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Green microalgae in polar lakes: diversity, biogeography and methodological comparison

Zelené mikrořasy v polárních jezerech: diverzita, biogeografie a porovnání metodiky

Diploma thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 15.7.2021 Bc. Tereza Šamšulová

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Abstrakt

V polárních oblastech jsou zelené řasy (Chlorophyta) důležitou skupinou primárních producentů. Doposud jim však byla věnována malá pozornost ve srovnání s jinými primárními producenty, jako jsou rozsivky a sinice. Cílem této práce bylo popsat diverzitu skupiny Chlorophyta v arktických a antarktických jezerech a získat nové poznatky o jejich taxonomickém složení a rozšíření.

Vzorky byly odebrány z litorální zóny 137 arktických a antarktických jezer z různých oblastí (Svalbard, severní Grónsko, jižní Grónsko, Antarktický poloostrov a kontinentální Antarktida) během expedic v letech 1993 až 2018. Zkombinovali jsme Sangerovo sekvenování izolovaných kmenů a amplikonové sekvenování přírodních vzorků. Pomocí obou molekulárních metod byly analyzovány markery 18S rDNA a ITS2 rDNA.

Získali jsme celkem 65 laboratorních kultur ze vzorků ze Svalbardu a Antarktického poloostrova. Pomocí Sangerova sekvenování těchto kmenů bylo odhaleno 15 Chlorophytních taxonů patřících do tříd Chlorophyceae a Trebouxiophyceae. Nejhojnější byly například *Chlorella* spp. a *Micractinium* spp. Pomocí amplikonového sekvenování 18S rDNA jsme získali celkem 202 OTU, z nichž 61.4 % bylo nalezeno v obou polárních oblastech. Použití ITS2 rDNA odhalilo celkem 1,104 OTU, přičemž 53.2 % se vyskytovalo jak v Arktidě, tak v Antarktidě. Alfa diverzita (OTU bohatost) byla v Arktidě mnohem vyšší (průměrně 106 OTU/vzorek) než v Antarktidě (průměrně 72), avšak tento trend se neprojevil v 18S rDNA datech. Analýzou sekvencí 18S rDNA bylo zjištěno, že nejhojnější třídou v Arktidě byla Ulvophyceae (56 % OTU), zatímco v Antarktidě převažovala třída Chlorophyceae (49 %). Podíl neklasifikovaných zelených řas byl velmi malý na rozdíl od ITS2 rDNA, kde bylo do této kategorie zařazeno zhruba 60 % OTU v obou polárních oblastech. Kanonická analýza odhalila významné oddělení jednotlivých biogeografických oblastí. Mezi nejčastější OTU s bipolární distribucí patřily např. *Chloromonas* sp. a *Ulothix zonata*, byly zde zastoupeny také druhy, které byly dříve považovány za endemické (např. *Chodatodesmus australis*, *Hazenia broadyi*). Hojně zastoupené byly také sněžné řasy (*Sanguina* spp., *Raphidonema nivale*). Počet taxonů detekovaných metodami závislými i nezávislými na kultivování byl poměrně nízký (13 taxonů), což ukazuje, že oba přístupy se navzájem doplňují. Pokud je nám známo, jedná se o první studii, která použila amplikonové sekvenování se skupinově specifickými primery k odhalení rozmanitosti zelených mikrořas v polárních jezerech.

Klíčová slova: polární oblasti, Arktida, Antarktida, Chlorophyta, biogeografie, amplikonové sekvenování

Abstract

In polar regions, green microalgae (Chlorophyta) are an important group of primary producers. However, little attention has been paid to them in comparison with other primary producers such as diatoms and cyanobacteria. The aim of this thesis was to describe the diversity of Chlorophyta in Arctic and Antarctic lakes, and to gain new insights into their taxonomic composition and biogeographic patterns.

Microbial mat samples were collected from the littoral zones of 137 Arctic and Antarctic lakes from various regions (Svalbard, North Greenland, South Greenland, Antarctic Peninsula, and Continental Antarctica) during expeditions performed between 1993 and 2018. We combined Sanger sequencing of isolated strains and amplicon sequencing of environmental samples. The 18S rDNA and ITS2 rDNA regions were analysed using both molecular methods.

In total, 65 laboratory cultures were established from Svalbard and Antarctic Peninsula samples. Sanger sequencing of strains revealed 15 taxa belonging to classes Chlorophyceae and Trebouxiophyceae. The most abundant were for instance *Chlorella* spp. and *Micractinium* spp. Amplicon sequencing of 18S rDNA yielded 202 OTUs in total, of which 61.4% of OTUs were present in both polar regions. The use of ITS2 rDNA revealed 1,104 OTUs in total, with 53.2% being present in both the Arctic and Antarctica. The alpha diversity (OTU richness) was much higher in the Arctic (average 106 OTUs/sample) than in Antarctica (average 72). However, this trend was absent when 18S rDNA data were considered. 18S rDNA data showed that the most abundant class in the Arctic was Ulvophyceae (56% of OTUs), while in Antarctica Chlorophyceae prevailed (49%). There was a very low amount of unclassified Chlorophyta in contrast to ITS2 rDNA (roughly 60% in both polar regions). Canonical analysis revealed high separation of samples from particular biogeographic regions. Among the most frequent OTUs with bipolar distribution (e.g., *Chloromonas* sp., *Ulothix zonata*) there were also species which were previously considered as presumably endemic (e.g., *Chodatodesmus australis*, *Hazenia broadyi*). Several species of snow algae were also abundant (*Sanguina* spp., *Raphidonema nivale*). Number of taxa detected by both culture dependent and independent methods was quite low (13 taxa). This shows that both approaches complement each other. To our knowledge, this is the first study that used amplicon sequencing with group specific primers to uncover green microalgae diversity in polar lakes.

Key words: polar regions, Antarctica, Arctic, Chlorophyta, biogeography, amplicon sequencing

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1 Introduction

The polar regions represent some of the most extreme conditions on Earth. They are characterized by generally low temperatures, large seasonal fluctuation in terms of light availability, and can even exhibit marked differences throughout the day (Mock & Thomas, 2008; Peck et al., 2005; Thomas et al., 2008). Despite the harsh conditions present there, these regions are rich with various types of lakes with an abundance of liquid water, which is an essential condition to any life on Earth.

Lakes in polar regions provide various, unique environmental conditions for biological communities, and can be collectively considered oases for life in these otherwise inhospitable environments. They represent a dynamic aquatic environment, and each of them can vary in chemical, physical and biological properties (Priscu et al., 1998; Vincent & Laybourn-Parry, 2008). There are many factors which affect biological production and composition of organisms, such as water and nutrient supply, temperature, distance from the sea, depth, size, irradiance, etc. This thesis will describe basic lake types of polar regions along with their primary characteristics.

Chlorophyta (green microalgae) show a great diversity, tolerance and adaptability to a wide range of environmental conditions. Thus, they are distributed in various types of ecosystems all over the globe and play a significant role in carbon, nitrogen and phosphorus cycles (Mock and Thomas 2008; Hong et al. 2015). In polar regions, microalgal communities exist as dominant life forms, and contribute a major component of the total ecosystem biomass (Broady 1996; Vincent 2006).

Green algae, together with cyanobacteria and diatoms (Bacillariophyceae), are important components of lakes in polar regions. However, in polar regions green algae are poorly studied in comparison to cyanobacteria and diatoms (Vyverman et al., 2010). It can be assumed based on Finlay's theory about the ubiquitous distribution of microorganisms, which is determined by their size (Finlay, 2002), that green microalgae, with their small size, simple morphology, and capability to adapt to various and extreme conditions, would be distributed worldwide. However, a higher degree of endemism is expected in Antarctica due to its greater isolation (Elster & Benson, 2004; Vyverman et al., 2010). This is supported, for example, by the study of De Wever et al. (2009) and newly discovered species, such as *Hazenia broadyi* or *Chloromonas polyptera*, that are so far found only in Antarctica (Sciuto et al., 2015; Škaloud

et al., 2013). However, more studies are needed to support or confirm the cosmopolitan distribution of green algae.

In the last few decades, molecular methods have provided us with much deeper insights into the diversity of microorganisms. Despite this, microscopy and culturing techniques still have its place among often used methods for the study of microorganisms (Izaguirre et al., 2015). Even Sanger sequencing and next-generation sequencing have their own limitations, despite their undisputed benefits. The choice of suitable sequencing markers for groups of studied organisms is also crucial.

These technological advances, and the lack of studies on the biodiversity in algae from the polar regions prompted the origin of this thesis. We studied 137 lake samples from various localities in the Arctic and Antarctic regions, which were examined by traditional and molecular methods. Sanger and amplicon sequencing of the 18S rDNA gene, and the more specific ITS2 rDNA marker, were used. A comparison of detected species by each method was also performed.

Aims and hypotheses:

Aims:

- Study diversity patterns, taxonomic composition, and provide insights into the biogeography of green microalgae (Chlorophyta) from microbial mats of lakes in polar regions with the use of Sanger sequencing of newly isolated strains and amplicon sequencing of environmental samples
- Compare 18S rDNA and ITS2 rDNA as molecular markers to study the diversity of Chlorophyta
- Compare the data obtained by Sanger and amplicon sequencing

Hypotheses:

- Higher biodiversity of green microalgae will be found in the Arctic than in Antarctica
- There will be fewer endemic species in the Arctic than in Antarctica
- Isolation of strains obtained from incubation will yield low number of species
- A low proportion of genera will be detected by both amplicon sequencing and a combination of culturing, strain isolation, and Sanger sequencing

2 Literature review

2.1 Lakes in polar regions

The polar regions[1] cover considerable parts of the Earth, and represent large areas with relatively extreme conditions. They are generally defined by low temperatures throughout the year with large seasonal fluctuations – continuous irradiance in summer and a lack of sunlight in winter, with substantial differences even throughout the day (Thomas et al., 2008). The regions also have the fastest changing climates on Earth (Peck et al., 2005; Seehaus et al., 2015)

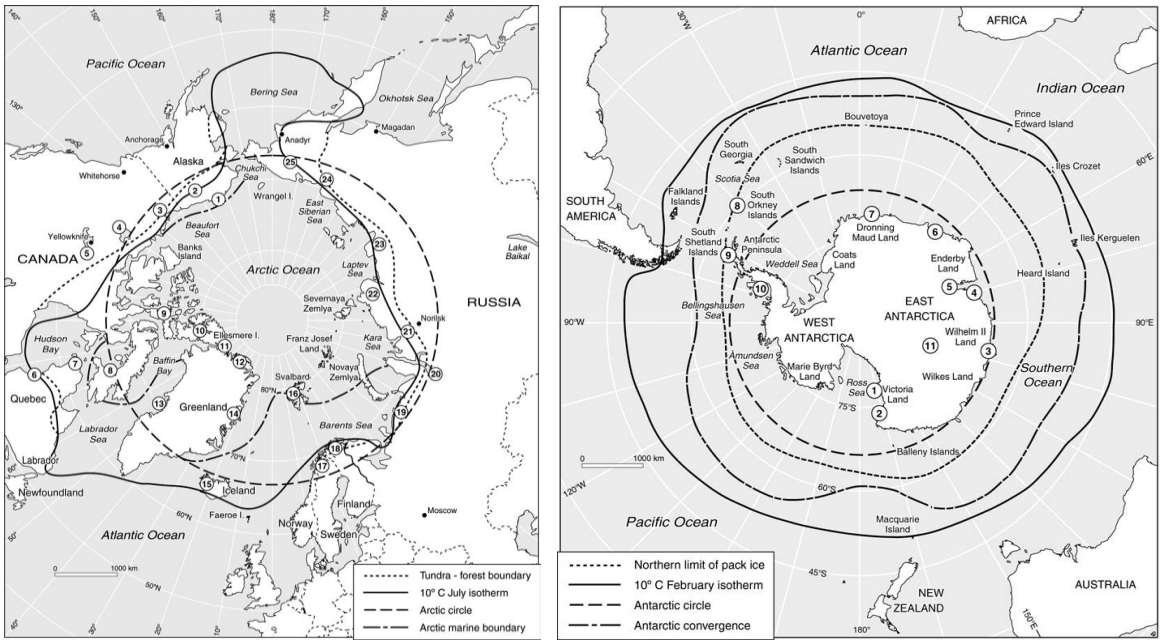

Fig. 1: Delimitation of Polar regions: the Arctic (left) and Antarctica (right, Vincent & Laybourn-Parry, 2008).

The availability of liquid water is a necessary condition for life (Vincent et al., 2009). In polar regions, this is strongly limited by sub-zero temperatures and freeze-thaw cycles. In deeper lakes, liquid water persists throughout the year under a thick layer of ice and a snow cover. There are many factors controlling biological production at high-latitude lakes, including

[1] Polar regions are not clearly defined. The Antarctic region is usually defined as an Antarctic continent which is surrounded by the Southern Ocean, but the Arctic can be defined by a variety of ways: by the northern treeline, the 10 °C July isotherm, or southern extent of discontinuous permafrost (Fig. 1, Vincent & Laybourn-Parry, 2008).

water and nutrient supply, irradiance and temperature. The seasonality of light is important, because it determines the rate of photosynthesis and subsequently the biomass of photosynthetic organisms over the year (Elster et al., 1994).

Arctic and Antarctic lacustrine habitats provide various types of environments for biological communities (Laybourn-Parry and Pearce, 2007). Thanks to the long-term presence of liquid water, polar lakes are considered to be important oases of life and biodiversity, hosting diverse communities, sometimes with a significant share of endemic species (Priscu et al., 1998; Vincent & Laybourn-Parry, 2008).

Polar lakes are characterized by simplified food webs dominated by photoautotrophic microbes, which are the dominant primary producers (Pienitz et al., 2008). Food chain structure is usually more complex in the Arctic than in Antarctica due to a greater diversity of zooplankton and microbes, as well as the occasional presence of fish (Vincent et al., 2009). The following sections will further explore the basic characteristics of the main lake types.

2.2 Types of lakes in polar regions

The origin of most lakes in polar regions is connected with glacial activity, such as their retreat following the last glacial period. Some lake types, such as epishelf or subglacial, are found almost exclusively in polar regions. On the other hand, tectonic, volcanic or meteoric impact crater lakes are in general quite rare. Physical and chemical conditions in polar lakes are very diverse, ranging from freshwater to saline, from acidic to alkaline, from perennially ice-covered lakes to concentrated brines that never freeze (Pienitz et al., 2008). Accordingly, there are few distinguishing features which are the same for all polar lakes, though many exhibit long periods of ice cover and complete darkness in the winter with several months of 24 hour light in the summer (Kling, 2009). Shallow lakes, e.g. lakes on sea terraces, are thermally variable with great fluctuations throughout the year as well as over shorter temporal periods. Furthermore, when bird colonies are present, lakes may be richer in nutrients in comparison to the oligotrophic character of most lakes (Pienitz et al., 2008). The Antarctic Peninsula region is currently considered one of the fastest warming regions on Earth (Turner et al., 2005), and a large number of lakes have originated here from recent increases in temperature. However, in the Arctic, recent warming has resulted in the opposite effect, with a significant reduction in total lake area and abundance (Smith et al., 2005).

2.2.1 Wetlands

The origin of water in wetlands is rainfall and snowmelt, which is confined to the active layer above the permafrost. Wetlands can range from small strips to extensive plains, and are more common in the Low Arctic than in the High Arctic due to generally higher levels of precipitation at lower latitudes.

2.2.2 Ice-dependent lakes

Many polar lakes are formed in the vicinity of glaciers or ice caps, on and between ice sheets, and in ice-filled moraines (Fig. 2). Some of them can exist for a long time and are supplied by annual influxes of meltwater, while others are temporary, flooded only during the summer season. This type of lake is the most common in the Arctic. For example, in Greenland all the large lakes are usually of this type. **Epiglacial lakes** occur next to glacier fronts in mountainous terrain or beside ice shelves. They are common in Antarctica where they are fed by glacial meltwater and can exist for many years with frequent changes in water level and morphology (Hodgson et al., 2004). **Supraglacial lakes** are found in depressions on glaciers, ice-sheets and ice-shelves, and are generally ephemeral, forming during the summer melt season. Proglacial lakes, which are dammed by floating ice shelves so that fresh water cannot escape to the ocean, are called **epishelf lakes**. They are tidally forced because of their connection to the ocean. Some can have a saline bottom, while others are freshwater throughout, and are becoming increasingly rare in the Arctic (Pienitz et al., 2008).

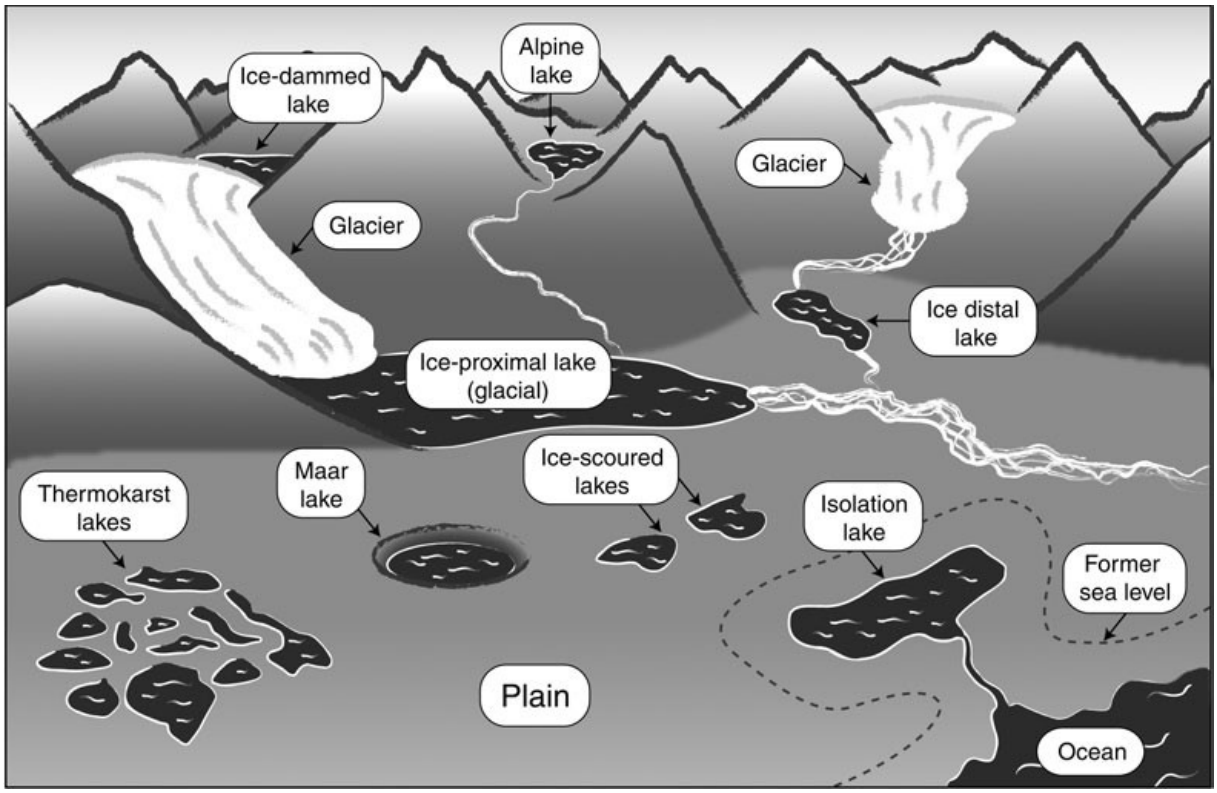

Fig. 2: Examples of lake types in polar regions (Pienitz et al., 2008).

2.2.3 Postglacial lakes

Glacial erosion and deposition of continental and local ice masses create a great number of lakes in polar and temperate regions. When glaciers deposit moraine material, sedimentary dams are created which form lakes behind the former ice margin. Since different bedrock types have a different resistance to erosion, lakes of various shapes and morphometries can develop. They are typically long-lasting due to the impact of glaciation on landscapes.

2.2.4 Thermokarst lakes

The thawing of permafrost results in depressions, resulting in the development of the thermokarst lake type. They are also called “thaw lakes”, are usually shallow (less than 1 m depth), and are very common in the Arctic.

2.2.5 Coastal uplift systems

New lakes can be formed as the sea level drops. Therefore, lakes which are closer to the present-day sea level are usually younger than those located at higher elevations or more distant from the sea (Pienitz et al., 2008). For example, sea level was previously decreasing at a rate of about 1 m per century, but due to future warming it is likely to increase by approximately 0.5 m by 2100 (Allard & Tremblay, 1983; Knutti & Stocker, 2000). This lake type can range from fully freshwater to hypersaline, due to very different possible paths in their evolution (Van Hove et al., 2006).

2.3 Lacustrine green algae in polar regions

Green algae have an importance as primary producers on our planet comparable to that of the rainforests (Naselli-Flores & Barone, 2009). Chlorophytes are often used as aquatic bioindicators, and due to their fast growth are considered important resources for biotechnology applications such as biofuel production, nutritional supplements, or in the cosmetics industry (Kvíderová et al., 2017). Some representatives of the green algae are extremophiles[2], which can withstand extreme conditions in terms of temperature, salinity, or pressure. Psychrophiles and psychrotrophs are also considered extremophiles. Psychrophiles are cold-loving microorganisms with an optimal temperature for growth at 15 °C, with a maximum around 20 °C and minimum around 0 °C. Psychrotrophic organisms are cold-tolerant, which have the ability to grow at low temperatures, but they have an optimal and maximal temperature for growth above 15 and 20 °C, respectively (Moyer & Morita, 2007).

Green algae (Chlorophyta) are photosynthetic eukaryotes belonging to the Viridiplantae within the kingdom Plantae, and are characterized by the presence of chloroplasts with two thylakoid membranes and chlorophyll *a* and *b*. They produce starch as the main reserve of polysaccharides, which is stored inside their plastids. They are distributed worldwide in aquatic and soil habitats, ranging from arid to tropical regions, and from oceans to the freshwater lakes of polar regions (Brodie & Lewis, 2007). The group is ancient and taxonomically diverse with approximately 8,000 species, and it is assumed that another 5,000 species have not been described yet (Guiry, 2012). Green algae show great morphologic variability, but those living in polar lakes are mostly unicellular flagellates or coccoid cells (Fig. 3, Brodie & Lewis, 2007).

[2] "Photosynthetic microorganisms such as algae occupy most environments on Earth that are illuminated with visible light. Among these habitats are several places which are, from the anthropocentric view, inhospitable and different from the "normal" places. The microbes occurring in those environments are referred to as "extremophiles". We could assume that those extremophiles regard their harsh habitats as an oasis or a paradise." (Seckbach, 2007)

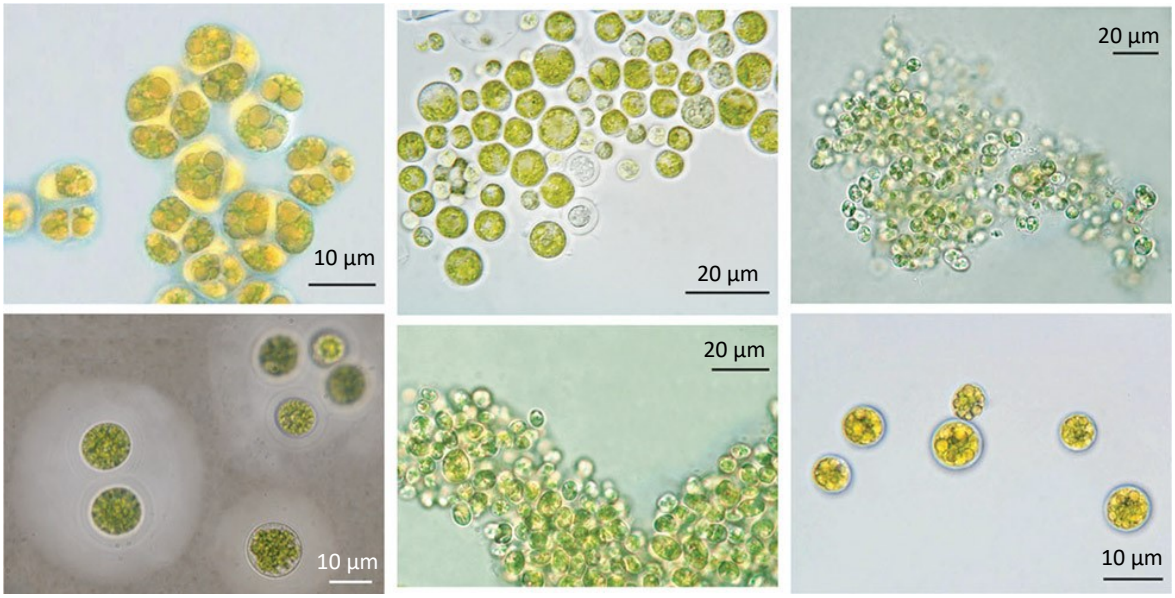

Fig. 3: Microphotography of Antarctic Chlorophyta strains isolated from lake sediment. Modified according to De Wever et al. (2009).

