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ARCTIC BRYOSPHERE: accessing hidden moss carpet biodiversity via DNA Metabarcoding

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BRIOSFERA ÁRTICA: acessando biodiversidade escondida em tapete de musgo com a técnica de DNA Metabarcoding

1. INTRODUÇÃO

O aquecimento global tem intensificado o degelo, facilitando a exploração de recursos naturais e o tráfego marítimo, o que aumenta a relevância geopolítica do Ártico (CÂMARA, 2023). Esse avanço humano ameaça a biodiversidade local, ampliando a presença de turistas e atividades extrativistas, e favorecendo a introdução de espécies invasoras (MAIA, 2023; HALL et al., 2010). Com o aumento das temperaturas, barreiras ambientais deixam de existir, permitindo que novas espécies se estabeleçam no Ártico (GILG et al., 2012). A maior disponibilidade de energia e água pode alterar a dinâmica ecológica (HANSELL et al., 1997) e favorecer o avanço de espécies temperadas, que competem com espécies endêmicas, potencialmente causando efeitos em cascata (HANSELL et al., 1997; CALLAGHAN et al., 2004; GILG et al., 2012).

O Ártico abriga aproximadamente 5.900 espécies de plantas, majoritariamente criptógamas, além de 315 vertebrados e mais de 3.000 invertebrados (CALLAGHAN et al., 2004). A briosfera, composta por musgos e sua biota associada, constitui um sistema ecologicamente complexo (LINDO & GONZALEZ, 2010) e exerce papel crucial no aporte de nitrogênio nas florestas boreais (LINDO & GONZALEZ, 2010; TURETSKY et al., 2012; KARDOL et al., 2016). Estudos mostram que tapetes de musgos abrigam uma grande diversidade de organismos (SCHUTER & GRAVEN, 2007; BOWSER et al., 2020), ampliada quando analisada por metabarcoding (CÂMARA et al., 2021).

Diante disso, o estudo em questão busca caracterizar a biodiversidade críptica presente em tapetes de musgos em dois locais de Svalbard. A comparação dos resultados permitirá avaliar o impacto humano sobre espécies endêmicas e atualizar o conhecimento sobre a biodiversidade frequentemente negligenciada da briosfera.

2. METODOLOGIA

Amostras de *Sanionia uncinata* foram coletadas em dois pontos com diferentes níveis de impacto antrópico: Longyearbyen, uma cidade que recebe turistas e tem elevado fluxo de pessoas; e Diabasodden, um ponto isolado, acessível apenas com transporte aquático. As amostras foram congeladas e transportadas para a Universidade de Brasília, onde o DNA foi extraído e amplificado utilizando o FastDNA Spin Kit for Soil (MP Biomedicals, California, EUA), utilizando os marcadores Cox1 e ITS2. A etapa de sequenciamento foi feita pela empresa Macrogen Inc. (Coreia do Sul). A etapa de bioinformática foi feita utilizando os programas BBDuk version 38.34 (BBMap – Bushnell B. –sourceforge.net/projects/bbmap/) e QIIME2 version 2019.10 (https://qiime2. org/).

3. RESULTADOS

Foram encontradas 1023 ASVs (*amplicon sequence variants*), das quais apenas 148 ocorreram em ambos os pontos. Em Diabasodden, Viridiplantae foi o reino mais abundante, enquanto Fungi foi o reino mais rico. Em Longyearbyen Fungi também foi o reino mais rico, enquanto Metazoa foi o mais abundante. No reino Chromista, foram encontradas 107 ASVs distribuídas em 5 filos, sendo Ochrophyta e Ciliophora os filos mais ricos, com 33 ASVs cada, e Ochrophyta o mais abundante. No reino Fungi, com 313 ASVs, o filo mais rico foi Ascomycota e o mais abundante foi Basidiomycota. Em Metazoa foram encontradas 135 ASVs, com Arthropoda sendo o filo mais rico e mais abundante. No reino Protista foram encontradas apenas 10 ASVs, a maioria pertencendo ao filo Amoebozoa. Por fim, no reino Viridiplantae, foram encontradas 73 ASVs, com Chlorophyta sendo o filo mais rico e Bryophyta o mais abundante.

Foram encontradas 36 espécies exóticas em Diabasodden e 46 em Longyearbyen, oriundas de todas as regiões biogeográficas. Além disso, 384 ASVs não puderam ser identificadas a nível de reino, sendo classificadas como desconhecidas. A diferença entre as comunidades e a maior ocorrência de espécies exóticas em Longyearbyen indicam maior impacto na briosfera próxima à cidade, sendo provável que o impacto seja de origem antrópica. Por fim, o alto número de ASVs desconhecidas é alarmante, levantando a necessidade de futuras investigações sobre os organismos da região em ordem de evitar possíveis patógenos e proteger a segurança alimentar e de saúde da população.

Palavras-chave: Briosfera, DNA ambiental, ecossistema polar.

Abstract

The accelerated warming and fast anthropic advance in the Arctic, the region's ecosystem is going through major changes, which can present consequences not only at a local, but also on a global scale. The bryosphere, composed of living and dead moss tissue, as well as all organisms living inside the moss carpet, is a complex ecosystem which is often ignored. Therefore, the aim of this work is to investigate the bryosphere community in two different sites in Svalbard. The first site was Longyearbyen, a city with high touristic flux, while the second site was Diabasodden, an isolated spot only accessed by aquatic transportation. Samples of *Sanionia uncinata* moss carpets were collected and processed with the DNA Metabarcoding method for ambiental DNA. We targeted five kingdoms: Chromista, Fungi, Metazoa, Protista e Viridiplantae. We found a total of 1023 ASVs (*amplicon sequence variants*), from which only 148 were present in both sites. In Diabasodden, the most abundant kingdom was Viridiplantae and the richest was Fungi. Fungi was also the richest kingdom in Longyearbyen, while the most abundant kingdom was Metazoa. In the Chromista Kingdom there were 107 ASVs, with Ochrophyta and Ciliophora being the most abundant phylums, and Ochrophyta the richest. In Fungi there were 313 ASVs, Ascomycota being the richest and Basidiomycota the most abundant phylums. In the Kingdom Metazoa, there were 135 ASVs, and Arthropoda was both the most abundant and richest phylum. In the Protista kingdom, there were only 10 ASVs, most of it belonging to the phylum Amoebozoa. At least, in the Viridiplantae kingdom, there were 73 ASVs, Chlorophyta being the richest and Bryophyta the most abundant phylum. There were 23 non-native species in Diabasodden and 46 in Longyearbyen, which originated from all biogeographic regions. Furthermore, there were 384 ASVs that could not be identified at any taxonomic level, classified as unknown. The dramatic difference between both communities and the greatest number of non-native species in Longyearbyen indicates greater impact in the bryosphere located near the town, hence probable anthropic origin. Nevertheless, there were an alarming number of unknown ASVs, which highlights the urgent need for further investigations on the region's organisms in order to avoid possible pathogens that could threaten population’s health and food security.

Keywords: Bryosphere, ambiental DNA, polar ecosystem.

1. INTRODUCTION

Since there are more than one Arctic definition, for this essay we used the Arctic Circle definition, which defines the region as the land and sea area north of the Arctic Circle, located at the latitude 66° 34′ North (O'ROURKE, 2011). Its territory belongs to eight different countries: Canada, USA, Russia, Norway, Denmark, Finland, Iceland and Sweden (MAIA, 2023). With the current climate change scenario, where the defrosting facilitates the exploration of natural resources as well as the ship traffic in the region, its geoeconomics and geopolitics importance is even more prominent (CÂMARA, 2023).

The anthropogenic advance is a great threat to the local biodiversity and ecosystem. With the reduction of the frozen areas, the exploration of natural resources and tourism, human presence and influence is increasing in the region (MAIA, 2023; HALL et al., 2010). This growing presence of people makes the Arctic environment more vulnerable to invasive species (HALL et al., 2010).

Furthermore, with global climate change, temperature is no longer a barrier to new species to inhabit and thrive in the Arctic region (GILG et al. 2012). Warmer temperatures and more UV light disponibility might change the region's ecosystem dynamics by providing greater water and energy availability (HANSELL et al., 1997) and allowing exotic species to establish themselves. Such processes may result in an advance of temperate species Northward, threatening the endemic Arctic species habitat, since this species would likely have a competition disadvantage (HANSELL et al., 1997; CALLAGHAN et al., 2004; GILG et al., 2012). Due to the endemic species functional redundancy, the introduction of invasive species might lead to a cascade effect, reducing the survival rate of these individuals (HANSELL et al., 1997; GILG et al., 2012).

In this context, the Arctic endemic biodiversity conservation is a great priority. The Arctic environment is the natural habitat to, approximately, 5900 plant species, most of them being represented by cryptogams (CALLAGHAN et al., 2004). According to Callaghan et al. (2004), the area also houses 315 species of vertebrates, and more than 3000 invertebrate species. Even though these species are specialized to survive the extreme conditions from its habitat, it made them more vulnerable to changes in the biotic and abiotic surroundings, such as temperature changes and invasive species (GILG et al., 2012).

Because of this cascade effect, knowing the region 's total biodiversity has a great value to conservation efforts. Although the vertebrates and plants biodiversity are well known, there are few studies concerning the Arctic microorganisms and invertebrates

(RIPPIN et al., 2018). Rippin et al. (2018) used the DNA Metabarcoding tool to better understand the biodiversity present in Arctic soil samples. Similar studies using this tool has been widely used to understand Antarctic biodiversity (CÂMARA et al., 2021; CARVALHO-SILVA et al., 2021; DA SILVA et al., 2022; DE MENEZES et al., 2022; DE SOUZA et al., 2022; ROSA et al., 2022; ROSA et al., 2022b; CÂMARA et al., 2023b; DE SOUZA et al., 2023; CÂMARA et al, 2023; DE MENEZES et al., 2024).

The bryosphere is defined as "the combined complex of living and dead moss tissue and associated organisms, representing a tightly coupled system of both above and belowground processes" (LINDO and GONZALEZ, p.613, 2010). As it inhabits a great diversity of N-fixing cyanobacteria, the bryosphere has a great role in the Nitrogen input in the boreal forests, hence its primary production (LINDO and GONZALEZ, 2010; TURETSKY et al., 2012; KARDOL et al., 2016). It also provides habitat to a large group of species (CÂMARA et al., 2021) and these organisms form a complex ecosystem, even though this trophic interaction are not fully explored (LINDO and GONZALEZ, 2010; TURETSKY et al., 2012).

Câmara et al. (2021) made a study using the DNA Metabarcoding as a tool to better understand the diversity living in a transplanted moss carpet, in the surroundings of the Brazilian Antarctic station (Comandante Ferraz). In his essay, they found individuals from 6 Kingdoms, 33 Phyla and a total of 263 amplicon sequence variants (ASVs). This study shows that there is an even greater biodiversity living in the bryosphere than what was found in previous studies using traditional methods (CÂMARA et al., 2021), although studies using traditional methods have already shown that moss carpets are habitat to a great diversity of species (SCHUTER e GRAVEN, 2007; BOWSER et al., 2020).

Therefore, the present study aims to understand the cryptic biodiversity present in moss carpets in two different sites in Norway's archipelago of Svalbard . Is expected that, with comparative analysis of the found biodiversity, we could have a better understanding of the human impact in the endemic species and local biodiversity. Furthermore, it is expected to update the database of the cryptic biodiversity, mostly ignored, living in the bryosphere.

2. BIBLIOGRAPHY REVIEW

2.1. Arctic

2.1.1. History and Geopolitics

There are a few different definitions of Arctic. For this Essay, we will use the Arctic Circle definitions, which considers “Arctic” as all land and sea above latitude 66° 34′ North (O’ROURKE, 2011). Unlike its Polar opposite, the Antarctic, which is regulated by an International Treaty, Arctic territories belong to eight countries: Russia, U.S.A., Iceland, Finland, Sweden, Norway, Canada and Denmark (CÂMARA et al., 2023).

Because of its borders, the Arctic had received a special spotlight during the Cold War. The conflict between the U.S. and the late Soviet Union gave the region a great strategic status, and the Arctic was heavily militarized (OSTERUD and HONNELAND, 2014). Only in the 90s, after the Soviet Union dissolution, did the strategic importance of the Arctic weakened, opening the door to new economics and environmental interests (OSTERUD and HONNELAND, 2014). However, with the recent conflicts, particularly the war in Ukraine, the region seems to have regained unwanted attention.

The war conflicts were replaced by conflicts of jurisdiction, new space for science development and extractivist interest (OSTERUD and HONNELAND, 2014; KALTENBORN et al., 2019; ZAIKOV et al., 2019). Great reserves of oil, gas and minerals came into focus, with technologies advancing and global climate change simplifying the access to these natural resources (OSTERUD and HONNELAND, 2014; ZAIKOV et al., 2019). The warmer conditions and associated ice melt, also opened the door to new commercial routes in the Arctic Ocean (OSTERUD and HONNELAND, 2014), leaving the countries with new prospects of traffic development (U.S. Department of Transportation, 2019).

Another growing industry in the Arctic is tourism. With the easier accessibility and the so-called “Last-Chance Phenomenon” (STEIGER et al., 2013), tourism in the Arctic has increased (MANSON, 2010; KALTENBORN et al., 2019). The pristine environment is a great attraction in the Arctic to worldwide tourists, and most of the activities are nature related (MANSON, 2010; VARNAJOT and LUNDÉN, 2024). The lack of an unified code or regulation dealing with this fast growing flux of people is a great concern for the preservation of the natural environment, and the anthropogenic impact it may have in the ecosystem is widely discussed amongst the scientific community (CHAPIN et al., 2001; MANSON, 2010; CONVEY et al., 2012; HALL et al., 2014; VARNAJOT and LUNDÉN, 2024).

Alongside the environmental implications of this fast anthropogenic advance in the Arctic, another issue concerns the vulnerability of Indigenous People living in the region. Indigenous Peoples have been living in the Arctic for thousands of years, with the first written register of the Saami people dating back to the Roman Empire (KELMAN and NÆSS, 2013). Beyond the Saami, there are Inuits, Aleut, Athabascans, Gwith'n, Nenets, amongst others (STEPHEN et al., 2014). Their traditional way of life is intimately connected to nature, which is threatened by the new industry and climate change advance (MANSON, 2010; STEPHEN et al., 2014; GASSIY, 2018).

The Indigenous People, alongside nature, represent one of the main tourist attractions in the Arctic, raising the fear of a "cultural zoo", as said Mason (2010). Stephen (2014) said that:

> "Discriminatory practices, acculturation, usurpation of ownership and management of natural resources, and rise in poverty are undeniable and persistent parts of the Arctic's historical and political landscape." - Stephen, p. 73, 2010

Kelman and Næss (2013) highlights that there is no data of the financial returns of the touristic industry to the Indigenous People. Gassiy (2018) brings out the great impact of climate change in the traditional way of life, where the shifts in the environment lead to disease, hunger, freezing and abandonment of tradition to search for a better life in the city. The author call attention to the central importance of those people in understanding the fast changes occurring in the Arctic:

> "Indigenous people also have their own observations related to climate change, since no one can see better what is happening in the North, and there are significant shifts in their strategies for adapting to these changes." - Gassiy, p. 2, 2018

To try to coordinate and cooperate in the efforts for environmental preservation, the Arctic Environmental Protection Strategy (AEPS) was formed in 1991, and it evolved to the Arctic Council in 1996 (CONVEY et al., 2012). All the eight Arctic countries participate as Permanent Members of the Council, alongside 6 representatives of Indigenous Peoples (Arctic Council, 2024). However, it haveinternational forum status rather as an "international organization", leave the Council with no International Law, thus its members face no legal implication by choosing to decline its approved decisions and policies (BLOOM, 1999).

2.1.2. Environment

Walker (2000) divided its Tundra environment in four sub-zones: the cushion-forb subzone, prostate dwarf-shrub subzone, erect dwarf-shrub subzone and low-shrub subzone. The cushion-forb subzone is the coldest zone, representing 4.6% of the Arctic, its vegetation being composed mainly by bryophytes and lichens. The prostate dwarf-shrub subzone is warmer, thus having more presence of vascular plants, and representing about 35% of Arctic. The erect dwarf-shrub subzone has a significant difference in soil components, therefore resulting in a change in the vegetation, with 50-80% of vascular plants covering and representing about 33% of the region. And the low-shrub subzone, in the lowest latitude, is the warmer region, bordering and merging the Boreal Forest (WALKER, 2000).

The Arctic flora is composed of an estimated 5900 species, most of them being cryptogams. About 4% of the world's bryophytes and 11% of lichens are from the Arctic (CALLAGHAN et al., 2004). Mainly because of the cold, cryptogams dominate the environment in higher latitudes since they are more adapted to the cold and dryness of winter, and there are only about 1800 species of vascular plants (CALLAGHAN et al., 2004; TURETSKY et al., 2012).

The Arctic is populated by many terrestrial and maritime mammals and birds, with few to none reptiles and amphibians. There are registers of 75 maritime and terrestrial mammals and about 300 species of birds, including the ones in forest Tundra (CHENOV, 1995). One of the most important animals in the local human community is the reindeers, since the caribou reindeers are one of the main sources of food for the region Indigenous People (KELMAN and NÆSS, 2013). These species have an intimate dependence on the native vegetation, once they feed in very specific plants. Thus, a change in the vegetation could cause a great scale impact in the whole trophic cascade (JEFFERIES and BRYANT, 1995).

Gerson (1982) made an extensive review of the invertebrate species living in the Arctic moss. According to the author, there are registers of species of the Class Turbellaria, the Phyllum Rotifera, Nematoda, Anellida, Mollusca, Tardigrada and Arthopoda. In general, Callaghan et al. (2004) estimated 3300 invertebrate species only within the Class Insecta, 50% being from the Diptera Order and 10% of each Order Coleoptera, Lepidoptera and

Hymenoptera. All Arctic invertebrates have specific adaptation to deal with the harsh climate condition, the three main strategies being freeze tolerance, avoiding freezing by physical dehydration (BALE, 2002).

The Arctic microbiota is also rich in species. Using molecular approach, Cutler et al. (2017) sampled moss shoots and found 895 Operational Taxonomic Units (OTUs) from 9 phyla of bacteria in the boreal forest, most of them belonging to the phyla Proteobacteria, Acidobacteria and Actinobacteria. The authors also found 839 OTUs in the Fungi kingdom, with three phyla being abundant: Ascomycota, Basidiomycota and Zygomycota. Jassey et al. (2022), also using molecular approach, found 1049 non-singleton prokaryotic OTUs and non-singleton eukaryotic 746 OTUs in the peat-lands, being 165 OTUs for photosynthetic bacteria and 499 OTUs for photosynthetic protists.

The Arctic environment has a key-role in regulating the global climate, storing 32% of the earth C and taking 20% of the produced atmospheric C, functioning as an important global C-sink (ALVARENGA and ROUSK, 2022). The Arctic is also responsible for about 35% of the worlds Net primary production (NPP), and its ecosystem has a important role N cycle, P cycle and methane oxidation (CHAPIN et al., 1987; TURETSKY et al., 2012; ALVARENGA and ROUSK, 2022). Most of these processes occur within the Tundra moss layer, which has a vast community with complex trophic interactions that are still not completely understood (LINDO and GONZALES, 2010).

Because of the low winter temperatures and dryness, Arctic organisms are highly adapted to deal with harsh abiotic pressures (CALLAGHAN et al., 2004). Therefore, there is a functional redundancy in the ecosystem, leading species to have a high dependance to one another (CALLAGHAN et al., 2004). The alteration of the microbiota could result in a disequilibrium that could affect the nitrogen and carbon cycle, altering the ecosystems productivity (SCHMEL, 1995). Changes in vegetation could affect pollinators that are species specific, thus affecting herbivores and the whole environment energy flux (CHAPIN et al., 1995). Even in aquatic environments, such as lakes, the productivity is highly dependent on terrestrial biodiversity (KLENG, 1995). Therefore, the function of the ecosystem depends on the preservation of the native biodiversity.

2.1.3. Climate change

Currently, one of the main challenges in the Arctic concerns climate change as the region is getting warmer at an alarming rate compared with the rest of the globe

(GRIGORIEVA, 2024). Since temperature is a key aspect of the Arctic ecosystem, there is a great apprehension about the full extend of the impact that these changes bring on the regions environment, not only at a local, but also at a global scale (WALKER, 1995; CHAPIN et al., 2001; CALLAGHAN et al., 2004; CONVEY et al., 2012).

The warmer environment may result in a series of changes, the main changes being in the precipitation regime and soil fertility (CALLAGHAN and JONASSON, 1995). According to the authors, the “Arctic biota might be particularly sensitive to such changes because life in the Arctic is strongly regulated by climate constraints” (p. 151, 1995). The harsh conditions of the natural environment lead to a biota adapted to specific conditions, and these species may not be resilient enough to deal with such changes (WALKER, 1995; CHAPIN et al., 2001; CALLAGHAN et al., 2004).

According to Walker (1995):

> “Temperature has strong effects on almost all aspects of Arctic ecosystems, including soil stability, moisture, and nutrient availability, and anyone or many of these may effectively limit an individual species presence in the ecosystem.” Walker, p. 6, 1995

Since the Arctic ecosystem has a great functional redundancy, the loss of species may lead to a cascade effect, resulting in the whole ecosystem to collapse (CONVEY et al., 2012). Arctic pollinators may be high species-specific, or depend on a specific flowering sequence to survive (CHAPIN et al., 1995). The loss of species, may lead to the death of native pollinators, and consequently affect the pollination of other plants (CHAPIN et al., 1995). Subsequently, changes in the vegetation may affect native vertebrates that rely on native species to feed, leading to starvation (CHAPIN et al. 1995; JEFFERIES and BRYANT, 1995) and the melting of the permafrost may impact the migration of these species (CONVEY et al., 2012). The decrease of these populations may also strongly affect the predator species (JEFFERIES and BRYANT, 1995). Therefore, the preservation efforts face a great challenge, since many native species heavily rely on each other.

Another great concern involving climate change in the Arctic ecosystem, and maybe the most alarming, is the introduction of invasive species. The low temperature was the main barrier preventing invasive species from thriving in the Arctic and warmer temperatures may open the door to new species to survive and spread (GILG et al., 2012). Due to the before mentioned adaptations of native species to the Arctic abiotic traits, these species have a disadvantage to deal with the new biotic pressions, such as competition (CALLAGHAN and

JONASSON, 1995; CALLAGHAN et al., 2004; CONVEY et al., 2012). Callaghan (2004), wrote that:

> "The indirect effect of warming and UV-B increases, and particularly those mediated by species interactions such as competition with more aggressive immigrants from the South, are likely to dominate Arctic species responses to environmental change." - Callaghan et al, p. 414, 2004

The anthropogenic advance towards the Arctic, either for exploiting natural resources or tourism, may represent one of the main risk factors for the introduction of invasive species (CONVEY et al., 2012; HALL et al., 2014). Even though the vulnerabilities of the ecosystem are much alike in the Antarctic and Arctic, the biosecurity regime in the Arctic is much less restricted, and the extractivism and exploitation of the environment mentality leave the ecosystem in an even more vulnerable position (MANSON, 2010; HALL et al., 2012; ØSTERUD and HONNELAND, 2014). The increasing tourism stands as a great threat and, according to Hall et al. (2014):

> "In the case of the Arctic, cruise ships, as well as other forms of tourist transport, pose a significant biosecurity threat, not only because they are starting to visit previously little or unvisited areas, but also because the frequency of visits is increasing." - Hall et al., p.354, 2014

Therefore, all of these increasing human activities pose a great threat to native biodiversity. As mentioned before, the Arctic environment have a great value not only in its raw nature, but also because of its function as a C-sink and contribution to primary production (TURETSKY et al., 2012; JASSEY et al., 2022; ALVARENGA and ROUSK, 2022; SLATE et al., 2024). A lot of these processes happen within the moss microbiome, mostly on the peatlands, and there is a very delicate community balance for this ecosystem to function (KARDOL et al., 2016; JASSEY et al., 2022). Since the community within the moss is responsible not only to help in the C uptake, but also in its immobilization, changes in these microbiota or, in more extreme cases, loss of the moss may shift these microbiomes from C-sink to C-source (LINDO and GONZALES, 2010; JASSEY et al., 2022; ALVARENGA and ROUSK, 2022). According to Schimel (1995):

> "Several properties of tundra systems may be particularly sensitive to microbial community structure: plant nitrogen uptake, vegetation response to changing climate, and the effects of

> Arctic pollution. Nutrient supply to plants may be more sensitive to microbial community structure in tundra than in any other systems." - Schimel, p. 249, 1995

Therefore, even small scale changes in the environment may cause global scale consequences.

2.2. Svalbard

2.2.1. History and Geopolitics

Svalbard is a Norwegian archipelago, located in the High Arctic, the desertic Arctic area. The archipelago was discovered in 1596, by the Dutch explorer Willem Barentsz. Despite early sovereignty conflicts, in 1920 the Svalbard Treaty was implemented, securing Norway's claim and opening space to other countries (KALTENBORN et al., 2019). The treaty is open to all nations to sign, and by signing it, have the right to exert research and explore local resources (Svalbard Treaty, 1920).

The economy of Svalbard was established around coal mining, and for many years it sustained the local business, bringing reasonable profit (KALTENBORN et al., 2019). The consistent extraction resulted in an exhaustion of the resources, and nowadays the local economy relies strongly on tourism and scientific research. In fact, Svalbard became a great scientific pole for Arctic research, for its heavy response to climate change (KALTENBORN et al., 2019).

In the current year of 2024, there are 2617 habitants in the archipelago (Statistics Norway, 2024). Construction, accommodation and food services have a great part of the employment, followed by administrative and support service activities (Statistics Norway, 2024). Until June, 2024, a total of 1.530.362 tourists from Norway went to Svalbard and 963.204 tourists from other nations (Statistics Norway, 2024).

The Svalbard Treaty, alongside securing Norway's sovereignty, affirms Norway's responsibilities in preserving the local environment (KALTENBORN et al., 2019). In this sense, 120.730km² of the archipelago are protected areas, from which 41.558km² are located in land (Statistics Norway, 2024). Even so, the Environmental Law is considered static, and the national and international pressure about the environmental decisions is concerning, considering the rapid changes that are happening in consequence of climate changes (KALTENBORN et al., 2019). The nature of these changes give science an unique environment to better understand climate change not only locally, but on a global scale, and also understand the anthropogenic impact of the growing people flux that come with the increasing tourism industry (KALTENBORN et al., 2019).

2.2.2. Environment

The terrestrial fauna of Svalbard is composed by an endemic reindeer — *Rangifer tarandus platyrhynchus* — alongside arctic foxes and sibling voles, the last being an invasive species (Statistics Norway, 2016). There are also marine mammals, the most famous being the polar bear, that is under total protection since 1973, alongside walruses, harbour seals, bearded seals, ringed seals, belugas, narwhals and Greenland right whale (Statistics Norway, 2016). Around 36 species of birds breeds in Svalbard, however, only one — *Lagopus muta hyperborea* — stay during winter, and is restricted to the area (Norwegian Polar Institute, 2024).

Svalbard has a rich invertebrate fauna, having 1089 registered species (COULSON et al., 2024). According to the authors dataset, the Phylum Arthropoda is the richest, with nearly half of the species, 212 from the Subphylum Chelicerata and 287 from Mandibulata. All 212 Chelicerata are from the Arachnida Class, most of them (156) belonging to the Acariforme Superorder. In Mandibulata, the richest class is Insecta, with 220 species, and Diptera is the richest Order, with 128 species (COULSON et al., 2024).

The flora of the archipelago counts with more than 200 vascular plants (LEE, 2020). According to the Svalbard Flora (http://svalbardflora.no/), there are 6 of these species that are critically endangered, and other 14 are endangered. Bryophytes are not present on the website, but according to the website Statistics Norway, there are 380–390 species of moss (https://www.ssb.no/en, 2015). Also in the cryptogams, there are 742 lichen species in the region (ØVSTEDAL et al., 2009).

In the microbiome, recents studies using molecular approach detected a high number of species. In soil samples, Dziurzynski et al. (2022) found over 830 genera of bacteria, with the ASVs generally dominated by *Proteobacteria* and *Actinobacteria*. Using culture and morphological analysis, Davydov (2020), found 292 species of cyanobacteria in both terrestrial and aquatic samples. Dziurzynski et al. (2022) also found 880 Fungi ASVs, with 62.61% of identified ASVs belonging to *Ascomycota*, followed by *Mortierellomycota* with 14.25% and *Basidiomycota* with 13.07% of the ASVs, tough only 106 ASVs could be identified using culture approach. Statistics Norway (https://www.ssb.no/en, 2015) registers over 750 Fungi species. Besides the few studies, there are records of 48 species of Protozoa (BALIK, 1994) and 29 taxa of algae (KIM, 2008).

2.3. Bryosphere

The Bryosphere was first defined by Lindo and Gonzales (2010) as the live and dead moss tissue, as well as all associated organisms within it. It has an important role in nutrient cycling, and the strong interaction from diverse and abundant organisms facilitates the energy and nutrient flux (LINDO and GONZALES, 2010). The invertebrate fauna is rich, and it is divided into microfauna (<100 μm) and mesofauna (>100 μm) (LINDO and GONZALES, 2010). The authors also highlight its bacteria diversity, from which the cyanobacteria mediate a great N_2 input in the ecosystem.

