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RESEARCH

# Investigating the diversity contained in an established moss carpet in Admiralty Bay, King George Island (maritime Antarctic), using DNA metabarcoding

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## Abstract

Moss carpets are a common feature in the maritime Antarctic. They provide habitats for a diverse community of organisms, including microbial groups, micro-invertebrates, and micro-arthropods, all of which depend on the moss for their survival. Despite recognition of this important role, studies of the moss-associated biodiversity to date have rarely involved the application of modern molecular tools. This study focused on an extensive and long-established moss carpet primarily comprising the moss *Saniona uncinata*, located adjacent to a reconstructed whale skeleton on a coastal beach and of historical importance, located near the Brazilian Antarctic Station (Admiralty Bay, King George Island), and is a popular attraction for tourists and scientists alike. We employed DNA metabarcoding to investigate the biodiversity of organisms potentially associated with the moss carpet, employing three well-established genetic markers, 16S, ITS2, and COX1. The marker generating the greatest number of amplicon sequence variants (ASVs, a proxy for taxon diversity) was 16S with 199 assigned ASVs, followed by COX1 (121) and ITS2 (26). In total, 346 ASVs were assigned, representing two Domains, five Kingdoms, and 38 phyla. As well as representing a diverse range of likely native taxa, a proportion of the assignments were exotic taxa not forming part of Antarctica's native biodiversity. These assignments, while they cannot be proven to represent the presence of living organisms or established populations, may be an important indication of otherwise invisible human influence on the Antarctic environment, such as the introduction of DNA originating in human food and associated microbes, pests, and parasites.

**Keywords** High throughput sequencing · Invasive species · Bryosphere · eDNA

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## Introduction

Antarctica's extreme environmental conditions are widely recognized to impose severe limits on the continent's terrestrial diversity, including that of its vegetation. It hosts only two native angiosperm species and approximately 116 species of Bryophyta (Convey and Biersma 2024). At present, molecular phylogenetic approaches have not been widely applied in studies of Antarctic bryophytes, and understanding of their genetic diversity remains limited, and molecular tools have just started to be used for such purposes (Biersma et al. 2018 and Câmara et al. 2019, 2021b). Some recent studies suggest considerable reconsideration of the true identity of currently accepted species records in some genera may be required (e.g., *Bryum*, Cannone et al. (2024) and *Bartramia*, Câmara et al. (2019)).

The Antarctic continent is broadly separated into two large-scale biogeographic regions, the more extreme continental Antarctic and the generally milder and damper maritime Antarctic (Convey and Biersma 2024). Mosses are present in both regions, but recorded species-level diversity is around five times greater in the latter region (~116 species) and their spatial extent considerably greater. A well-known feature of the milder maritime Antarctic relative to the continental Antarctic, especially in parts of the South Shetland Islands and South Orkney Islands, is the scale of moss development, including extensive moss carpets, which play a vital role in soil formation and nutrient cycling in these Antarctic ecosystems (Smith 1972; Ochyra et al. 2008). However, even in the maritime Antarctic the absolute extent of vegetation growth remains limited, not least through the restricted availability of ice-free ground (Hughes et al. 2016).

Moss carpets often primarily consist of extensive and nearly uniform patches of mosses such as *Sanionia* sp. and other pleurocarpous mosses and are commonly characterized by low genetic diversity (Shaw et al. 2003; Biersma et al. 2017). Studies of Antarctic *Sanionia* populations have confirmed minimal genetic variation (Hedenäs 2012; Hebel et al. 2018), which is further compounded by the nearly total absence of sexual reproduction in this genus in Antarctica (Lewis Smith and Convey 2002; Ochyra et al. 2008; Hebel et al. 2018), although Vargas et al. (2024) recently reported an exceptional increase in sporophyte production by *Sanionia uncinata* on King George Island. Moss carpets play an important role in providing habitats for a potentially diverse community of organisms, including multiple microbial groups and micro-invertebrates, all of which inherently depend on the moss for their survival.

Despite the dominance of bryophyte vegetation in the maritime Antarctic and their key contribution to habitat formation (Block et al. 2009), the overall biodiversity of the "bryosphere" (Lindo and Gonzalez 2010) has yet to be extensively explored using molecular tools. To date, studies of the contained biodiversity have focused on traditional extraction approaches and classical taxonomy for, primarily, micro-arthropods (e.g., Convey and Lewis Smith 1997) and, to a lesser extent, micro-invertebrates (e.g., Maslen and Convey 2006), and have largely ignored the microbial groups. While molecular phylogenetic approaches are now starting to be applied to specific micro-invertebrate groups (e.g., nematodes, Kagoshima et al. (2019); tardigrades, Short et al. (2022)), our understanding of the genetic diversity and ecological dynamics within these moss-carpet ecosystems remains limited (Câmara et al. 2021a; Hebel et al. 2018). Now, the application of modern and rapidly developing DNA metabarcoding tools in studies of environmental DNA (eDNA) offers a promising approach to improving knowledge of the currently invisible biological diversity potentially present in natural ecosystems and providing insights into the intricate relationships they contain.

Recent metabarcoding studies focusing on microbial groups in diverse habitats across the South Shetland Islands and northern Antarctic Peninsula have reported the detection of DNA representing a significantly greater diversity than previously recognized using conventional methods (Câmara et al. 2021a, 2022, 2023; Czechowski et al. 2022; Fraser et al. 2018; Garrido-Benavent et al. 2020; Lebre et al. 2023; Ogaki et al. 2021; Rosa et al. 2020a). Câmara et al. (2021c) analyzed a sample of moss carpet transplanted due to the construction of the new Brazilian Antarctic Comandante Ferraz Station on King George Island, reporting a total of 263 DNA sequence assignments to different taxa representing five kingdoms. Such studies highlight the remarkable hidden diversity that is potentially present in these moss carpet habitats. However, the Câmara et al. (2021c) study specifically focused on a transplanted moss carpet (henceforth referred to as TMC) and, therefore, the data obtained may not be directly applicable to other moss carpets in the region. In order to address this limitation, in the current study, we seek to provide a more detailed representation of the taxonomic diversity and ecological dynamics of an in situ natural Antarctic moss carpet ecosystem.

