Received: 11 January 2023 | Revised: 11 April 2023 | Accepted: 16 April 2023

DOI: 10.1111/ddi.13703

RESEARCH ARTICLE

Diversity and Distributions WILEY

Spatio-temporal patterns of eukaryotic biodiversity in shallow hard-bottom communities from the West Antarctic Peninsula revealed by DNA metabarcoding

Carlos Angulo-Preckler[1,2,3] | Marta Turon[1] | Kim Præbel[1,4] | Conxita Avila[5] | Owen S. Wangensteen[1,5]

[1]Norwegian College of Fishery Science, UiT The Arctic University of Norway, Tromsø, Norway

[2]Marine Science Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Kingdom of Saudi Arabia

[3]Red Sea Research Center (RSRC), King Abdullah University of Science and Technology (KAUST), Thuwal, Kingdom of Saudi Arabia

[4]Department of Forestry and Wildlife Management, Inland Norway University of Applied Sciences, Campus Evenstad, Elverum, Norway

[5]Department of Evolutionary Biology, Ecology, Environmental Sciences, and Biodiversity Research Institute (IrBIO), Faculty of Biology, University of Barcelona, Catalonia, Spain

Correspondence
Carlos Angulo-Preckler, Norwegian College of Fishery Science, UiT The Arctic University of Norway, Tromsø, Norway.
Email: carlospreckler@hotmail.com; carlos.preckler@kaust.edu.sa

Funding information
Ministerio de Ciencia y Tecnología; Universitetet i Tromsø; Ramon Areces Foundation

Editor: Yanhua Qu

Abstract

Aim: We studied molecular eukaryotic biodiversity patterns in shallow hard-bottom Antarctic benthic communities using community DNA metabarcoding. Polar ecosystems are extremely exposed to climate change, and benthic macroinvertebrate communities have demonstrated rapid response to a range of natural and anthropogenic pressures. However, these rich and diverse ecosystems are poorly studied, revealing how little is known about the biodiversity of the Antarctic benthos associated with hard-bottom habitats.

Location: West Antarctic Peninsula and South Shetland Islands.

Methods: Using data collected in seven localities along the western Antarctic Peninsula, we calculated spatial patterns of alpha and beta diversities. Furthermore, we analysed temporal changes in benthic composition in one location (Deception Island) over 3 years. We calculated the temporal alpha and beta diversities to reveal changes in this community over time.

Results: We obtained a final list of 2057 molecular operational taxonomic units. We found significant differences in benthic community composition between localities and among years. Our dataset revealed a total of 10 different kingdom-level lineages and 34 different phyla in the samples. The most diverse phylum was Arthropoda, followed by Bacillariophyta, and Annelida, while the highest relative read abundances belonged to Annelida, Porifera and Echinodermata. Benthic community compositions changed between 2016 and 2018 in Deception Island, and decreasing species richness was the main component of temporal beta diversity.

Main Conclusions: Direct sampling methods are required for monitoring these complex communities. Informative biodiversity patterns can be retrieved even though most of the benthic biodiversity found in Antarctic habitats is yet to be taxonomically described and barcoded. Hard-bottom assemblages exhibit high spatial variability and heterogeneity, not related to depth, which represent a huge challenge for large-scale studies in the Southern Ocean. Local patchiness and structure within these

communities are probably a consequence of a combination of several biotic and abiotic factors (i.e. ice disturbance, food supply and competition).

KEYWORDS
benthic invertebrates, bulk DNA, community DNA, community ecology, eukaryotic, metabarcoding, Southern Ocean

1 | INTRODUCTION

The human perception of cold waters as an unpleasant extreme environment is not shared by polar organisms. Despite sub-zero water temperatures, the biodiversity and biomass of polar organisms in Antarctica are high, thanks to their adaptation and evolution over 300 million years of isolation (Peck, 2018). However, Antarctica is a fragile environment under threat from increased human activities (Pertierra et al., 2021; Tin et al., 2009). The importance of conserving global biodiversity has come to the public's attention in recent decades due to unprecedented rates of species extinctions driven by human activities (Duarte et al., 2020; Luypaert et al., 2020). The West Antarctic Peninsula (WAP) has experienced warming at significantly greater rates than the rest of the Antarctic continent and possibly greater than any other region on Earth over the past 50 years (Turner et al., 2005; van Wessem et al., 2015). These changes have driven habitat and community shifts with significant impacts on biodiversity and ecosystem functioning. Although large-scale ecological conditions (i.e. low and relatively stable temperatures, seasonality of primary production and low terrigenous input) are similar around most parts of the continent (Bullivant, 1959; Clarke & Leakey, 1996), the benthic biodiversity in the Antarctic region is remarkably patchy (Almond et al., 2021) and depends on complex interactions between physical and biological factors that are not easily defined (Smale & Barnes, 2008).

In Antarctica, marine habitats shallower than 100 m occupy an estimated total area of approximately 25,000 km^2 (Clark et al., 2015). Antarctic benthos has been categorized as a relatively homogenous biological unit (Downey et al., 2012; Smale, 2008). The benthos is the richest element of the food web in terms of numbers of macrospecies, dominated by suspension feeders in the shallows and deposit feeders in deeper waters (Griffiths, 2010), although their roles and interactions are poorly known. Over 4100 benthic species have been reported from the Southern Ocean (SO), with polychaetes, gastropods and amphipods being the taxa with highest number of described species (Clarke & Johnston, 2003). Gutt et al. (2004) estimated 11,000–17,000 macrozoobenthic species alone using statistical techniques to extrapolate species diversity from a sampled area in the Weddell Sea, pointing out that even this large figure may be an underestimate of true diversity. Other taxa with high species richness include bryozoans and sponges (Arntz et al., 1994). Bivalve molluscs and isopods show lower species richness in the SO than in equivalent areas of shelf elsewhere, while some groups of decapod crustaceans are completely absent, and pycnogonids, echinoderms and many suspension feeders are rich and diverse (Clarke, 1990; Clarke & Johnston, 2003). All marine phyla are present in Antarctic waters (de Broyer et al., 2014). Recent estimates suggest that between 33% and 91% of all marine species around the world have never been named (Appeltans et al., 2012; Mora et al., 2011), and taxonomic knowledge gaps have limited our ability to investigate patterns of diversity beyond a few indicator groups (Tittensor et al., 2010).

Nearshore benthic communities of Antarctica have demonstrated a rapid response to a range of natural and anthropogenic pressures and therefore, their assessment can be used as an indicator of ecosystem health (Magni, 2003). Unfortunately, visual morphology, widely used for species identification in coastal and marine communities, is cumbersome and entails limitations (time-consuming, expensive, requires extensive taxonomic expertise; Aylagas et al., 2016; Wood et al., 2013). Despite hard-bottoms usually support higher abundance and diversity than soft-bottoms, very few studies have consistently evaluated the benthic diversity of hard-bottom habitats at different spatial scales along the WAP. The real extent of biodiversity and its temporal and spatial patterns remain unknown for the majority of the WAP, despite being one of the most studied areas in Antarctica. Hard-substrate habitats in shallow coastal Antarctic waters tend to display a gradient of benthic communities related to ice cover (Clark et al., 2013, 2015). Furthermore, iceberg scouring causes a significant reduction in benthic biomass and biodiversity at small spatial scales (Conlan & Kvitek, 2005). Shallow areas of heavily disturbed sites are characterized by assemblages of low diversity and biomass able to be rapidly re-colonized after impacts (Gutt & Piepenburg, 2003; Peck et al., 1999; Smale, 2007; Teixidó et al., 2004). Intermediate frequencies of ice disturbance are thought to enhance diversity by preventing species domination and creating a patchwork of habitat and communities in various stages of recovery (Brown et al., 2004; Conlan & Kvitek, 2005; Smale et al., 2007). Thus, evaluations of biodiversity in benthic fauna are of critical importance for understanding ecosystem functioning, sustainability and resilience.

Documenting the diversity of marine life is challenging because many species are cryptic, small and/or rare, and belong to poorly known groups (Leray & Knowlton, 2015). Complex hard substrates, particularly in Antarctica, provide huge challenges for consistent sampling, because the communities are largely inaccessible to exhaustive qualitative or quantitative biodiversity assessments (de Broyer et al., 2014), and also because of the abundance of small epibionts, and the massive, colonial or modular morphology of many

species. Furthermore, an important constrain is that monitoring is typically focussed on macrofauna (>1 mm size), which is only a part of a community that also comprises microeukaryotes and other inconspicuous taxa. Surveys focussing only on macrofauna may underestimate the sensitivity to changes in the whole community (Lanzén et al., 2016; Leray & Knowlton, 2015). Thus, meiofauna assessments are needed to generate a more complete and mechanistic understanding of marine benthic ecosystems (Bourlat et al., 2013). Meiofauna (the microscopic taxa generally between 45–500 μm) are important members of the benthic ecosystems, playing a critical role in carbon transfer and nutrient cycling (Fonseca et al., 2017; Schratzberger & Ingels, 2018).

Another limitation to improve our understanding of Antarctic marine benthic ecosystems is the ability to generate comparable biological time-series data. Time series are essential tools for studying changes within ecosystems and could be particularly useful for understanding the effects of anthropogenic impacts and global change, but the remoteness of Antarctic habitats represents a big challenge to sample such long-time series (Fonseca et al., 2022; Schratzberger & Ingels, 2018).

Molecular biodiversity assessment methods are promising not only for the fundamental understanding of diversity but also for biodiversity monitoring in the context of global change (Bourlat et al., 2013). DNA barcoding is a technique that uses a short gene sequence, from a standardized region of the genome as a diagnostic 'biomarker' for species (Hebert et al., 2003). Natural variability of the mitochondrial cytochrome c oxidase I gene (COI) enables the taxonomic resolution of metazoan taxa at the species level and allows to resolve cryptic species complexes (Leray & Knowlton, 2016; Wangensteen et al., 2018). DNA metabarcoding has emerged as a powerful tool for quantifying biodiversity using genetic sequences extracted from an environmental or bulk sample (i.e. water, sediment, community; Taberlet et al., 2012). Community DNA from bulk samples can be defined as the organismal DNA extracted from whole individuals that were presumably alive (or recently dead) at the time of sampling (Rodriguez-Ezpeleta et al., 2021). One of the main advantages of community-DNA studies is that they may uncover larval, small (meiofaunal) or rare taxa that may be missed by traditional surveys, being a powerful tool for biodiversity monitoring. Bulk samples often contain many different taxa that vary several orders of magnitude in biomass. (Rodriguez-Ezpeleta et al., 2021). Few specimens of high biomass will dominate the dataset, potentially leading to smaller specimens remaining undetected. Sorting taxa by size and pooling them proportionately according to their abundance leads to a more equal amplification of taxa compared with the processing of complete samples without sorting (Elbrecht et al., 2017; Wangensteen & Turon, 2017). Thus, metabarcoding provides a cost-effective, ecosystem-wide method for the assessment of biodiversity. Molecular methods are particularly powerful when combined with standardized sampling, allowing for direct comparisons across space and through time. The resulting barcode sequence reads can be subsequently matched with sequences assigned to taxon names accessed from databases such as NCBI GenBank (Sayers et al., 2020) and the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007). Although the use of extra-organismal DNA (the DNA released from cell lysis; Rodriguez-Ezpeleta et al., 2021) is increasingly applied to water samples, there is not robust evidence that the entire macroinvertebrate community can be detected using exclusively extra-organismal DNA (Antich, Palacin, Cebrian, et al., 2021; Antich, Palacín, Wangensteen, et al., 2021; Rey et al., 2020). Hence, metabarcoding of benthic communities is needed for providing valuable information on unknown benthic biodiversity (Fonseca et al., 2010; Leray & Knowlton, 2015), and it can therefore be used to assess Antarctic species richness. Comparisons of benthic community composition between different Antarctic regions are difficult due to the fact that researchers have used different collection methods, depths, sieve sizes and temporal ranges. Furthermore, Antarctic biodiversity has not so far been comprehensively explored at the molecular diversity level, with the exception of some specific groups (Grant et al., 2011). Many marine shelf, deep water and polar areas are notoriously undersampled.