The moss itself presents some key characteristics that not only ensure its survival in harsh conditions, but also enables the bryosphere community. The most important trait is the moss water absorption and retention in the ecosystem (JACKSON et al., 2013; SLATE et al., 2024). Mosses are able to absorb water from many sources besides the rain, including dew, mist and snow, therefore increasing the water input in the ecosystem (SLATE et al., 2024). By retaining it, the moss turns into a humid microhabitat, unlike the usually dry conditions outside the bryosphere in the Arctic, functioning as a refuge from desiccation for other organisms and preventing the soil beneath from humidity fluctuations (ALVARENGA and ROUSK, 2022).

The humidity within the moss carpet is also important in maintaining a stable microclimate in the ecosystem. The water prevents it from having great temperature fluctuations within the moss layer and in the covered soil, while also maintaining the temperature higher than in the bare soil (TURETSKTY et al., 2010; TURETSKY et al., 2012). Therefore, it grants its community more reliable and secure temperature conditions, enhancing the productivity and decomposition within the bryosphere (GRAUS-ANDRÉS et al., 2022; SLATE et al., 2024). By maintaining a stable temperature, the moss layer may also prevent the permafrost from melting, which may have an influence in the ecosystems resilience (TURETSKY et al., 2011).

The disposition of the moss layer is also a factor to the bryosphere's biodiversity. The formation of microtopography – elevated mounds (hummocks) separated by low hollows – enhances the microhabitat diversity, thus creating more ecological niches (TURESTKY et al., 2012). This way, the water gradient between the humid hummocks and dry hollows enables a higher diversity in the community, by opening space to diverse functional traits in the ecosystem (TURESTKY et al., 2012).

Gerson (1982) made a very complete literature review about the invertebrates living in the moss, as mentioned in the previous section using morphology alone. The authors list registers the Phylum Platyhelminthes, Nematoda, Rotifera, Annelida, Mollusca, Tardigrada and Arthropoda. The Phylum Arthropoda was better described, having species from the Maxillopoda, Malacostraca and Insecta Class, the Myriapoda Superclass and the Arachnida Subclass (GERSON, 1982). In their article, Schuster and Greven (2007) collected moss samples to study Tadigrada population and found 19.909 individuals from 24 species in a period of 54 months, showing that it represents an abundant and rich community living in the moss. There is also a register of the Collembola Class and a great variety of mites (ERLUDÓTTIR, 2023).

The small scale of the ecosystem stands as a challenge to the understanding of the bryosphere (LINDO and GONZALES, 2010). To overcome this barrier, Fontaneto et al. (2019) proposes the use of the new molecular tools, with emphasis in the DNA Metabarcoding. Studies using this tool have been widely done in the Antarctic environment in a variety of substracts, such as soil, ice and snow (CÂMARA et al., 2021; ROSA et al., 2022a; ROSA et al., 2022b; CÂMARA et al., 2023a; CÂMARA et al., 2023b). In the moss subtract, Câmara et al. (2021) found a total of 263 taxa in the Bacteria Domain, with other taxa distributed between Chromista, Fungi, Metazoa, Protista and Viridiplantae. All of these data shows the high potential of the DNA Metabarcoding tool in uncovering hidden biodiversity associated with mosses.

In the Arctic, most specifically in bryosphere subtract, molecular tools were already used to identify a series of microorganisms. Cutler et al. (2017) used these tools to identify Fungi in moss samples in the boreal forest. According to the authors, there were three abundant Phylums: 62% were Ascomycota, 23% Basidiomycota and 3.8% Zygomycota. Jassey et al. (2022) used the same tools to identify photosynthetic Bacteria and Protozoa in the peatland moss, and found that 7.7% of Bacteria reads and 21% of Protozoa reads were from this type of organisms. Hamard et al. (2021) have done similar work in peatlands with photosynthetic Bacteria and Protozoa, where they found, respectively, 74 Operational Taxonomic Units (OTUs) and 277 OTUs.

The presence of these photosynthetic organisms highlights their active nature in the energy input in the ecosystem. As mentioned, the bryosphere community have an important role in the N_2 and C fixation in the moss, and the structure of the community may have a significant impact in these processes (LINDO and GONZALES, 2010; KARDOL et al., 2016; HAMARD et al., 2021; ALVARENGA and ROUSK, 2022). Kardol et al., (2016) demonstrated that the N_2 fixation rate in the moss is really low without the presence of cyanobacteria. The authors also emphasize the need of a complex trophic net, that generates a top-down control in the bacteria feeding microorganisms, to assure high rates of N_2 fixation in the bryosphere. The nitrogen fixed by cyanobacteria is readily incorporated in the ecosystem, either absorbed by the moss or consumed by other organisms in the community, playing an important part in the trophic net (ARRÓNIZ-CRESPO et al., 2022).

The C uptake by the bryosphere and its immobilization have impact not only on a local, but at a global scale (LINDO and GONZALES, 2010). In some ecosystems, the gross C uptake and retention by bryophytes may even exceed vascular plants, turning this ecosystem into an important global C-sink (STREET et al., 2013; SLATE et al., 2024). This is especially true to high latitude regions, since bryophytes may persist productive longer than vascular plants, with the water availability being the main factor to its productivity (SLATE et al., 2024). Therefore, a climate change driven succession leading to the replacement of the bryosphere by vascular plants, or even compromising the structure of its community, may shift the ecosystem from C-sink to a C-source (LETT et al., 2021; SLATE et al., 2024).

The presence of detritivore and saprophytes organisms in the bryosphere is also a factor in the decomposition, C-cycling and C-retention within the ecosystem (LINDO and GONZALES, 2010; JACKSON et al. 2013; CUTLER et al, 2017; GRAU-ANDRÉS et al., 2022; SLATE et al., 2024). Studies analyzing decomposition rates in the Arctic found that the absence of the moss layer has a negative effect on the abundance of those organisms, leading to a decrease in the decomposition (JACKSON et al. 2013; GRAU-ANDRÉS et al., 2022). Consequently, the presence of the bryosphere have a positive effect in the decomposition rates and the loss or removing of this layer may have a negative impact in decomposition, hence nutrient availability, in the Arctic ecosystem (GRAU-ANDRÉS et al., 2022).

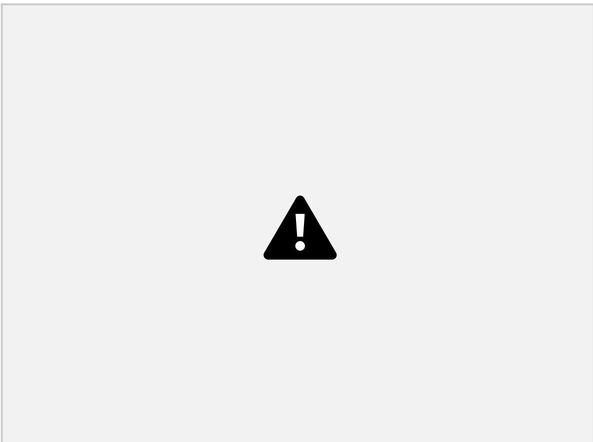
> “It is in the colony form that bryophytes most strongly affect the environment through their physical presence, as well as biogeochemically and biotically through interactions with other organisms in the ecosystem.” - Lett et al., p. 612, 2021

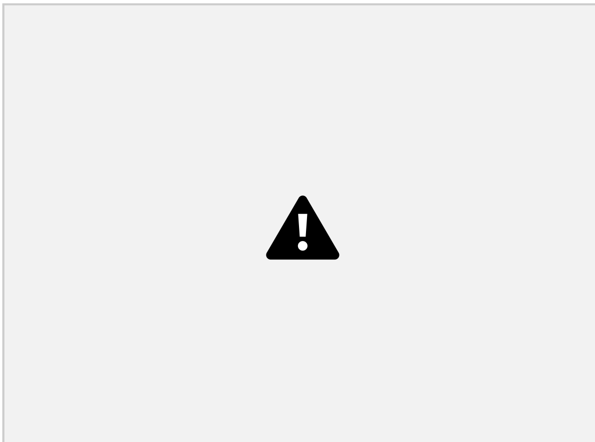
Therefore, in this work we aim to uncover the community living in the bryosphere ecosystem in Svalbard and investigate the differences between a highly affected by human activity community and a community with low contact with human disturbances.

3. METHODOLOGY

Figure 1. Svalbard location.

Samples were collected in two sites ─ three samples in each one ─, both in Svalbard (Fig. 1). The first site, Longyearbyen (Fig. 2) is the capital city of the islands (78°21’55’’N 15°40’29’’E). Diabasodden (78°21’40’’N 16°06’32’’E), the second site, is a more isolated spot, being at a distance of 20.33km, in a straight line, from Longyearbyean, having no connections with cities and only being assessed by sea, using boats, as shown in figure 3.





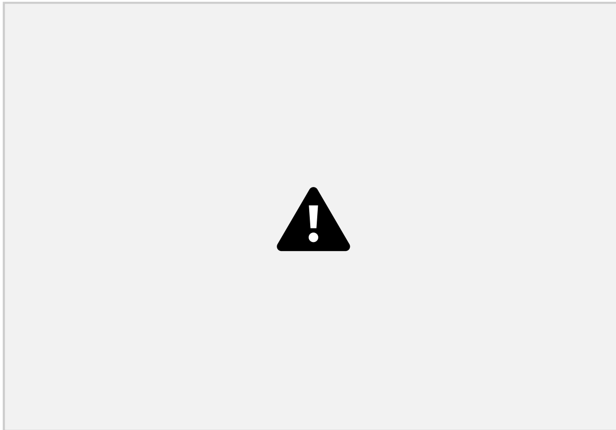


Figure 2. Close image from Diabasodden (above) and from Longyearbyen (below).

Three samples of moss carpet composed by *Sanionia uncinata* (Hedw.) Loeske were collected in each site using gloves and previously sterilized forceps and placed in a sterilized WhirlPak bag (Sigma-Aldric, USA) and kept frozen until processed. Extraction used the FastDNA Spin Kit for Soil (MP Biomedicals, California, USA), following the manufacturer’s instructions. DNA quality was analyzed by agarose gel electrophoresis (1% agarose in 1×Trisborate-EDTA) and then quantified using Quanti-iT™ Pico Green dsDNA Assay (Invitrogen). We aimed to target DNA from five groups of organisms: Chromista, Fungi, Metazoa, Protista and Viridiplantae.

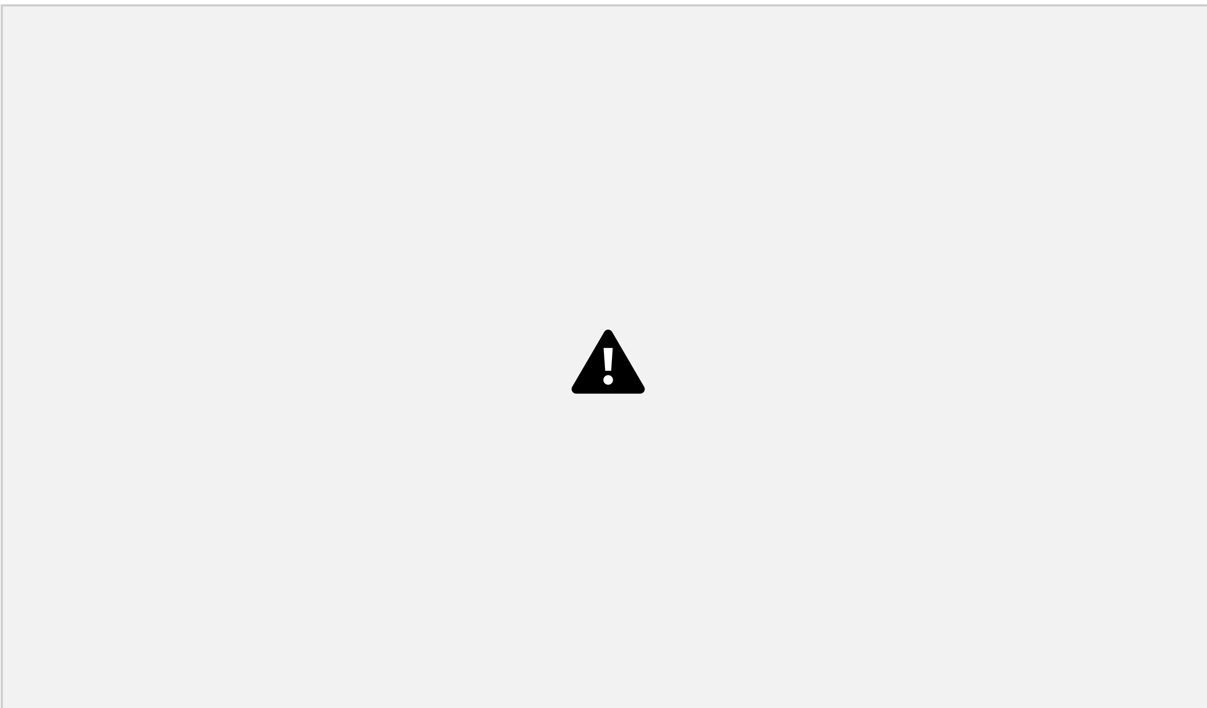


Figure 3. Site map showing the second collecting site, Diabasodden.

The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification of Chromista, Protista, Viridiplantae and Fungi (Chen et al. 2010) using the universal primers ITS3 and ITS4 (White et al. 1990). For Metazoa, we used Cox1 (Folmer et al. 1994; Câmara et al., 2024) with UEA3F and HCO2198R primers. Library construction and DNA amplification were performed using the Library kit Herculase II Fusion DNA PolymeraseNextera XT Index Kit V2, following Illumina 16S Metagenomic Sequencing Library Preparation Part #15,044,223 Rev. B protocol. Paired-end sequencing (2×300 bp) was performed on a MiSeq System (Illumina) by Macrogen Inc. (South Korea).

Raw fastq files were filtered using BBDuk version 38.34 (BBMap – Bushnell B. –sourceforge.net/projects/bbmap/) to remove Illumina adapters, known Illumina artifacts, and the PhiX Control v3 Library. Quality read filtering was carried out using Sickle version 1.33 -q 30 -l 50 (Joshi et al. 2011), to trim 3' or 5' ends with low Phred quality score, and sequences shorter than 50 bp were discarded. The remaining sequences were imported to QIIME2 version 2019.10 (https://qiime2. org/) for bioinformatics analyses (Bolyen et al. 2019). The qiime2-dada2 plugin is a complete pipeline that was used for filtering, dereplication, turn paired-end fastq files into merged, and remove chimeras (Callahan et al. 2016). Taxonomic assignments were determined for amplicon sequence variants (ASVs) using the qiime2-feature-classifier (Bokulich et al. 2018), UNITE Eukaryotes ITS database version 8.2 (Abarenkov et al. 2020) for Eukaryota, and MIDORI (Leray et al. 2018) for COX1, trained with Naïve Bayes classifier. We aimed to maximize resolution by obtaining data from specific and curated databases for the specific target groups.

Rarefaction calculations were carried out using the rarefaction analysis command in the platform MOTHUR (Schloss 2009), where we clustered sequences into OTUs by setting a 0.03 distance limit. Many factors, including extraction, PCR and primer bias, can affect the number of reads obtained (Medinger et al. 2010), and thus lead to misinterpretation of absolute abundance (Weber and Pawlowski 2013). However, Giner et al. (2016) concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are linked with higher abundance (Deiner et al. 2017; Hering, 2018). Therefore, for comparative purposes, we used the number of reads as a proxy for relative abundance. Ecological indices were calculated using PAST 1.90 (Hammer et al. 2001). Sequences from the Phyla Ochrophyta and Oomycota were obtained from Cox1 and the Midori database while Ciliophora used ITS and the UNITE database. The classification systems used were: Leliaert et al. (2012) for Viridiplantae; Cavalier-Smith (2007) for

Chromista, Protista and Metazoa; Kirk, (2011), Tedersoo et al. (2011), MycoBank (http://www.mycobank.org) and the Index Fungorum (http:// www.indexfungorum.org) for Fungi.

The Python software (https://www.python.org/) was used for statistical analysis. The main libraries utilized were pandas (https://pandas.pydata.org/), xlrd (https://pypi.org/project/xlrd/), matplotlib (https://matplotlib.org/) and seaborn (https://seaborn.pydata.org/). Pandas and xlrd libraries were used to organize data so it could be better handled in future analysis. Graphics were made using pyplot (https://matplotlib.org/3.5.3/api/_as_gen/matplotlib.pyplot.html). All the rows with unknown kingdom were disconsidered for the richness and abundance graphs. The remaining lines, for the richness graphs, the function groupby (https://pandas.pydata.org/docs/reference/api/pandas.DataFrame.groupby.html) was used to cluster the kingdoms and the function size was used to count taxa. For the abundance graphics, stead of the size, sum function was used to sum the number of reads from each taxa.

To seek numerical evidence and comparability of the diversity within the samples collected, diversity indices were used: Shannon index, Simpson's index (1-D), Pielou's evenness, Fisher's alpha and Margalef index. For diversity indices, R software (https://www.r-project.org/) was chosen, specifically the Vegan library (https://cran.r-project.org/web/packages/vegan/index.html). All indices used (besides Margalef's index) followed the methods within vegan library and can be checked on its documentation (https://cran.r-project.org/web/packages/vegan/vegan.pdf). It used a rarefaction curve, with an interval of 95% confidence, to verify if the curves stabilized and the samples collected were satisfactory enough. The rarefaction curve was made with the Past software.

The table was produced summing the reads of the triplicates of each site. Geographic distributions were found using the GBIF (Global Biodiversity Information Facility) searching tool, or, when not found, directly in the literature. For the distribution areas, the principal biogeographic regions were used as reference points (SCLATER, 1858; WALLACE, 1876). Species were considered cosmopolitan when present in four or more regions. For biodiversity analysis Shannon, Simpson, Fisher-α, and Pielou indexes were made.

4. RESULTS

The manuscript of the paper produced with the results of this work is present in the appendix 1. The paper was submitted in the periodic Polar Biology (Springer), as shown in the appendx 2.

4.1. Diabasodden

With the COX1 marker, 444896 raw sequences were found in Diabasodden. After bbduk - Input Qiime, there remained 202595 sequences. Then the sequences went through Qiime quality control. After being filtered there remained 202535 sequences, then it was denoised, remaining 191155 sequences, merged, remaining 144574 sequences and lastly the out-put sequences (non-quimeric) was 140976 sequences, or 31.69% of the raw sequences.

A total of 586 ASVs were found in Diabasodden summing both markers, in which 39.7% could not be identified at Kingdom level. In the remaining ASVs, 45.3% belong to the Fungi Kingdom (Fig. 4 and 5). Nevertheless, Viridiplantae were the most abundant, followed by Chromista and Fungi, respectively.

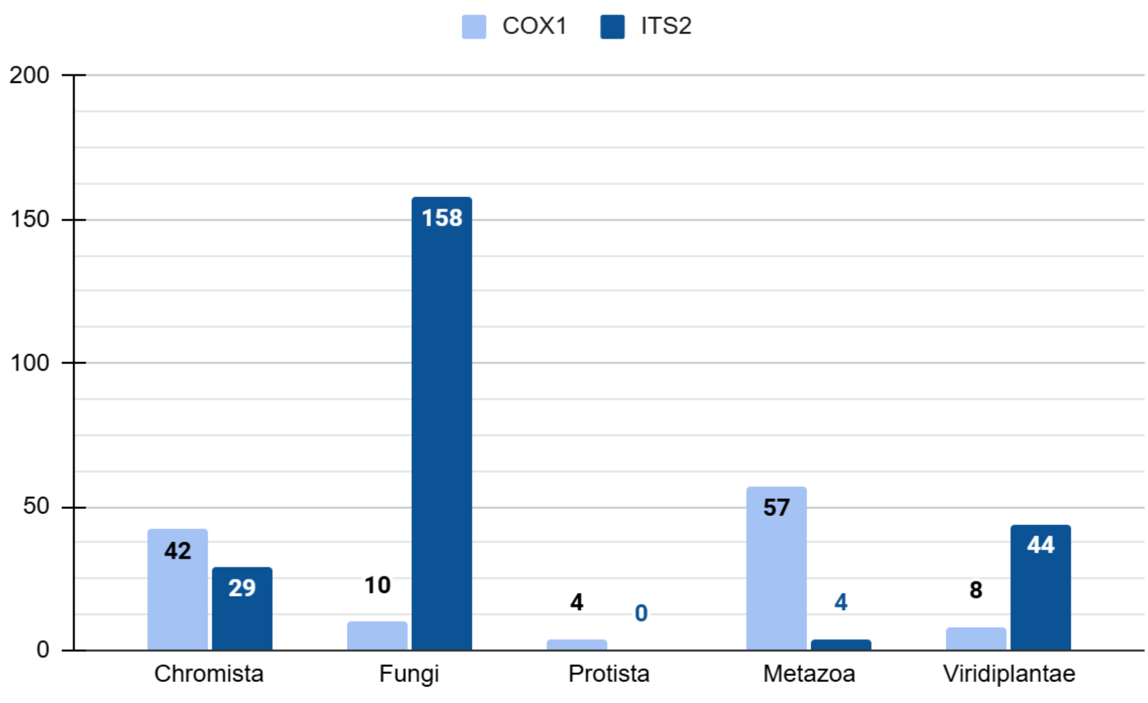


Figure 4. Number of ASVs of each Kingdom, in Diabasodden with each marker.

Without the reads unidentified at Kingdom level, there were 124477 reads using COX1 marker in Diabasodden. The readings had ASVs from all five Kingdoms investigated, with 59785 Chromista reads, 7552 Fungi reads, 2692 Protista reads, 53845 Metazoa reads and

603 Viridiplantae reads. The richest Kingdom was Metazoa, while the most abundant was Chromista (Fig. 4 and 5).

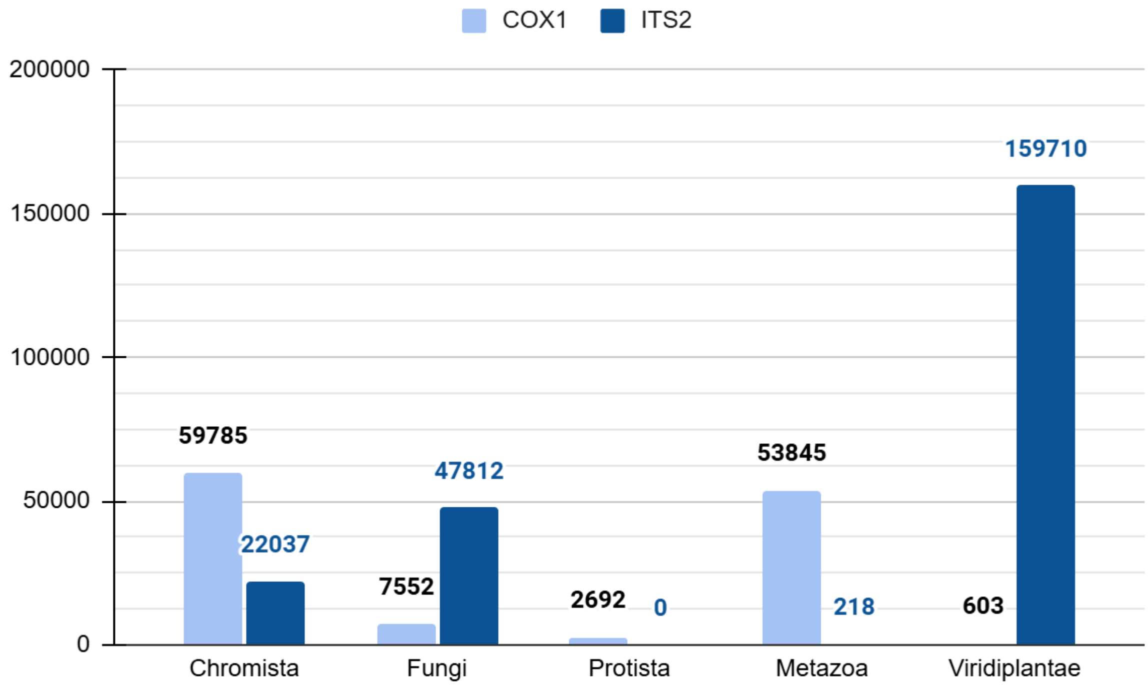


Figure 5. Number of reads of each Kingdom with each marker, in Diabasodden.

With the ITS2 marker, there were 229777 reads in Diabasodden. They were distributed in four Kingdoms, with no Protista found, 22037 Chromista reads, 47812 Fungi reads, 218 Metazoa reads and 159710 Viridiplantae reads. The most abundant Kingdom was Viridiplantae, while the richest was Fungi, with 158 ASVs (Fig. 4 and 5). Metazoa was both the less rich and abundant Kingdom when using the ITS2 marker, with only 4 ASVs. Regarding the non-native species, summing the five Kingdoms there were 41 ASVs in Diabasodden.

4.2. Longyearbyen

In Longyearbyen, there were 728240 raw sequences. After bbduk - Input Qiime, there were 343781 sequences remaining. Then the sequences went through Qiime quality control. After being filtered there remained 343599 sequences, then it was denoised, remaining

333308 sequences, merged, remaining 296412 sequences and lastly the out-put sequences (non-quimeric) was 285363 sequences, or 39.19% of the raw sequences.

A total of 581 ASVs and 522561 reads were found in Longyearbyen, summing both markers, from which 28.57% couldn't be identified at Kingdom level. Fungi was, again, the richest Kingdom. However, the most abundant was Metazoa, with 197824 reads, followed by Viridiplantae, with 143867 reads, Fungi, with 124766, and Chromista, with 48336 (Fig. 7). Protista was both the less abundant and rich Kingdom, with 9 ASVs and 7768 reads (Fig. 6 and 7).

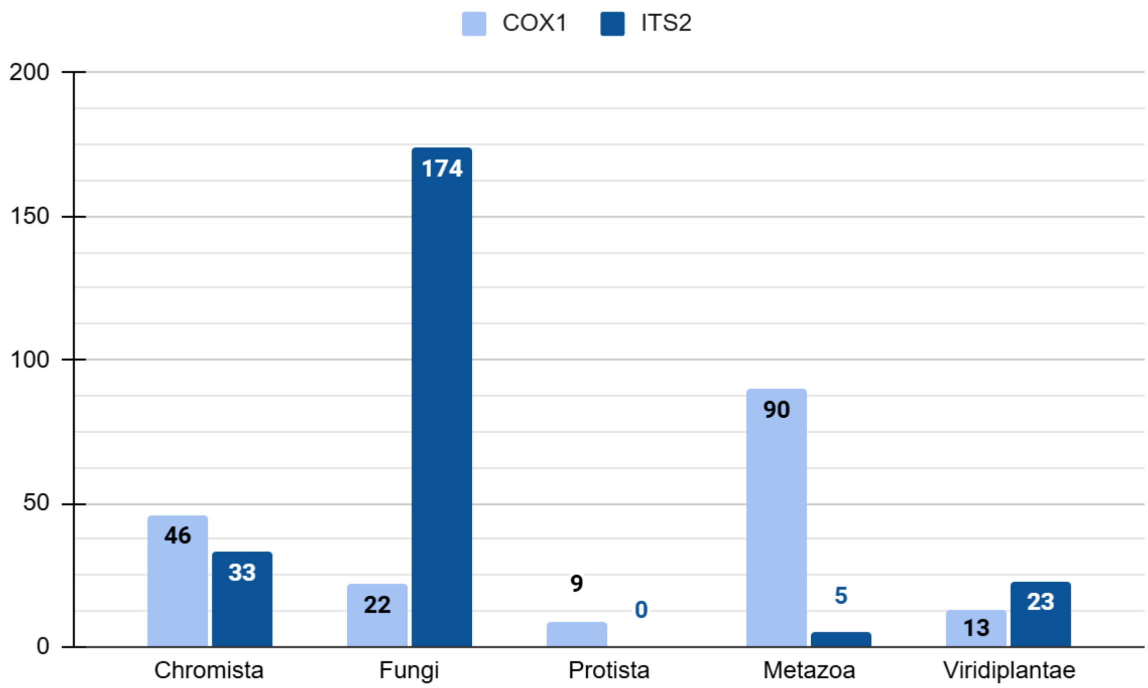


Figure 6. Number of ASVs of each Kingdom, with each marker, in Longyearbyen.

With the COX1 marker, there were 243152 reads found in Longyearbyen, divided among all five Kingdoms investigated. The most abundant Kingdom was Metazoa, with 197532 reads, followed by Chromista, with 32413 reads, Protista, with 7768 reads, Fungi, with 3397 reads and lastly Viridiplantae, with 2042 reads (Fig. 7). Metazoa was also the richest Kingdom, while Protista was the less rich, with only 9 ASVs (Fig. 6).