This study focused on the "whale moss carpet" (henceforth WMC) located adjacent to the so-called Jacques Cousteau Whale, a site with significant historical importance located on the Keller Peninsula, King George Island. In 1972, during an expedition to Antarctica, the French explorer Jacques-Yves Cousteau (1910–1997) and his research team reconstructed a complete whale bone skeleton using bones from multiple individuals and species placed on a moss carpet, as a memorial to the historical whale-hunting era (Kittel 2001). This monument, located on a raised beach terrace near the Brazilian Comandante

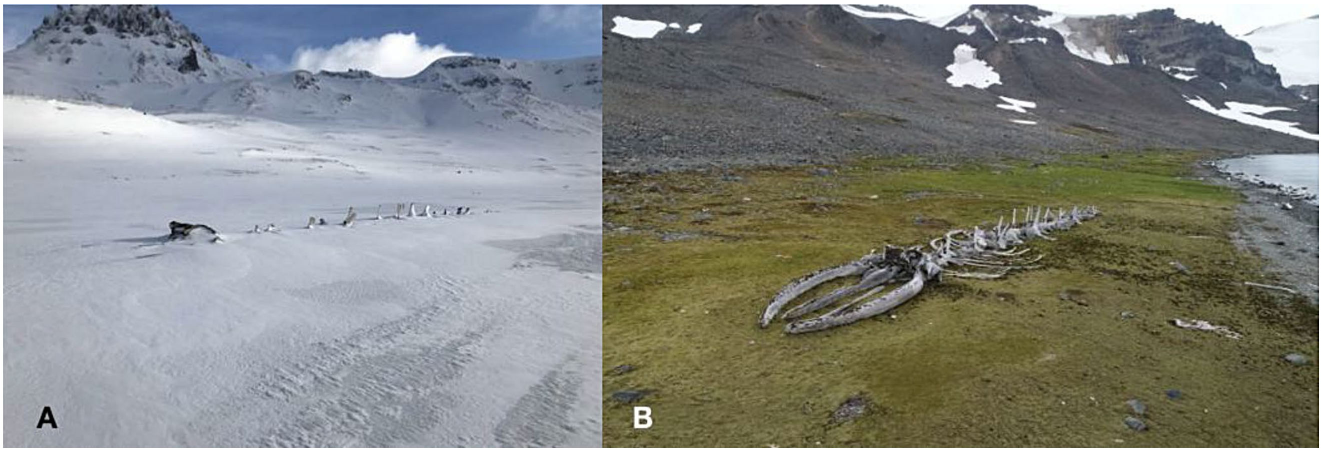

**Fig. 1** The Whale Moss Carpet (WMC), covered by snow (**A**) and ice free (**B**)

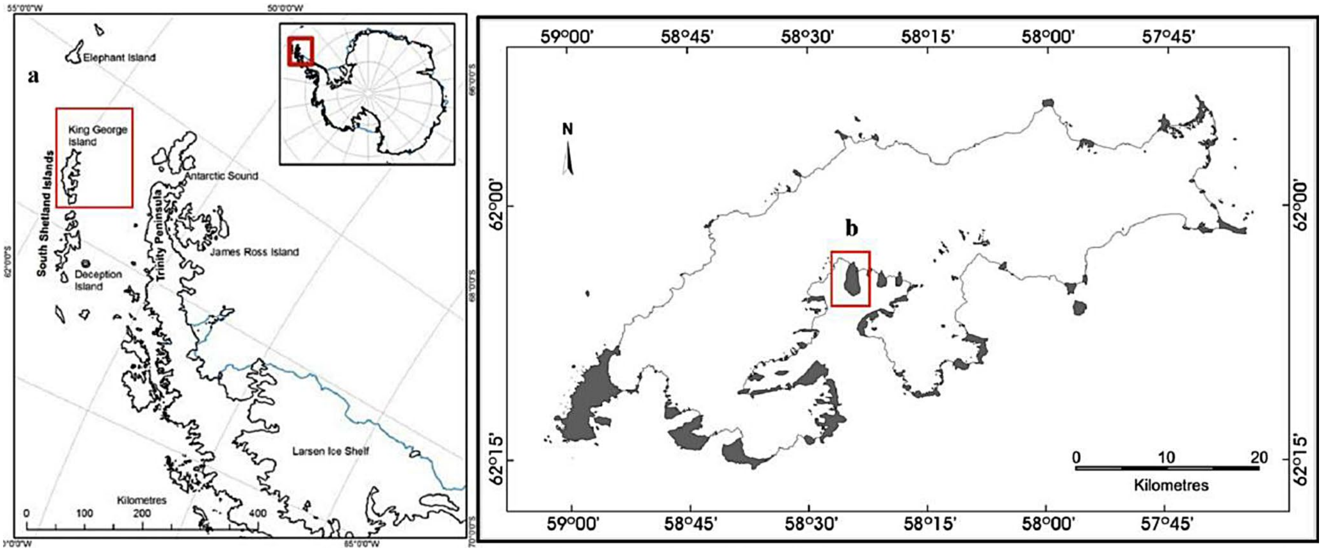

**Fig. 2** Maps showing the location of King George Island (**a**) and the Keller Peninsula (**b**)

Ferraz Antarctic Station, is completely covered by snow and ice during winter and is snow free during summer and has become a popular attraction for tourists and scientists alike (Fig. 1). We employed DNA metabarcoding to investigate the biodiversity of organisms found in the WMC, expanding the results reported previously by (Chen et al. 2010) and also examined the potential impacts of the moss carpet transplantation event on the contained genetic biodiversity of moss carpets.

## Materials and methods

### Sampling

In order to make a comparison possible, the sampling procedure followed that described by Câmara et al. (2021c), and a single shoot of *S. uncinata* was removed using gloves and previously sterilized forceps and placed in a sterilized WhirlPak bag (Sigma-Aldric, USA). A sample was collected during the austral summer of 2020/21 from the WMC (62°05.163′S, 058°24.784′W) (Fig. 2). From this sample, a shoot of *Sanionia uncinata* (Hedw.) Loeske was removed using gloves and previously sterilized forceps and placed in a sterilized WhirlPak® bag (Sigma-Aldric, USA). The shoot sampled was about 4 cm long and included both aerial parts as well as rhizoids (underground), and was taken immediately to the molecular biology laboratory at Comandante Ferraz Station, where DNA extraction was completed in a flow hood under sterile conditions. DNA Extraction was carried out using 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 the Quanti-iT™ Pico Green dsDNA Assay (Invitrogen). We aimed to target the DNA of six groups of organisms: Prokaryotes, Chromista, Fungi, Metazoa, Protista and Viridiplantae. For this purpose, we used the internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA as a barcode for Chromista, Protista, Viridiplantae and Fungi (Chen et al. 2010) using the universal primers ITS3 and ITS4 (White et al. 1990). For Prokaryotes, we used the 16S rRNA gene V3–V4 region (Klindworth et al. 2013) and the primers Bakt_341F and Bakt_805R (Herlemann et al. 2011). For Metazoa, we used Cox1 and primers UEA3F and HCO2198R (Folmer et al. 1994). DNA sequencing was carried out commercially by Macrogen Inc. (South Korea) using the Illumina MiSeq platform.

