Cryptic eukaryotic diversity in Svalbard moss carpets (Sanionia uncinata (Hedw.) Loeske) under different anthropogenic pressures

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Research Article

Keywords: Bryosphere, Arctic, exotic species, climate change

Posted Date: June 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9802157/v1

Additional Declarations: No competing interests reported.

Abstract

The bryosphere is the microhabitat within the moss layer, a community that is still poorly understood and is of great importance in polar ecosystems. As for other native communities, the climate is a key factor allowing it to thrive and limiting the survival of foreign species in the Arctic, thus the diminishing of this barrier may be a threat to native biodiversity, which is not adapted to biotic pressures such as competition. The rapid climate changes, alongside the expanding human flux in Svalbard, may present dire repercussions for the moss layer ecosystems. Since the lack of knowledge of the bryosphere is a limiting factor in understanding its community, which is fundamental for efficient preservation efforts, in this work we aimed to investigate it with the DNA metabarcoding approach. Samples were collected in two sites with different rates of human interference in the Svalbard archipelago. Four kingdoms were detected: Chromista, Metazoa, Protista and Viridiplantae, with two primers (ITS2 and Cox1). A similar number of ASVs — amplicon sequencing variants — were detected in each site, approximately 400 ASVs. However, only 97ASVs occurred in both Longyearbyen and Diabasodden. There were also a high number of exotic species ASVs in both sites, with 56 detected in Longyearbyen and 37 in Diabasodden. This data highlights the need for further documentation of the native community and for the investigation of the actual presence of living exotic organisms, alongside the implementation of an effective biosecurity regimen in the Svalbard's region.

INTRODUCTION

Climate change is a primary contemporary challenge for Arctic ecosystems, encompassing warming, changes in timing form and quantity of precipitation, and 'polar amplification' of global climate change means that the region is warming considerably more rapidly (up to four times; Rantanen et al. 2022) than the global average (Pedersen et al. 2022; Grigorieva 2024). The impact of these changes are already and will continue to cause changes in the weather, precipitation and water availability in Arctic ecosystems, which is a matter of serious concern (Chapin et al. 2001; Walther et al. 2002; Callaghan et al. 2004; Convey et al. 2012). Native biota adapted to typical Arctic conditions may not have the resilience to cope with these ongoing changes (Chapin et al. 2001; Callaghan and Jonasson 1995; Callaghan et al. 2004), leading to unforeseen consequences for local biota.

Another stressing factor acting in tandem with climate change in Arctic ecosystems is the anthropogenically-assisted introduction of non-native species. Rapidly increasing anthropogenic activity in the region, with increasing use of trade northern shipping routes, exploitation of living and geological natural resources and rapidly increasing tourism, is likely to be the main driver for the future introduction of non-native species (Hall et al. 2014; Convey et al. 2012; Kaltenborn et al. 2020; Coulson et al. 2024). The combined influence of these factors lowers the barriers preventing non-native species arriving in the Arctic from establishing, as well as opening the door for a wider range of species to survive and spread (Gilg et al. 2012; Coulson et al. 2015; Bartlett et al. 2021). Due to the generally stress tolerant adaptations and low competitive abilities that characterise life history strategies of many polar terrestrial organisms (Convey 1997), native species are likely to be at a competitive disadvantage with these new

invaders (Callaghan et al. 2004; Convey et al. 2012). In this context, the conservation of native and endemic Arctic biodiversity is a high priority. The loss of species may lead to cascade effects, ultimately resulting in ecosystem collapse (Convey et al. 2012).

The Arctic environment plays a key-role in regulating the global climate, storing 32% of the earth's carbon in its soils and permafrost and uptaking 20% of the produced atmospheric C, thereby functioning as an important global carbon sink (Alvarenga and Rousk 2022). The Arctic is also responsible for about 35% of global net primary production (NPP), as well as its ecosystems playing important roles in the nitrogen and phosphorous cycles and methane oxidation (Turetsky et al. 2011; Alvarenga and Rousk 2022). Processes occurring within the Arctic tundra, and particularly its moss carpet communities, a complex community that is still poorly understood, make important contributions to these global processes (Lindo and Gonzales 2010).

The Arctic flora is composed of an estimated 6000 species (Meltofte 2013). Though there is no conclusive data of the number of species of non-vascular plants in Arctic, it is estimated 900 species of bryophytes, representing 6% of global bryophyte diversity (Turetsky et al. 2012; Meltofte 2013), with an estimated number of 400 bryophytes living in Svalbard (Frisvoll and Elvebakk 1996). Lindo and Gonzales (2010) referred to the moss layer and its associated organisms as the "bryosphere". The moss itself provides a moist microhabitat and buffers the underlying soil from humidity fluctuations, helping maintain a stable microclimate (Alvarenga and Rousk 2022). It also buffers temperature fluctuations within the moss layer and soil, typically maintaining temperatures higher than in surrounding bare soil (Turetskty et al. 2010, 2012). Gerson (1982) in a review of invertebrates associated with moss, noted seven phyla (Platyhelminthes, Nematoda, Rotifera, Annelida, Mollusca, Tardigrada and Arthropoda). Callaghan et al. (2004) noted that the Arctic bryosphere hosts more than 3,000 invertebrate species, with high diversities of springtails (Collembola) and mites (Acari) (Erludóttir 2023). Notably, to date, these biodiversity studies have been based on morphology alone.