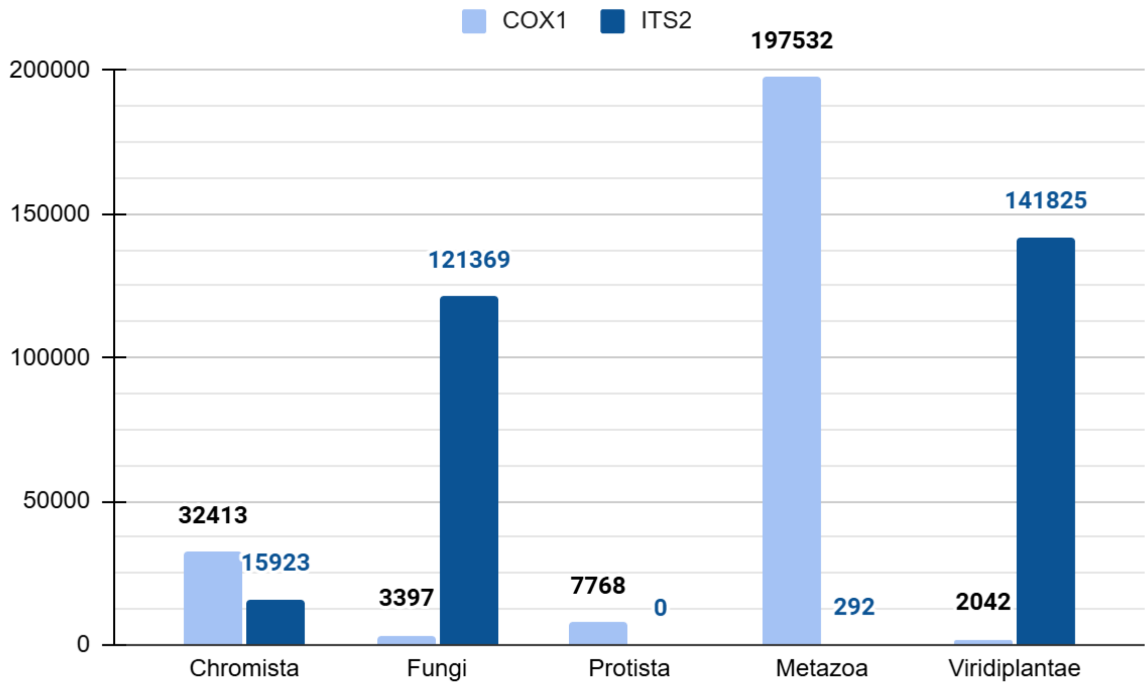


Figure 7. Number of reads of each Kingdom with each marker, in Longyearbyen.

Using ITS2 marker, 279409 reads were found in Longyearbyen. The most abundant Kingdom was Viridiplantae, with 141825 reads, followed by Fungi, with 121369 reads, Chromista, 15923 reads and Metazoa, with only 292 reads (Fig. 7). Fungi was by far the richest Kingdom, with 174 ASVs, followed by Chromista and Viridiplantae, respectively with 33 and 23 ASVs, and the last rich was Metazoa, with only 5 ASVs (Fig. 6). Regarding the non-native species, summing the five Kingdoms there were 71 ASVs in Longyearbyen.

Despite the high number of ASVs in both sites, there were only 144 ASVs shared between them (Fig. 8), which represent 32.6% of the ASVs in Diabasodden and 33.2% of Longyearbyens ASVs. Even though there are a high number of species in Longyearbyen, it is lower than Diabasodden, an environment with less human disturbances.

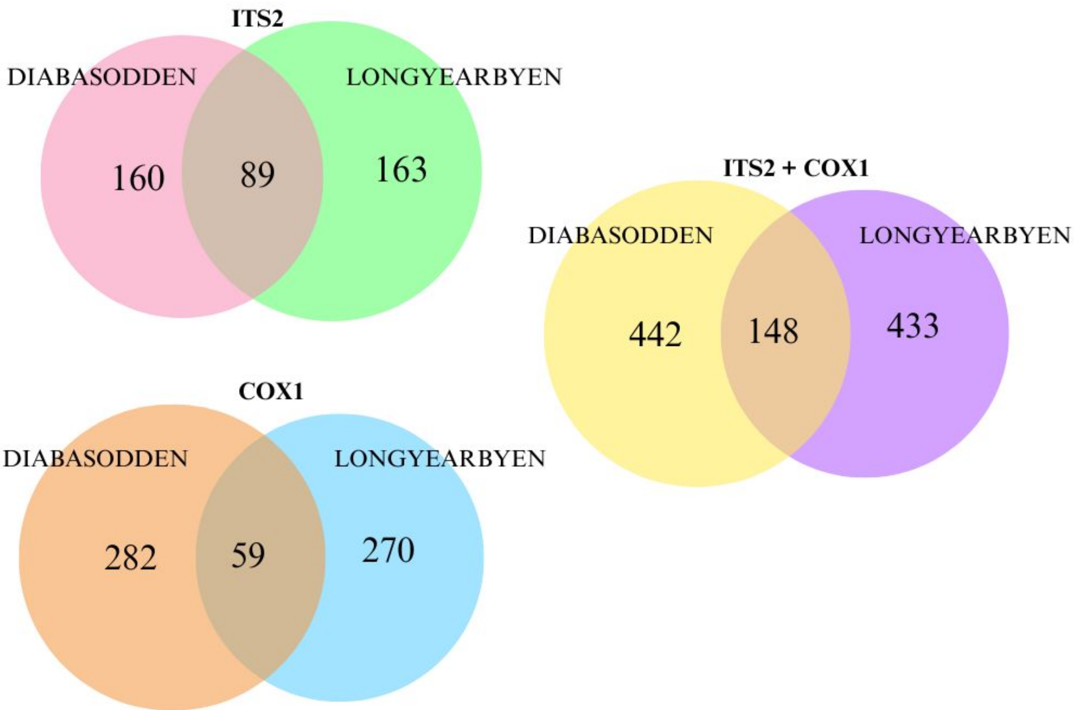


Figure 8. Venn diagrams of all ASVs found in the two sites.

Results of all five indices are present in Table 1. Diabasodden showed higher scores than Longyearbyen in all five indices, with 3.759839 in Shannon's, 0.9404809 in Simpson's, 68.25429 in Fisher's α, 0.5893052 in Pielou's Evenness and 45.78 in Margalef's. Longyearbyen scored 3.084356 in Shannon's index, 0.8662317 in Simpson's, 63.93366 in Fisher's α, 0.4845997 in Pielou's Evenness and 43.79 in Margalef's.

Table 1. Biostatistics indices.

	Shannon	Simpson (1-D)	Fisher's α	Pielou's Evenness	Margalef
Diabasodden	3.759839	0.9404809	68.25429	0.5893052	45.78
Longyearbyen	3.084356	0.8662317	63.93366	0.4845997	43.79

The rarefaction graphic (Fig. 9) stabilized in both sites, indicating maximum sample effort, thus no need to further collect.

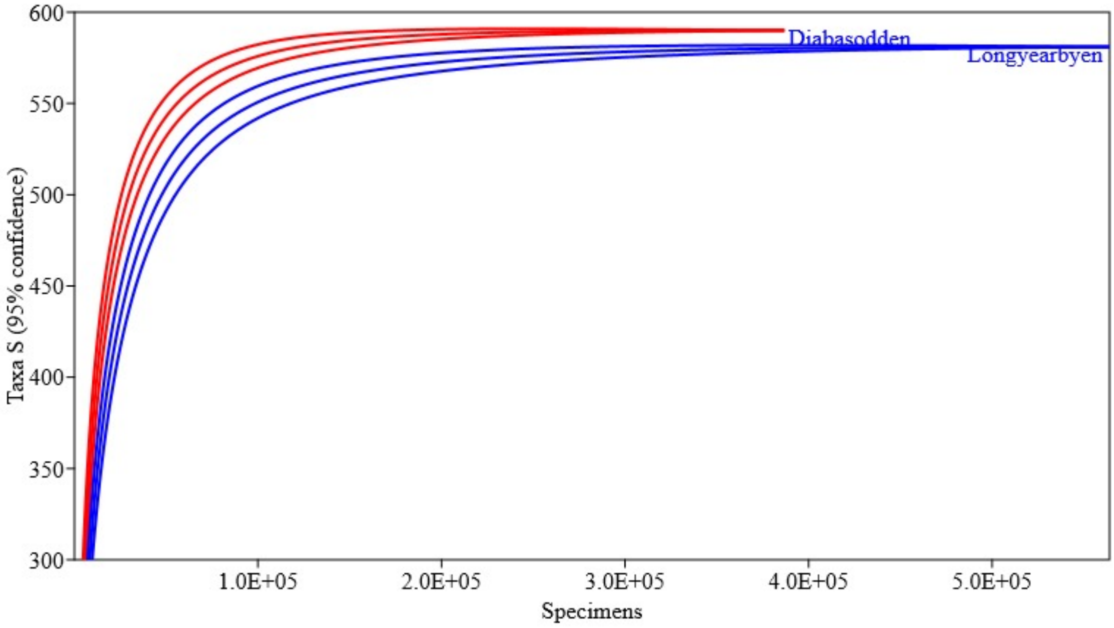


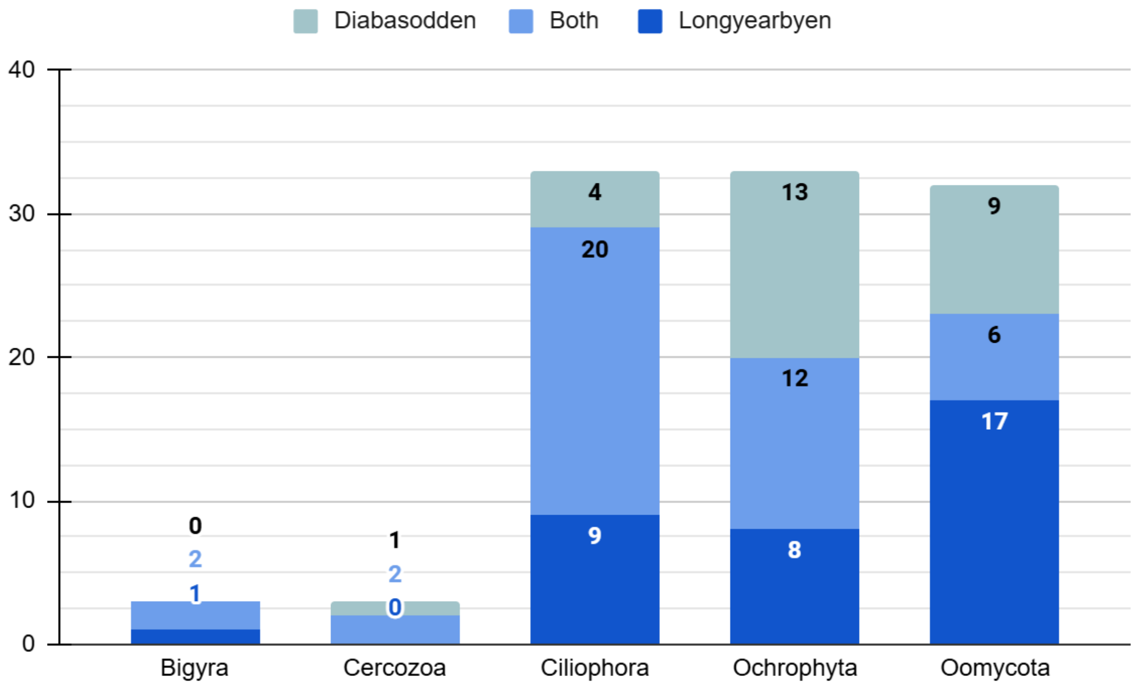
Figure 9. Rarefaction graphic for the samples of the two sites. Diabasodden in red and Longyearbyen in blue, with the superior and inferior line indicating the confidence interval for each site.

4.3. Results per Kingdom

From now on, all results will be shown summing data found with both COX1 and ITS2 markers.

4.3.1. Chromista

Within the Kingdom Chromista, a total of 107 ASVs were found, from which 68 ASVs were identified at species level and 23 ASVs identified only at genus level. There were 1.9% — 2 ASVs — that only could be identified at Kingdom level (Fig. 10). These species are distributed in 5 Phyla, 16 Classes, 27 Orders and 44 Families. The most abundant Phylum was Ochrophyta, with 55.1% of the reads (Fig. 11), and the richests were Ochrophyta and Ciliophora, both with 33 ASVs (Fig. 10). All Ciliophora ASVs were found with ITS2 marker, while the remaining ASVs were found with COX1.



Figures 10. Number of ASVs of each Phylum within the Chromista Kingdom in each site.

In general, the most abundant Order was Ectocarpales, with 30% of the reads, followed by Mischoccoales, with 27.2% and Sporadotrichida, with 12.5%. In terms of number of species, the richest order was Peronosporales, with 16 different species, followed by Pythiales, with 13 species and Ectocarpales, with 8 species. The most abundant Family was Mischococcaceae, with 23.3% of the reads, even though it was represented by only one species. It was followed by Chordariaceae, 16.6%, and Botrydiopsidaceae, 11.5%. The family Peronosporaceae was the richest, with 16 species, followed by Pythiaceae, with 13 species. The remaining families had around the same number of species.

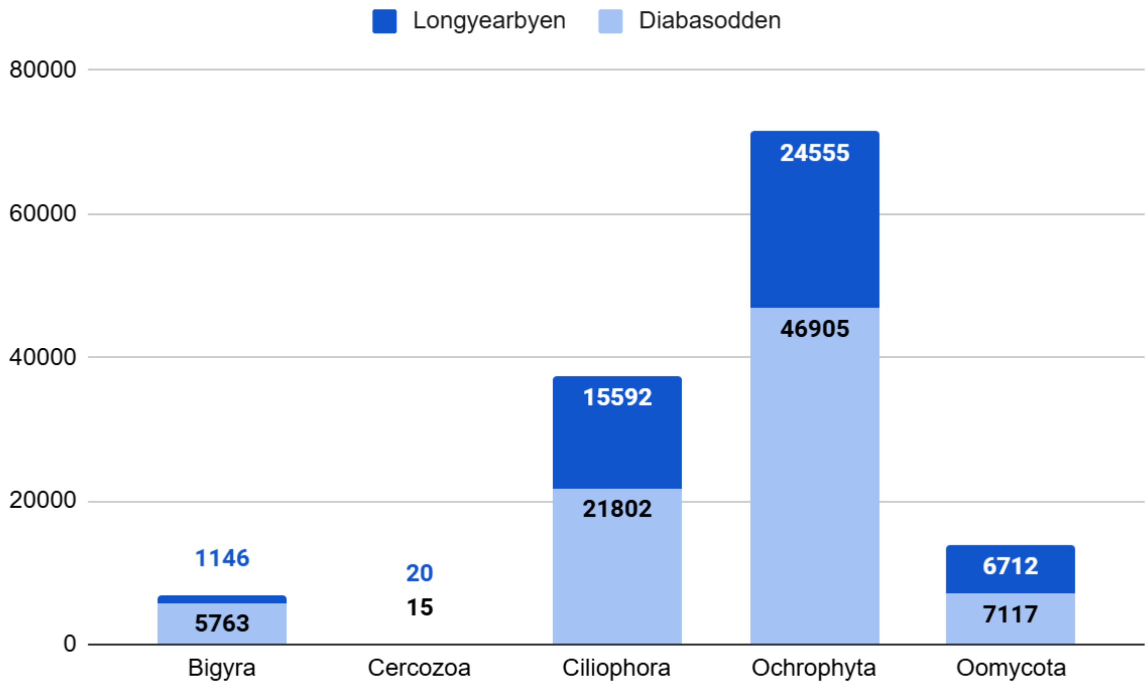


Figure 11. Number of reads of each Chromista Phylum.

In Diabasodden exclusively, 28 ASVs were found. These species were distributed in 4 Phyla, 8 Classes, 22 Orders and 34 Families. There were also 2 ASVs that could only be identified at higher taxonomic levels. Ochrophyta was both the richest and most abundant Phylum, with 25 ASVs and 57.5% of the reads (Fig. 10 and 11). The most abundant Order in Diabasodden was Ectocarpales, with 74.1% of the reads, followed by Peronosporales with 10.2%. The richest Orders were Peronosporales and Pythiales, with 4 species each.

In Longyearbyen, 36 ASVs occur exclusively, with 8 of the ASVs only identified at genus level and 1 at Order level. There were 5 Phylum, with 7 Classes, 20 Orders and 34 Families. The most abundant Phylum was Ochrophyta, with 51.1% of the number of reads, while the richest was Ciliophora, with 29 ASVs (Fig. 10 and 11). The most abundant Orders were Ectocarpales with 52.4% of reads, and Pythiales 21.2%. The richest Orders were 10 species of Peronosporales and 6 of Pythiales.

There were 20 species that occurred in both sites, 12 ASVs that could be identified at Genus level and 10 that could only be identified at higher taxonomic levels. The shared species belong to 6 different Phylum, 11 Classes, 19 Orders and 24 Families.

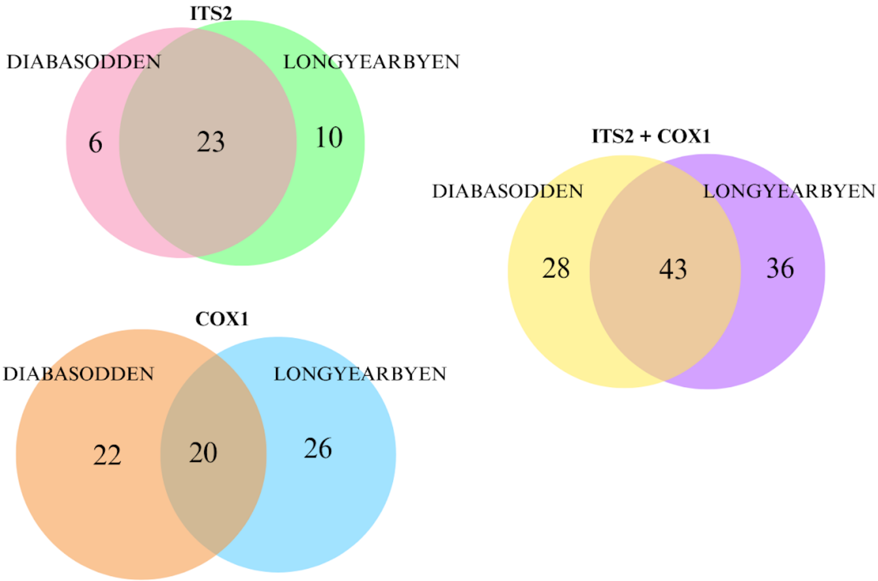


Figure 12. Venn diagram of Chromista ASVs in both sites.

Within the 34 Families in Diabasodden, Mischococcaceae was the most abundant, with 31.9% of the reads, followed by Scytosiphonaceae, with 18.6%. In terms of species, in Diabasodden, Peronosporaceae and Pythiaceae stands out again, with 4 species each.

In Longyearbyen there's a different scenario, where Chordariaceae was the most abundant, with 26.9%, followed by Botrydiopsidaceae, 21.8%, and Mischoccaceae antanding in third, with 12.7%. In Longyearbyen there were 10 species of Peronosporaceae and 6 species of Pythiaceae. With shared species, the richest Families were Chordariaceae, Pythiaceae and Vorticellidae, with 3 species each (Fig. 12).

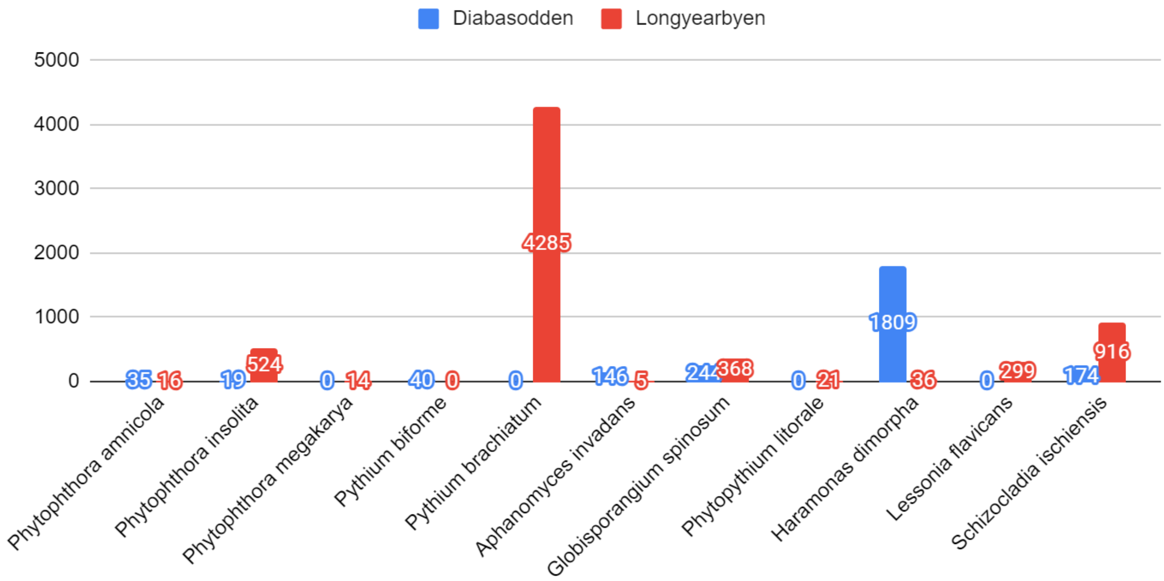


Figure 13. Number of reads and location of each non-native Chromista species.

Exclusively in Diabasodden was found only one non-native species, *Pythium biforme*, a japanese endemic. In Longyearbyen there were four new species, *Phytophthora megakarya*, common in the african continent, *Phytopythium litorale*, natural from warmer climates, *Pythium brachiatum*, endemic to Japan and *Lessonia flavicans*, common in south of South America and Antarctica. Shared between two sites, there were also five new species: New Zealands *Phytophthora amnicola*, Japaneses *Globisporangium spinosum*, *Aphanomyces invadans* that is common to the oriental region, Greeces *Schizocladia ischiensis* and Australians *Haramonas dimorpha* (Fig. 13).

Table 2. Name of each Chromista taxa found, indicating which maker used (ITS2 or COX1), which site it was found (Diabasodden, Longyearbyen or both), and the distribution of each taxa.
C = Cosmopolitan, HA = Holarctic, PA = Palearctic, NA = Nearctic, NT = Neotropical, E = Ethiopian, O = Oriental, A = Australian, An = Antarctic; BGR = Bulgaria; CHN = China; GRL = Germany; ITA = Italy; JPN = Japan; NZ = New Zealand; RUS = Russia; USA = United States of America.

TAXA	Hab/ Distr.	Marker	D.	L.
KINGDOM CHROMISTA			**N° of**	**reads**
Phylum Bacillariophyta				
Class Bacillariophyceae				
Order Bacillariales				

Family Bacillariaceae				
Tryblionella apiculata	C	COI	0	27
Order Cymbellales				
Family Gomphonemataceae				
Didymosphenia geminata	HA	COI	59	10
Gomphonema parvulum	C	COI	0	4
Order Naviculales				
Family Pinnulariaceae				
Pinnularia borealis	C	COI	104	19
Family Phaeodactylaceae				
Phaeodactylum tricornutum	C	COI	26	0
Class Coscinodiscophyceae				
Order Coscinodiscales				
Family Coscinodiscaceae				
Coscinodiscus granii	C	COI	744	0
Order Thalassiosirales				
Family Skeletonemataceae				
Skeletonema marinoi	PA	COI	50	0
Phylum Bigyra				
Class Labyrinthulea				
Order Thraustochytrida				
Family Thraustochytriaceae				
Aurantiochytrium acetophilum		COI	5377	863
Botryochytrium radiatum		COI	0	23
Thraustochytrium aureum	HA	COI	386	260
Phylum Ciliophora				
Class Nassophorea				
Order Nassulida				
Family Nassulidae				

Nassula sp.	HA, O, A	ITS	52	641
Family Furgasoniidae				
Parafurgasonia sp.	C	ITS	349	170
Class Litostomatea				
Order Cyclotrichida				
Family Mesodiniidae				
Askenasia sp.	C	ITS	97	0
Class Oligohymenophorea	C	ITS	2218	66
Oligohymenophorea ord Incertae sedis				
Family Epistylidae				
Epistylis sp.	C	ITS	143	27
Family Opisthonectidae				
Aristerostoma sp.	C	ITS	0	7
Opisthonecta sp.	C	ITS	0	41
Family Vorticellidae		ITS	0	47
Epicarchesium sp.	HA, E, A	ITS	0	11
Pseudovorticella sp.	C	ITS	13	450
Vorticella sp.	C	ITS	76	829
Vorticellides sp.	HA	ITS	1121	436
Family Zoothamniidae				
Zoothamnium sp.	C	ITS	295	63
Order Peniculida				
Family Stokesiidae				
Stokesia sp.	C	ITS	914	1329
Order Philasterida				
Family Loxocephalidae				
Cardiostomatella sp.	C	ITS	134	0
Family Uronematidae				
Homalogastra sp.	C	ITS	54	64

Class Spirotrichea		ITS	443	192
Order Sporadotrichida	C	ITS	10624	1416
Family Oxytrichidae	C	ITS	3582	3060
Cyrtohymena sp.	C	ITS	675	394
Stylonychia sp.	C	ITS	414	97
Order Stichotrichida				
Family Amphisiellidae				
Onychodromus sp.		ITS	0	8
Family Schmidingerotrichidae				
Schmidingerothrix sp.	PA	ITS	0	264
Family Kahliellidae				
Kahliella sp.	C	ITS	84	126
Order Urostylida	C	ITS	157	407
Family Holostichidae				
Anteholosticha sp.	C	ITS	316	370
Family Urostylidae	C	ITS	28	403
Uroleptus sp.	C	ITS	0	2327
Urostyla sp.	C	ITS	0	290
Phylum Cercozoa				
Class Imbricatea				
Order Thaumatomonadida		COI	5	17
Family Thaumatomonadidae				
Thaumatomonas sp.	C	COI	3	0
Phylum Ochrophyta				
Class Eustigmatophyceae				
Order Eustigmatales				
Family Monodopsidaceae				
Nannochloropsis limnetica	PA	COI	988	105
Class Pelagophyceae				

Order Pelagomonadales				
Family Pelagomonadaceae				
Aureococcus anophagefferens	HA	ITS	224	0
Class Phaeophyceae				
Order Ectocarpales				
Family Chordariaceae				
Leathesia difformis	C	ITS	0	12401
Myriotrichia claviformis	C	ITS	4605	2592
Punctaria latifolia	C	ITS	191	244
Sauvageaugloia divaricata	PA	ITS	128	19
Family Ectocarpaceae				
Ectocarpus siliculosus	C	ITS	0	16
Family Scytosiphonaceae				
Colpomenia peregrina	C	ITS	73	0
Colpomenia sinuosa	C	ITS	13328	0
Dactylosiphon bullosus	C	ITS	0	22
Order Laminariales				
Family Agaraceae				
Alaria praelonga	HA	ITS	13	23
Thalassiophyllum clathrus	HA	ITS	31	0
Family Laminariaceae				
Hedophyllum nigripes	HA	ITS	19	0
Saccharina coriacea	HA	ITS	94	181
Family Lessoniaceae				
Lessonia flavicans	NT, An	ITS	0	299
Order Fucales				
Family Fucaceae				
Fucus vesiculosus	HA	ITS	27	0
Order Dictyotales				

Family Dictyotaceae				
Dictyota flabellata	HA, NT	ITS	0	97
Order Scytothamnales				
Family Bachelotiaceae				
Bachelotia antillarum	C	ITS	67	0
Class Raphidophyceae				
Order Chattonellales				
Family Chattonellaceae				
Chattonella marina	HA, A, NT	COI	0	3
Haramonas dimorpha	A	COI	1809	36
Heterosigma akashiwo	C	COI	67	0
Class Schizocladiophyceae				
Order Schizocladiales				
Family Schizocladiaceae				
Schizocladia ischiensis	GRL, ITA	COI	174	916
Class Xanthophyceae				
Order Botrydiales				
Family Botrydiaceae				
Botrydium granulatum	HA, A	ITS	608	174
Order Mischococcales				
Family Botrydiopsidaceae				
Botrydiopsis alpina	C	ITS	38	0
Family Mischococcaceae				
Mischococcus sphaerocephalus	BGR	ITS	23008	7367
Order Tribonematales				
Family Heteropediaceae				
Heterococcus caespitosus	PA, O	ITS	352	0
Phyllum Oomycota				
Class Peronosporea				

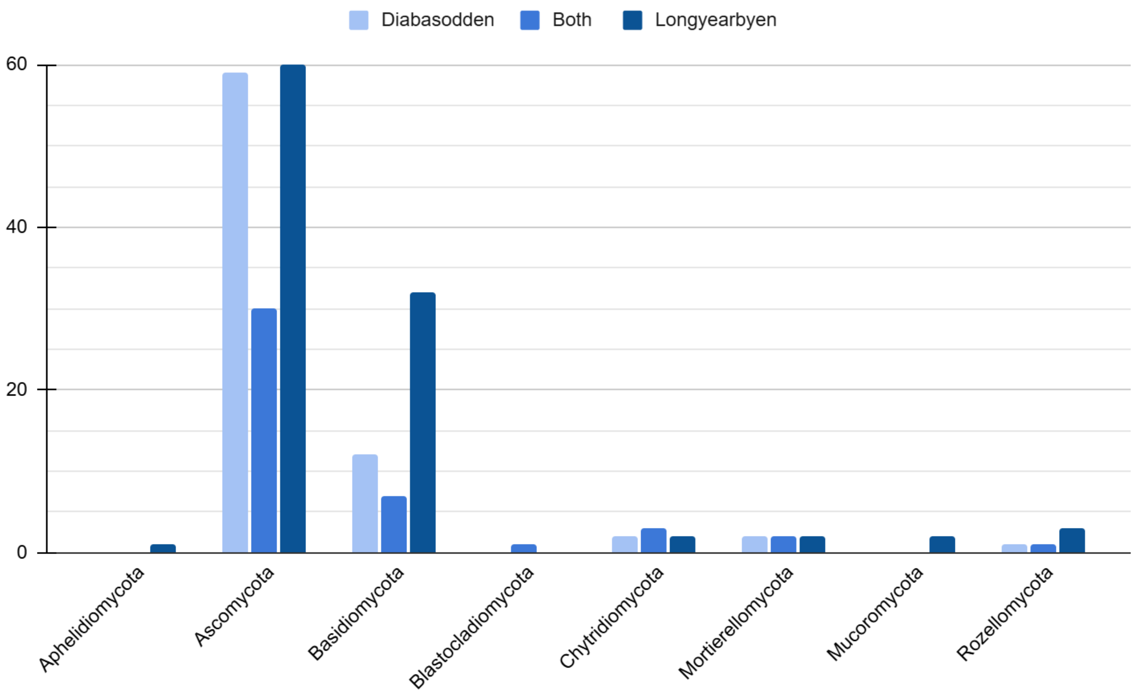
Order Peronosporales				
Family Peronosporaceae				
Halophytophthora mycoparasitica	O, PA	COI	0	16
Phytophthora amnicola	NZ	COI	35	16
Phytophthora caryae	NA	COI	0	113
Phytophthora gregata	NA	COI	0	14
Phytophthora idaei	PA	COI	14	0
Phytophthora insolita	O	COI	0	524
Phytophthora megakarya	E	COI	19	0
Phytophthora morindae	NA	COI	0	14
Phytophthora pseudotsugae	NA	COI	52	0
Phytophthora psychrophila	HA	COI	0	65
Phytophthora tropicalis	NT	COI	0	99
Phytophthora virginiana	NA, O	COI	0	4
Phytophthora sp. 'docynia'		COI	711	0
Phytophthora sp. 'kelmania'		COI	0	146
Phytophthora x stagnum	NA, NT	COI	1067	0
Phytophthora thermophila x Phytophthora moyootj		COI	0	45
Phytophthora x vanyenensis		COI	0	90
Order Pythiales				
Family Pythiaceae				
Globisporangium echinulatum	A, HA	COI	8	0
Globisporangium irregulare	HA, E, A	COI	0	219
Globisporangium spinosum	A, JPN	COI	244	368
Globisporangium sylvaticum	HA, A	COI	0	20
Pythium aphanidermatum	HA, O, A	COI	32	86
Pythium brachiatum	JPN	COI	0	4285
Pythium biforme	JPN	COI	40	0

Pythium jasmonium	NA	COI	707	0
Pythium oopapillum	HA, NT	COI	3953	205
Phytopythium boreale	PA	COI	58	0
Phytopythium litorale	O, E, A	COI	0	21
Phytopythium mercuriale	PA, E	COI	32	0
Order Saprolegniales				
Family Saprolegniaceae				
Achlya bisexualis	HA	COI	0	278
Aphanomyces invadans	CHN, USA	COI	146	5
Leptolegnia caudata	PA	COI	57	0

4.3.2. Fungi

A total of 313 ASVs belonging to the Fungi Kingdom were found, from which 40.7% of the ASVs could be identified at genus level. The species are allocated in 6 different Phylum, 27 Classes, 58 Orders and 96 Families. 48 ASVs could only be identified in Kingdom level, while 50 others could be identified in higher taxonomic levels, but not in species level. From the 313 ASVs found in both sites, 284 were found with ITS2 marker and 29 with COX1 marker. All Unknown Fungi were found with ITS2 marker.

