype was characterized by the presence of false branching. The characters allowed assignment to *Plectolyngbya* cf. *hodgsonii*, which was recorded by Taton et al. (2011) in Antarctica. Mph10 was present in five samples.

### Morphotype 11 (Fig. 3c1-c4)

**Description:** Filaments long, flexuous or slightly curved, sometimes irregularly coiled, richly and repeatedly branched, somewhat

constricted at the cross-walls. Sheath thick, slightly widened from trichomes. Cells always shorter than wide.

**Remarks:** Morphological features were consistent with the genus *Plectolyngbya*. The major characteristic distinguishing Mph 11 from Mph 10 was the cell width, which was wider in Mph 11. Mph 11 was present in only two samples.

### Morphotype 12 (Fig. 3d)

**Description:** Trichome straight, distinctly constricted at the cross-wall, cell rounded or up to barrel-shaped, shorter than wide to isodiametric, with prominent granules, apical cells widely rounded.

**Remarks:** This morphotype may represent the genus *Leptolyngbya*, with resemblance to *Phormidium priestleyi* as described by Komárek & Anagnostidis (2005). It was present in 17 samples.

### Morphotype 13 (Fig. 3e)

**Description:** Filaments densely joined (filamentous-colonial) and entangled with one another forming irregularly wavy tallus. Trichome not attenuated towards the ends, slightly constricted at the cross-walls. Cells cylindrical, mostly longer than wide, apical cells mainly conical.

**Remarks:** This strain showed characteristics possibly consistent with the genus *Schizothrix* and was present in three samples.

### Morphotype 14 (Fig. 3f)

**Description:** Trichome cylindrical, straight or slightly wavy, sometimes with screw-like coiling at the ends, not constricted, motile with gliding and oscillation. Filament cell very short, wider than long, without sheaths.

**Remarks:** The morphological characteristics allowed the assignment of this morphotype to the genus *Ocillatoria*. It was present in three samples.

### Morphotype 15 (Fig. 3g)



**Description:** Trichome straight, cylindrical, not constricted, slightly attenuated at the end (outer cell). Filaments cells were always shorter than wide, with somewhat thickened outer cell wall.

**Remarks:** The possession of consistent characters in this morphotype allowed assignment to the genus *Ocillatoria*. Although the cell width of Mph 15 was only a little broader than that of Mph 14, the cell length was constantly longer than that of Mph 14. Moreover, the shape of apical cell also differs comparing the two morphotypes. Apical cell of Mph 15 ranged from round to slightly elongated while in Mph 14 it was broadly rounded. Mph 15 was present in only one sample.

**Morphotype 16** (Fig. 3h)

**Description:** Tallus olive green, thick, weakly curved, not constricted at the cross-walls, not attenuated at the ends. Filament always wider than long. Apical cell rounded with slightly thickened outer cell wall.

**Remarks:** The shape and dimensions of Mph 16 correspond with the genus *Oscillatoria*. The main feature separating Mph 16 from other morphotypes assigned to *Oscillatoria* in the present study was the cell width, which was wider in Mph 16 than in the other morphotypes (Mph 14, Mph 15, Mph 17). This morphotype was recorded in only two samples.

**Morphotype 17** (Fig. 3i)

**Description:** Filament straight, not constricted, slightly attenuated and somewhat hooked at the ends. Cell were always shorter than wide.

**Remarks:** This morphotype was assigned to the genus *Oscillatoria* and was distinguished from Mph 14, Mph 15 and Mph 16 by the apical cell and trichome width. Mph 17 was thinner compared to the other morphotypes and, additionally, the apical cell possessed a greatly

thickened outer cell wall. Mph 17 was recorded in two samples.

**Morphotype 18** (Fig. 3j)

**Description:** Filament single, trichome straight or slightly wavy, cylindrical, not attenuated towards ends, slightly constricted at cross-walls, cells very short, always shorter than wide, terminal cells widely-rounded.

**Remarks:** Mph 18 was assigned to *Crinalium* cf. *glaciale* and was present in only one sample. *Crinalium* sp. was previously described by Broady & Kibblewhite (1991) from the surface of an Antarctic glacier.

**Morphotype 19** (Fig. 4a)

**Description:** Filament long, variously curved but sometimes straight, not constricted, sheath present. Cells were short, consistently wider than long, with rounded apical cell.