Morphological identification of organisms in the bryosphere requires specific taxonomic knowledge of highly diverse and taxonomically complex groups of microinvertebrates and microorganisms, which provides a challenge in its understanding. To overcome this barrier, Fontaneto et al. (2019) highlighted the potential of the recent and rapid developments in molecular tools, with emphasis on DNA metabarcoding. Studies using these tools have been widely applied in the Antarctic environment in a variety of substrata, such as vegetation, soil, ice and snow (Rippin et al. 2018; Fraser et al. 2018; Câmara et al. 2021; Câmara et al. 2023; Czechowski et al. 2022; Rosa et al. 2022). Câmara et al. (2021) reported DNA sequence assignments from a total of 263 taxa (including Bacteria, Chromista, Fungi, Metazoa, Protista and Viridiplantae) associated with a moss carpet on King George Island (South Shetland Islands), supporting the potential of the DNA metabarcoding for uncovering hidden biodiversity. In the Arctic, Cutler et al. (2017) used molecular tools to identify to identify fungi present in moss carpets in boreal forests, while Jessey et al. (2022) and Hamard et al. (2021) applied them to identify photosynthetic bacteria and protozoa in peatland moss. Therefore, in this study we applied DNA metabarcoding to help characterize communities associated with moss carpets on High Arctic Svalbard.

This way, we aimed to have a better understanding of the native community and the current non-native species scenario, while also comparing the communities of a pristine site and a highly anthropic influenced site.

METHODS

Study Sites and Sampling— Located in the High Arctic (74º-81º N; 10º-34º E), Svalbard is a Norwegian archipelago, surrounded by the Arctic Ocean to the north, Barents Sea to the east and Greenland Sea to the west. Moss samples were collected from two locations on Svalbard (n = 3 at each location) (Fig. 1). The first was within Longyearbyen (78°21'55"N 15°40'29"E); Longyearbyen, is the administrative centre and largest settlement on Svalbard, has ca. 2,500 inhabitants, and it is estimated that 67,000 cruise passengers visited Longyearbyen in 2024 (n.visitsvalbard.com 2025). The second site was Diabasodden (78°21'40"N 16°06'32"E), an isolated site, which presents a more pristine environment. Three moss carpet samples of *Sanionia uncinata* (Hedw.) Loeske were collected at each location using gloves and previously sterilized forceps, placed in sterilize WhirlPak bags (Sigma-Aldric, USA) and kept frozen until processing. The sites were chosen based on an assumption that human impact was likely higher in the settlement of Longyearbyen) than in the more isolated and less visited Diabasodden. The sites are separated by ca. 20 km, with no direct land connections.

DNA extraction, amplification and sequencing— DNA extraction from the moss samples (3 from each site), used the QIAGEN Power Soil Kit (QIAGEN, Carlsbad, 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 four groups of eukaryotic organisms: Chromista, Metazoa, Protista and Viridiplantae.

The internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular sequence assignments of Chromista and Viridiplantae (Chen et al. 2010, Dharmayanti et al. 2019), using the universal primers ITS3 and ITS4 (White et al. 1990). For Metazoa and Protozoa, we used Cox1 (Folmer et al. 1994, Kher et al. 2011) 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).

Data analyses and taxon assignment— Raw fastq files were filtered and trimmed using BBDuk version 38.37 (BBMap – Bushnell B. –sourceforge.net/projects/bbmap/) with the following parameters: Illumina adapters removing (Illumina artefacts and the PhiX Control v3 Library); ktrim ¼ l; k ¼ 23; mink ¼ 11; hdist ¼ 1; minlen ¼ 50; tpe; tbo; qtrim ¼ rl; trimq ¼ 20; ftm ¼ 5; maq ¼ 20. The remaining sequences were imported to QIIME2 version 2024.5 (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 to remove chimeras using default parameters (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 10.0 (Abarenkov et al. 2020) for Eukaryota, and MIDORI database version 265 (Leray et al. 2018) for COX1, trained with Naïve Bayes classifier using a confidence threshold of 98.5%. We aimed to maximize resolution by obtaining data from specific and curated databases of the target groups.