## Data analyses and taxa identification

Extracted DNA quality analysis was carried out using BBDuk v. 38.87 in BBmap software (Bushnell 2014) with the following parameters: Illumina adapters removing (Illumina artefacts and the PhiX Control v3 Library); ktrim=l; k=23; mink=11; hdist=1; minlen=50; tpe; tbo; qtrim=rl; trimq=20; ftm=5; maq=20. The remaining sequences were imported to QIIME2 version 2021.4 (https://qiime2.org/) for bioinformatics analyses (Bolyen et al. 2019). The qiime2-dada2 plugin was used for filtering, dereplication, turning paired-end fastq files into merged, removing chimeras, and creating amplicon sequence variants (ASVs) with default parameters (Callahan et al. 2016). ASVs are inferred from 16S rRNA gene sequences that can be understood as taxonomic units of a certain number of reads (Callahan et al. 2017; Eren et al. 2013). Taxonomic assignments of ASVs were derived using the qiime2-feature-classifier (Bokulich et al. 2018), *classify-sklearn* against SILVA 138 Ref NR 99 (Quast et al. 2012) for the 16S rRNA gene, UNITE Eukaryotes ITS database version 8.2 (Abarenkov et al. 2020b) for Eukaryota, and MIDORI (Leray et al. 2018) for COX1, trained with Naïve Bayes classifier. For ITS, firstly, ASVs were classified against the PLANiTS2 database (Banchi et al. 2020). After this step, ASVs that were not classified were filtered, and *classify-sklearn* classified against the UNITE Eukaryotes ITS database version 8.3 (Abarenkov et al. 2020a). Finally, remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (October 2023) using Blast (Camacho et al. 2009) with default parameters; the nt database was filtered with the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer”, and “internal transcribed spacer”. Taxonomic assignments were performed using MEGAN6 (Huson et al. 2016). Venn diagrams were prepared using the package JVenn (Bardou et al. 2014), and ecological indices were calculated using the package “vegan” (Oksanen et al. 2022) in the software R (R Core Team 2023). Rarefaction calculations were carried out using PAST (Hammer et al. 2001).

Although all ASVs were classified, it is generally accepted that the 16S rRNA gene can correctly assign sequences up to the genus level. Frequently, sequences can only be assigned with certainty to higher taxonomic levels. Finally, only taxa recognized in classification systems described in Whitman et al. (2012) and Yilmaz et al. (2014) were reported. ASVs classified as uncultured or using numbers, for example, were grouped together into higher taxonomic levels with recognized classification. Sequences assigned to chloroplast or mitochondrial DNA were not included. The denomination “Candidatus” indicates a taxonomic status for uncultured bacteria following the International Code of Nomenclature of Bacteria (Murray and Stackebrandt 1995). DNA analyses were carried out as described by Câmara et al. (2021c), and the classification systems used were: Whitman (2012) and Yilmaz et al. (2014) for Bacteria; Leliaert et al. (2012) for Viridiplantae; Kirk et al. (2008), Tedersoo et al. (2018), the Index Fungorum (http://www.indexfungorum.org) and MycoBank (http://www.mycobank.org) for Fungi; Cavalier-Smith (1998) for Chromista, Protista, and Metazoa.

# Results

The 16S marker generated 199 associated ASVs, followed by COX1 with 121 assigned taxa and ITS2 with 26 taxa. All rarefaction curves (Online Resource 1) reached a plateau, indicating that the sampling effort was sufficient to represent the diversity present at the sampled site. Some ASVs could only be assigned at higher levels (Class, Order, Family). Across all markers, 346 taxa were assigned, representing five Kingdoms and 38 phyla (Table 1). A comparison between the assigned diversity previously reported in the TMC (Câmara et al. 2021c) and the WMC is presented as a Venn diagram in Online Resource 2 and 3, illustrating the numbers of overlapping and unique assigned taxa in each carpet and taxonomic group. Câmara et al. (2021c) reported 263 taxa from the TMC, while the current study reports 346. For more details on taxa found see Online Resource.

## 16S marker

A total of 86,407 reads were obtained, of which 61,683 remained after quality control. A total of 61,664 reads were assigned to ASVs representing 14 phyla in the Domain Bacteria: *Acidobacteriota*, *Actinobacteriota*, *Armatimonadota*, *Bacteroidota*, *Chloroflexi*, *Cyanobacteria*, *Deinococcota*, *Firmicutes*, *Gemmatimonadota*, *Myxococcota*,

**Table 1** Habitat: B=Brackish, G=Generalist, F=Freshwater, M=Marine, T=Terrestrial, A=Aerial, pa.=Parasitic. Distribution: Eu=Europe, NA=North America, SA=South America, ME=Middle East, As =Asia, NZ=New Zealand, W=Widespread, CA=Central America, PI=Pacific Islands, SEA=Southeast Asia, AU=Australia, IO=Indian Ocean, PO=Pacific Ocean, Car=Caribbean, CA=Central Atlantic, WP=Western Pacific, Alp=alpine, Arc=Arctic, Ant=Antarctica, AI=Atlantic Islands, Af=Africa, Saf=South Africa

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| **Domain Bacteria** | | | | |
| **Phylum *Acidobacteriota*** | 16S | G | W | |
| *Granulicella* | 16S | G | W | 128 |
| *Granulicella aggregans* | 16S | G | W | 161 |
| *Granulicella tundricola* | 16S | G | W | 22 |
| *Terriglobus* | 16S | G | W | 192 |
| *Candidatus_Solibacter !* | 16S | G | W | 86 |
| Fam. *Acidobacteriaceae* ! | 16S | G | W | 16 |
| Fam. *Blastocatellaceae* ! | 16S | G | W | 79 |
| Fam. *Vicinamibacteraceae* | 16S | G | W | 34 |
| Ord. *Vicinamibacterales* | 16S | G | W | 168 |
| Class *Vicinamibacteria* ! | 16S | G | W | 27 |
| **Phylum*Actinobacteriota*** | 16S | | | |
| *Iamia* ! | 16S | G | W | 15 |
| *Mycobacterium* ! | 16S | G | W | 273 |
| *Rhodococcus* ! | 16S | G | W | 356 |
| *Nocardia* | 16S | G | W | 27 |
| *Jatrophihabitans* ! | 16S | G | W | 592 |
| *Nakamurella* | 16S | G | W | 898 |
| *Cellulomonas* ! | 16S | G | W | 20 |
| *Oryzihumus* ! | 16S | G | W | 24 |
| *Nocardioides* ! | 16S | G | W | 60 |
| *Pseudonocardia* ! | 16S | G | W | 16 |
| Fam. *Ilumatobacteraceae* | 16S | G | W | 450 |
| Fam. *Microtrichaceae* | 16S | G | W | 58 |
| Fam. *Kineosporiaceae* | 16S | G | W | 393 |
| Fam. *Microbacteriaceae* | 16S | G | W | 1,418 |
| Fam. *Intrasporangiaceae* | 16S | G | W | 478 |
| Fam. *Solirubrobacteraceae* | 16S | G | W | 394 |
| Order *Microtrichales* | 16S | G | W | 46 |
| Order *Frankiales* | 16S | G | W | 643 |
| Order *Gaiellales* | 16S | G | W | 44 |
| Order *Solirubrobacterales* | 16S | G | W | 193 |
| Class *Acidimicrobiia* | 16S | G | W | 191 |
| Class *Actinobacteria* | 16S | G | W | 150 |
| **Phylum*Armatimonadota*** | 16S | | | |
| *Armatimonadales* | 16S | G | W | 104 |
| *Chthonomonas* ! | 16S | G | W | 55 |
| *Fimbriimonadaceae* | 16S | G | W | 24 |
| **Phylum*Bacteroidota*** | 16S | | | |
| *Flavobacterium tegetincola* | 16S | G | W | 30 |
| *Flavobacterium ovatum* | 16S | G | W | 8 |
| *Dinghuibacter* | 16S | G | W | 34 |
| *Ferruginibacter* | 16S | G | W | 552 |
| *Parafilimonas* ! | 16S | G | W | 25 |
| *Terrimonas* ! | 16S | G | W | 54 |
| *Hymenobacter* ! | 16S | G | W | 110 |
| *Arcicella* ! | 16S | G | W | 34 |
| *Fluviicola* | 16S | G | W | 90 |
| *Flavobacterium* ! | 16S | G | W | 457 |
| *Chryseobacterium* | 16S | G | W | 240 |
| *Pedobacter* ! | 16S | G | W | 65 |