Polar microorganisms are characteristically able to withstand the cold environment, as their photosynthetic activity starts at temperatures near 0 °C. Algae take advantage of the long and intensive photoperiod for exponential growth. Most of them are also able to utilize nutrients present at low concentrations, as long as there is a steady supply. They are also adapted to periodic drying associated with large changes in temperature (Elster et al., 1994). As experimentally found by Morgan-Kiss et al. (2005) on *Chlamydomonas raudensis* (isolated from Lake Bonney, a permanently ice-covered lake in Antarctica), Chlorophyta are able to adapt to various conditions, such as growth at low temperatures in combination with low irradiance.

In polar lakes, Chlorophyta can be found both in plankton and in the benthos. In both environments they are usually not dominant. However, some genera, such as *Ankistrodesmus* and *Monoraphidium* or filamentous *Hazenia broadyi* are exceptions (Butler et al., 2000; Nedbalová et al., 2017; Škaloud et al., 2013).

Together with cyanobacteria and diatoms (Bacillariophyceae), green algae are important components of these communities. For example, Broady (1979) studied terrestrial algae on Signy Island, Antarctic Peninsula, which are able to colonize a wide range of habitats. In saline Antarctic ponds, filamentous chlorophytes (or e.g. *Chlamydomonas* cf. *snowiae*) often dominate alongside abundant diatoms (Vincent & James, 1996). However, in contrast

to cyanobacteria and diatoms, green algae are poorly studied in polar regions (Vyverman et al., 2010), especially compared to temperate regions.

Table 1: Overview of green algae genera occurring in the lakes of polar regions.

Class/Genera	Source:	**Class**/Genera	Source:	**Class**/Genera	Source:
Chlorophyceae		**Trebouxiophyceae**		**Zygnematophyceae**	
Ankistrodesmus	22.	*Botryococcus*	22.	*Closterium*	22.
Ankyra	22.	*Gloeotila*	22.	*Cosmarium*	10., 22.
Carteria	5.	*Chlorella*	1., 8., 23.	*Cylindrocystis*	5.
Coelastrella	1.	*Nannochloris*	1.	*Euastrum*	22.
Dunaliella	1.	*Oocystis*	22.	*Mougeotia*	3., 22.
Gloeococcus	1.	*Paradoxia*	1.	*Spirogyra*	2.
Haematococcus	7.	*Prasiola*	5., 9.	*Staurastrum*	12.
Chlamydomonas	1., 4., 8., 20.	*Raphidonema*	1., 5., 9., 10., 22.	*Zygnema*	14.
Chlorococcum	1., 9., 21.	*Stichococcus*	10., 21., 23.	**Charophyceae**	
Chlorogonium	5.	*Trebouxia*	1.	*Nitella*	6.
Chloromonas	1.	**Ulvophyceae**		**Pedinophyceae**	
Chodatodesmus	17.	*Binuclearia*	22.	*Pedinomonas*	22.
Monoraphidium	5., 9., 11.	*Cladophora*	7.	**Prasinophyceae**	
Oedogonium	22.	*Hazenia*	19.	*Monomastix*	22.
Pediastrum	22.	*Ulothrix*	5., 12.	*Pyramimonas*	8.
Scenedesmus	1.				
Tetraspora	15.				

Sources to the table 1: 1. (De Wever et al., 2009), 2. (Hawes et al., 1988), 3. (Hawes, 1989), 4. (Izaguirre et al., 2001), 5. (Izaguirre et al., 2015), 6. (Jónasson, 1992), 7. (Kim et al., 2008), 8. (Säwström et al., 2002), 9. (Mataloni et al., 2005), 10. (Müller et al., 1998), 11. (Nedbalová et al., 2017), 12. (Pavlova et al. , 2016), 14. (Pichrtová et al. , 2016), 15. (Richter et al., 2017), 17. (Sciuto et al., 2015), 19. (Škaloud et al., 2013), 20. (Stibal et al., 2007), 21. (Stibal et al., 2006), 22. (Vincent & Laybourn-Parry, 2008), 23. (Hodač et al., 2016).

2.4 Biogeography of green algae

The ubiquitous distribution observed for some microorganisms may suggest the absence of dispersal limitation (Vyverman et al., 2010). Yet, whether or not microorganisms display biogeographical patterns remains an open question. Nearly a century ago, Baas-Becking (1934) claimed that "Everything is everywhere, but the environment selects." More recently, Finlay's Ubiquity Hypothesis argued that organisms smaller than 2 mm are both sufficiently abundant and easily dispersed to have a worldwide distribution (Finlay, 2002). Research shows that the smaller the organism is, the larger the population size it may have (Martiny et al., 2006). That means that very small organisms are capable of huge population sizes and are relatively

easy to disperse, and thus should only be limited by the availability of suitable habitat. Finlay (2002) predicts that there should be some size limit where microorganisms still have biogeographies. He estimated the microorganism size for a cosmopolitan distribution to be 1-10 mm (Fig. 4). Nevertheless, more studies will be necessary to determine the exact organism size and other important factors determining microorganism distributions.

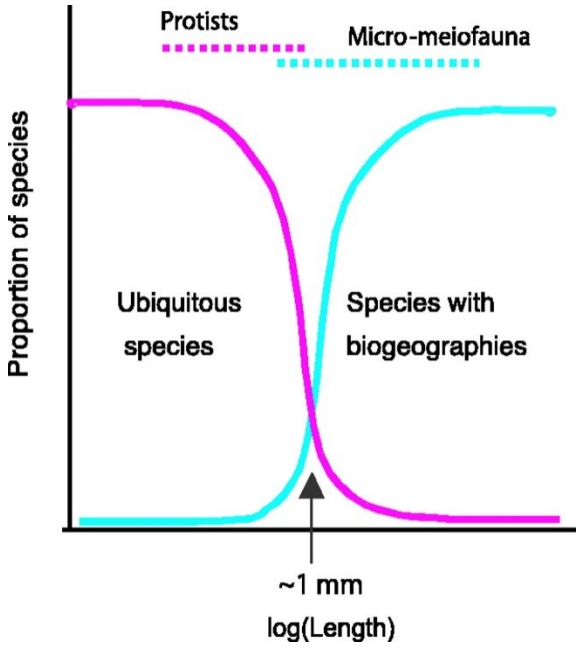

Fig. 4: Hypothetical distribution of organisms according to their size. It shows transition between ubiquitous species and species with biogeographies, while the marginal size of organisms is in range of 1-10 mm according to Finlay (2002). The real slopes of the "ubiquity" and "biogeography" curves are not yet known.

The biogeography of microorganisms has long been a popular research topic, and many studies from the 19th century already pointed out the cosmopolitan distribution of many microbes. One of these studies, which supported Finlay's hypothesis, was the study of Martinus Willem Beijenrick, who observed that species found in specific habitats from different geographical places are very similar if not the same (van Iterson et al., 1983). Microorganisms should be randomly distributed everywhere, due to the fact that they are dispersed by random forces, but sometimes it is not obvious if non-random population growth in a particular environment overlaps with the random distribution of microorganisms (Finlay, 2002).

The statement "everything is everywhere, but the environment selects" assumes that microorganisms are highly stress-tolerant, due to the fact that many of them are able to create resting stages. Chlorophytes can create cysts or spores capable of surviving freezing or desiccation, and they are able to remain in this state for long periods of time (Remias et al.,

2016; Vincent et al., 2000). The main ecological features of microorganisms, such as large population sizes, short generation times, and high rates of dispersion, suggest that biological and physical factors are more important than historical factors (Dolan, 2005). Yet, it seems that environmental selection is at least partly responsible for spatial variation in microbial biodiversity, and many studies argue against the idea that "everything is everywhere" (Martiny et al., 2006). For example, Coesel & Krienitz (2007) found that some morphologically highly different microorganism taxa are actually closely affiliated based on molecular analysis, while on the other hand, some taxa which are morphologically very similar had only little relationship. They claim that despite the ability of microbes to disperse easily, cosmopolitan species are still relatively scarce.

Broady (1996) mentioned in a morphological study on terrestrial algae from Antarctica, that there was only a small proportion of endemic species, and that they only slightly differed from related species in other regions. Based upon this, it would seem that a significant portion of species are cosmopolitan, even though it is usually only possible to identify them at the genus level. They also suggest that there could be dispersal barriers both to and within Antarctica. Some green coccoid algae, like genera *Chlorella* and *Stichococcus*, which are morphologically simple and easily dispersed by wind, are considered to be distributed worldwide. Hodač et al. (2016) studied the distribution of the representants of these two genera in polar, temperate, and tropical regions using a phylogenetic analysis of both 18S rDNA and ITS2 rDNA. When using a morphological approach, they found differences across regions. On the other hand, when they used a molecular approach, they found similarities in the sequences from different regions. Another example of a green alga with a confirmed widespread geographic distribution is a strain of *Monoraphidium* isolated from lakes on James Ross Island, Antarctic Peninsula, which was very similar in its 18S rDNA sequence to another strain isolated from a stream in Germany (Nedbalová et al., 2017). The ubiquitous distribution theory was also supported by Rippin et al. (2018) with the finding of the globally-distributed genus *Chloromonas* in soil crusts of polar regions using 18S rDNA gene.

It is suggested that there are more endemic species in Antarctica than in the Arctic due to the fact that the Antarctic continent is more isolated (Elster & Benson, 2004; Vyverman et al., 2010). Using high-throughput sequencing of 18S rDNA from 16 glaciers in the Arctic (Svalbard, Greenland, Iceland and Sweden) it was found that the snow algal community composition and their relative abundance in all studied sites was highly similar, even though they were well separated and physico-chemically diverse (Lutz et al., 2016). Therefore, they

proposed that snow algae are cosmopolitan, which is in contrast to previous molecular studies suggesting that micro-eukaryotes within a specific habitat have strong biogeographies. However, Segawa et al. (2018) claims that sequencing only 18S rDNA is not suitable for the biogeographical study of snow algae due to low variability of this gene. In their study, they used variable ITS2 rDNA and found that some snow algae are locally endemic, and therefore exhibit some dispersal limitation. Hence, Remias et al. (2013) suggest that the use of the ITS2 rDNA marker should aid in the clarifying the identity of various geographical populations.

De Wever et al. (2009) supports the claim that 'morphologically similar taxa don't need to be relatives' in their study of Chlorophyta in lacustrine habitats from maritime and continental Antarctica. They found 14 distinct chlorophycean and trebouxiophycean lineages using sequencing of 18S rDNA from five distinct regions. No sequences were found in more than three regions, and besides a *Chlorella vulgaris* strain identical to an isolate from eutrophic pond in the Netherlands, none of the Antarctic sequences were identical to non-Antarctic sequences available in the GenBank database at the time of publication. In fact, its conclusions could be significantly affected by the limited sampling efforts and database entries. Other species thought to be potentially endemic were *Chodatodesmus australis*, isolated from a lake in Victoria Land (Sciuto et al., 2015), and the filamentous alga *Hazenia broadyi* described from a lake on James Ross Island, Antarctic Peninsula (Škaloud et al., 2013).

2.5 Approaches used for the study of microalgal diversity

It all started in 1676 when Antoni van Leeuwenhoek used a microscope for observing microorganisms (Fig. 5, Schierbeek, 1959), which subsequently became an important and main tool for studying microorganisms. In 1888, Robert Koch introduced solid media suitable for growing of microorganisms and the use of agar as a base for culture media. Later, in 1931, Winogradsky studied microorganisms and their environmental roles, which gave birth to microbial ecology (Ackert, 2013). Thus, for almost 300 years, the study of microorganisms was based only on morphology and life cycles based on environmental samples or laboratory cultures (Fott, 1974). Then in the 1970s, Carl Woese proposed the ribosomal RNA genes as molecular marker for taxonomic classification (Woese & Fox, 1977). Afterwards, a significant milestone for the study and classification of microorganisms came with the idea of Sanger automated sequencing (Sanger et al., 1977) and the development of the Polymerase Chain Reaction (PCR). At the beginning of the new millennium, Next Generation Sequencing

(NGS) technologies were released, which are capable sequencing millions of DNA molecules in parallel with different yields and sequencing lengths, with the possibility of sequencing entire microbial communities (Logares et al., 2012). NGS technology offers shorter read lengths, but higher a yield for lower cost than Sanger sequencing. The theoretical collection of all genomes from members of a microbial community from a specific environment helped to define a new area called metagenomics (Handelsman et al., 1998). Nevertheless, cloning and sampling biases are problematic, even though these approaches have led to identification of new microbial communities members.

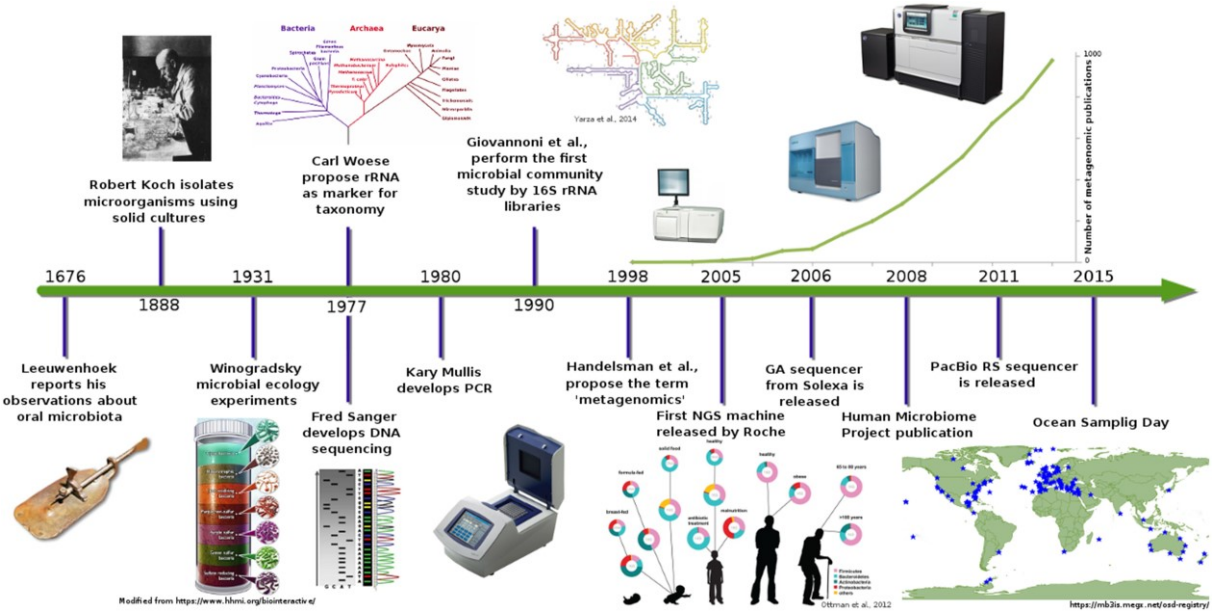

Fig. 5: Timeline and milestones of microbial studies (Escobar-Zepeda et al., 2015).

2.5.1 Microscopy

Light microscopy was and still is traditionally used in many studies describing species composition, as well as for water quality analysis via indicator organisms. In comparison with molecular methods, determination using light microscopy is cheap and fast, but due to the fact that cells of green algae are usually very small and morphologically very simple, it is not easy (or impossible) to determine representatives of this group (Neustupa et al., 2011; Rippin et al., 2018; Xiao et al., 2014). For example, the genera *Chloromonas* and *Chlamydomonas* have been traditionally separated based on the presence-absence of pyrenoids. However, molecular phylogenetic analyses show that some strains of *Chloromonas* and *Chlamydomonas* belong to the same clade (as in the case of *Chloromonas reticulata*, Pröschold, 2001). Even though this classification problem is very common, it is often overlooked (Anesio et al., 2017).

To conclude, light microscopy observations are still a very important tool for the basic description of samples and taxonomic determinations, but in many cases they have to be combined with molecular methods to ensure the correct identification of green algal taxa.

Electron microscopy allows for the observation of microorganisms under great magnification (up to 1,000,000x), thus enabling the study of external and internal structures of the cell in detail. The great advantage of scanning electron microscopy (SEM) is the possibility of examining the detailed structure of the cell, thanks to a large depth of field, which makes the object appear to be three-dimensional. The differences in green algae cell morphologies visible by SEM can be crucial for determining individual species (Procházková et al., 2018). Transmission electron microscopy (TEM) is used for the detailed study of the cell wall and the inner cell structure, e.g. ultrastructure of chloroplast, mitochondria, nucleolus, flagella, pyrenoid, Golgi apparatus or starch grains (Nishikawa et al., 2003). For green algae, TEM is crucial in genera determination, where the cell structures are compared (Neustupa et al., 2011). For both SEM and TEM, the samples have to be prepared. In the case of SEM, sample have to be dried, fixed, and coated in a layer of heavy metal, usually gold. In the case of TEM, the sample must be dried, fixed, and cut in thin slices of max 100 nm (Nebesářová, 2002).

2.5.2 Cultivation and isolation techniques

For green algae cultivation, liquid or solid media are used, many of which were developed around 1900 (Preisig & Andersen, 2005). Some media are based on the chemical composition of water in the cell's natural habitat, while others were developed based on the detailed study of a species' nutrient requirements or other ecological parameters. In order to enable growth in the laboratory, it is sometimes necessary to create conditions that are as close as possible to the habitat where they naturally appear, considering optimal temperature, nutrient composition, light intensity and photoperiod. There are many types of media, and the selection of one for a given study depends mainly of which taxonomic group will be cultured. One of the most frequently used freshwater media is Bold's Basal Medium (BBM, Bischoff & Bold, 1963), while other examples include BG-11 Medium (Allen & Stanier, 1968) or C Medium (Ichimura, 1971). These media can be used as a liquid or solidified by using 1-1.5% agar. Before use, they should be sterilized by autoclaving (usually at 121 °C for 15 minutes) and then they may be stored in the refrigerator for several weeks (Watanabe, 2005).

The key to successful cultivation and isolation is to understand and mimic the natural environment, to know the taxonomy of the target species and to avoid contamination.

The sample collection itself and the storage of the sample shortly after collection can be also crucial. It is necessary to get a one-species culture for Sanger sequencing and to study life cycles, and there are several methods to achieve this.

One technique is single-cell isolation, which is performed with a Pasteur pipette or a glass capillary. The Pasteur pipette is heated over a flame and then carefully extended and broken, so its diameter is a little bigger than the isolated cell itself. The isolation is performed by picking up the cell without damage using the pipette and transferring it into a sterile droplet, and then transferring it into a second sterile droplet. This is repeated until we get the algal cell, without any other organisms, which can then be placed into the culture medium. A microscope is necessary to observe and isolate the cell.

An old and common method of isolation is the use of agar plates, which is very suitable for coccoid algae. The concentration of agar is usually 1.5%. The sample is spread on an agar plate, and then it is incubated until colonies of cells begins to appear, which can take a few weeks. When the colonies are present, it is possible to transfer them, using a pipette or sterile loop, into another agar plate or liquid medium. Contamination is a major disadvantage of this method due to the ability of fungi and bacteria to quickly grow on agar.

Another method is the dilution technique, which was also used for many years and is still common. It is very effective for species that are rather abundant in samples. The goal is to get only one or a few cells of one species into a test tube or the well of a multi-well plate, until a single-species isolate is achieved. This technique is often used when we would like to get random algal species from environment samples, often with the goal of discovering new species (Andersen & Kawachi, 2005).

2.5.3 Molecular methods for green algae study

In last few decades, molecular methods have brought us deeper insights into the diversity of microorganisms (Izaguirre et al., 2015). Sanger sequencing (Sanger et al., 1977) has been the most widely used sequencing method for about 40 years. Lately, it has been almost replaced by Next Generation Sequencing (NGS) techniques. DNA sequencing technologies have been driving and changing the genome research field in recent years, and their progress in development has enabled the production of massive high-quality sequencing reads at decreasing costs (Wong et al., 2019). Both sequencing approaches have their benefits and disadvantages, which are summarized and compared below.

Four parallel sequencing reactions were originally used to sequence a single sample with the Sanger sequencing method. Nowadays, the automated Sanger sequencing method uses fluorescent labelling of each deoxynucleotides (dNTPs) and they are mixed in a single reaction. It can achieve long sequencing reads with high raw read accuracy, but for a relatively high cost and significant time investment. Because it can generate only one sequence in a run, it is inappropriate for the study of environmental samples. The strain must be obtained by incubation and isolation. In general, the study of microorganisms is limited by the fact that some of them are not easily cultivable or even resist completely any cultivation attempt. This also applies to Chlorophyta (Watanabe, 2005). The use of NGS besides the study of complex microbial communities, for instance, gives us the opportunity to study these uncultivable species (Shokralla et al., 2012). In contrast to Sanger sequencing, NGS methods are capable of sequencing multiple samples in parallel for a relatively low cost. The sequence length is usually shorter (e.g. about 300 bp for Illumina MiSeq), and NGS datasets are generally much larger in size, which requires better computing facilities for bioinformatic analysis. Amplicon sequencing enables the selective sequencing of DNA fragments from a specific genomic region. There are many NGS platforms which are used, including QIAGEN, Illumina, ArcherDX and Ion Torrent systems. Each of them is based on a different sequencing method and could be suitable for different types of study. The inconvenience of using amplicon sequencing is the scarcity of reference sequences in databases (Escobar-Zepeda et al., 2015).

The cultivation of microorganisms combined with light microscopy and Sanger sequencing can provide a detailed analysis of individual strains, including those used in laboratory experiments. However, the unculturable taxa will be neglected. In contrast, amplicon sequencing enables the investigation of the entire species pool in an environmental sample. Nevertheless, this approach is always dependent on the reference database because it is difficult (if not impossible) to correctly annotate taxa which are misidentified or absent in the databases. The selection of one or the other approach should be based on the individual research focus, but it is recommended to use multiple techniques to validate results (Rippin et al., 2018).

3 Materials and methods

3.1 Field sampling

Samples from Arctic and Antarctic lakes were collected in the boreal and austral summers between 1993 and 2018. In 2017, Antarctic samples were collected on Ulu Peninsula, James Ross Island, and on Dundee Island and around Hope Bay, Antarctic Peninsula in 2018. Samples from Svalbard were collected in Adventfjorden and Billefjorden in 2017 and during a boat expedition from Ny-Ålesund along north-west part of Svalbard up to the Sjuøyane Archipelago in 2018. The remaining samples, which were used only for amplicon sequencing, were collected on field expeditions in context of the Protistology and Aquatic Ecology lab's (UGent, Belgium) national and international (paleo)limnological research programs between 1993 and 2014. These samples were already provided as DNA isolates, and in case of 18S rDNA were provided as sequence data. In total, 137 samples were collected. A list of all samples is presented in Supplement Table S1 and their location is shown on the map on Fig. 6.

Microbial mat sample were collected from the littoral zone of lakes by scraping epilithon from stones and collecting sediment into sterile tubes. From Svalbard (2017) and the Antarctic Peninsula (2018), three replicate samples were collected in parallel. One of them was fixed in formaldehyde, the second in LifeGuard Soil Preservation solution (Qiagen, Germany), and the third was without fixative. Non-fixed samples and samples fixed in LifeGuard were stored cold and moved to the freezer as soon as possible. Samples fixed in formaldehyde were kept at outside temperatures in the vicinity of the collection area. The remaining samples were collected into sterile tubes as two replicates - fixed in LifeGuard solution and one without fixation. During sampling, the GPS position was recorded, and a photo of the sampling site was taken. After transport to the Czech Republic, subsamples were observed under an Olympus BX43 light microscope type (Olympus corporation, Japan) equipped with a digital camera DXM 1200F (Nikon, USA). Microphotographs were processed using the QuickPHOTO Camera 3.0 software (Promicra, Czech Republic). The average conductivity of Arctic lakes was 0.35 mS cm^{-1} (N = 39) and 0.48 mS cm^{-1} in Antarctic lakes (N = 33).