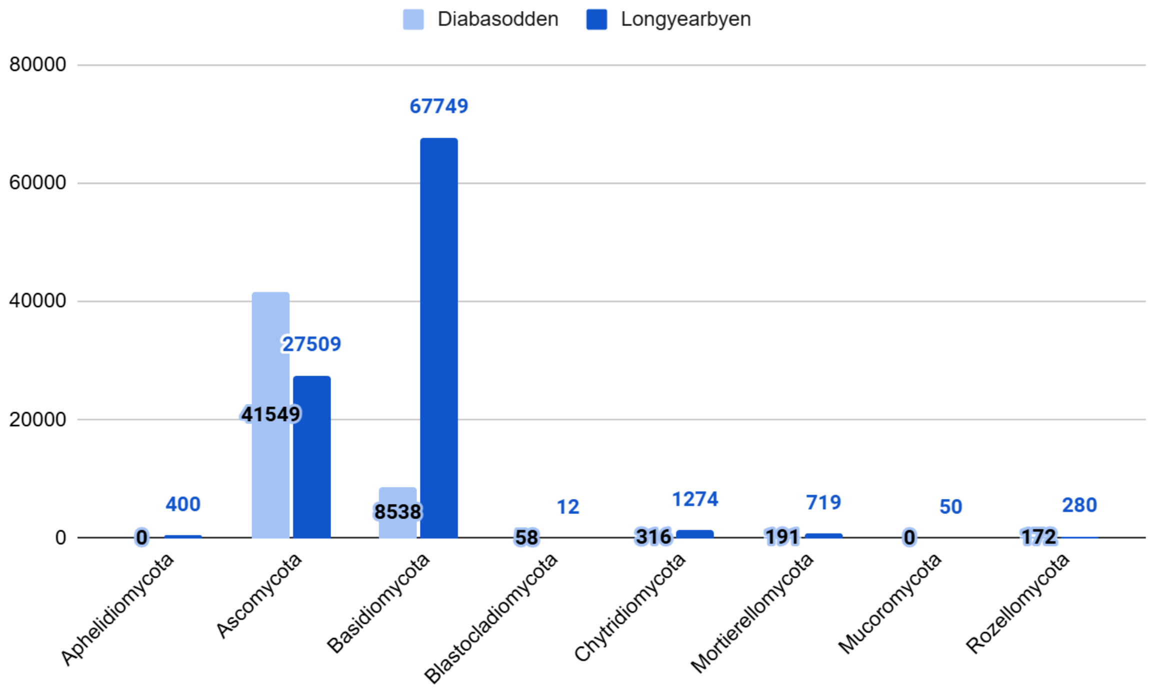
The richest Phylum in general was Ascomycota, with 149 ASVs (Fig. 14), while the most abundant was Basidiomycota, with 51.3% of the reads (Fig. 15). The richest Order was Helotiales, with 26 species. Nevertheless, the most abundant Order was Thelephorales, with 28.9% of the total reads. The richest Family was Verrucariales, with 11 species and the most abundant Family was Thelephoraceae, with 14.8% of reads.



Figures 14. Number of ASVs of each Fungi Phylum in each site.

A total of 168 ASVs were found in Diabasodden and 22.2% of the ASVs could be identified at Genus level. Nonetheless, 7.7% of the area's ASVs could only be identified at a higher taxonomic level and 35.9% could be identified only at Kingdom level. These species were distributed in 4 Phylum, 16 Classes, 41 Orders and 55 Families.

Ascomycota was both the richest and most abundant Phylum in Diabasodden (Fig. 14 and 15). The most abundant Order was Kriegeriales, with 31.8% of the reads number, and the richest was Verrucariales, with 8 species. In the Families, Camptobasidiaceae was the most abundant, with 31.8%, while Verrucariaceae was the richest, with 8 species also.



Figures 15. Number of reads of each Fungi Phylum.

In Longyearbyen, there were 196 ASVs. However, 15.9% of the ASVs were identified in higher taxonomic levels and 29% only at kingdom level. The found species were allocated in 5 Phylum, 16 Classes, 32 Orders and 74 Families. Basidiomycota was the most abundant Phylum, with 69.1% of the number of reads (Fig. 15), and Ascomycota was the richest, with 90 ASVs (Fig. 14). Helotiales was the richest Order, with 16 species and the most abundant was Thelephorales, with 23.1% of the reads. Within Families, the species were well distributed, the richest Family being Helotiaceae, with only 4 species. The most abundant was Thelephoraceae, with 23.1% of reads.

There were 12 identified species that occurred in both sites (Fig. 16), out of 50 ASVs. Furthermore, 31.4% of the ASVs could be identified at genus level, 11.8% could be identified solely in higher taxonomic levels and 35.3% only at kingdom level. There were 4 Phylums, 10 Classes, 17 Orders and 21 Families. The richest and most abundant Order was Helotiales, with 5 species and 39% of reads. The richest Family was Helotiaceae, with 3 species, and the most abundant was Hygrophoraceae, with 22% of reads.

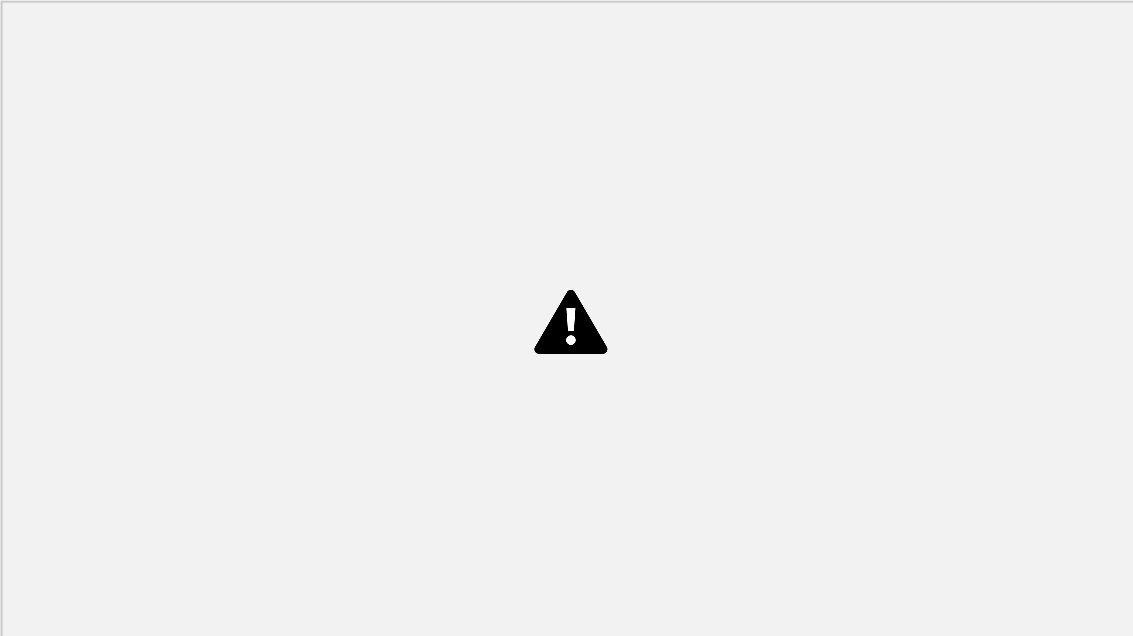


Figure 16. Venn diagram of Fungi Kingdom ASVs in both sites.

There was one non-native species found exclusively in Diabasodden, *Coniozyma leucospermi*, from ethiopian region. Exclusively in Longyearbyen, there were 3 invasive species, *Leohumicola levissima*, common to warmer environments, Indian's *Ophiostoma himal-ulmi* and *Russula griseocarnosa,* from the Oriental part of Asia. Shared between sites, there was *Arthrocladium fulminans*, found naturally in Mayotte and South Africa (Fig. 17).

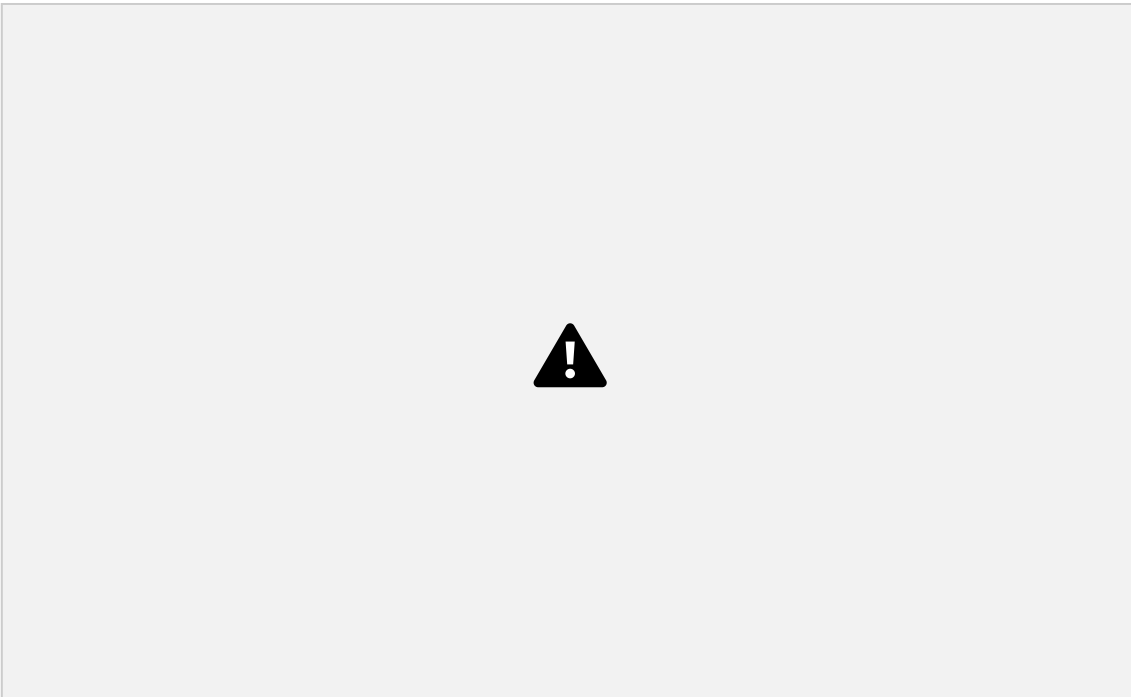


Figure 17. Number of reads and location of each non-native Fungi species.

Table 3. Name of each taxa found, indicating which maker used (ITS2 or COX1), which site it was found (Diabasodden, Longyearbyen or both), and the distribution of each taxa.

C = Cosmopolitan, HA = Holarctic, PA = Palearctic, NA = Nearctic, NT = Neotropical, E = Ethiopian, O = Oriental, A = Australian, An = Antarctic; IND = India; MYT = Mayotte; NOR = Norway; RUS = Russia; USA = United States of America.

TAXA	Hab/ Distr.	Marker	D.	L.
KINGDOM FUNGI			0	400
Phylum Aphelidiomycota	C	ITS	0	400
Phylum Ascomycota	C	ITS	0	105
Class Arthoniomycetes				
Order Arthoniales				
Family Lecanographaceae				
Alyxoria varia	C	ITS	13	0
Class Dothideomycetes	C	ITS	1196	873
Order Capnodiales	C	ITS	6	48
Family Cladosporiaceae	C	ITS		25
Cladosporium herbarum	C	ITS	479	468
Rachicladosporium sp.	C	ITS	53	0
Family Neodevriesiaceae	C	ITS	405	38
Neodevriesia sp.	C, An	ITS	17	0
Family Paradevriesiaceae				
Paradevriesia americana	HA	ITS	0	47
Order Cladosporiales				
Family Davidiellaceae				
Cladosporium sphaerospermum	C	COI	109	0
Order Dothideales				
Family Dothideaceae				
Coniozyma leucospermi	E	ITS	2	0
Order Mycosphaerellales				
Family Mycosphaerellaceae				

Zasmidium cellare	HA, E, A	COI	0	19
Zymoseptoria tritici	C	COI	37	0
Family Teratosphaeriaceae				
Elasticomyces elasticus	PA, NT, An	ITS	0	15
Oleoguttula mirabilis	HA, An	ITS	11	28
Capnobotryella sp.	C	ITS	7	0
Constantinomyces sp.	HA, NT	ITS	0	22
Friedmanniomyces sp.	HA, NT, An	ITS	96	0
Order Mytilinidales				
Family Gloniaceae				
Cenococcum sp.	C, An	ITS	0	466
Order Pleosporales	C	ITS	2362	20
Family Didymellaceae	C	ITS	554	1252
Family Lentitheciaceae				
Keissleriella cladophila	PA, A	ITS	8	0
Lentithecium sp.	HA, A	ITS	0	129
Family Leptosphaeriaceae	C	ITS	149	349
Leptosphaeria doliolum	C	ITS	0	32
Leptosphaeria sclerotioides	HA	ITS	122	0
Family Melanommataceae				
Herpotrichia sp.	C, An	ITS	0	678
Family Sporormiaceae	C	ITS	32	37
Sporormiella intermedia	C	ITS	111	0
Family Phaeosphaeriaceae	C	ITS	0	184
Comoclathris sp.	C	ITS	96	98
Phaeosphaeria sp.1	C, An	ITS	192	10
Phaeosphaeria sp.2	C, An	ITS	28	0
Phaeosphaeriopsis sp.	C	ITS	19	0
Order Venturiales				

Family Venturiaceae				
Venturia sp.	C, An	ITS	0	64
Class Eurotiomycetes				
Order Chaetothyriales	C	ITS	1204	285
Family Epibryaceae				
Epibryon sp.1	HA, A, NT	ITS	0	266
Epibryon sp.2	HA, A, NT	ITS	0	341
Family Herpotrichiellaceae				
Cladophialophora minutissima	C	ITS	0	3677
Cladophialophora nyingchiensis	C	ITS	0	41
Cladophialophora sp.	C	ITS	0	39
Rhinocladiella sp.	C, An	ITS	705	36
Family Trichomeriaceae				
Arthrocladium fulminans	MYT	COI	326	105
Knufia petricola	PA, NT, O	ITS	247	0
Order Eurotiales				
Family Aspergillaceae				
Penicillium sp.	C	ITS	92	184
Order Mycocaliciales				
Family Mycocaliciaceae				
Mycocalicium subtile	C	COI	20	0
Order Onygenales				
Family Arthrodermataceae				
Arthroderma uncinatum	HA, A	COI	18	0
Order Sclerococcales	C	ITS	30	0
Order Verrucariales	C	ITS	378	12
Family Verrucariaceae	C	ITS	275	0
Atla tibelliorum	NOR	ITS	105	0
Polyblastia bryophila	HA	ITS	30	0

Staurothele drummondii	HA	ITS	24	0
Thelidium minutulum	HA, NT, O	ITS	11	0
Verrucaria aethiobola	HA, E, An	ITS	16	0
Verrucaria alpicola	HA	ITS	0	18
Atla sp.	HA	ITS	278	0
Agonimia sp.	C, An	ITS	26	65
Verrucaria sp.1	HA, An	ITS	166	0
Verrucaria sp.2	HA, An	ITS	192	0
Class Lecanoromycetes				
Order Caliciales				
Family Physciaceae				
Rinodina sophodes	HA, O, NT	ITS	54	0
Physcia sp.1	C	ITS	49	0
Physcia sp.2	C	ITS	140	0
Order Lecanorales	C	ITS	57	0
Family Catillariaceae				
Catillaria nigroclavata	HA, O	ITS	31	0
Family Lecanoraceae				
Lecidella sp.	C	ITS	16	0
Family Porpidiaceae				
Mycobilimbia microcarpa	HA	ITS	105	0
Mycobilimbia tetramera	HA	ITS	164	0
Family Ramalinaceae				
Bacidina sp.	C	ITS	861	0
Biatora sp.	C	ITS	0	26
Lecania sp.	C	ITS	48	0
Family Stereocaulaceae				
Lepraria neglecta	C	ITS	59	0
Order Peltigerales				

Family Collemataceae				
Scytinium subtile	HA, A	ITS	41	0
Leptogium sp.	C	ITS	16	0
Family Pannariaceae				
Psoroma hypnorum	C, An	ITS	0	6
Family Peltigeraceae				
Peltigera extenuata	C	ITS	0	21
Order Rhizocarpales				
Family Rhizocarpaceae				
Rhizocarpon geminatum	HA, A, An	ITS	50	0
Rhizocarpon intermediellum	HA, O	ITS	17	0
Order Teloschistales				
Family Teloschistaceae	C	ITS	148	0
Caloplaca sp.	C	ITS	30	0
Rusavskia sp.	HA	ITS	786	0
Class Leotiomycetes	C	ITS	96	1617
Order Helotiales	C	ITS	10480	4774
Family Dermateaceae				
Coleophoma sp.	C	ITS	0	310
Family Discinellaceae				
Gyoerffyella entomobryoides	C	ITS	0	217
Family Helotiaceae	C	ITS	65	164
Stamnaria persoonii	HA, A	ITS	0	8
Tetracladium apiense	C	ITS/COI	6740	0
Tetracladium maxilliforme	PA, A	COI	0	68
Crocicreas sp.	C	ITS	0	181
Filosporella sp.1	HA	ITS	0	161
Filosporella sp.2	HA	ITS	216	0
Gorgoniceps sp.	C	ITS	0	30

Rhizoscyphus sp.	C	ITS	989	513
Tetracladium sp.1	C	ITS	352	30
Tetracladium sp.2	C	ITS	14	124
Tetracladium sp.3	C	COI	0	21
Family Pezizellaceae				
Chalara pseudoaffinis	C	ITS	89	3321
Family Hyaloscyphaceae	C	ITS	0	1318
Clathrosphaerina zalewskii	C	ITS	0	74
Heliscella stellata	PA	ITS	0	5
Lachnellula fuscosanguinea	C	ITS	32	0
Unguicularia carestiana	C	ITS	238	0
Hyaloscypha sp.	C	ITS	63	0
Family Leotiaceae	C	ITS	0	59
Alatospora acuminata	C	ITS	0	112
Gorgomyces honrubiae	C	ITS	6	42
Pezoloma ericae	C	ITS	0	347
Articulospora tetracladia	HA, O, A	COI	0	11
Family Sclerotiniaceae	C	ITS	0	3
Septotinia podophyllina	NA	ITS	0	16
Family Ploettnerulaceae				
Cadophora sp.	C	ITS	0	50
Helotiales fam Incertae sedis				
Rhexocercosporidium panacis	C	ITS	102	0
Leohumicola minima	C	COI	0	3
Leohumicola levissima	NT, E, O	COI	0	22
Leohumicola verrucosa	C	COI	0	43
Leohumicola sp.	C	ITS	0	1139
Order Phacidiales				
Family Helicogoniaceae				

Xenosphaeropsis pyriputrescens	RUS, USA	ITS	0	154
Family Phacidiaceae				
Eleutheromyces sp.	HA	ITS	0	91
Phacidiales ord Incertae sedis				
Family Pseudeurotiaceae				
Pseudogymnoascus pannorum	C	COI	0	14
Order Thelebolales				
Family Thelebolaceae				
Thelebolus sp.	C	ITS	91	0
Class Lichinomycetes	C	ITS	1729	0
Order Lichinales				
Family Lichinaceae				
Lempholemma polyanthes	HA, A	ITS	48	0
Class Orbiliomycetes	C	ITS	0	479
Order Orbiliales	C	ITS	0	48
Family Orbiliaceae	C	ITS	62	108
Hyalorbilia sp.	C	ITS	335	0
Orbilia sp.	C	ITS	0	309
Class Pezizomycetes				
Order Pezizales				
Family Pyronemataceae				
Geopora nicaeensis	HA	ITS	15	0
Geopora sp.	C	ITS	1240	13
Family Tuberaceae				
Tuber sp.	C	ITS	19	0
Pezizales fam Incertae sedis				
Trichobolus sp.	C	ITS	14	0
Class Saccharomycetes				
Order Saccharomycetales	C	ITS	5	0

Class Sordariomycetes				
Order Coniochaetales				
Family Coniochaetaceae	C	ITS	0	259
Order Glomerellales				
Family Plectosphaerellaceae				
Plectosphaerella sp.	C	ITS	8	0
Order Hypocreales	C	ITS	5600	20
Family Bionectriaceae				
Nectriopsis rexiana	HA, A	ITS	25	24
Family Cordycipitaceae				
Akanthomyces muscarius	HA, A, An	ITS	0	18
Leptobacillium leptobactrum	C	ITS	0	34
Family Nectriaceae				
Cosmospora gigas	RUS	COI	62	0
Neonectria sp.	C	ITS	150	21
Family Ophiocordycipitaceae				
Purpureocillium lilacinum	C	COI	0	111
Sordariomycetes ord Incertae sedis				
Oncopodiella trigonella	HA, A	ITS	47	0
Order Ophiostomatales				
Family Ophiostomataceae				
Ophiostoma himal-ulmi	IND	COI	0	132
Order Xylariales				
Family Sporocadaceae	C	ITS	0	348
Microdochium phragmitis	C	ITS	0	13
Phylum Basidiomycota				
Class Agaricomycetes				
Order Agaricales				
Family Amanitaceae				

Amanita thiersii	HA	COI	0	12
Family Clavariaceae				
Clavulinopsis sp.	C	ITS	0	7
Hyphodontiella sp.	HA, A, NT	ITS	0	399
Family Cortinariaceae				
Cortinarius sp.	C	ITS	74	23
Family Hygrophoraceae				
Arrhenia peltigerina	HA, O	ITS	0	151
Arrhenia retiruga	HA, A	ITS	0	56
Arrhenia sp.	C	ITS	124	2899
Family Hymenogastraceae				
Hebeloma sp.	HA	ITS	0	185
Family Inocybaceae				
Inocybe filiana	HA	ITS	0	20
Inocybe sp.	C	ITS	14	0
Family Mycenaceae	C	ITS	0	31
Family Psathyrellaceae				
Coprinellus micaceus	C	COI	0	135
Family Squamanitaceae				
Cystoderma tuomikoskii	HA	ITS	0	4
Family Tricholomataceae				
Mycenella trachyspora	HA, O	ITS	0	104
Rimbachia sp.	C, An	ITS	0	1070
Order Boletales				
Family Gomphidiaceae				
Chroogomphus rutilus	C	COI	0	42
Family Paxillaceae				
Paxillus rubicundulus	PA	COI	0	27
Order Cantharellales				

Family Cantharellaceae				
Cantharellus lutescens	HA, O	COI	160	0
Order Russulales				
Family Russulaceae				
Russula griseocarnosa	O	COI	0	69
Order Sebacinales	C	ITS	0	28
Family Serendipitaceae				
Serendipita sp.	C	ITS	135	6449
Order Thelephorales				
Family Thelephoraceae	C	ITS	576	5028
Thelephora caryophyllea	C	ITS	0	69
Tomentella galzinii	C	ITS	0	894
Tomentella muricata	HA	ITS	0	3486
Order Trechisporales	C	ITS	0	44173
Order Tremellodendropsidales	C	ITS	0	71
Class Cystobasidiomycetes				
Order Cystobasidiales				
Family Cystobasidiaceae				
Cystobasidium sp.	C	ITS	0	10
Order Sakaguchiales				
Family Sakaguchiaceae				
Sakaguchia sp.	C	ITS	0	34
Class Exobasidiomycetes				
Order Exobasidiales				
Family Exobasidiaceae				
Arcticomyces warmingii	HA, NT	ITS	0	2
Class Malasseziomycetes				
Order Malasseziales				
Family Malasseziaceae				

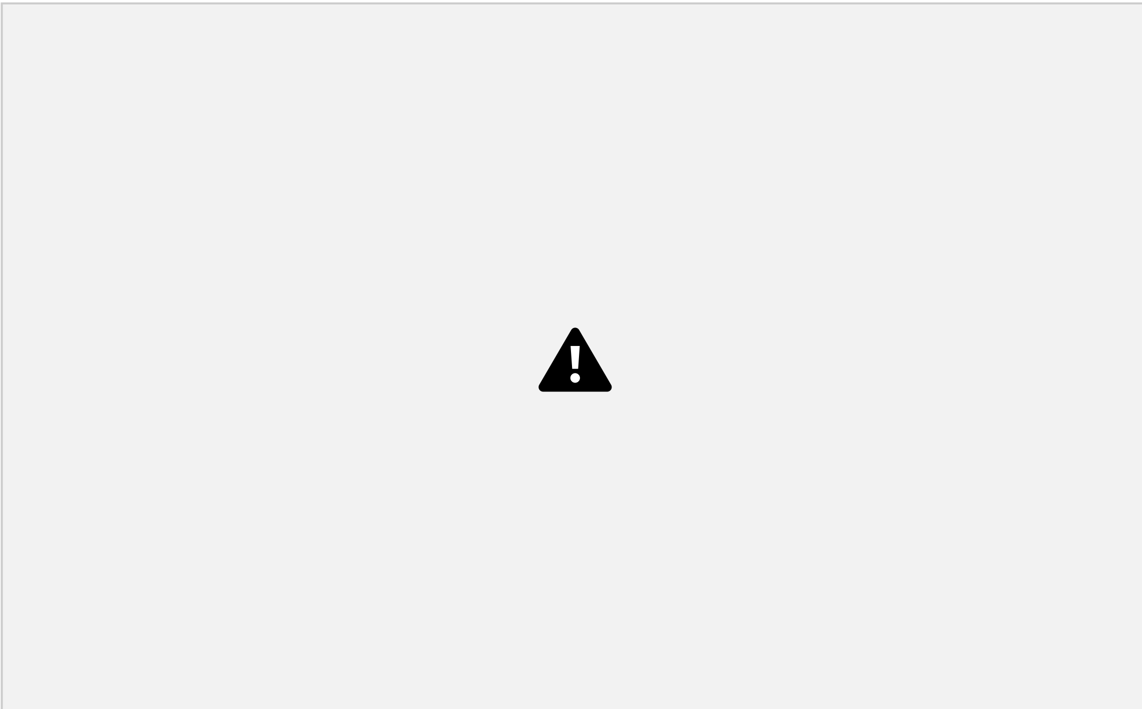
Malassezia japonica	C	COI	22	0
Class Microbotryomycetes				
Order Kriegeriales				
Family Camptobasidiaceae	HA, O, NT, An	ITS	0	31
Glaciozyma antarctica	HA, An	ITS	680	0
Order Leucosporidiales				
Family Leucosporidiaceae				
Leucosporidium muscorum	C	ITS	0	83
Order Microbotryales				
Family Microbotryaceae				
Microbotryum pustulatum	HA	ITS	47	0
Order Sporidiobolales				
Family Sporidiobolaceae				
Rhodotorula mucilaginosa	C	COI	0	126
Class Tremellomycetes	C	ITS	6	0
Order Cystofilobasidiales				
Family Mrakiaceae				
Mrakia frigida	HA, An	ITS	28	0
Mrakia niccombsii	PA, An	ITS	5	0
Order Filobasidiales				
Family Filobasidiaceae				
Goffeauzyma gastrica	C	ITS	19	20
Naganishia randhawae	C	ITS	11	0
Family Piskurozymaceae				
Piskurozyma cylindrica	HA, NT	ITS	0	34
Order Tremellomycetes ord Incertae sedis	C	ITS	107	0
Order Tremellales				
Family Bulleribasidiaceae				
Dioszegia fristingensis	PA, NT, E	ITS	12	0