**Table 1** (continued)

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| *Bdellovibrio* ! | 16S | G | W | 37 |
| *Oligoflexus* | 16S | G | W | 65 |
| *Mucilaginibacter* ! | 16S | G | W | 303 |
| Fam. *Prolixibacteraceae* | 16S | G | W | 6 |
| Fam. *Saprospiraceae* | 16S | G | W | 14 |
| Fam. *Chitniohagaceae* | 16S | G | W | 633 |
| Fam. *Microscillaceae* | 16S | G | W | 33 |
| Fam. *Lentimicrobiaceae* | 16S | G | W | 6 |
| Fam. *Sphingobacteriaceae* | 16S | G | W | 37 |
| Order *Sphingobacteriales* | 16S | G | W | 153 |
| Class *Oligoflexia* | 16S | G | W | 5 |
| **Phylum*Chloroflexi*** | 16S | G | W | 219 |
| Fam. *Anaerolineaceae* | 16S | G | W | 5 |
| Fam. *Caldilineaceae* | 16S | G | W | 15 |
| Fam. *Chloroflexaceae* | 16S | G | W | 8 |
| Fam. *Roseiflexaceae* | 16S | G | W | 399 |
| Class *Anaerolineae* | 16S | G | W | 36 |
| **Phylum*Cyanobacteria*** | 16S | | | |
| *Nostoc PCC-73,102* | 16S | G | W | 46 |
| *Geitlerinema LD9* | 16S | G | W | 59 |
| *Pseudanabaena PCC-7429* | 16S | G | W | 129 |
| *Sericytochromatia* | 16S | G | W | 18 |
| **Phylum*Deinococcota*** | 16S | | | |
| *Deinococcus* | 16S | G | W | 20 |
| **Phylum*Firmicutes*** | 16S | | | |
| *Clostridium sensu stricto 12* | 16S | G | W | 561 |
| *Clostridium sensu stricto 13* | 16S | G | W | 108 |
| *Desulfosporosinus* ! | 16S | G | W | 121 |
| **Phylum*Gemmatimonadota*** | 16S | | | |
| *Gemmatimonas* ! | 16S | G | W | 911 |
| Fam. *Gemmatimonadaceae* | 16S | G | W | 62 |
| **Phylum*Myxococcota*** | 16S | | | |
| *Haliangium* ! | 16S | G | W | 173 |
| *Pajaroellobacter* ! | 16S | G | W | 92 |
| *Aetherobacter* ! | 16S | G | W | 11 |
| Fam. *Sandaracinaceae* | 16S | G | W | 33 |
| Order *Polyangiales* | 16S | G | W | 313 |
| **Phylum*Planctomycetota*** | 16S | | | |
| *Tepidisphaera* | 16S | G | W | 56 |
| *Gemmata* ! | 16S | G | W | 33 |
| *Tundrisphaera* | 16S | G | W | 63 |
| *Pirellula* ! | 16S | G | W | 12 |
| Fam. *Pirellulaceae* | 16S | G | W | 3 |
| Order *Tepidisphaerales* | 16S | G | W | 213 |
| **Phylum*Proteobacteria (Pseudomonadota)*** | 16S | | | |
| **Class*Alphaproteobacteria*** | 16S | G | W | 17 |
| *Acidiphilium* ! | 16S | G | W | 223 |
| *Rhodovastum* ! | 16S | G | W | 134 |
| *Inquilinus* | 16S | G | W | 20 |
| *Asticcacaulis* ! | 16S | G | W | 54 |
| *Phenylobacterium* ! | 16S | G | W | 115 |
| *Hirschia* ! | 16S | G | W | 13 |
| *Dongia* ! | 16S | G | W | 28 |
| *Reyranella* | 16S | G | W | 21 |

**Table 1** (continued)

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| *Devosia* ! | 16S | G | W | 95 |
| *Hyphomicrobium* ! | 16S | G | W | 23 |
| *Aureimonas* | 16S | G | W | 7 |
| *Bauldia* ! | 16S | G | W | 52 |
| *Pseudolabrys* ! | 16S | G | W | 174 |
| *Rhodobacter* ! | 16S | G | W | 72 |
| *Sphingomonas* ! | 16S | G | W | 813 |
| *Polymorphobacter* ! | 16S | G | W | 642 |
| *Novosphingobium* ! | 16S | G | W | 237 |
| *Rhizorhapis* ! | 16S | G | W | 231 |
| Fam. *Acetobacteraceae* | 16S | G | W | 104 |
| Fam. *Caulobacteraceae* | 16S | G | W | 192 |
| Fam. *Hyphomonadaceae* | 16S | G | W | 24 |
| Fam. *Micropepsaceae* | 16S | G | W | 171 |
| Fam. *Paracaedibacteraceae* | 16S | G | W | 12 |
| Fam. *Beijerinckiaceae* | 16S | G | W | 99 |
| Fam. *Rhizobiaceae* | 16S | G | W | 112 |
| Fam. *Xanthobacteraceae* | 16S | G | W | 72 |
| Order. *Ferrovibrionales* | 16S | G | W | 3 |
| **Class*Gammaproteobacteria*** | 16S | G | W | 295 |
| *Limnobacter* ! | 16S | G | W | 952 |
| *Ralstonia* | 16S | G | W | 28 |
| *Rhizobacter* ! | 16S | G | W | 2,136 |
| *Rhodoferax* ! | 16S | G | W | 192 |
| *Polaromonas* ! | 16S | G | W | 85 |
| *Aquicella* | 16S | G | W | 37 |
| *Acidibacter* ! | 16S | G | W | 32 |
| *Rhodanobacter* | 16S | G | W | 273 |
| *Dokdonella* ! | 16S | G | W | 21 |
| *Arenimonas* ! | 16S | G | W | 31 |
| Fam. *Comamonadaceae* | 16S | G | W | 1,659 |
| Fam. *Nitrosomonadaceae* | 16S | G | W | 158 |
| Fam. *Steroidobacteraceae* | 16S | G | W | 5 |
| Order *Burkholderiales* | 16S | G | W | 495 |
| **Phylum*Verrucomicrobiota*** | 16S | | | |
| *Neochlamydia* ! | 16S | G | W | 10 |
| *Chthoniobacter* ! | 16S | G | W | 314 |
| *Lacunisphaera* ! | 16S | G | W | 55 |
| *Opitutus* ! | 16S | G | W | 112 |
| Candidatus *Udaeobacter* | 16S | G | W | 62 |
| Candidatus *Xiphinematobacter* | 16S | G | W | 31 |
| Fam. *Pedosphaeraceae* | 16S | G | W | 659 |
| Fam. *Chthoniobacteraceae* | 16S | G | W | 17 |
| **Phylum*WPS-2*** | 16S | G | W | 19 |
| **Cadidatus Phylum*Patescibacteria*** | 16S | | | |
| *Parcubacteria* | 16S | G | W | 6 |
| *Candidatus Kaiserbacteria* | 16S | G | W | 27 |
| Order *Saccharimonadales* | 16S | G | W | 2,234 |
| **DOMAIN EUKARYA** | | | | |
| **KINGDOM FUNGI** | ITS2 | T, F, A, M | W | 378 |
| **Phylum*Ascomycota*** | | | | |
| Order *Helotiales* | ITS2 | T, F | W not tropical | 14,928 |
| *Chalara pseudoaffinis* | ITS2 | T, F | W | 619 |
| **Phylum*Basidiomycota*** | | | | |

