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MICROBIOLOGY

DNA metabarcoding reveal hidden diversity of periphytic eukaryotes on marine Antarctic macroalgae

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Abstract: Polar marine macroalgae thrive in extreme conditions, often displaying geographic isolation and high degree of endemism. The "phycosphere" refers to the zone around the algae inhabited by microrganisms. Our study used DNA metabarcoding to survey the eukaryotic communities associated with seven seaweed species obtained at King George Island (South Shetland Islands, maritime Antarctic), including two Rhodophyta, two Chlorophyta and three Phaeophyceae. The ITS2 region was used as a barcode and our analysis yielded 77 eukaryotic ASVs spanning five Kingdoms (Fungi, Metazoa, Chromista, Protozoa, and Viridiplantae) and ten phyla (Ascomycota, Basidiomycota, Cercozoa, Ciliophora, Ochrophyta, Amebozoa, Chlorophyta, Rhodophyta, Bryophyta and Cnidaria). Additionally, we identified 14 potential new occurrence records for Antarctica. Ciliates and green algae were the most species-rich groups. The most abundant assigned associated species was *Monostroma angicava* (Chrorophyta). Within the macroalgal, the Chlorophyceans *Ulothrix* sp. hosted the greatest number of taxa, followed by *Monostroma hariotii*. Our data suggested that Antarctic macroalgae host a rich diversity of associated organisms and the biodiversity associated with the phycosphere remains underestimated.

Key words: chlorophyta, high throughput sequencing, King George Island, Phaeophyceae, Rhodophyta, seaweeds.

INTRODUCTION

The Southern Ocean harbors a rich variety of macroalgae, including numerous endemic species. Macrophycological studies have established an extensive biodiversity database for this region (Wiencke et al. 2014). Oliveira et al. (2020) presented a checklist of 151 macroalgal species covering the entire Antarctic region, while Pellizzari et al. (2017) provided a comparative taxonomic list of 104 species for only eight islands in the South Shetland Islands (SSI). Recently, Pellizzari et al. (2023) reported eight new records from Vega Island in the north-western Weddell Sea east of the tip of the Antarctic Peninsula and five from the SSI. Studies on King George Island (KGI), particularly in Admiralty Bay, have contributed the most to seaweed taxonomic research in the SSI, with 74 species currently identified (Zieliński 1990, Quartino et al. 2005, Oliveira et al. 2009, Yoneshigue-Valentin et al. 2013, Valdivia et al. 2014, Pellizzari et al. 2017). Considerable progress has been made in developing knowledge of Antarctic macroalgae (reviewed by Gómez & Huovinen 2020). However, the identification of some, particularly cryptic, taxa is now being revisited using molecular tools in addition to the

traditional morphological approaches. The SSI, including KGI, is an ecotone between the sub-Antarctic islands and the Antarctic Peninsula, and is therefore of particular interest for studies of species distributions, particularly in the context of regional climatic changes (Zieliński 1990, Sanches et al. 2016).

Macroalgae are primary producers and represent a polyphyletic assemblage of organisms possessing chlorophyll and other photosynthetic pigments. Polar representatives are adapted to extreme and stressful environmental conditions, such as wide seasonal variations in photoperiod (long periods of continuous light or dark seasonally), irradiance and UV radiation exposure. Polar macroalgal distributions reflect adaptations to distinct environmental conditions, such as temperature, salinity, pH, ice dynamics and substrate availability for recruitment Pellizzari et al. (2017). Due to the region's geographic isolation and typically extreme abiotic stresses, the Antarctic macroalgal flora is characterized by a high degree of endemism (Wiencke & Clayton 2002), and attracts interest as producers of diverse bioactive compounds that may be used in, for instance, the cosmetic and pharmaceutical industries (Barbosa et al. 2014, Martins et al. 2018, Olasehinde et al. 2019, Negreanu-Pirjol et al. 2022).

Symbiotic microorganisms often live in association with macroalgae, and the term "phycosphere" refers to the zone that extends outwards from the algal surface, analogous to the rhizosphere in soils around terrestrial plant roots, where microbial growth can be stimulated by extracellular products of the algae (Bell & Mitchell 1972). This zone plays an important role in nutrient fluxes (Bell & Mitchell 1972, Amin et al. 2012). Many bioactive compounds isolated from marine organisms originate from enzymatic interactions with symbiotic microorganisms (Variem & Kizhakkedath 2021). Additionally, microorganisms such as microalgae that live in association with the macroalgal phycosphere can produce their own distinct bioactive compound (Metting & Pyne 1986, Singh et al. 2005). Microorganisms can also modify the chemical environment in their immediate vicinity, including oxygen and pH levels, and release a wide variety of organic compounds (Azam & Malfatti 2007, Philippot et al. 2013, Seymour et al. 2017).

Although the term phycosphere was originally developed with reference to bacterial studies, many other microorganisms are also present in the phycosphere, including Fungi, Protozoans, Chromista and microalgae. However, little is yet known about these microbial communities as they are both difficult to sample and challenging to identify. The phycosphere may represent a poorly explored niche of marine diversity, where certain species may thrive or even be unable to survive beyond its boundaries.

