PHENOTYPIC DIVERSIFICATION OF TERRESTRIAL CYANOBACTERIA FROM SELECTED HABITATS ON SIGNY ISLAND, SOUTH ORKNEY ISLANDS, ANTARCTICA

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by

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LIST OF ABBREVIATIONS

µg/mL	micrograms per milliliter
µL	microlitre
16S	Small Ribosomal Subunit
BBM	Bold's Basal Medium
BG-11	Blue Green Medium
BI	Bayesian Inference
BP	Bootstrap
bp	Base pair
CHX	cycloheximide
cm^3	cubic centimeter
DNA	Deoxyribonucleic Acid
g	gram
ITS	Internal transcribed spacer
M	Molar
mA	milliamps
mL	millilitre
ML	Maximum-Likelihood
mm	millimetre
mm^3	cubic millimetre
MPN	Most probable number
OTU	Operation taxonomic unit
PCR	Polymerase Chain Reaction
PP	Posterior probability

ppt	Parts per thousand
rDNA	Ribosomal Deoxyribonucleic Acid
rpm	revolutions per minute
TEM	Transmission Electron Microscopy
V	volt
µm	micrometre
µmol m^{-2} s^{-1}	micromole per second and square meter

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DIVERSIFIKASI FENOTIPIK TERESTRIAL SINOBAKTERIA DARIPADA HABITAT TERPILIH DI PULAU SIGNY, PULAU ORKNEY SELATAN, ANTARTIKA

ABSTRAK

Satu kajian floristik telah dijalankan ke atas sinobakteria daripada habitat terestrial di Pulau Signy (60.7170° S, 45.6000° W), Pulau Orkney Selatan, Antartika pada musim panas 2015/2016, semasa ekspedisi Antartika bersama British Antarctic Survey (BAS). Sampel telah diambil mengikut 120 lokasi yang telah direkodkan oleh Broady (1979). Tiga puluh tujuh daripada 120 lokasi persampelan yang telah direkodkan oleh Broady (1979) berjaya diperolehi dan dibandingkan untuk kajian ini. Perubahan komuniti sinobakteria yang telah direkodkan oleh Broady (1979) akan memberikan maklumat asas mengenai status kepelbagaian dan penyebaran mikroflora Antartika. Ini sangat penting untuk memahami bagaimana kehidupan Antartika bertindak balas terhadap perubahan persekitaran semasa dan bagaimana benua pada masa yang lalu. Kajian ini merangkumi pendekatan taksonomi morfologi tradisional dan molekul moden yang memberikan pandangan baru mengenai sinobakteria. Tujuh belas morfospesies daripada 14 genera: *Chamaesiphon, Cyanosarcina, Desmonostoc, Leptolyngbya, Microcoleus, Nodosilinea, Nostoc, Oscillatoria, Phormidium, Phormidesmis, Pseudanabaena, Synechocystis, Trichocoleus* dan *Wilmottia* telah direkodkan dalam kajian ini. Kajian ini telah merekodkan sepuluh morfospesies yang menyamai rekod terdahulu yang diperolehi oleh Broady (1979). Tujuh morfospesies baru telah direkodkan dari Pulau Signy; *Leptolyngbya* cf. *subcapitata, Nodosilinea signiensis, Oscillatoria* cf. *subsala, Phormidium uncinatum, Trichocoleus* cf. *hospitus, Wilmotia murrayi* Morfotip 1 dan

Wilmottia murrayi Morfotip 2. Penemuan spesis baru daripada genus *Nodosilinea, Nodosilinea signiensis* sp. nov. R. Radzi & F. Merican 2019 telah dilaporkan berdasarkan klasifikasi polifasa yang telah dijalankan keatas strain di dalam kultur. Kedudukan *Nodosilinea* ini terpisah daripada spesis *Nodosilinea* yang lain di dalam pokok filogenetik 16S rDNA. Secara morfologi, strain ini juga menunjukkan perbezaan terutamanya dari segi saiz sel, bentuk sel, pengecilan filamen, morfologi sarung dan granulasi. Helix DI-D1' dari analisis 16S-23S ITS juga menunjukkan bahawa *N. signiensis* secara genetik berbeza daripada spesies *Nodosilinea* lain yang telah direkod sebelum ini. Dua morfotip sinobakteria telah diasingkan dan dikategorikan dengan menggunakan pendekatan polifasa. Kedua-duanya menunjukkan ciri morfologi yang sama dengan genus *Phormidium*, khususnya dengan trikom yang ringkas, uniseriat dan tidak bercabang. Walaubagaimanapun, analisis filogenetik 16S rDNA menunujukkan bahawa mereka jelas berbeza daripada klad *Phormidium*. Kedua-dua morfotip dikelompokkan dalam klad utama *W. murrayi*. Menariknya, klad utama *W. murrayi* telah membentuk dua kelompok yang memisahkan spesies dari kawasan sejuk daripada spesies di kawasan tropika. Sehingga kini, genus ini hanya mengandungi tiga spesies, *W. murrayi, W. stricta* dan *W. koreana.* Dalam kajian ini, penemuan kedua-dua *W. murrayi* M1 dan M2 merupakan rekod pertama yang dilakukan di Pulau Signy. dan ini telah memperluaskan taburannya di Antartika. Oleh itu, morfospesies yang telah direkodkan dalam kajian ini secara signifikannya telah memberikan maklumat yang berharga terhadap kepelbagaian dan penyebaran sinobakteria terrestrial di Antartika.

PHENOTYPIC DIVERSIFICATION OF TERRESTRIAL CYANOBACTERIA FROM SELECTED HABITATS ON SIGNY ISLAND, SOUTH ORKNEY ISLANDS, ANTARCTICA

ABSTRACT

A floristic study was conducted on cyanobacteria from terrestrial habitats in Signy Island (60.7170° S, 45.6000° W), South Orkney Islands during the expedition of British Antarctic Survey (BAS) in austral summer of 2015/2016. Samples were collected following the previous 120 locations that was provided by Broady (1979). Thirty seven out of 120 collection sites established by Broady (1979) were successfully obtained and compared in this study. Changes in the cyanobacteria community at sites previously studied by Broady (1979) will provide excellent baseline information on the status of Antarctic terrestrial cyanobacteria diversity and dispersal. It is crucial to understand how the Antarctic life form is responding to current environmental change and what the continent was like in the past. This study has integrated traditional morphological and modern molecular taxonomic approaches providing new insights into the taxonomy of Antarctic cyanobacteria. Seventeen morphospecies belonging to 14 genera; *Chamaesiphon, Cyanosarcina, Desmonostoc, Leptolyngbya, Microcoleus, Nodosilinea, Nostoc, Oscillatoria, Phormidium, Phormidesmis, Pseudanabaena, Synechocystis, Trichocoleus* and *Wilmottia* have been recorded with robust descriptions by using streaking and most probable number techniques. Ten morphospecies recorded were similar to records by Broady (1979). Seven of the morphospecies; *Leptolyngbya* cf. *subcapitata, Nodosilinea signiensis, Oscillatoria* cf. *subsala, Phormidium uncinatum, Trichocoleus* cf. *hospitus, Wilmotia murrayi* M1 and *Wilmottia murrayi* M2 are new