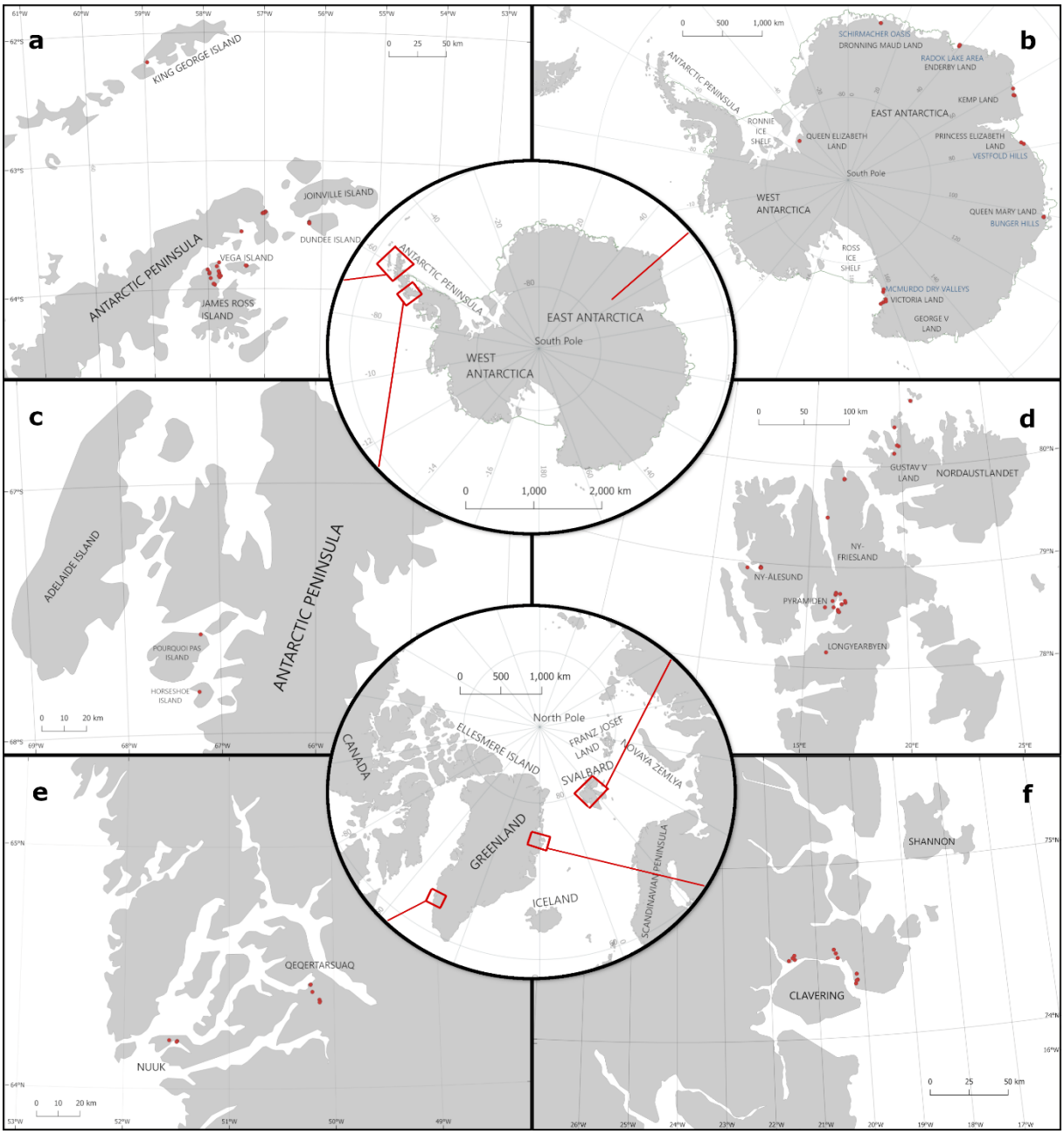

Fig. 6: Locations of sampling sites (a,c – Antarctic Peninsula, b – Continental Antarctica, d – Svalbard, e – South Greenland, f – North Greenland). Map with localities was created in QGIS v3.16.0 (QGIS.org, 2021) with Nature Earth maps and merged using GNU Image Manipulation Program v2.10.22 (GIMP, http://gimp.org).

3.2 Sample incubation and strain isolation

Frozen samples from the 2017 and 2018 seasons were gently defrosted, homogenised, inoculated into Bold's Basal Medium (BBM) (Bischoff & Bold, 1963) on 24-well plates, and placed in an incubator and at 7 °C and 12 °C respectively, with continuous light provided by LED lamp (~ 20 µmol m^{-2} s^{-1}). The serial dilution method was used in order to achieve an unialgal strain from the bulk sample. The process consisted of transferring a small amount

of cultivate, once algae had grown sufficiently, into the 24-well plate with BBM medium (approx. 2 ml in each well) which was repeated until unialgal strains were obtained (Andersen & Kawachi, 2005).

3.3 Sanger sequencing

3.3.1 DNA extraction, PCR and sequencing

DNA was extracted from unialgal cultures following the standard protocol in the DNeasy Plant Mini Kit (Qiagen, Germany), with minor modifications: At the beginning of the procedure, cells were mechanically disrupted by shaking for 20 min (30 Hz) in the presence of glass beads (1.25-1.65 mm diameter, Carl Roth GmbH, Karlsruhe, Germany) and 0.5 mm diameter (BioSpec Products, Bartlesville, OK, USA) in the Mixer Mill MM 400 (Retsch, Germany). Then, DNA concentration was measured with the NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA). The 18S small subunit ribosomal DNA gene (SSU 18S rDNA) and the internal transcribed spacer region 2 (ITS2 rDNA) were amplified from DNA isolates by PCR using primers shown in Table 2.

Amplification reactions for the 18S rDNA gene were performed using the following cycle parameters: 10 min at 95 °C, followed by 35 cycles (1 min at 95 °C, 1 min at annealing temperature of 56 °C or 59 °C (depending on primer set), 2 min at 72 °C) and 10 min at 72 °C. Each 20 µl PCR reaction for 18S rDNA amplification contained 5 µl of DNA isolates (diluted to concentration of 5 ng/µl), 0.8 µl of each 10 µM primer, 1.6 µl of 25 mM $MgCl_2$, 1.5 of 2 mM dNTP´s, 2 µl of 10X Taq Buffer + KCl-$MgCl_2$, 8.2 µl of sterile water and 0.1 µl of Taq polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Amplification reactions for ITS2 rDNA region were performed using the following cycle parameters: 5 min at 97 °C, followed by 37 cycles (1:25 min at 95 °C, 1 min at annealing temperature of 56 °C, 4 min at 72 °C) and 7 min at 72 °C. Each 36 µl PCR reaction for ITS2 rDNA amplification contained 1 µl of DNA isolates (diluted to concentration of 5 ng/µl), 1.4 µl of each 10 µM primer, 2.8 µl of 25 mM $MgCl_2$, 2.63 of 2 mM dNTP´s, 3.5 µl of 10x Taq Buffer + KCl-$MgCl_2$, 23.18 µl of sterile water and 0.1 µl of Taq polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

The PCR products were stained with bromophenol loading dye, quantified on 1.5% agarose gel, stained with Red gel, and underwent electrophoresis for 40 mins (120 V and 400 A). The gel was photographed on a UV transillumination table with a CCD camera.

The products were purified and sequenced at Macrogen Europe B.V. (Amsterdam, the Netherlands).

Table 2: List of primers used for the amplification of 18S rDNA and ITS2 regions for Sanger sequencing.

Marker	Primer	Sequence 5´-3´	Source
18S rDNA	18F1	CTGCTTTATACTGCGAAACTGC	(Hoham et al., 2002)
	18R1	CTTCACCAGCACACCCAATC	
	18F	AACCTGGTTGATCCTGCCAGT	(Katana et al., 2001)
	18R	TGATCCTTCTGCAGGTTCACCTACG	
	P2	CTGGTTGATTCTGCCAGT	(De Wever et al., 2009)
	P4	TGATCCTTCYGCAGGTTCAC	
	FA	AACCTGGTTGATCCTGCCAGT	(Matsuzaki et al., 2015)
	RF	CCCGTGTTGAGTCAAATTAAG	
	FE	GGGAGTATGGTCGCAAGGCTG	(Matsuzaki et al., 2015)
	RB	TGATCCTTCTGCAGGTTCACCTAC	
ITS2 rDNA	TW	GGGATCCGTTTCCGTAGGTGAACCTGC	(Goff et al., 1994)
	AB	GGGATCCATATGCTTAAGTTCAGCGGGT	
	ITS5	GGAAGTAAAAGTCGTAACAAGG	(White et al., 1990)
	ITS4	TCCTCCGCTTATTGATATGC	
	ITS1	TCCGTAGGTGAACCTGCGG	(White et al., 1990)
	ITS4	TCCTCCGCTTATTGATATGC	
	LSU	AGTTCAGCGGGTGGTCTTG	(Piercey-Normore & DePriest, 2001)
	SSU	CTGCGGAAGGATCATTGATTC	
	AL	GCGCGCTACACTGATGC	(Helms et al., 2001)
	LR3	GGTCCGTGTTTCAAGACGG	(Vilgalys & Hester, 1990)

Sequencing data were checked and trimmed using SeqMan (Swindell & Plasterer, 1997) and FinchTV 1.4.0 (Geospiza, USA). The sequences were compared against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the names for the 18S rDNA and ITS2 rDNA sequences were recorded. For the ITS2 region, the ITS2 database was used (http://its2.bioapps.biozentrum.uni-wuerzburg.de/) and the Annotate tool was utilized to annotate the sequence using 5.8S and 28S borders. The ITS2 sequences were then compared to the NCBI database. The best hits were recorded, along with the reference number, culture collection/reference strain number, identities, and query cover (Supplement Tables S2 and S3). Based on these data, alga identities in the established cultures were determined at the species level using the protocol proposed in Fawley & Fawley (2020). The final determination was performed by integrating taxonomic assignments based on both 18S rDNA and ITS2 rDNA data.

3.4 Amplicon sequencing

3.4.1 DNA extraction, PCR and sequencing

DNA was extracted from microbial mat samples fixed in LifeGuard Soil Preservation solution (Qiagen, Germany) following the standard protocol in DNeasy Power Soil Kit (Qiagen, Germany). The isolated DNA was stored at -20 °C until further processing. The 18S rDNA was amplified from DNA isolates by PCR using primers shown in Table 3. Amplification reactions for the 18S rDNA region were performed using the following cycle parameters: 5 min at 95 °C, followed by 10 touchdown cycles (1 min at 94 °C, 1 min at annealing temperature starting at 57 °C [-0.5 °C every cycle], 3 min at 72 °C), followed by 25-30 cycles (1 min at 95 °C, 1 min at annealing temperature of 52 °C, 3 min at 72 °C) and 20 min at 72 °C.

Touchdown is a method for increasing specificity of the PCR reaction. It uses a cycling program where the annealing temperature is gradually reduced (in this case by 0.5 °C). The initial annealing temperature should be several degrees above the estimated melting temperature (Tm) of the primers (Korbie & Mattick, 2008).

Table 3: List of primers used for the amplification of 18S rDNA and ITS2 regions for amplicon sequencing.

Marker	Primer	Sequence 5´-3´	Source
18S rDNA	TAReuk454FWD1	CCAGCASCYGCGGTAATTCC	(Stoeck et al., 2010)
	TAReukREV3	ACTTTCGTTCTTGATYRA	
ITS2 rDNA	5.8SbF	CGATGAAGAACGCAGCG	(Mikhailyuk et al., 2008)
	TS4R	TCCTCCGCTTATTGATATGC	(White et al., 1990)
	TChl9140F	AACGATGAAGAACGCAG	own

The primer TChl9140F was designed by the following procedure: 17 702 ITS2 Chlorophyta sequences obtained in the NCBI database were aligned using the MUSCLE algorithm in MEGA X Software (Kumar et al., 2018). Using the guidelines for designing primers (Elsalam, 2003), few short sequences were found in the alignment and the best one with the most hits and variable, in terms of hits in more Chlorophyta classes, was chosen.

Each 25 µl PCR reaction for 18S rDNA and ITS2 rDNA amplification contained 1 µl of DNA isolates, 2 µl of each 10 µM primer, 2.5 µl of 2 mM dNTP´s, 2.5 µl of FastStart High Fidelity Reaction Buffer, 14.75 µl of sterile water and 0.25 µl of FastStart Taq polymerase (FastStart High Fidelity PCR system, Roche).

Amplification reactions for the ITS2 region were performed using the following cycle parameters: 5 min at 95 °C, followed by 10 touchdown cycles (0.5 min at 95 °C, 0.5 min

at annealing temperature starting at 60 °C [-0.5 °C per cycle], 3 min at 72 °C), followed by 15-30 cycles (0.5 min at 95 °C, 0.5 min at annealing temperature of 55 °C, 3 min at 72 °C) and 20 min at 72 °C.

The PCR products were stained with bromophenol loading dye, quantified on 1.5% agarose gel, and underwent electrophoresis for 30 mins (100 V and 400 A=variable). The gels were stained with ethidium bromide and photographed on a UV transillumination table with a CCD camera. Purification and library preparation was then performed, and concentrations were measured by Qubit (Invitrogen, Waltham, Massachusetts, USA) with the QUBIT dsBROAD range kit and diluted to the same concentration. The products were sequenced at Base Clear (Leiden, the Netherlands) on Illumina MiSeq 2x250 bp paired-end.

3.4.2 Data processing

The MetaCentrum server was used for data processing and analyses. Computational resources were supplied by the project "e-Infrastruktura CZ" (e-INFRA LM2018140) provided within the program Projects of Large Research, Development and Innovations Infrastructures.

18S rDNA sequences were processed by the following procedure: Forward and reverse reads were merged using algorithms (Paired-End reAd merger, PEAR version 0.9.11, Zhang et al., 2014) with a minimum overlap size of 15 bp, minimum and maximum assembled length of assembled sequences of 220 and 400 bp, respectively, and with a 15 threads to use. Quality filtering was performed using Usearch11 [v11.0.667_i86linux32] (Edgar, 2010), using a Q30 quality threshold and minimum sequence length of 220 bp. Samples collected in Greenland and Svalbard (except for B1, H1, M3, M4, G1, O2, S1, S9, S13, S17, S18, S21, S24 and S26) were trimmed only to a length of 220 bp due to their low quality. Unique sequences were then clustered into Operational Taxonomic Units (OTUs) with a 97% identity threshold. An OTU table was made, and taxonomy classification was assigned using Mothur [v1.39.5] (Schloss et al., 2009) and the PR2 database [v 4.12.0] (Guillou et al., 2013). Samples with less than 2,000 reads/sample were not used for subsequent analyses.

Processing of ITS2 sequences followed the same procedure as the 18S rDNA sequences. The ITS2 region was extracted from the sequences using ITSx software (Bengtsson-Palme et al., 2013). Unique sequences were then clustered into OTUs with a 97% identity threshold. An OTU table was made, and taxonomy classification was assigned using Mothur [v1.39.5] (Schloss et al., 2009) and the UNITE database (Nilsson et al., 2019).

ITS2 sequences acquired using primer set 5.8SbF and TS4R and the second primer set TChl9140F and TS4R were processed together, and reads were treated by the following process: For samples, which were sequenced by both primer sets, the numbers of OTU reads were averaged. Samples which were sequenced by only one primer set, the number of reads in OTUs remained the same.

Visual processing of Venn diagrams was made using MyDraw (https://www.mydraw.com/). Species accumulation curves and Canonical Analysis of Principal coordinates (CAP) analysis were made in R Studio (RStudio Team, 2020). To investigate the community composition of green algae in more detail, canonical analysis of principal coordinates (Anderson & Willis, 2003) was utilized. CAP analysis was performed on a Bray–Curtis dissimilarity matrix followed by a canonical discriminant analysis of predefined groups with the 'CAPdiscrim' function in the R package *BiodiversityR* (Kindt & Coe, 2005). This analysis assesses the extent to which samples are effectively classified into *a priori* defined groups using the correct classification rate (CCR) statistic. In order to account for non-linear relationships between OTU richness and mean annual temperature, generalized additive models (GAM) were developed using the R package *mgvc* (Wood, 2011), selecting the 'REML' method and the quasipoisson error distribution. Mean annual air temperature data were extracted from publicly available databases corresponding to the nearest weather station for the given sampling year. To visualize differences in the distribution of particularly influential OTUs, the top 50 OTUs by abundance were plotted (log10 +1-transformed) in a heatmap. A dendrogram was produced with the 'heatmap.2()' function in the gplots package (Warnes et al., 2015) using the 'average' clustering method and Euclidean distance in R. The remaining analyses and graphs were performed in GraphPad Prism version 9.0.2 for Windows, GraphPad Software, San Diego, California, USA (https://www.graphpad.com/). To test for significant differences in OTU richness between polar regions, an unpaired t-test was used, and between main biogeographical regions a one-way ANOVA was used.

ITS2 rDNA Sanger sequences from the strains established during this study were used to create a custom reference database in SEED (Větrovský et al., 2018). In the next step, the automatic BLAST search (Kent, 2002) of each unique ITS2 rDNA OTU Illumina sequence (this study) against this local database was performed in SEED. The minimum percent similarity of 97% identity and 100% coverage were used as the threshold to consider a database match as a hit.

4 Results

4.1 Strain isolation and Sanger sequencing

In total 65 algal cultures were established and sequenced (30 from Svalbard and 35 from the Antarctic Peninsula), see Supplementary Table S1.

The overall view of the taxonomic diversity detected in the isolates from the Arctic and Antarctica are shown in the Venn diagram (Fig. 7). Ten taxa were detected in Antarctic cultures and eight in cultures from the Arctic. There were only three taxa shared by both polar regions.

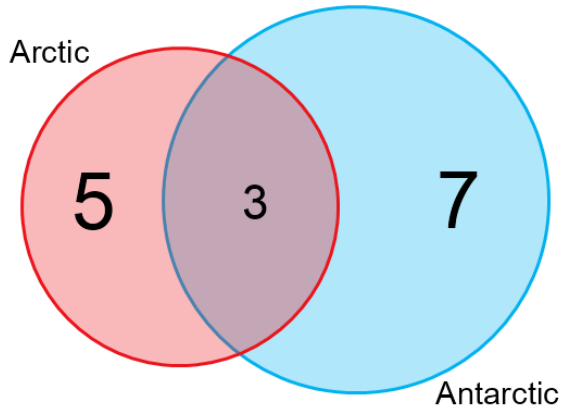

Fig. 7: Comparing the number of Arctic (red) and the Antarctic (blue) taxa found using Sanger sequencing of cultures.

In total, there were 15 taxa belonging to the classes Chlorophyceae and Trebouxiophyceae (Fig. 8). Of these, *Chlorella* spp., *Micractinium* spp. and *Neochlorosarcina negevensis* f. *ferruginea* were the most represented (Figs. 8, 9). From the seven taxa belonging to the class Trebouxiophyceae, five of them were classified in the order Chlorellales, family Chlorellaceae (*Chlorella* spp., *Micractinium* spp.). The other two taxa belonged to the family Coccomyxaceae (*Coccomyxa subelipsoidea*) and Stichococcaceae (*Pseudostichococcus* sp.). From the eight taxa belonging to the class Chlorophyceae, six of them were classified in the order Chlamydomonadales (*Deasonia granata*, *Chlorococcum* spp., *Chloromonas reticulata*, *Macrochloris* sp., *Neochlorosarcina negevensis* f. *ferruginea* and cf. *Sphaerocystis* sp.) and two in order Sphaeropleales (*Chodatodesmus* spp.).

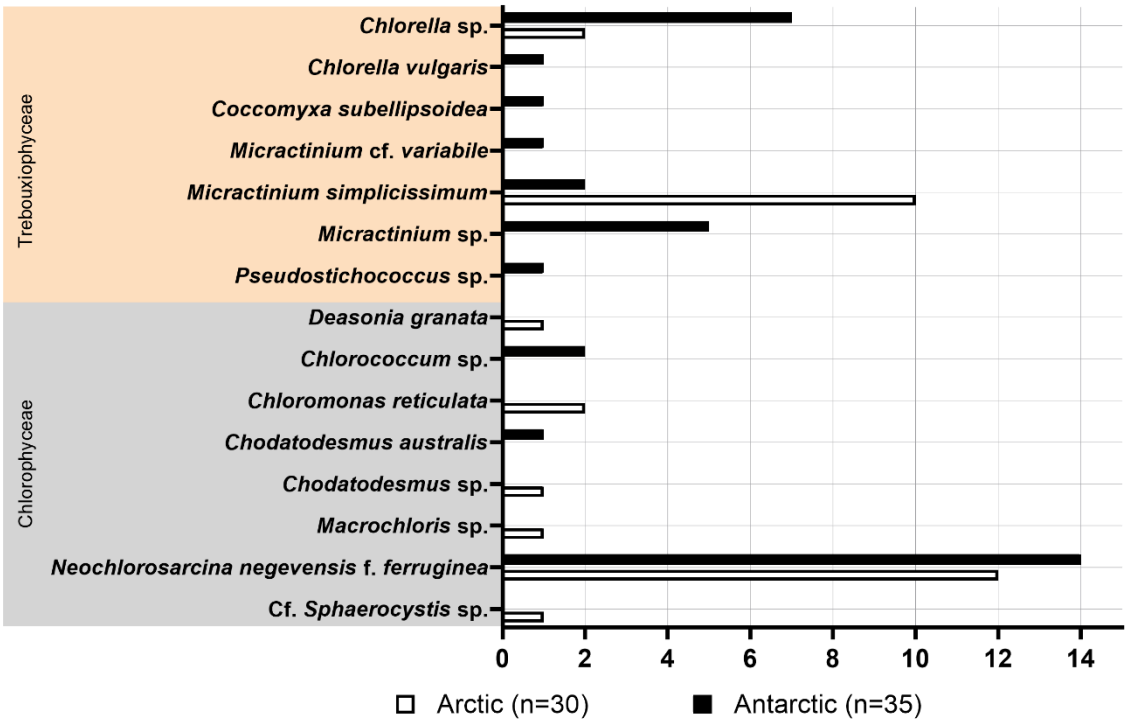

Fig. 8: Representation of taxa in all cultures isolated from Arctic and Antarctic newly established strains.

All cultures isolated from samples collected in the year of 2017 in both polar regions were classified as *Neochlorosarcina negevensis* f. *ferruginea* from the class Chlorophyceae (Figs. 8 and 9). There were 14 cultures of this species from Antarctica and 12 cultures from the Arctic.

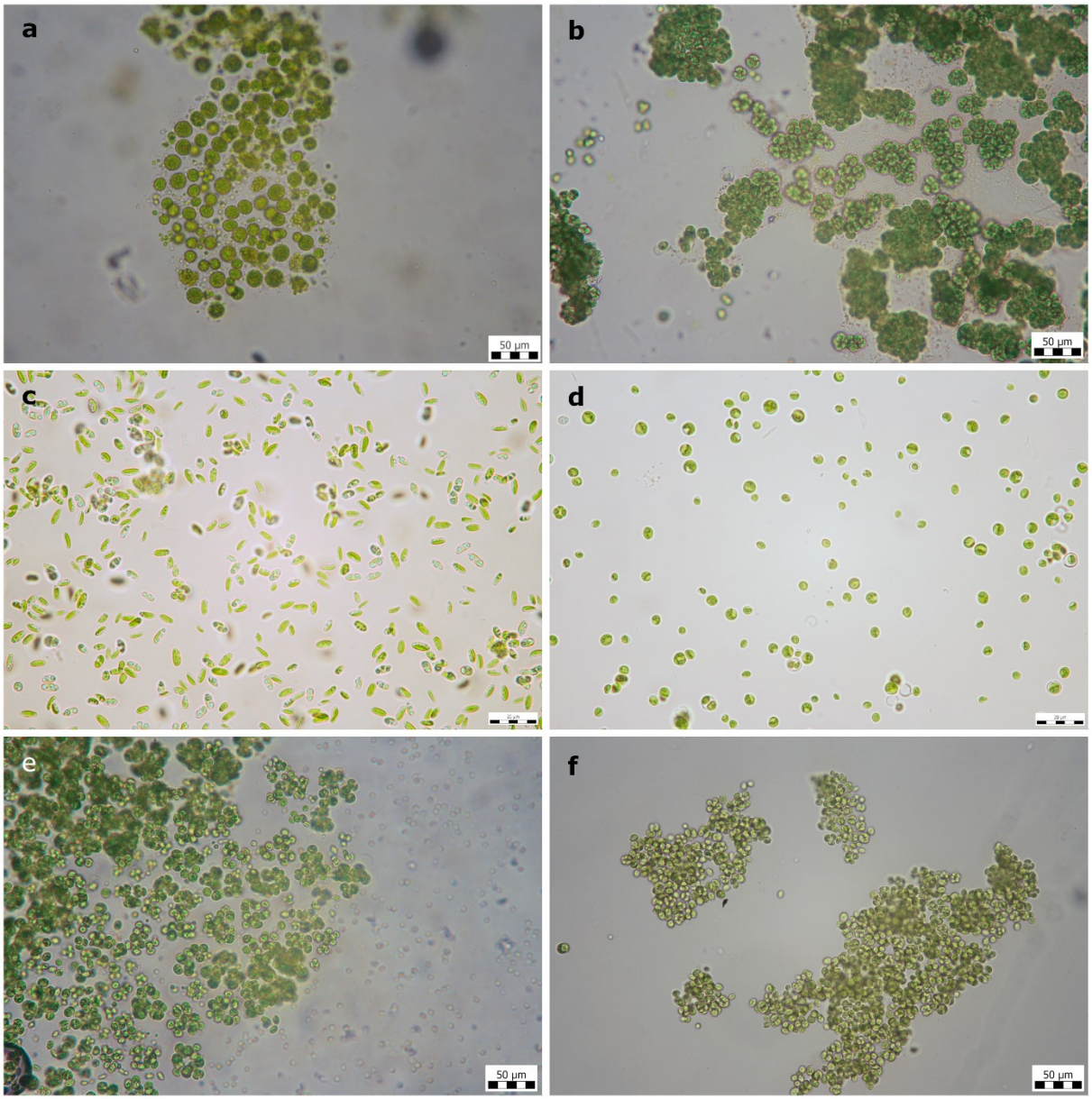

Fig. 9: Microphotographs of unialgal strains (a – *Chlorococcum* sp., b – *Macrochloris* sp., c – *Coccomyxa subelipsoidea,* d – *Chlorella* sp., e – *Micractinium* sp., f - *Neochlorosarcina negevensis* f. *ferruginea*).