Microorganisms associated with macroalgae and responsible for the synthesis of potentially valuable metabolites are difficult to identify due to the current lack of knowledge of these communities. Culturing approaches are often selective towards generalist species and may exclude rare or specialist species, rendering identification even more challenging (Broady 1996, Coêlho et al. 2019). Recent developments in molecular biology have allowed considerable advances in the assessment of diversity in environmental samples obtained from various ecosystems. DNA metabarcoding using high-throughput sequencing (HTS) provides an accessible method for the detection of the DNA of different organisms (Rippin et al. 2018, Ruppert et al. 2019). At the same time, advances in DNA sequencing allow the direct evaluation of the sequence diversity and species diversity present in environmental samples (eDNA; Taberlet et al.

2012). Câmara et al. (2021b) used this approach to compare periphytic diversity between two lakes in the South Shetland Islands, but this approach has not yet been used widely in the assessment of marine macroalgal periphytic biodiversity in Antarctica. In this study we used DNA metabarcoding to survey eukaryotic communities associated with marine macroalgae obtained from Punta Plaza, Admiralty Bay, King George Island.

MATERIALS AND METHODS

Sampling and species identification

Samples of seven species of macroalgae were collected, one individual of each species, from one site at the intertidal zone of a rocky shore, amongst boulders in tidal pools during the austral summer of 2021/22. Collections were made at Punta Plaza, at the tip of the Keller Peninsula, King George Island, South Shetland Islands (62°05'03'' S; 58°23'30''W), ca. 1 km from the Brazilian Comandante Ferraz Antarctic Station (Fig. 1). Samples of two red (Rhodophyta), two green (Chlorophyta) and three brown (Phaeophyta) seaweed species were collected using sterilized gloves and sealed in sterile plastic bags (Whirl Pack®/US). Samples were rapidly returned to the research station where they were kept frozen (-20 °C) until DNA was extracted under sterile conditions in the molecular biology laboratory at Ferraz Station. Species were selected in order to reflect different taxonomic groups and different morpho-functional groups. The macroalgae were morphologically identified based on

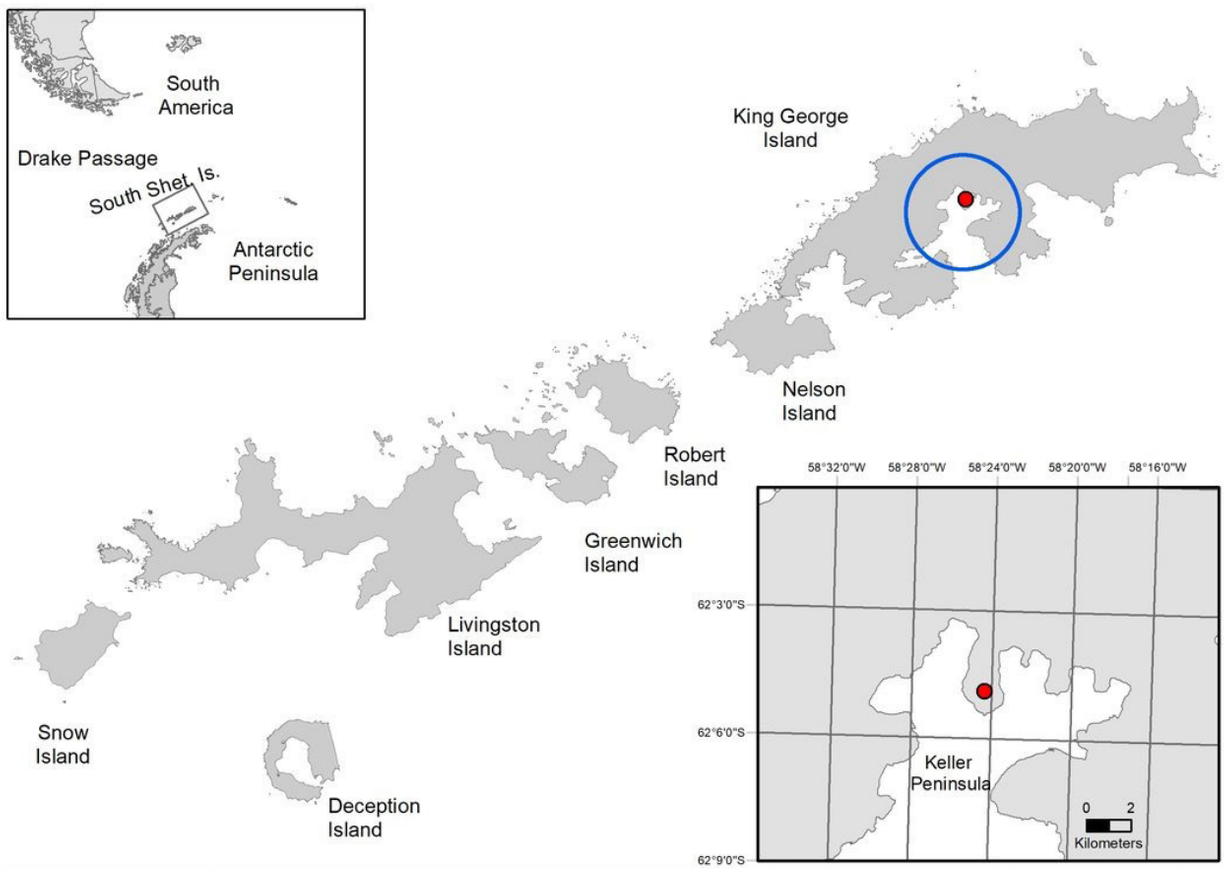

Figure 1. Sampling area of Punta Plaza (dot), Admiralty Bay (circle), King George Island, South Shetland Islands.