records for Signy Island. A novel species of *Nodosilinea*, *Nodosilinea signiensis* sp. nov. R. Radzi & F. Merican 2019 have been reported based on a polyphasic assessment of the strain in culture. This member of the genus *Nodosilinea* is well separated from the other *Nodosilinea* species in the 16S rDNA phylogenetic tree. Morphologically, the strain also showed difference in its morphological characteristics especially in cell size, cell shape, filament attenuation, sheath morphology and granulation. The D1-D1' helix of the 16S – 23S ITS region analyses showed that *N. signiensis* is genetically distinct from other recorded species of *Nodosilinea.* Two cyanobacterial morphotypes were also successfully isolated and characterized using a polyphasic assesment. Both showed similar morphological characteristic to the genus *Phormidium*, in particular with simple, uniseriate and unbranched trichomes. However, 16S rDNA phylogenetic analyses showed a clear separation from members in the *Phormidium*. Both strains were grouped within the major clade of the *Wilmottia murrayi*. Interestingly, there are two lineages within the major clade of *W. murrayi* separating tropical species with those from the cold regions. To date, this genus at present contains only three species, *W. murrayi*, *W. stricta* and *W. koreana.* In this study, the occurrence of both *W. murrayi* M1 and M2 were first described from Signy Island. Therefore, the morphospecies recorded in this study have significantly provide a valuable information towards the diversity and dispersal of terrestrial cyanobacteria in Antarctica.

CHAPTER 1

GENERAL INTRODUCTION

1.1 The phylum Cyanobacteria

Cyanobacteria or blue-green algae are an ancient group that has existed on the Earth from 3.5 billion years ago (Knoll, 2008; Rasmussen *et al.*, 2008). The largest known calcareous cyanobacteria microbialites were discovered in Lake Van, Turkey, and are believed to be 700 million years old (Kempe *et al.*, 1991). Fossil evidence of cyanobacterial mats existing 440 million years ago from Early Silurian in Virginia USA indicate that cyanobacteria were the dominant colonizers in the oceans (Tomescu *et al.*, 2006). Examples of these ancient fossils have included both unicellular and multicellular morphotypes (Amard & Bertrand-Sarfati, 1997), some having specialized cells (Graham & Wilcox, 2000). For eons, they have been present in most sun-exposed environments on Earth (Whitton & Potts, 2012) and were the sole photosynthesizers generating oxygen (Seckbach & Oren, 2007; Broady & Merican, 2012). Cyanobacteria were key in the "Great Oxygenation Event" 3 billion years ago allowing the rise of other eukaryotic organisms (Bekker *et al.*, 2004; Blakenship, 2010).

Oxygenic phototrophic cyanobacteria are well-known for their ability to survive and thrive in a wide range of conditions of pH, salinity, radiation and temperature (Seckbach & Oren, 2007). Cyanobacteria have been recorded to be present in acidic lakes, Bavaria with pH as low as pH of 2.9 (Steinberg *et al.*, 1998). Some members of the group can tolerate high salt concentrations and are able to grow in salinity up to

180 ppt in Solar Lake, Sinai (Padan, 1979; Padan & Cohen, 1982). The highest temperature at which active growth of cyanobacteria has been recorded is 84°C in Yellowstone (Copeland, 1936; Papke *et al.*, 2003; Yilmaz-Sariozlu & Yilmaz-Cankilic, 2018). Cyanobacteria are abundant in cold environments such as in both Polar Regions, where they occur both in and on ice and snow, in freshwater and saline lakes and streams, in soils and below and within rocks (Taton *et al.*, 2003; Vincent & Quesada, 2012).

1.2 Antarctic region

Antarctica has been physically isolated from the rest of the world since its separation from southern South America in the final stages of the breakup of Gondwana 30-35 million years ago and the subsequent formation of the oceanic Antarctic Polar Front around 18 million years ago (Clarke *et al.*, 2005; Convey *et al.*, 2008; 2018). It hosts amongst the most extreme environments on Earth, with persistent low temperatures, associated fluctuations in water availability and desiccation, exposure to repeated freeze-thaw cycles and highly variable light environment (Vincent, 2000; Namsaraev *et al.*, 2010). According to Vincent & Quesada (2012), cyanobacteria often dominate extreme cold environments, occupying restricted habitats such as under or within rocks, where they are physically protected to some degree from the harsh ambient conditions. Cyanobacteria are well suited to the cryosphere because of their broad tolerance towards low temperatures with the added ability to survive prolonged dormancy, and their resistance to many natural environmental stressors (Seckbach & Oren, 2007).

Cyanobacteria taxonomy and diversity in Antarctica is currently uncertain, despite the acknowledged functional importance of the group (Broady, 1996; Vincent, 2000; Vincent & Quesada, 2012). There is evidence that some cyanobacteria taxa are endemic to Antarctica (e.g. Taton *et al.*, 2006; Komárek *et al.*, 2008). However, Jungblut *et al.*, (2010) reported that Antarctic cyanobacteria genetic diversity was similar by (>99 %) to that was present in Arctic and Alpine regions. Some studies have also reported that strains isolated from both Polar Regions overlap geographically with others isolated from temperate regions (Vincent & Quesada, 2012). The application of molecular tools to Antarctic communities, such as the use of PCR fingerprinting and phylogenetic analyses such as of the 16S rDNA and 16S – 23S ITS are of particular utility in determining the genetic relationships of Antarctic cyanobacteria communities (Vincent, 2000). Utilization of these techniques will help reveal the true diversity of cyanobacteria inhabiting the continent.

Not all parts of the continent have been subjected to a thorough cyanobacteria diversity assessment. Most of the studies on cyanobacteria to date have been centred on Antarctic Peninsula, West Antarctica and Ice shelves (Šabacká, 2004; Komárek & Anagnostidis, 2005; Casamatta *et al.*, 2005; Taton *et al.*, 2006; Comte *et al.*, 2007; Strunecký *et al.*, 2011). These studies have been conducted using various methods; light microscopy (Broady, 1979; Komárek, 2007) and molecular genetics (Taton *et al.*, 2006; Strunecký *et al.*, 2012). Only recently, studies have been conducted by utilizing the combination of both morphological and molecular assessment in determining the identity of cyanobacteria present in Antarctica (Comte *et al.*, 2007; Taton *et al.*, 2011; Strunecký *et al.*, 2011; Radzi *et al.*, 2019). Hence, more work is required to achieve a full inventory of all the species present in Antarctica.