Rarefaction calculations were carried out using the rarefaction analysis command in the platform MOTHUR (Schloss et al. 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 et al. 2018). Therefore, for comparative purposes, we used the number of reads as a proxy for relative abundance (Câmara et al. 2021; Ferrera et al. 2024; Bones et al. 2025). Ecological indices were calculated using PAST 1.90 (Hammer et al. 2001); we calculated Shannon-Weaver to understand and compare the diversity of the two sites, Simpson's index to evaluate dominance and Pielou's evenness to evaluate if the communities were uniform; t-test were performed to assess if the difference in the indices result were significantly different. Sequences from the Phyla Ochrophyta and Oomycota were obtained from Cox1 and the Midori database while those for Ciliophora used ITS and the UNITE database. The classification systems used were, Leliaert et al. (2012) for Viridiplantae, Cavalier-Smith (2018) for Chromista, Protista and Metazoa.

All descriptions of geographical distributions were based on information obtained from available databases such as GBIF (www.gbif.org), Catalogue of Life (https://www.catalogueoflife.org/), Tropicos (www.tropicos.org), AlgaeBase (www.algaebase.org) and the relevant literature cited, where was considered non-native species with no previous records in the hollarctic region.

RESULTS

Overall, across both markers and sampling locations, a total of 706 ASVs were detected, representing four Kingdoms (Chromista, Metazoa, Protista, Viridiplantae), and 23 Phyla (Bigyra, Cercozoa, Ciliophora, Ochrophyta, Oomycota, Annelida, Arthropoda, Chordata, Cnidaria, Gastrotricha, Mollusca, Nematoda, Onychophora, Placozoa, Platyhelminthes, Porifera, Rotifera, Tardigrada, Loukozoa, Amebozoa, Chlorophyta, Rhodophyta, Streptophyta). A total of 384 ASVs were not identified at Kingdom level (Fig. 2a).

For this analysis, the reads of *S. uncinata* were excluded due to it being the collected substrate. Across both markers, there were 321 ASVs assigned exclusively in Diabasodden, from which 217 ASVs could not be identified at any taxonomic level (Fig. 2a). Of the remainder, the richest kingdoms were Metazoa and Viridiplantae — 38 ASVs and 37 ASVs, respectively. The most abundant kingdom was Viridiplantae, followed by Chromista, with 105643 and 81822 reads, respectively. In Longyearbyen, there were 288

ASVs, exclusively, of which 151 could not be identified at any taxonomic level. In the remainder, the richest kingdom was Metazoa, with 74 ASVs, followed by Chromista, with 36 ASVs. Metazoa was also the most abundant, with 197824 reads, Viridiplantae being the second, with 67766 reads.

For this study, ASVs that were native to areas outside the holarctic regions were considered exotic. There was a high number of exotic DNA reads (212.396 reads), especially in Longyarbyen (69,09% of the number of reads) and the group Metazoa, with 95,18% of the reads when summing both sites (Fig. 3a). The same tendency was also found in the number of assignments to taxa not native to Svalbard, with the highest number assigned to Metazoa (59 ASVs), followed by Chromista (11 ASVs), Viridiplantae (8 ASVs) and Protista (1 ASV) (Fig. 4b).

The rarefaction curves (Fig. 4) stabilized for both sampling locations, indicating sufficient sampling effort to provide an accurate representation of the local diversity. Details of all five diversity indices are present in Table 1, with Diabasodden showing higher values than Longyearbyen in all indices. The *p* values for Simpson and Shannon indexes were < 0.001.

Table 1
Ecological indexes. The *p* value for Simpson's and Shannon's indexes was < 0.001, indicating a statistically significant difference between communities

Diabasodden	**Shannon**	**Simpson (1-D)**	**Pielou's Evenness**
	2.974542	**0.9037970**	**0.5680465**
Longyearbyen	2.065310	0.7458899	0.3832404

Chromista

A large proportion of the assigned taxa in this Kingdom have widespread or cosmopolitan distributions, with 11 of the 107 taxa assigned not being recorded from holarctic regions. Amongst these, six were recorded in both sampling locations, one only in Diabasodden and four only in Longyearbyen. The most abundant Phylum was Ochrophyta, representing 55.1% of the reads obtained, and the most diverse phyla were Ochrophyta and Ciliophora (Suppl. Figures 2 and 3).

Metazoa

The most abundant and diverse phylum represented was Arthropoda, with 69.3% of reads and 63 ASVs, followed by Tardigrada in terms of abundance and Mollusca in terms of richness (Suppl. Figures 6 and 7). the potential numbers of non-native metazoan species appear alarming (Suppl. Figures 9 and 10), with 25 previously unrecorded taxa in Diabasodden and 39 in Longyearbyen (see supplemental material). This gave a total of 59 assignments to taxa native to other regions. Of these, 24 assignments were to taxa with more than 100 reads.

Protozoa

In this study only 10 protozoan ASVs were assigned, representing two phyla, two classes, six orders and four families — including four ASVs at unassigned family level. Sequences of Amebozoa represented 98.4% of reads (Suppl. Figure 11a) and 90% of ASVs (Suppl. Figure 11b). The richest orders were Centramoebida and Himatismenida, with three assigned species each. The most abundant was Himatismenida, with 71.4% of reads. All Protista ASVs were identified using the COX1 marker.