Vishniacozyma tephrensis	C, An	ITS	44	0
Vishniacozyma victoriae	C, An	ITS	474	113
Family Cuniculitremaceae				
Kockovaella sp.	C	ITS	0	22
Family Tremellaceae	C	ITS	0	78
Tremella sp.	C	ITS	0	12
Class Ustilaginomycetes				
Order Ustilaginales				
Family Ustilaginaceae				
Macalpinomyces bursus	O, NT, NA	COI	0	14
Phyllum Blastocladiomycota				
Class Blastocladiomycetes				
Order Blastocladiales				
Family Blastocladiaceae				
Blastocladiella emersonii	C	COI	58	12
Phyllum Chytridiomycota				
Class Chytridiomycetes				
Order Spizellomycetales				
Family Powellomycetaceae				
Powellomyces hirtus	HA	COI	0	584
Chytridium sp.	HA, O, A	ITS	3	0
Geranomyces sp.	C	ITS	0	46
Class Lobulomycetes				
Order Lobulomycetales	C	ITS	258	570
Class Rhizophydiomycetes				
Order Rhizophydiales	C	ITS	18	97
Family Alphamycetaceae				
Betamyces sp.	C, An	ITS	12	23
Family Protrudomycetaceae				

Protrudomyces sp.	HA, NT	ITS	25	0
Phyllum Mortierellomycota				
Class Mortierellomycetes				
Order Mortierellales				
Family Protrudomycetaceae	C	ITS	0	51
Dissophora globulifera	C	ITS	0	67
Linnemannia sclerotiella	C	ITS	9	532
Mortierella alpina	C	ITS	9	0
Mortierella turficola	C	ITS	163	69
Podila humilis	C	ITS	10	0
Phyllum Mucoromycota				
Class Mortierellomycetes				
Order Mortierellales				
Family Mortierellaceae				
Entomortierella parvispora	C	COI	0	18
Class Mucoromycetes				
Order Mucorales				
Family Mucoraceae				
Mucor lusitanicus	C	COI	0	32
Phyllum Rozellomycota		ITS	128	162

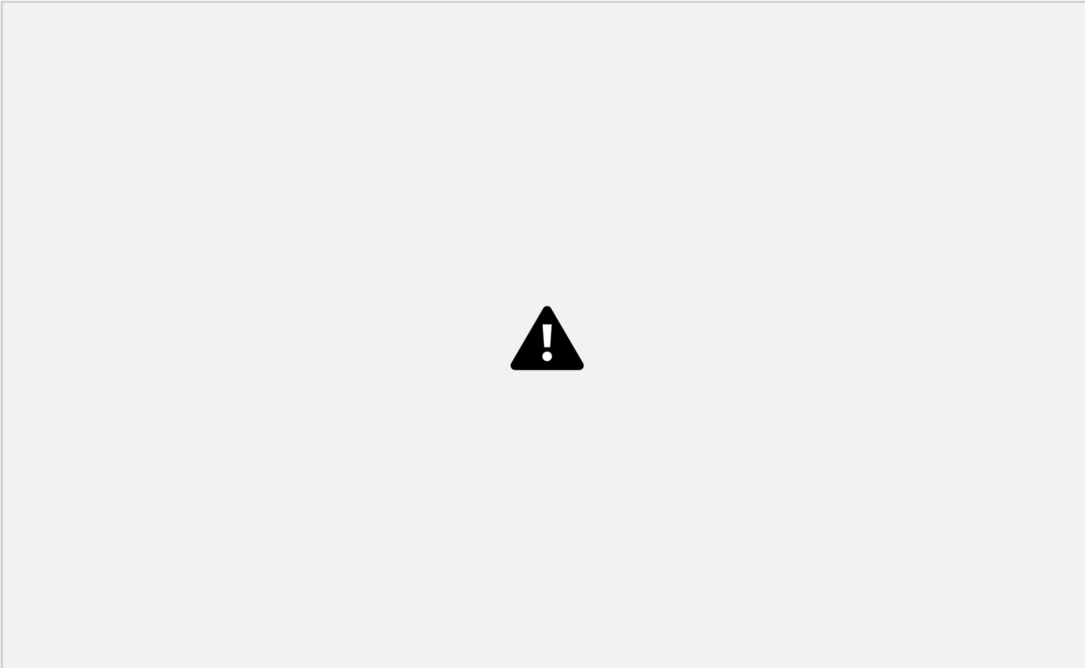
4.3.3. Metazoa

The total number of ASVs found were 135, distributed in 13 Phylum, 28 Classes, 64 Orders and 106 Families, and 3% — 4 ASVs — could only be identified at Kingdom level. All Metazoa ASVs were found with COX1 marker. The most abundant and richest Phylum was Arthropoda, with 69.3% of the reads and 63 ASVs, followed by Tardigrada in abundance and Mollusca in richness (Fig. 18 and 19). The richest Order were Diptera and Lepidoptera, with 13 species each. The most abundant was Parachela, with 55.8% of reads. The richest Families were Culicidae and Conidae, with 4 species each, and the most abundant was Eupodidae, with 55.8% of reads.



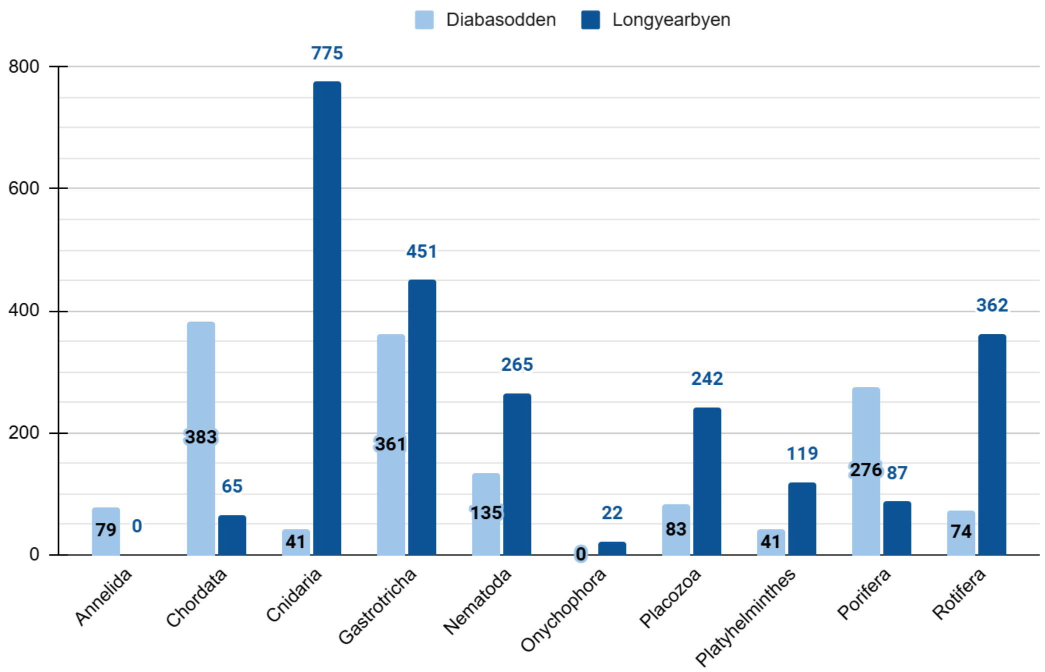
Figures 18. Number of ASVs of each Metazoa Phylum in each site.

There were 58 ASVs that occurred in Diabasodden. These ASVs were part of 7 Phylums, 17 Classes, 29 Orders and 36 Families. The Phylum Tardigrada was the most abundant, with 49.7% of the number of reads (Fig. 19), while Arthropoda was the richest Phylum (Fig. 18). The richest Order was Lepidoptera, with 4 species. The species were well distributed among Families, only Erebidae and Turridae having more than one species. The most abundant Orders were Parachela and Lepidoptera, with 53.5% and 38.3% of the reads, respectively, even though Parachela was represented by only one species. The percentages repeat at Family level, being 53.5% Hypsibiidae and 38.3% Riodinidae.



Figures 19. Number of reads of the most abundant Metazoa Phylum.

Longyearbyen had 94 ASVs. They were allocated in 9 Phylum, 17 Classes, 39 Orders and 63 Families. Arthopoda was both the richest and most abundant Phylum (Fig. 18 and 19). The richest Order was Diptera, with 9 species, followed by Coleoptera, with 6 species. The most abundant was Trombidiformes, with 72.5% of the reads, followed by Parachela, with 25.2%. The most abundant Family was Eupodidae, that had 72.4% reads — represented solely by *Eupodes voxencollinus* —, followed by Richtersiidae, that had the same number of reads of its Order, Parachela. The species were well distributed in Family terms, Culicidae being the richest, with 4 species.



Figures 20. Number of reads of the remaining Metazoa Phylums.

Only 20 species were shared between both sites, distributed in 9 Phylum, 13 Classes, 16 Orders and 17 Families (Fig. 21). There were also 1 ASVs identified only at Kingdom level and 1 at Class level. The richest clades were the Order Macrodasyida and its Family Turbanellidae, with 3 species, the remaining species being well distributed among Orders and Families. Diptera was the most abundant Order, with 58% of reads, and it had only one Family, Cecidomyiidae, and one species, *Asteromyia modesta.*

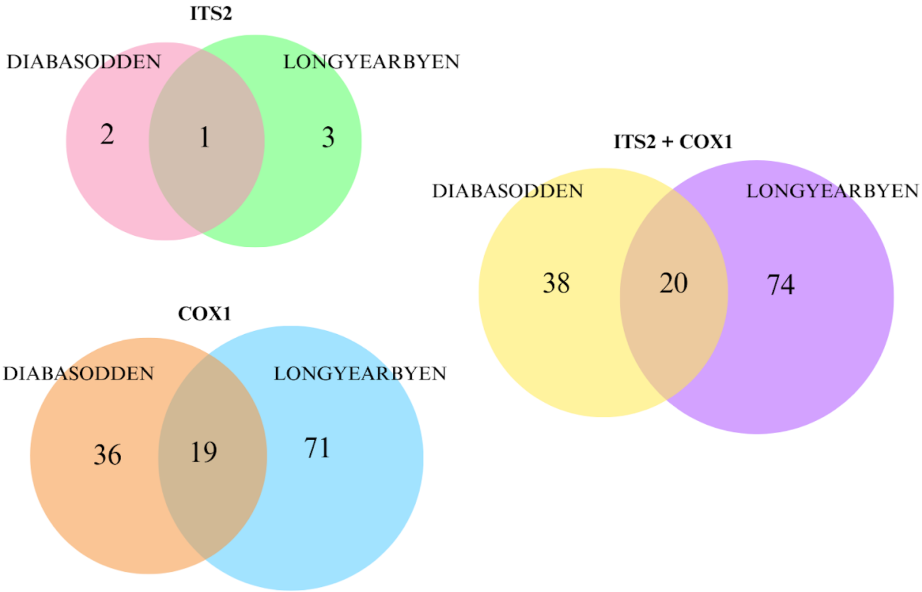


Figure 21. Venn diagram of Metazoa ASvs in both sites.

In the Metazoa Kingdom, the non-native species situation is more alarming (Fig. 22 and 23). In Diabasodden, there were 20 unrecorded species. There were 6 species common to Oriental Asia, 5 from the Australian region, 3 from Antarctica, which 2 occurs also in south South America and south Africa and 4 from the Neotropical region. There were also 2 species endemic to Japan, North Korea and South Korea.

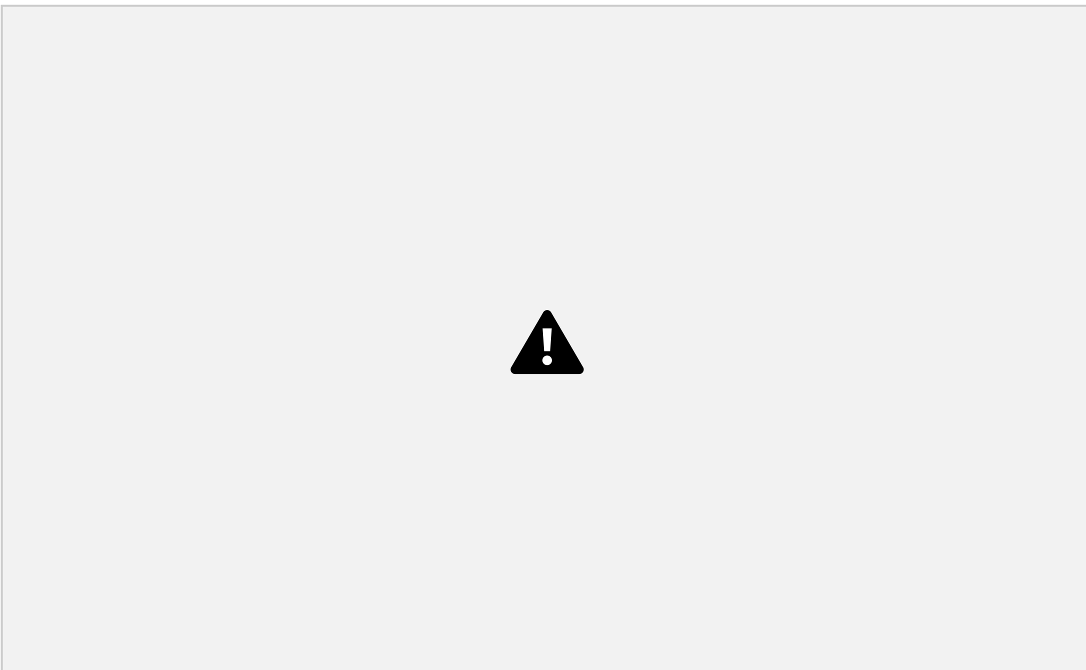


Figure 22. Number of reads and location of the Metazoa non-native species with >1000 reads.

In Longyearbyen there were 34 non-native species. There were 6 of them from the Oriental area, 3 from the Australian region, 1 from the Ethiopian region and 4 from the Neotropical region. Two species occur in three different areas, and 1 in two areas. There were 4 species endemic to North and South Korea and Japan. There was also 1 species endemic to the Korean area and 1 occurring only in Japan. There were 2 species endemic to Japan and Taiwan. There were 5 endemic species, Colombia’s *Smithagathis davidsmithi*, South Africa’s *Larentioides cacothemon,* Hawaii’s *Manduca blackburni*, Greece’, *Dolichopoda kiriakii* and Spain’s *Iberozospeum biscaiense.* There were also 2 species from the West Coast of the USA, from the states of California, Oregon and Washington.

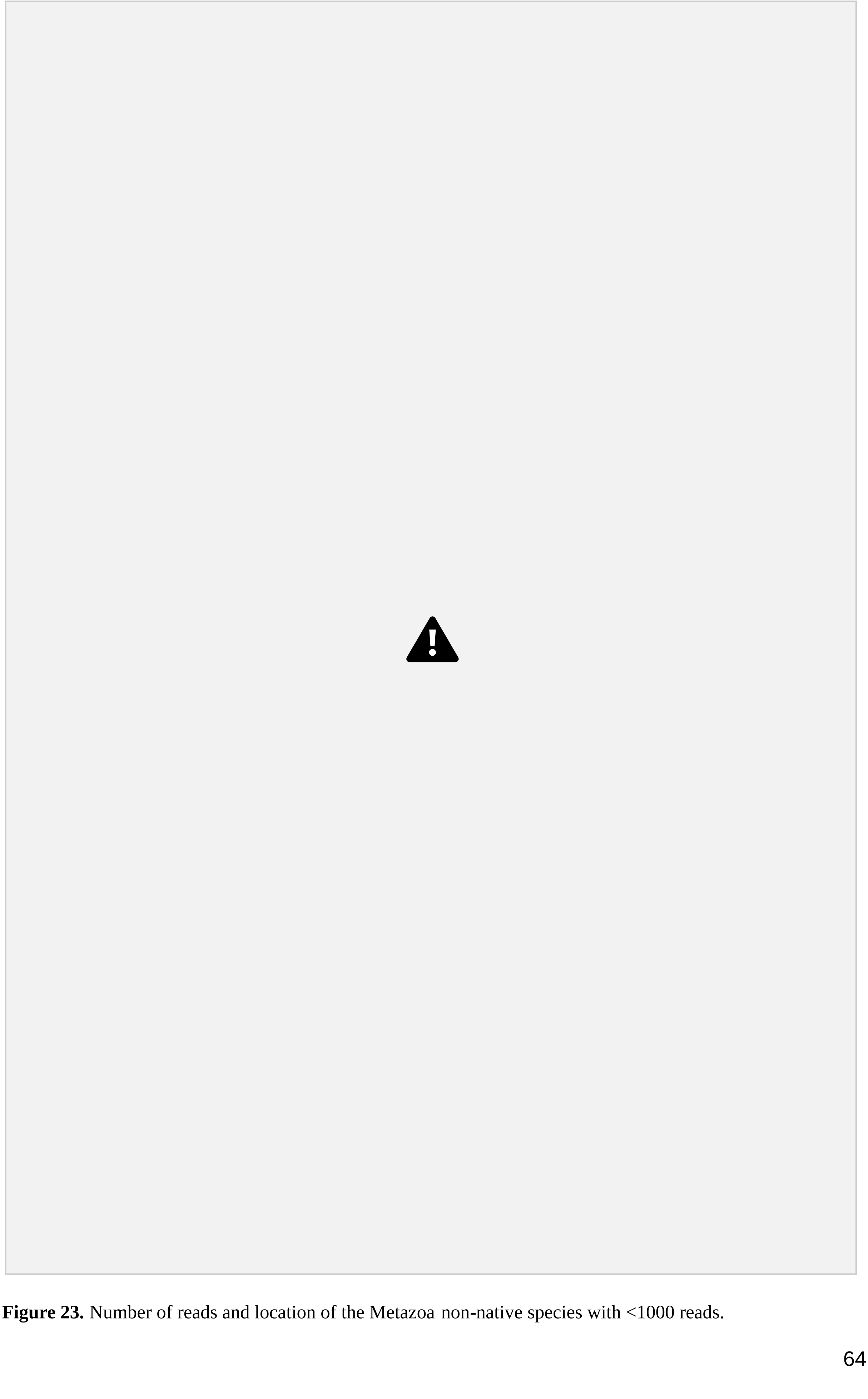


Figure 23. Number of reads and location of the Metazoa non-native species with <1000 reads.

Shared between sites there were 5 new species. One species was endemic to France, another 1 occurs in France and the USA. There was one Japanese species *Nipponacmea nigrans*, one Caribbean species *Acropora palmata*, and one species that occurs in warmer environments *Periglypta puerpera*.

Table 4. Name of each Metazoa taxa found, indicating which maker used (ITS2 or COX1), which site it was found (Diabasodden, Longyearbyen or both), and the distribution of each taxa.
C = Cosmopolitan, HA = Holarctic, PA = Palearctic, NA = Nearctic, NT = Neotropical, E = Ethiopian, O = Oriental, A = Australian, An = Antarctic; BGR = Bulgaria; CHN = China; COL = Colombia; ESP = Spain; FRA = France; GRC = Greece; GRL = Germany; IND = India; ITA = Italy; JPN = Japan; KOR = South Korea; MDG = Madagarcar; MYT = Mayotte; MYS = Malaysia; NZ = New Zealand; RUS = Russia; RPDC = North Korea; THA = Thailand; TWN = Taiwan; USA = United States of America; ZAF = South Africa.

TAXA	Hab/ Distr.	Marker	D.	L.
KINGDOM METAZOA				
Phylum Annelida				
Class Clitellata				
Order Hirudinida				
Family Haemopidae				
Haemopis terrestris	NA	COI	28	0
Class Polychaeta				
Order Sabellida				
Family Serpulidae				
Protula pacifica	NA	COI	51	1
Phylum Arthropoda				
Class Arachnida				
Order Araneae				
Family Hypochilidae				
Hypochilus gertschi	NA	COI	0	138
Family Leptonetidae				
Neoleptoneta paraconcinna	NA	COI	21	24
Leptonetela hangzhouensis	CHN	COI	0	66

Family Linyphiidae				
Syedra oii	KOR	COI	0	14
Family Salticidae				
Hakka himeshimensis	HA	COI	0	16
Phintella arenicolor	JPN, KOR, RPDC	COI	0	36
Order Mesostigmata				
Family Laelapidae				
Tropilaelaps mercedesae	O	COI	0	44
Order Sarcoptiformes				
Family Oppiidae				
Oppiella nova	C	COI	180	0
Order Trombidiformes				
Family Eriophyidae				
Acalitus vaccinii	C	COI	0	35
Family Eupodidae				
Eupodes voxencollinus	GRM	COI	0	139356
Class Branchiopoda				
Order Notostraca				
Family Triopsidae				
Triops longicaudatus	HA, NT	COI	138	413
Class Collembola				
Order Entomobryomorpha				
Family Isotomidae				
Folsomia quadrioculata	HA	COI	226	0
Parisotoma notabilis	HA	COI	0	40
Order Poduromorpha				
Family Hypogastruridae				
Gomphiocephalus hodgsoni	An	COI	186	0

Class Diplopoda				
Order Spirostreptida				
Family Cambalopsidae				
Trachyjulus phylloides	THA	COI	10	0
Class Insecta				
Order Coleoptera				
Family Chrysomelidae				
Cerotoma arcuata	NA	COI	0	19
Family Cleridae				
Phlogistomorpha croesus	A	COI	148	0
Family Dytiscidae				
Hydaticus vittatus	A, O	COI	0	2305
Hydaticus quadrivittatus	A	COI	0	205
Family Lampyridae				
Pteroptyx tener	O	COI	0	40
Family Silvanidae				
Dendrophagus cygnaei	NA	COI	0	9
Family Tenebrionidae				
Gonocephalum pubens	JPN, RPDC, KOR	COI	0	58
Order Diptera				
Family Agromyzidae				
Ophiomyia nasuta	HA	COI	0	771
Family Cecidomyiidae				
Asteromyia carbonifera	NA	COI	0	80
Asteromyia modesta	NA	COI	2678	2945
Family Chironomidae				
Procladius crassinervis	HA	COI	0	93
Family Culicidae				

Aedes aegypti	C	COI	0	66
Anopheles messeae	PA	COI	0	20
Anopheles quadriannulatus	NA	COI	0	64
Culex sitiens	O, A, E	COI	0	108
Family Muscidae				
Haematobia irritans	C	COI	25	0
Family Nycteribiidae				
Nycteribia schmidlii	NA, E	COI	338	0
Family Simuliidae				
Simulium maculatum	HA	COI	41	0
Family Tephritidae				
Acanthiophilus helianthi	PA	COI	0	81
Philophylla caesio	PA	COI	0	47
Order Ephemeroptera				
Family Heptageniidae				
Maccaffertium pudicum	NA	COI	0	70
Order Hemiptera				
Family Miridae				
Deraeocoris claspericapilatus	JPN, KOR, RPDC	COI	0	28
Family Cicadidae				
Gaeana maculata	O	COI	0	93
Order Hymenoptera				
Family Agaonidae				
Wiebesia pumilae	JPN, TWN	COI	0	8
Family Apidae				
Euglossa rufipes	NT	COI	0	6
Family Braconidae				
Smithagathis davidsmithi	COL	COI	0	157

Family Formicidae				
Lasius balearicus	PA	COI	0	556
Order Lepidoptera				
Family Erebidae				
Cyana pratti	O	COI	41	0
Polypogon obscuripennis	NA	COI	357	0
Family Geometridae				
Eupithecia exiguata	PA	COI	178	0
Larentioides cacothemon	ZAF	COI	0	43
Family Hesperiidae				
Celaenorrhinus victor	PA	COI	0	24
Family Lecithoceridae				
Scythropiodes barbellatus	PA	COI	0	35
Family Lycaenidae				
Lampides boeticus	C	COI	79	195
Tomares callimachus	PA, E	COI	0	31
Family Nymphalidae				
Mycalesis visala	O	COI	565	0
Family Pieridae				
Moschoneura pinthous	NT	COI	0	46
Family Riodinidae				
Dodona eugenes	O	COI	18928	0
Family Saturniidae				
Archaeoattacus edwardsii	O	COI	67	258
Family Sphingidae				
Manduca blackburni	USA	COI	0	947
Order Neuroptera				
Family Osmylidae				
Gryposmylus pennyi	MYS	COI	68	0

Order Odonata				
Family Epiophlebiidae				
Epiophlebia superstes	JPN	COI	0	11
Order Orthoptera				
Family Rhaphidophoridae				
Dolichopoda kiriakii	GRC	COI	0	201
Class Malacostraca				
Order Amphipoda				
Family Lysianassidae				
Scopelocheirus schellenbergi	O	COI	181	0
Order Cumacea				
Family Nannastacidae				
Campylaspis sulcata	PA	COI	0	100
Order Decapoda				
Family Atyidae				
Caridina typus	O, E, A	COI	0	23
Order Isopoda				
Family Armadillidiidae				
Armadillidium vulgare	C	COI	0	37
Class Entognatha				
Order Protura				
Family Fujientomidae				
Fujientomon dicestum	PA	COI	0	97
Class Thecostraca				
Order Balanomorpha				
Family Chthamalidae				
Chthamalus dalli	HA	COI	21	0
Phylum Chordata				
Class Aves				

Order Passeriformes				
Family Thamnophilidae				
Pithys albifrons	NT	COI	66	0
Class Actinopteri				
Order Kurtiformes				
Family Apogonidae				
Apogon binotatus	NT	COI	27	0
Class Lepidossauria				
Order Squamata				
Family Dipsadidae				
Oxyrhopus guibei	NT	COI	290	0
Class Mammalia				
Order Primates				
Family Cebidae				
Sapajus nigritus	NT	COI	0	65
Phylum Cnidaria				
Class Anthozoa				
Order Actiniaria				
Family Actiniidae				
Actinia tenebrosa	A, NA	COI	0	7
Order Pennatulacea				
Family Virgulariidae				
Acanthoptilum gracile	NA	COI		4
Order Scleractinia				
Family Acroporidae				
Acropora palmata	NT	COI	41	182
Class Hydrozoa				
Order Anthoathecata				
Family Corynidae				

Codonium proliferum	PA	COI	0	42
Family Corymorphidae				
Corymorpha nutans	HA, E	COI	82	13
Family Pandeidae				
Leuckartiara octona	C	COI	55	0
Family Ptilocodiidae				
Thecocodium quadratum	NA, E	COI	25	0
Order Leptothecata				
Family Clytiidae				
Clytia hemisphaerica	C	COI	0	80
Family Campanulariidae				
Orthopyxis integra	C	COI	21	0
Order Siphonophorae				
Family Agalmatidae				
Nanomia cara	HA	COI	0	158
Family Athorybiidae				
Athorybia rosacea	C	COI	38	0
Family Rhizophysidae				
Rhizophysa filiformis	C	COI	0	21
Class Scyphozoa				
Order Semaeostomeae				
Family Cyaneidae				
Cyanea nozakii	O	COI	0	37
Phylum Gastrotricha				
Class Macrodasyida				
Order Macrodasyida				
Family Turbanellidae				
Turbanella ambronensis	PA	COI	127	74
Turbanella hyalina	PA	COI	153	120

Turbanella mustela	FRA	COI	81	257
Phylum Mollusca				
Class Bivalvia				
Order Mytiloida				
Family Mytilidae				
Mytilus edulis	C	COI	171	109
Order Ostreida				
Family Ostreidae				
Saccostrea mordax	JPN, TWN	COI	0	1490
Order Pterioida				
Family Pinnidae				
Atrina pectinata	C	COI	53	0
Order Venerida				
Family Veneridae				
Leukoma jedoensis	JPN, KOR, RPDC	COI	110	0
Mercenaria mercenaria	HA	COI	0	5
Periglypta puerpera	O, E, A	COI	109	13
Saxidomus purpurata	JPN, KOR, RPDC	COI	0	12
Class Cephalopoda				
Order Octopoda				
Family Octopodidae				
Octopus variabilis	O	COI	0	8423
Class Gastropoda				
Order Hygrophila				
Family Acroloxidae				
Acroloxus lacustris	PA	COI	0	27
Order Pylopulmonata				
Family Amphibolidae				

Salinator rosaceus	A	COI	42	0
Order Cephalaspidea				
Family Haminoeidae				
Bullacta exarata	PA	COI	0	72
Order Cycloneritida				
Family Neritidae				
Nerita japonica	JPN, KOR, RPDC	COI	10	0
Order Ellobiida				
Family Ellobiidae				
Iberozospeum biscaiense	ESP	COI	0	28
Order Littorinimorpha				
Family Cassidae				
Casmaria erinaceus	C	COI	0	25
Order Patellogastropoda				
Family Lottiidae				
Nipponacmea nigrans	JPN	COI	375	16
Order Neogastropoda				
Family Conidae				
Conus capitaneus	C	COI	72	0
Conus dalli	NA, NT	COI	0	182
Conus laterculatus	O, A	COI	0	37
Conus miles	Pacific and Indic Oceans	COI	0	20
Conus obscurus	C	COI	28	55
Family Muricidae				
Ocinebrellus inornatus	HA	COI	0	16
Family Turridae				
Turris babylonia	A	COI	13	0
Turridrupa jubata	PA, A	COI	14	0

Order Neomphalida				
Family Neomphalidae				
Lamellomphalus manusensis	A	COI	27	0
Class Planorbidae				
Bulinus globosus	E	COI	0	211
Order Stylommatophora				
Family Helicarionidae				
Mysticarion hyalinus	A	COI	0	29
Family Helminthoglyptida				
Helminthoglypta hertleini	USA	COI	0	16
Helminthoglypta talmadgei	USA	COI	0	31
Family Camaenidae				
Mesodontrachia fitzroyana	A	COI	70	0
Phylum Nematoda		COI	113	40
Class Chromadorea				
Order Rhabditida				
Family Aphelenchoididae				
Bursaphelenchus cocophilus	C	COI	22	41
Eucephalobus sp.	C	ITS	0	184
Phylum Onychophora				
Class Udeonychophora				
Order Euonychophora				
Family Peripatopsidae				
Kumbadjena shannonensis	A	COI	0	22
Phylum Placozoa				
Polyplacotoma mediterranea		COI	83	242
Phylum Platyhelminthes				
Class Rhabditophora				
Order Tricladida				

Family Geoplanidae				
Caenoplana coerulea	HA, A	COI	41	15
Obama carinata	NT	COI	0	83
Tetracladium sp.	C	ITS	0	21
Phylum Porifera				
Class Demospongiae				
Order Bubarida				
Family Dictyonellidae				
Scopalina ruetzleri	HA, NT	COI	70	0
Order Tetractinellida				
Family Ancorinidae				
Stellettinopsis megastylifera	NT	COI	196	0
Class Homoscleromorpha				
Order Homosclerophorida				
Family Oscarellidae				
Pseudocorticium jarrei	FRA	COI	10	87
Class Eurotatoria				
Order Adinetida				
Family Adinetidae				
Bradyscela clauda	CHN	COI	0	288
Order Ploima				
Family Brachionidae				
Brachionus calyciflorus	C	COI	74	15
Family Synchaetidae				
Synchaeta stylata	HA, E, NT	COI	0	59
Phylum Tardigrada				
Class Eutardigrada				
Order Parachela				
Family Hypsibiidae				

Acutuncus antarcticus	An, NT, E	COI	26455	0
Family Richtersiidae				
Richtersius coronifer	HA, E	COI	0	34855
Class Heterotardigrada				
Order Echiniscoidea				
Family Echiniscidae				
Testechiniscus spitsbergensis	HA, E	COI	229	0

4.3.4. Protista

Only 10 ASVs of Protista were found. These ASVs were allocated in 2 Phylum, 2 Classes, 6 Orders and 4 Families — 4 ASVs in Family *icertae sedis*. Amebozoa had 98.4% of reads (Fig. 24a) and 90% of ASVs (Fig. 24b). The richest Orders were Centramoebida and Himatismenida, with 3 species each. The most abundant was Himatismenida, with 71.4% of reads. All Protista ASVs were found with COX1 marker.