external and internal features of the vegetative and reproductive (when present) structures, and classified with respect to functional groups (Steneck & Dethier 1994) (Table I, Fig. 2). Also, DNA data helped to confirm its identity. Seaweed distribution and nomenclature follows Guiry & Guiry (2023).

DNA extraction and sequencing

A total of 1 cm^2 of macroalgal thallus was placed in a sterile plastic tube for DNA extraction. Total DNA was extracted using the FastDNA Spin Kit for Soil (MPBIO, Ohio, 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). Negative controls did not contain any detectable DNA. We used the internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA (Richardson et al. 2015, Chen et al. 2010, Câmara et al. 2021a, b, 2022) as a barcode, as it has been widely applied to identify a diverse range of eukaryotic organisms including fungi, animals, protozoans, chromists and plants (Ruppert et al. 2019), and has proved effective in recent studies of Antarctic diversity using environmental samples (Rosa et al. 2020, Câmara et al. 2021a, b, 2022, Carvalho-Silva et al. 2021, Ogaki et al. 2021). For this step we used the universal primers ITS3 and ITS4 (White et al. 1990). Library construction and DNA amplification were performed using the library kit Herculase II Fusion DNA Polymerase Nextera 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 taxa identification

Quality analysis was carried out using BBDuk v. 38.87 in BBmap software (BBMap - Bushnell B.; sourceforge.net/projects/bbmap/) with the following parameters: Illumina adapters were removed (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, turn paired-end fastq files into merged, and remove chimeras,

Table I. Macroalgal species sampled at Punta Plaza, King George Island, including reference code and taxonomic and morpho-functional group classification.

Taxa	Code	Taxonomic group	Morpho-functional group
Desmarestia menziesii J. Agardh	A	Ochrophyta/Phaeophyceae/Desmarestiales	Leathery branched
Monostroma hariotii Gain	B	Chlorophyta/Ulotrichales	Foliose, balloon single layered
Ulothrix sp.	C	Chlorophyta/Ulotrichales	Filamentous
Sarcopeltis skottsbergii (Setchell & N.L.Gardner) Hommersand, Hughey, Leister & P.W.Gabrielson	D	Rhodophyta/Gigartinales	Terete/fleshy; rough
Adenocystis utricularis (Bory) Skottsberg	E	Ochrophyta/Phaeophyceae/Ectocarpales	Balloon
Iridaea sp.	F	Rhodophyta/Gigartinales	Terete/fleshy
Ascoseira mirabilis Skottsberg	G	Ochrophyta/Phaeophyceae/Ascoseirales	Leathery

using default parameters (Callahan et al. 2016). Taxonomic assignments of ASVs (amplicon sequence variants) were determined using the qiime2-feature-classifier (Bokulich et al. 2018) classify-sklearn against different databases, using a sequence similarity threshold of 97%. First, ASVs were classified against the PLANiTS2 database (Banchi et al. 2020). After this step, ASVs that remained unclassified were filtered and classify-sklearn classified against the UNITE Eukaryotes ITS database version 8.3 (Abarenkov et al. 2020). Finally, remaining unclassified ASVs were filtered and aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (October 2021) using BLASTn (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). For simplicity we henceforth refer to the assigned ASVs as "taxa". Venn diagrams were prepared as described by Bardou et al. (2014). For comparative purposes, we consider reads as a proxy for relative abundance (Deiner et al. 2017, Hering et al. 2018, Câmara et al. 2021a, b, Carvalho-Silva et al. 2021, Rosa et al. 2021).

Diversity analyses

Rarefaction calculations were carried out using the rarefaction analysis command in the software PAST 4.03 (Hammer et al. 2001). The Simpson index was used to estimate the probability that two individuals selected at random from the sample would belong to the

Figure 2. Macroalgal species sampled in this study. a) *Desmarestia menziesii* (Phaeophyta), b) *Monostroma hariotii* (Chlorophyta), c) *Ulothrix* sp. (Chlorophyta), d) *Sarcopeltis skottsbergii* (Rhodophyta), e) *Adenocystis utricularis* (Phaeophyta), f) *Iridaea* sp. (Rhodophyta), g) *Ascoseira mirabilis* (Phaeophyta). Scale bar 1, 2, 3, 5, 7 = 1 cm; 4 and 6 = 0.5 cm. Images by F. Pellizzari.

same species. The Shannon index was used to assess the degree of uncertainty in predicting the species identity of an individual chosen at random from the sample. We also calculated the Equitability (Pielou's evenness) index by dividing the Shannon diversity index by the logarithm of the number of taxa present in the sample. This index reflects the evenness with which individuals are distributed among the taxa present. Additionally, we used the Margalef index to estimate the biodiversity of the community based on the numerical distribution of individuals from different species relative to the total number of individuals in the sample (Hammer et al. 2001, Magurran 2021). Geographical distributions are based on Guiry & Guiry (2023). DNA reads from host species are presented in table but excluded from ecological analysis.