Viridiplantae

A total of 73 ASVs representing this kingdom were assigned, belonging to three phyla, 13 classes, 29 orders and 37 families. Of these, three ASVs (4.1%) could only be identified at Kingdom level. Of the 73 ASVs, 54 were identified using ITS2 and 19 using COX1. All Rhodophyta ASVs were identified using COX1. Bryophyta was the most abundant phylum, with 90.2% of reads, followed by Chlorophyta (Suppl. Figure 14), while the richest was Chlorophyta, with 37 ASVs (Suppl. Figure 13). The richest Orders were Trebouxiales and Chlamydomonadales, with 10 and 6 species, respectively. The most abundant was Hypnales, with 87.6% of reads. The most abundant family was Amblystegiaceae, with 69.1% of reads, while the richest was Trebouxiaceae, with 10 species.

Discussion

Studies relying on metabarcoding alone inherently do not confirm the presence of living organisms or viable propagules, but rather the assignment of a ‘most similar’ DNA fragment based on information deposited in the publicly available databases that exist, the relatively high number of DNA sequences not assigned even at high taxonomic level here could represent organisms not present in the consulted databases, although some could also represent new or undescribed species. Nevertheless, we acknowledge the markers bias, where different markers possibly showing different results.

Longyearbyen is the largest human settlement in Svalbard, with a considerable tourism rate (n.visitsvalbard.com 2025). These high human numbers, which has been specifically associated with the inward transport of a diversity of non-native propagules (Palmer 2013) has the potential to have a great impact on native biological communities. Diabasodden, in contrast, while only ~ 20 km from Longyearbyen, is much more isolated with much lower levels of human presence and interaction. Our data suggest clear differences in the bryosphere communities present at each location. Even though the numbers of assigned taxa were similar, only a small number, 97/706 ASVs, were detected in both locations (Supl. Figure 1), possibly consistent with the greater impacts of human activity in Longyearbyen (Post et al. 2009).

Diabasodden displayed higher values of all three diversity indices than Longyearbyen, indicating a more diverse and even distribution of the community (Thukral 2017). Shannon index (*H*’) values usually range between 1.5 and 3.5 for a diverse community, with Diabasodden exhibiting a significantly higher level of Shannon diversity (Lillie et al. 2003). The value of Simpson’s D ranges from 0 to 1, with 0 representing high diversity and low dominance and 1 representing the oposite. For this study we used its complement (1-D), and the communities of both locations had a high level of diversity, with low dominance (Kiernan

2014), with Diabasodden score, again, significantly greater. Pielou's evenness also ranges from 0 to 1. A value of 0 indicates low diversity and low evenness and 1 represents high diversity and high evenness (Magurran 2004). Diabasodden had a slightly greater value than Longyearbyen, but both had values close to 0.5, indicating a non-even distribution among the species (Table 1).

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. Most of the assigned taxa that are currently not known from Svalbard are from tropical and sub-tropical regions. While a greater number of non-native taxa were assigned in Longyearbyen than Diabasodden, the number in the latter, much less human impacted location, is striking. However, it should also be noted that, while contemporary human interaction is limited largely to a small number of local residents visiting summer cabins, as well as winter snowmobile travel (including tourism), historically the entire area was subject to various levels of impact from the local coal mining industry. As noted previously, the increasing number of visitors to Svalbard, including tourists, may be responsible for the import of non-native propagules. Some of these potentially may have negative impacts on native species and communities and we provide here some comments on relevant data relating to each major taxonomic group, with more information provided in the Supplemental Material. Marine taxa are expected as the sites are very near the shore and the strong winds do blow seaspray over the sampling sites.

Climate change is dramatically affecting the ecology of extreme environments like the Arctic. One factor that cannot be overlooked is the potential risk of new organisms emerging, including those pathogenic to animals, plants, and humans, related to permafrost thawing (Gartler et al. 2025). In this scenario of surveillance of emerging pathogens, pandemics related to zoonoses, and the One Health approach, the occurrence of 39.7% of specimens that could not be identified to the Kingdom level in Diabasodden deserves attention (Wilson 2025).

Chromista

Only one non-native species, the Japanese endemic *Pythium biforme*, was assigned exclusively in Diabasodden. In the absence of any confirmed specimen, such assignments seem likely to reflect the incomplete nature of available sequence databases rather than a true occurrence record. Similarly, the four 'new records' from Longyearbyen seem likely to represent database limitations. Of these, *Phytophthora megakarya* is common in the African continent, *Phytopythium litorale* also from warmer climates, *Pythium brachiatum* is another Japanese endemic and *Lessonia flavicans* is an Ochrophyta, common in southern South America and Sub-Antarctic region (Rothman et al. 2014). Similarly again, of the five new assigned taxa shared between two sampling locations, *Phytophthora amnicola* occurs naturally in New Zealand, *Globisporangium spinosum* in Japan, *Aphanomyces invadans* is widespread in the oriental region, *Schizocladia ischiensis* in Greece and *Haramonas dimorpha* in Australia (Suppl. Figure 5).