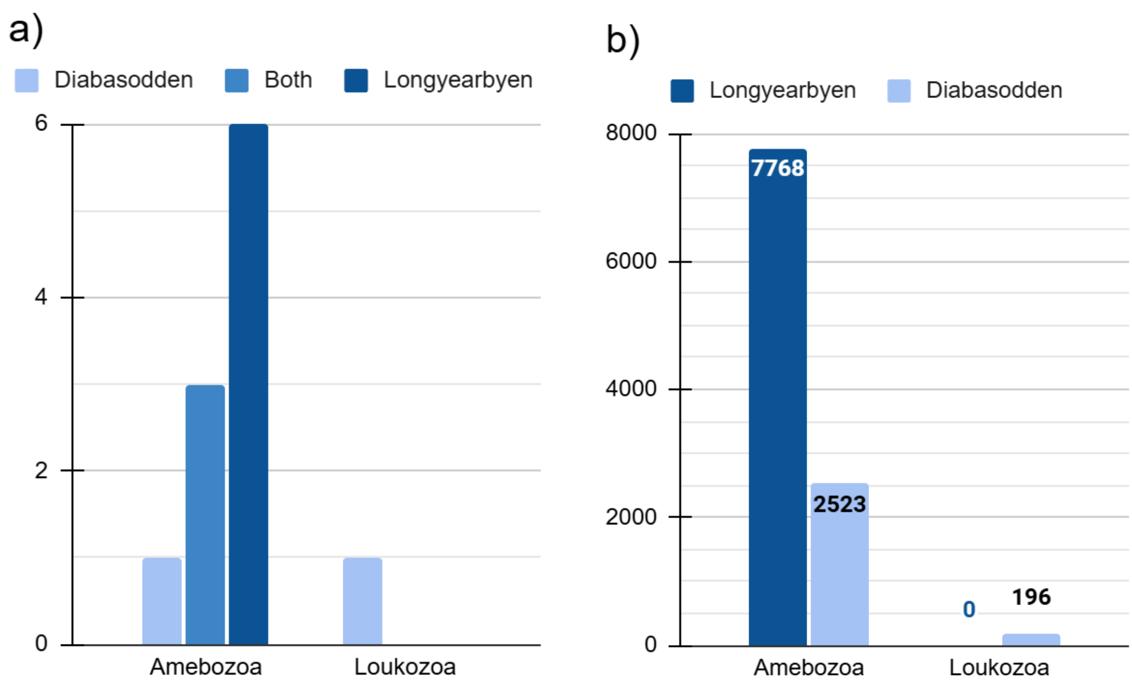


Figure 24. Number of ASVs of each Protista Phylum in each site (a) and number of reads (b).

Only one species was found exclusively in Diadasodden, *Reclinomonas americana*, Order Jakobida and Family Histionidae. There were 6 species found exclusively in Longyearbyen, all from the same Phylum and Class, and well distributed between 4 Orders. 3 species were shared between sites, belonging each one to a different Order (Fig. 25).

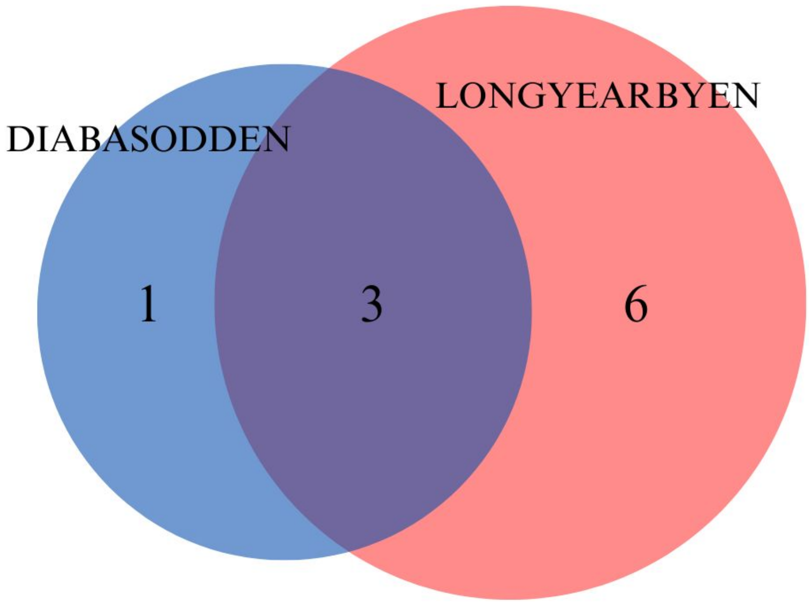


Figure 25. Venn diagram of Protista ASVs in both sites.

Only one species of Protista was not from the region, *Acanthamoeba tubiashi*, which occurs in the Australian and Oriental regions.

Table 5. Name of each Protista taxa found, indicating which maker used (ITS2 or COX1), which site it was found (Diabasodden, Longyearbyen or both), and the distribution of each taxa.
C = Cosmopolitan, HA = Holarctic, PA = Palearctic, NA = Nearctic, NT = Neotropical, E = Ethiopian, O = Oriental, A = Australian, An = Antarctic; RUS = Russia; TWN = Taiwan.

TAXA	Hab/ Distr.	Marker	D.	L.
KINGDOM PROTISTA				
Phylum Amoebozoa				
Class Discosea				
Order Dactylopodida				
Family Paramoebidae				
Paramoeba branchiphila	C	COI	0	7
Dactylopodida incertae sedis				
Squamamoeba japonica	PA	COI	1106	0
Family Vannellidae				
Clydonella sawyeri	TWN, RUS	COI	0	76

Order Himatismenida				
Family Cochliopodiidae				
Cochliopodium marrii	HA	COI	0	664
Cochliopodium pentatrifurcatum		COI	0	118
Family Parvamoeba				
Parvamoeba rugata	C	COI	1124	5562
Order Longamoebia				
Family Acanthamoebidae				
Acanthamoeba byersi	C	COI	0	72
Acanthamoeba comandoni	HA, A	COI	0	1229
Acanthamoeba tubiashi	O, A	COI	293	12
Phylum Loukozoa				
Class Jakobea				
Order Jakobida				
Family Histionidae				
Reclinomonas americana		COI	169	0

4.3.5. Viridiplantae

A total of 73 ASVs were found, belonging to 3 Phylum, 13 Classes, 29 Orders and 37 Families. There were 4.1% — 3 ASVs — that could only be identified at Kingdom level. From the 73 ASVs, 54 were found with ITS2 and 19 with COX1 marker. All Rhodophyta ASVs were found with COX1 marker.

From the found ASVs, 13.7% could only be identified in higher taxonomic levels. 26.1% of the found ASVs could only be identified at Genus level. Bryophyta was the most abundant Phylum, with 90.2% of reads, followed by Chlorophyta (Fig. 27), while the richest was Chlorophyta, with 37 ASVs (Fig. 26). The richest Orders were Trebouxiales and Chlamydomonadales, with 10 and 6 species, respectively. The most abundant was Hypnales, with 87.6% of reads. When it comes to Family, the most abundant was Amblystegiaceae, with 69.1% of reads, while the richest was Trebouxiaceae, with 10 species.

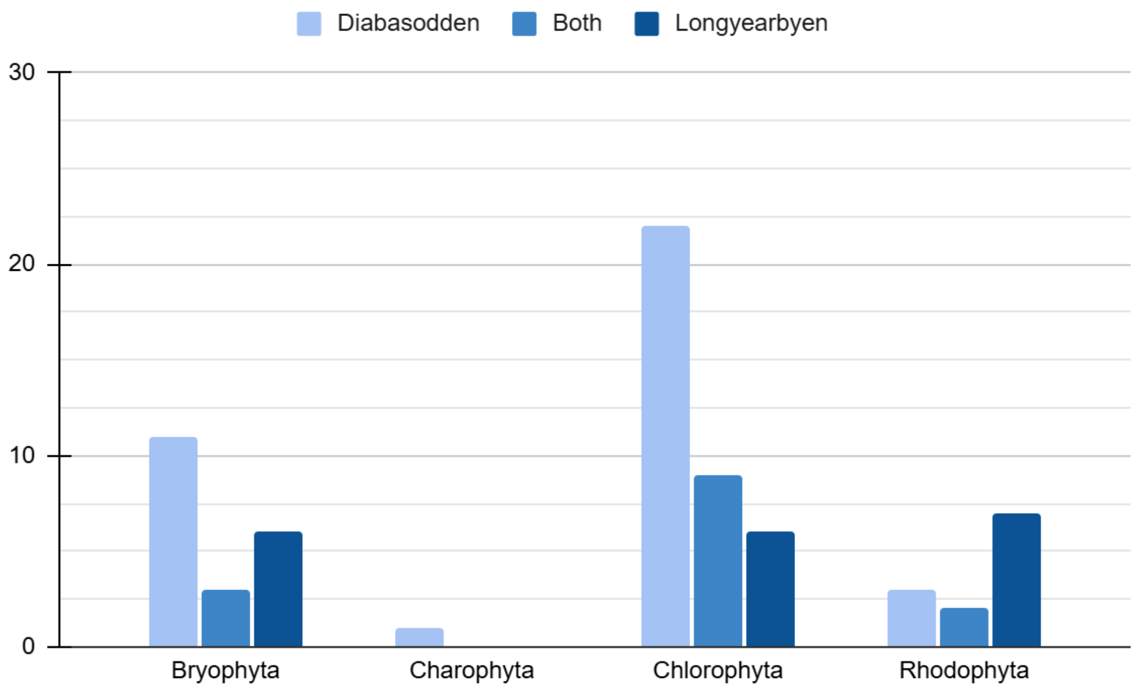


Figure 26. Number os ASVs of each Viridiplantae Phylum in each site.

There were 37 ASVs found in Diabasodden. They were allocated in 3 Phylum, 8 Classes, 18 Orders and 22 Families. There were two ASVs that only could be identified in higher taxonomic levels. The richest Phylum was Chlorophyta, while the most abundant was Bryophyta (Fig. 26 and 27). The ASVs were well distributed between Orders and Chlamydomonadales, Trebouxiales, Hypnales and Pottiales had four species each. The most abundant was Hypnales, with 88.1% of reads. The most abundant Family was Pylaisiaceae, with 53.4%, and the richest were Pottiaceae and Trebouxiaceae, with four species each.

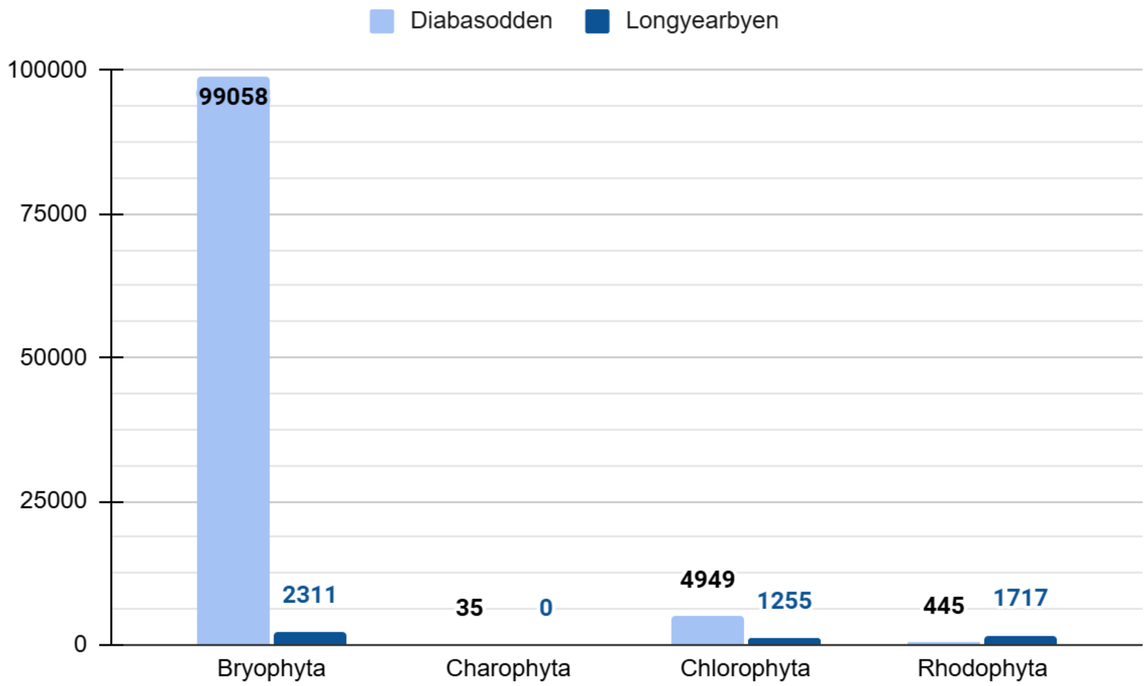


Figure 27. Number of reads of each Viridiplantae Phylum in each site. As *Sanionia uncinata* was the collected subtract, its reads were disconsidered for this figure.

In Longyearbyen, there were 21 ASVs. It belongs to 3 Phylum, 8 Classes, 13 Orders and 13 Families. There were 1 ASVs that could only be identified at Kingdom level and 1 that was identified at Order level. The most abundant and richest Phylum was Chlorophyta (Fig. 26 and 27). All were well distributed between Orders and Families. The most abundant Order was Rhizogoniales, with 39.8% of reads, and the most abundant Family was Aulacomniaceae, with 39.8%.

There were 15 ASVs shared between both sites (Fig. 28). 9 could be identified at species level and 2 in genus level. There are 2 ASVs that could only be identified at Kingdom level. The richest Order and Family were Trebouxiales and Trebouxiaceae, with 5 species. The most abundant Order and Family were Hypnales and Amblystegiaceae, with 96.9% of reads.

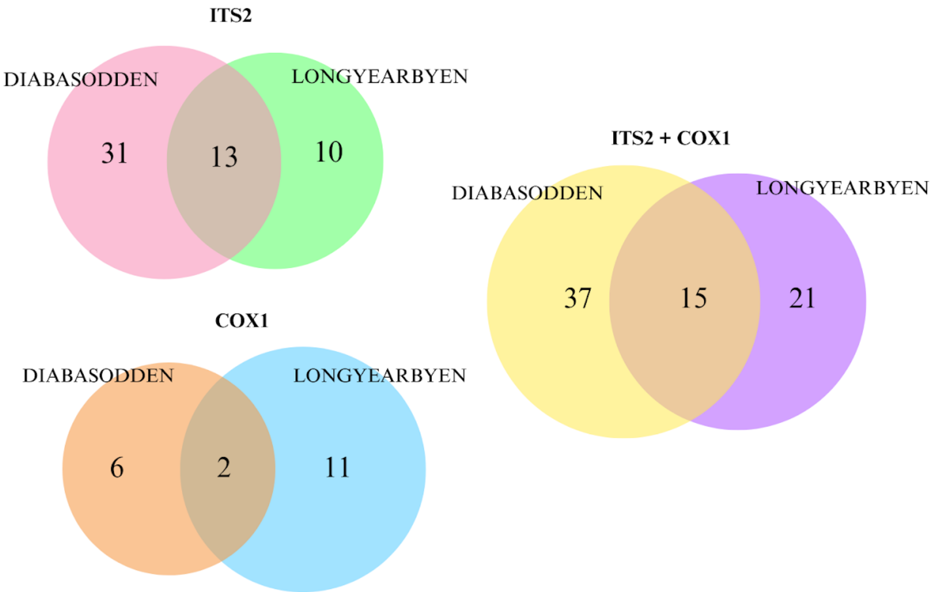


Figure 28. Venn diagram of Viridiplantae ASVs in both sites.

In Diabasodden, there were 3 non-native species. Two species, *Trebouxia brindabellae* and *Tayloria longiseta*, are from the Australian area and one species, *Tupiella akineta*, are from Oriental region. In Longyearbyen, there were 4 non-native species. One of them is a endemic Japanese species, *Gloiopeltis compressa*, one is endemic do Madagascar, *Ptilophora malagasya*, one came from Neotropical region, *Steereobryon subulirostrum*, and one is present in Ethiopian and Oriental regions, *Hydropuntia rangiferina*. Only one new species was found in both sites, *Cephalocystis furcellata*, from the Australian region (Fig. 29).

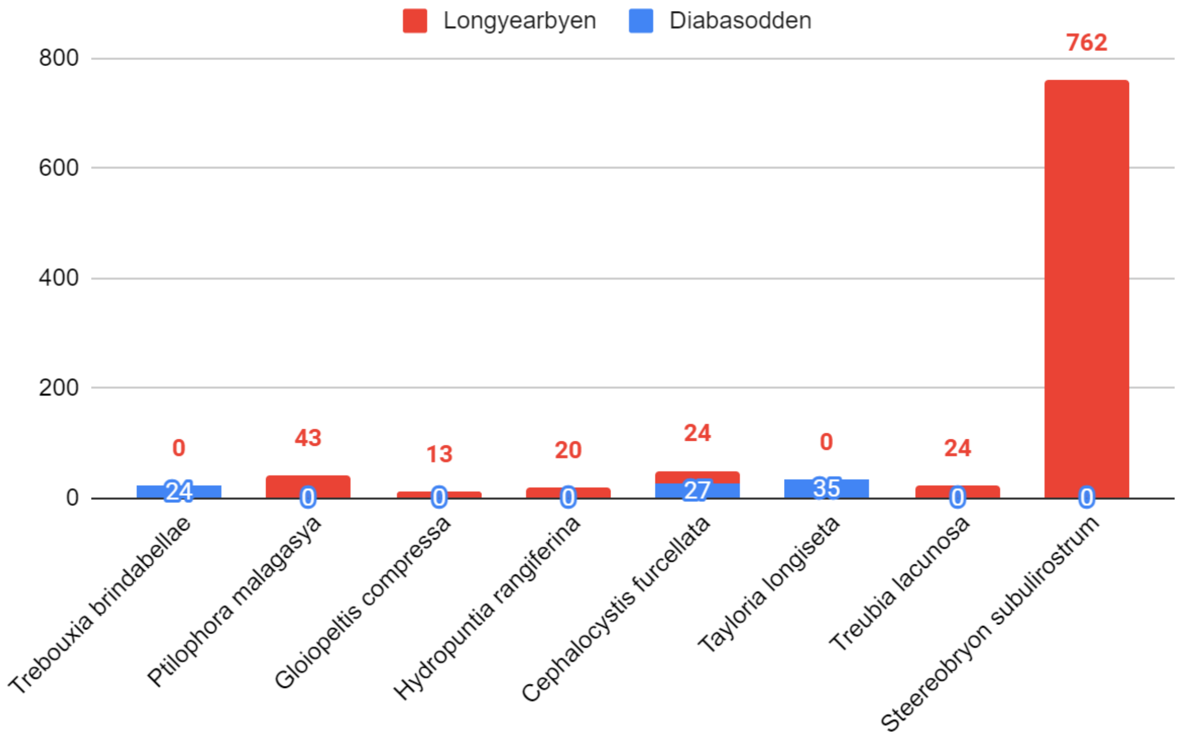


Figure 29. Number of reads and location of each non-native Viridiplantae ASV.

Table 6. Name of each Viridiplantae taxa found, indicating which maker used (ITS2 or COX1), which site it was found (Diabasodden, Longyearbyen or both), and the distribution of each taxa.
C = Cosmopolitan, HA = Holarctic, PA = Palearctic, NA = Nearctic, NT = Neotropical, E = Ethiopian, O = Oriental, A = Australian, An = Antarctic; JPN = Japan; MDG = Madagascar.

TAXA	Hab/ Distr.	Marker	D.	L.
KINGDOM VIRIDIPLANTAE				
Phylum Charophyta				
Class Coleochaetophyceae				
Order Chaetosphaeridiales				
Family Chaetosphaeridiaceae				
Chaetosphaeridium globosum	C	COI	35	0
Phylum Chlorophyta	C	ITS	260	385
Class Chlorophyceae	C	ITS	37	0
Order Chlamydomonadales				
Family Chlorococcaceae				

Chlorococcum microstigmatum	HA, An	ITS	1155	0
Chlorococcum rugosum		ITS	21	0
Family Chlamydomonadaceae				
Chloromonas arctica	Svalbard	ITS	13	0
Ostravamonas meslinii	PA, A	ITS	55	0
Chloromonas sp.	C	ITS	0	12
Chlamydomonas sp.	C	ITS	47	0
Order Chaetophorales				
Family Characiaceae				
Diplosphaera sp.	C	ITS	836	0
Order Sphaeropleales				
Family Chlamydomonadaceae				
Schroederia sp.	C	ITS	12	0
Class Mamiellophyceae				
Order Mamiellales				
Family Mamiellaceae				
Mantoniella squamata	C	COI	0	122
Class Pleurastrophyceae				
Order Pleurastrales				
Family Pleurastraceae				
Pleurastrum sp.	C	ITS	37	0
Class Trebouxiophyceae				
Order Chlorellales				
Family Chlorellaceae				
Coenochloris signiensis	PA, An	ITS	161	39
Coccomyxa sp.	C	ITS	38	153
Family Oocystaceae				
Pseudococcomyxa sp.	C	ITS	0	10
Order Prasiolales				

Family Koliellaceae				
Koliella longiseta	HA, NT, An	ITS	489	73
Pseudochlorella signiensis	PA, An	ITS	0	12
Pseudochlorella sp.	C	ITS	11	0
Family Stichococcaceae				
Desmococcus olivaceus	C, An	ITS	628	0
Stichococcus bacillaris	C, An	ITS	312	24
Stichococcus mirabilis	HA, NT, An	ITS	36	0
Stichococcus sp.1	C	ITS	124	0
Stichococcus sp.2	C	ITS	42	0
Incertae sedis				
Elliptochloris subsphaerica	HA, O, A	COI	0	35
Order Trebouxiales				
Family Trebouxiaceae				
Asterochloris echinata	PA	ITS	24	0
Lobosphaera incisa	HA, An	ITS	35	61
Myrmecia bisecta	HA, A, An	ITS	16	47
Myrmecia pyriformis	HA	ITS	53	136
Symbiochloris pauciautosporica	PA	ITS	52	0
Trebouxia asymmetrica	PA	ITS	217	11
Trebouxia brindabellae	A	ITS	0	8
Trebouxia flava	PA, An	ITS	263	15
Trebouxia sp.	C	ITS	89	0
Parietochloris sp.	C	ITS	29	0
Incertae sedis				
Family Coccomyxaceae				
Coccomyxa subellipsoidea	PA, An	COI	0	124
Order Watanabeales				
Family Watanabeaceae				

Chloroidium engadinensis	PA, An	ITS	15	0
Class Nephroselmidophyceae				
Order Nephroselmidales				
Family Nephroselmidaceae				
Nephroselmis olivacea	HA, NT	COI	46	0
Class Pleurastrophyceae				
Order Pleurastrales				
Family Pleurastraceae				
Pleurastrum sp.	C	ITS	37	0
Class Ulvophyceae				
Order Ulotrichales				
Family Ulotrichaceae				
Tupiella akineta	O	COI	77	0
Hazenia sp.	PA, O, NT	ITS	14	0
Planophila sp.	C	ITS	704	0
Planophila sp.		ITS	100	0
Phylum Rhodophyta				
Class Bangiophyceae				
Order Cyanidiales				
Family Bangiaceae				
Neoporphyra haitanensis	HA	COI	0	969
Family Cyanidiaceae				
Cyanidioschyzon merolae	PA	COI	93	0
Class Florideophyceae				
Order Bonnemaisoniales				
Family Bonnemaisoniaceae				
Asparagopsis taxiformis	C	COI	122	0
Order Ceramiales				
Family Rhodomelaceae				

Eutrichosiphonia paniculata	HA, NT	COI	0	332
Pterosiphonia spinifera	PA, NT, E	COI	0	49
Family Spyridiaceae				
Spyridia filamentosa	C	COI	12	0
Order Gigartinales				
Family Endocladiaceae				
Gloiopeltis compressa	JPN	COI	0	13
Family Gracilariaceae				
Agarophyton vermiculophyllum	HA	COI	191	215
Hydropuntia rangiferina	O, E	COI	0	20
Order Gelidales				
Family Gelidiaceae				
Gelidium elegans	PA	COI	0	52
Ptilophora malagasya	MDG	COI	0	43
Order Rhodymeniales				
Family Rhodymeniaceae				
Cephalocystis furcellata	A	COI	27	24
Phylum Bryophyta				
Class Bryopsida				
Order Bryales				
Family Bryaceae	C	ITS	2980	0
Ptychostomum sp.	C	ITS	0	20
Order Dicranales				
Family Dicranaceae	C	ITS	3378	46
Dicranum sp.	C	ITS	0	7
Order Hypnales				
Family Amblystegiaceae				
Campylium bambergeri	HA	ITS	26848	0
Drepanocladus sp.	C	ITS	5845	0

Sanionia sp.	C	ITS	55711	13849 2
Family Plagiotheciaceae				
Myurella sp.	C	ITS	4397	0
Family Pylaisiaceae				
Roaldia revoluta	C	ITS	52539	0
Order Pottiales				
Family Pottiaceae		ITS	0	200
Didymodon icmadophilus	C	ITS	2015	0
Didymodon johansenii	HA, O	ITS	349	0
Syntrichia sp.	C	ITS	54	0
Trichostomum sp.	C	ITS	120	0
Order Pseudoditrichales				
Family Ditrichaceae				
Distichium sp.	C	ITS	514	0
Order Rhizogoniales				
Family Aulacomniaceae				
Aulacomnium sp.	C	ITS	0	1237
Order Splachnales				
Family Splachnaceae				
Tayloria longiseta	A	ITS	19	0
Class Polytrichopsida				
Order Polytrichales				
Family Polytrichaceae				
Steereobryon subulirostrum	NT	ITS	0	762
Phylum Marchantiophyta				
Class Haplomitriopsida				
Order Treubiales				
Family Treubiaceae				

Treubia lacunosa	A	COI	0	24
Class Jungermanniopsida				
Order Jungermanniales				
Family Scapaniaceae				
Scapania mucronata	HA	ITS	0	15

5. DISCUSSION

Longyearbyen is the capital city of Svalbard,with 2617 habitants but it is estimated that "40,000 tourists arrived in Svalbard by plane in 2008 and around 30000 came by sea" (Statistics Norway, 2009). This high human flux may have a great impact in the moss community. Diabasodden, however, is more isolated and less accessible; resulting in a more pristine environment, with less human impact, hence a more preserved local biodiversity.