RESULTS

A total of 1,789,704 paired-end DNA reads were generated in the sequencing run and 553,618 reads remained after quality filtering, 6,778 were unknown eukaryotes and 546,840 reads represented 73 eukaryotic ASVs. These included five Kingdoms (Fungi, Metazoa, Chromista, Protozoa and Viridiplantae) and ten phyla (Ascomycota, Basidiomycota, Cercozoa, Ciliophora, Ochrophyta, Amebozoa, Chlorophyta, Rhodophyta, Bryophyta and Cnidaria) (Fig. 3, Table II). Some sequences could only be assigned at higher taxonomic level (family, order or division). The calculated rarefaction curves indicated that the DNA reads gave an accurate representation of the local diversity in all seven samples (Fig. 4).

The highest numbers of ASVs were present in samples obtained from *Desmarestia menziesii* (A), *Monostroma hariotii* (B), *Ulothrix* sp. (C) and *Sarcopeltis skottsbergii* (D). The most species-rich groups detected based on assigned sequences in this study were the ciliates, with 21 taxa and the green algae, with 20 taxa. The most abundant assigned species was *Monostroma angicava* Kjellman. Amongst the macroalgae sampled, *Desmarestia menziesii*, a leathery branched brown alga, hosted the highest number of assigned taxa (41), followed by *Ulothrix* sp., a filamentous green alga with 37 (Table II) Species with balloon shape, as well as those with terete fleshy surfaces such as *Adenocystis utricularis* (a brown alga), hosted fewer assigned taxa with the lowest number (07). Table III provides the ecological indices associated with the various macroalgae. Specific taxon assignments included 14 potential new occurrence records for Antarctica (Table II).

The Simpson index (Table III) showed higher values for *Ulothrix* sp. and *D. menziesii* (> 0.5) than for *M. hariotii* and *S. skottsbergii*, indicating that these substrata hosted the most diverse communities. Similarly, the Shannon index showed the highest diversity for *Ulothrix* sp. and *D. menziesii*, followed by *M. hariotii* and *S. skottsbergii*. The Equitability analysis showed low values (<0.4) for all samples, indicating that some taxa have a much higher abundance of reads than others, even in areas with higher Simpson and Shannon values, suggesting that certain species are dominant in these samples. Margalef's diversity index also showed the highest values for the communities obtained from the four macroalgae mentioned above, with *Ulothrix* sp. having the highest value. However, as the sampling number (n) was low, these results should be taken carefully as purely descriptive as they may have been occurred by chance.

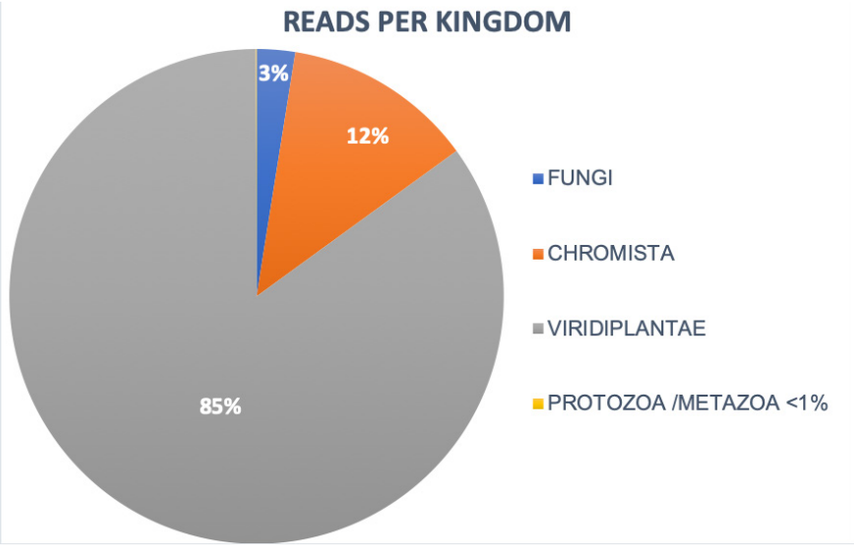

Figure 3. Percentage of the Eukaryotic kingdoms detected by applying a metabarcoding approach to the phycosphere of seven different Antarctica macroalgae.

Table II. Taxa associated with the sampled macroalgal species (A to G, as listed in Table I), bases on assigned sequences. * Taxon not previously recorded from Admiralty Bay; ** taxon not previously recorded from Antarctica.