Different DNA metabarcoding approaches have been recently applied to assess biodiversity from the SO. These studies have mainly focussed on microbial communities (Flaviani et al., 2018; Luria et al., 2014), fungal diversity (Ogaki et al., 2021), sponge microbiomes (Castro-Fernández et al., 2023; Sacristán-Soriano et al., 2020), metazoans (Clarke et al., 2021; Vause et al., 2019) and benthic meiofauna (Brannock et al., 2018; Fonseca et al., 2017, 2022). However, studies analysing community DNA extracted from hard-bottom communities are lacking, which would provide a powerful tool to complement existing approaches, and a timely opportunity to gain insight into alpha and beta-diversity patterns of Antarctic hard-bottom communities, by providing the needed baselines to assess the resilience of these ecosystems in the context of global warming.

The goal of this study is to provide a global description of the shallow benthic communities associated with hard-bottoms along the WAP using high-throughput sequencing of community DNA, to provide a baseline for future biomonitoring studies in hard-bottom ecosystems. Additionally, we evaluated the temporal patterns of two nearby communities during three consecutive years in Deception Island.

2 | METHODS

2.1 | Sample collection

Samples were collected along the WAP and South Shetland Islands (SSI) on both sides of the Bransfield passage (Figure 1, Table 1) by specialized divers during the austral summer of 2016. All samples were collected in rocky bottoms, mostly rocky walls or boulders, at 20 m to avoid the influence of depth in the communities. Three stations were located in the continent and four in the South

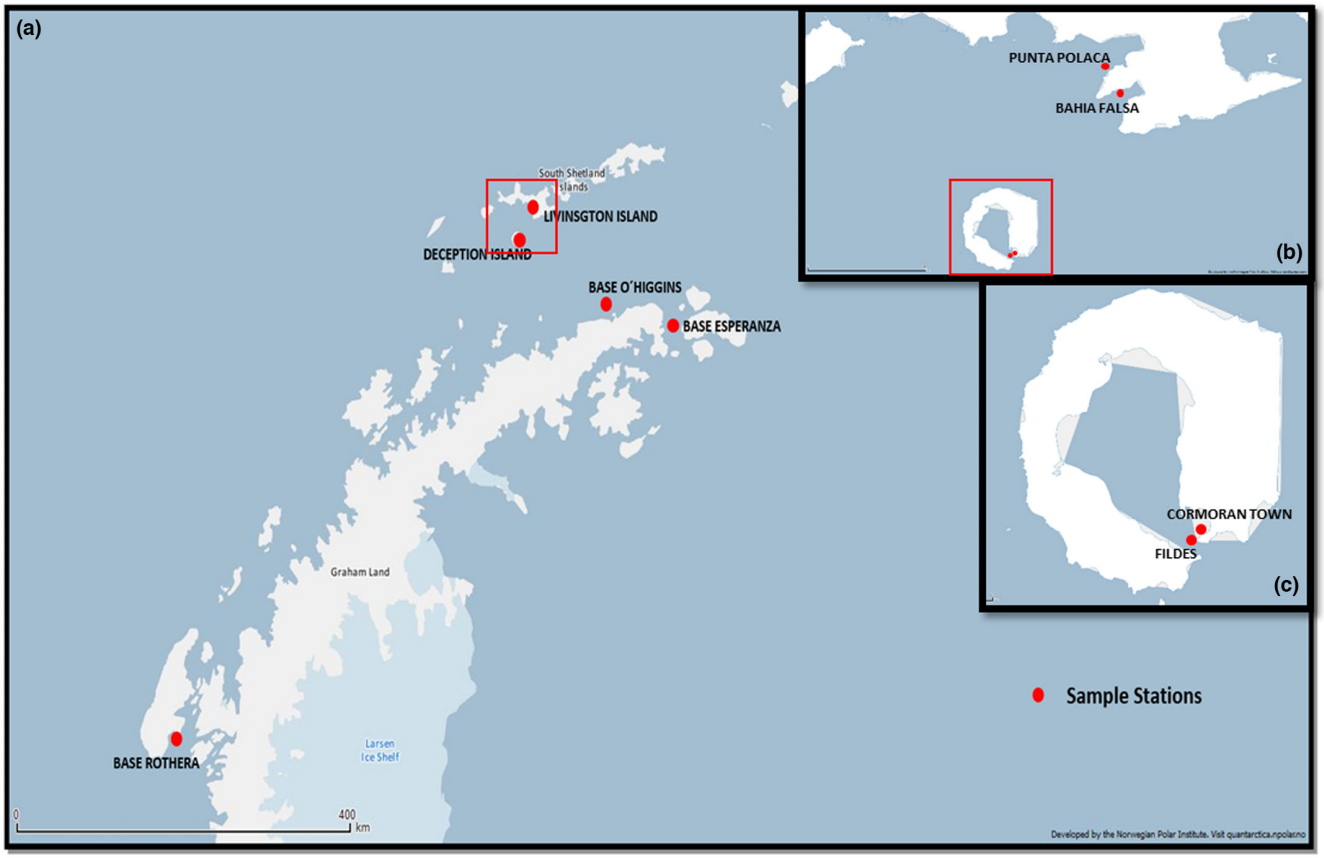

FIGURE 1 Map of the study area in the Antarctic Peninsula (a), South Shetland Island (b) and detail of Deception Island sampling stations (c).

Shetland Islands. Furthermore, the two stations inside Deception Island were sampled during three consecutive years to cover annual variability (2016, 2017 and 2018). All stations were selected in order to minimize the ice-scouring (vertical walls when possible). Three replicates per station were collected by scraping to bare rock quadrats of 25 × 25 cm with a scraper. All the material was collected underwater in zip-lock bags. Two divers performed the sampling, with one keeping the plastic bag open just below the zone being scraped to avoid small pieces of organisms and/or motile fauna scaping.

2.2 | Sample processing

Benthic samples were filtered through a 63 μm mesh to discard the seawater and immediately kept at −20°C until processed in the laboratory. Then, the samples were separated into three different size fractions (A: >10 mm; B: 1–10 mm; C: 63 μm–1 mm) using a stainless-steel mesh sieve column. Each fraction was homogenized with a blender and stored in ethanol at −20°C until DNA extraction. All the equipment was carefully bleached between samples. Our sample dataset thus consisted of 99 benthic samples (7 communities × 3 replicates × 3 fractions and 2 communities × 3 years × 3 replicates × 3 fractions).

2.3 | DNA extraction, PCR amplification and library preparation

All procedures were performed in a laminar flow cabinet sterilized with UV light between samples. DNA from benthic samples was extracted using 10 g of homogenized material using the DNeasy PowerMax Soil Kit (Qiagen). Extraction of negative controls was included for each extraction event. We amplified the partial COI 'Leray-XT fragment' (313 bp), using the mlCOIintF-XT/jgHCO2198 primer pair. mlCOIintF-XT (5'-GGWACWRGWTGRACWITITA YCCYCC-3′; Wangensteen et al., 2018) is a modified version of the mlCOIintF forward primer (Leray et al., 2013) used together with the reverse primer jgHCO2198 (5'-TAIACYTCIGGRTGICCRAA RAAYCA-3'; Geller et al., 2013). All primers had an 8-base specific tag attached. The tags had a minimum difference of three bases from each other. Forward and reverse primers used for the amplification of each sample had the same tag to minimize the tag jumping. A variable number of degenerated (N) bases were also attached to the primers to improve sequence diversity for Illumina processing. PCR blanks were run by amplifying the PCR mixture without any DNA template. Also, negative controls were added by processing sand samples that were charred in a furnace (400°C for 24 h) and then sieved and processed as the benthic samples. PCR condition for COI amplification followed (Wangensteen

TABLE 1 Coordinates of each sampling location.

Station	Coordinates		Total reads	MOTUs	Phylum	Reads ($\bar{x}$ ± SD)	S ($\bar{x}$ ± SD)	H ($\bar{x}$ ± SD)	Simp ($\bar{x}$ ± SD)
	Lat (S)	Lon (W)							
Bahia Falsa (LI)	−62.699444	−60.369444	1,241,175	589	28	137.908 ± 80.999	170 ± 98.3	2.33 ± 0.68	0.78 ± 0.11
Punta Polaca (LI)	−62.660556	−62.3975	954,868	859	31	106.096 ± 39.306	255 ± 119.0	2.62 ± 0.69	0.835 ± 0.08
Cormoran Town (DI)	−62.988989	−60.558353	3,760,481	930	32	139.277 ± 101.413	122 ± 87.7	1.61 ± 0.61	0.637 ± 0.18
Fildes (DI)	−62.990864	−60.565122	3,407,956	1413	32	126.221 ± 73.679	281 ± 126.0	1.96 ± 0.61	0.707 ± 0.13
Esperanza	−63.371775	−56.982144	1,644,206	730	27	182.690 ± 78.094	223 ± 108.0	2.24 ± 0.58	0.78 ± 0.13
O'higgins	−63.330567	−57.97365	1,389,322	897	30	154.369 ± 58.541	272 ± 92.7	1.92 ± 0.36	0.756 ± 0.07
Rothera	−67.595067	−68.213267	1,881,047	527	24	209.005 ± 87.935	190 ± 61.9	1.86 ± 0.33	0.721 ± 0.08
Total			14,285,651	2057	34	144.233 ± 83.686			

Note: Total reads. Total number of different MOTUs and phyla. Reads; total reads. S, MOTUs richness; H, Shannon diversity index; Simp, Simpson diversity index. Values are given as mean ± standard deviation.