**Table 1** (continued)

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| Class *Tremellomycetes* | ITS2 | T | W | 1,570 |
| *Leucosporidium* sp.! | ITS2 | T, F | Ant | 762 |
| *Phenoliferia psychrophila* | ITS2 | T, F | Alp, Ant | 242 |
| *Piskurozyma* sp. | ITS2 | | | 25 |
| **Phylum*Chytridiomycota*** | | | | |
| Order *Spizellomycetales* | ITS2 | T, F, M | W | 63 |
| *Chytridium* sp. | ITS2 | T, F, M | W | 32 |
| *Sanchytrium* sp. | ITS2 | T, M, F | | 37 |
| **Phylum*Rozellomycota*** | ITS2 | T, F, A, F | W | 41 |
| **KINGDOM CHROMISTA** | | | | |
| **Phylum Ciliophora** | | | | |
| Class Oligohymenophorea | ITS2 | F, M | W | 365 |
| Fam. Oxytrichidae | ITS2 | F, M | W | 69 |
| *Stylonychia lemnae* | ITS2 | F, T | W | 381 |
| Fam. *Vorticellidae* | ITS2 | F, B, M | W | 365 |
| **Phylum Bacillariophyta** | COX1 | F, M | W | 4 |
| Class Bacillariophyceae | COX1 | F, M | W | 8 |
| **Phylum Ochrophyta** | | | | |
| Class Chrysophyceae | | | | |
| *Cryptomonas curvata** | COX1 | F | Af, As, Eu, ME, NA, SA | 6 |
| *Segregatospumella* sp. | COX1 | F | Eu | 100 |
| *Spumella lacusvadosi** | COX1 | F | NZ | 9 |
| *Spumella* sp. | COX1 | F | W | 963 |
| Class Phaeophyceae | COX1 | M | W | 1,872 |
| Fam. Acinetosporaceae | COX1 | M | W | 21 |
| Fam. Agaraceae | COX1 | M | W | 88 |
| Fam. Chordariaceae | COX1 | M | W | 82 |
| Fam. Scytosiphonaceae | COX1 | M | W | 43 |
| *Botrydiopsis alpina* | COX1 | F, T | Eu | 65 |
| *Botrydium granulatum** | COX1 | F, T | W | 12 |
| *Dictyota hamifera** | COX1 | M | Af, As, CA, PI, SA | 16 |
| *Diplura* sp. * | COX1 | M | As, NA | 275 |
| *Durvillaea* sp. | COX1 | M | W | 9 |
| *Hydroclathrus tenuis** | COX1 | M | Af, As, PI, SEA | 135 |
| *Lobophora guadeloupensis** | COX1 | M | CA | 7 |
| *Mesospora* sp.* | COX1 | M | W | 4 |
| *Neoralfsia expansa** | COX1 | M | W | 216 |
| *Planosiphon nakamurae** | COX1 | M | As, SA | 19 |
| *Poterioochromonas malhamensis** | COX1 | F | Eu | 1,492 |
| *Stypopodium multipartitum** | COX1 | M | Af, As | 74 |
| **Phylum Oomycota** | COX1 | F, M, T | W | 10,083 |
| Fam. Peronosporaceae | COX1 | T, p.a. | W | 4 |
| Fam Phytiaceae | COX1 | T, p.a. | W | 295 |
| Fam. Saprolegniaceae | COX1 | F, M, T | W | 490 |
| *Achlya radiosa* | COX1 | F, p.a. | W | 5 |
| *Anisolpidium rosenvingei** | COX1 | M, p.a. | W | 346 |
| *Aphanomyces* sp. | COX1 | F, p.a. | W | 483 |
| *Bremia* sp. | COX1 | T, p.a. | W | 26 |
| *Crypticola clavulifera** | COX1 | T, p.a. | Au | 37 |
| *Phytophthora cactorum** | COX1 | T, p.a. | W | 59 |
| *Phytophthora meadii** | COX1 | T, p.a. | W | 6 |
| *Phytophthora* sp. ! | COX1 | T, p.a. | W | 178 |
| *Globisporangium mamillatum** | COX1 | T, p.a. | W | 101 |
| *Globisporangium marsipium** | COX1 | T, p.a. | W | 7 |