Members of the genera *Aphanomyces, Phytophthora, Pythium, Phytopythium* and *Globisporangium*, all assigned to sequences obtained in this study, are known to cause a large number of plant diseases that lead to extensive damage or even death of the plant (Akrofi 2015; Bregant et al. 2023). Some also cause Epizootic Ulcerative Syndrome (EUS) in fishes (Iberahim et al. 2018), with records of *A. invadans* affecting 87 different fish genera, hence representing a great threat to the aquaculture industry (Oidtmann 2012; Iberahim et al. 2018). Crops of kiwifruit (*Actinidia chinensis* Planch.) and ginseng (*Panax ginseng* C.A.Mey.) in China attacked by *Globisporangium* suffered 30–70% product loss (Huo et al. 2023; Feng et al. 2024). However, at present, there are no records of these cited genera causing diseases on Svalbard, and it is possible that they have also saprophytic roles (Uzuhashi et al. 2015).

Metazoa

Our assigned Metazoa included a high proportion of taxa representing groups known from Svalbard, although the total number of taxa assigned represents only 6.5% of the Svalbard invertebrate fauna, with 71 ASVs (Coulson et al. 2024). There were 59 non-native Metazoa ASVs summing both sites and, though is not possible to confirm the presence of living representatives of these taxa at the two sampling sites, the high number of reads must raise concern in terms of threat to the native fauna (Gilg et al. 2012; Convey et al. 2012), highlighting a need for carefully targeted confirmatory ground survey.

Amongst the non-native species assigned here, *Tropilaelaps mercedesae* is a vector of the honeybee (*Apis mellifera* L.) virus DWV (Dainat et al. 2009), being a major threat to *A. mellifera*, thus honey production (Forsgren et al. 2009), although bees do not occur on Svalbard. *Culex sitiens* can feed on human blood and is a vector of the Japanese encephalitis virus, a flavivirus related to the dengue, yellow fever and West Nile viruses (Vythilingam et al. 2003), *Deraeocoris claspericapilatus* (Kulik 1965) is a plant-bug, representing a reasonably visible group. Nevertheless, neither of the two genus had been observed on Svalbard. As an example of a marine taxon assigned in the current study, not in itself unlikely given the proximity of the sampling locations to the coast, *Acropora palmata* (Cnidaria) is facing a massive decline due to climate and environmental changes in its native distribution in the Caribbean (Willians et al. 2007), however may again be indicative of database limitations given that other cnidarians occur in the Svalbard region. Nevertheless, no species from the *Acropora* genus had been previously recorded in Svalbard. The same may apply to the assignment of *Cyanea nozakii* (ghost jellyfish), another species of warmer waters, whose blooms may have serious impacts in the marine ecosystem including on fisheries (Dong et al. 2008). The assignment of the freshwater snail, *Bulinus globosus*, a group otherwise unknown from Svalbard (Coulson et al. 2024), represents the host and vector of the platyhelminth *Schistosoma haematobium*, capable of transmitting the urogenital schistosomiasis disease (Davies et al. 1999; Kalinda et al. 2017; Pennance et al. 2022).

DNA from important mosquito vectors of serious human diseases has been detected in Svalbard, including *Anopheles messeae* and *Anopheles quadriannulatus* — known vectors of malaria — and *Aedes aegypti*. The latter is a vector of the world's major endemic arboviruses, such as dengue, Zika, Chikungunya, yellow fever, Mayaro, Ross River, and Rift Valley fever, which cause millions of cases and

tens of thousands of deaths annually (Cheong et al. 2025). Schilling et al (2025) performed metagenomic analysis of RNA extracted from pools of *Aedes* species found in Greenland and detected a number of novel RNA viruses belonging to a variety of different virus families, including Flaviviridae, Orthomyxoviridae, Bunyavirales, Totiviridae, and Rhabdoviridae, originally known to harbor viruses causing serious human diseases such as hemorrhagic fevers (Dengue, yellow fever, Crimean-Congo, Rift Valley), neurological diseases (congenital Zika, Japanese encephalitis, WNV), pulmonary and renal syndromes (Hantavirus), influenza, and rabies.

Protozoa

Few taxa were assigned to the Kingdom Protozoa in this study, most likely due to the use of an inappropriate marker. Forty Protozoa species have previously been recorded from Svalbard (Balik 1994). Further studies using different markers are required to allow for robust assessment of this group. Only one assigned taxon is not known from the region of Svalbard, *Acanthamoeba tubiashi*, which is native to the Australian and Oriental regions although, again other representatives are known from this region, likely another example of database limitations. Members of the genus are known as opportunistic pathogens, causing keratitis, sinusitis and encephalitis, although there are no records of these diseases in Svalbard to this day (Rayamajhee et al. 2022; Halim et al. 2023).