Abbreviations: DI, Deception Island; LI, Livingston Island.

et al., 2018) and the success of PCR amplification was checked through gel electrophoresis. Then, DNA was purified and concentrated using MinElute PCR Purification Kit (Qiagen) and measured with a Qubit 3.0 fluorometer (Thermo Fisher Scientific). Amplification products were pooled to build two Illumina libraries using Nextflex PCR-free library preparation kit (BIOO Scientific). Both libraries were sequenced together in an Illumina MiSeq V3 run using 2×250 bp paired-end sequencing.

2.4 | Bioinformatic analyses

The initial bioinformatic steps used the OBItools package (Boyer et al., 2016). *Illuminapairedend* was used to align paired-end reads and keep only those with >40 alignment quality scores. Reads were demultiplexed and primer sequences were removed using *ngsfilter*. Those with mismatched primer tags at any end were discarded. *Obigrep* and *obiuniq* were used to perform a length filter (retaining only those between 310 and 317 bp) and dereplicate sequences. *Uchime-denovo* algorithm from VSEARCH v2.7.1 (Rognes et al., 2016), was used to remove chimeric amplicons. Sequences were then clustered into molecular operational taxonomic units (MOTUs) with SWARM v2.1.7 using a distance value of $d = 13$, which is the optimal value for this marker as explained in Antich, Palacin, Cebrian, et al. (2021) and Antich, Palacín, Wangensteen, et al. (2021). Singletons (MOTUs with just one read) were removed after this step to minimize data loss (Atienza et al., 2020). Taxonomic assignment was performed using *ecotag* (Boyer et al., 2016) and a custom reference database containing sequences from the EMBL and from the Barcode of Life Database (BOLD). This reference database is publicly available from https://github.com/uit-metabarcoding/DUFA. Assignment of some metazoan sequences was further improved by querying the BOLD database. Sequences with a species name assigned and with an identity match >95% in BOLD were kept, whereas matches below this threshold, even if assigned to the species level by *ecotag*, were downgraded to genus level. The final refining steps consisted of deleting any MOTU for which reads in blank or negative controls represented more than 10% of total reads for that MOTU across all samples (Wangensteen & Turon, 2017). A minimum relative abundance filter was also applied, removing, for a given PCR replicate, the MOTUs that represented <0.005% of total reads of that replicate. We also removed MOTUs that had a combined total of <6 reads across all samples after the previous steps. All MOTUs assigned to Prokaryotes or classified as evident nontarget, contamination taxa (e.g. Insecta, Mammalia, Arachnida) were removed.

2.5 | Data analyses

All analyses were performed in R version 4.0.5 (https://www.r-project.org/), and graphic visualizations were done with the *ggplot2* package (Wickham et al., 2016). Rarefaction, subsampling and biodiversity estimates were calculated using the R package *vegan* (Oksanen

et al., 2019). A Venn diagram was delineated with the *VennDiagram* package (Chen, 2018), to represent the MOTUs overlap between fractions. The three fractions of each biological sample were analysed separately as different replicates. To account for differences in total number of reads, samples were rarefied to the lowest number of sequences (7000 reads) before calculating MOTU richness. Total reads were then transformed to relative abundance. Alpha diversities were compared using different metrics: species richness, Shannon and Simpson diversity indices, and tested for significant differences (one-way analysis of variance (ANOVA) on sqrt-transformed data for the factor *station*). Potential dissimilarities between regions (beta diversity) in terms of MOTU composition were estimated using the Jaccard dissimilarity index to minimize the weight given to 'absence' values and implemented using *vegdist* function. Also, the mantel function was used to assess the correlation (Pearson correlation coefficient) between community dissimilarities and geographical distance. The Mantel approach is appropriate for testing the variation in beta diversity among groups of sites (Legendre et al., 2005). To assess the taxonomic composition of the communities in each station, MOTUs were combined at the phylum level. The composition was depicted via donut plots with *ggplot2*. Patterns of sample dissimilarity were visualized using nonmetric multidimensional scaling (nMDS) with the *metaMDS* function with 500 iterations. All calculations of Bray–Curtis dissimilarities were performed using fourth square root transformed values of relative frequencies of read numbers of MOTUs in each sample. Permutation analysis of variance was performed on Bray–Curtis distances with function *adonis* to test differences between relevant factors: A one-way analysis was performed between stations to evaluate the spatial patterns, and a two-way analysis was done with *stations* and *years* as main factors for temporal patterns. To study the change in community composition through time in Deception Island (from 2016 to 2018), we calculated the temporal beta-diversity index (TBI; Legendre, 2019). TBI was computed for both stations by measuring the change in MOTU composition between the first (2016) and last survey (2018). TBI was decomposed into MOTU loss (B) and gain (C) components of these dissimilarities. Analyses were performed using the R package *adespatial* (Dray et al., 2021).

3 | RESULTS

We metabarcoded a total of 99 samples. After quality filtering, demultiplexing, dereplicating and chimaera removal, we had a total of 17,967,165 reads in 4,944,922 unique COI sequences. Initially, 14,216 nonsingleton MOTUs were obtained by the clustering step but, after the removal of low-abundance MOTUs and those not assigned to marine eukaryotes, we obtained a final list of 2057 MOTUs. Rarefaction curves (Figure S1) showed that a plateau was reached for the number of MOTUs with the sequencing depth obtained in most samples. The final average number of eukaryotic reads by the sample was 144,233 ± 83,686 (mean ± SD), while the number of reads per MOTU ranged from six to 2,362,527. In addition, there was

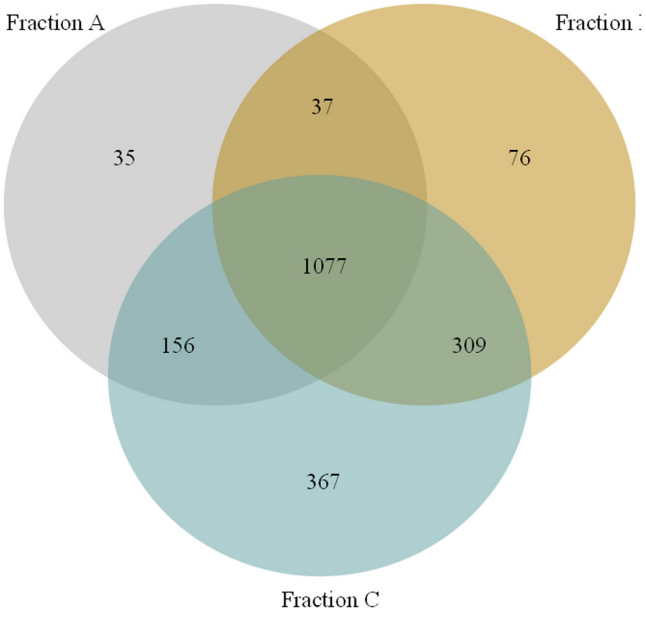

FIGURE 2 Venn diagram showing the overall MOTU overlap between the three fractions of the samples.

a linear relationship between the frequency of occurrence and the total number of reads (Figure S2). Sorting the samples into three size categories showed a great overlap between fractions (1078 MOTUs shared by the three fractions) and a higher number of detected MOTUs in the smallest size fractions (Figure 2). Fractions were not significantly different for the total reads (ANOVA: F value = 2.297; $p = .106$) but significantly different for the MOTU richness (ANOVA: F value = 20.28; $p < .0001$).

Our dataset revealed a total of 10 different kingdom-level lineages (Table S1). Most MOTUs (1328) and reads (13,236,723) belonged to Metazoa, (representing 64.56% and 92.65%, respectively), Stramenopiles (including diatoms and brown algae, among others), with 177 MOTUs and 512,963 reads (representing 3.59% and 8.61%, respectively, of the total dataset), and the third most abundant taxon were Rhodophyta with 45 MOTUs and 422,095 reads (2.95% and 2.19%, respectively, of the total dataset). Taxonomic assignment showed a total of 34 phyla in the samples, of which the most diverse was Arthropoda (275 MOTUs, 1,065,441 reads, all samples combined), followed by Bacillariophyta (99 MOTUs, 107,241 reads), and Annelida (88 MOTUs, 5,278,300 reads; Table 2, Figure 3). Other phyla, although not so diverse, showed high read abundances such as Porifera (18 MOTUs, 3,031,876 reads), Echinodermata (23 MOTUs, 1,721,283 reads) and Nemertea (30 MOTUs, 612,006 reads). Six phyla showed a unique representative MOTU (Acanthocephala, Blastocladiomycota, Brachiopoda, Cephalorhyncha, Chaetognatha and Percolozoa). A total of 938 of 2057 MOTUs (45.6%) were assigned at least at the phylum level, grouping 14,082,565 reads (98.6%), while only 100 MOTUs were assigned to species level (5.1%) representing 5,837,084 reads (Table S2). Moreover, 20 phyla did not show any MOTU identified at the species level (Table 2). The four most abundant MOTUs belonged to two polychaete species (Terebellidae sp. and *Thelepus antarcticus*),

TABLE 2 Total number of MOTUs and reads by phylum.