Our data suggested a high difference in the community living in the bryosphere in each site. Even though the number of taxa is similar, there is just a small number, with only 148 out of 1023 ASVs, that occurs in both sites (Fig. 10). This could be explained by the greater impact of human activity in Longyearbyen (CONVEY et al., 2012).

In all five indices, Diabasodden had greater numbers than Longyearbyen, indicating a more diverse and even distribution of the community (THUKRAL, 2017). The H' (Shannon index) values usually range between 1.5 and 3.5 for a diverse community, Diabasodden overcoming this range, having a higher level of diversity (LILLIE et al., 2003). The value of Simpson's D ranges from 0 to 1, with 0 representing infinite diversity and 1 representing no diversity. For this essay we used its complement (1-D), and both communities had a high level of diversity (KIERNAN. 2014). Pielou's evenness also ranges from 0 to 1. A value 0 indicates low diversity and low evenness and 1 represents high diversity and high evenness (MAGURRAN, 2003). Diabasodden had a greater level than Longyearbyen, but both sites had numbers close to 0.5, indicating a non-even distribution among the species.

There is little previous knowledge about the bryosphere community in Svalbard, especially with the use of DNA, therefore it is hard to know what its structure was before human interference. However, our results showed a similar fauna and microbiota found in other Arctic sites in continental Europe, both with molecular and morphological approaches (GERSON, 1982; CUTLER et al., 2017; HAMARD et al., 2021; JASSEY et al., 2022; ERLUDÓTTIR, 2023).

In Longyearbyen, we found DNA of 67 species non-native from holarctic regions, most of them belonging to the Kingdom Metazoa. In Diabasodden, even with less flux of people, we found DNA of 39 non-native species. The presence of the DNA, however, does not necessarily confirm the presence of the living organism in the moss, but it could also indicate that somehow fragments or dead organisms, single cells, pollen, spores or cists may be finding its way into the ecosystem. Nevertheless, it reinforces the alarming vulnerability of the native community and the need for a more restricted biosecurity regime to avoid introduction of new species (HALL et al., 2014).

The most abundant Kingdom was Viridiplantae, which is a reflection of the subtract collected for molecular analysis that consisted in *Sanionia uncinata* moss carpets. The richest Kingdom was Fungi. There was a high number of ASVs that could not be identified at species level, some of them only identified at Kingdom level. The situation was especially alarming in the Kingdom Fungi, in which 29% of the ASVs could only be identified at Kingdon level. These data may reflect two scenarios: the databases are incomplete, hence the need to constantly update it with new information, or there are in fact new undescribed species in the sites. Further studies are necessary in exploring these species (CAMÂRA et al., 2021).

In general, the least rich and abundant Kingdom was Protista. It may have happened because of the use of an unfitting marker or methodology, meaning that our data may be underestimating the actual diversity. Jassey et al. (2022) used high-throughput sequencing with the marker 18S to identify protists in boreal peatlands and found a high number of ASVs.

There were also 384 ASVs that could not be identified at Kingdom level, to which further investigation is needed to learn if it was a methodological issue — incomplete database, low quality of the DNA or a pipeline error — or new unknown organisms. From these Unknown ASVs, 15 were found in the samples of both sites, the number of reads ranging from 30 to up 8000 reads, with the most abundant ASV having 8892 reads. In total, there were 13 Unknown ASVs with >1000 reads.

If those reads are from new, unidentified organisms, it is of vital importance to invest in new studies to better understand if it represents any danger to humans or other organisms. With climate change, there were already records of organisms that were suspended in the recently melted permafrost causing disease in the Arctic (GASSIY, 2018). Since the nature of these potential new organisms is unknown, all efforts are necessary to uncover these data, in order to avoid possible future impact on human health and food security.

5.1. Chromista

We found no previous works on the Kingdom Chromista in Svalbard to compare data. However, we found a great number of ASVs of Chromista in both sites, including 23 identified only in genus level and 10 in higher taxonomic levels. The microscopic nature of these organisms may be a limiting factor in producing studies about it, but we showed that there is a vast field to be investigated in order to understand their community and role in the ecosystem.

Summing both sites, the Phylum Ciliophora was the second most abundant and had 29 ASVs, although none of it could be identified at species level. It could be a taxonomic issue, with unknown species, or solely a database issue. Taxonomic information about this Phylum is usually blurry. However, Cavalier-Smith (2018) emphasizing periplastid protein targeting reallocated the Phylum from the Protista to the Chromista Kingdom. However, further investigation in Ciliophora is necessary to enrich the database in order to understand the Svalbard bryosphere community.

There was a large amount of Chromista species that had a cosmopolitan distribution. If those species are native, it is highly possible that those species evolved unique characteristics to survive the harsh environment. McGraw (1995) discussed the importance of genetic diversity within the same species, which enables it to habit a wider range of habitats. The presence of this cryptic biodiversity is also highlighted by Bálint et al. (2011), who states that the climate change driven biodiversity loss could be far greater than expected. Since Chromista have species of interest both as highly virulent pathogens and to the biotechnological industry, investigating its diversity in a extreme environment may be a great opportunity to have new insights in the species potential and function (FELDMAN et al., 1999; AKROFI, 2015; SEO et al., 2018; KIM et al., 2022; BREGANT et al., 2023).

There were 11 species of Chromista found distributed in both sites that are non-native to Svalbard. From them, 6 occurred in both sites, 1 only in Diabasodden and 4 only in Longyearbyen. The species were from two Phylums: Oomycetes and Ochrophyta. The Ochrophyta, the richest and most abundant Phylum, are composed of photosynthetic stramenopiles. They are closely related to Oomycetes, but work as primary producers in the ecosystem (BROWN and SORHANNUS, 2010). Oomycetes, second richest and third in abundance, are fungi-like organisms that are usually known as plant pathogens, representing a great threat to the agriculture industry (HARDHAM, 2007).

5.1.1. Most abundant taxa

There were three dominant species from the Phylum Ochrophyta: *Leathesia difformis* L., with 17.4% of the Ochrophyta reads, *Colpomenia sinuosa* (Mert. ex Roth) Derbès & Solier, with 18.6%, and *Mischococcus sphaerocephalus* Vischer, with 42.5%. From the least to the most abundant, *L. difformis* is a cosmopolitan species and had 12401 reads. It is widely studied by its bioactive properties, which turns it into a species of interest to the pharmaceutical and cosmetic industry (FELDMAN et al., 1999; SEO et al., 2018; KIM et al., 2022). For instance, the species was already investigated, all with positive results, as a potential antiviral, its inhibitory effect on melanin biosynthesis (possible to use in cosmetic and treatment of skin-pigmentation related diseases) and UV-protective agent (FELDMAN et al., 1999; SEO et al., 2018; KIM et al., 2022).

The second most abundant species was *C. sinuosa,* with 13328 reads. The species is cosmopolitan and was also extensively studied for industry purposes. Kiran and Murugesan (2014) investigated the synthesis of silver nanoparticles with *C. sinuosa*, and had a positive result, which indicates that it may be a viable solution to produce antidiabetics. It is also research in antitumor properties of the species, with positive outcomes in the laboratory tests, thus potential in cancer treatment (MONLA et al., 2020; MONLA et al., 2022).

The most abundant species was *Mischococcus sphaerocephalus,* with 23008 reads, being also the most abundant in the Chromista Kingdom, with 19% of the reads. There is not much information in the literature about the species, the first register being the only register of it in nature, in the algaebase website (https://www.algaebase.org/). The *Mischococcus* genus has a palearctic distribution and belongs to the class Xanthophyceae, which often produce carotenoid pigments (WHITTLE and CASELTON, 1995).

There were two other dominant species within the Kingdom Chromista, *Aurantiochytrium acetophilum* E.Ganuza & R.A.Andersen, from the Phylum Bigyra, with 5377 reads and *Pythium oopapillum* Bala & Lévesque, from the Phylum Oomycota and had 3953 reads. Location of each species in Figure 32.

The oomycete *Pythium oopapillum* have registers in the holarctic and neotropical regions and is known to cause plant root rot disease. There are reports of it infecting foxglove in Italy, common weeds living alongside cultures in Brazil and soybeans in the USA (BARBOSA et al., 2022; GARIBALDI et al., 2022; VARGAS et al., 2022).

The Bigyra *Aurantiochytrium acetophilum* have no natural distribution records, but is used in the agriculture industry (YANG et al., 2022; RODJAROEN et al., 2024). *Aurantiochytrium* sp. are used as alimentary supplement, improving growth in African sharptooth catfish, Zebrafish and broiler chickens (YANG et al., 2022; KALIA et al., 2023; RODJAROEN et al., 2024). The species *A. acetophilum* are used to improve growth in salmon culture, being an important species to maintain high production in the industry (RUIZ et al., 2022; RUIZ et al., 2024).

5.1.2. Non-native species

There were 11 non-native Chromista species in our samples, some with less than 100 reads, which means that the chance of false positives is high. Consequently, the chances of the organism's presence increases as the number of reads increase, even though it is not possible to know in fact if there are living organisms in the samples.

There were 3 species belonging to genus *Phytophthora.* This genus is known to cause a large number of plant diseases that cause extreme damage or even death of the plant (BRASIER et al., 1995; AKROFI, 2015; TRZEWIK et al., 2015; BREGANT et al., 2023). Another important characteristic of the genus is its capacity for hybridization, which allows them to adapt to new environments and conditions when in contact with local species (AKROFI, 2015).

The first species, *Phytophthora amnicola* T.I.Burgess & T.Jung (51 reads), has registers of occurrence in the GBIF database only in the Australian region, mostly in New Zealand. However, there are registers of *P. amnicola* hybrids occurring in South Africa and Europe (NAGEL et al., 2013; AGHIGHI et al., 2016; BREGANT et al., 2023). In Europe, the species is causing great loss of *Alnus glutinosa* (L.) Gaertn., the symptoms involving canopy dieback, stem bleeding cankers, necrotic bark lesions at the collar and root rot (BREGANT et al., 2023). Some hybrids also seem to affect blackberries (*Rubus anglocandicans* A. Newton), serving as a potential control to this invasive species in Australia (AGHIGHI et al., 2016).

The species *Phytophthora insolita Ann* & W.H.Ko (543 reads) is natural to the oriental region. There are no records of *P. insolita* natural hosts, but its pathogenicity was tested several times. The results shows that it can not affect unwounded fruits, but causes watersoaked dark brown lesion when inoculated in wounded fruits, having this effect on apple, avocado, cucumber, eggplant, mango, green pepper and tomato (HO et al., 2002; DAS et al., 2012; BAWAGE et al., 2013)

The second species was *Phytophthora megakarya* Brasier & Griffin (14 reads), common in the African continent. This species is highly pathogenic, and one of the biggest threats to the cocoa (*Theobroma cacao* L.) culture in the continent (AKROFI, 2015). The symptoms of the *P. megakarya* infection includes root rot, pod rot and canker. Even though the plant may be affected by other *Phytophthora* species, *P. megakarya* is the most virulent, and causes great impact on the economy of West and Central Africa (AKROFI, 2015).

There were also 2 species of the genus *Pythium, Pythium biforme* Uzuhashi & G. Okada (40 reads) and *Pythium brachiatum* Uzuhashi & G. Okada (4285 reads), both of them found in the Nagano Lake, Japan (UZUHASHI et al., 2015). The genus species are usually pathogenic, causing diseases in algae species (HERRERO et al., 2020). However, there is still no register of the found species causing diseases, and it is possible that they have saprophytic habits (UZUHASHI et al., 2015). Nevertheless, *P. brachiatum* was the most abundant species in the Phylum Oomycota, counting with 31% of the reads.

We found three other Oomycete species, from three different genus. The first species is *Aphanomyces invadans* David & Kirk (151 reads). *A. invadans* is known to cause Epizootic Ulcerative Syndrome (EUS) in fishes, infecting damaged skin tissue, resulting in inflammation, ulceration, tissue necrosis and death (IBERAHIM et al., 2018). The species is common in the oriental and ethiopian region, but there are also records of EUS outbreaks in Canada, USA and Australia (OIDTMANN, 2011). So far, there are records of *A. invadans* affecting 87 different fish genera, representing a great threat to the aquaculture industry (OIDTMANN, 2011; IBERAHIM et al., 2018).

The second species was *Globisporangium spinosum* (Sawada) Uzuhashi (612 reads), Tojo & Kakish, with reports from Australian and oriental regions. According to Huo et al. (2023), *G. spinosum* causes diseases in a lot of plants around the world, leading to great loss in cultivated species. Root rot is a consistent symptom, and the period that takes until the symptoms can be observed varies according to the infected species (HUO et al., 2023; FENG et al., 2024). In the last reports, *G. spinosum* was causing root rot disease in kiwifruit (*Actinidia chinensis* Planch.) and ginseng (*Panax ginseng* C.A.Mey.) cultures in China, resulting in 30-70% of product loss (HUO et al., 2023; FENG et al., 2024).

Lastly, *Phytopythium litorale* (Nechw.) Abad, de Cock, Bala, Robideau, A.M.Lodhi & Lévesque (21 reads), is natural to warmer climates, with occurrences in australian, ethiopian and oriantal regions. It also causes plant diseases, causing a variety of symptoms, even ultimately leading to the death of the plant (DERVIŞ et al., 2020). There are recent reports of *P. litorale* affecting kiwifruit, *Rhododendron pulchellum* Salisb., *Cornus kousa* Bürger and *Platanus orientalis* L. (DERVIŞ et al., 2020; LI et al., 2021; POLAT et al., 2023; OKSEL et al., 2024).

There were three non-native Ochrophyta species found in our samples. The first was *Haramonas dimorpha* Horiguchi (1845 reads), a marine raphidophyte, from Australia (HORIGUCHI, 1996). Second was *Lessonia flavicans* Bory (299 reads), a macroalgae natural from sub-Antarctic region, and lastly *Schizocladia ischiensis* Henry, Okuda et Kawai (1090 reads), a filamentous algae from Greece (KAWAI et al., 2003; MANSILLA et al., 2013).

5.2. Fungi

Our results in the Fungi Kingdom were consistent with previous studies both in Svalbard and other Arctic site bryosphere. Both Cutler et al. (2020) and Dziurzynski et al. (2022) found a high number of Fungi ASVs with both markers, with Ascomycota being the most prominent Phylum, followed by Basidiomycota. However, Dziurzynski et al. (2022) found a high number of Mortierellomycota species, which had a small number of species that were not abundant in our study.

Between all Kingdoms investigated in this essay, Fungi was the richest, with 313 ASVs, 284 with ITS2 marker and 29 with COX1 marker. There were 145 ASVs in Longyearbyen, 117 in Diabasodden and 51 shared between sites. The similar number of species in each site may reflect a substitution in the community, with native species being replaced by more aggressive species in Longyearbyen (CALLAGHAN et al., 2004). Most of the species in both sites are cosmopolitan, making it hard to infer which species were naturally there and which may have been brought afterwards. Also, in both sites Fungi is the richest Kingdom, with over 50% of ASVs, implicating a high importance of these organisms in the bryosphere.

From found Fungi ASVs, 29% could only be identified at Kingdom level, all found with ITS2 marker. Dziurzynski et al. (2022) found that only a small part of the Fungi ASVs could be identified using culture approach. Therefore, it is possible that the Unknown Fungi ASVs are new species, which we could not investigate before with traditional methods. However, since there are also limitations in the molecular approach, it is possible that this high number only reflects the need to update in the existing databases. Nevertheless, further investigation is needed to better understand if this new data shows a hidden biodiversity or a methodological issue.

5.2.1. Most abundant taxa

The most abundant Ascomycete species was *Tetracladium apiense* R.C.Sinclair & Eicker, with 16.2% of Ascomycota reads in Diabasodden (Fig. 34), found with both IT2 and COX1 markers. It was first described in South Africa, being now considered as a cosmopolitan species (SINCLAIR and EICKER, 1981; GBIF, 2023). It is considered an aquatic hyphomycete, participating in leaf decomposition in streams (AL-RIYAMI et al., 2009; LETOURNEAU et al., 2009).

Two other Ascomycota species were dominant: *Cladophialophora minutissima* M.L.Davey & Currah and *Chalara pseudoaffinis* Koukol. Both species are cosmopolitan and they contributed with, respectively, 13.4% and 12.1% of the Ascomycota reads in the site. *Cladophialophora minutissima*, found in Longyearbyen (Fig. 34) were also found in Antarctic, both in soil samples and associated with moss, suggesting a bipolar distribution (GESHEVA and NEGOITA, 2012; HIROSE et al., 2016. Both were found only with ITS2 marker.

The second species, *Chalara pseudoaffinis,* was found in both sites. It was also found in the Antarctic continent, in infected moss samples that presented fairy rings (ROSA et al., 2019). However, according to the authors, it is not a common species in the region. The genus *Chalara* also has a role as a saprophyte in coniferous forests and is usually found in coniferous needles and litter (KOUKOL, 2010).

The dominant Basidiomycota species in Diabasodden was *Glaciozyma antarctica* (Fell, Statzell, I.L.Hunter & Phaff) M.Groenew. & Q.M.Wang, with 78.2% of the Basidiomycota reads, with ITS2 marker. It is usually found in the Antarctic and the holarctic region, indicating a bipolar distribution (GBIF, 2023). The species is often investigated by its antifreezing proteins, which are substances of interest for the food industry and other biotechnological applications (RAMLI et al., 2011; HASHIM et al., 2014).

In Longyearbyen there were three dominant taxa: *Serendipita* sp. (9.5% of the reads), *Tomentella muricata* (5.1% of the reads) and *Arrhenia* sp. (4.3% of the reads), all with ITS2 marker. The genus *Serendipita* is known as an endophytic fungi, forming a symbiotic relation with the host plant (RIESS et al., 2014; TYAGI et al., 2022). Previous studies observed *Serendipita* forming association with liverworths in Svalbard and the Sub-Antarctic region, although there was no identification at species level (NEWSHAM and COPSTAKE, 2021; NEWSHAM et al., 2023).

The species *Tomentella muricata* (Ellis & Everh.) Wakef. is natural to the holarctic region. We did not found much information in the literature about the species, but the genus *Tomentella* is known to form ectomycorrhizas, with the hosts being usually gymnosperms (JAKUCS and HONTI, 2008; ZHANG et al., 2024).

Although we could not identify the *Arrhenia* species, there are registers in Svalbard of *Arrhenia auriscalpium* (Fr.) Fr., a species common to the Arctic region (HØILAND, 1996; CRIPPS and HORAK, 2006). The genus live in different forms, usually found in lichenicolous form and sometimes having a mutualistic relationship with bryophytes, also having saprophyte habits (BARRASA and RICO, 2003; RICO, 2010; WANG et al., 2023).

5.2.2. Non-native species

There were 5 non-native Fungi species distributed among sites, but all had low numbers of reads, which could mean a false positive. The number of reads of each non-native Fungi ASV is listed in figure 35. Four of these species (*Arthrocladium fulminans*, *Leohumicola levissima*, *Ophiostoma himal-ulmi* and *Russula griseocarnosa*) were found with COX1 marker, while the other one (*Coniozyma leucospermi*) was found with ITS2.

The first was *Arthrocladium fulminans* M.M.F.Nascim., Vicente & de Hoog, an Ascomycete from the Trichomeriaceae family, native from Mayotte. Although this family is usually non-parasitic, *A. fulminans* seems to work as a human opportunist (NASCIMENTO et al., 2015). Therefore, for a human infection to happen, the patient is usually in an immunosuppressed condition. Although infection cases are rare, there are reports of *A. fulminans* causing osteoarticular chromoblastomycosis and disseminated mycosis (DIALLO et al., 2017; EGENLAUF et al., 2018). In both cases the infection was aggressive, ultimately resulting in the patient's death.

There is little information in the literature about *Coniozyma leucospermi* (Crous & Denman) Crous. It belongs to the Ascomycota Phylum and there are records of its occurrence in warmer climates, specifically in Iran, south-Russia and Mexico (OLIVARES et al., 2013; MIRZAEI et al., 2015; CLEARY et al., 2016).

Leohumicola levissima H.D.T.Nguyen & Seifert is an Ascomycete, found in South Africa, Colombia and the USA (NGUYEN and SEIFERT, 2008; NICOLA et al., 2021). This species seems to function as root endophytic, as a mycorrhizal fungi (ADEOYO et al., 2021). The authors also highlighted the need for further studies investigating possible antibacterial properties of *Leohumicola* species. However, there were only 2 reads for this species, making it highly possible to be a false positive.

The genus *Ophiostoma* is known to cause Dutch elm disease (DED) across Europe and North America (BRASIER et al., 1995; PIPE et al., 1997; BRASIER, 2000; MATISONE et al., 2019; KATANIĆ et al., 2020). The species *Ophiostoma himal-ulmi* Brasier & M.D.Mehrotra, natural to the Himalaya, is an Ascomycete and follows the same thread and is suspect of being the origin of more virulent species, such as *O. ulmi* and *O. novo-ulmi* (BRASIER et al., 1995; PIPE et al., 1997; BRASIER, 2000). The DED disease affects trees from the *Ulmus* genus, and cause yellowing and wilting of leaves on individual branches, spreading rapidly among the canopy, the fall of branches and leaves and finally leads the trees to death (D’ARCY, 2000).

Lastly there was *Russula griseocarnosa* X.H.Wang, Zhu L.Yang & Knudsen, a saprophyte native from the oriental region of China (LIU et al., 2024). It is consumed as a medicinal mushroom and is a source of many important minerals, such as potassium, magnesium, calcium, iron and zinc, also presenting phenols, flavonoids, ergosterols, carotenoids and antioxidant properties (CHEN et al., 2010; YUAN et al., 2017). Laboratory essay also showed that *R. griseocarnosa* extract presents anti-tumor properties and its investigation could be of interest for the pharmaceutical industry (LIU et al., 2017; YUAN et al., 2017).

5.3. Metazoa

In Metazoa, our study found a lot of species of the main groups described in the region, however the number of species we found represent only 6.5% of the Svalbard invertebrate fauna, with 71 ASVs (COULSON et al., 2024). Another 57 ASVs of non-native species were found, although only 22 had more than 100 reads, thus indicating a possible false-positive. The Kingdom also stood out in Longyearbyen, being the most abundant with 37.9% of the reads in the site, with 70.5% belonging to one mite species, *Eupodes voxencollinus* (Thor, 1934) and 17.6% to *Richtersius coronifer* (Richters, 1903). All Metazoa ASVs were found with COX1 marker.

From the 135 Metazoa ASVs, 128 could be identified at species level, indicating a high knowledge and very complete database in this Kingdom. There were only 4 Unknown Metazoa ASVs, all of them with less than 50 reads, which could indicate a methodological issue leading to a false positive. However, further research is necessary to confirm this hypothesis.

Arthropoda was both the richest and the most abundant Phylum, which is consistent with the long list of these organisms in the Gerson (1982) work describing the moss fauna and Callaghan et al. (2004) estimation of a high number of Arthropoda species. The second most abundant was the Phylum Tardigrada, which is also consistent with the extensive work of Schuster and Greven (2007), studying Tardigrada communities living in the moss. The second richest Phylum was Mollusca, although only 8 from the 29 species had more than 100 reads, which again could indicate a false positive or, since a lot of these species are consumed as human food, particles of these organisms may have ended up in the moss and caught in the sample.

The community structure was highly different between sites, with only 20 ASVs happening in both sites, out of 134 ASVs. However, there are a great number of non-native species in both sites, which could mean that both communities are compromised. Also only 47 Metazoa ASVs had more than 100 reads, and further investigation is needed in order to understand if the species with <100 reads are actually living in the sites.

Metazoa was, by far, the Kingdom with the great number of non-native species, with a total of 59 ASVs native to other regions. From these, there were 24 ASVs with more than 100 reads and, though is not possible to affirm the presence of the living organism in the sites, the high number of reads raises this possibility, which could endangered the natural fauna (GILG et al., 2012; CONVEY et al., 2012). In Diabasodden, there were three unregistered species that are native to the Antarctic and Sub-Antarctic region, which could indicate a bipolar distribution. Lastly, there were 10 species that are common in Japan and nearby regions, mostly of them in Longyearbyen, and the reason for it remains unknown.

There were 8 species that were dominant in our data, 3 in Diabasodden, 6 in Longyearbyen and 1 in both sites. In Diabasodden they were *Dodona eugenes* (Bates, 1867), *Acutuncus antarcticus* (Richters, 1904) and *Asteromyia modesta* (Felt, 1907), which summ 88.9% of the Diabasodden reads. In Longyearbyen, there were *E. voxencollinus, R. coronifer, A. modesta, Saccostrea mordax* (Gould, 1850), *Octopus variabilis* (Sasaki, 1929) and *Hydaticus vittatus* (Fabricius, 1775), summing 95.7% of the site site reads.

5.3.1. Most abundant taxa

The most abundant species was *Eupodes voxencollinus* with 139356 reads and it happened only in Longyearbyen. It has a palearctic distribution, though there are records of its occurrence in Egypt and Iran (ABOU-AWAD, 1984; KALMOSH and YASIN, 2018; LANIECKI et al., 2021, BARON, 2022). It inhabits diverse subtracts, but is usually found in mosses and lichens (LASK et al., 2022). The high number of reads suggests the actual presence of the organism in the Longyearbyen moss. Since there were no previous records of this species in Svalbard, altogether with the presence of the mite only in Longyearbyen, it is possible that it came from tourists. Another possibility is that it is a pipeline error or absence of other species in the database, since there are other species of the same genus (*Eupodes variegatus* C.L.Koch, 1838) already recorded in Svalbard.

The second most abundant species was *Dodona eugenes* with 18928 reads, occurring only in Diabasodden. It is a butterfly, common in the Oriental region, with records in

Vietnam, Nepal and India but no records northwards (CALLAGHAN, 2009; MIYA, 2012; LIMBU and ACHINT, 2024). There are, however, records of *D. eugenes* living in the Indian Himalayan Region, which have more similar environmental conditions with Svalbard (ARYA et al., 2020; PARVEEN et al., 2023). Therefore, it is possible that the species already had adaptations to deal with harsh environmental conditions, which could smooth its way in Svalbard as an invasive species.

The species *Asteromyia modesta* had a high number of reads both in sites (2678 in Diabasodden and 2945 in Longyearbyen). It has a nearctic distribution and is known by causing galls (BORKENT and BISSETT, 1985; HEATH et al., 2013). It has a close host-plant relation with plants of the family Asteraceae, which lead to *Asteromyia* also being a megadiverse group (STIREMAN III et al., 2009). According to the Svalbard Flora website (svalbardflora.no), the archipelago has 10 Asteraceae species in 5 different genus, which could give this species an advantage in colonizing the new environment. There are no previous records of any *Asteromyia* species in Svalbard, and further investigation is necessary to know if they were brought to the Island from the mainland or it is a native variation of the species.

Lastly was *Prodaticus vittatus*, with 2305 reads in Longyearbyen. It is an aquatic beetle, native from the Australian and oriental region (CROSSLAND, 1998; DASH and ROY, 2017; GHOSH and GRUPTA, 2024). There are no previous records in Svalbard for this or any species from this genus.

The most abundant Mollusca species was *Octopus variabilis,* with 8423 reads and occurring only in Longyearbyen, and it is a small octopus natural to tropical region in the Pacific Ocean, specially in China coast (YULAN et al., 2016). It feeds on gastropods, bivalve mollusks, and crustaceans, also being prey of sharks, eels and other carnivorous fishes (YING et al., 2013; YULAN et al., 2016). It is consumed by humans, being a common food in Japan, Korea and China (YANLI et al., 2017). *Octopus variabilis* microbiota often presents bacteria that are potential pathogens, such as *Vibrio vulnificus,* a causative agent of foodborne diseases (LEE et al., 1997; LEE et al., 2017). Since they are natural to sea water, it is unlikely that the living organism is present in the moss, rather possible that the DNA is present by human food districts or the DNA came from the sea, since the site is near the coast.

The second species was *Saccostrea mordax,* with 1490 reads, natural from the Japan and Taiwan coasts and also occurred only in Longyaerbyen. These rock oysters were used as pollutants bioindicators in Japan (MUKAI et al., 2020). The genus inhabits rocky shores in tropical regions, needing saline water to survive (CUI et al., 2021). Therefore, it is also unlikely the presence of the living organism in the Svalbard moss. However, *S. mordax* are not used as humansfood to or any terrestrial organisms, neither a species with industry interest, thus leaving no explanation to the presence of its DNA in our samples.

The Phylum Tardigrada had only three species, from which two were highly abundant, both from the Order Parachela. First there was *Richtersius coronifer*, with 34855 reads, present only in Longyearbyen. The species was previously recorded in Svalbard, and is already long adapted to survive the harsh environmental conditions (PEDERSEN et al., 2021).

The second species, *Acutuncus antarcticus*, had no previous records in any Arctic or Northern territory, being natural to the Antarctic and Sub-Antarctic region (CESARI et al., 2016). It had 26455 reads, and occurred only in Diabasodden. The species presents phenotypic plasticity, and studies indicate that it will be able to deal with new climate change related pressures, at least in the small term, while in the long term the increase of UV radiation may represent a risk to its survival (ALTIERO et al., 2015; GIOVANNINI et al., 2018).

Acutuncus antarcticus is already adapted to the dry and cold polar environment and could thrive in the Arctic as an invasive species. However, there is also the possibility that this species, as many others, have a bipolar distribution, therefore needing forward investigation to verify its condition. Finally it is possible that it is a methodological error, and the DNA may belong to other species of the same genus, *Acutuncus mariae* Zawierucha, 2020, recorded in Svalbard.