In contrast, cultivation efforts based on samples collected in 2018 resulted in the establishment of unialgal strains of many different taxa (Fig. 8). In total, fourteen taxa were detected, seven from each of the classes Trebouxiophyceae and Chlorophyceae. The most represented taxa were *Chlorella* sp. and *Micractinium simplicissimum* (Fig. 9). There were 21 cultures from Antarctica and 18 cultures from the Arctic. The species *Neochlorosarcina negevensis* f. *ferruginea* was not detected in cultures incubated from 2018 samples.

4.2 Amplicon sequencing of environmental samples

In total, 107 samples were sequenced for amplicons using Illumina MiSeq: 90 samples were sequenced for 18S rDNA and 100 samples for ITS2 rDNA.

4.2.1 18S rDNA

For 18S rDNA amplicon sequencing, we obtained 753,629 Chlorophyta reads out of 3.3 million reads in total. 202 Chlorophyta OTUs were found in 90 samples, of which 35 were from the Arctic and 55 were from Antarctica.

Regional species richness was standardized to the lowest number of samples present in one of the polar regions (N = 35 in the Arctic) using rarefaction curves (100 permutations) obtained from 18S rDNA sequences (Fig. 10). The number of OTUs was only slightly higher in the Arctic compared to Antarctica.

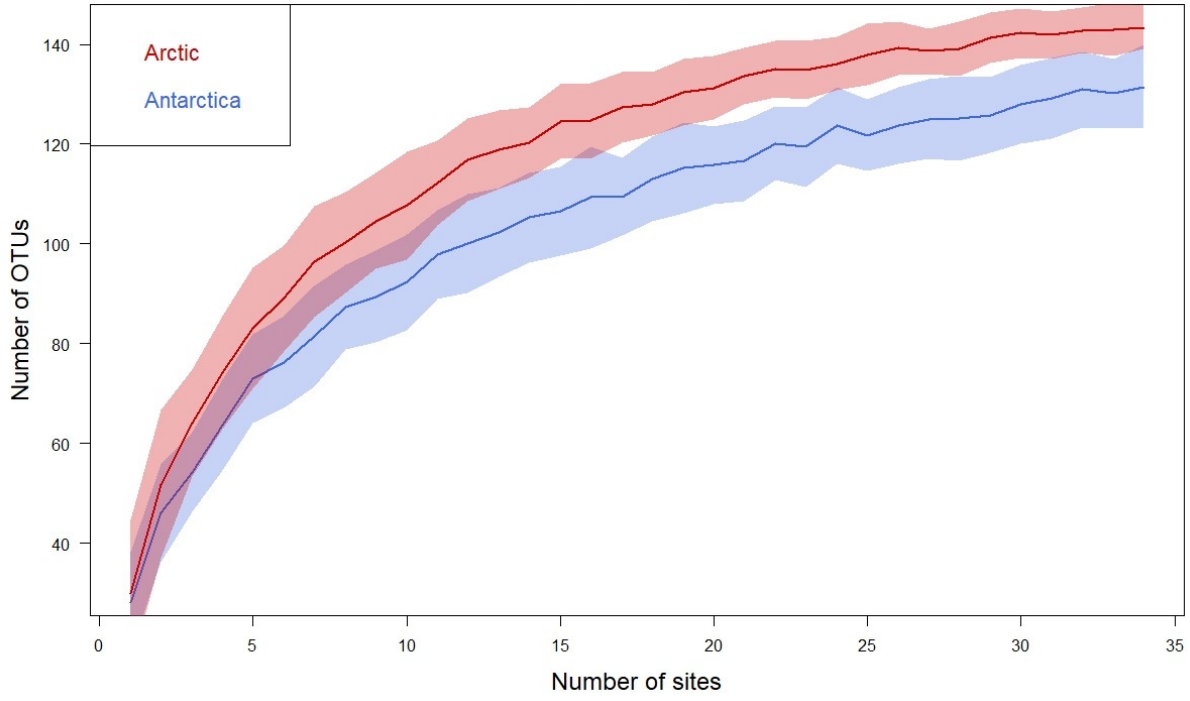

Fig. 10: Rarefaction curves based on number of OTUs (18S rDNA), with the Arctic in red and Antarctica in blue. The shaded area around the lines indicates 95% confidence intervals.

The Venn diagram in Fig. 11 shows the overall distribution of taxonomic diversity in Chlorophyta detected for the Arctic and Antarctica. In total, 160 OTUs were detected in the Arctic and 166 in Antarctica, with 124 OTUs (61.4%) being present in both polar regions. 17.8% of OTUs were present only in the Arctic and 20.8% in Antarctica.

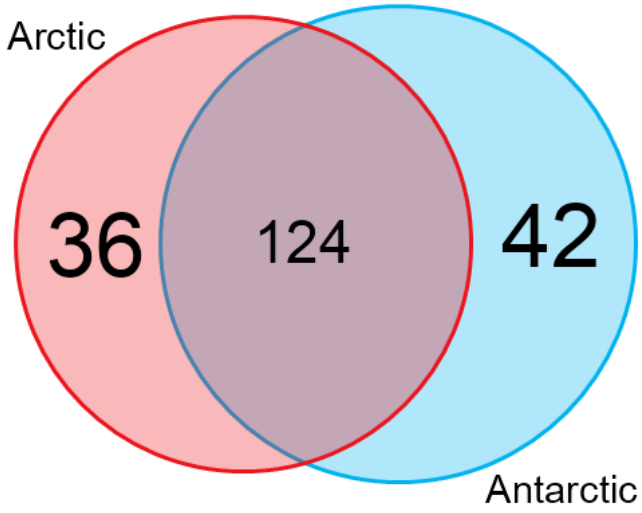

Fig. 11: Comparing the number of Arctic (N = 35, red) and Antarctic (N = 55, blue) OTUs found using 18S rDNA amplicon sequencing.

Fig. 12 shows the numbers of OTUs per sample detected in both polar regions. Average values were almost the same – 30 OTUs per sample in the Arctic and 32 OTUs per sample in Antarctica. The differences were not significant.

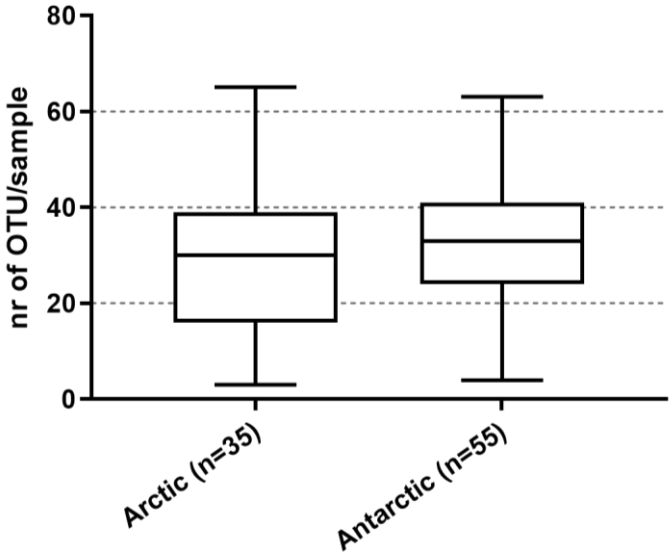

Fig. 12: Comparison of the number of OTUs per sample (alpha diversity) in the Arctic and Antarctic based on 18S rDNA (p = 0.48, unpaired t-test).

Fig. 13 shows the percent abundance of OTUs belonging to particular Chlorophyta classes in both polar regions. The most abundant class in the Arctic was Ulvophyceae with 56% relative abundance (38% in Antarctica). In the case of Antarctica, the most abundant class was Chlorophyceae with 49% of the OTUs (29% in the Arctic). Class Trebouxiophyceae was

represented by 15% and 13% in the Arctic and Antarctic, respectively. Other classes, including Mamiellophyceae, Pedinophyceae, Chlorodendrophyceae, Pyramimonadales and unclassified Chlorophyta, were represented in both regions by less than 1% (0.9% in the Arctic and 0.5% in the Antarctic).

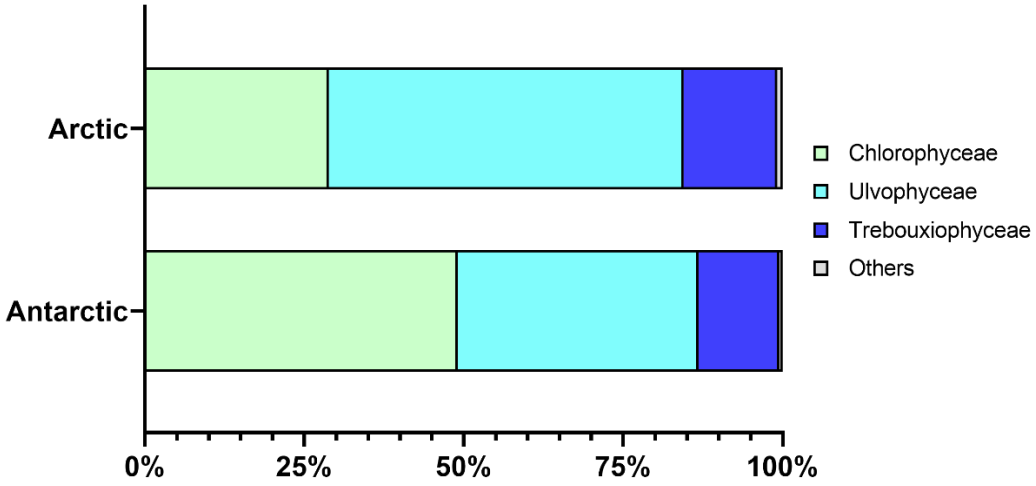

Fig. 13: Percent abundance of OTUs belonging to particular Chlorophyta classes based on the 18S rDNA sequences in the Arctic and Antarctic. 'Others' represents Mamiellophyceae, Pedinophyceae, Chlorodendrophyceae, Pyramimonadales and unclassified Chlorophyta.

Fig. 14 shows the results of the CAP analysis for the 18S rDNA dataset focused on differences between both polar regions (35 Arctic samples and 55 Antarctic samples). CCR in Antarctica was 100% and 82.9% in the Arctic, suggesting a high level of separation of samples between the two regions.

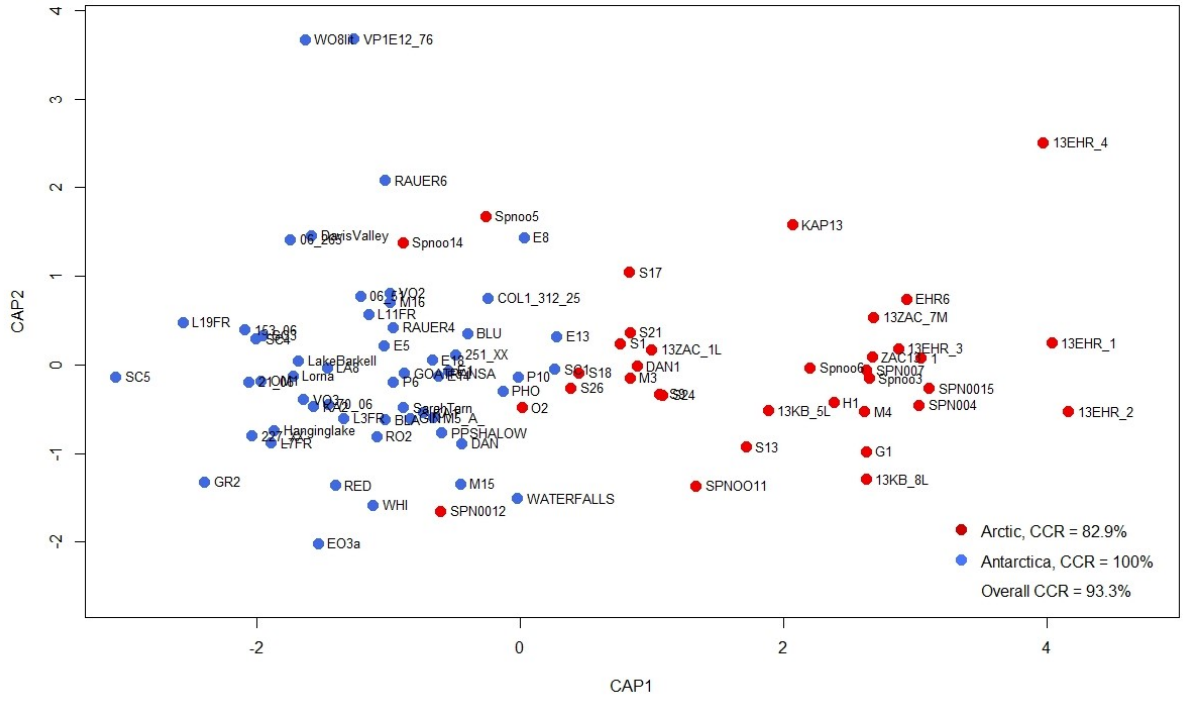

Fig. 14: Canonical analysis of principal coordinates (18S rDNA) grouped by Arctic and Antarctic regions. Overall CCR was 93.3%.

Fig. 15 shows the result of the CAP analysis for the 18S rDNA dataset focused on the main biogeographical regions (23 Svalbard samples, 9 North Greenland samples, 3 South Greenland samples, 26 samples from Continental Antarctica, and 29 samples from Antarctic Peninsula). The highest CCR (less shared species between groups) was in the Antarctic Peninsula (82.8%) and the lowest (more shared species between groups) was in Svalbard (65.2%). The overall CCR was 74.4%. This indicates slight differences between how species are shared among biogeographical regions.

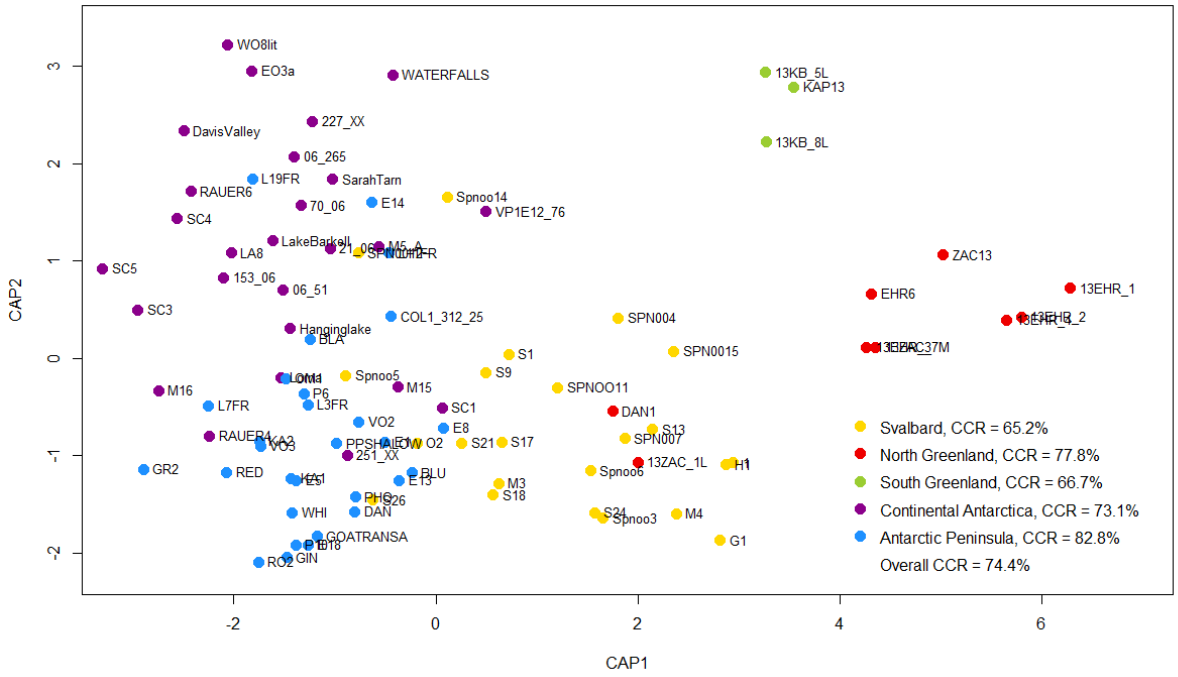

Fig. 15: Canonical analysis of principal coordinates (18S rDNA) grouped in main biogeographical regions. The overall CCR was 74.4%.

4.2.2 ITS2 rDNA

For the ITS2 rDNA amplicon dataset, we obtained 1.5 million Chlorophyta reads out of almost 5 million in total. 1,104 Chlorophyta OTUs were found in 102 samples, of which 48 were from the Arctic and 54 were from Antarctica.

Regional species richness was again standardized to the lowest number of samples present in one of the polar regions (N = 48 in the Arctic). The rarefaction curves (100 permutations) obtained from ITS2 rDNA sequences differed among Arctic and Antarctic lakes (Fig. 16). There is no visible dominance of one or a few species as indicated by the steeply rising curve, and because the curve has not completely flattened at the end, there are still many species to be found with increased sampling intensity or sequencing depth. The number of OTUs was almost two times higher in the Arctic than in Antarctica at N = 48.

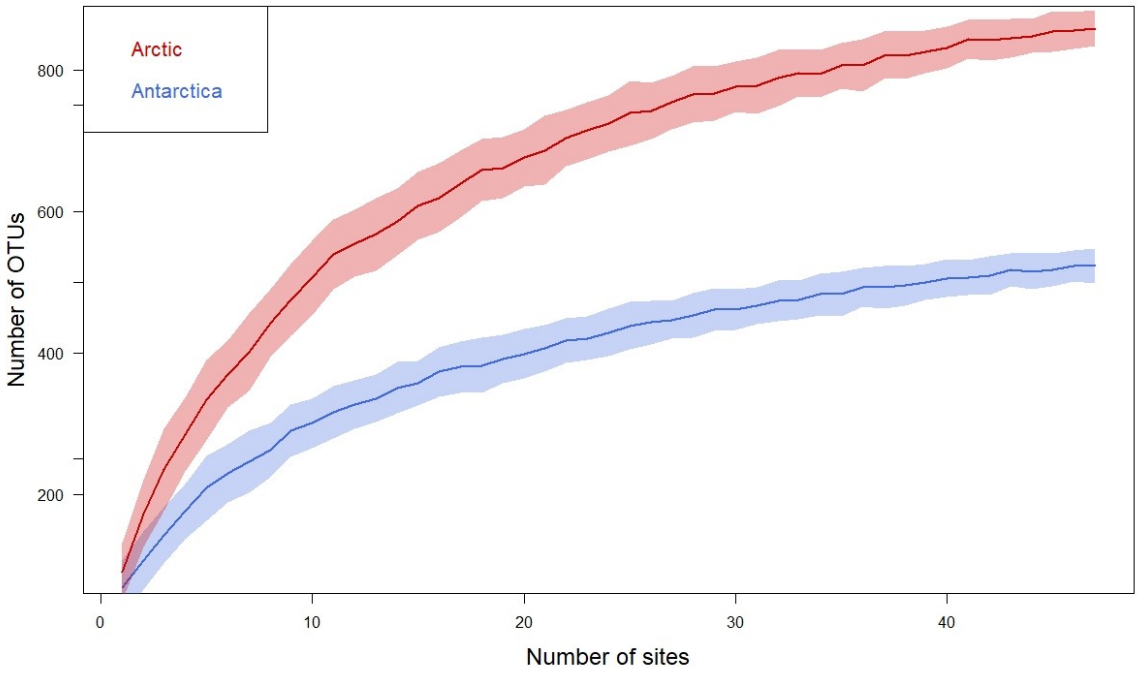

Fig. 16: Rarefaction curves based on the number of OTUs (ITS2 rDNA), separated by Arctic (red) and Antarctic (blue) samples. The shaded area around the lines indicates 95% confidence intervals.

In total, we found 1,104 Chlorophyta OTUs in the ITS2 rDNA dataset (1,020 in the Arctic and 671 in Antarctica). Of these 92.4% (1,020) were detected in the Arctic and 60.8% (671) were present in Antarctica. 53.2% (587) of Chlorophyta OTUs were shared among both polar regions, while 39.2% (433) were unique to the Arctic and only 7.6% (84) were unique to Antarctica (Fig. 17).

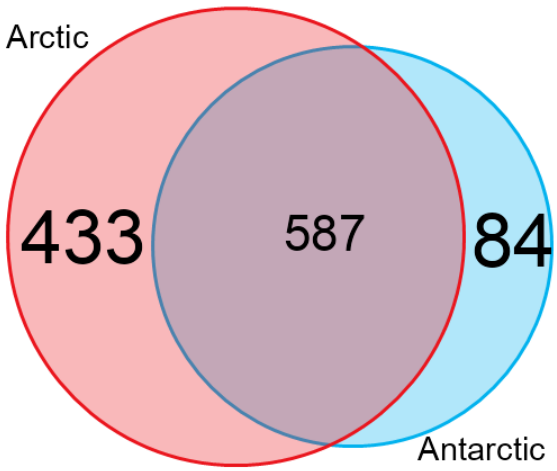

Fig. 17: Comparing OTU richness in the Arctic and Antarctic based on ITS2 rDNA.

Fig. 18 depicts a more detailed comparison of the main biogeographical regions. There were only 114 Chlorophyta OTUs shared between all biogeographic regions (Svalbard, North Greenland, South Greenland, Antarctic Peninsula, and Continental Antarctica), which is only

10.3% of the total richness. It is clear that more OTUs were shared between the particular regions within the Arctic or within Antarctica than between both main polar regions. For example, 42.7% (286) of OTUs were shared between the Antarctic Peninsula and Continental Antarctica. The amount of OTUs shared between North Greenland and South Greenland was 37.8% (315), and between Svalbard and North Greenland and Svalbard and South Greenland 45.5% (404) and 34.3% (314), respectively. 19.4% of OTUs were shared between all of the Arctic regions.