	Bahia Falsa		Punta Polaca		Cormoran town		Fildes		Esperanza		Ohiggins		Rothera		Total		% of identification			
Phylum	MOTUs	Reads	MOTUs	Reads	MOTUs	Reads	MOTUs	Reads	MOTUs	Reads	MOTUs	Reads	MOTUs	Reads	MOTUs	Reads	Order	Family	Genus	Species
Annelida	33	298,661	51	353,733	44	1,243,347	49	1,688,422	31	204,727	38	360,840	32	1,127,860	88	5,278,300	75	45.5	20.5	12.5
Porifera	10	585,859	14	271,094	16	1,257,563	14	494,484	5	31	10	418,794	4	79	18	3,031,876	88.9	61.1	55.6	27.8
Echinodermata	7	2518	8	4070	12	339,526	12	697,662	8	27,358	8	89,560	9	560,542	23	1,721,283	95.7	73.9	69.6	56.5
Arthropoda	108	64,695	118	32,223	121	79,180	158	191,025	123	554,072	137	95,348	90	48,406	275	1,065,441	59.6	26.5	17.5	6.9
Nemertea	16	4380	15	114,099	9	53,849	15	141,571	11	235,369	16	60,665	7	2065	30	612,006	56.7	26.7	26.7	10
Cnidaria	10	353	12	5176	23	389,683	27	48,639	9	65	8	86	12	2411	37	446,838	70.3	32.4	24.3	8.1
Mollusca	27	46,184	24	43,648	26	227,319	30	19,924	28	21,554	24	14,438	21	56,557	56	429,683	64.3	37.5	28.6	16.1
Bryozoa	20	160,404	23	81,212	15	24,262	17	29,807	17	90,476	20	27,385	9	12,771	26	426,363	88.5	50.0	42.3	3.8
Rhodophyta	22	3482	21	3850	27	52,392	30	6890	26	296,833	28	4094	17	54,402	45	422,095	91.1	68.9	64.4	33.3
Ochrophyta	14	24,848	15	870	19	25,226	17	7605	13	106,401	15	237,029	6	769	21	402,748	85.7	71.4	42.9	23.8
Unidentified	238	20,192	430	18,481	443	27,173	798	46,305	328	65,854	452	11,940	247	12,359	1117	202,879				
Bacillariophyta	27	3069	37	1294	49	3041	81	10,545	65	21,440	62	67,424	25	428	99	107,241	59.6	40.4	32.3	4
Platyhelminthes	2	24,762	3	16,789	3	74	1	12	2	264	3	829	2	1526	4	44,256	50	25	25	0
Dinoflagellata	5	176	7	231	21	14,929	19	9393	2	18,808	8	399	4	109	23	44,211	30.4	30.4	21.7	0
Chordata	4	472	5	4244	5	344	4	8502	2	232	1	69	3	323	10	14,194	100	40	20	10
Chlorophyta	1	176	4	211	7	9043	4	2571	0	0	2	12	2	30	7	12,266	100	100	100	57.1
Brachiopoda	1	86	1	2379	1	7025	1	12	0	0	0	0	0	0	1	9637	100	0	0	0
Haptophyta	5	273	6	205	17	4773	11	1597	3	6	8	143	3	3	17	7000	88.2	88.2	82.4	17.6
Discosea	11	44	27	173	26	153	47	1336	17	44	19	59	11	25	51	1834	100	45.1	100	0
Nematoda	7	195	7	84	5	179	10	411	8	277	6	38	6	148	19	1332	89.5	10.5	5.3	0
Oomycota	9	52	11	91	12	213	23	376	8	60	11	52	7	48	28	893	0	0	0	0
Basidiomycota	0	0	1	498	1	119	2	34	1	2	1	8	0	0	2	661	100	50	50	0
Bigyra	3	35	5	68	4	208	12	229	5	12	5	37	3	41	16	630	100	100	93.75	0
Rotifera	2	25	3	13	6	168	10	207	9	101	3	6	1	9	12	529	100	83.3	75	0
Xenacoelomorpha	1	106	2	58	1	7	3	31	2	117	3	11	0	0	7	330	100	14.3	14.3	0
Cryptophyta	1	2	1	3	2	191	1	78	0	0	1	10	0	0	2	284	100	100	100	0
Apicomplexa	1	8	1	9	3	24	4	93	1	1	2	4	4	133	8	272	100	87.5	87.5	0
Ascomycota	1	71	1	1	3	107	3	55	0	0	1	7	0	0	5	241	60	20	20	0
Thecomonadea	2	25	1	2	1	2	3	50	3	13	2	25	1	2	3	119	100	100	100	0
Acanthocephala	0	0	1	2	0	0	0	0	1	87	1	1	0	0	1	90	0	0	0	0
Blastocladiomycota	0	0	1	1	1	2	1	21	1	1	1	6	0	0	1	31	100	100	0	0
Mucoromycota	0	0	0	0	1	2	2	28	0	0	0	0	0	0	2	30	100	50	50	0
Percolozoa	1	1	0	0	0	0	1	18	1	1	1	3	0	0	1	23	100	100	100	0
Cephalorhyncha	0	0	1	9	1	8	1	3	0	0	0	0	1	1	1	21	100	100	100	0
Chaetognatha	0	0	0	0	1	14	0	0	0	0	0	0	0	0	1	14	100	100	100	0

Note: Percentage of identification depending on the taxonomic rank by phylum.

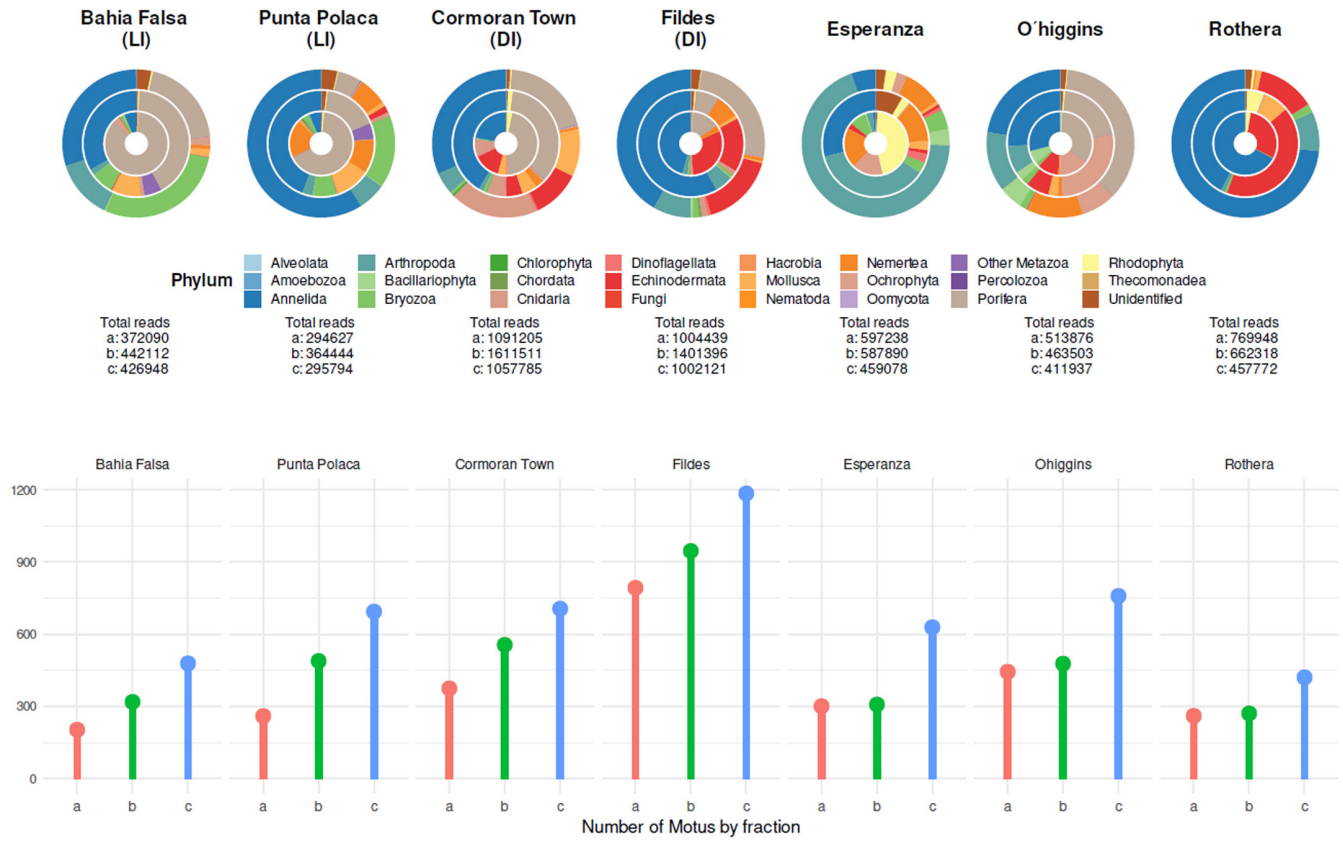

FIGURE 3 (Top) Donut charts by stations representing the regional composition by size fraction (a; outer rings, b; medium rings and c; inner rings) of the average relative read abundance by phylum and (Bottom) lollipop charts showing the mean MOTU richness by fraction and station. Some minor phyla were grouped (Alveolata: apicomplexan and bigyra. Fungi: ascomycota, basidiomycota, blastocladiomycota and mucoromycota. Hacrobia; cryptophyta and haptophyta. Other Metazoa; acanthocephala, braquiopoda, cephalorhyncha, chaetognatha, platyhelminthes, rotifera and xenacoelomorpha).

a sponge species (*Dendrilla antarctica*) and the sea urchin (*Sterechinus neumayeri*), and combined represented 5,677,431 reads accounting for 40% of the total reads.

3.1 | Spatial patterns

3.1.1 | Alpha diversity

Average MOTU richness per locality varied significantly across stations (ANOVA: $F_{6,92}$ value = 6.41; $p < .0001$), ranging between 122 and 281 MOTUs in Cormoran Town and Fildes, respectively (Table 1), although the number of unique MOTUs per locality showed a higher diversity. Moreover, there were significant differences in Shannon diversity across localities (ANOVA: $F_{6,92}$ value = 4.52, $p < .0005$). Cormoran Town had the lowest diversity (Figure 4).

3.1.2 | Beta diversity

A clear differentiation in community composition between stations was observed (Figure 5a, PERMANOVA $p < .001$), albeit with some overlap due to the O'Higgins samples. The Bray–Curtis dissimilarity matrix has a significant relationship with the geographical separation of the samples (Mantel statistic *R*: .2226, $p = .0002$). Moreover, spatial patterns of benthic beta diversity showed a marked difference between Rothera and the rest of the stations. The highest similarity was observed between both stations at Deception Island (Fildes and Cormoran Town, probably due to the larger number of samples from these stations). O'Higgins appeared as a transitional station showing the highest Jaccard index among all the stations (Figure 6).