5.3.2. Non-native species

All the next species had <1000 number of reads, thus less trustful diagnostic. There were 26 Arthropoda, 4 Chordata, 2 Cnidaria, 16 Mollusca, and each of Onychophora, Platyhelminthes, Porifera, Rotifera and Tardigrada had 1 ASV. The Classes Insecta and Gastropoda had the highest number of ASVs, with 18 and 13, respectively. Because of the high number of species, this section will be written in sections, divided by Phylum.

5.3.2.1. Phylum Arthropoda

The species *Leptonetela hangzhouensis* (Chen, Shen & Gao, 1984) is a small spider from the Leptonetidae Family, native from the Zhejiang province, in China (WANG et al., 2011). There is little information about this species in the literature, all found studies focusing on taxonomic description.

Syedra oii (Saito, 1983) is a spider from the Family Linyphiidae, native from South Korea and nearby nations. Is described as a common species in the region, and there is little information about it other than distribution and taxonomic traits (KWON et al., 2023).

The last spider was *Phintella arenicolor* (Grube, 1861), from the Family Salticidae. It has records from Korea, China, Japan, Russia (JUNG et al., 2007). No further information besides taxonomic description was found.

The last species from the Arachnida Class was *Tropilaelaps mercedesae* (D.L.Anderson & M.J.Morgan, 2007), a mite from the Oriental region. It is a vector of the honeybee (*Apis mellifera* L.) virus DWV (DAINAT et al., 2009). When infected, the honeybees exhibit symptoms of crippled wings, which could lead to a colony collapse, being a major threat to *A. mellifera*, thus honey production (FORSGREN et al., 2008). None of the four genus had no records of other species in Svalbard.

There was only one Collembola species, *Gomphiocephalus hodgsoni* (Carpenter, 1908). The springtail is considered endemic to the Antarctic region, therefore having a high tolerance to cold and dry conditions, similar to the Arctic (SINCLAIR and SJURSEN, 2001; STEVENS and HOGG, 2003). There are no previous records of the genus *Gomphiocephalus* in Svalbard, and further investigation is necessary to identify a possible bipolar distribution.

The Class Diplopoda had also only one species, *Trachyjulus phylloides* (Golovatch, Geoffroy, Mauriès & VandenSpiegel, 2012). The millipede was only found in Thailand, living specifically in karst caves (LIKHITRAKARN et al., 2020). There are no previous records of the genus *Trachyjulus* in Svalbard.

The beetle *Phlogistomorpha croesus* (Blackburn) is a Cleridae native from the Australian region. No further information was found besides the anatomical description of the species (OPITZ, 2003).

The second beetle species was *Pteroptyx tener* (E.Olivier, 1907), a Lampyridae native from the oriental region. The firefly depends on its natural environment to survive, having a straight relation with the tropical plants of their habitat (KIRTON et al., 2006; KHOO et al., 2012; CHENG et al., 2017).

The last beetle was *Gonocephalum pubens* (Marseul), a Tenebrionidae with records in China, South Korea, Japan, and Taiwan. It was one of the most abundant species in a previous study in the coastal sand dunes in South Korea, and is often found in warmer tropical regions (MIN et al., 2018; HEDGE, 2019).

We only found one mosquito in our study, *Culex sitiens* (Wiedemann) from the family Culicidae. It has reports from Oriental, Australian and Ethiopian regions and feeds mostly of birds and pigs blood (VYTHILINGAM et al., 1994). However, it can also feed of human blood, and the great concern about this species is that it is a potential vector of the Japanese encephalitis (JE) virus, a flavivirus related to dengue, yellow fever and West Nile viruses (VYTHILINGAM et al., 2003; World Health Organization, who.int). The disease is usually asymptomatic, but can cause permanent neurologic, cognitive and behavioural sequelae and, in some cases, death (World Health Organization, who.int).

The Hemiptera *Gaeana maculata* (Drury, 1773) is a cicada with Oriental distribution. Most of the studies found in the literature discurred about its ultrastructure, cytochemistry and morphology (CHEUNG and MARSHALL, 1973; RUSCHEL et al., 2019). Also from the Order Hemiptera, *Deraeocoris claspericapilatus* (Kulik, 1965) is a plant-bug from Japan and Korea area, that belongs to the Miridae Family (JUNG and LEE, 2011; KIM et al., 2023).

There were 3 Hymenoptera species, the first being *Wiebesia pumilae* (Hill, 1967) is a wasp, with records from China, Japan and Taiwan. It is known by its obligate mutualism with *Ficus pumila* L., working as their pollinators, showing a classic co-evolutionary system (CHEN et al., 2015; LO et al., 2021).

The second Hymenoptera species was *Euglossa rufipes* (Rasmussen & Skov, 2006), native from the Neotropical region. The genus *Euglossa* is known for its orchid bees (FERRARi et al., 2017; KHADEMI, 2017). Being natural to tropical climate, there are no orchids in Svalbard (Svalbard Flora, svalbardflora.no).

The last Hymenoptera species was *Smithagathis davidsmithi* (Sharkey). It is a parasitic wasp, endemic to the Colombian Andes, and we found no further information in the literature about the species (SHARKEY and CHAPMAN, 2017; SHARKEY, 2024).

There were 6 Lepidoptera species. The first was *Cyana pratti* (Elwes, 1890), a moth from the Oriental region. It is usually found in China, although there was a record in Indonesia (VOLYNKIN et al., 2023; DAAWIA et al., 2024). Also from the Oriental region, there was *Mycalesis visala* (Moore, 1857), a butterfly usually found in India (DEEPIKA et al., 2014; PONMANICKAM et al., 2022).

The third Lepidoptera species from the same region was *Archaeoattacus edwardsii* (White, 1859), a silk moth usually found in India. Since it inhabits mountainous environments, such as Khasi Hills and Arunachal Pradesh, with colder winters, it is likely that this species already have adaptations that could help them survive in Svalbard (NARANG and GRUPTA, 1982; GOGOI et al., 2014).

Still in Lepidoptera Order, there was *Larentioides cacothemon* (Prout, 1917), another moth, from Ethiopian region (SIVOHNEN et al., 2017). It is a cycad-moth, that are one of the main pollinator of the cycad plants, mostly because of its ability to resist to the plants toxins (SCHNEIDER et al., 2002; SIVOHNEN et al., 2017).

Moschoneura pinthous (L.) is a species of butterfly, natural to South America. *Moschoneura* is a small genus, all species having Neotropical distribution (LAMAS, 2004; NEILD et al., 2021). The last Lepidoptera was *Manduca blackburni* (Butler, 1880), an endemic moth from Hawaii islands. Before human contact, the moth is thought to depend on endemic solanaceous trees, nowadays feeding on tobacco weed (RUBINOFF and JOSE, 2010).

Gryposmylus pennyi (Winterton & Y.-j.Wang, 2016) is a Neuroptera, distributed in northern Vietnam and adjoining southern China. There is not much information in the literature about the species other than its description and distribution (WINTERTON and WANG, 2016).

The dragonfly *Epiophlebia superstes* (Selys, 1889) is endemic to Japan. It is part of the clade Anisozygoptera and have similar features to the other suborders Zygoptera and the Anisoptera (RÜPPEL and HILFERT, 1993; APPEL and GORB, 2011; WANG et al., 2013).

The last member of the Class Insecta was *Dolichopoda kiriakii* (Rampini & Di Russo, 2008), is a cricket endemic to Greece. The genus *Dolichopoda* is composed by cave crickets, and *D. kiriakii* was only found in the Kiriaki Cave, in the district of Preveza (RAMPINI et al., 2008; ALLEGRUCCI et al., 2009) None of the Insecta species had any species of the same genus recorded in Svalbard.

There were only two species in the Class Malacostraca: *Scopelocheirus schellenbergi* (Birstein & Vinogradov, 1958) and *Caridina typus* (H.Milne Edwards, 1837). The first species, *S. schellenbergi* is an amphipod, usually found in deep sea, with two records in the Kermadec Trenches and one register in the New Hebrides Trench, being most abundant at ~6500–7500m (BLANKENSHIP et al., 2006; LACEY et al., 2017).

The second species, *C. typus* is a freshwater shrimp, common in the tropical regions. Although it is widely spread, present in Neotropical, Oriental and Ethiopian regions, it has no records Northward, indicating that it needs warmer climates (SOOMRO et al., 2011; BERNARDES et al., 2017). Again, no species of similar genus have records in Svalbard in the Class Malacostraca.

There were four species from the Phylum Chordata, all with neotropical distribution. The first was *Apogon binotatus* (Poey, 1867), a fish from Central America. It lives associated with coastal reefs, hiding in cracks and crevices during the day (Fishbase, fishbase.se). The genus is cosmopolitan, although there is no register up the Arctic Circle parallel.

The second species was *Pithys albifrons* (L.), a bird from the South America rainforest getting close to the Andes. Common in the amazon forest, they feed mostly on ants, therefore landing close to the ground (eBird.org). Its genus is also endemic to South America, with no species in any other region.

The third species was *Oxyrhopus guibei* (Hoge & Romano, 1977), also known as false coral snake, native to South America, most common in southeastern Brazil. It is a non-venomous snake, having a constrictor behavior and feeding mostly on rodents (ANDRADE and SILVANO, 1996). The false coral is known to present mimicry as a defensive mechanism, having similar color pattern as the coral snakes, highly venomous snakes of the genera *Leptomicrurus, Micrurus* and *Micruroides* (BUCARETCHI et al., 2016). All species of the genus *Oxyrhopus* have neotropical distribution.

The last Chordata species was *Sapajus nigritus* (Goldfuss, 1809), a monkey species endemic to the Atlantic Forest in Brazil and the Argentinean provinces of Iguazú and Missiones (MARTINS et al., 2019). It is insecto-frugivorous, and usually live in groups of about 20 animals (MARTINS et al., 2019).

Since all Chordata species are from South America, mostly Brazil, it is possible a contamination of the collected samples. However, if it is not the case, it is important to investigate if the DNA of these animals came to Svalbard via tourism or other means.

The Phylum Cnidaria had two species, *Acropora palmata* (Lamarck, 1816) and *Cyanea nozakii* (Kishinouye, 1891). The first species, *A. palmata*, is a coral that was usually found in the Caribbean, Bahamas and Florida. Its population, however, is facing a massive decline due to climate and environmental changes (WILLIANS et al., 2007). The genus *Acropora* had no species in Svalbard, most of them distributed among warmer regions.

The second species was *C. nozakii*, also known as ghost jellyfish, with oriental distribution. Its blossoms caused a serious impact in the Chinese marine ecosystem and in the fishery economy (DONG et al., 2008). It prefers warmer waters, with a high salinity level (DONG et al., 2008). *C. nozakii* is also venomous, presenting cytotoxicity and hemolytic activity, although is yet to understand the full mechanism of its venom (YANG et al., 2020). The genus *Cyanea* had one species with records in Svalbard, *Cyanea capillata* (Linnaeus, 1758), and one species with holarctic distribution, *Cyanea lamarckii* (Péron & Lesueur, 1810), therefore it is possible a misidentification.

5.3.2.2. Phylum Mollusca

There were three species from the Class Bivalvia, *Leukoma jedoensis* (Lischke, 1874), *Periglypta puerpera* (L). and *Saxidomus purpurata* (G.B.Sowerby II, 1852). None of the three genus have any other species recorded in Svalbard.

The first Bivalvia, *L. jedoensis,* is common in Japan and Korea sea coasts. It is produced for human consumption, being an important source of dietary-essential amino acids, lipids, vitamins, minerals and other bioactive nutrients (RISTIVOJEVIĆ et al., 2019; XIN et al., 2024). Its shell is also used in traditional Chinese medicine to treat ecthyma (AHMAD et al., 2017). The culture of these important Bivalves is threatened by various factors, including pollution driven saturation states in sediments, hydrodynamics, temperature and parasitic copepods (GREEN et al., 2007; XIN et al, 2024).

The second species, *P. puerpera* has a Oriental distribution. No understandable information other than taxonomic description was found about this species. Lastly, *S. purpurata* is also common in the Japanese, Korean and Chinese coasts. It inhabits shallow sea waters and is also a common fishery for those nations' diets (SELIN, 2014). It has i-type lysozymes of high bacteriolytic activities, that might be interesting for future studies, while still a rich nutrient source (MIYAUCHI et al., 2006; LIU et al., 2017).

There were 13 species of Gastropoda in our samples. Only the genus *Turris* was already recorded in Svalbard, although there is no identification at species level.

The first species was *Salinator rosaceous* (Golding, Ponder & Byrne, 2007) is an amphiboloidean, only recorded in Australia. It was found on mud between mangroves (GOLDING et al., 2007). No further information was found in the literature. The *Lamellomphalus manusensis* (S.-Q.Zhang & S.-P.Zhang, 2017) is a neomphalid also from the Australian region. It is an extremophile, found in deep sea-waters, with records of it at 1,603m deep (ZHONG et al., 2022).

Then there was *Nerita japonica* (Dunker, 1860), with records from Japan and Korea. The genus is known to have internal fertilization and producing egg capsules (PARUNTU and TOKESHI, 2003). It lives by the sea, usually found on boulder shores (TAKADA, 2008). Also from Japan, there was *Nipponacmea nigrans* (Kira, 1961), a limpet found in Japanese seashores and an important part of the community on rocks and boulders in Japan coast (TAKADA, 1997).

Iberozospeum biscaiense (Gómez & Prieto, 1983) is a snail endemic to Spain. All species of its genus are from Spain or nearby nations, mostly found in caves (KNEUBÜHLER et al., 2022).

There were two species of the *Conus* genus: *Conus laterculatus* (G.B.Sowerby II, 1870), *Conus miles* (L.). The genus is widely studied by its venom, the conotoxins, that have potential for the production of new drugs, promising therapeutic resources for the treatment of chronic pain, among other diseases (HEMANI et al., 2018; JERGOVA et al., 2021; MARGIOTTA et al., 2022). An already known medication produced with its venom is Ziconotide (Prialt®), a non-opioid used to treat severe chronic pain. The species used to produce the drug (*Conus geographus* (L.) and *Conus magus* (L.)) were not found in our work.

The cone snail *C. miles* is widely spread among Indian and Pacific Oceans, with 1,330 georeferenced records in the GBIF database. The second species, *C. laterculatus,* from australian and oriental regions, also produces insulin as a part of its venom, having a high potential to improve diabetes treatment in humans (XIONG et al., 2022).

In the genus *Turris* we found the species *Turris babylonia* (L.), a shallow-water marine cone snail natural to the Australian region (KILBURN et al., 2012). Its genus also produces venom and the toxins, although still not used, also have the potential to be used in new therapeutic medicine (HEMANI et al., 2018; HILARIO and HERALDE III, 2020).

The freshwater snail *Bulinus globosus* (Morelet, 1866) is natural from the Ethiopian region. It is host and vector of the platyhelminth *Schistosoma haematobium* ((Bilharz, 1852) Weinland, 1858), thus capable of transmitting the urogenital schistosomiasis disease (DAVIES et al., 1999; KALINDA et al., 2017; PENNANCE et al., 2022). According to the World Health Organization (https://www.who.int/) the symptoms include haematuria, kidney damage and fibrosis of the bladder and ureter, genital lesions, vaginal bleeding, pain during sexual intercourse, nodules in the vulva, pathology of the seminal vesicles, prostate and other organs, infertility and bladder cancer. There are records of the disease in the African continent, the Middle East, Corsica (France) (WHO, https://www.who.int/, 2024).

The species *Mesodontrachia fitzroyana* (Solem, 1985) is an Australian endemic land snail. It was found in the Victoria River District, belonging to a small genus, all species of the genus being endemic (CRISCIONE and KOHLER, 2013). *Mysticarion hyalinus* (L.Pfeiffer, 1855) is another land snail native from Australia. These species, as long as a lot of Australian terrestrial vertebrates, were deeply impacted by the 2019–2020 Bushfires on New South Wales, and are still recovering (HYMAN et al., 2020).

Lastly, the genus *Helminthoglypta* had two species: *Helminthoglypta hertleini* (G.D.Hanna & A.G.Smith, 1937) and *Helminthoglypta talmadgei* (B.Roth, 1988). Both species are native from the USA, endemic to the States of Washington, Oregon and California (ROTH and SADEGHIAN, 2006). Their common names are Oregon shoulderband and Trinity shoulderband and its natural environment are the coniferous forests (DUNK et al., 2002).

5.3.2.3. Phylum Onychophora

There was only one species of the Phylum Onychophora: *Kumbadjena shannonensis* (Reid, 2002), native from Australia. The species of this Phylum are commonly called velvet worms, they usually are restricted to temperate and tropical regions, due to its incapacity in retaining water, and are usually endemic (BLAXTER and SUNNUCKS, 2011). Currently, there are only 216 valid species described in this Phylum spread worlwide (OLIVEIRA, 2023).

5.3.2.4. Platyhelmintha

There was one species of Platyhelminthes: *Obama carinata* ((Riester, 1938) Carbayo et al., 2013). Its genus is composed by Neotropical land planarians, with the only records located in Brazilian rainforests (CSEH et al., 2015; ITURRALDI et al., 2021). They feed on a wide range of organisms, including snails, slugs, acari, insects, earthworms and others (CSEH et al., 2015).

5.3.2.5. Phylum Porifera

Stellettinopsis megastylifera (Wintermann-Kilian & Kilian, 1984) is a sea sponge, with neotropical distribution, being an important component of the region's coastal community (SANDES et al., 2019). No further information was found other than taxonomic description and phylogeny.

5.3.2.6. Rotifera

Bradyscela clauda (Bryce, 1893) is a bdelloid native from Korea and China. It is able to survive in environmental extremes, thus are widely spread (SONG and MIN, 2015).

5.4. Protozoa

As mentioned before, the Kingdom Protozoa had only 11 species, probably due the use of an unfitting marker. Previous works in Svalbard recorded 48 Protozoa species, so our data may be underrating the actual Protozoa community. Further studies in the area using a different marker are necessary to verify if our data is compatible with the community.

There were only 10460 Protozoa reads, 6686 belonging to one species, *Parvamoeba rugata* (Rogerson, 1993), a cosmopolitan species that occurred in both sites. It is a marine amoebozoa, with no previous records of it living in terrestrial environments (ROGERSON, 1993; TURON et al., 2022; ZENG et al., 2024).

Acanthamoeba tubiashi (Lewis & Sawyer, 1979) was the only non-native species in our samples. It is usually found in Oriental and Australian regions, living in freshwater environments (PUTAPORNTIP et al., 2021). The genus *Acanthamoeba* is known as an opportunistic pathogen, causing keratitis, sinusitis and encephalitis, although there is no records of *A. tubiashi* causing any disease (RAYAMAJHEE et al., 2022; HALIM et al., 2023).

5.5. Viridiplantae

In the Viridiplantae Kingdom we found 73 ASVs, 37 only from the Chlorophyta Phylum. We haven't found much information about Svalbard's algae. Kim et al. (2008) made a list with 29 taxa of cyanobacteria and algae in Svalbard. Our work exceeded this number, indicating a greater and little studied diversity. There were 3 ASVs that could only be identified at Kingdom level. However, all had a small number of reads (26, 15, 166), which could indicate a methodological issue. Most ASVs were found with ITS2 marker, although there were 23 ASVs found with COX1, most of them (15) belonging to the Phylum Rhodophyta.

The ASV with most reads was *Sanionia* sp., with 55711 in Diabasodden and 138492 in Longyearbyen. Since the substrate collected to our study were shoots from a moss carpet of the species *Sanionia uncinata*, this result was expected. The richest Phylum was Chlorophyta, with 37 ASVs. From these, there were 13 species that were recorded before in the Antarctic and/or Sub-Antarctic region, indicating a potential high hate of bipolar distribution within this Phylum. Further studies are necessary in order to better understand this bipolarity.

In general, the richest Orders were Trebouxiales and Chlamydomonadales, with 10 and 6 species, respectively. The Order Trebouxiales is composed mostly by unicellular species lichen symbionts, sometimes also found in freshwater, including Antarctic lakes (VANČUROVÁ et al., 2015; CÂMARA et al., 2021b). Chlamydomonadales is a more ubiquitous Order, with records on snow, freshwater and soil samples (TSEGMID, 2021; GAO et al., 2022; ÇIFTÇI et al., 2024).

Longyearbyen had 15 species less than Diabasodden, with an overlap of 15 species between sites. That difference of species in each site could indicate a substitution of the community, where Diabasodden, with less human interference had a greater number of species. There were 20 ASVs that could be identified only at genus level, 14 of them only in Diabasodden. It could mean the presence of new unidentified species, considering that Diabasodden are far from human activity, thus have less studies. But our data are not able to confirm this theory, and further investigation is necessary to confirm if they are in fact new undescribed species or incomplete databases.

5.5.1. Most abundant taxa

Though Chlorophyta was the richest Phylum, there was only one species with >1000 reads, using ITS2 marker. It was *Chlorococcum microstigmatum* (P.A.Archibald & Bold), with 1155 reads and occurring only in Diabasodden. It was previously recorded in Holarctic and Antarctic regions, usually in soil microbiota (MALTSEV and KONOVALENKO, 2017). In Antarctic it was found in Esmeralda, Copépodo and Pan Negro Lakes (FONSCECA et al., 2022). For a long time it was synonym to *Chlorococcum oleofaciens* (Trainor & H.C.Bold, 1954), and they are the closest one to each other, in terms of molecular phylogeny (MALTSEV and KONOVALENKO, 2017). *C. oleofaciens* is widely studied by its capacity to accumulate oil, having a great biotechnological potential (MALTSEV and KONOVALENKO, 2017).

Other than *Sanionia* sp. there were 6 Bryophyta ASVs with >1000 reads, 3 identified only at genus level, all found with ITS2 marker (Location of each species in Fig. 37). The first was *Roaldia revoluta* (Mitt.) P.E.A.S. Câmara & Carv.-Silva, with 52539 reads. It is a moss with a cosmopolitan distribution and already recorded as a bipolar species by Câmara et al (2023).

Second there was *Campylium bambergeri* (Schimp.) Hedenäs, Schlesak & D.Quandt, with 26848 reads. It is a common species, with Holarctic distribution, and was already recorded in Svalbard. The third species was *Didymodon icmadophilus* (Schimp. ex Müll.Hal.) K.Saito, with 2015 reads and a cosmopolitan distribution. It was not previously recorded in

Svalbard, but there was one species of the same genus: *Didymodon acutus* (Brid.) K.Saito. It is possible that the moss carpet had more than one species, though *Sanionia uncinata* was the predominant species.

Then, identified only at genus level, there were *Drepanocladus* sp. (5845 reads), *Myurella* sp. (4397 reads) and *Aulacomnium* sp. (1237 reads). All of them are mosses with cosmopolitan distribution.

In Svalbard, there are records of 9 *Drepanocladus* species: *D. aduncus* (Hedw.) Warnst., *D. angustifolius* (Hedenäs) Hedenäs & Rosborg, *D. arcticus* (R.S.Williams) Hedenäs, *D. brevifolius* (Lindb.) Warnst., *D. lycopodioides* (Brid.) Warnst., *D. polycarpos* (Blandow ex Voit) Warnst., *D. polygamus* (Schimp.) Hedenäs, *D. sendtneri* (Schimp. ex H.Müll.) Warnst., *D. trifarius* (F.Weber & D.Mohr) Broth. and *D. turgescens* (T.Jensen) Broth.

The *Myurella* genus have records of two species in Svalbard: *M. julacea* (Schwägr.) Schimp. and *M. tenerrima* (Brid.) Lindb.

Aulacomnium species were studied and it was shown that they have flavonoids, antioxidants, antimicrobial and cytotoxic molecule(s), which could be interesting for new biotechnological research (HANH et al., 1995; VATAN et al., 2017). There are records of two species of the genus *Aulacomnium* in Svalbard: *A. palustre* (Hedw.) Schwägr and *A. turgidum* (Wahlenb.) Schwägr.

5.5.2. Non-native species

There were 8 non-native Viridiplantae species in our samples. Only one had >100 reads, therefore this data being possibly a methodological issue. From these, 4 species belonged to the Phylum Rhodophyta, 3 from Bryophyta and 1 from Chlorophyta. The number of reads of all Viridiplantae non-native species is shown in figure 38.

The only non-native Chlorophyta found in our samples was *Trebouxia brindabellae* (A.Beck 2002), native from the Australian region. It is known to form association with Fungi, in a lichen symbiosis (SADOVSKA-DEŚ et al., 2013; SINGH et al., 2016).

There were 4 Rhodophyta non-native species, all found with COX1 marker, none of them with previous records of the genus in Svalbard. The first species was *Ptilophora malagasya* (G.H.Boo, L.Le Gall, I.K.Hwang, K.A.Miller & S.M.Boo 2018), a small agar-producing red algae, endemic to Madagascar (BOO et al., 2018). No further information besides taxonomic description was found. The second was *Gloiopeltis compressa* (Harvey) H.Kawai, K.Yamamura & T.Hanyuda 2019, a red algae from Japan and Korea region. It was previously called *Caulacanthus compressus*, the name changing after phylogenetic analysis (HANYUDA et al., 2019).

The third was *Hydropuntia rangiferina* (Kützing) Gurgel & Fredericq 2004, with records from Oriental, Ethiopian and Neotropical regions. It was first collected in Brazil and its taxonomy still not fully resolved (GURGEL et al., 2020). The last Rhodophyta was *Cephalocystis furcellata* (J.Agardh) A.J.K.Millar, G.W.Saunders, I.M.Strachan & Kraft 1996, native from Australian region. It usually grows in rocks either in deep intertidal pools or subtidally at 2-20 m depths (MILLAR et al., 1995).

From the Bryophyte Phylum there were 3 species, from which 2 (*Tayloria longiseta* and *Steereobryon subulirostrum*) were found with ITS2 marker and 1 (*Treubia lacunosa*) was found with COX1 marker. The first species was *Tayloria longiseta* E.B. Bartram, a moss endemic to Papua New Guinea and Indonesia. It seems to be a rare species, having only 14 occurrences in the Consortium of Bryophyte Herbaria website (https://bryophyteportal.org/) and 17 occurrences in the GBIF (https://www.gbif.org/) database. There are 3 species of the *Tayloria* genus recorded in Svalbard: *T. froelichiana* (Hedw.) Mitt. ex Broth., *T. lingulata* (Dicks.) Lindb. and *T. acuminata* Hornsch., which could mean a potential misidentification of the found species.

The second Bryophyte was *Treubia lacunosa* (Colenso) Prosk., a liverwort from the Australian region, as long as all species in the *Treubia* genus. Because its Class, Haplomitriopsida, is considered an ancient clade, it is studied to understand plant evolution (LIU et al., 2011). It also was investigated by forming mutualistic partnerships with Mucoromycotina fungi, opening space to research on evolution of land plant–fungus symbioses and their role in the colonization of terrestrial environments by plants (FIELD et al., 2015).

The last non-native species was *Steereobryon subulirostrum* (Schimp. ex Besch.) G.L.Sm., with Neotropical distribution, mostly in Central America, being the only species in this genus. It is from the Class Polytrichopsida, a separate lineage commonly called “hair-cap mosses”, with about 200 species, easily distinguished from the Bryopsida mosses by its relatively large size, the gametophyte able to reach 65cm high and usually have greatest degree gametophyte and sporophyte complexity (BELL et al., 2021, BIPPUS et al., 2017).

6. CONCLUSION

Arctic species are highly adapted to extreme abiotic conditions, creating a complex and fragile ecosystem in which organisms heavily rely on one another. These environmental characteristics make the Arctic ecosystem particularly vulnerable to new biotic stresses, such as competition. Due to the accelerated rate of climate change in the Arctic, the climatic barrier that once prevented the establishment of new species is receding. As a result, the native community is becoming increasingly vulnerable to the introduction of exotic species.

These new species may cause significant imbalances, potentially leading to the loss of native biodiversity and, in extreme cases, the collapse of the ecosystem. In this study, we aimed to explore the biodiversity of the bryosphere—a small and poorly studied ecosystem within the moss layer—at Arctic sites, while also comparing the communities of two locations with differing levels of anthropogenic influence: Longyearbyen, which experiences higher human disturbance, and Diabasodden, a more pristine environment.

Our results revealed a clear difference between the sites, with a higher number of non-native species in Longyearbyen. This finding suggests that the risk of exotic species introduction increases with greater human activity. However, even in the nearly undisturbed environment of Diabasodden, we also detected DNA from non-native species. How these organisms—or fragments of organisms—reach such remote locations remains unknown, underscoring the urgency for further studies on this topic and the need for more comprehensive environmental regulations in the Arctic.

Nevertheless, both sites exhibited a high and comparable number of ASVs, highlighting the bryosphere as a rich field for future research into its diverse and complex ecosystem. Given that Arctic organisms are known to possess unique features and adaptations that allow them to endure extreme abiotic conditions, the rich community identified in this study also indicates the bryosphere's considerable biotechnological potential, offering a wide range of opportunities for the discovery of new substances and applications.

Finally, we identified a significant number of ASVs that could not be classified at any taxonomic level. Although this result may be due to methodological limitations, the possibility that these DNA sequences belong to previously unknown organisms warrants further investigation—for both scientific and biosecurity purposes.

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APPENDICES

1. Manuscript

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2. Submission receipt

Your submission

Title

Sanionia uncinata bryosphere: DNA metabarcoding as a tool to investigate cryptic eukaryotic diversity in Svalbard moss carpets

Type

Research

Journal

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Submission ID

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