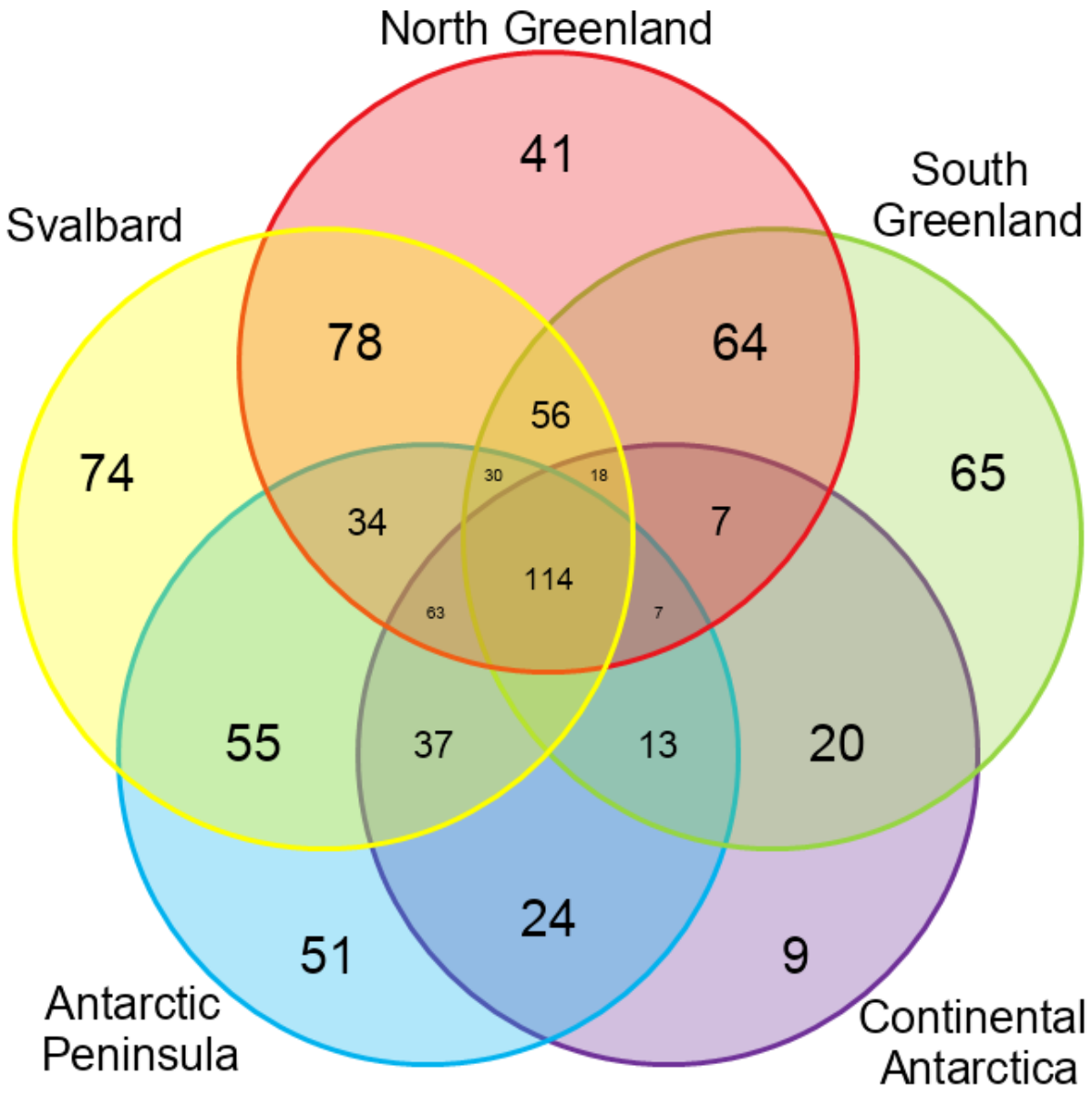

Fig. 18: Venn diagram of OTU richness (ITS2 rDNA) detected in each region, including Svalbard (yellow), North Greenland (red), South Greenland (green), Continental Antarctica (violet) and the Antarctic Peninsula (blue).

Fig. 19 shows the overall number of OTUs in each biogeographic region. The number of samples in each region was set to 11 based on the lowest number of samples (South Greenland). Eleven samples from each region were randomly chosen. The highest number (595 OTUs) was found in North Greenland, followed by 544 OTUs in South Greenland and 543 OTUs in Svalbard. Significantly lower numbers of OTUs were found in the Antarctic regions, with 383 in the Antarctic Peninsula and 229 in Continental Antarctica.

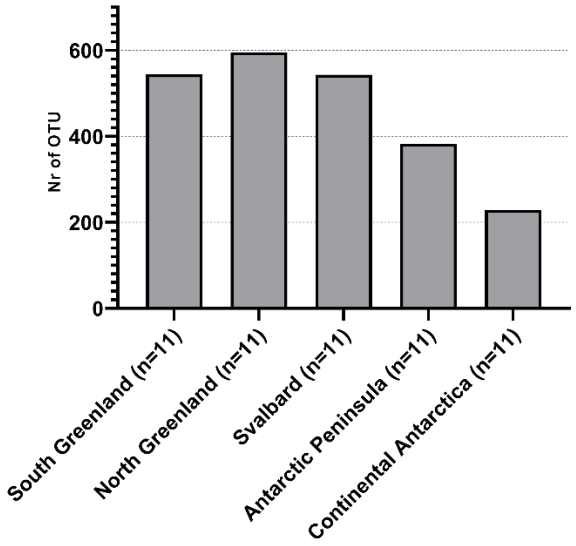

Fig. 19: Comparison of overall OTU richness based on ITS2 rDNA present in each biogeographic region.

Fig. 20 shows that the alpha diversity (OTU richness) from the 48 Arctic samples and 54 Antarctic samples was much higher in the Arctic (average 105.8) than in Antarctica (average 71.9).

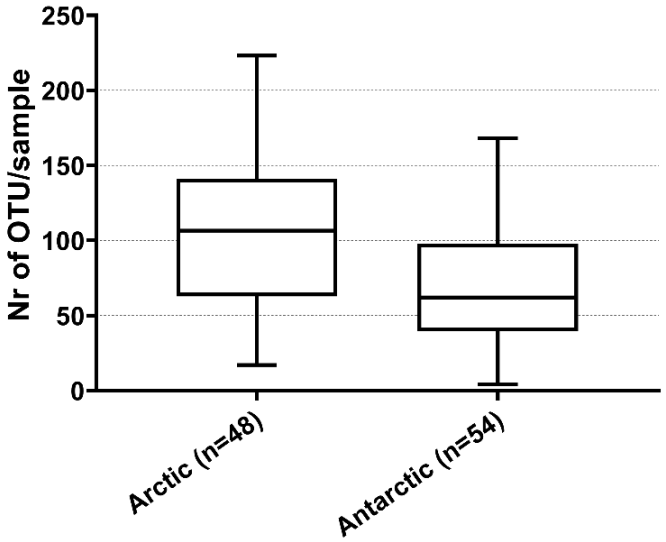

Fig. 20: Comparison of the number of OTUs per sample (alpha diversity) in the Arctic and Antarctic based on ITS2 rDNA ($p = 0.0003$, unpaired t-test).

When the main biogeographical regions were compared, OTU richness (Fig. 21) was lowest in Continental Antarctica (average 49.2), and the highest OTU richness (average 115.7) was in South Greenland. In North Greenland OTU richness averaged 114, in Svalbard 97.6, and in the Antarctic Peninsula 91.6.

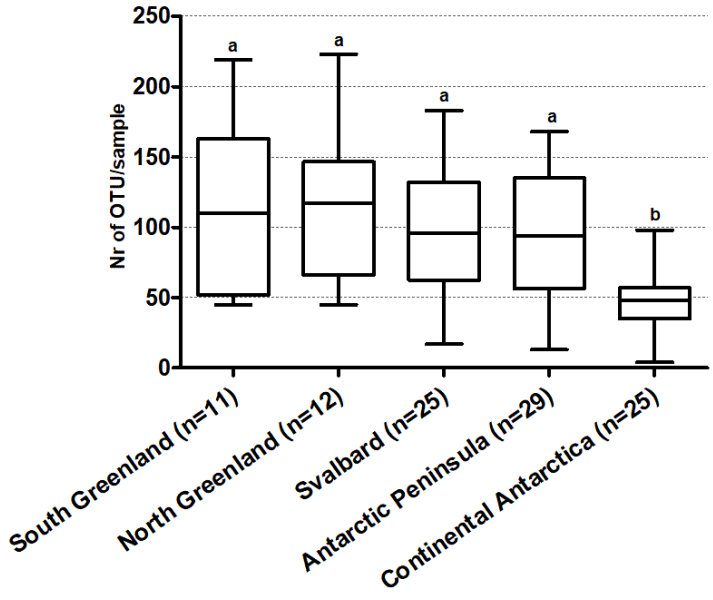

Fig. 21: Comparison of the number of OTUs per sample (alpha diversity) in each biogeographic region based on ITS2 rDNA ($p < 0.0001$, one-way ANOVA). Significant differences (at $p = 0.05$, post hoc Tukey test) are indicated by different letters.

The relationship between OTU richness per sample and the mean annual air temperature is shown on Fig. 22. The proportion of OTUs per sample increases with increasing mean annual air temperature, which was confirmed by multiple non-linear regression (GAM).

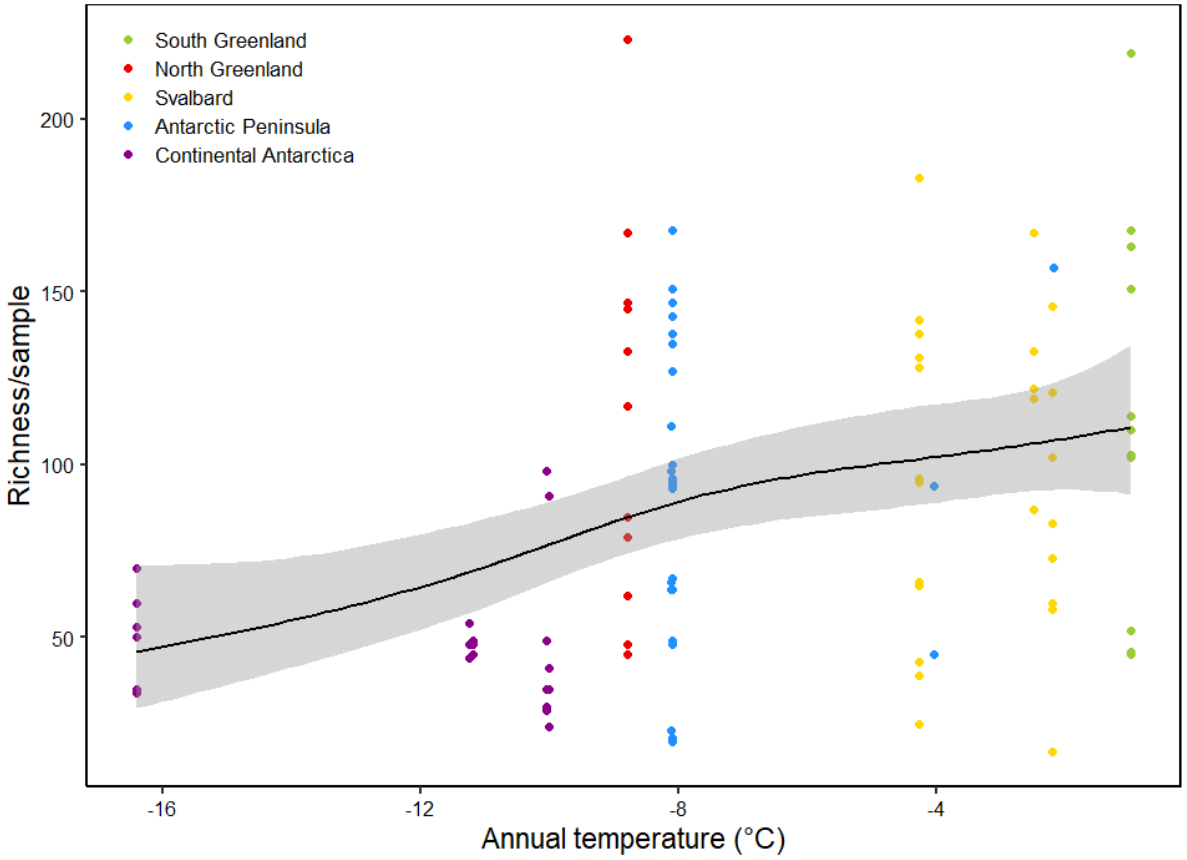

Fig. 22: Mean annual air temperature and OTU richness in polar regions based on ITS2 rDNA. Each point represents one sample and its colour represents a given biogeographic region ($R^2 = 0.13$, $p = 0.045$).

Fig. 23 shows the result of a CAP analysis for the ITS2 rDNA dataset focused on the differences between the two polar regions (48 Arctic samples and 52 Antarctic samples). The CCR in the Arctic is 91.7% and 94.2% in Antarctica, which shows that Antarctic samples are slightly more isolated. The overall CCR is 93%.

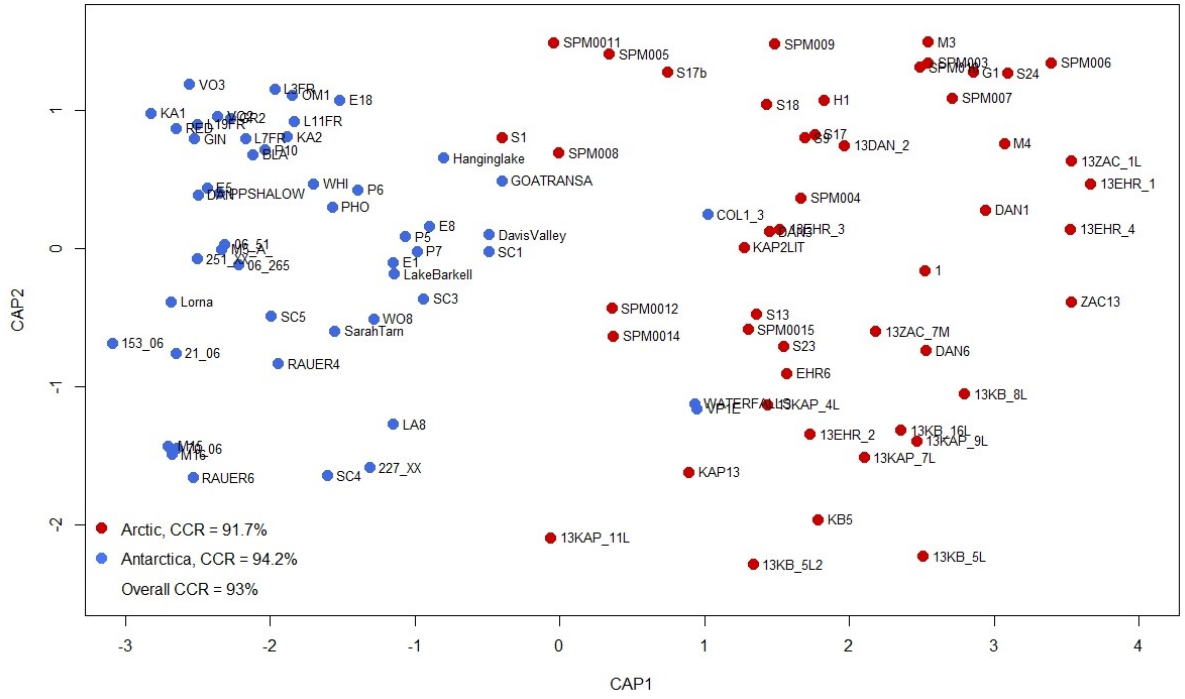

Fig. 23: Canonical analysis of principal coordinates (ITS2 rDNA) grouped by the Arctic and Antarctic regions. Overall CCR was 93%.

The result of CAP analysis for the ITS2 rDNA dataset focused on the five biogeographical regions is shown in Fig. 24. There are 25 Svalbard samples, 12 North Greenland samples, 11 South Greenland samples, 24 samples from Continental Antarctica, and 28 from the Antarctic Peninsula. The highest CCR was in North Greenland (100%), which shows that there were less species shared. The lowest CCR was in Continental Antarctica (79.2%).

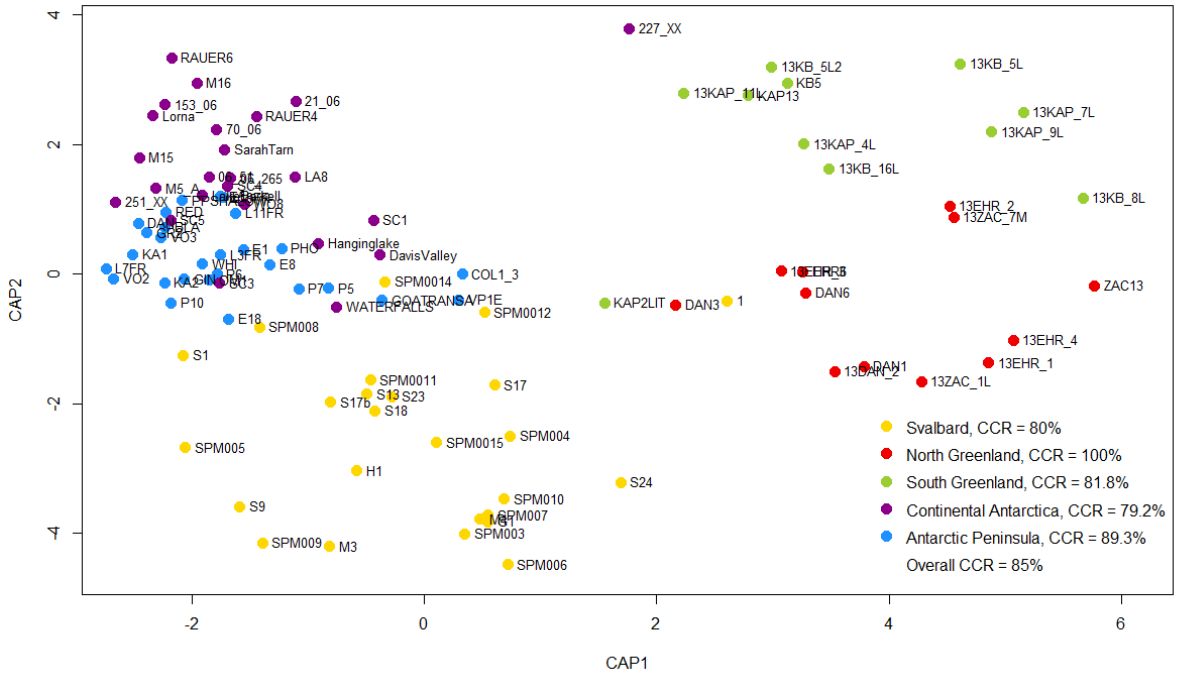

Fig. 24: Canonical analysis of principal coordinates (ITS2 rDNA) grouped by the five biogeographic regions. Overall CCR was 85%.

Fig. 25 shows the percent abundance of OTUs belonging to particular Chlorophyta classes between the two polar regions. The most abundant OTUs were classified only to the phylum Chlorophyta, and included 37% of the OTUs in case of the Arctic and 32.8% for the Antarctic. The second most represented class were Chlorophyceae with 21.7% in the Arctic and 29.9% in Antarctica. 27.9% of OTUs in the Arctic and 16.9% in the Antarctic were classified as Chlorophyta Incertae sedis. Noticeably more abundant in Antarctica was the class Ulvophyceae with 12.1% of the OTUs, while only 2% of OTUs from the Arctic belonged to this class. Trebouxiophyceae were represented in the Arctic and Antarctica with 7.3% and 2.6%, respectively. 'Other' classes, including Pleurastrophyceae and Prasinophyceae, were represented by 4.2% in the Arctic and 5.7% in Antarctica.

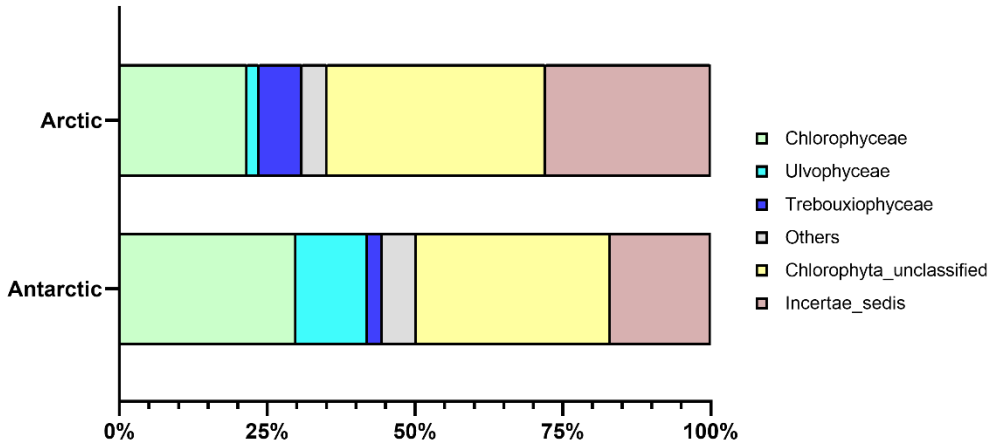

Fig. 25: Percent abundance of classes present in sequences (ITS2 rDNA) from the Arctic and Antarctica. 'Others' are represented by Pleurastrophyceae and Prasinophyceae.

Fig. 26 shows the percent abundance of OTUs belonging to particular Chlorophyta classes within the five biogeographical regions. The most abundant in all regions were OTUs classified only to the phylum Chlorophyta, with the highest representation for South Greenland with 45.9% and the lowest for the Antarctic Peninsula with 30.5%. The second most abundant class was Chlorophyceae, with around 30% in Antarctic regions and around 20% in Arctic regions, and only about 14% in South Greenland. Chlorophyta incertae sedis were also abundant, with the highest (34.4%) in South Greenland, and the lowest (9.5%) in Continental Antarctica. The class Ulvophyceae was better represented in Antarctic regions (16.2% in Continental Antarctica and 9.6% in Antarctic Peninsula), but was more sparse among Arctic regions with only 3% in Svalbard, 1.4% in South Greenland, and 0.3% in North Greenland. On the other hand, the class Trebouxiophyceae was more abundant in Arctic regions with 8.3% in North Greenland, 7.9% in Svalbard, and 4.6% in South Greenland. In Antarctic regions, the class Trebouxiophyceae was represented by around 2%. 'Others' (Pleurastrophyceae and Prasinophyceae) were quite well represented in North Greenland, Continental Antarctica, and the Antarctic Peninsula, with 11.5%, 7.6%, and 4.6%, respectively, predominantly from the class Pleurastrophyceae.

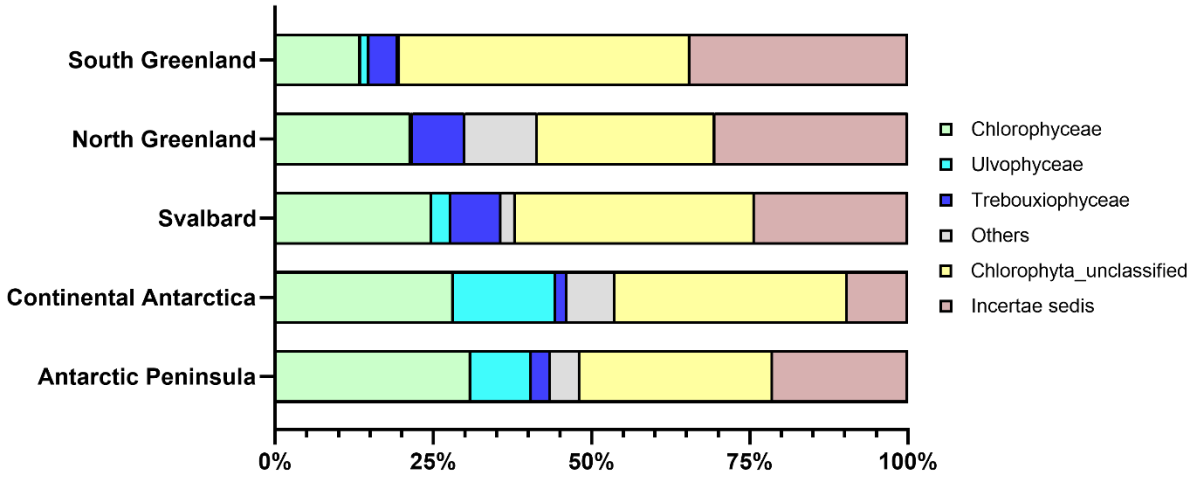

Fig. 26: Percent abundance of classes present in sequences (ITS2 rDNA) among the five biogeographic regions. 'Others' represents Pleurastrophyceae and Prasinophyceae.

The heatmap (Fig. 27) shows the 50 most common OTUs within all the samples, with sample name colour indicating their biogeographical region. There were six significant clusters created, named a - f and indicated by colour. Antarctic samples are more similar than samples from the Arctic in comparison to the CAP analysis (Fig. 24). The most abundant taxa occurring in more than 70 samples out of 100 and in all biogeographic regions were *Chloromonas* sp., *Ulothrix* sp. and *Hazenia broadyi*. Among the top 50 OTUs, there were 24 OTUs which were present in at least one sample from each biogeographic region (for instance OTU_8 *Chloromonas* sp., OTU_1085 *Ulothrix* sp., OTU_1002 Chlamydomonadales, OTU_100 *Ulothrix* sp., OTU_1050 *Chlamydomonas* sp., and OTU_6 *Planophila laetevirens*). Taxa which were not present in at least one biogeographical region included OTU_9 *Chodatodesmus australis*, OTU_1224 Chlamydomonadales, OTU_15 *Pleurastrum* sp., OTU_5 *Aplanochaete* sp., OTU_2018 *Asterococcus* sp. and OTU_16 *Dunaliella* sp.