3.1.3 | Taxonomic composition

Differences between stations were observed not only in the lowest taxonomic resolution but also in the phylum level within macrobenthic organisms (Figure 7a). Nonetheless, 100 of all MOTUs were present in all localities, forming a benthic Antarctic core community. Five phyla encompassed more than 80% of the total reads (Annelida, Porifera, Echinodermata, Arthropoda and Nemertea), forming different macrobenthic settlements at each locality (Figure 8). Due to the low species-level resolution, the calculation of the indicator value scores (IndVal) used to gather information about specific

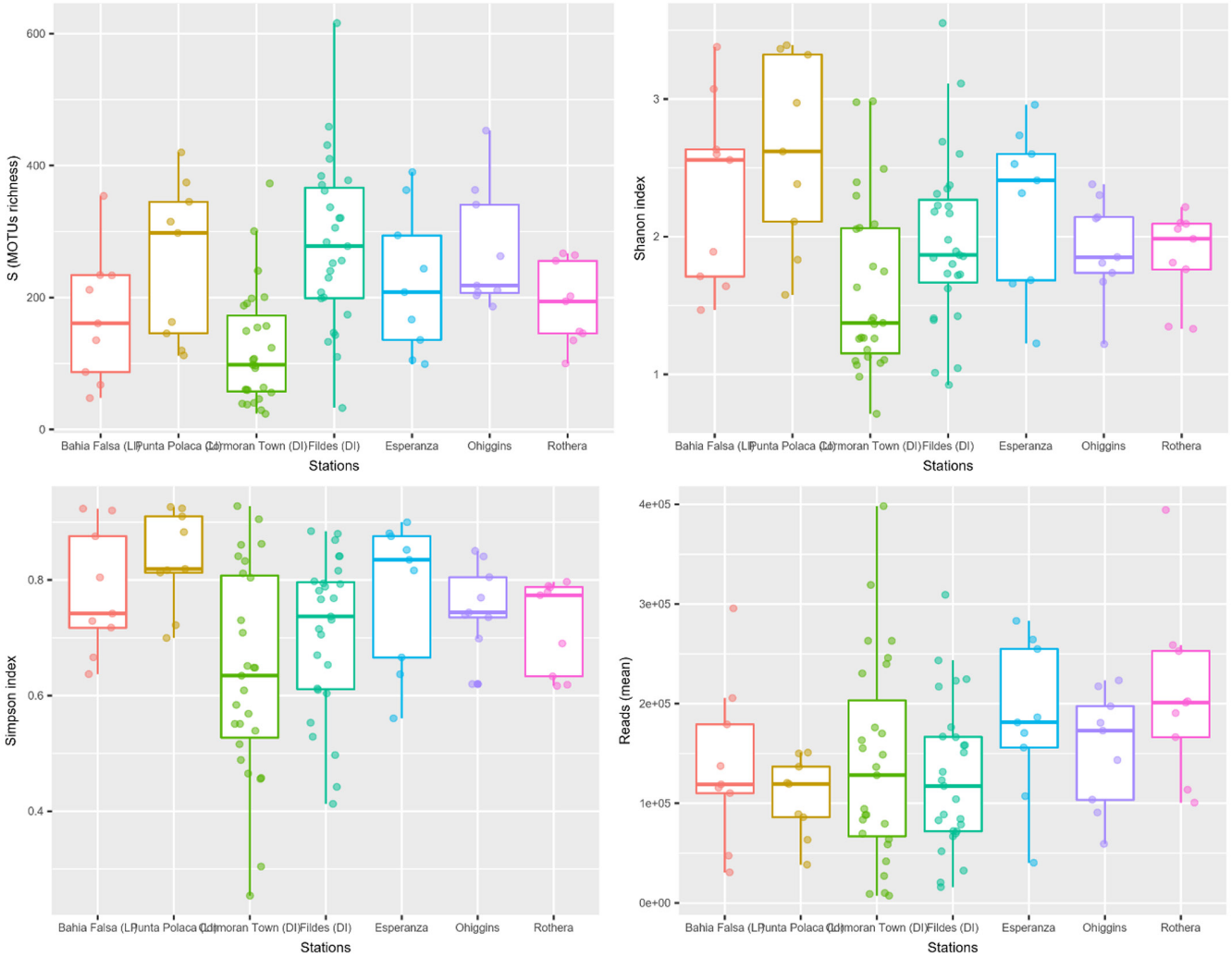

FIGURE 4 Diversity indices by station. MOTUs richness. Shannon diversity index. Simpson diversity index. Number of reads.

characteristic habitats was less informative. Stations in Deception Island had no locality indicator species while for Livingston Island, only three MOTUs per station were consistently associated with these stations (the pycnogonid *Achelia hoekii*, the diatom *Skeletonema* sp. and an unidentified metazoan in Bahia Falsa; whereas a polychaete from the Syllidae family, the nemertean *Nipponnemertes* sp. and an Amphipoda represented Punta Polaca). The same number of MOTUs were observed for O'Higgins (a red algae from Florideophyceae class, a brown algae from Desmarestiaceae and another Amphipoda). On the other hand, several MOTUs characterized Esperanza (19) and Rothera (15) stations (Figure S3).

3.2 | Species composition in each locality

3.2.1 | Bahia Falsa (Livingston Island)

This location is a pristine habitat dominated by the proximity of the Huntress glacier. Steep slopes of soft-bottoms characterized by several boulders and dropped stones unevenly distributed. The main characteristic of this community is the large abundance of Porifera (*Tedania* sp., *Dendrilla antarctica*, Poecilosclerida, *Haliclona* sp., *Fibulia maeandrina* and *Artemisina plumosa*) and bryozoa (*Antarctothoa* sp. and some Cheilostomatida) as three-dimensional biostructures, settling an important population of polychaetes (Phyllodocidae, Lumbrineridae, Terebellidae), a chitonid mollusc and some ostracoda and isopoda. There is an important contribution of the brown algae *Himantothallus* sp., *Himantothallus grandifolius*, *Desmarestia menziesii* and *Ascoseira mirabilis*. Less abundant but with noticeable diversity is the community of red algae (*Pantoneura plocamioides*, *Gigartina skottsbergii*, *Gymnogongrus turquetii*, *Iridaea cordata*, *Plocamium cartilagineum*, *Rubrointrusa membranacea*, *Callophyllis* sp., *Myriogramme manginii* and *Wildemania amplissima*).

3.2.2 | Punta Polaca (Livingston Island)

Locality with two research stations in the vicinity and very exposed to the waves with no apparent influence of ice. Several sponges form the main structure of this community (*Haliclona* sp., *Dendrilla*

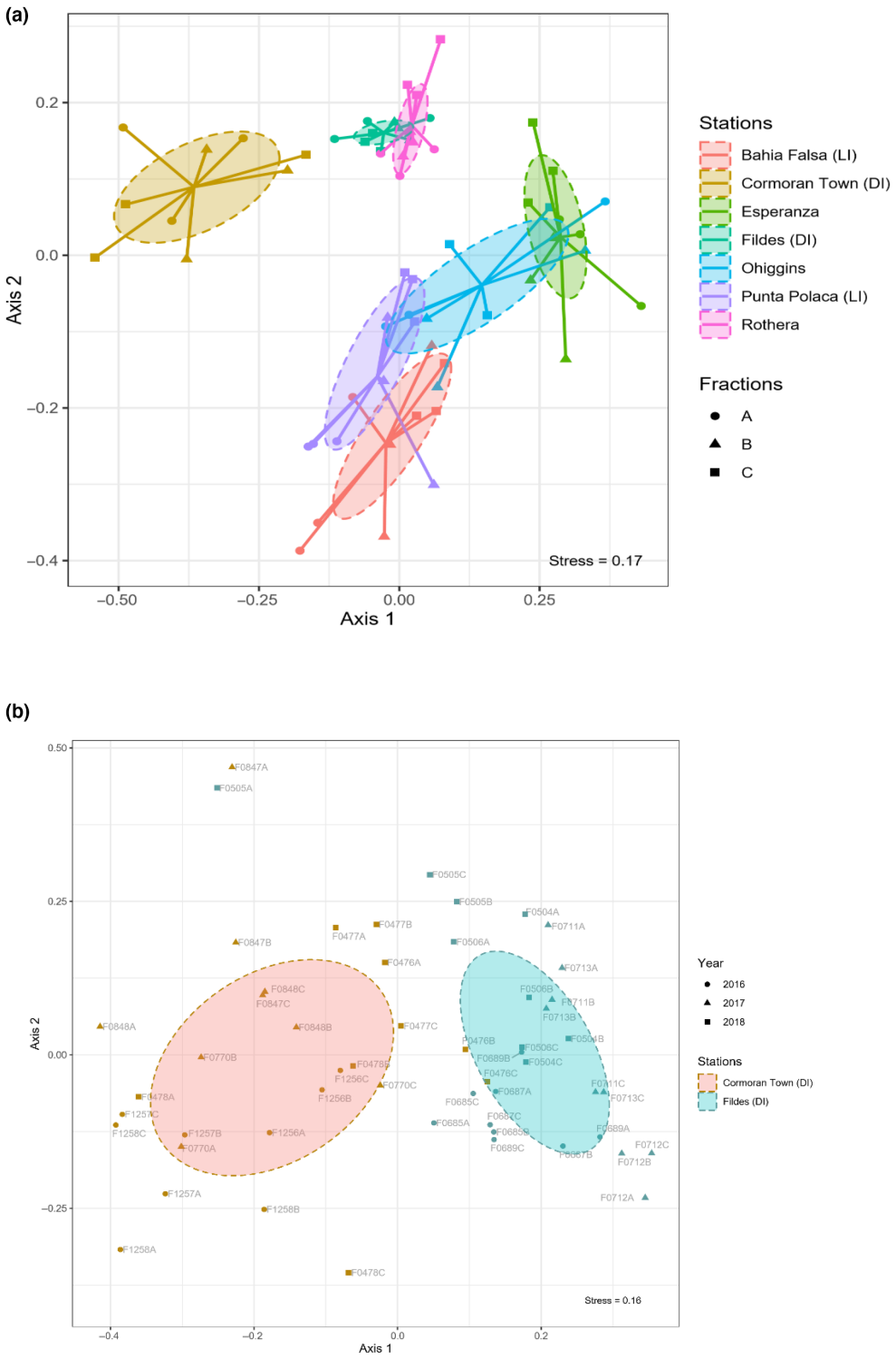

FIGURE 5 Nonmetric multidimensional scaling representation using Bray–Curtis indices based on relative read abundances. (a) Spatial data where samples were separated in three different size fractions: A (>10 mm); B (between 10 and 1 mm); and C (between 1 mm and 63 μm). (b) Temporal data for Deception Island. Stations are coded by colours and fractions and years by symbols.

antarctica, *Tedania* sp., *Fibulia maeandrina*, *Sphaerotylus antarcticus*, two unidentified Poecilosclerida and one Acarnidae), altogether with several bryozoa (*Antarctothoa* sp., *Antarctothoa antarctica*, *Arachnopusia unicornis*, *Beania* sp. and Cheilostomatida) that give structural complexity that allows many polychaetes to settle (mainly Syllidae and Terebellidae), along with nemerteans (*Parborlasia corrugatus* and *Nipponnemertes* sp.), a brachiopod (probably *Liothyrella uva*) and several molluscs (Chitonida and Gastropoda). Less abundant but also important was the presence of some red algae (*Pantoneura plocamioides*, *Iridaea cordata*, *Rubrointrusa membranacea*, *Callophyllis*