	Number of DNA reads						
Taxa	**A**	**B**	**C**	**D**	**E**	**F**	**G**
KINGDOM FUNGI							
Phylum *Ascomycota*							
Chaetomium sp.	16	0	0	8	0	0	0
Aureobasidium pullulans	0	0	0	0	0	0	12560
Cladosporium sp.	310	0	0	0	0	0	0
Candida parapsilosis	181	0	0	0	0	0	0
Hortaea werneckii	56	0	49	0	0	0	23
Tetracladium sp.	61	0	0	0	29	0	0
Debaryomyces hansenii	44	0	0	0	0	0	0
Helotiales sp.	0	26	0	0	0	0	0
Ciliophora sp.	0	0	0	0	0	0	10
Saccharomyces sp.	7	0	0	0	0	0	0
Phylum *Basidiomycota*							
Cystofilobasidium infirmominiatum	129	0	0	0	0	0	0
Malassezia restricta	43	32	0	0	0	0	0
Curvibasidium rogersii	53	0	0	0	0	0	0
Xylodon flaviporus	0	50	0	0	0	0	0
Malassezia globosa	21	0	0	0	0	0	0
Glaciozyma litoralis	17	0	0	0	0	0	0

Table II. Continuation.

Cutaneotrichosporon debeurmannianum	0	0	4	0	0	0	9
Vishniacozyma victoriae	11	0	0	0	0	0	0
KINGDOM CHROMISTA							
Phylum Cercozoa	42	0	0	0	0	0	0
Phylum Ciliophora							
Class Oligohymenophorea	449	7	717	4	4	0	0
Class Phyllopharyngea	31	0	0	0	0	0	0
Class Spirotrichea	351	0	0	0	0	0	0
Order Dysteriida	3	56	109	131	0	0	0
Order Sporadotrichida	0	0	13	43	0	0	0
Order Tricladida	0	0	0	1140	0	0	0
Fam. Kyaroikeidae	607	0	0	0	0	0	0
Aspidisca sp.	0	0	18	0	0	0	0
Chlamydonella sp.	0	32	0	0	0	0	0
*Dysteria brasiliensis***	0	12	0	11	0	0	0
*Dysteria derouxi***	0	19	0	0	0	0	0
Dysteria sp.	309	215	220	142	0	20	0
Holosticha sp.	4408	385	5771	2728	0	12	0
Lacrymaria sp.	0	0	5	0	0	0	0
Mesanophrys sp.**	55	32	0	13	0	0	0
Parauronema sp.	448	0	67	47	0	0	0
Pseudovorticella sp.**	0	179	0	0	0	0	0
Strombidium sp.	36	19	221	292	0	24	0
*Urceolaria mitra***	0	0	27	0	0	0	0
Uronema sp.	0	0	80	0	0	0	0
Zosterodasys sp.	0	0	38	4	0	0	0
Phylum Ochrophyta							
Class Fragilariophyceae	0	20	0	0	0	0	0
Fam. Bacillariaceae	1932	226	466	134	0	0	0
Colpomenia sp.**	08	43435	1232	270	0	0	0
Navicula perminuta	798	90	0	0	0	0	0
Navicula sp.	32	0	0	0	0	0	0
Porosira glacialis	0	0	0	11	0	0	0
KINGDOM PROTOZOA							
Phylum Amebozoa							

Table II. Continuation.

Fam. Paramoebidae	0	0	2	0	0	0	0
Fam. Vannellidae	72	0	16	11	0	0	0
KINGDOM VIRIDIPLANTAE							
Phylum Chlorophyta							
Order Ulvales	61	250	89	708	0	0	0
Order Ulotrichales	0	89	0	0	0	0	0
Fam. Ulotrichaceae	686	0	220	115	0	0	0
Blidingia sp.	0	0	573	0	86	0	0
*Collinsiella tuberculata***	0	1165	104	0	0	0	0
Desmococcus olivaceus	0	14	0	18	0	0	0
Kornmannia leptoderma	161	293	20	24	0	0	0
Lithotrichon sp.**	0	27	0	0	0	0	0
*Monostroma angicava***	107	1468	459	29	88180	88663	95249
Monostroma sp.	95	252	131	52	0	0	0
Paulbroadya petersii	0	0	22	0	0	0	0
*Prasiola delicata **	0	51	207	58	0	0	0
Prasiola sp.	0	78	57	346	0	109	25
Protomonostroma sp.	0	0	156	75	0	0	0
Pseudendoclonium submarinum	0	0	88	0	0	0	0
Pseudendoclonium sp.	40	0	111	23	0	0	16
*Pseudothrix groenlandica***	926	216	3908	1755	355	2657	593
Ulothrix sp.	8546	185	11383	4282	637	104	461
Umbraulva japonica	43817	1243	0	0	0	11	50
Urospora sp.	1945	2725	34218	53497	47	143	7769
Phylum Rhodophyta							
*Laurencia thyrsifera***	0	92	183	69	0	0	0
Laurencia sp.	270	1346	213	0	0	0	0
Phylum Bryophyta							
Sanionia sp.	0	49	17	0	0	0	0
KINGDOM METAZOA							
Phyllum Cnidaria							
*Edwardsia timida***	12	0	0	0	0	0	0
*Sarsia lovenii***	0	166	0	0	0	0	0
Unknown Eukarya	2480	2458	377	221	658	517	67
Total Reads	69676	57002	61591	66261	89996	92260	116832