1.3 Project aims and an overview of the structure of this thesis

This study examined in detail the present-day diversity of terrestrial cyanobacteria on Signy Island, South Orkney Islands, maritime Antarctic. The findings were compared with prior detailed records from the 1970s from the same island by Broady (1979), a unique opportunity to make inferences about changes over time in Antarctic microbial communities. Descriptions of the cyanobacteria diversity in terrestrial habitats across the island were compiled.

Chapter 2 describes the diversity of cyanobacteria found on the island. Morphospecies diversity was assessed through light microscopy of cultures derived from a range of newly collected soil samples and from mixed cultures established in Antarctica.

Chapter 3 considers the molecular phylogenetic of selected cyanobacteria. It recognises the need for use of polyphasic approaches in order to fully characterize and identify specific morphospecies. Ultrastructural analyses, 16S rDNA phylogenies, and 16S-23S internal transcribed spacer (ITS) compositions were completed for three selected morphotypes and integrated with identification made using traditional morphological approaches (Chapter 2).

The study has considerably increased knowledge of terrestrial cyanobacteria in Signy Island, building on the baseline provided by Broady (1979). Chapter 4 presents a short concluding discussion for the study, with suggestions for further work.

CHAPTER 2

THE DIVERSITY OF TERRESTRIAL CYANOBACTERIA

2.1 Introduction

2.1.1 Prior research on terrestrial cyanobacteria in Antarctica

Cyanobacteria are an ancient group of photosynthesising prokaryotes found in most sun-exposed ecosystems on Earth (Whitton & Potts, 2012), including Antarctica. Cyanobacteria are present in all habitats in high latitude environments, including soils, rocks, glaciers, ice shelves, streams, ponds and lakes (Vincent, 2000). The first discovery of Antarctic cyanobacteria was made during the Shackleton's expedition (1907-1909) to Ross Island, Antarctica (Murray, 1910), which documented the presence of benthic mats consisting of filamentous cyanobacteria under the ice of a frozen lake. More discoveries on the occurrence of cyanobacteria were made during Scott's Terra Nova Expedition 1910-1913 to Ross Island and Victoria Land (Fritsch, 1917). These identified 12 morphospecies of cyanobacteria including *Anacystis marginata, Chroococcus minutus, Gleocapsa shuttleworthiana, Merismopedia tenuissima, Microcystis marginata, Nostoc fuscescens, Phormidium autumnale, P. fragile, P. laminosum, P. priestleyi, Oscillatoria autumnalis* and *Schizothrix antarctica*, mainly from freshwater lakes at Cape Sustruzi and Cape Adare (Figure 2.1). These earlier collections of cyanobacteria from Antarctica gave the first confirmation of the presence of the group in this environmentally challenging continent.

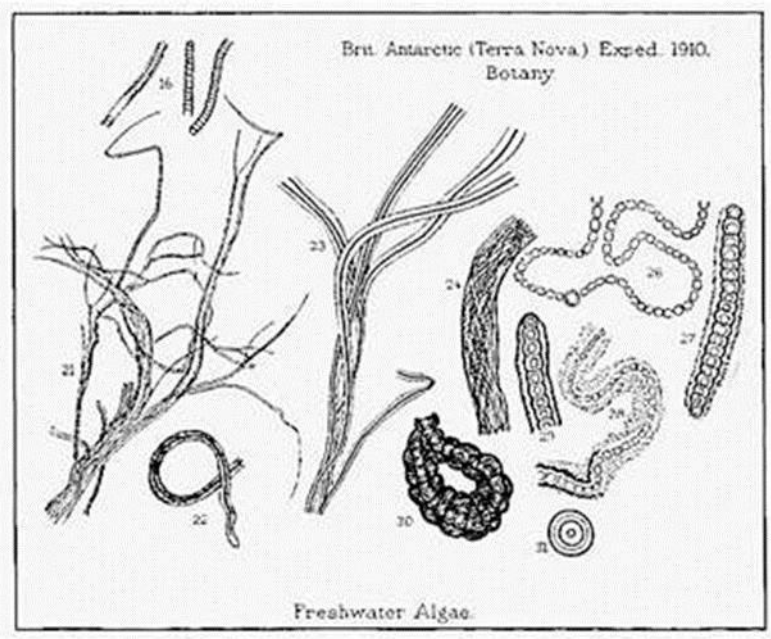

Figure 2.1: Drawings of cyanobacteria by Fritsch collected from Ross Island, Antarctica during Scott's Terra Nova expedition (Fritsch, 1917).

Less than 0.3% of Antarctic land surface area is free of ice, with this area mostly consisting of frigid deserts, nunataks (mountain summits protruding through surrounding ice sheets) and coastal oases (Convey, 2017). Previous studies have indicated that cyanobacteria are the dominant photosynthesising microorganisms in the regions of Antarctica that are ice-free during the polar summer (Broady, 1989; Pandey *et al.*, 1992; 1995). Recent warming trends across the Antarctic Peninsula have been reported to contribute to the expansion of vegetated areas, as well as changing the distribution patterns throughout this region (Fowbert & Smith, 1994; Smith, 1994; Convey, 2003; Convey & Peck, 2019).

According to Vincent (2000), cyanobacteria are amongst the most widely distributed microorganisms in Antarctic soils, and are primary colonizers as ice and snow cover retreat. They play a vital role in soil stabilization, photosynthetic carbon fixation and

release of fixed nitrogen, whilst forming the base of the terrestrial food web (Vincent, 2000; Gaydon et al., 2012). They can form visible dark crusts on the rock (epilithic) or thin biofilms under translucent rock (hypolithic) and even grow within rock fissures (chasmoendolithic) in Antarctic terrestrial ecosystems (Friedmann, 1982; Broady, 1996; Mur *et al.*, 1999; Vincent, 2000; Vincent & Quesada, 2012). *Nostoc commune* was reported from terrestrial habitats of Ross Island and Victoria Land by Holm-Hansen (1963). *Schizothrix calcicola* was recorded from the soil of cinder cones on Deception Island, South Shetland Islands (Cameron & Benoit, 1970). However, despite the existing work on terrestrial cyanobacteria in Antarctica, the overall diversity of the group in terrestrial habitats remains poorly unknown. Several hundred species of cyanobacteria have been recorded from terrestrial habitats worldwide. However, in Antarctica, less attention has been given to assessing terrestrial cyanobacteria diversity other than pioneering studies such as those of Broady (1979), Castenholz (1992), Davey & Clarke (1992), Broady & Weinstein (1998) and Nadeau *et al.* (2001), which mostly focussed on specific locations.