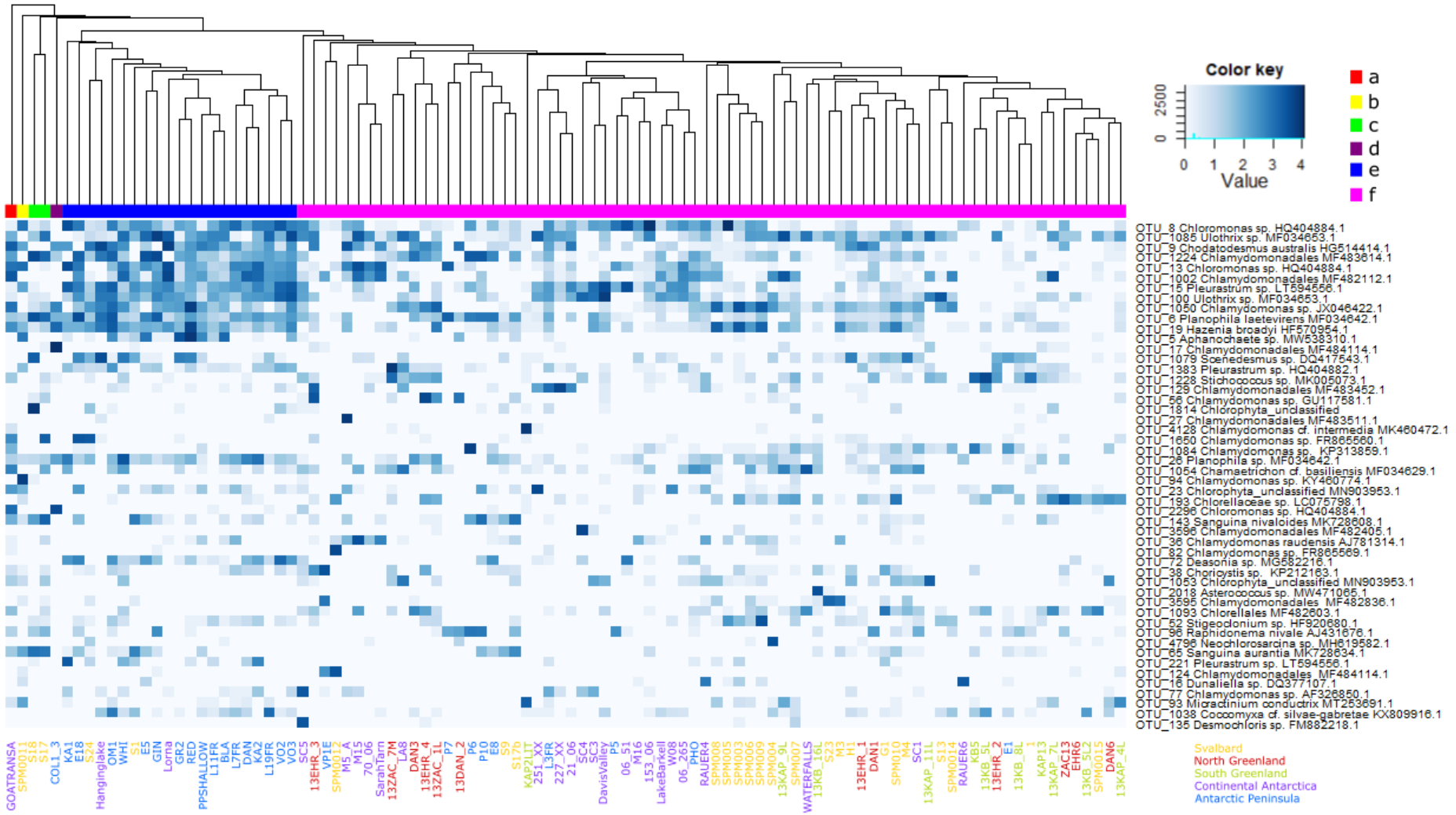

Fig. 27: Heatmap of the 50 most common OTUs (ITS2 rDNA). Significant clusters are marked with colour and named a-f. Sample names have different colour depending on from which biogeographical region they were sampled from.

4.3 Comparison of Sanger and amplicon sequencing data

In total, 65 newly established algal cultures were processed by Sanger sequencing and 102 environmental samples by amplicon sequencing. Strains sequenced with the Sanger sequencing method were only from Svalbard and the Antarctic Peninsula. Seven highly similar sequences (>97%) originating from six strains processed by Sanger sequencing were found in Svalbard amplicon sequencing data, and ten sequences from eight strains processed by Sanger sequencing were found in the Antarctic Peninsula amplicon sequencing data. No similar sequences were found in any sample sequenced by both methods. The proportion of taxa in acquired cultures detected in amplicon sequencing data is shown in Figs. 28 and 29. The darker column represents the biogeographical region where samples were collected, and 100% would indicate that all sequences from acquired cultures were detected in samples from a particular region by amplicon sequencing. In both cases, the proportion of detected taxa was the highest in the region of origin. Detailed results are shown in Supplementary Table S4.

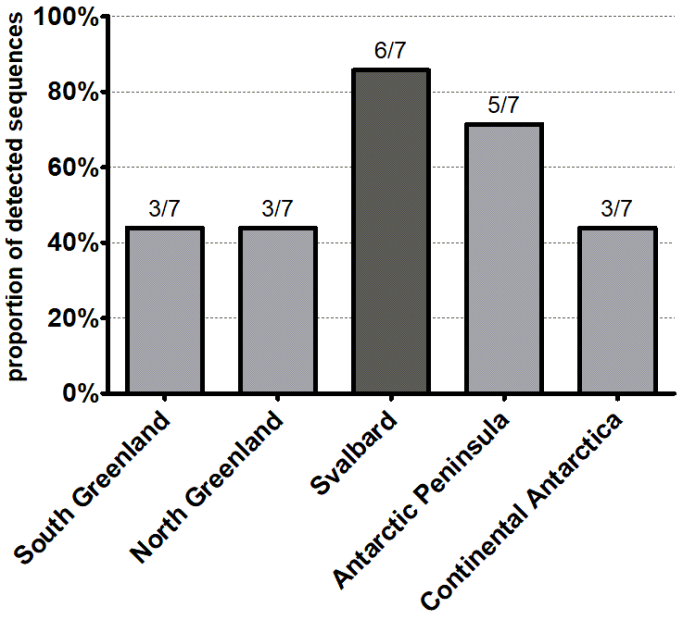

Fig. 28: Proportion of taxa in acquired cultures from Svalbard (darker column) detected in amplicon sequencing data from a given region.

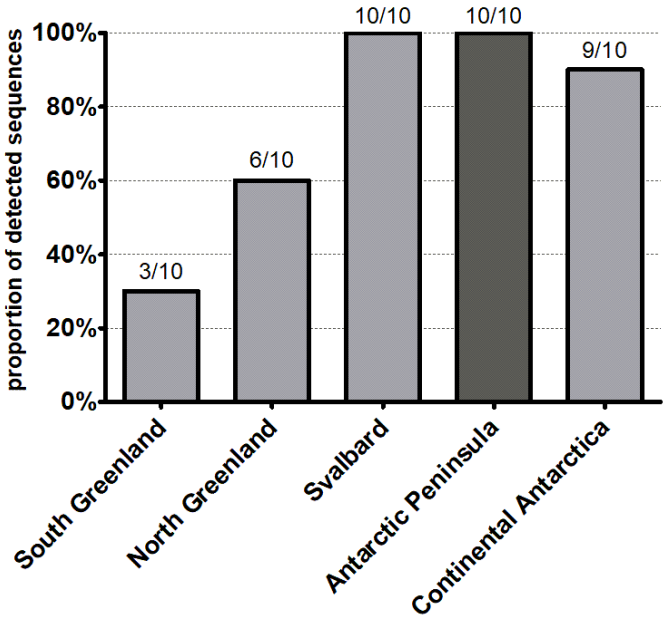

Fig. 29: Proportion of taxa in acquired cultures from the Antarctic Peninsula (darker column) detected in amplicon sequencing data from a given region.

5 Discussion

5.1 Sanger sequencing

Until recently, the identification of green algae relied on traditional microbiological approaches and the Sanger sequencing method, which is still a very valuable tool for the study of this group (Procházková et al., 2019; Sciuto et al., 2015; Škaloud et al., 2013).

Broady (1996) assumed that most of the Antarctic terrestrial green algae are cosmopolitan, and the endemic species which are present genetically differ only slightly from related species elsewhere. He supposed that dispersal by wind seems readily accomplished, but he also admits that there are certain dispersal barriers operating both to and within Antarctica. Therefore, it is rather assumed that there are more endemic species in Antarctica than in the Arctic (De Wever et al., 2009; Vyverman et al., 2010). The overall view of the taxonomic diversity detected in cultures from Antarctica and the Arctic is shown in the Fig. 7 Venn diagram. Ten taxa were detected in Antarctic cultures and eight in Arctic cultures, while three taxa were detected in both polar regions. For instance, *Chlorella* sp., which is known to be widespread, was found in our cultures from both polar regions (Hodač et al., 2016).

5.1.1 Trebouxiophyceae

The class Trebouxiophyceae is rich in species, and their members show remarkable ecological variation, being distributed among both aquatic and terrestrial habitats. Their members are also valuable for possible biotechnological use, such as biofuel production (Lemieux et al., 2014). Trebouxiophycean taxa *Chlorella* sp., *Chlorella vulgaris*, *Coccomyxa subellipsoidea*, *Micractinium* cf. *variabile*, *Micractinium simplicisimum*, *Micractinium* sp., and *Pseudostichococcus* sp. were all identified in our cultures.

Algae from the genus *Chlorella* are only coccoid, solitary with spherical morphology, and reproduce quickly and asexually by autospores (Luo et al., 2010). They are morphologically simple airborne microalgae which occur in terrestrial and aquatic habitats, and are able to grow under various environmental conditions including a large range of temperatures (Cao et al., 2016). Yet, their distribution was previously inferred only from ambiguous morphology-based evidence (Cao et al., 2016; Chae et al., 2019; Hodač et al., 2016; Luo et al., 2010; Morgan-Kiss et al., 2008; Yang et al., 2016). Hodač et al. (2016) performed a phylogenetic analysis of an expanded SSU and ITS2 rDNA sequence dataset from terrestrial habitats of polar, temperate, and tropical regions. They found that species closely related to *Chlorella vulgaris*, recovered from extreme environments of polar regions and hot deserts, are particularly widespread, suggesting that they might be capable of intercontinental dispersal.

Cultures E7, E9 and E16 from Antarctica identified as *Chlorella* sp. (Fig. 9) were identical to Antarctic strain L3 (under accession number KX355545.1) from Hodač et al. (2016). The ITS2 sequence of Arctic strain S16 from Svalbard identified as *Chlorella* sp. was identical to strain BJ4_1_1 isolated from a glacier in the Arctic (accession number KM514860.1) and the 18S rDNA sequence of this strain was identical to strain JL 2-2 (accession number AY195979.1) isolated from lakes in North Dakota, USA (Fawley et al., 2004; Zou et al., 2016). Cultures E1, P5, P6, P10 from Antarctica and S8 from the Arctic, which were identified as *Chlorella* sp. were identical to strain Ru-0-4 isolated from soil crusts in sand dunes on the Baltic sea coast, Germany, and their ITS2 sequence can be found under accession number MH703753.1 (Mikhailyuk et al., 2019). Collectively, these results support the claim that the genus *Chlorella* is widespread, due to identifying newly established strains identical to strains previously found in different continents.

Strain P9 isolated from a lake on Dundee Island, Antarctic Peninsula, was identified as *Coccomyxa subellipsoidea*. Its ITS2 sequence was identical to a strain under the accession number MN871439.1 isolated from snow in Eastern Antarctica, Ross Island, Antarctica (HG972971), Russia (MW013811) or Czech Republic (HE586527). *Coccomyxa subellipsoidea* has adapted to extreme cold conditions through greater versatility of its lipid metabolism that allows it to synthesize a greater diversity of cell membrane components (Blanc et al., 2012). Due to this fact, *Coccomyxa subellipsoidea* is also a biotechnologically interesting species (Wang et al., 2017).

Micractinium sp. described in Hong et al. (2015) was isolated from Antarctic lakes, and cells were round to slightly ellipsoid in shape, being approximately 3-5 μm in diameter. Their isolates are able to survive and grow in a temperature range of 5-30 °C. These *Micractinium* strains also showed a higher concentration of unsaturated fatty acids (PUFAs) and tolerance to lower temperatures. They believe that they could be a potential biological resource to produce compounds of biochemical interest such as omega-3 and omega-6 fatty acids.

ITS2 sequences of strains isolated from cultures E12, E13 isolated from lakes near Esperanza base on the tip on Antarctic Peninsula, and 18S rDNA sequences of strain S7 from Svalbard, were identified as *Micractinium* sp. (Fig. 9) identical to KNUA029 (ITS2 sequence under accession number LR215792.1 and 18S rDNA sequence under KM243319.1) isolated from lakes in West Antarctica (Hong et al., 2015). Strain E11 identified as *Micractinium* cf. *variabile* and E14 identified as *Micractinium* sp. were similar to KNUA036 (accession number

KT883910.1) based on analyses of ITS2 rDNA isolated by Hong et al (2017) from freshwater microalgae mass cultivation facility in South Korea. Their strain KNUA036 was identical to 18S rDNA sequence of KNUA034 from a lake in West Antarctica isolated by Hong et al. (2015). *Micractinium simplicissimum* (strains E2-E5 from Antarctica and S1-S3, S5-S7, S9, S10, S15 and S17 from the Arctic) were identical based on analyses of ITS2 rDNA to strain KSF0112 (accession number MN414470.1) isolated from lakes on King George Island, Antarctica (Chae et al., 2019).

Culture from sample E18 collected from the Antarctic Peninsula was identified as *Pseudostichococcus* sp. (Fig. 9), and was identical based on an analysis of both 18S rDNA and ITS2 rDNA to strain LH08SW8044 originally isolated by Hodač et al. (2016) from soil in Germany.

5.1.2 Chlorophyceae

Chlamydomonadales, is the largest order of Chlorophyceae with approximately 1,700 species, which are distributed in aquatic and terrestrial environments worldwide (Buchheim et al., 1996; Guiry & Guiry, 2019) and widely recognized for their potential applications in biotechnology (Mazumdar et al., 2018). Taxa *Chlorococcum* sp., *Chloromonas reticulata*, *Macrochloris* sp., *Neochlorosarcina negevensis* f. *ferruginea* and cf. *Sphaerocystis* sp. isolated from cultures in this work belong to this order.

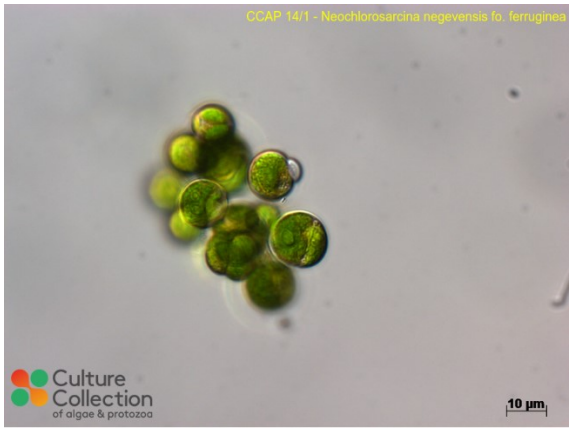

Fig. 30: *Neochlorosarcina negevensis* f. *ferruginea* (Gachon et al., 2007)

Neochlorosarcina negevensis (Figs. 9 and 30) is a coccoid alga producing zoospores covered by a single-layered cell wall, and pyrenoids are penetrated by tubular thylakoids (Watanabe et al., 2006). It seems to have wide environmental adaptations, and has been found in biological soil crust from all dry land regions in the world as well as, lakes in China (Büdel et al., 2009; Chen et al., 2008; Flechtner et al., 2013; Shi et al., 2020). Its strategy to survive

harsh conditions include the development of dormant stages or avoidance through motility, though high relative humidity is necessary to induce metabolic activity (Vimercati et al., 2020).

Of the 26 cultures isolated from samples collected in 2017, 14 from Antarctica and 12 from the Arctic were identified as *Neochlorosarcina negevensis* f. *ferruginea* (Fig. 8). They were identical to strain CCAP 14/1 originally isolated from soil in Negev Desert, Israel (Friedmann & Ocampo-Paus, 1965; Watanabe et al., 2006). The strain isolated from culture B1 was closely related to strain UPMC-A0066 based on analysis of 18S rDNA, and was identified as *Neochlorosarcina sempervirens* isolated from Sekinchan Paddy field, Ipoh, Malaysia (Khaw et al., 2020). This unexpected outcome of cultivation efforts from 2017 could be caused by algal contamination in the lab or influenced by lower temperature during their cultivation (7 °C) in contrast to samples collected in 2018 (12 °C). These altered conditions could theoretically support the growth of this species such that it became dominate, and massively overgrown all other species originally present in the environmental sample. As discussed in Thomas et al. (2017), underappreciated interactions between environmental factors (temperature, nutrients and light) can alter growth and patterns of occurrence of microorganisms.

Macrochloris sp. identified in culture S22 from Svalbard was previously found in Petuniabukta, Svalbard (Kvíderová, 2012), and due to its production of monosaturated fatty acids could be valuable for applications in industrial production (Hulatt et al., 2017).

Culture S12 from Svalbard was identified as cf. *Sphaerocystis* sp. identical to culture CCCryo 133-01/135-01 which was also isolated on Svalbard, and was described on Signy Island, South Orkney Islands, Antarctica (Broady, 1976). The cultures were previously named *Coenochloris signiensis*, but then it was reclassified as cf. *Sphaerocystis* sp. However, its attribution to genera *Sphaerocystis* and *Coenochloris* remains unclear due to the slightly different characteristics of the strains (Procházková et al., 2019).

The culture from sample P4 isolated from a lake on Dundee Island, Antarctic Peninsula, was identified as *Chodatodesmus australis* and based on 18S rDNA analysis (accession number FJ946898.1) was identical to strain EO2-14 isolated from various localities in Antarctica (Trinity Peninsula, Lützow Holm Bay and Prydz Bay (De Wever et al., 2009)). Together with a culture isolated from sample S24 collected on Svalbard, which was identified as *Chodatodesmus* sp., they were identical to strain CCCryo 424-14 based on analysis of ITS2 rDNA (accession number AM419228.1) collected in Terra Nova Bay, Victoria Land, Antarctica (Andreoli et al., 1996) and described by Sciuto et al. (2015). *Chodatodesmus australis* was so

far known only from Antarctica, but strain S24 isolated from sample collected in the north of Svalbard, which was identical to *Chodatodesmus australis* in 216 out of 227 bases in ITS2 region (accession number AM419228.1), shows that this species could be distributed in both polar regions.

Chloromonas reticulata is widely distributed unicellular alga occurring in snow and freshwater habitats (Barcytė et al. , 2020; Novakovskaya et al., 2018; Pröschold et al., 2001). It was detected in two strains from Svalbard (S21 and S25). ITS2 sequences of strain S21 was identical to strain CAUP G 1302 (accession number MK912127.1), which was found in Bjørndalen, Svalbard (Barcytė et al., 2020). Strain S25 was similar to strain CCCryo 338-08 (accession number HQ404900.1) isolated on Mt. Blanc, France.

To conclude, taxa obtained from cultures from Arctic and Antarctic lake samples were mostly common species already known from one or both polar regions. They were predominantly coccoid algae with simple morphology. Due to their small size, they are usually easily distributed but difficult to identify (Callieri & Stockner, 2002; Fawley et al., 2004). They have resistant vegetative cells or cysts and can adapt to various environmental conditions (Dolhi et al., 2013; Fuentes et al., 2016; Masojídek et al., 1999; Morgan-Kiss et al., 2006). Taxa (*Chlorella* sp., *Micractinium simplicissimum*, *Pseudostichococcus* sp., etc.) which were previously detected in various biogeographical regions worldwide were found in cultures obtained from Arctic and Antarctic samples. This reinforces the claim about their ubiquitous distribution. However, some taxa which were previously considered to be endemic to Antarctica were detected in cultures obtained from Svalbard samples (*Chodatodesmus australis* and cf. *Sphaerocystis* sp.). It could be expected that there are more globally distributed taxa which have been until now considered as potentially endemic.

5.2 Amplicon sequencing

The use of NGS technology for studying algal biodiversity has the potential for a more automated, complete, and accurate assessment of natural communities (Manoylov, 2014). Amplicon sequencing is an especially useful tool allowing us to study the whole microbial community in one run unlike Sanger sequencing. NGS methods also allow us to study species which are not easily cultivable or are under-represented in the sample (Rippin et al., 2018), and to reconstruct their population structure (Brown & Tucker, 2020)

Unfortunately, there is no research engaged in the study of green algae from lakes in polar regions using amplicon sequencing as of today. There are a few studies of Chlorophyta from other polar habitats using the amplicon sequencing method (Luo et al., 2020; Lutz et al., 2015; Novis et al., 2015; Rippin et al., 2018; Segawa et al., 2018), and the use of amplicon sequencing on other microbial groups in the polar regions (for example Obbels et al., 2016; Rippin et al., 2018; Theroux et al. , 2012) and other biomes (for example Bock et al., 2020; Boenigk et al., 2018; Olefeld et al., 2020) is widespread.

The samples used in this thesis cover a wide geographical range – especially those from Antarctica. Terauds et al. (2012) suggested that there are 15 Antarctic Conservation Biogeographic Regions delineated by comparing biologically distinct groups formed by the cluster analyses. In this study we used the traditional division of Antarctica to Continental Antarctica and Antarctic Peninsula (Convey, 2013) due to few or missing samples from some Antarctic Biogeographic regions suggested by Terauds et al. (2012).

5.2.1 18S rDNA

18S rDNA is a widely-used marker for (micro)organism identification (Hoham et al., 2002; Jungblut et al., 2012; Sciuto et al., 2015;Watanabe et al., 2006) with its own benefits and disadvantages. Among the advantages, a large coverage of database sequences is available for Chlorophyta, although this gene usually only allows us to identify Chlorophyta to the genus level (Segawa et al., 2018). In contrast to the ITS2 rDNA marker, 18S rDNA primers are more "universal", meaning that they can capture more groups of organisms (Fawley & Fawley, 2020).

For defining green algae taxa, there is no generally accepted threshold of 18S rDNA sequence divergence (Vyverman et al., 2010). The threshold of 97% has been widely applied in metagenomic studies of microalgae from various habitats (Rii et al., 2016; Tragin et al., 2018; Zhu et al., 2018). However, a far stricter threshold is necessary when studying closely related taxa (Lutz et al., 2018). The value of 97% used in this study could therefore cause blurring of some trends in less variable 18S rDNA marker (see Fig. 12 *vs.* Fig. 20 based on ITS2 rDNA), and this strategy resulted in a far lower number of Chlorophyta OTUs that were detected in comparison with the ITS2 rDNA data. Also, a higher proportion of taxa found in both polar regions characterised the 18S rDNA dataset (Fig. 11 *vs.* Fig. 17). There are not many biogeography studies that include the whole Chlorophyta group based on 18S rDNA sequencing, although there are a few studies that examine the distribution of Chlorophyta

genera (Coesel & Krienitz, 2007; Hodač et al., 2016; Lutz et al., 2016; Metz et al., 2019; Rippin et al., 2018; Schmidt & Darcy, 2015; Segawa et al., 2018).

In the Arctic, there is an expected lower number of endemic species than in Antarctica (Kim et al., 2011; Richter et al., 2018). Until recently, most data on Antarctic green algae depended on morphology-based taxonomic inventories, and a high degree of cosmopolitanism was assumed (Broady, 1996; Mataloni & Pose, 2001; Zidarova, 2007). In contrast, the study of more than 40 microchlorophyte strains (Sanger sequencing of 18S rDNA) from maritime and continental Antarctica (De Wever et al., 2009) found that except for one strain, all Antarctic sequences were different from non-Antarctic sequences currently available in the GenBank database. Vyverman et al. (2010) investigated the distribution of green algae within Antarctica and found only two taxa belonging to the genera *Chlorella* and *Scenedesmus* were detected in three of five regions. These taxa are considered to be easily dispersed and distributed worldwide (Guiry & Guiry, 2019; Hodač et al., 2016). Studies of eukaryotes in Arctic and Antarctic cyanobacterial mats using 18S rDNA indicate that there are close genetic similarities between certain taxa in the Arctic and Antarctica (Jungblut et al., 2012). However, our data showed that the overlap in OTU diversity was high when comparing Antarctica and the Arctic (Fig. 11), with slightly more OTUs observed only in Antarctica (42) compared to those observed only in the Arctic (36), suggesting that Antarctica potentially has more endemic species. A more strict threshold for OTU clustering could provide more support for such a trend (see above).