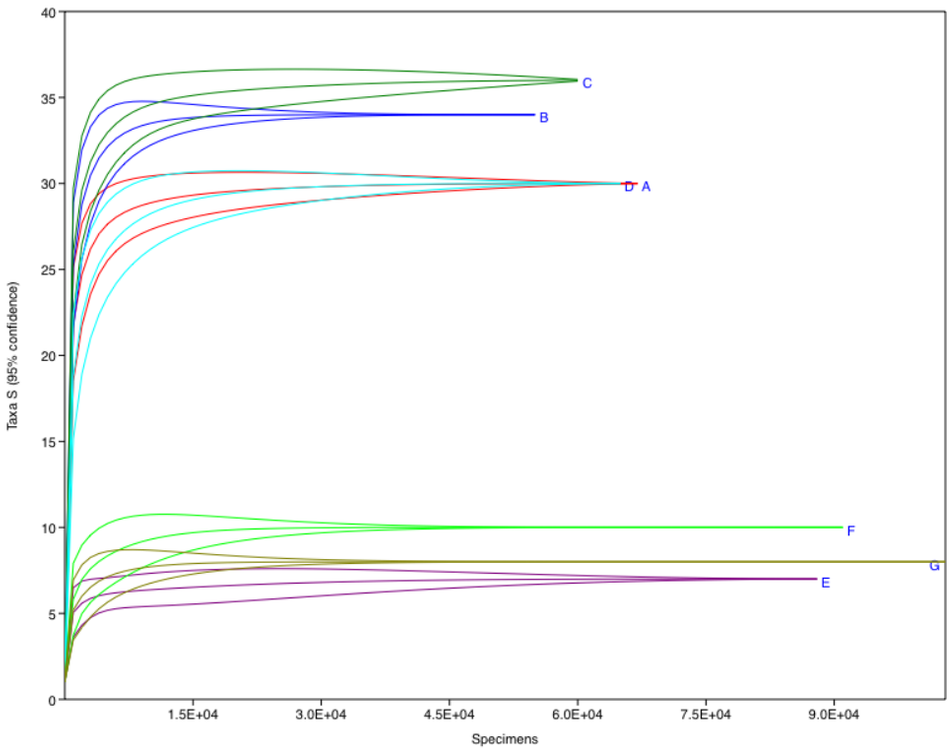

Figure 4. Rarefaction curves and 95% confidence limits, based on taxa profile (0.03 similarity) obtained from the seven sampled macroalgal species. a) ***Desmarestia menziesii***, b) ***Monostroma hariotii***, c) ***Ulothrix*** sp., d) ***Sarcopeltis skottsbergii***, e) ***Adenocystis utricularis***, f) ***Iridaea*** sp. and g) ***Ascoseira mirabilis.***

DISCUSSION

Ecological analysis

The macroalgal group with the greatest periphyton diversity (including primarily other micro- or macroscopic algae) was the delicate Chlorophyta/Ulotrichales, represented here by *M. hariotii* and *Ulothrix* sp., a monostromatic vesicular foliaceous thallus and a filamentous thallus, respectively. These were followed by the dichotomous leathery brown *D. menziesii*, and the red algae *S. skottsbergii* (Rhodophyta, terete/fleshy and rough). These observations may suggest that the macroalgal morphofunctional group has limited influence on the occurrence of certain faunal or algal groups in the associated periphyton, although further studies using increased sample sizes are required to fully assess the levels of variability within and between macroalgal species. It is notable that ciliates and protozoans were not detected in the sample from *A. mirabilis* and almost absent in that from *A. utricularis*, although the reasons underlying this are unclear. As the study was designed as preliminary investigative survey of overall periphyton diversity associated with marine macroalgae, the low number of samples and lack of replicates did not allow deeper analysis of the variation in epiphytic communities between individuals and macroalgal species.

Species distribution

Fungi

The relationships between fungi and marine macroalgae have been little studied in detail and range from parasitism to mutualism. We detected the DNA of 18 fungal taxa representing the phyla *Ascomycota* and *Basidomycota*. *Aureobasidium pullulans* (*Ascomycota*) was the only abundant fungal taxon assigned and was recorded on the thalli of *A. mirabilis*. *Ascomycota* and *Basidiomycota* are the most common fungi reported from different regions in Antarctica,

Table III. Ecological indices of the assemblages present in the phycospheres of seven macroalgae sampled at Punta Plaza, King George Island, South Shetland Islands.

Diversity indices	A	B	C	D	E	F	G
Number of taxa	41	35	37	30	7	9	11
Number of DNA reads	69,676	57,002	61,591	66,261	89,996	92,260	116,832
Simpson's	0.55	0.36	0.64	0.34	0.03	0.06	0.32
Shannon H	1.43	1.02	1.53	0.87	0.08	0.17	0.64
Margalef's	3.56	3.11	3.226	2.61	0.52	0.70	0.86
Equitability	0.38	0.29	0.42	0.26	0.04	0.08	0.29

including in association with macroalgae (Rosa et al. 2019). *Aureobasidium pullulans* is a cosmopolitan yeast-like polymorphic fungus present in cold ecosystems (Ruisi et al. 2007, Buzzini et al. 2018), including Antarctica (Da Silva et al. 2022). In addition, as a cosmopolitan fungus, *A. pullulans* has been described as endophyte of macroalgae at the Atlantic coast of Canada (Flewelling et al. 2013).