In the Antarctic maritime, the first collection of cyanobacteria was made from wet soils, moss-covered rocks, damp moss and mud from a penguin rookery on Laurie Island, South Orkney Islands, during the Scottish National Antarctic Expedition 1902-04 (Fritsch, 1912). Fritsch (1912) recorded the presence of approximately 18 morphospecies of cyanobacteria; *Synechococcus aeruginosa*, *Entophysalis granulosa, Aphanothece saxicola*, *Microcystis olivacea, M. merismopedia, Clathrocystis reticulate, Gomphosphaeria aponina, Coelosphaerium kutziangianum, Merismopedia glaucum, M. tenuissimum, Oscillatoria brevis, O. splendida, O. subtilissima, O. tenuis, Spirulina subtilissima*, *Isocystis infusionum, Nostoc minutissimum,* and *Calothrix*

aeruginea. Although the early expeditioners initiated studies on the maritime Antarctic islands, subsequent studies have been limited. The only extensive study of terrestrial cyanobacteria was that of Broady (1976, 1977, 1979a, b). He investigated samples from over 120 sites around Signy Island, also in the South Orkney Islands, and reported the presence of 49 morphospecies of cyanobacteria. The family Oscillatoriaceae was the most prominent cyanobacteria family recorded.

2.1.2 Approaches to cyanobacteria taxonomy

There have been numerous approaches to the taxonomy of cyanobacteria. The 'traditional approach' in cyanobacteria taxonomy developed in the 19th century. At that point, cyanobacteria were classified solely based on morphological features of field-collected samples observed under light microscopy (Geitler, 1932; Desikachary, 1959). Samples with similar phenotypic appearance were placed in the same species. Almost 2000 morphospecies in about 150 genera have been described this way (Waterbury, 2006; Broady & Merican, 2012). Drouet (1968, 1978, 1981) later reviewed the morphological classification approach, and proposed that many morphospecies recorded previously were invalid due to phenotypic plasticity in response to environmental variation. This reduced the number of cyanobacteria morphospecies recognised drastically (Castenholz, 2001) and led to confusion in identification as well as loss of ecological information (Whitton, 2008).

Later, Stanier *et al.* (1971), a bacteriologist who was convinced that cyanobacteria are bacteria established an approach that includes morphospecies described from cultures. This "bacteriological approach" was based on morphological, physiological and

genetic characteristics of axenic and clonal strains. Although providing additional information that can be very useful in identification, this approach was later found to be biased towards cultured morphospecies (Whitton, 2011). The use of bacteriological nomenclature further complicated the taxonomy of the group as it conflicted with earlier classifications that were based on the International Code of Botanical Nomenclature (ICBN) (Oren, 2004).

At present, a combination of data from morphological, ultrastructural, ecological and molecular evaluation are being used in the renaming and reorganisation of families and genera (Anagnostidis & Komárek 1999; Komárek & Anagnostidis 2005; Komárek, 2013, 2014, 2016, 2018). This is known as a polyphasic approach (Anagnostidis & Komárek 1999; Komárek & Anagnostidis 2005; Komárek, 2013, 2014, 2016, 2018). This wide-ranging approach enhances the credibility of species identification for cyanobacteria (Yu *et al.*, 2015). Polyphasic approaches in cyanobacteria classification are now widely used to define new taxa (Komárek *et al.*, 2014; Komárek, 2016, 2018; Mares, 2018). Based on this approach, the phylum Cyanobacteria is currently divided into eight orders as shown in Table 2.1 (Komárek *et al.*, 2014; Komárek, 2016, 2018).

Table 2.1: Cyanobacteria classification system for Orders and Families (Komárek *et al.*, 2014; Komárek, 2016, 2018).

No.	Order	Families
I	Gloeobacterales	Gloeobacteraceae
II	Synechococcales	Synechococcaceae, Merismopediaceae, Prochloraceae, Coelosphaeriaceae, Acaryochloridaceae, Chamaesiphonaceae, Romeriaceae, Pseudanabaenaceae, Leptolyngbyaceae, Heteroleibleiniaceae, Schizotrichaceae
III	Spirulinales	Spirulinaceae
IV	Chroococcales	Microcystaceae, Aphanothecaceae, Cyanobacteriaceae, Cyanothrichaceae, Stichosiphonaceae, Chroococcaceae, Gomphosphaeriaceae, Entophysalidaceae
V	Pleurocapsales	Hydrococcaceae, Dermocarpellaceae, Xenococcaceae, Pleurocapsaceae,
VI	Oscillatoriales	Cyanothecaceae, Borziaceae, Coleofasciculaceae, Microcoleaceae, Homoeotrichaceae, Oscillatoriaceae, Gomontiellaceae
VII	Chroococcidiopsidales	Chroococcidiopsidaceae
VIII	Nostocales	Scytonemataceae, Symphyonemataceae, Rivulariaceae, Tolypothrichaceae, Godleyaceae, Chlorogloeopsidaceae, Hapalosiphonaceae, Capsosiraceae, Stigonemataceae, Gloeotrichiaceae, Aphanizomenonaceae, Nostocaceae

Up-to-date information on cyanobacteria diversity can be retrieved from AlgaeBase (Table 2.2). This is an online database resource for algae that provide free access to the latest authoritative information on taxonomic, distributional and nomenclatural data (Guiry & Guiry, 2019).

Table 2.2: Most recent cyanobacteria classification system for Orders and Families retrieved from AlgaeBase (Guiry & Guiry, 2019).

Order	Families
Chroococcales	Aphanothecaceae, Chroococcaceae, Cyanobacteriaceae, Cyanothrichaceae, Entophysalidaceae, Microcystaceae, Stichosiphonaceae
Chroococcidiopsidales	Chroococcidiopsidaceae
Gloeobacterales	Gloeobacteraceae
Gloeomargaritales	Gloeomargaritaceae
Nostocales	Aphanizomenonaceae, Capsosiraceae, Cyanomargaritaceae, Dapisostemonaceae, Fortieaceae, Geitleriaceae, Gloeotrichiaceae, Godleyaceae, Hapalosiphonaceae, Microchaetaceae, Nostocaceae, Nostochopsidaceae, Rhizonemataceae, Rivulariaceae, Scytonemataceae, Stigonemataceae, Symphyonemataceae, Tolypothrichaceae
Oscillatoriales	Ammatoideaceae, Borziaceae, Camptothricaceae, Coleofasciculaceae, Cyanothecaceae, Desertifilaceae, Gomontiellaceae, Homoeotrichaceae, Microcoleaceae, Oscillatoriaceae, Phormidiaceae
Pleurocapsales	Dermocarpellaceae, Hydrococcaceae, Hyellaceae, Xenococcaceae
Pseudanabaenales	Schizotrichaceae
Spirulinales	Spirulinaceae
Synechococcales	Acaryochloridaceae, Chamaesiphonaceae, Coelosphaeriaceae, Heteroleibleiniaceae, Leptolyngbyaceae, Merismopediaceae, Oculatellaceae, Prochlorotrichaceae, Pseudanabaenaceae, Romeriaceae, Trichocoleusaceae

2.2 Objectives

The diversity of cyanobacteria morphospecies have been investigated from 37 sites from Signy Island, South Orkney Islands, maritime Antarctic, to achieve the following aims:

1. To provide robust descriptions of morphospecies, using selected culture techniques to aid identification.
2. To compare morphospecies recorded from this study with previous records provided by Broady (1979).