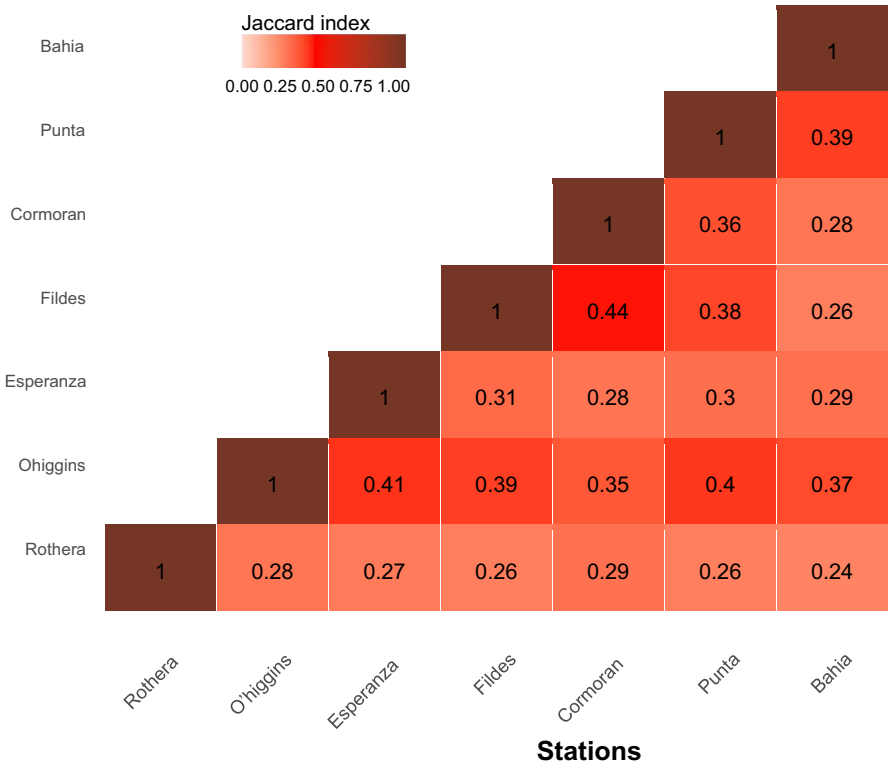

FIGURE 6 Heatmap showing the similarity (Jaccard index) between pairs of regions. Darker colours show communities that are more similar than those with lighter colours.

sp.), the mollusc *Margarella antarctica*, the cnidarian *Lafoea dumosa* and the pycnogonida *Nymphon brevicaudatum* and *Austrodecus glaciale*.

3.2.3 | Cormoran Town (Deception Island)

Inside this active Antarctic volcano, we found the less diverse habitat of our study. The entrance of the island and the east part of Whaler Bay, represent the only hard-bottom habitat found inside Deception Island (Angulo-Preckler et al., 2018). Despite some similar sponges as in LI were present (*Dendrilla antarctica*, *Tedania* sp., *Sphaerotylus antarcticus* and one Poecilosclerida), they were less relevant in the overall community. An important contribution of different cnidarian species was observed (some Anthozoa such as Actinaria, *Edwardsia* sp., *Urticinopsis antarctica* and some hydrozoan such as *Obelia* sp., *Lafoea dumosa*, Sertulariidae). Some common Antarctic invertebrates were also present, such as echinoderms (*Sterechinus neumayerii*, *Odontaster meridionalis* and *Odontaster validus*), the ascidian *Cnemidocarpa verucosa*, the nudibranch *Doris kerguelenensis*, the nemertean *Parborlasia corrugatus*, the pycnogonids *Pentanymphon antarcticum*, one brachiopod (probably *Liorythella uva*) and several polychaetes (Terebellida, *Antarctinoe ferox*, *Flabelligera mundata*, *Lanicides bilobata*) and isopoda (such as *Iathrippa sarsi*). There was also a macroalgal community represented by the red algae *Iridaea cordata*, *Pentanymphon antarcticum*, *Phycodrys quercifolia* and the brown algae *Cystosphaera jacquinotii*, *Desmarestia menziesii* and *Ascoseira mirabilis*. Two different sporadic dinoflagellates had an important contribution in some samples in this station, probably as parasites or symbionts.

3.2.4 | Fildes Point (Deception Island)

Station is located at the entrance of Deception Island (Neptune Bellows), characterized by the strong currents crossing the narrow channel, and the absence of ice-scouring as the main Antarctic disturbance factor. The main structure of this community is built up by sponges (*Mycale* sp. *Dendrilla antarctica*, *Kirkpatrickia variolosa*, *Haliclona* sp. and a Poecilosclerida), hydrozoa (Campanulariidae), the bryozoan *Antarctothoa* sp. and the ascidian *Cnemidocarpa verrucosa* creating microhabitats for many polychaetes (*Polyeunoa laevis*, *Lanicides bilobata*, *Antarctinoe ferox*, *Aglaophamus trissophyllus* and terebellida), the nemertean *Parborlasia corrugatus*, the sipunculida *Golfingia margaritacea*, the sea urchin *Abatus agassizii*, the nudibranch *Doris kerguelenensis* and several amphipoda and isopoda (such as *Iathrippa sarsi*). In Deception Island, the sea urchin *Sterechinus neumayerii* and the sea star *Odontaster validus* are always present in high abundance and biomasses (Angulo-Preckler et al., 2017).

3.2.5 | Esperanza (Hope Bay)

This is the northernmost location of the study, located at the tip of the Antarctic Peninsula. It is characterized by the presence of huge icebergs grounding the seafloor. This community is mainly formed by

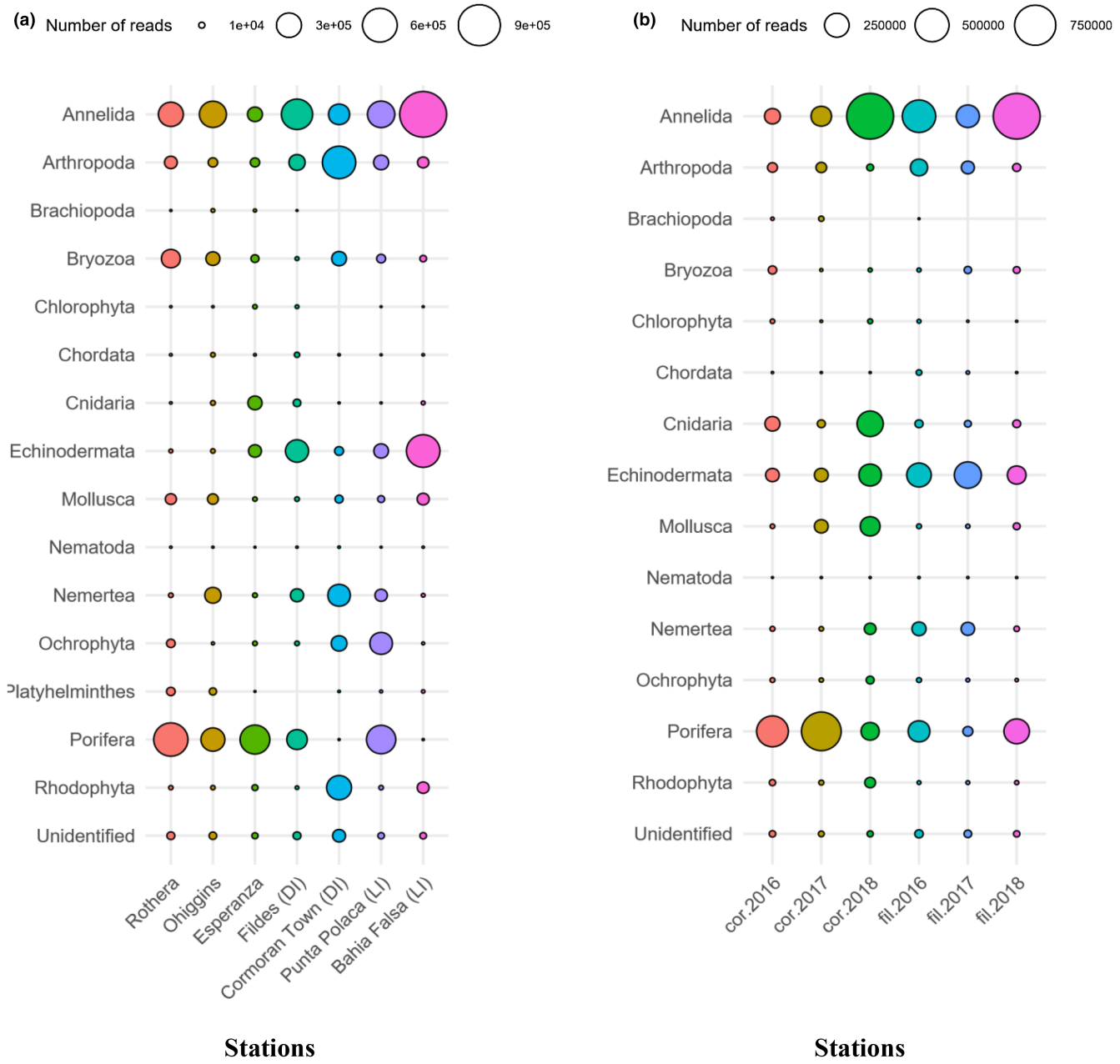

FIGURE 7 Bubble plot representing the spatial and temporal patterns of relative read abundance (average values per station) for macroscopic phyla. (a) Spatial pattern along the WAP. (b) Temporal pattern in Deception Island from 2016 to 2018. Cor, Cormoran Town; Fil, Fildes Point.

macroalgae, unidentified bryozoans and vagile organisms. The brown algae include species such as *Ascoseira mirabilis*, *Himantothallus grandifolius* and *Desmarestia menziesii* and red algae such as *Plocamium cartilagineum*, *Picconiella plumose*, *Callophyllis* sp., *Myriogramme manginii*, *Iridaea cordata* and *Pantoneura plocamioides*. This algal community supports different species composition than those dominated by sponges. Despite the presence of the cosmopolite terebellida *Thelepus antarcticus*, biodiversity was dominated by several amphipoda, nemertean (*Parborlasia corrugatus*, *Antarctonemertes valida*, *Antarctonemertes* sp., *Oerstedia* sp.), echinodermata (*Odontaster validus*, *Diplasterias brucei* and *Sterechinus neumayeri*) and mollusca (*Margarella antarctica*, Littorinidae, Chitonida). In addition, some microeukaryotes such as dinoflagellates (*Hematodinium* sp.) and diatoms contribute to the diversity in this station.