Viridiplantae: Eight non-native Viridiplantae were assigned from sequences obtained from our samples, of which four species represented Rhodophyta, three Bryophyta and one Chlorophyta. The latter was *Trebouxia brindabellae* (A.Beck 2002), native to the Australian region, although other representatives of the genus are known from Svalbard and northern latitudes. Along with other members of the genus *Trebouxia*, it is known to associate with fungi in a lichen symbiosis (Singh et al. 2017). The four Rhodophyta assigned were *Cephalocystis furcellata, Gloiopeltis compressa, Hydropuntia rangiferina* and *Ptilophora malagasya*, none of them previously recorded in Svalbard. *Ptilophora malagasya*, a small agar-producing red algae, endemic to Madagascar (Boo et al. 2018). *Gloiopeltis compressa* is a red algae from Japan and Korea region. *Hydropuntia rangiferina*, is recorded from Oriental, Ethiopian and Neotropical regions and *Cephalocystis furcellata* is native from Australian region, usually growing in rocks either in deep intertidal pools or subtidally at 2–20 m depths (Millar et al. 1996). The three Bryophyta found were *Tayloria longiseta, Treubia lacunosa* and *Steereobryon subulirostrum*. *Tayloria longiseta* is a rare species, with 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 *Tayloria* recorded in Svalbard: *T. froelichiana*, *T. lingulata* and *T. acuminata*, which could mean a potential misassignment of the found species. *Treubia lacunosa*, is a liverwort from the Australian region, as well as all species in the genus. The last non-native species was *Steereobryon subulirostrum*, with Neotropical distribution, mostly in Central America.

CONCLUSIONS

There has been little previous research effort directed at the bryosphere community on Svalbard, or application of DNA metabarcoding approaches. Our eDNA sequence assignments suggest that the High Arctic *S. uncinata* bryosphere hosts a diverse community of taxa representing different kingdoms. We recognize that the assignment of a sequence in an eDNA study does not confirm the presence of a living or viable organism, not least as a result of the considerable database limitations that exist, although it could still indicate that fragments or dead organisms, single cells, pollen, spores or cysts may be arriving by various natural or anthropogenic routes into the region. Our findings reinforce the need to monitor biodiversity using modern molecular techniques, particularly in concert with developing more thorough and robust baseline sequence databases as, in the face of contemporary climate change, organisms for which environments are not suitable now, may gain the ability to colonise in the near future, highlighting both the urgency of accurately documenting the native community and the need for application of effective biosecurity regimes in order to avoid introduction of new species.

Declarations

Funding information

This research was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the brazilian science, technology and innovation ministry (MCTI).

Ethics declaration: not applicable

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Figures

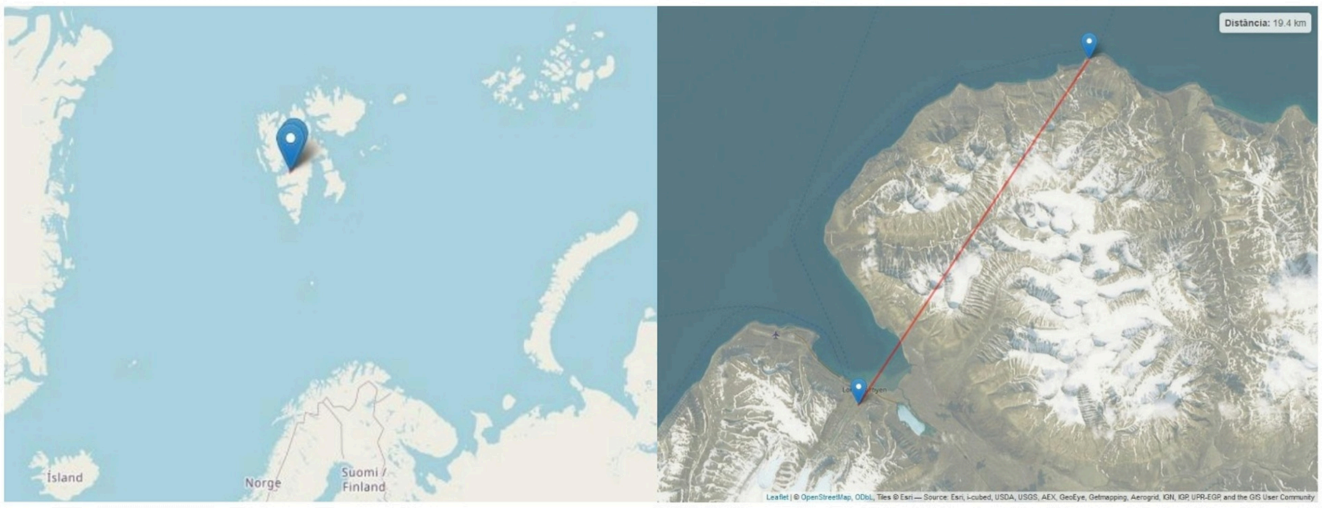

Figure 1

A The Svalbard archipelago, the blue points indicating the sampling locations. B Higher scale illustration of the sampling sites.