**Table 1** (continued)

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| *Globisporangium sylvaticum** | COX1 | T, p.a. | W | 24 |
| *Globisporangium ultimum** | COX1 | T, p.a. | W | 36 |
| *Globisporangium* sp. | COX1 | T, p.a. | W | 137 |
| *Pythium aphanidermatum* | COX1 | T, p.a. | W | 21 |
| *Pythium cucurbitacearum* | COX1 | T, p.a. | W | 134 |
| *Pythium insidiosum* | COX1 | T, F, p.a. | W | 653 |
| *Pythium monospermum* ! | COX1 | T, p.a. | W | 229 |
| *Pythium myriotylum* | COX1 | T, p.a. | W | 579 |
| *Pythium* sp.! | COX1 | T, p.a. | W | 94 |
| *Saprolegnia* sp. | COX1 | B, F, T | W | 154 |
| *Sapromyces elongatus* | COX1 | F | W | 152 |
| **KINGDOM PROTOZOA** | | | | |
| **Phylum Discosea** | | | | |
| *Cochliopodium crystalli** | COX1 | F | NA | 27 |
| *Cochliopodium kieliense** | COX1 | F | Eu | 4 |
| *Cochliopodium* sp. ! | COX1 | B, F, M | W | 5,530 |
| *Korotnevella* sp.* | COX1 | B, F | W | 11 |
| *Parvamoeba rugata** | COX1 | M | Eu | 162 |
| *Ripella* sp.* | COX1 | F | Eu, ME | 38 |
| *Vannella simplex* ! | COX1 | B, F | As, Eu | 290 |
| **Phylum Loukozoa** | | | | |
| *Reclinomonas americana* ! | COX1 | F | NA | 86 |
| **KINGDOM METAZOA** | COX1 | | | 14,247 |
| **Phylum Annelida** | | | | |
| *Chloeia parva** | COX1 | M | PO, IO | 19 |
| **Phylum Arthropoda** | COX1 | F, M, T | W | 25 |
| Class Arachnida | COX1 | F, M, T | W | 9 |
| Class insecta | COX1 | F, T | W | 10 |
| Class Mandibulata | COX1 | F, M, T | W | 24 |
| Order Diptera | COX1 | F, T | W | 218 |
| Order Hemiptera | COX1 | T | W | 19 |
| Order Hymenoptera | COX1 | F, T | W | 36 |
| Fam. Culicidae | COX1 | F, T | W | 9 |
| Fam. Desmosomatidae | COX1 | M | W | 25 |
| Fam. Entomobryidae | COX1 | T | W | 5,178 |
| Fam. Ichneumonidae | COX1 | F, T | W | 113 |
| Fam. Lysianassidae | COX1 | M | W | 4 |
| *Atergatis integerrimus** | COX1 | M | Au, IO, PO | 38 |
| *Campylaspis sulcata* | COX1 | M | Eu, SEA | 6,955 |
| *Cicadatra atra** | COX1 | T | As, Eu | 43 |
| *Culex atratus** | COX1 | F, T | CA, Car, NA, SA | 27 |
| *Eriocheir japonica** | COX1 | F, M | As | 298 |
| *Eurythenes gryllus* | COX1 | M | Ant, Arc, CA, WP | 2,695 |
| *Maccaffertium modestum** | COX1 | T | NA | 11 |
| *Mayetiola hordei** | COX1 | F, T | As, EU | 13 |
| *Neoplocaederus bicolor** | COX1 | T | As | 19 |
| *Ocyptamus* sp.* | COX1 | T | NT | 101 |
| *Ozestheria lutraria** | COX1 | F, T | Au | 15 |
| *Scopelocheirus schellenbergi** | COX1 | M | NZ, P, | 97 |
| *Spathius* sp.* | COX1 | T | As | 15 |
| **Phylum Chordata** | | | | |
| Class Actinopterygii | COX1 | F, M | W | 80 |
| *Engraulis japonicus** | COX1 | M | As, SEA, WP | 245 |
| *Homo sapiens* | COX1 | T | W | 135 |

**Table 1** (continued)

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| *Leptoclinides* sp.* | COX1 | M | W | 42 |
| *Neacomys* sp.* | COX1 | T | NT | 28 |
| *Xenopus tropicalis** | COX1 | F | Af | 15 |
| **Phylum Cnidaria** | | | | |
| Class Hydrozoa | COX1 | F, M | W | 91 |
| Order Zoantharia | COX1 | M | W | 4 |
| *Crambionella stuhlmanni** | COX1 | M | IO | 608 |
| **Phylum Echinodermata** | COX1 | M | W | 17 |
| **Phylum Gastrotricha** | | | | |
| *Turbanella cornuta** | COX1 | B, M | Eu | 13 |
| *Turbanella* sp.* | COX1 | B, M | W | 54 |
| **Phylum Mollusca** | | | | |
| Fam. Tegulidae | COX1 | M | W | 124 |
| *Anadara broughtonii** | COX1 | M | As, PO | 178 |
| *Atrina pectinata** | COX1 | M | W | 38 |
| *Bellamya* sp.* | COX1 | F | W | 5 |
| *Crassostrea virginica** | COX1 | B, M | NA, SA | 22 |
| *Mytilus edulis* | COX1 | M | W | 32 |
| *Mytilus trossulus** | COX1 | M | W | 32 |
| *Nipponacmea nigrans** | COX1 | M | P | 29 |
| *Pareledone charcoti* | COX1 | M | AS, Ant. | 5 |
| *Pulsellum salishorum** | COX1 | M | NA, PO | 7 |
| *Radix* sp. | COX1 | F | Palearctic | 405 |
| **Phylum Nematoda** | | | | |
| *Plectus murrayi* | COX1 | T | Ant | 2,511 |
| **Phylum Rotifera** | | | | |
| Class Eurotatoria | COX1 | B, F, M, T | W | 2,080 |
| Fam. Philodinidae | COX1 | B, F, M, T | W | 2,394 |
| *Adineta* sp. | COX1 | F | W | 44,512 |
| **KINGDOM VIRIDIPLANTAE** | | | | |
| **Phylum Charophyta** | | | | |
| *Chlorokybus atmophyticus** | COX1 | A, T | Eu, CA, SA | 60 |
| **Phylum Chlorophyta** | | | | |
| Class Trebouxiophyceae | COX1 | A, F, T | W | 34 |
| Order Chlamydomonadales | ITS2 | F, T | W | 10 |
| Fam Ulotrichaceae | ITS2 | M | W | 106 |
| *Chlamydomonas proboscigera*! | ITS2 | F | Eu, Saf, SEA, As | 68 |
| *Coenochloris* sp. | ITS2 | F | W | 37 |
| *Cymbomonas tetramitiformis** | COX1 | M | AU, Eu, NZ | 38 |
| *Lobochlamys segnis* | ITS2 | F | Eu, AU, NZ, As, SA | 19 |
| *Micromonas pusilla** | COX1 | M | AU, Eu, Na, NZ, SA | 512 |
| *Planophila* sp. | ITS2 | M | W | 60 |
| *Tetracystis* sp. | ITS2 | F | W | 63 |
| *Tupiella akineta* | COX1 | F | SEA | 3 |
| **Phylum Rhodophyta** | COX1 | F, M | W | 872 |
| Class Florideophyceae | COX1 | M | W | 89 |
| Fam. Rhodomelaceae | COX1 | M | W | 406 |
| *Arthrocardia carinata** | COX1 | M | Af, AI, | 12 |
| *Cyanidium caldarium** | COX1 | F | AU, As, Eu, NZ | 10 |
| *Leiomenia* sp.* | COX1 | M | AU, NZ, SEA | 2,605 |
| *Riquetophycus* sp. | COX1 | M | Eu | 28 |
| **Phylum Bryophyta** | | | | |
| Order Hypnales | ITS2 | T | W | 89,163 |
| *Sanionia* sp.! | ITS2 | T | Bipolar | 98 |

**Table 1** (continued)

| Taxa | Marker | Habitat | Distribution | Number of Reads |
|---|---|---|---|---|
| **UNKNOWN** | 16S | | | 197 |
| **UNKNOWN** | COX1 | | | 13,702 |
| **UNKNOWN** | ITS2 | | | 1,169 |

Asterisks indicate assigned taxa not previously recorded from Antarctica.! indicates taxa reported by Câmara et al. (2021c) from TMC

*Planctomycetota*, *Proteobacteria* (*Pseudomonadota*), *Verrucomicrobiota*, and *WPS-2*. A total of 2,267 ASVs were assigned to the phylum *Patescibacteria* (Candidate Phylum Radiation - CPR), which as yet are represented only by DNA sequences, and no isolate has yet been cultured (Rinke et al. 2013). The taxonomic rank of *Candidatus Patescibacteria* is yet to be determined. A total of 19 ASVs could only be assigned to the Domain Bacteria (Table 1). The phylum Proteobacteria contributed 36% of the assigned ASVs, followed by *Actinobacteriota* with 24% and *Bacteroidota* with 11%. The remaining phyla each contributed < 5% of assigned ASVs.