2.3 Materials and Methods

2.3.1 Study Sites

A study was conducted on cyanobacteria obtained from terrestrial habitats on Signy Island (60° 43.02'S, 45° 36.00'W), South Orkney Islands, during British Antarctic Survey (BAS) supported fieldwork in the austral summer of 2015/2016. The samples were collected from previously sampled locations as listed by Broady (1979) (Figure 2.2).

Due to Malaysian import permit conditions that allow a maximum of only 1 kg to be imported, only samples from 37 sites that are similar with Broady (1979) (Table 2.3 and Figure 2.2) were transported back to the laboratories of Universiti Sains Malaysia. The remaining soil samples are held at the British Antarctic Survey.

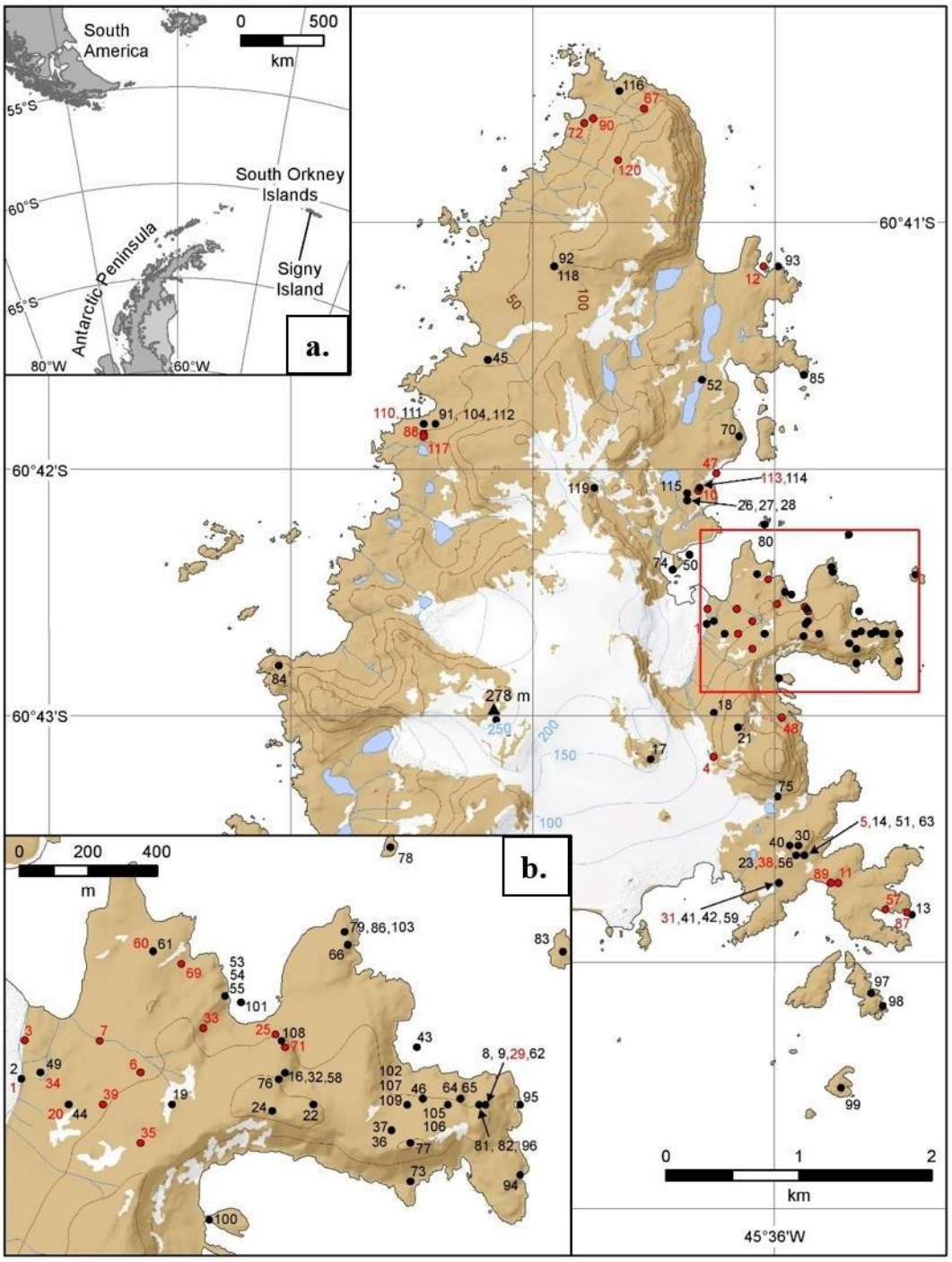

Figure 2.2: Map showing the study sites on Signy Island, South Orkney Islands, Antarctica; a., Location of Signy Island, South Orkney Islands, in relation to the Antarctic Peninsula and southern South America. **b.,** Enlargement of sampling areas within the red box on the main figure. All dot represented the total of 120 sites that were previously collected by Broady (1979). Red dots indicated 37 sites present collection. Map prepared by Laura Gerrish (BAS).

Table 2.3: Location and description of the sampling sites with site numbers for sites that were resampled in the 2015/16 season.