Chromista

Three phyla representing Chromista were present. Only higher rank taxa were recognized amongst the Cercozoa, limiting interpretation as this group that includes more than 700 species (mostly defined based on molecular studies) and is very common in terrestrial, freshwater and marine environments, with many known to live in the phycosphere (Bass & Cavalier-Smith et al. 2004).

Taxa representing Ciliophora include many cosmopolitan taxa that are known to occur in Antarctica. A number of genus-level assignments made may represent known Antarctic species. *Aspidisca* is a genus with 41 described species, including *A. antarctica* reported from the Weddell Sea (Petz et al. 1995) and *A. terranovae* from Terra Nova Bay (Valbonesi 1996). Members of the genus *Chlamydonella* are also reported from Antarctica, *C. prostomata* from the Weddell Sea (Song & Wilbert 2000) and *C. pseudochilodon* from the Ross Sea (Petz et al. 1995). *Dysteria* is a genus including about 45 species (Zhao et al. 2022) but, due to the lack of morphological features that can be used for reliable species identification, there have been many misidentifications and synonyms (Gong et al. 2007, Zhao et al. 2022). Two species, *D. calkinski* and *D. monostyla*, have been reported from the Atlantic sector of the Southern Ocean and from the Ross Sea (Petz et al. 1995, Song & Wilbert 2000). The two specific taxa assigned in this study represent new records, *D. brasiliensis* known from tropical waters in Brazil and *D. derouxi* from the Yellow Sea (Gong et al. 2007, Zhao et al. 2022). However, caution is required in interpreting this finding, as sequences of the Antarctic species *D. calkinski* are not available in the databases used. *Holosticha* is a genus containing more than 70 species, with three reported from the Ross Sea: *H. foissneri, H. pullaster* and *H. spindleri* (Petz et al. 1995). *Lacrymaria* also includes more than 40 species, with *L. lagenula* and *L. spiralis* also reported from the Ross Sea (Petz et al. 1995). *Metanophrys* is a poorly-known genus, that can live freely in the shallow surface of tissues of newly dead juvenile crustaceans and can also invade the hemolymph of living animals (crabs, lobsters and shrimps), leading to death (Small 2012). The genus is reported from Europe and

the Pacific coast of North America, and this is the first putative record from Antarctica. *Parauronema virginianum* (= *Uronema acutum*) and *P. antarcticum* (= *U. antarcticum*) have both been reported from the Atlantic sector of the Southern Ocean (Thompson & Croom 1978) and the Weddell Sea (Petz et al. 1995). The genus *Pseudovorticella*, with 60 species, is very hard to separate from the closely related *Vorticella* (Zhang et al. 2022), and is a very difficult genus to study. According to Thompson et al. (2019), five species of *Vorticella* have been recorded from Antarctica (*V. astyliformis, V. companula, V. infusionum, V. microstoma* and *V. striata*). The genus *Strombidium* includes 13 species reported from Antarctica (Petz 2005), mostly from the Ross Sea and Weddell Sea, but its taxonomic complexity, the many existing synonyms and its poorly known morphology again hamper better assessment.

Urceolaria mitra is an epizoic species that lives on the surface of flatworms (Bowen 1994, Rataj & Vdačný 2019) Many Oligohymenophorea are endo- or ectosymbionts and some can cause diseases in invertebrates, while other *Urceolaria* are free-living. The genus *Uronema*, which contains more than 15 species, has four known Antarctic representatives, with *U. acutum* and *U. antarcticum* reported from the Weddell Sea (Petz et al. 1995) and Atlantic sector of the Southern Ocean (Thompson & Croom 1978), *U. marinum* from the Ross Sea (Rataj & Vdacný 2019) and the Atlantic sector of Southern Ocean (Thompson & Croom 1978) and *U. elegans* from the Atlantic sector of Southern Ocean (Thompson et al. 2019). Ciliates belonging to the class Scuticociliates, such as *Uronema*, are obligate parasites causing significant economic losses in aquatic animals, amongst other diseases, (Piazzon et al. 2014). The genus *Zosterodasys* has nine described species (Vdačný & Tirjaková 2012) one of which, *Z. kryophilus*, has been reported from the Weddell Sea (Petz et al. 1995).

Amongst the Ocrophyta, the class Fragilariophyceae, family Bacillariaceae, includes many species that are widely distributed both globally and in the Antarctic. The genus *Colpomenia* includes 11 currently described species widely distributed in tropical and temperate waters, with no previous records from Antarctica (Guiry & Guiry 2023). Of these, only *C. sinuosa* (Mertens ex Roth) Derbès & Solier, occurs as far south as Iles Kerguelen in the sub-Antarctic (Papenfuss 1964, Féral et al. 2021).

Protozoa

Protozoa could only be assigned at family rank (Paramoebidae and Vannellidae), with both assignments from the phylum Amebozoa. However, the assigned taxa include hundreds of marine species, including both potentially dangerous and free-living representatives.