The regional OTU richness based on our 18S rDNA dataset was only slightly higher in the Arctic than in Antarctica. There were no significant differences between OTU richness in both polar regions. However, the use of the 18S rDNA marker only for studying the geographic distribution of algae could erase subtle differences between samples due to the fact that 18S rDNA is likely to determine algae on genus level (Segawa et al., 2018). Therefore, the high similarity between Arctic and Antarctic diversity could be a consequence of using 18S rDNA. Average alpha diversity was almost identical for both polar regions (Fig. 12), with 30 OTUs/sample in the Arctic and 32 OTUs/sample in Antarctica. In contrast, a study of bacterial communities from polar lakes based on 16S rRNA (Liu et al., 2021) revealed that OTU richness was significantly higher in the Arctic than Antarctica. However, Lozupone & Knight (2005) compared bacterial communities (16S rRNA) from sea ice, seawater, and sediment. Their data showed similarities between communities in each polar region from particular environment types and that environment types dominated differences

between communities. Their analyses support the hypothesis that geography plays a minimal role in structuring bacterial communities and that bacterial taxa are dispersed widely in similar habitat types across the globe.

There was no visible dominance of one or several OTUs in our dataset, as can be seen from the steep rise of the rarefaction curves (Fig. 10). Canonical analysis of principal coordinates for investigating community composition performed on the 18S rDNA dataset for both polar regions showed that Antarctic samples were more similar to each other (CCR = 100%) than Arctic samples (CCR = 82.9%, Fig. 14). Nevertheless, the CAP analysis for the five main biogeographical regions (North Greenland, South Greenland, Svalbard, Antarctic Peninsula and Continental Antarctica, Fig. 15) revealed that samples in each region were not very distinct. For instance, a NMDS analysis based on Bray-Curtis distances across lake samples from Liu et al. (2021) showed structuring based on geographic location, while bacterial communities in Arctic lakes showed clear segregation and Antarctic lake samples were more dispersed. However, a CAP analysis of diatoms from Antarctica (Verleyen et al., 2021) revealed a clear separation between samples from sub-Antarctic, Maritime and Continental regions.

With regard to the distribution of Chlorophyta classes among polar regions, the most abundant class in the Arctic was Ulvophyceae, in contrast with Antarctica where the most abundant class was Chlorophyceae. The third most abundant class in both polar regions was Trebouxiophyceae (Fig. 13). The low amount of unclassified Chlorophyta reflected the large coverage of 18S rDNA Chlorophyta sequences in the databases. Similarly, as in a study of Arctic and Antarctic mats (Jungblut et al., 2012), the most abundant Chlorophyta class in the Arctic was Ulvophyceae and in Antarctica was Chlorophyceae. Their data suggests that the abundant sequences could indicate habitat preferences by certain groups. Chlorophyceae, which are known to be rich in carotenoid pigments that protect against UV stress, were found in exposed ice shelf mats, while Ulvophyceae were most prominent phototrophic group in thicker more cohesive mats. When comparing our data with an overview of the genera occurring in lakes of polar regions (Table 1), Chlorophyceae are comparably represented, Trebouxiophyceae are quite underrepresented in our data, and there is a high representation of Ulvophyceae in our data. Surprisingly, there were no OTUs classified to the classes Zygnematophyceae, Charophyceae, or Prasinophyceae (Jónasson, 1992; Säwström et al., 2002; Vincent & Laybourn-Parry, 2008), which may be due to non-amplification of these genera

by the used primers. Overall, the intersection between our data and the detected genera from publications is limited, and more studies of green algae biodiversity are clearly needed.

5.2.2 ITS2 rDNA

Use of the ITS2 rDNA marker allows us to study Chlorophyta at the species level. Nevertheless, the representation of ITS2 sequences in databases is still quite low compared with 18S rDNA. In this work, the OTU similarity threshold was set to 97% as performed in other microbial and algal studies (Frey et al., 2016; Tragin et al., 2018). However, due to the fact that the ITS2 rDNA marker is more variable, it could potentially be clustered at lower similarity threshold (e.g. 94%, Lutz et al., 2019).

The total biodiversity of polar limnetic systems is generally considered to be low (Pearce & Galand, 2008). However, the regional species richness was approximately two times higher in the Arctic than in Antarctica as shown in the rarefaction curves (Fig. 16). The was not seen dominance of few species in Antarctica, however, the Arctic communities were more diverse. Antarctic samples are therefore more similar to each other than the Arctic ones, as can be seen in Figs. 17, 18 and 27. From the rarefaction curves, it is also visible that there are still species which were not captured in both polar regions and could be found by increasing the sampling intensity or sequencing depth. Alpha diversity was higher in the Arctic (average 106 OTU/sample) than in Antarctica (72, Fig. 20). Upon closer inspection of the five biogeographic regions, all Arctic regions exhibited a similar average number of OTUs per sample (Fig. 21). On the other hand, alpha diversity in the Antarctic Peninsula (92) was closer to the Arctic regions than Continental Antarctica (49). This can be explained by the harsher conditions present in the latter region. Most of our sampled Arctic and Antarctic lakes are postglacial lakes. The average conductivity in Arctic lakes was 0.35 mS cm^{-1} (N = 39) and in Antarctic lakes 0.48 mS cm^{-1} (N = 33), which indicates that lakes in both polar regions were freshwater and that differences were probably not sufficient to affect diversity patterns between polar regions.

Schiaffino et al. (2016) studied microbial eukaryote communities along a gradient of Patagonian and Antarctic lakes, and among other things, they found that the number of autotrophic and heterotrophic eukaryotic taxa significantly increased with temperature similar to our data shown on Fig. 22. This trend was also confirmed within a study of Antarctic diatoms, which was based on the morphological identification of species (Verleyen et al., 2021).

A comparison of OTUs present in the five biogeographic regions (Fig. 18) shows that more OTUs are shared between the particular regions within the Arctic or within Antarctica than between the two polar regions. This could be due to the shorter distances between biogeographical regions situation at a given Earth's pole. In a study of freshwater protist communities from European lakes (Boenigk et al., 2018), community similarity generally decreased with increasing distance. In our study there were 19.4% shared OTUs among all Arctic regions and 42.7% of OTUs were shared among Antarctic regions. This could be explained by more pronounced differences in environmental conditions among Arctic regions, or due to the fact that Arctic regions are not part of one mainland like Antarctica. Rippin et al. (2018) studied genus-level richness of microalgae and cyanobacteria in biological soil crusts from Svalbard and Livingston Island, Antarctica. They found that 52.5% of the genera were shared between Svalbard and Livingston Island, while in Livingston Island the richness was two times lower than in Svalbard. In the CAP analysis from our dataset, the overall CCR in comparing Arctic and Antarctic samples (Fig. 23) was 93%, which shows that both polar regions are quite isolated. When comparing all five biographic regions (Fig. 24) North Greenland showed both the greatest isolation (CCR = 100%) as well as the highest diversity (595 OTUs, Fig. 19). For instance, the Antarctic freshwater diatom study showed clear separation between the canonical biogeographic regions (Verleyen et al., 2021).

The most abundant Chlorophyta class in both polar regions was the class Chlorophyceae with 21.7% in the Arctic and 29.9% in Antarctica (Fig. 25). Class Ulvophyceae was noticeably more abundant in Antarctica with 12.1% than in the Arctic (2%), while Ulvophyceae was the most abundant class in the Arctic using 18S rDNA data. 64.9% of OTUs in the Arctic and 49.7% in Antarctica were unclassified Chlorophyta and Chlorophyta Inc. sed. There was quite a high number of unclassified Chlorophyta in comparison with 18S rDNA (Fig. 13), where there was less than 1% unclassified Chlorophyta in both polar regions. This suggests that there is still a scarcity of reference sequences in the databases, and therefore more Chlorophyta studies using ITS2 rDNA marker are needed.

When searching whether OTUs detected only in the Arctic or Antarctica were already detected elsewhere or could be considered as potentially endemic for one polar region, we found, for example, that OTU_2018 is only found in the Arctic and was similar to a sequence with the accession number MW471065.1 which was found in United Kingdom (88% identity only, unpublished data). On the other hand, OTU_1990 was only detected in the Arctic in our data, and was most similar to the sequence HQ404867.1 (*Ploeotila* sp.) from Svalbard

(96% identity, unpublished data). From the OTUs only detected in Antarctica, for example, OTU_55 was similar to a sequence under the accession number MG952962.1 (*Dunaliella* sp.) that was found in the Great Salt Lake, USA (Henley et al., 2018), and OTU_345 was similar to a sequence under the accession number KM514860 (*Chlorella* sp.) found on a glacier in the Arctic. Therefore, based on these findings, there could be more species with worldwide distributions which are currently considered to be potentially endemic, and therefore amplicon sequencing data must be handled with caution.

The heatmap in Fig. 27 shows the 50 most common OTUs. Giner et al. (2016) concluded that more reads are genuinely linked to a higher proportion of cells of the species, and therefore we used the number of reads as a proxy for the relative abundance of individual OTUs to compare samples. Within this dataset, there were species which were previously considered to be endemic. For example, *Chodatodesmus australis* was previously described only from Antarctica (Gondwana Station, Terra Nova Bay, Victoria Land - Sciuto et al., 2015; Rauer Islands, Trinity Peninsula and Lützow Holm Bay - De Wever et al., 2009). OTU 9 was 100% identical to a sequence of *Chodatodesmus australis* and was frequently found in samples from all biogeographical regions in Antarctica and the Arctic. Moreover, the presence of this genus in the Arctic was also confirmed by Sanger sequencing. *Hazenia broadyi* described by Škaloud et al. (2013) isolated from Green Lake 1 and 2 (James Ross Island, Antarctic Peninsula) was so far found only there. We found OTU 19, which was 99% identical to ITS2 sequence of *Hazenia broadyi*, in all biogeographical regions in large numbers. Sample GR2 collected from Green Lake 2 in 2017 also contained sequence of *Hazenia broadyi*. Species which were previously detected in many localities worldwide were present among the most frequent OTUs. For instance, *Chloromonas* sp. was the most frequent OTU and *Chlamydomonas* sp. (both occurring several times among the 50 most frequent OTUs) were previously recorded in many localities worldwide from cold-tolerant habitats (Hoham et al., 2002; Pröschold et al., 2001). Sequences (accession number HQ404884.1) similar to our *Chloromonas* sp. (OTU_8, OTU_13 and OTU_2296) were found in Svalbard, although, in our data it was detected in abundance from both polar regions. Sequences identical to *Chlamydomonas* sp. from our data were detected in Argentina (JX046422.1) and Minnesota, USA (FR865560.1). *Scenedesmus* sp. (OTU_1079) was identical to sequence DQ417543.1 detected in Minnesota, USA (Johnson et al., 2007). From these data it can be assumed that among the most frequent OTUs in our data, many of them have a worldwide distribution. The Antarctic psychrophile, *Chlamydomonas raudensis*, which grows under extreme conditions including low temperature and irradiance,

was described from Lake Bonney, Antarctica (Dolhi et al., 2013; Morgan-Kiss et al., 2005). OTU 36 was 100% identical to sequence of *Chlamydomonas raudensis*, and was found in samples from many lakes in Antarctica and Svalbard. The snow alga *Sanguina aurantia* has been only recorded from snow habitats in the Northern Hemisphere with a circumpolar and temperate alpine distribution (Brown & Tucker, 2020; Procházková et al., 2019). However, OTU 65, which was 100% identical to the ITS2 sequence of *Sanguina aurantia*, was found in all biogeographic regions in this study. *Micractinium conductrix* was previously described only from Europe and North America (Guiry & Guiry, 2019). OTU 93 was 100% identical to sequences of *Micractinium conductrix*, and was found in all biogeographical regions except for Continental Antarctica. OTU 1038 was 98% identical to *Coccomyxa silvae-gabretae*, which was previously found and described only from Hromnice Lake in Czech Republic by Barcyte & Nedbalová (2017). This OTU was present in several samples from all biogeographic regions. These species can thus be described as cosmopolitan based on their recovery from different locations. Most abundant OTUs typically have resistant vegetative cells or can produce cysts; thus, they are probably well-dispersed and adapted to various conditions (Sciuto et al., 2015).

Among the most abundant OTUs were several taxa which primarily occur in the liquid water film on snow and glacier surfaces (*Sanguina aurantia*, *Sanguina nivaloides*, *Raphidonema nivale*) which create macroscopically visible coloration (Procházková et al., 2019). It is not assumed that they would actively grow in the lake water environment. However, there are difficulties with their cultivation (Hoham, 1973; Nedbalová & Komárek, 2007), such as the germination of the field collected cysts, and we were therefore not able to isolate any snow algae strains despite their frequent occurrence in the samples. Scattered snow algal cysts were previously reported from high-alpine lakes, where they are most probably flushed from the surrounding snowfields during snowmelt into the lakes (Fott et al., 1994; Hořická et al., 2006; Nedbalová et al., 2006; Procházková, 2010; Procházková et al., 2018). As polar ecosystems are generally oligotrophic (Vincent & Laybourn-Parry, 2008), autochthonous algal biomass is usually low. The input of cells from blooming snowfields can thus be rather significant for the ecosystem as a whole (Fott et al., 1994). As shown in recent metagenomic studies, sequences of snow algae were found in French Alps soils (Stewart et al., 2021) and on gravel bars in Swiss Alps rivers (Vančurová et al., 2020). Snow algae cells are likely to reach these localities after snowmelt. These results show, among other things, that amplicon sequencing is a suitable tool for detecting these organisms even outside their growth period and to monitor their occurrence.

5.3 Comparison of Sanger and amplicon sequencing data

There are not many microalgae studies comparing Sanger sequences of cultured material and amplicon sequences of environmental samples. For example Câmara et al. (2021) compared data on terrestrial green algae obtained by ITS2 amplicon sequencing with data based on cultivation (Fermani et al., 2007) from two localities on Deception Island, Antarctic Peninsula. The algal richness detected by amplicon sequencing was roughly nine times higher than the richness obtained based on cultivation. They also found that 35 out of 65 (53%) taxa have not been previously recorded in Antarctica. Surprisingly, in our data originating from Svalbard and the Antarctic Peninsula, no highly similar sequence was found by both methods in one sample. However, taxa characterized by Sanger sequencing were found in samples from all biogeographic regions including the original one. While in both cases the proportion of detected taxa was the highest in the region of origin (Figs. 28 and 29). The possible reason that no identical sequence was found by both methods may be that the species was not present in the isolated DNA of the environmental sample, or it could not be amplified due to a low number of cells in the sample. Several technical biases of amplicon sequencing, such as primer selection, library preparation, or annealing temperature should be taken in account as discussed in Lutz et al. (2019). For instance, Škaloud et al. (2020) pointed out drawbacks when using metabarcoding to estimate species richness, such as the generation of short single loci sequences which makes it difficult to determine appropriate species boundaries and the creation of chimeras. Therefore, richness estimates may rely on the completeness and quality of the reference databases, and results should be interpreted with caution. Another possibility of using culture independent methods may be single-cell Sanger sequencing of environmental samples, which should show a high similarity with Sanger sequencing data of cultures (Bock et al., 2014; Segawa et al., 2018).

Rippin et al. (2018) compared data obtained by morphology and molecular (amplicon sequencing) approaches characterizing cyanobacteria and microalgae from Arctic and Antarctic soil crusts. They reported more genera with the molecular survey than by using morphology-based methodology. Despite this fact, molecular methods did not recover all genera determined by light microscopy. In our data, we found more genera via amplicon sequencing than with a combination of strain isolation from incubated samples and Sanger sequencing. Six out of eleven genera obtained by Sanger sequencing of unialgal strains were detected among the 50 most frequent OTUs that resulted from amplicon sequencing of environmental samples

(Fig. 27). This indicates that the genera obtained by Sanger sequencing of newly isolated strains are widespread.

The use of NGS methods allow us to study complex microbial communities unlike Sanger sequencing (Shokralla et al., 2012). Similarly to the results of Câmara et al. (2021), our data shows that the use of amplicon sequencing allows for the identification of a higher proportion of diversity (including uncultivable species) than traditional culture methods and Sanger sequencing. Nonetheless, our study confirmed that light microscopy and/or Sanger sequencing can also recover taxa not obtained by amplicon sequencing.

6 Conclusion

This thesis examined the diversity and biogeography of green microalgae (Chlorophyta) from 137 lakes of both polar regions, using strain isolation combined with Sanger sequencing and amplicon sequencing of environmental samples. This enabled a comparison of the Chlorophyte diversity between both polar regions, and well as a comparison of these different methodological approaches.

Taxa recovered from laboratory cultures mostly belonged to common genera of Chlorophyta – coccoid algae with simple morphologies, which are expected to be easily dispersed and widely distributed. Most of them are already known from both polar regions, and some taxa (such as *Chlorella* sp., *Micractinium simplicissimum*, and *Pseudostichococcus* sp.) have been previously detected from various biogeographical locations worldwide, thus strengthening the claim about their ubiquitous distribution. On the other hand, some taxa (*Chodatodesmus australis* and cf. *Sphaerocystis* sp.) which were previously considered to be Antarctic endemics were detected in the set of newly isolated strains from Svalbard. This suggests that probably more presumably endemic taxa of green algae are in fact distributed worldwide.

We confirmed our hypothesis that higher biodiversity of green microalgae will be found in the Arctic than in Antarctica. Using 18S rDNA amplicon sequencing, a total of 160 and 166 OTUs were recovered in the Arctic and Antarctica, respectively, while ITS2 rDNA revealed 1,020 and 671 OTUs in the Arctic and Antarctica. A steeper rarefaction curve for Antarctic samples revealed the dominance of several OTUs. Alpha diversity based on the ITS2 rDNA marker was almost two times higher in the Arctic, while the lowest alpha diversity was detected in Continental Antarctica. The use of 18S rDNA did not confirm this trend, which is likely result of its lower specificity in comparison with ITS2 rDNA. The most abundant class in the Arctic was Ulvophyceae, while in Antarctica the most abundant class was Chlorophyceae.

There were more OTUs shared between the Arctic and Antarctica when 18S rDNA data were considered (61.4%). In contrast, the ITS2 rDNA marker revealed a higher proportion of potentially endemic taxa (46.8%). 10.3% of OTUs were shared between all biogeographic regions. The CAP analysis showed high isolation in all biogeographic regions (ITS2 rDNA). However, the use of the non-specific 18S rDNA marker revealed that samples in each region

were not so distinct. Our data showed that there is a positive relationship between the number of OTUs in the sample and the annual air temperature. Among the most abundant OTUs there were species which were previously considered to be endemic (for instance *Chodatodesmus australis*, *Hazenia broadyi* and *Sanguina aurantia*) and many taxa which primarily occur in snow (for example *Sanguina aurantia*, *Sanguina nivaloides* and *Raphidonema nivale*) and were most probably flushed from snowfields into lakes.

The use of both 18S rDNA and ITS2 rDNA is a valuable tool not only in a study focusing on Chlorophyta. 18S rDNA has the advantage of having a large representation of database sequences, and that the primer is "universal" in terms of its ability to capture many groups of organisms, although it only allows us to study Chlorophyta at the genus level, and therefore some important trends might be overlooked when using this marker. On the other hand, ITS2 rDNA provides us with the opportunity to study Chlorophyta at the species level. While the primer is indeed more specific, the identification of OTUs suffers from a scarcity of reference database sequences. From our data, it is visible that there are significant differences between results obtained using each marker on the same samples (e.g. OTU richness). The use of 18S rDNA produced a better picture of composition at the class level, while ITS2 rDNA gives a more precise classification at the species level due to its variability. The given OTU threshold (97%) could also affect the resulting data. In case of 18S rDNA, a less strict threshold could result in a blurring of trends, while a stricter threshold in case of ITS2 rDNA might not capture some species due to its variability. Consequently, the use of both markers together reveals a better picture of the whole algal community.

When comparing data obtained by Sanger and amplicon sequencing from Svalbard and the Antarctic Peninsula, there was no identical sequence found by both methods in one sample. However, taxa characterized by Sanger sequencing were found in samples from the same or different biogeographic regions. Amplicon sequencing yielded many more species than strain isolation and Sanger sequencing. These results support the use of a combination of methods as a recommended strategy to help improve the precision of studies on microbial communities.

To our knowledge, this is the first study on the diversity and biogeography of Chlorophyta from polar lakes using amplicon sequencing. Additionally, the use of more methods when studying this group is quite rare. Our results provided valuable insight into the biogeography of many species of green microalgae. Nonetheless, more studies on the biogeography of green microalgae are required to support their ubiquitous distribution or the endemism of some species.

7 References

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8 Supplementary data

Table S1: List of samples with their geographical location. Processing of samples by Sanger sequencing (strain isolation) and amplicon sequencing (18S rDNA and ITS2 rDNA – primer sets P2 and P3) is also indicated.

Polar region	Biogeographical region	Sample name	GPS coordinates		Date of collection	Sanger	Ill ITS2		Ill 18S
							P2	P3	
Antarctica	Antarctic Peninsula	DAN	63°52'44.00"S	57°58'43.00"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	BLA	63°57'58.00"S	57°52'59.00"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	BLU	63°54'54.00"S	57°57'24.00"W	2017	x			x
Antarctica	Antarctic Peninsula	GIN	63°52'28.98"S	57°48'10.02"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	GR2	63°53'54.00"S	57°46'33.00"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	KA1	63°52'22.98"S	57°58'31.98"W	2017	x	x		x
Antarctica	Antarctic Peninsula	VO2	63°57'36.00"S	57°54'22.98"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	KA2	63°52'16.02"S	57°58'33.00"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	LA1	63°47'34.98"S	57°48'13.98"W	2017	x			
Antarctica	Antarctic Peninsula	OM1	63°51'40.02"S	57°48'49.98"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	PHO	63°50'49.02"S	58° 0'28.02"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	RED	63°54'33.00"S	57°47'46.02"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	RO2	63°49'22.98"S	57°50'40.98"W	2017	x			x
Antarctica	Antarctic Peninsula	WHI	63°53'49.98"S	57°48'45.00"W	2017	x	x	x	x
Antarctica	Antarctic Peninsula	VO3	63°57'33.00"S	57°54'7.98"W	2017		x	x	x
Antarctica	Antarctic Peninsula	E1	63°24'14.30"S	56°59'49.80"W	05.01.2018	x	x	x	x
Antarctica	Antarctic Peninsula	E2	63°24'17.00"S	56°59'58.50"W	05.01.2018	x			
Antarctica	Antarctic Peninsula	E3	63°24'17.00"S	56°59'58.50"W	05.01.2018	x			
Antarctica	Antarctic Peninsula	E4	63°24'29.50"S	57° 1'22.50"W	07.01.2018	x			
Antarctica	Antarctic Peninsula	E5	63°24'32.60"S	57° 1'37.60"W	07.01.2018	x	x	x	x
Antarctica	Antarctic Peninsula	E7	63°24'35.40"S	57° 1'53.90"W	07.01.2018	x			
Antarctica	Antarctic Peninsula	E8	63°24'37.00"S	57° 1'58.50"W	07.01.2018		x	x	x
Antarctica	Antarctic Peninsula	E9	63°24'38.90"S	57° 2'7.00"W	07.01.2018	x			
Antarctica	Antarctic Peninsula	E10	63°24'38.10"S	57° 2'0.60"W	20.01.2018	x			