**Remarks:** The presence of a thick hyaline sheath suggests that Mph 19 is a representative of the genus *Lyngbya*. It was present in three samples.

**Morphotype 20** (Fig. 4b1-b3)

**Description:** Filamentous trichomes, straight or slightly curved, not or only slightly constricted at cross-walls, sheath present (Fig. 4b3) or absent (Fig. 4b1-b2). Cells quadratic or longer than wide, occasionally with large granules. Terminal cells conical-shaped (Fig. 4b1) or rounded (Fig. 4b2).

**Remarks:** The regular occurrence of characters observed in this morphotype allowed assignment to *Phormidium* cf. *murrayi*. This species has previously been reported by Mataloni et al. (2005) and Komárek & Elster (2008). However, subsequent studies have separated Antarctic strains previously identified as *P. cf. murrayi* to a new genus, renaming them as *Wilmottia* cf. *murrayi* (Strunecký et al. 2011). Additionally, the new genus was separated genetically from all related oscillatorialean genera and consisted of only one species

(Strunecký et al. 2011). Recently, the number of *Wilmottia* species and its distribution were extended. This genus currently includes three species (*W. murrayi*, *W. stricta*, and *W. koreana*) and new records of its occurrence in Antarctica (Radzi et al. 2021).

**Morphotype 21** (Fig. 4c)

**Description:** Thick filament, mostly straight but sometimes slightly curved (especially at the ends) with somewhat hooked end, not constricted. Cells mostly wider than long, rarely longer than wide, with dispersed granules.

**Remarks:** Mph 21 was assigned to *Phormidium cf. autumnale*, a taxon documented from many Antarctic systems (Broady & Kibblewhite 1991, Mataloni et al. 2005, Komárek & Elster 2008, Corrêa D et al., unpublished data). It was present in 17 samples, of our studied area.

**Morphotype 22** (Fig. 4d)

**Description:** Filament straight or slightly curved, not constricted, slightly attenuated at the ends.

**Remarks:** Mph 22 was also assigned to the genus *Phormidium*. Distinction between Mph 21 and Mph 22 were made using the cell shape and size. Filaments designated to Mph 22 were mostly thinner than those assigned to Mph 21. Additionally, Mph 22 contains cells that are nearly quadratic or sometimes longer than wide while Mph 21 possesses cells mostly wider than long. The characteristics of Mph 22 suggest assignment to *Phormidium cf. setchellianum*. It was present in six samples.

## Nostocales

**Morphotype 23** (Fig. 4e)

**Description:** Filamentous, isopolar, more or less straight or curved. Trichomes uniseriate, cylindrical, constricted at cross-walls, presence of heterocytes more or less regularly spaced distances from each other. Cells slightly barrel-shaped with the length never exceeding the

width. Heterocytes slightly larger than vegetative cells.

**Remarks:** Mph 23 was assigned to the genus *Nodularia*. Different morphospecies belonging to this genus have been described from various regions of Antarctica by Broady (2005), Taton et al. (2006a) and Komárek & Elster (2008). It was recorded in six samples.

**Morphotype 24** (Fig. 4f1-f4)

**Description:** Filamentous tallus, widely constricted with intercalary heterocytes, slightly coiled, long, in cluster forming dense mat/colony. Colony morphology changes during development. The mucilage of the colony is firm, wide and sometimes yellowish-green to brownish. Cells are barrel shaped with a uniform shape and size along trichome, bright blue-green (after growing in culture medium, Fig. 4f1, 4f4) or olive-green (field sample, Fig. 4f2, 4f3).

**Remarks:** From the trichome characterization Mph 24 was assigned to the genus *Nostoc* and was present in nine samples. Although representatives of *Nostoc* were present in only nine samples in this study, the genus has been documented from a wide range of Antarctic habitats (Broady 2005, Taton et al. 2006a, Komárek & Elster 2008, Fernández-Carazo et al. 2012, Corrêa D et al., unpublished data).

**Morphotype 25** (Fig. 4g1-g2)

**Description:** Filaments heteropolar, differentiated into basal and apical parts, simple, solitary or in small groups but not in common mucilage. Trichome unbranched, constricted at the cross-wall with terminal heterocytes and widened basal part. Apical part composed of narrow, long, hyaline cells. Hair formation at the apical ends of filaments was observed. Sheaths always present. Cells slightly barrel-shaped.