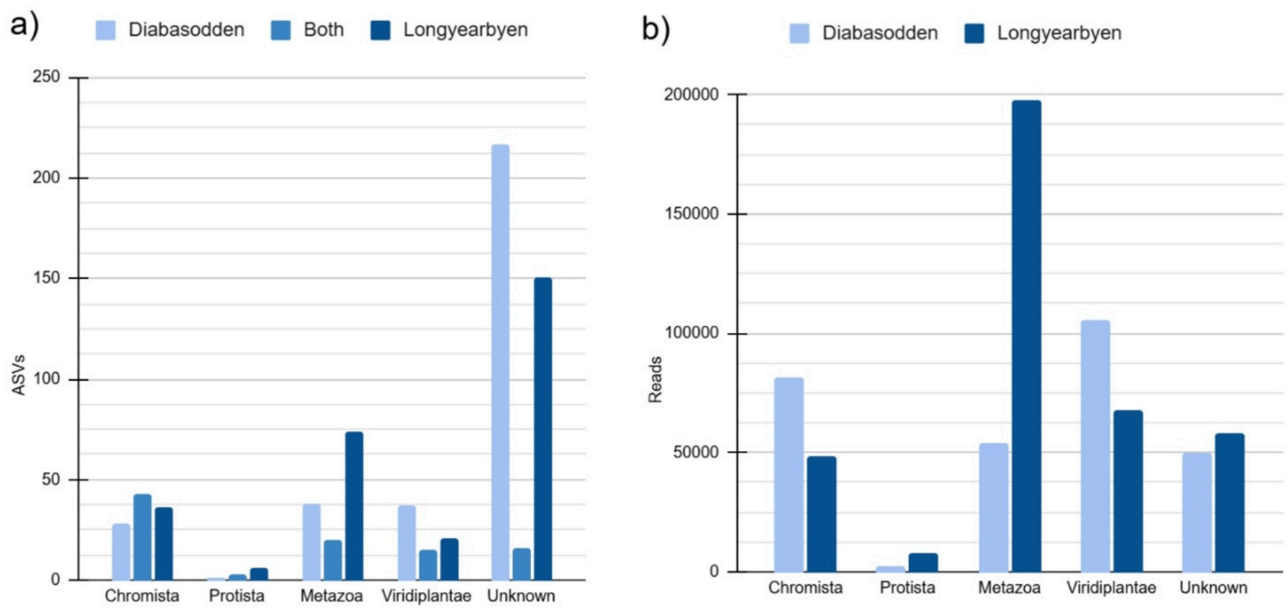

Figure 2

A) Numbers of reads, and **B)** assigned ASVs of each Kingdom in samples comparing both sample locations and the ASVs found in both sites. In A), the number of reads indicates the number of DNA sequences assigned to each Kingdom. For this analysis, the substrate *S. uncinata* reads were excluded, hence it being the subtract collected. In B) the number indicates the number of taxa (ASVs) detected in each Kingdom, with a high number of unidentified ASVs, which represent the reads that could not be assigned at kingdom level.