Num.	Site Number	Locality	Coordinates	Description
1.	**1**	Lateral moraine of Orwell Glacier	60° 42.63'S, 45° 36.56'W	Mineral soil
2.	**3**	Northern end of lateral moraine of Orwell Glacier in Cemetery Bay	60° 42.57'S, 45° 36.55'W	Moss
3.	**4**	Col between Garnet Hill and Rusty Bluff	60° 43.17'S, 45° 36.50'W	Lichen on soil
4.	**5**	Summit of hill south-west of Rethval Point	60° 43.57'S, 45° 35.75'W	Fine soil
5.	**6**	West-facing slopes at northern end of Moraine Valley	60° 42.62'S, 45° 36.18'W	Fine soil
6.	**7**	North west-facing slope above east side of Cemetery Bay	60° 42.57'S, 45° 36.31'W	Fine soil / lichen on rock
7.	**8**	Pinder Gully	60° 42.67'S, 45° 35.08'W	Raw fine-grained mineral soil on rock.
8.	**9**	Pinder Gully	60° 42.67'S, 45° 35.08'W	Mineral soil mixed with organic debris
9.	**10**	Summit of marble knoll above eastern slopes of Three Lakes Valley	60° 42.09'S, 45° 36.63'W	Nodular marble soil
10.	**11**	Summit of marble outcrop on Gourlay Peninsula	60° 43.68'S, 45° 35.47'W	Nodular marble soil
11.	**12**	Sea-washed rock in Tern Cove	60° 41.18'S, 45° 36.09'W	Raw fine-grained mineral soil on rock
12.	**20**	West-facing slope of Moraine Valley	60° 42.67'S, 45° 36.41'W	Moss soil
13.	**25**	South-east coast of Factory Cove	60° 42.56'S, 45° 35.75'W	Moss pale blue-green
14.	**29**	North-facing slope below Observation Bluff	60° 42.67'S, 45° 35.08'W	Cushion of *Pottia austro-georgica* Card. From immediately below the nest of a cape pigeon
15.	**31**	Hillier moss, bordering site	60° 43.68'S, 45° 35.96'W	Moss/soil
16.	**33**	Summit of cliffs above west side of Factory Cove	60° 42.55'S, 45° 35.98'W	Moss/soil
17.	**34**	Floor of Moraine Valley, near Orwell Glacier moraines	60° 42.62'S, 45° 36.50'W	Moss/soil

18.	**35**	West-facing slopes of Moraine Valley	60° 42.73'S, 45° 36.18'W	Moss/soil
19.	**38**	North-west facing slope below Rethval Point	60° 43.57'S, 45° 35.82'W	*P. alpestre* bordering a small melt runnel
20.	**39**	Northern end of Moraine Valley	60° 42.67'S, 45° 36.30'W	Moss/soil
21.	**47**	Slope north-west of Waterpipe Beach	60° 42.02'S, 45° 36.48'W	Moss/soil/lichen
22.	**48**	Lower slopes above Paal Harbour	60° 43.01'S, 45° 35.94'W	Moss
23.	**57**	Above Rock Haven, Gourlay Peninsula	60° 43.79'S, 45° 35.08'W	Moss/lichen
24.	**60**	Close to cemetery near Mooring Point	60° 42.43'S, 45° 36.14'W	*C. sarmentosum* carpet on which elephant seals had wallowed and killed the living moss surface
25.	**64**	North facing slope below Observation Bluff west of Pinder Gully	60° 42.66'S, 45° 35.16'W	Moss/soil
26.	**67**	North facing slope below Robin Peak	60° 40.54'S, 45° 37.08'W	Receiving some water from marble outcrops above moss
27.	**69**	Mooring Point	60° 42.45'S, 45° 36.05'W	Along the sloping sides of a temporarily water-filled melt runnel moss
28.	**71**	South-west of the British Antarctic Survey station; below Factory Bluffs	60° 42.58'S, 45° 35.72'W	Flushed with water run-off from cliffs above on which many nesting birds moss
29.	**72**	West side of Mirounga Flats cliffs 500m south-south-west of North Point	60° 40.60'S, 45° 37.57'W	Lichen on rock/no grass
30.	**87**	Pageant Point	60° 43.80'S, 45° 34.90'W	Deep gully in cliff. Algae/53.38m from real point real sample in water/sea
31.	**88**	Marble outcrop of Foca cave	60° 41.86'S, 45° 38.90'W	Aerial site: Exposed rock face Algae
32.	**89**	Marble outcrop Gourlay Peninsula	60° 43.68'S, 45° 35.53'W	Rock fragments small rock

33.	**90**	Marble outcrop 400m south of North Point	60° 40.58'S, 45° 37.50'W	Rock fragments subaerial alga mat
34.	**110**	Coastal rocks in Foca Cave	60° 41.82'S, 45° 38.90'W	Damp rock faces damp rock
35.	**113**	Shore of Foca Cove 100m west of Waterpipe Beach	60° 42.08'S, 45° 36.62'W	Algae on rock, cracks and cervices in rocks and beneath loose fragments of stone.
36.	**117**	North facing slope above Foca Cove	60° 41.87'S, 45° 38.90'W	Algae on rock, cracks and cervices in rocks and beneath loose fragments of stone.
37.	**120**	West facing slope below Robin Peak	60° 40.75'S, 45° 37.29'W	Algae on rock, cracks and cervices in rocks and beneath loose fragments of stone.

2.3.2 Sample collection

Samples were collected by Dr. Japareng Lalung, who participated in the expedition with British Antarctic Survey (Figure 2.3). Samples consisted of moss, soil, lichen and small rocks were collected from sites as close as possible to the original location that remained accessible. Collections were made of all visible growth of cyanobacteria. At each sampling location, about 10 cm^3 samples were collected and stored in zip-lock plastic bags (Figure 2.3a).

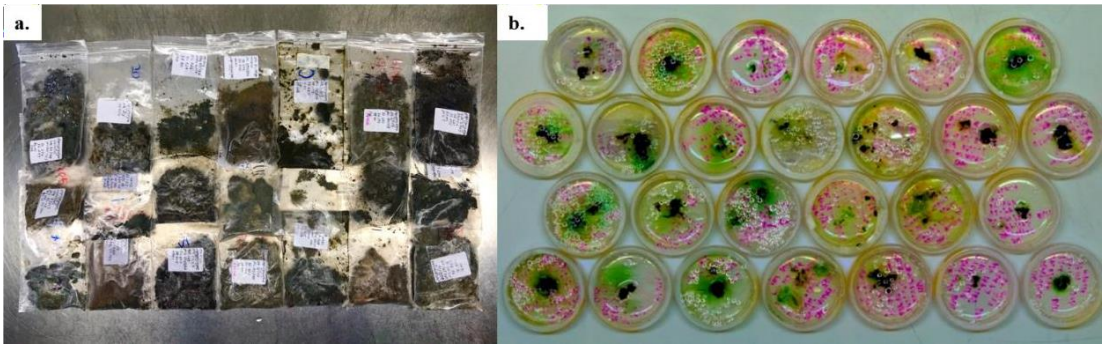

Figure 2.3: Sample collection from Signy Island; a. soil samples stored in zip-lock plastic bags. **b.** culture establishment in Antarctica by using streaking technique.

2.3.3 Culture establishment in Antarctica

While on Signy Island, Dr. Japareng Lalung managed to establish 27 mixed cultures by using streaking techniques (Figure 2.3b). Freshly collected field samples were used to inoculate these cultures. However, no further isolation was carried out by Dr. Japareng Lalung in Antarctica. Hence, the cultures received for this study are all mixed cultures from the initial inoculation of the field materials. Of these, only nine cultures were utilised by using light microscope in this study as these plates contained cyanobacteria. Both BBM and BG-11 media culture plates were used. The samples were kept frozen (-20 °C) in sterile containers during subsequent transport to the United Kingdom and then on to Malaysia.