**Remarks:** The characteristics of Mph 25 suggest assignment to the genus *Calothrix*. It was present in only one sample. Different representatives of the genus have previously

been reported from various Antarctic regions (Broady 2005, Komárek & Elster 2008, Martineau et al. 2013).

## Chroococcales

### Morphotype 26 (Fig. 4h)

**Description:** Groups of cells (only few-celled) surrounded by mucilaginous envelopes; colonial slime fine, diffluent, homogeneous and colourless. Cell widely oval, bright blue-green with homogeneous or granular content.

**Remarks:** The persistent characteristics of Mph 26 allow assignment to the genus *Chroococcus*. It was present in two samples.

### Morphotype 27 (Fig. 4i)

**Description:** Groups of cells forming colonies with numerous, sparsely arranged, cells; colonial mucilage colourless, indistinct margin, formless, cells with individual gelatinous envelopes. Cells widely rounded.

**Remarks:** The characteristics of Mph 27 are similar to those of the genus *Aphanocapsa*. It was present in seven samples.

### Morphotype 28 (Fig. 4j)

**Description:** Groups of cells forming colonies, mucilage colourless, indistinct margin, formless, cells with individual gelatinous envelopes. Cells rounded.

**Remarks:** Mph 28 was distinguished from Mph 27 mainly by colony shape. Mph 28 colonies were characterised by densely arranged cells while those of Mph 27 were sparse. Mph 28 was also assigned to genus *Aphanocapsa* and was present in five samples.

### Morphotype 29 (Fig. 4k)

**Description:** Groups of agglomerated cells forming colonies, colonial mucilage colourless, indistinct margin, formless. Cells range between round and slightly elongated.

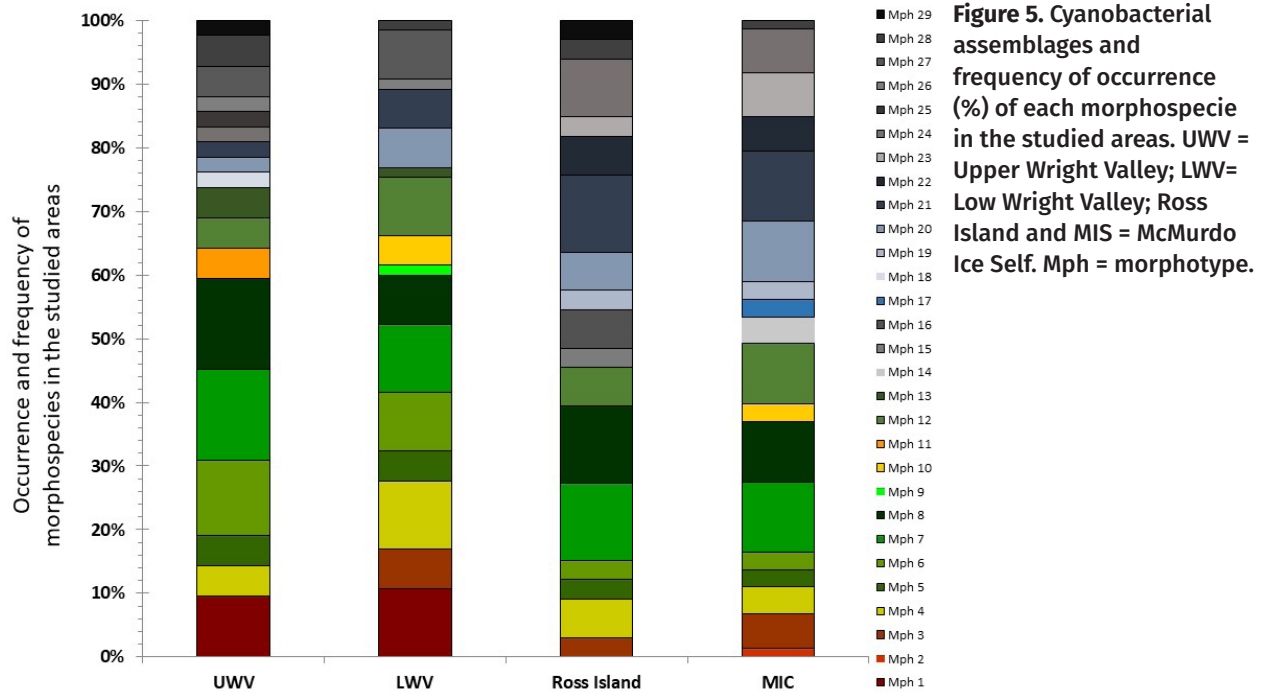
**Remarks:** Mph 29 may also represent the genus *Aphanocapsa*. The major characteristic distinguishing Mph 29 from Mph 27 and Mph