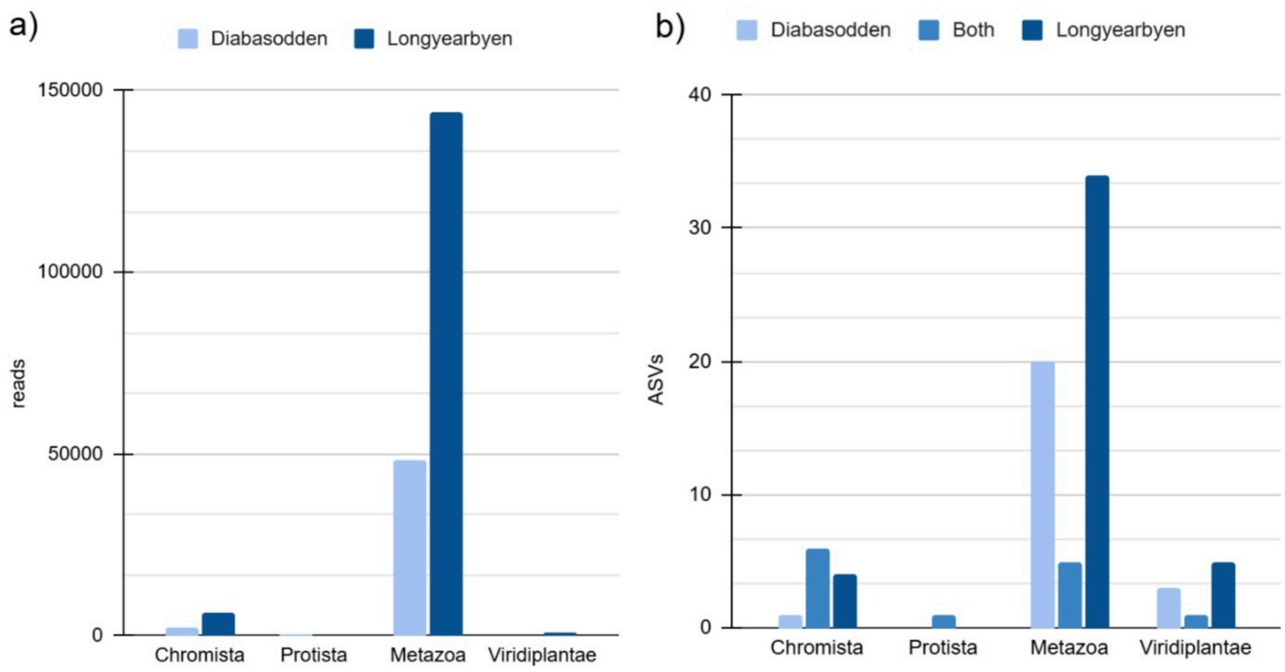

Figure 3

A) Numbers of assigned reads representing species not currently recorded on the Arctic in the kingdoms surveyed from both sampling locations. B) Numbers of taxa assigned that are currently not native to Svalbard in all five kingdoms surveyed from both sampling locations

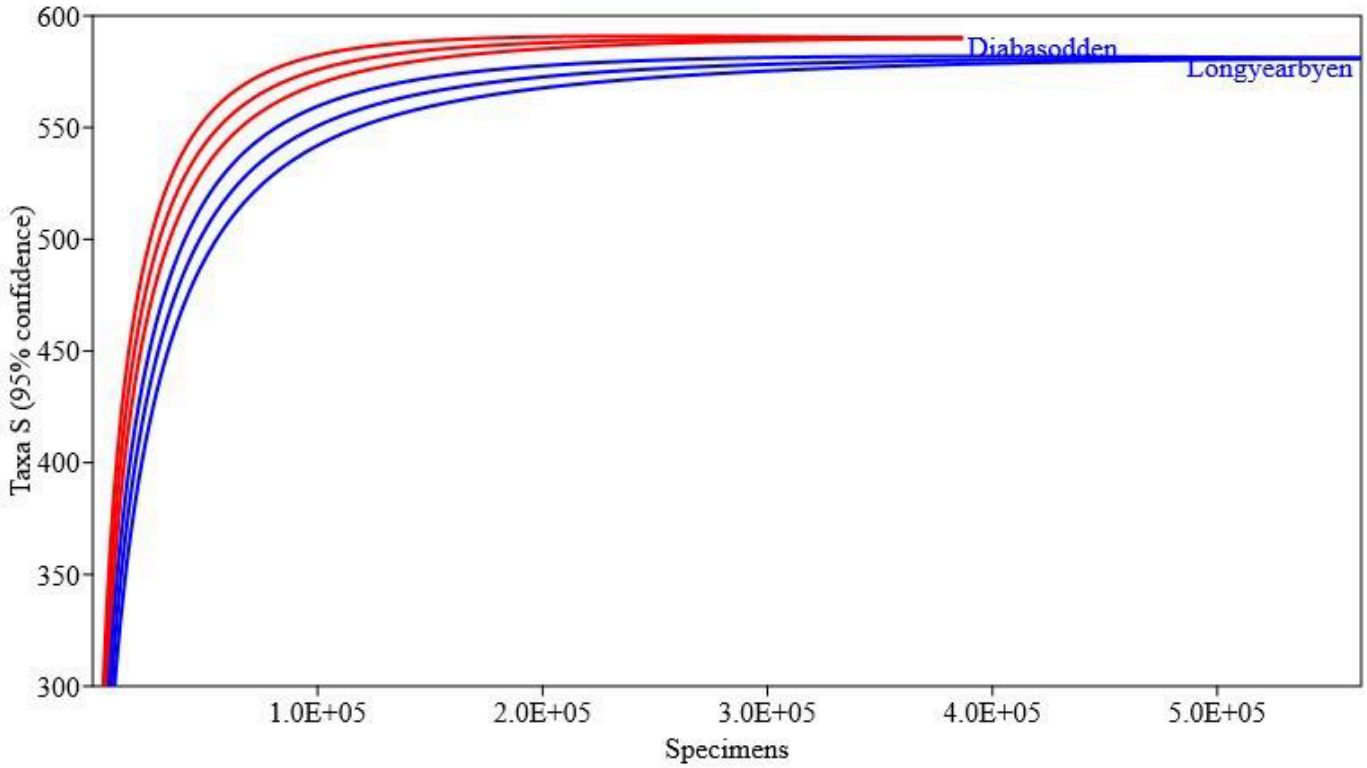

Figure 4

Rarefaction curves for the two sampling locations, Diabasodden in red and Longyearbyen in blue, including 95% confidence intervals.

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