3.2.6 | O'Higgins (Rada Covadonga)

Station at similar latitude to the SSI stations located in the WAP. As all the stations in the Antarctic Peninsula, it is heavily influenced by ice disturbance due to icebergs. It is the station with less consistency between replicates. Two samples were very similar, but

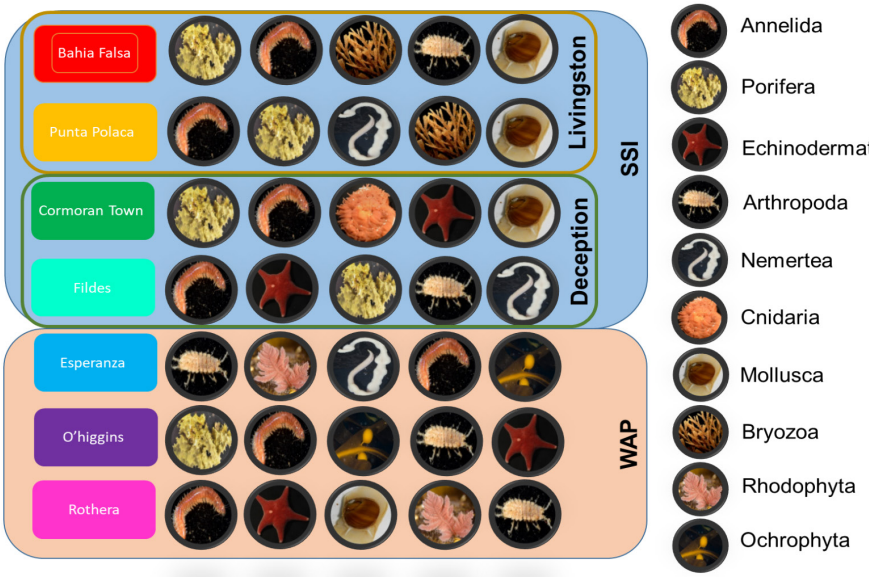

FIGURE 8 Diagram representing the five most abundant phyla per station (together they account for 85%–95% of the total reads by station).

one of them showed high dissimilarities showing a completely different community. We mainly found a community represented by sponges (*Dendrilla antarctica*, Acarnidae, *Artemisina plumosa* and Poecilosclerida) and bryozoans (*Antarctothoa* sp., *Hippomenella* sp., *Arachnopusia unicornis* and other cheilostomatids), hosting different polychaetes species (*Thelepus antarcticus* and other phyllodocids), nemerteans and arthropoda (amphipoda and copepoda). On the other hand, we found a different community mainly formed by brown algae from Acinetosporaceae and Desmaresticeae families, echinodermata (Holothurian and *Odontaster validus*) and mollusca (*Margarella antarctica*). This last community presented an important contribution of diatoms to the total composition, probably as epibionts.

3.2.7 | Rothera (Trolval Island)

This is the furthest and southernmost station of our study, composed of a bedrock substratum with a topography that prevents most icebergs from reaching the site. It represents a different benthic community characterized by the absence of sponges. The sessile component is clearly dominated by red algae (*Phyllophora* sp., *Trematocarpus antarcticus*, *Phycodrys quercifolia*, *Hymenocladiopsis prolifera* and *Wildemania amplissima*) and some unidentified bryozoans. The vagile community is formed by several polychaetes (*Lanicides bilobata*, *Thelepus antarcticus*, *Antarctinoe ferox*, *Polyeunoa laevis*, *Polycirrus* sp. and *Flabelligera* sp.), echinodermata (*Sterechinus neumayeri*, *Cucumaria georgiana*, *Heterocucumis steineni*, *Diplasterias brucei*, *Lysasterias perrieri* and *Odontaster validus*), arthropoda (the isopod *Iathrippa sarsi*, some ostracoda and the pycnogonida *Pentanymphon antarcticum* and *Austrodecus glaciale*) and mollusca (the bivalve *Philobrya barbata*, one Chitonida and some Gastropoda).

3.3 | Temporal patterns

Despite the close proximity between the two stations on Deception Island, they showed a completely different community composition and biodiversity patterns. Alpha diversity (MOTUs richness (*S*) and Shannon index (*H*)) displayed significant differences (*S*; ANOVA: F value = 28.99, $p < .0001$, and *H*; F value = 4.58, $p = .0371$). We also found significant differences in *S* and *H* among the years within stations (Fildes: *S*; ANOVA: F value = 5.74, $p = .0244$, and *H*; F value = 7.38, $p = .0118$, and Cormoran Town: *S*; ANOVA: F value = 7.37, $p = .0119$, and *H*; F value = 5.17, $p = .0319$), with opposite diversity trends (Figure S4b). Fildes showed higher values of alpha diversity along the years with an important decline trend in all the diversity indices measured, while Cormoran Town displayed a negative relationship between species richness and evenness, with an important increase in the last year resembling Fildes values (see Table S3). In addition, a slight but important decrease in the evenness was observed along the years in both stations (Figure S5). Moreover, no differences were found related to the number of reads between these two stations. The community composition showed significant differences between stations (PERMANOVA $p < .0009$) and years (PERMANOVA $p < .0009$). Although the two communities were grouped separately in the nmMDS (Figure 5b), there was a tendency to converge among the years, probably due to the drastic annual decrease of the MOTUs richness in Fildes.

3.3.1 | Beta diversity

Overall, there were changes in benthic community compositions between 2016 and 2018. Species loss was the main component of beta diversity in Deception Island between 2016 and 2018 ($t = -0.85$,

$p < .05$). Benthic communities lost MOTUs in Fildes (B = 424, C = 213) and gained MOTUs in Cormoran Town (B = 118, C = 468; Figure S6), but only Cormoran Town showed significant differences in the MOTUs assemblage over the years ($t = 0.902$, $p = .001$).

4 | DISCUSSION

The logistic difficulties of accessing the remote Antarctic ecosystems call for cost-effective and comprehensive tools for the evaluation of community diversity facing a changing climate. Despite the relatively easy way in which eDNA water sampling can be performed, water eDNA has been proved to be a poor surrogate for benthic structure and composition (Antich, Palacin, Cebrian, et al., 2021; Antich, Palacín, Wangensteen, et al., 2021; Cowart et al., 2018) and direct sampling methods are required for monitoring these complex communities via metabarcoding techniques. The sampling of benthic hard-bottom habitats requires direct access to the environments and involves more effort than sampling sediments or water, which can be accessed remotely.

An important challenge for molecular methods is the presence of large taxonomic gaps in global reference sequence databases. Kvist (2013), indicated that bryozoans, platyhelminths and nematodes were highly underrepresented globally in the reference sequence databases, while echinoderms, cnidarians and Porifera had moderately good barcode coverage. Focussing on Antarctic waters, around 70 classes of organisms (SCAR-MarBIN's Register of Antarctic Marine Species, RAMS) have no sequence information yet. These include important classes such as Calcarea (calcareous sponges), Stauromedusae (stalked jellyfish), Pterobranquia, Ciliophora, Monoplacophora and Aplacophora Mollusca, Priapulida and Tardigrada (Grant et al., 2011). Here we present a pioneer study to establish the molecular biodiversity baselines of shallow hard-bottom habitats from the WAP. This preliminary study, similar to what Fonseca et al. (2017, 2022) found in soft-bottom habitats, reveals that most of the benthic biodiversity found in Antarctic habitats is yet to be taxonomically described in regard to the identification and development of associated barcodes. Only around 5% of our MOTUs had a full taxonomical match at species level (>99% similarity) against public databases. Despite limitations due to taxonomic assignment at the species level, this study shows interesting insights into the extend of biodiversity in Antarctic shallow benthos. Similar levels of diversity were found when compared to other marine studies carried out in temperate regions using the same methodology (Antich, Palacin, Cebrian, et al., 2021; Antich, Palacín, Wangensteen, et al., 2021). Additionally, our study pinpoints the importance of MOTU diversity and the relative read abundance of metazoans in samples from the Antarctic benthos. These findings are fundamental to identifying key species in the benthic communities, understanding trophic relationships and evaluating ecosystem dynamics.

Metabarcoding of benthic communities, using a broad range of eukaryotic marker (COI), retrieved substantial differences in community structure among localities at different latitudes and years. Our study provides a framework for the assessment of biodiversity associated with shallow rocky bottoms along the WAP and South Shetland Islands (SSI), including the often neglected 'hidden biodiversity' of small organisms contributing to the cryptic community. The spatial patterns of MOTU richness and relative read abundance showed a clear dominance of macrobenthic taxa (mainly metazoans, rhodophytes and ochrophytes), but meiobenthic eukaryotes (such as diatoms, dinoflagellates and haptophytes) also formed an important component of these communities. Quantifying biodiversity in highly diverse habitats raises questions about the sampling effort. Here, rarefaction and the accumulation curve of MOTUs versus number of samples flattens to asymptotes, so the dataset is very close to reflect the sampling habitat to represent the natural diversity. Furthermore, the sorting of samples into three size categories reduces substantially the dominance of large specimens, allowing for a better detection of the smallest associated fauna, as also shown in Antich, Palacin, Cebrian, et al. (2021), Antich, Palacín, Wangensteen, et al. (2021), Elbrecht et al. (2017), and Wangensteen et al. (2018) for temperate communities. The natural heterogeneity and complexity of communities imply that accurate monitoring requires high resolution, both temporally and spatially, as well as more complete assessments of taxa.

The amplification efficiency of the COI gene varies among groups and species, and this might bias the quantitative results (Descôteaux et al., 2021; Hajibabaei et al., 2011). However, even though correlations between read abundance and species biomass are not perfect, the relative read abundance from community-DNA metabarcoding datasets has shown to be significantly associated with the relative biomass of the species, which adds support to the development of assessment tools based on semiquantitative approaches for this kind of metabarcoding data (Elbrecht & Leese, 2015; Ershova et al., 2021). Our benthic samples are community or bulk DNA (Rodriguez-Ezpeleta et al., 2021). This type of DNA is typical of significant quantity and high quality. In addition, the mesh size used here (63 μm), guarantees that most prokaryotes and the smallest fraction of the microeukaryotes were washed out, along with viruses, cell debris and extracellular DNA, which explains the high number of reads assigned to eukaryotes. When using quantitative metrics based on relative read abundances, replicates of each ecological location always clustered together and thus the combined replicate samples accurately reflected alpha and beta diversities from the WAP. Benthic community composition can be extremely variable even within small spatial scales, but all localities maintained a similar core community. Local patchiness and structure within these communities are probably a consequence of a combination of several biotic and abiotic factors (i.e. ice disturbance, food supply, competition, seabed topography and life-cycle strategies; Clarke et al., 2021; Fonseca et al., 2022). Understanding spatial–temporal changes in ecological communities of coastal ecosystems is crucial in the context of climate change.

4.1 | Spatial patterns

Although no clear trends in MOTUs richness or Shannon diversity index were observed with latitude, a correlation between the geographical distance and the dissimilarity of the community

composition at each locality was detected. Therefore, as samples became physically more separated their community composition became more dissimilar. Furthermore, some differences in community composition were found between the WAP stations and the SSI stations and even between localities within areas. The contribution of each site to beta diversity was positively correlated with MOTU richness. This is especially remarkable for Rothera station, the most geographically distant station, which has comparatively lower values for both alpha and beta diversity compared with other localities. The lowest diversity values were found in Cormoran Town, at Deception Island. Surprisingly, Fildes Point, also in Deception Island, and separated only by a few tens of metres from Cormoran Town, was the station with the highest MOTU richness in our study. This is a striking example of the well-documented patchiness in benthic Antarctic communities (Gutt & Piepenburg, 2003; Smale, 2008) and may be related to the exposure to strong currents in this site, closer to the Neptune Bellows (bay entrance).