2.3.4 Preparation of culture media in USM laboratory

Both BBM and BG-11 media were prepared. Distilled water was added at the end to make up to a final volume of 500 mL. Both were supplemented with 100 µg/ml cycloheximide to prevent growth of eukaryotes (Bolch & Blackburn, 1996).

Agar was prepared by adding 10 g of agar powder (NextGene, Malaysia) to 250 mL of distilled water. The prepared media were autoclaved in separate Schott bottles for both solutions of 500 mL of media and 250 mL of agar. Then, the two autoclaved solutions were cooled to approximately 58 °C and were aseptically mixed together in a Schott bottle. Then, 0.1 g of cycloheximide was added to 250 mL of distilled water. The cycloheximide solution was filter-sterilised using a 0.25 µm pore filter. Then, the 250 mL filtered cyloheximide solution was added into the agar mixture solution to give a final volume of 1 L medium. The prepared media were then poured into sterilised 90 mm Petri dishes and allowed to set. Once the agar had solidified, the plates were inverted and stored in airtight plastic bags at 4 °C. Agar preparation was carried out in a laminar flow cabinet for sterility.

2.3.5 Isolation techniques

In this study, two different isolation methods were used to obtain unialgal cultures. These were the streaking technique (Anderson & Kawachi, 2005) and the dilution technique (Meynell & Meynell, 1965).

2.3.5(a) Streaking technique

The cyanobacteria isolation process is similar to standard bacterial isolation methods. This method was used only for 27 mixed culture plates initially prepared in Signy Island by Dr. Japareng Lalung. First, a small quantity of sample was scraped from the culture plate by using a sterile streaking loop to examine the samples under the light microscope. Selected colonies were picked and introduced onto a new agar plate. The colonies were then spread across the agar plates by using the streaking loop to separate

the cells thoroughly. Plates were prepared in triplicates, and were sealed using Parafilm (Bemis, United State) to avoid evaporation. They were then incubated with the same settings as Broady (1979) for three to four weeks at 15 ± 2 °C with 24 h light supplied by cool white fluorescent lamps at 27 μmol m^{-2} s^{-1}. This process was repeated several times in order to achieve unialgal cultures.

2.3.5(b) Most probable number

The dilution technique is used to obtain cultures of algal species from field samples, commonly from mineral soil (Anderson & Kawachi, 2005). It is effective for organisms that are abundant in the sample but is largely ineffective for rare organisms (Kufferath, 1929; Droop, 1954; Meynell & Meynell, 1965; Throndsen, 1978; Anderson, 2005). This method was applied to the 37 sites of soil samples collected and returned from Signy Island. A schematic illustration of the approach is presented in Figure 2.4.

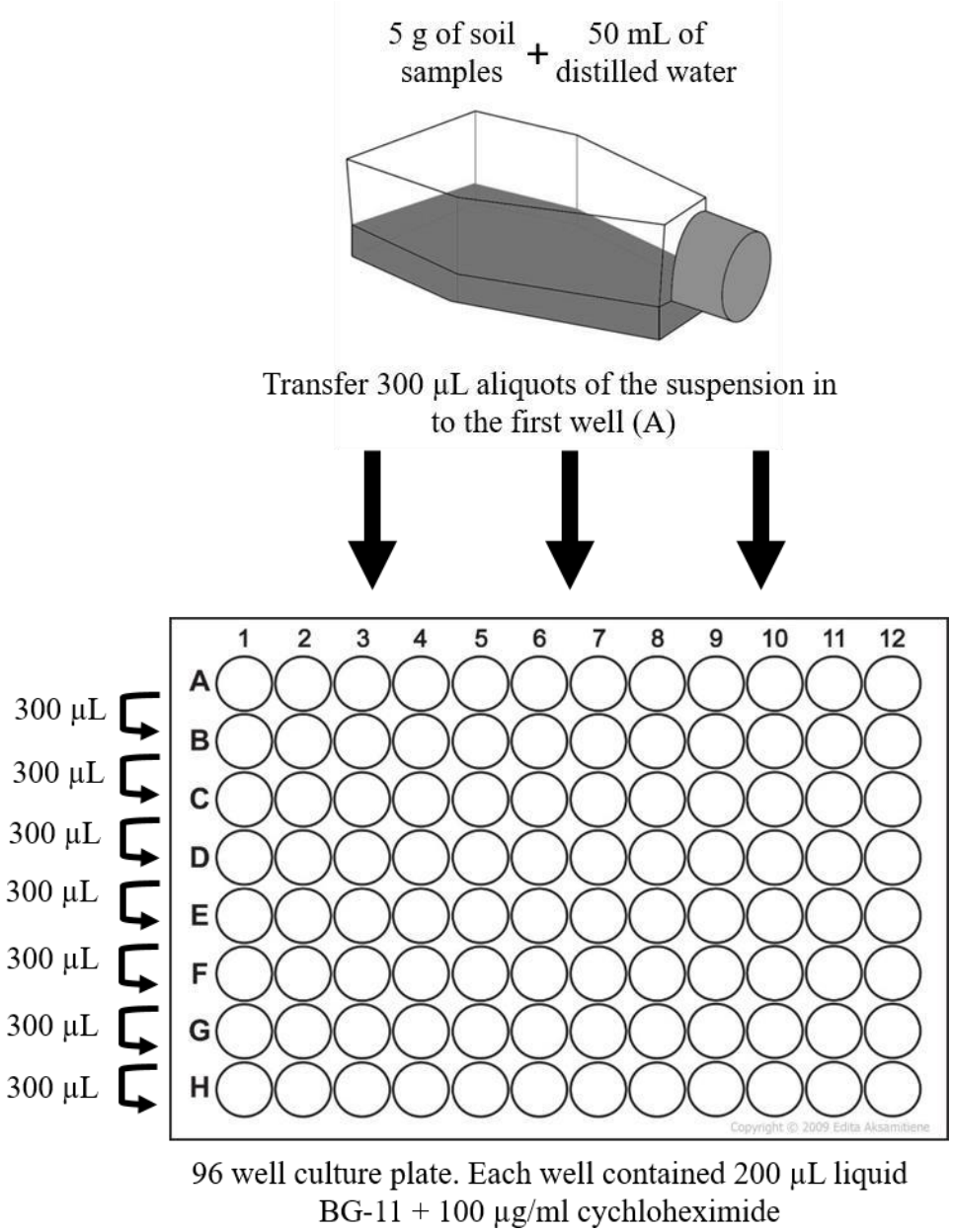

Figure 2.4: Illustration of the dilution technique; BG-11 + 100 µg/mL CHX was diluted by transferring 300 µL of the solution to the subsequent well containing BG-11 + 100 µg/mL CHX of the same volume, successively reducing the concentration by half in each well.