Polar region	Biogeographical region	Sample name	GPS coordinates		Date of collection	Sanger	Ill ITS2		Ill 18S
							P2	P3	
Antarctica	Antarctic Peninsula	E11	63°23'58.50"S	56°58'52.30"W	10.01.2018	x			
Antarctica	Antarctic Peninsula	E12	63°23'53.50"S	56°58'58.80"W	10.01.2018	x			
Antarctica	Antarctic Peninsula	E13	63°23'56.70"S	56°58'48.10"W	10.01.2018	x			x
Antarctica	Antarctic Peninsula	E14	63°24'15.40"S	57° 0'40.30"W	20.01.2018	x			x
Antarctica	Antarctic Peninsula	E16	63°24'18.10"S	56°59'28.50"W	23.01.2018	x			
Antarctica	Antarctic Peninsula	E18	63°24'33.90"S	57° 0'28.20"W	26.01.2018	x	x	x	x
Antarctica	Antarctic Peninsula	P3	63°28'39.90"S	56°13'20.70"W	07.02.2018	x			
Antarctica	Antarctic Peninsula	P4	63°28'39.30"S	56°13'19.40"W	07.02.2018	x			
Antarctica	Antarctic Peninsula	P5	63°28'41.00"S	56°13'14.10"W	07.02.2018	x	x		
Antarctica	Antarctic Peninsula	P6	63°28'46.80"S	56°13'12.40"W	07.02.2018	x	x	x	x
Antarctica	Antarctic Peninsula	P7	63°28'50.30"S	56°13'7.50"W	10.02.2018		x		
Antarctica	Antarctic Peninsula	P8	63°29'14.80"S	56°13'13.10"W	10.02.2018	x			
Antarctica	Antarctic Peninsula	P9	63°29'14.30"S	56°13'20.80"W	10.02.2018	x			
Antarctica	Antarctic Peninsula	P10	63°29'19.60"S	56°13'0.20"W	12.02.2018	x	x	x	x
Antarctica	Antarctic Peninsula	GOATRANSA	62°13'15.60"S	58°57'15.40"W	2006		x	x	x
Antarctica	Antarctic Peninsula	L11FR	63°49'16.00"S	57°19'33.40"W	2013		x	x	x
Antarctica	Antarctic Peninsula	L19FR	63°49'10.90"S	57°18'59.10"W	2013		x	x	x
Antarctica	Antarctic Peninsula	L3FR	63°49'17.50"S	57°19'30.20"W	2013		x	x	x
Antarctica	Antarctic Peninsula	L7FR	63°49'6.70"S	57°20'1.60"W	2013		x	x	x
Antarctica	Antarctic Peninsula	PPSHALOW	67°36'3.24"S	67°12'26.94"W	NA		x	x	x
Antarctica	Antarctic Peninsula	VP1E12_76	63°33'9.30"S	57°24'10.98"W	NA		x	x	x
Antarctica	Antarctic Peninsula	COL1_312_25	67°49'52.20"S	67°13'56.22"W	NA		x	x	x
Antarctica	Continental Antarctica	06_265	75°53'55.68"S	162°34'45.91"E	2005		x	x	x
Antarctica	Continental Antarctica	153_06	74°42'36.00"S	164° 3'29.99"E	2005		x	x	x
Antarctica	Continental Antarctica	21_06	74°36'45.65"S	165°12'49.61"E	2005		x	x	x
Antarctica	Continental Antarctica	227_XX	76° 9'44.57"S	161°59'50.17"E	2004		x	x	x
Antarctica	Continental Antarctica	251_XX	72°35'60.00"S	169°20'60.00"E	2004		x	x	x
Antarctica	Continental Antarctica	70_06	74°58'4.44"S	162°30'59.04"E	2005		x	x	x

Polar region	Biogeographical region	Sample name	GPS coordinates		Date of collection	Sanger	Ill ITS2		Ill 18S
							P2	P3	
Antarctica	Continental Antarctica	DavisValley	82°28'0.00"S	51° 9'0.00"W	2003		x	x	x
Antarctica	Continental Antarctica	EO3A	69° 0'59.22"S	39°35'1.20"E	2003			x	x
Antarctica	Continental Antarctica	Hanginglake	67°41'33.79"S	63° 1'13.87"E	2001		x	x	x
Antarctica	Continental Antarctica	LA8	69°12'5.34"S	39°39'2.82"E	2003			x	x
Antarctica	Continental Antarctica	LakeBarkell	67°27'58.02"S	60°58'1.14"E	2001		x	x	x
Antarctica	Continental Antarctica	Lorna	67°47'4.45"S	62°47'17.48"E	2001		x	x	x
Antarctica	Continental Antarctica	M15	66°14'24.42"S	100°42'29.52"E	2002		x	x	x
Antarctica	Continental Antarctica	M16	66°15'2.28"S	100°36'18.00"E	2002		x	x	x
Antarctica	Continental Antarctica	M5_A_	66°17'47.22"S	100°41'29.10"E	2002		x	x	x
Antarctica	Continental Antarctica	RAUER4	68°49'0.00"S	77°42'0.00"E	1993		x	x	x
Antarctica	Continental Antarctica	RAUER6	68°52'60.00"S	77°50'60.00"E	1993		x	x	x
Antarctica	Continental Antarctica	SC1	70°46'25.68"S	11°48'45.18"E	2003		x	x	x
Antarctica	Continental Antarctica	SC3	70°46'0.84"S	11°45'7.74"E	2003		x	x	x
Antarctica	Continental Antarctica	SC4	70°45'57.00"S	11°45'35.10"E	2003		x	x	x
Antarctica	Continental Antarctica	SC5	70°45'57.12"S	11°45'53.40"E	2003		x	x	x
Antarctica	Continental Antarctica	SarahTarn	69°22'60.00"S	76°22'60.00"E	1993		x	x	x
Antarctica	Continental Antarctica	WATERFALLS	68°32'57.00"S	78°20'8.00"E	2004		x	x	x
Antarctica	Continental Antarctica	06_51	74°43'23.41"S	162°41'37.79"E	2005		x	x	x
Antarctica	Continental Antarctica	WO8lit	69° 1'44.40"S	39°31'22.80"E	2003		x	x	x
Arctic	North Greenland	13EHR_1	74°27'21.40"N	21°36'13.90"W	2013		x	x	x
Arctic	North Greenland	13EHR_2	74°27'41.60"N	21°28'38.90"W	2013			x	x
Arctic	North Greenland	13EHR_3	74°28'16.80"N	21°31'20.60"W	2013		x	x	x
Arctic	North Greenland	13EHR_4	74°29'6.80"N	21°28'56.80"W	2013		x	x	x
Arctic	North Greenland	13ZAC_1L	74°27'35.20"N	20°33'13.30"W	2013		x	x	x
Arctic	North Greenland	13ZAC_7M	74°30'31.40"N	20°37'41.40"W	2013		x	x	x
Arctic	North Greenland	DAN1	74°18'25.50"N	20°11'48.40"W	2013		x	x	x
Arctic	North Greenland	DAN3	74°19'40.50"N	20° 9'11.10"W	2013		x	x	x
Arctic	North Greenland	DAN6	74°21'44.90"N	20°10'7.60"W	2013		x	x	x

Polar region	Biogeographical region	Sample name	GPS coordinates		Date of collection	Sanger	Ill ITS2		Ill 18S
							P2	P3	
Arctic	North Greenland	EHR6	74°30'54.30"N	21°28'60.00"W	2013		x	x	x
Arctic	North Greenland	ZAC13	74°29'11.70"N	20°35'19.20"W	2013		x	x	x
Arctic	North Greenland	13DAN_2	74°19'26.60"N	20°10'42.30"W	2013		x		x
Arctic	South Greenland	13KAP_11L	64°21'33.24"N	50° 8'4.80"W	2013			x	x
Arctic	South Greenland	13KAP_4L	64°25'41.87"N	50°13'22.68"W	2013		x	x	x
Arctic	South Greenland	13KAP_7L	64°21'57.09"N	50° 8'21.66"W	2013		x	x	x
Arctic	South Greenland	13KAP_9L	64°21'18.40"N	50° 8'16.05"W	2013		x	x	x
Arctic	South Greenland	13KB_16L	64°12'1.00"N	51°34'15.15"W	2013		x	x	x
Arctic	South Greenland	13KB_5L	64°11'38.09"N	51°30'0.30"W	2013		x	x	x
Arctic	South Greenland	13KB_8L	64°11'49.30"N	51°29'40.77"W	2013		x	x	x
Arctic	South Greenland	KAP13	64°23'54.93"N	50°12'22.73"W	2013		x	x	x
Arctic	South Greenland	KAP2LIT	64°25'46.18"N	50°13'16.54"W	2013		x	x	x
Arctic	South Greenland	KB5	64°11'38.09"N	51°30'0.30"W	2013		x	x	
Arctic	Svalbard	1	78° 9'23.76"N	16° 2'2.04"E	11.08.2017		x	x	
Arctic	Svalbard	O1	78°39'22.98"N	16°55'4.98"E	20.08.2017	x			
Arctic	Svalbard	B1	78°38'12.00"N	16°43'49.98"E	19.08.2017	x			x
Arctic	Svalbard	H1	78°44'55.57"N	16°26'8.04"E	14.08.2017	x	x	x	x
Arctic	Svalbard	CH	78°39'9.39"N	16°55'20.30"E	14.08.2017	x			
Arctic	Svalbard	M1	78°33'48.00"N	16°33'58.98"E	19.08.2017	x			
Arctic	Svalbard	M3	78°33'46.98"N	16°35'12.00"E	19.08.2017	x	x	x	x
Arctic	Svalbard	M4	78°33'34.02"N	16°36'54.00"E	19.08.2017	x	x	x	x
Arctic	Svalbard	B2	78°38'10.98"N	16°44'19.02"E	19.08.2017	x			
Arctic	Svalbard	G1	78°36'15.00"N	16°20'13.02"E	19.08.2017	x	x	x	x
Arctic	Svalbard	O2	78°39'16.98"N	16°55'21.00"E	20.08.2017	x			x
Arctic	Svalbard	S1	78°57'6.10"N	12°29'21.40"E	31.07.2018	x	x	x	x
Arctic	Svalbard	S2	78°57'6.10"N	12°29'21.40"E	31.07.2018	x			
Arctic	Svalbard	S3	78°57'6.10"N	12°29'21.40"E	31.07.2018	x			
Arctic	Svalbard	S5	78°57'14.90"N	12°29'53.50"E	04.08.2018	x			

Polar region	Biogeographical region	Sample name	GPS coordinates		Date of collection	Sanger	Ill ITS2		Ill 18S
							P2	P3	
Arctic	Svalbard	S6	78°57'35.30"N	12°30'18.60"E	04.08.2018	x			
Arctic	Svalbard	S7	78°57'16.30"N	12°32'1.20"E	04.08.2018	x			
Arctic	Svalbard	S8	78°57'16.30"N	12°32'1.20"E	04.08.2018	x			
Arctic	Svalbard	S9	78°57'4.10"N	12°31'44.00"E	04.08.2018	x	x	x	x
Arctic	Svalbard	S10	78°56'30.20"N	11°49'26.30"E	05.08.2018	x			
Arctic	Svalbard	S12	80° 7'43.02"N	19°35'7.50"E	18.07.2018	x			
Arctic	Svalbard	S13	80° 7'43.02"N	19°35'7.50"E	18.07.2018	x		x	x
Arctic	Svalbard	S15	80° 7'43.02"N	19°35'7.50"E	18.07.2018	x			
Arctic	Svalbard	S16	80°12'5.04"N	19°51'26.52"E	19.07.2018	x			
Arctic	Svalbard	S17	80°12'37.38"N	19°45'35.88"E	19.07.2018	x	x	x	x
Arctic	Svalbard	S18	80°23'23.76"N	19°38'27.72"E	20.07.2018		x	x	x
Arctic	Svalbard	S21	80°38'49.56"N	20°39'28.68"E	21.07.2018	x			x
Arctic	Svalbard	S22	79°52'44.28"N	16°45'30.30"E	22.07.2018	x			
Arctic	Svalbard	S23	79°52'31.38"N	16°43'55.20"E	22.07.2018			x	
Arctic	Svalbard	S24	79°52'43.38"N	16°43'54.30"E	22.07.2018	x	x	x	x
Arctic	Svalbard	S25	79°29'29.52"N	15°52'24.42"E	23.07.2018	x			
Arctic	Svalbard	S26	79°29'28.68"N	15°52'7.56"E	23.07.2018				x
Arctic	Svalbard	SPN0015	78°40'1.00"N	16°16'36.00"E	2014		x	x	x
Arctic	Svalbard	SPN004	78°44'21.00"N	16°38'53.00"E	2014		x	x	x
Arctic	Svalbard	SPN007	78°33'33.00"N	16°36'54.00"E	2014		x	x	x
Arctic	Svalbard	SPN008	78°38'16.00"N	16°44'11.40"E	2014		x	x	x
Arctic	Svalbard	Spnoo10	78°36'16.00"N	16°20'14.00"E	2014		x	x	x
Arctic	Svalbard	Spnoo14	78°36'7.00"N	15°54'9.00"E	2014			x	x
Arctic	Svalbard	Spnoo3	78°43'47.00"N	16°24'40.00"E	2014		x	x	x
Arctic	Svalbard	Spnoo5	78°34'52.00"N	16°33'0.00"E	2014		x	x	x
Arctic	Svalbard	Spnoo6	78°33'47.00"N	16°35'14.00"E	2014		x	x	x
Arctic	Svalbard	Spnoo9	78°40'13.00"N	16°54'4.00"E	2014		x	x	x
Arctic	Svalbard	SPN0012	78°44'13.00"N	16°26'14.00"E	2014		x		x

Polar region	Biogeographical region	Sample name	GPS coordinates		Date of collection	Sanger	Ill ITS2		Ill 18S
							P2	P3	
Arctic	Svalbard	SPNOO11	78°36'7.20"N	15°54'9.70"E	2014		x		x

Table S2: List of the final determination of isolated strains sequenced by Sanger sequencing method with information of blasted 18S rDNA sequences.

Sample name	Final determination	18S rDNA			
		Accession number	Reference strain code	Identities	Query cover
O1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1588/1591(99%)	100%
B1	*Neochlorosarcina negevensis* f. *ferruginea*	MK834575.1	UPMC-A0066	569/576(99%)	99%
H1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1552/1555(99%)	100%
CH	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1078/1078(100%)	100%
M1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1656/1658(99%)	100%
M3	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1585/1587(99%)	100%
M4	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1387/1389(99%)	100%
B2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1632/1672(98%)	100%
G1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	880/880(100%)	94%
O2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1545/1553(99%)	100%
O3	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1437/1439(99%)	100%
3	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1434/1436(99%)	100%
DAN	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	620/620(100%)	100%
BLA	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1580/1582(99%)	100%
BLU	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1474/1476(99%)	100%
GIN	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1213/1213(100%)	100%
GR2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1404/1406(99%)	100%
KA1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	696/698(99%)	100%
VO2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	601/601(100%)	100%
KA2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	629/629(100%)	100%
LA1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	704/704(100%)	100%
OM1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	935/937(99%)	100%
PHO	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	601/601(100%)	100%
RED	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	646/646(100%)	100%
RO2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1545/1547(99%)	100%

Sample name	Final determination	18S rDNA			
		Accession number	Reference strain code	Identities	Query cover
WHI	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	1394/1398(99%)	100%
E1	*Chlorella* sp.	MK541792.1	CCAP 211/19	1074/1079(99%)	100%
E2	*Micractinium* sp.	MN414472.1	KSF0100	661/661(100%)	100%
E3	*Micractinium* sp.	MH683925.1	YACCYB505	1456/1466(99%)	100%
E4	*Micractinium* sp.	MH683925.1	YACCYB505	1250/1250(100%)	100%
E5	*Micractinium* sp.	MN414472.1	KSF0100	662/662(100%)	100%
E7	*Chlorella* sp.	LC505542.1	NKH6	631/631(100%)	100%
E9	*Chlorella* sp.	MH683925.1	YACCYB505	480/480(100%)	100%
E10	*Chlorococcum* sp.	AY220092.1	Pic 9/21 P-2w	500/512(98%)	100%
E11	*Micractinium* cf. *variabile*	LC505542.1	NKH6	266/273(97%)	100%
E12	*Micractinium* sp.	MN414469.1	KSF0094	662/663(99%)	100%
E13	*Micractinium simplicissimum*	MN414472.1	KSF0100	661/661(100%)	100%
E14	*Micractinium* sp.	LC505542.1	NKH6	1365/1374(99%)	100%
E16	*Chlorella* sp.	LC505542.1	NKH6	397/397(100%)	100%
E18	*Pseudostichococcus* sp.	KX094812.1	LH08SW8044	1380/1380(100%)	100%
P3	*Chlorococcum* sp.	MK231277.1	KZ-5-4-8	652/661(99%)	100%
P4	*Chodatodesmus australis*	FJ946898.1	EO2-14	1094/1095(99%)	100%
P5	*Chlorella* sp.	KM020033.1	isolate BS775	604/604(100%)	100%
P6	*Chlorella* sp.	KM020033.1	isolate BS775	661/661(100%)	100%
P8	*Chlorella vulgaris*	MK541792.1	CCAP 211/19	622/623(99%)	100%
P9	*Coccomyxa subellipsoidea*	AC277064.1	FAHX137-B20	1047/1047(100%)	100%
P10	*Chlorella* sp.	KM020033.1	isolate BS775	1637/1641(99%)	100%
S1	*Micractinium* sp.	MN414472.1	KSF0100	655/655(100%)	100%
S2	*Micractinium* sp.	MN414472.1	KSF0100	656/656(100%)	100%
S3	*Micractinium* sp.	MH683925.1	YACCYB505	1073/1073(100%)	100%
S5	*Micractinium* sp.	MN414472.1	KSF0100	1267/1271(99%)	100%
S6	*Micractinium* sp.	MN414472.1	KSF0100	670/671(99%)	100%
S7	*Micractinium* sp.	KM243319.1	KNUA029	1103/1103(100%)	100%

Sample name	Final determination	18S rDNA			
		Accession number	Reference strain code	Identities	Query cover
S8	*Chlorella* sp.	KM020033.1	isolate BS775	603/603(100%)	100%
S9	*Micractinium simplicissimum*	LC505542.1	NKH6	602/602(100%)	100%
S10	*Micractinium simplicissimum*	LC505542.1	NKH6	589/589(100%)	100%
S12	cf. *Sphaerocystis* sp.	MK728648.1	CCCryo 133-01	1687/1687(100%)	100%
S13	*Deasonia granata*	KM020105.1	SAG 213-1a	647/647(100%)	100%
S15	*Micractinium simplicissimum*	LC505542.1	NKH6	603/603(100%)	100%
S16	*Chlorella* sp.	AY195979.1	JL 2-2	647/652(99%)	100%
S17	*Micractinium* sp.	MN414472.1	KSF0100	1602/1602(100%)	100%
S21	*Chloromonas reticulata*	JN904006.1	SAG 32.86	1669/1670(99%)	100%
S22	*Macrochloris* sp.	AB983624.1	CCCryo 340b-08	1590/1590(100%)	100%
S24	*Chodatodesmus* sp.	MK764918.1	Zhalong Salt Lake 2	603/605(99%)	100%
S25	*Chloromonas reticulata*	MK912145.1	SAG 25.87	1354/1354(100%)	100%

Table S3: List of the final determination of isolated strains sequenced by Sanger sequencing method with information of blasted ITS2 rDNA sequences.

Sample name	Final determination	ITS2 rDNA			
		Accession number	Reference strain code	Identities	Query cover
O1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
B1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
H1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
CH	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
M1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
M3	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
M4	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
B2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
G1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
O2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
O3	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
3	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
DAN	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
BLA	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
BLU	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
GIN	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	210/211(99%)	100%
GR2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
KA1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
VO2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
KA2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
LA1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
OM1	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
PHO	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
RED	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%
RO2	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/216(99%)	100%

Sample name	Final determination	ITS2 rDNA			
		Accession number	Reference strain code	Identities	Query cover
WHI	*Neochlorosarcina negevensis* f. *ferruginea*	MG022670.1	CCAP 14/1	215/217(99%)	100%
E1	*Chlorella* sp.	MH703753.1	Ru-0-4	215/246(87%)	96%
E2	*Micractinium* sp.	MN414470.1	KSF0112	236/245(96%)	100%
E3	*Micractinium* sp.	MN414470.1	KSF0112	244/244(100%)	100%
E4	*Micractinium* sp.	MN414470.1	KSF0112	243/244(99%)	100%
E5	*Micractinium* sp.	MN414470.1	KSF0112	236/245(96%)	100%
E7	*Chlorella* sp.	KX355545.1	L3	244/244(100%)	100%
E9	*Chlorella* sp.	KX355545.1	L3	242/244(99%)	100%
E10	*Chlorococcum* sp.	KM020023.1	SAG 2467	150/154(97%)	97.0%
E11	*Micractinium* cf. *variabile*	KT883910.1	KNUA036	242/243(99%)	100%
E12	*Micractinium* sp.	LR215792.1	KNUA029	244/244(100%)	100%
E13	*Micractinium simplicissimum*	LR215792.1	KNUA029	244/244(100%)	100%
E14	*Micractinium* sp.	KT883910.1	KNUA036	238/243(98%)	100%
E16	*Chlorella* sp.	KX355545.1	L3	240/244(98%)	100%
E18	*Pseudostichococcus* sp.	KX094837.1	LH08SW8044	260/260(100%)	94%
P3	*Chlorococcum* sp.	MH683988.1	YACCYB421	210/231(91%)	94%
P4	*Chodatodesmus australis*	AM419228.1	CCCryo 424-14	253/255(99%)	100%
P5	*Chlorella* sp.	MH703753.1	Ru-0-4	215/246(87%)	96%
P6	*Chlorella* sp.	MH703753.1	Ru-0-4	215/246(87%)	96%
P8	*Chlorella vulgaris*	MT137382.1	ATFG2	250/250(100%)	100%
P9	*Coccomyxa subellipsoidea*	MN871439.1	CUPVOUCHER_CS_2019_1	221/221(100%)	100%
P10	*Chlorella* sp.	MH703753.1	Ru-0-4	215/246(87%)	96%
S1	*Micractinium* sp.	MN414470.1	KSF0112	226/226(100%)	100%
S2	*Micractinium* sp.	MN414470.1	KSF0112	244/244(100%)	100%
S3	*Micractinium* sp.	MN414470.1	KSF0112	244/244(100%)	100%
S5	*Micractinium* sp.	MN414470.1	KSF0112	244/244(100%)	100%
S6	*Micractinium* sp.	MN414470.1	KSF0112	244/244(100%)	100%
S7	*Micractinium* sp.	MN414470.1	KSF0112	244/244(100%)	100%

Sample name	Final determination	ITS2 rDNA			
		Accession number	Reference strain code	Identities	Query cover
S8	*Chlorella* sp.	MH703753.1	Ru-0-4	207/236(88%)	92%
S9	*Micractinium simplicissimum*	MN414470.1	KSF0112	244/244(100%)	100%
S10	*Micractinium simplicissimum*	MN414470.1	KSF0112	244/244(100%)	100%
S12	cf. *Sphaerocystis* sp.	HQ404871.1	CCCryo 135-01	218/218(100%)	100%
S13	*Deasonia granata*	MG582216.1	ACSSI 152	243/245(99%)	100%
S15	*Micractinium simplicissimum*	MN414470.1	KSF0112	243/244(99%)	100%
S16	*Chlorella* sp.	KM514860.1	NUA-2014-3	238/251(95%)	100%
S17	*Micractinium* sp.	MN414470.1	KSF0112	217/217(100%)	100%
S21	*Chloromonas reticulata*	MK912127.1	CAUP G 1302	262/262(100%)	100%
S22	*Macrochloris* sp.	AB983643.1	CCCryo 340b-08	125/132(95%)	100%
S24	*Chodatodesmus* sp.	AM419228.1	CCCryo 424-14	216/227(95%)	100%
S25	*Chloromonas reticulata*	HQ404900.1	CCCryo 338-08	265/268(99%)	100%

Table S4: Sanger sequences of laboratory cultures, which were found in amplicon sequencing data with 100% query cover and >97% identity. Numbers in each biogeographical region represent number of samples in which the similar sequence was found.

Biogeographical region	Sample	Determination based on Sanger sequencing of strain	Amplicon sequencing	Similarity (%)	South Greenland	North Greenland	Svalbard	Antarctic Peninsula	Continental Antarctica
Antarctic Peninsula	E11	*Micractinium* cf. *variabile*	OTU_724	97.5		2	3	5	4
Antarctic Peninsula	E18	*Pseudostichococcus* sp.	OTU_229	98.2	2		6		1
Antarctic Peninsula	E18	*Pseudostichococcus* sp.	OTU_477	98.3			4	3	2
Antarctic Peninsula	E18	*Pseudostichococcus* sp.	OTU_633	98.7		2	2	7	1
Antarctic Peninsula	P10	*Chlorella* sp.	OTU_2372	99.6			3	2	
Antarctic Peninsula	P3	*Chlorococcum* sp.	OTU_316	100		1	2	14	3
Antarctic Peninsula	P4	*Chodatodesmus australis*	OTU_9	98.8	4	10	13	21	14
Antarctic Peninsula	P8	*Chlorella vulgaris*	OTU_301	100		3	3	1	2
Antarctic Peninsula	P9	*Coccomyxa subellipsoidea*	OTU_740	100		3	11	9	2
Antarctic Peninsula	RO2	*Neochlorosarcina negevensis* f. *ferruginea*	OTU_341	99.5	1		8	11	6
Svalbard	S10	*Micractinium simplicissimum*	OTU_222	98	1	7	7	12	7
Svalbard	S10	*Micractinium simplicissimum*	OTU_5549	98			3	4	
Svalbard	S12	cf. *Sphaerocystis* sp.	OTU_816	99.5			4	2	2
Svalbard	S13	*Deasonia granata*	OTU_2370	100	1		2		1
Svalbard	S16	*Chlorella* sp.	OTU_2160	97.1		4			
Svalbard	S21	*Chloromonas reticulata*	OTU_1900	99.2			3	1	
Svalbard	S25	*Chloromonas reticulata*	OTU_3069	98.1	3	3	4	2	