### ITS2 marker

A total of 116,630 reads were obtained of which 110,414 remained after quality control. A total of 109,245 reads represented three Kingdoms and eight phyla: Fungi (*Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Rozellomycota*), Chromista (Ciliophora), and Viridiplantae (Chlorophyta and Bryophyta). A total of 24 distinct taxa were assigned, all previously recorded from Antarctica. A total of 1,169 remained unassigned (Table 1).

### COX1 marker

A total of 204,203 reads were obtained, of which 114,399 remained after quality control. Of these, 100,697 reads represented four Kingdoms and 17 phyla: Chromista (Bacillariophyta, Ochrophyta and Oomycota) Protozoa (Discosea and Loukozoa), Metazoa (Annelida, Arthropoda, Chordata, Cnidaria, Echinodermata, Gastrotricha, Mollusca, Nematoda and Rotifera) and Viridiplantae (Charophyta, Chlorophyta and Rhodophyta). A total of 121 distinct taxa were assigned, of which 60 are not previously recorded from Antarctica. 13,702 reads remained unassigned (Table 1).

## Discussion

The generally higher diversities of most taxonomic groups found in the present study of the WMC when compared with the earlier study of the TMC (Câmara et al. 2021c) may suggest that the process of carrying out the first significant scale moss carpet transplant has been associated with a loss of contained diversity. Conversely, the finding of much higher fungal diversity in the TMC (Rosa et al. 2021) may be an indication of reduced moss health or increased fungal infection and subsequent decay processes, comparable with recent reports of fungal diversity linked with moss carpet 'fairy ring' disease (Rosa et al. 2020b; 2021). Whereas the current sampling is low and not enough for further conclusions we cannot fully rule out the possibility of the high level of impact on the WMC may also be related to species introduced due to human activity over decades in the surroundings of the sampling site, it is important to stress that the current low sampling was designed to facilitate comparison between the current study (WMC) and the previous one (TMC).

Multiple terrestrial and freshwater organisms have been reported as being deliberately or accidentally introduced to Antarctica (Frenot et al. 2005; Hughes et al. 2020; Leihy et al. 2023), although few have actually established in the natural environment (Hughes et al. 2016). The maritime Antarctic is identified as a region that is particularly at risk as it is experiencing considerable changes in climate, considerable intensification of human activity and is the closest part of Antarctica to another continental landmass (South America) (Chown et al. 2012; Siegert et al. 2019; Hughes et al. 2020). In common with other recent metabarcoding studies (Fraser et al. 2018; Câmara et al. 2021c, 2023, 2024), many of the taxa assigned here are not known to be resident in or even close to Antarctica. Given the overall poor documentation of Antarctic microbial diversity, it is plausible that considerable currently unrecognized native diversity could be detected by application of eDNA metabarcoding. However, as noted, the presence of either native or introduced taxa would require other approaches for confirmation that can identify viable living organisms. Some assignments, particularly of macroscopic plants and metazoans, could represent 'legacy' DNA or molecular pollution emanating from previous human activities, but many are so implausible as to represent practical limitations of the assignment methodologies and available sequence databases (see Fraser et al. (2018). On the other hand, Câmara et al. (2023) investigating aerosols from a latitudinal gradient of 40° (from Rio de Janeiro to King George Island), suggested that organisms do move across long distances.

As noted, the WMC is located immediately beneath the "Jacques Cousteau Whale", a site that has been visited since the mid 1970s, with tourist numbers increasing exponentially since the late 1980s. It is also very close to the Brazilian Comandante Ferraz Station and is regularly visited for both research and recreation by station staff. Ferraz Station was destroyed by a catastrophic fire in 2012, and rebuilding activities took place from 2013 until 2020, including the need for the significant and previously unattempted moss carpet transplant from specific sites (Câmara et al. 2021c). The site experienced particularly intense reconstruction activity in 2018–2020, involving hundreds of Chinese workers from the company CEIEC that carried out the reconstruction, and visits of multiple merchant vessels delivering cargo from China, Brazil, and Chile. While there is no direct evidence of importation of living organisms or their presence in the Antarctic (marine or terrestrial) environment, the association of non-native marine organisms with ship hull fouling, ballast water and sea chests, cargo, including their survival there during Antarctic deployments, is well known (Jensen and Armstrong 2004; Chown et al. 2012; McCarthy et al. 2019, 2022). A number of metagenomic studies have also postulated human foodstuffs as the source of exotic assigned DNA sequences obtained from the natural environment in the general vicinity of concentrations of human activity(Câmara et al. 2021a, 2023, 2024).

The overall assignment of marine taxa is unsurprising as the moss carpet is located very near the shoreline (<5 m) and both onshore winds and the movement of local marine birds and mammals provide effective means of moving marine particles (including DNA) into the moss carpet. Below we discuss briefly some of the most important findings; see Online Resource for more details.

## Bacteria

Bacteria form an important element of the moss microbiome where, particularly in harsh environments such as Antarctica, they make important contributions to nitrogen and carbon fixation (Klarenberg et al. 2023). Photosynthetic cyanobacteria are commonly found associated with mosses. In the present study, we assigned ASVs representing *Nostoc*, a nitrogen-fixing cyanobacterial genus that has been recorded from mosses, including in samples from the Arctic (Solheim et al. 2004). The *Acidobacteria* genus *Pseudanabaena* contributed the greatest proportion of ASVs, and along with the other genera, represents cosmopolitan genera that have previously been reported in Antarctic studies (Velichko et al. 2021). A total of 59 “uncultured Antarctic” ASVs were assigned here, related to the genus *Geitlerinema* which was also been reported from Antarctica (Lizieri et al. 2022). Other genera of nitrogen-fixing bacteria were assigned amongst the ASVs, such as members of the family *Beijerinckiaceae*. Methanotrophs are also commonly found associated with mosses (Park et al. 2013), but we did not detect the families *Methylococcaceae* (*Gammaproteobacteria/Pseudomonadota*), *Methylocystaceae* (*Alphaproteo bacteria*/*Pseudomonadota*) or *Methylacidiphilaceae* (*Verrucomicrobiota*) among the assigned ASVs. Acidophiles, another functional group of bacteria that feature in the moss microbiome, with representatives present in several phyla, such as the phyla *Verrucomicrobiota* and *Acidobacteriota*, contain many acidophilic bacteria and are known to be associated (Park et al. 2013) with mosses and lichens, being the third and fourth most common phyla in the present study.