Five grams of each soil sample was placed into separate capped culture flasks seeded with 50 mL sterile distilled water. The flasks were constantly agitated using an orbital shaker (150 rpm) overnight at 15 ± 2°C to allow for disintegration of large soil particles and homogenous mixing of soil in the solution (Prasanna *et al.*, 2006). As an

alternative to glass tubes often employed in dilution techniques, 96 well plates were used in this study. Each well was seeded with 200 µL liquid BG-11 + 100 µg/ml CHX. Aliquots (300 µL) of the suspension formed above the soil were taken from the overnight incubation and mixed with the 200 µL liquid BG-11 + 100 µg/ml CHX following the standard dilution techniques. The procedure was carried out aseptically in a laminar flow cabinet. The sample was placed into the first well (well A). The solution was pipetted repeatedly in order to mix well. Then, 300 µL was removed from the first well (well A) and dispensed into the next well (well B) using a new sterile pipette tip for each dilution. The process was repeated until the last well (well H). Six replicates were prepared for each dilution, and each experiment was repeated three times. All 96 well plates were shaken at 150 rpm and were sealed with Parafilm to avoid contamination and evaporation. All cultures were incubated with the same setting as Broady (1979) at 15 ± 2 °C with 24 h light supplied by cool white fluorescent lamps at 27 µmol m^{-2} s^{-1}.

2.3.6 Identification of morphospesies

For cultures growing on agar, small sub-samples were scraped from the agar medium for slide preparation. For liquid cultures, wells that had visible growth were observed after one to two weeks incubation. Twenty microliter samples were taken for slide preparation. Cover slips were sealed on the slides using nail polish in order to avoid evaporation during observation.

Observations were made using an Olympus BX-53 light microscope (Olympus America Inc., Center Valley, PA, USA) at 100 – 2000x magnification.

Photomicrographs were taken and illustrations were made with the aid of a *camera lucida*. Each entire preparation was examined and all morphospecies of cyanobacteria present were recorded. All relevant morphological features were recorded, including trichome colours, vegetative cell width and cell length, cell shape, motility, constriction at the cross wall, apical cells, presence of granules and sheath morphology. Measurements of specialised cells, heterocytes and akinetes were also made. Size measurements were made on 30 randomly chosen replicate samples for each morphospecies.

Taxonomic assessment was based on Broady (1979), and Komárek & Anagnostidis (1999, 2005) and Komárek (2013). Where uncertainty existed after assessment, this is indicated by "cf." (Latin conferature = to compare with). This is used for samples that resemble existing records but are not identical based on the description given in the main taxonomic source.

2.4 Results

2.4.1 Overview of morphospecies recorded

Isolations from samples obtained from 20 of the 37 sampling sites showed good visible growth and were selected for light microscopy. Seventeen morphospecies including one Chroococcales, one Nostocales, three Oscillatoriales and six Synechococcales, were recognised from the isolations. Each morphospecies was assessed as rare (+) or dominant (++) (Table 2.4). Morphospecies were considered dominant when they contributed >60% of the cultures examined. Of the 17 morphospecies obtained, three

were successfully grown in unialgal culture and the remaining 14 in mixed cultures. Seven of the morphospecies recognised are new records for Signy Island.

Table 2.4: List of cyanobacteria currently recorded from Signy Island, including comparison with previous records from the same locations reported by Broady (1979). Cultures from modified BG-11 + CHX agar medium are indicated by (M), and most probable number techniques by (MPN). Rarely presence is indicated by (+) and dominantly presence by (++). Blank indicates absent.

Family	Species	Cultures obtain from this study		Broady (1979)
		M	MPN	
Chamaesiphonaceae	*Chamaesiphon subglobosus*		+	+
Chroococcaceae	*Cyanosarcina chroococcoides*		+	+
Merismopediaceae	*Synechocystis minuscula*		+	+
Nostocaceae	*Desmonostoc muscorum*		+	+
	Nostoc cf. *commune*		+	++
	Nostoc punctiforme		+	++
Microcoleacea	*Microcoleus autumnalis*		+	++
Oscillatoriaceae	*Oscillatoria* cf. *subsala*		+	
	Phormidium uncinatum		++	
Leptolyngbyaceae	*Leptolyngbya foveolarum*		++	++
	Leptolyngbya cf. *subcapitata*		+	
	Phormidesmis priestleyi		++	++
Pseudanabaenaceae	*Pseudanabaena* cf. *catenata*		+	++
Trichocoleusaceae	*Trichocoleus* cf. *hospita*	+		
Prochlorotrichaceae	*Nodosilinea signiensis*	+		
Coleofasciculaceae	*Wilmottia murrayi* Morphotype 1	+		
	Wilmottia murrayi Morphotype 2	++		

2.4.2 Description of morphospecies

Compilations of photomicrographs (characteristic details are shown with an arrow), *camera lucida* illustrations and descriptions for each morphospecies from 37 sites are given based on Table 2.4. Morphological identifications were based on the literature indicated for each morphospecies. Genera are listed alphabetically within the family. Occurrence details were recorded for each morphospecies and comparison are given with previous records from Broady (1979).

2.4.2(a) Family Chamaesiphonaceae

Chamaesiphon subglobosus **(**Rostafinski) Lemmerman: Figure 2.5a, b, e

Broady (1979): Page 22; Figure 2o-q

Komárek & Anagnostidis (1999): Page 390; Figures 487 & 509

Description: Cell solitary or aggregated in small, irregular colonies, which later form two or more layers, or with several cells in irregular clusters (Figure 2.5a). Cell spherical to ovoid, pale grey, pale blue-green or yellow-brown to dark brown, (2.8)3 – 4.2(5) µm wide and 4.8 – 6.0 µm long. Sheath (pseudovaginae) colourless or sometimes completely lacking in culture. Reproduction by exocytes, usually one or two (Figure 2.5b, e), which differentiate at the cell apex and are attached to mother cell.

Occurrence: Dominant in site 69. Recorded also from sites 6, 10, and 39. Previously recorded from site 6 (Broady, 1979).

Remarks: The sample is consistent with the description of *C. subglobosus* (Komárek & Anagnostidis (1999). However, the cell length recorded by Broady (1979) was greater (6 – 7 µm) than in the present material.

The taxon has previously been collected mainly from the southern polar region: streams in the Dry Valleys (Broady, 1982); periphyton of lakes, Hope Bay (Vinocur & Pizarro, 1995); lakes and ponds of Victoria Land (Tell *et al.*, 1995); felt and ice bubble of Lake Otero, Cierva Point (Mataloni *et al.*, 1998; Mataloni & Pose, 2001); terrestrial location in Victoria Land (Broady, 2005). Elsewhere has been recorded from freshwater lakes, streams, waterfalls, in unpolluted water, from lowlands to mountains, mainly in central Europe (Komárek & Anagnostidis, 1999).