Viridiplantae

Amongst the Chlorophyta assigned in this study, most taxa have previously been reported in Antarctic studies. Amongst the new records, *Lithotrichon* is a genus containing two known species, with records from North America, the Middle East and Asia (Škaloud et al. 2018). Similarly, *Collinsiella tuberculata* Setchell & N. L. Gardner is a species only known from North America and Asia (Guiry & Guiry 2023). *Prasiola delicata* Setchell & N.L. Gardner is also only known from North America and Asia, but its close relative *P. crispa* (Lightfoot) Kützing, which is widely recorded in Antarctica (Dubrasquet et al. 2021).

Many members of the orders of green algae, Ulvales and Ulotrichales are opportunistic. Their taxonomy also remains very uncertain and is under revision (Hughey et al. 2019, Cui et al. 2022, Da Silva et al. 2022). The assignment of

Kornmannia is potentially notable, as this taxon, previously known as *Monostroma*, has only been recorded from the Northern Hemisphere, including the Arctic. Pellizzari et al. (2017) identified *M. grevillei* (Thuret) Wittrock from Deception Island using several markers, including ITS, another species previously only recorded from the Arctic and Northern Hemisphere, and speculated that it may have been introduced in association with the island's historical whaling industry. Sequences assigned to *K. leptoderma* (Kjellman) Bliding have also been reported in a terrestrial study on Deception Island (Câmara et al. 2021b). *Monostroma angicava* Kjellman is a further European and Asiatic species not previously recorded from Antarctica. *Urospora* and *Ulothrix* are cosmopolitan genera with many described taxa, including some that are common in the South Shetland Islands such as *Urospora penniciliformis* (Roth) Areschoug, *Ulothrix flacca* (Dillwyn) Thuret and *U. australis* Gain. *Umbraulva japonica* (Holmes) Bae & I.K.Lee is a strictly Asian species. While it could have been introduced by human activities, it is important to note that this genus is closely related to *Ulva* and the taxonomy of both is being revisited.

Monostroma angicava Kjellman, a delicate monostromatic foliaceous Ulothrichales, generated high numbers of ASV reads from several of the seaweeds examined here, including *Adenocystis utricularis, Iridaea* sp. and *Ascoseira mirabilis*. Available records of this species originate from Norway (Jaasund 1965), China (Ding & Luan 2013, Pellizzari et al. 2017), Japan (Yoshida et al. 2015, Horinouchi et al. 2019), Korea (Bae 2010), and Sakhalin Island (Tokida 1954).

Amongst the Rhodophyta, *Laurencia* is another genus whose taxonomy remains unclear, but *Laurencia thyrsifera* has a Southern Hemisphere distribution in Australian and New Zealand waters, including the Chatham Islands (Nelson et al. 2014). Within this genus of over 100 species, *L. chilensis* De Toni, Forte and M. Howe, has been reported as far south as Tierra del Fuego (Papenfuss 1964). Most other species in this genus are pantropical and only a few are recorded from temperate waters.

Finally, among the Bryophyta, *Sanionia* is one of the most widely distributed moss genera in maritime Antarctica and grows abundantly on the shores of Punta Plaza.

Metazoa

Only two Metazoa were assigned in this study. *Edwardsia timida* is known only from the Irish sea and English Channel but a close relative, *E. meridionalis*, has been reported from Antarctica (Williams 1981). This genus requires further taxonomic attention (Manuel 1977, Daly et al. 2012) *Sarsia lovenii* was originally described from Europe has and has also been reported from Canada and China (Prudkovsky et al. 2019).

In studies of this type, it is important to recognize that the assignment of a DNA sequence does not confirm the presence of the living organism or a viable propagule, and as noted in several instance above, is also limited by the quality and completeness of available databases, with many Antarctic species are also yet to be sequenced. There is also no universal DNA barcode for all living organisms, and the use of markers such as 18S, 28S and COX1 is more appropriate for assessing diversity of ciliates and protozoans but less effective for plants (Folmer et al. 1994, Elbrecht & Leese 2017, Pecina & Vdačný 2022), while our use of ITS2 allowed assessment of a wider range of taxonomic groups and ranks. This research approach has utility in informing new field surveys, the selection of samples for morphological analyses, and in confirming the presence of certain phycological taxa in Antarctica. Taxa assigned as 'unknown' are likely not yet to be present in available databases, and

could also include currently undescribed taxa that are new to science.

CONCLUSIONS

This descriptive preliminary survey presents the results of applying a recently available molecular approach to assess diversity present in the phycosphere of marine macroalgae, representing a poorly known niche from a very remote region. The DNA sequences assigned in this study included a range of taxa not previously recorded in this part of Antarctica or around the continent and Southern Ocean as a whole, suggesting that the biodiversity of the phycosphere in this region remains underestimated. Some of the newly reported taxa include potentially dangerous invasive and pathogenic taxa, which deserve monitoring and further investigation in Antarctica. Detailed surveys, using greater samples sizes, including other Antarctic islands, the use of multiple markers and linked with morphological analyses, ontogenetic, chemotaxonomic and macroecological studies are required to confirm the diversity and composition of microorganism communities present and the levels of variability within and between macroalgal species.

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