Proteobacteria (*Pseudomonadota*) contributed the highest number of assigned bacterial ASVs. The predominant known genus representing this phylum was *Rhizobacter*, generally considered a pathogen of vascular plants but also recently obtained in metagenomic studies of soil and freshwater environments in general (Jin et al. 2016). Some assigned gammaproteobacterial ASVs represented genera commonly found in cold environments, such as *Polaromonas* (Irgens et al. 1996). Within the *Actinobacteriota*, the second most dominant phylum, the taxon contributing the highest number of ASVs was the family *Microbacteriaceae*, which contains many spore-forming soil bacteria. Members of this family have been reported associated with mosses outside Antarctica (Zhou et al. 2016). The third most represented phylum, *Bacteroidota*, also includes many moss-associated members (Klarenberg et al. 2023).

## Fungi

Fungi are generally a highly diverse eukaryotic group reported in Antarctic studies (Rosa et al. 2019). However, only 10 taxa were assigned in the current study. This contrasts with the report of Rosa et al. (2021), who detected high richness and diversity of fungal taxa associated with the TMC in a metabarcoding study. In our current study, the carpet moss, despite being stable, was under anthropogenic influence that could impact the resident fungal diversity. On the other hand, the greater fungal diversity in the TMC might be an indication of stress on the carpet or poor moss health (i.e., increased fungal infection for instance, see Rosa et al. 2020b, 2021). *Helotiales* (*Ascomycota*) were the dominant taxon detected in the current study of the WMC. Newsham et al. (2020) considered that the *Helotiales* include members that are superabundant in the rhizosphere soils of Antarctic plants, and they have been reported in association with Antarctic mosses in both dependent culturing (Rosa et al. 2020b) and eDNA metabarcoding (Rosa et al. 2021) studies.

## Chromista

ASVs were only assigned to the phylum Ciliophora using the ITS2 marker. The higher rank groups of this phylum are cosmopolitan and widespread, with many representatives present in Antarctica (Thompson et al. 2019; Pertierra et al. 2024). The Ochrophyta assigned include algal pathogens (*Anisolpidium*), a dipteran endobiotic parasite (*Crypticola clavulifera*) and several Oomycota (e.g., *Phytophthora* and relatives) that are plant pathogens with hundreds of widespread species worldwide that are responsible for considerable economic losses in multiple crop plants (Chaithra et al. 2025).

## Protozoa

The ASVs assigned to this group include harmless, free-living organisms. The genus *Cochliopodium* has not previously been reported from Antarctica. *Parvamoeba*, the smallest amoeba known, has also not previously been recorded at high latitudes (Rogerson 1993).

## Metazoa

This group provides an example of group where most of the generic or specific ASV assignments were to taxa (or close relatives) that are certainly not present in Antarctica or even in cooler regions of southern South America. Amongst these assignments, the mosquito genus *Culex* is of public health importance being vectors of several serious human diseases (Sá et al. 2020). The midge *Mayetiola hordei* (barley stem gall) is a cereal pest from Europe, Mediterranean region and North Africa (Gagne et al. 1991; Bouktila et al. 2006). Two species of crab were included amongst the assignments, however, reptant decapods cannot survive in waters surrounding Antarctica and no species occur there (Aronson et al. 2015). *Atergatis integerrimus* (Red egg crab) is a poisonous crab from warm waters, while *Eriocheir japonica* (Japanese mitten crab) is a catadromous invasive species used as food (Guo et al. 1997). It has been reported as an invasive species in North America, introduced *via* shipping ballast water (Jensen and Armstrong 2004), while its close Chinese relative, *Eriocheir sinensis*, is invasive in Europe (Herborg et al. 2005).

## Viridiplantae

Most of the assignments represent widespread green algae, including some taxa previously not recorded from Antarctica (Câmara et al. 2020). Some taxa of the latter have previously only been reported from warmer waters at low latitudes. The two moss taxa assigned are amongst the most common in the maritime Antarctic region.

## Caveats

The diversity differences apparent between the TMC and WMC may be an indication of the consequences of the transplant process; however, it must be recognized that these differences are based on single samples from each carpet, so no assessment is possible of how much variability might exist between individual samples. The greater fungal diversity in the TMC could be an indication of stress on the carpet or poor health (Rosa et al. 2020b, 2021; Câmara et al. 2021d), which is also consistent with our findings of greater overall diversity in the WMC than TMC in the current study.

The greater fungal diversity in the TMC might be an indication of stress on the carpet or poor moss health (i.e., increased fungal infection for instance) — much as one argues in the fairy ring papers. This would also be consistent with the finding of greater overall diversity in the WMC than TMC in this study, though bear in mind the sample size and variation caveat mentioned earlier.

As with all metagenomic studies, the ability to generate reliable taxonomic assignments for the ASVs obtained is highly dependent on the quality and completeness of the available databases. While this is continuously increasing, robust Antarctic baseline sequence data for many of the taxa assigned in this study are simply not available. The high proportion of apparently exotic taxa assigned, particularly with the 16S and COX1 markers, very likely indicates this lack of completeness of the consulted databases but could also indicate that some remotely sourced diaspores may be transported longer distances into Antarctica as suggested by Câmara et al. (2023) where, even in the absence of viability, the DNA may remain detectable for an unknown period. We also recognize that, even if correct, a taxonomic assignment does not confirm the presence of a living or viable organism or propagule, as, for instance, epithelial single cells, dead organisms and parts thereof, pollen and feces will contain detectable DNA.

# Conclusions

DNA metabarcoding provides a powerful tool to help assess the cryptic diversity potentially present and associated with moss carpets, as well as in the detection of potential non-native and invasive species. Data generated here show a seemingly highly impacted moss carpet. In order to better understand levels of diversity in the bryosphere and the potential sources of impacts to this, further detailed studies are necessary and already undergoing, including the use of a wider geographical range, increasing the sampling site, the use of more molecular markers and multi-year sampling approaches to assess the presence of viable organisms/propagules and comparison with different moss carpets remote from human activity.

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**Author contributions** F.L.V.B. performed field and lab work, analyzed the data, reviewed and edited the manuscript final version. P.E.A.S.C. secure funds, wrote the main draft, performed lab and field work and analyzed the data. M.C.S. is the data curator, performed lab and field work and writing the paper, especially the plants. F.A.C.L. performed the bioinformatics, pipelines and data analysis. P.C. conceptualization, revised the whole manuscript and discussed the data, especially the animals. C.C.B. revised the paper and performed the prokaryote analysis. L.H.R., performed fieldwork and analyzed the fungi data.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Competing interests** The authors declare no competing interests.

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