28 was the cell size. Mph 29 was approximately 1.7  $\mu\text{m}$  wide, Mph 27 about 2.26  $\mu\text{m}$  and Mph 28 approximately 3  $\mu\text{m}$  (see Table SI). Mph 29 was present in two samples.

## Distribution of morphotypes

Seventy-five per cent of the morphotypes described in this study represented the cyanobacterial order Oscillatoriales. Morphotypes assigned to genus *Leptolyngbya* (Mph 7 and Mph 8) were widespread in the inland ponds sampled (ULW and LWV) and in coastal ponds (RI and MIS) and were the most commonly encountered, followed by Mph 21 which was related to *Phormidium cf. autumnale*. Ten percent of the morphotypes distinguished represented the order Nostocales. Morphotypes assigned to this order, representing the genera *Nostoc* and *Calothrix*, were mostly recorded on Ross Island and MIS sites, with only one record from the inland ponds (UWV) presented strains. Representatives of the order Chroococcales contributed 13% of morphotype diversity and were most frequent in UWV and LWV. Only two samples from Ross Island and one from MIS included morphotypes belonging to the order Chroococcales (Fig. 5).

Although molecular studies generally yield greater diversity than is recognised in studies based on classical morphological approaches (Taton et al. 2003, 2006a), our data are consistent with other studies of cyanobacterial diversity in Antarctica (Michaud et al. 2012, Zhang et al. 2015, Jungblut et al. 2016). According to Komárek (2007), the traditional definition and naming of species in agreement with the rules of botanical nomenclature continues to be necessary and the only acceptable method for characterisation of cyanobacterial taxonomic units (generic and subgeneric; ecologically as well as morphologically). We consider complementary molecular studies in future



work will be important to connect the genotypes with phenotypic characteristics, and to provide reliable generic and specific designation of different morphotypes.

The strong similarities found in cyanobacterial community composition across the four studied areas suggest that they represent the same metacommunity. However, some differences were present, which may represent the outcome of species sorting along the various environmental and physico-chemical gradients of the water bodies sampled, of the climatic characteristics of each site, or of different propagules dispersal limitations. Unravelling the factors involved in structuring cyanobacterial communities is a key challenge in microbial ecology, and it has been suggested that these include both deterministic and stochastic processes (Sloan et al. 2006).

## CONCLUSIONS

Using a classical morphological approach, we confirmed that the four studied areas possessed similar cyanobacterial richness (15-17 morphotypes) and shared many morphotypes. Each site also contained distinct floristic elements that were rare in or absent from the other sites. The combination of likely high degree of airborne connectivity between the four sites, general tolerance of cyanobacteria to desiccation and other physical environmental stresses, and the similarities of between their communities suggest that the four sites may belong to the same metacommunity. These findings contribute to extending knowledge of Antarctic cyanobacteria and should be integrated with future molecular studies to increase understanding of cyanobacterial diversity and distribution.

## Acknowledgments

We are grateful for the fellowship provided in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/PROANTAR) and Coordenação de

Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES). Antarctica New Zealand provided logistic support, in collaboration with the US National Science Foundation. We would like to acknowledge the National Institute of Water and Atmospheric Research (NIWA)-Christchurch for laboratory, technical and infrastructural support, particularly to Cathy Kilroy. Anne Jungblut, Antônio Galvão and Adriano Nunes for useful comments on earlier drafts. Dr. Paul Broady provided advice in cyanobacterial identification.

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## SUPPLEMENTARY MATERIAL

### Table S1.

**How to cite**

LIZIERI C, SCHAEFER CEGR & HAWES I. 2022. Morphological diversity of benthic cyanobacterial assemblages in meltwater ponds along environmental gradients in the McMurdo Sound region, Antarctica. *An Acad Bras Cienc* 94: e20210814. DOI 10.1590/0001-376520220210814.

*Manuscript received on June 2, 2021;  
accepted for publication on November 17, 2021*

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