Antarctic Peninsula sites can be remarkably rich in species, as well as having high biomass and abundance values relative to those in the Arctic or even temperate regions (Arntz et al., 1997; Brey & Clarke, 1993, Fonseca et al., 2017, 2022). Our results show that there is an Antarctic core community present in all localities mainly composed of terebellidae (i.e. *Thelepus antarcticus*), and other polychaeta, several classes of arthropoda such as ostracoda, amphipoda and copepoda, along with some mollusca (*Nacella concinna*, *Margarella antarctica*), echinodermata (*Odontaster validus*, *Sterechinus neumayeri*), nemertea (*Parborlasia corrugatus*) and oligochaeta, which seek shelter in the three-dimensional biotic components of these communities. Sponges (mainly *Dendrilla Antarctica*), bryozoans (*Antarctothoa* spp.) and several macroalgae (*Desmarestia menziesii*, *Phycodrys quercifolia*) formed the main structure shaping these communities. In addition, these communities were always sprinkled by several associations of diatoms (*Skeletonema* sp., *Navicula* sp., Sellaphoraceae, Mediophyceae), along with several unidentified metazoan (probably meiofaunal) and/or parasites and saprotrophs (from the Apicomplexa and Bigyra phyla). One hundred MOTUs were shared within all seven stations analysed here. This common core community in all these distant localities, separated by more than 500 km may be explained by the high number of Antarctic species with circumpolar distribution, the similarity of conditions in the sea around the continent and the circumantarctic current systems (Arntz et al., 1994), although the traditional view that many SO benthic invertebrate taxa have a circumpolar distribution has come recently under scrutiny (Harder et al., 2016; Hauquier et al., 2017; Janosik et al., 2011; Wilson et al., 2007). Previous studies in the region have described dense three-dimensional communities formed by sponges, hydrocorals, bryozoans and ascidians that are important hotspots of biodiversity (Angulo-Preckler et al., 2018; Cárdenas & Montiel, 2017; Gutt et al., 2017), the so-called 'Animal Forests' (AF; Rossi, 2013). The complexity of the AF depends on the ecological structuring organisms. Indeed, patches of sponges can be highly three-dimensionally complex and diverse, influencing hydrodynamics and supplying shelter and food to very diverse associated fauna (Rossi et al., 2017). A high geographical turnover of microbenthic assemblages within larger regions can generally be explained mainly by sea-ice conditions and proxies for food supply, such as current and pigments in the sediment (Gutt, 2007). All communities studied here present several MOTUs representing these habitat builders. Moreover, these species are represented with high relative read abundances in our dataset, probably due to the high relative biomass in the samples (sponges, bryozoans, macroalgae, annelids and echinoderms). We detected high diversity of some specific groups, mostly in accordance with general Antarctic diversity patterns, where pycnogonids, polychaetes, ascidians, amphipods and bryozoans are particularly species-rich around Antarctica (Clarke & Johnston, 2003). The most diverse taxa in our samples were Maxillopods (mainly copepods) with 108 MOTUs, followed by diatoms (99 MOTUs), amphipods (80 MOTUs) and polychaetes (67 MOTUs). On the other hand, we found an unexpected poor diversity of pycnogonids (6 MOTUs), sea stars (6 MOTUs) and sponges (18 MOTUs). Two main communities can be distinguished in this study, one dominated mainly by Porifera and to a lesser extent bryozoans (the four stations on the South Shetland Islands altogether with O'Higgins station), and the second one, mainly dominated by macroalgal species (both Rhodophyta and Ochrophyta) detected in Esperanza and Rothera stations, showing almost a complete absence of sponges. The composition of these structuring organisms (the trees of the marine animal forests) conditioned the associated fauna living on them. Furthermore, due to the high patchiness of Antarctic benthic communities, especially in shallow waters, the randomization of the sampling and the small surface sampled (625 cm^2) might greatly condition the obtained results. Therefore, we can assume that the communities obtained here are only representative of the sampled area at a local scale (few metres) but not at the regional scale (hundreds of metres). There were significant differences between stations, just a few metres apart, and variation at the scale of <5 m (replicates) was also high. The distribution of species in Antarctic shallow waters is inevitably highly heterogeneous due to the physical pressure of ice disturbance (Smale et al., 2007). The high heterogeneity found inside Deception Island, a place highly protected from ice-scouring, gives more weight to other physical factors such as current flows or food availability, or even to biological factors (niche-driven).

4.2 | Temporal patterns

Compared with traditional surveys, eDNA has increased the detection sensitivity of organisms (Bohmann et al., 2014). After several years of sampling and taxonomical studies at Deception Island, Angulo-Preckler et al. (2018) found high values of alpha diversity compared with previous studies (95 taxa in Cormoran Town and 97 taxa in Fildes), but those diversity results are far below the diversity values obtained here with metabarcoding (930 MOTUs in Cormoran Town and 1413 MOTUs in Fildes). Underestimates of species diversity are likely due, in part, to small and/or cryptic taxa. Moreover, metabarcoding allows for the detection of parasites and non-native

species that otherwise, would be easily overlooked (e.g. Descôteaux et al., 2021). To evaluate benthic Antarctic communities, traditional studies have focussed on macroorganisms, almost exclusively metazoans, neglecting the importance that the ecological role of smaller organisms, such as nematodes, diatoms or dinoflagellates, may have.

Our results show that benthic assemblages changed between 2016 and 2018 (temporal beta diversity), and these changes were linked to species loss rather than to species replacement. Due to the high degree of patchiness in the Antarctic hard-bottom communities, we cannot assert that the biodiversity is lower at the Fildes station (Table S3) or that biodiversity is accumulating at the Cormoran Town station. Although it may be a sampling artefact, a convergence between the most diverse and the least diverse stations was observed throughout the years. We detected a significant turnover in Cormoran Town (MOTU replacement; Figure S6) but an overall nestedness (MOTU loss) for Deception Island. Due to the oceanographic conditions, we should expect a higher biodiversity of sessile suspension feeders (habitat builders providing architectural complexity) in Fildes, which would allow a rich and diversified community of smaller organisms to settle. At the same time, the high rates of sedimentation inside Deception Island (Baldwin & Smith, 2003), can be the reason behind the less diverse community in Cormoran Town. The main abiotic factors affecting the benthic communities are common for both stations (ice disturbance, depth, sediment type). The main difference between stations is the currents (food supplied, sedimentation) since Fildes Point is located just in the entrance channel while Cormoran Town is inside the protected Whaler Bay. The highest values of alpha diversity of Fildes Point confirm the presence of an important biodiversity hotspot, probably due to the absence of physical disturbance such as ice-scouring and anchor ice at the entrance of Deception Island, which allows the development of these benthic assemblages, despite the background of recent volcanic eruptions (Angulo-Preckler et al., 2018, 2021). Future studies should focus on verifying if this decreasing trend in biodiversity is maintained and try to unmask the possible factors behind this process.

5 | CONCLUSION

Recent advances in molecular and sequencing methodologies enabled us to evaluate biodiversity levels from even the most remote habitats. Measuring species and biological diversity of the Antarctic shelf is notoriously difficult as a result of high community patchiness and complex hierarchical scales of spatial variation. Hard-bottom assemblages exhibit high spatial variability and heterogeneity, not related to depth, which is challenging to design large-scale sampling programmes to assess the state of the benthos in the Southern Ocean. There are many unknowns as to how communities will respond to climate change, and studies like this contribute to understanding the present spatial variability in Antarctic shallow hard-bottom communities and serve as a baseline for future comparisons. The identification of a remarkable core community shared within all the localities, regardless of the large distance between some stations, reinforces the traditional view of circumpolar distribution in several invertebrate taxa. As the accuracy of metabarcoding taxonomic assignment relies upon the completeness of public sequence databases, continued efforts in the improvement of these databases are essential to provide a holistic description of Antarctic marine ecosystems. An effort to increase the coverage of the sampled areas is necessary to achieve sufficient representability.

ACKNOWLEDGEMENTS

We thank all members of the DISTANTCOM and BLUEBIO research projects for their support. We especially thank the crews of the scientific vessels *BIO Hesperides* and Sarmiento de Gamboa, as well as the staff of the Spanish Antarctic Bases for their logistic support during the diving operations. We also want to thank Dr. Xavier Turon and Dr. Adria Antich for their help in processing the samples. CA-P was funded by the Ramon Areces Foundation. UiT the Arctic University of Norway is thanked for financial support to KP. This is an AntICON (SCAR) contribution. The publication charges for this article have been funded by a grant from the publication fund of UiT The Arctic University of Norway.

CONFLICT OF INTEREST STATEMENT

The research reported here has been conducted in an ethical and responsible manner and complies with all relevant legislation. The authors declare there is no potential conflict of interest.

PEER REVIEW

The peer review history for this article is available at: https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ddi.13703.

DATA AVAILABILITY STATEMENT

The raw sequencing data of this study have been deposited in the NCBI Short Read Archive (SRA). Project number: PRJNA914641.

ORCID

Carlos Angulo-Preckler https://orcid.org/0000-0001-9028-274X
Marta Turon https://orcid.org/0000-0002-3806-4937
Kim Præbel https://orcid.org/0000-0002-0681-1854
Conxita Avila https://orcid.org/0000-0002-5489-8376
Owen S. Wangensteen https://orcid.org/0000-0001-5593-348X

REFERENCES

Almond, P. M., Linse, K., Dreutter, S., Grant, S. M., Griffiths, H. J., Whittle, R. J., Mackenzie, M., & Reid, W. D. K. (2021). In-situ image analysis of habitat heterogeneity and benthic biodiversity in the Prince Gustav Channel, Eastern Antarctic Peninsula. *Frontiers in Marine Science*, *8*, 1–18. https://doi.org/10.3389/fmars.2021.614496

Angulo-Preckler, C., Figuerola, B., Núñez-Pons, L., Moles, J., Martín-Martín, R., Rull-Lluch, J., Gómez-Garreta, A., & Avila, C. (2018). Macrobenthic patterns at the shallow marine waters in the caldera of the active volcano of Deception Island, Antarctica.

Continental Shelf Research, *157*, 20–31. https://doi.org/10.1016/j.csr.2018.02.005

Angulo-Preckler, C., Pernet, P., García-Hernandez, C., Kereszturi, G., Alvarez-Valero, A. M., Hopfenblatt, J., Gomez-Ballesteros, M., Otero, X. L., Caza, J., Ruiz-Fernandez, J., Geyer, A., & Avila, C. (2021). Volcanism and rapid sedimentation affect the benthic communities of Deception Island, Antarctica. *Continental Shelf Research*, *220*(71), 104404. https://doi.org/10.1016/j.csr.2021.104404

Angulo-Preckler, C., Tuya, F., & Avila, C. (2017). Abundance and size patterns of echinoderms in coastal soft-bottoms at Deception Island (South Shetland Islands, Antarctica). *Continental Shelf Research*, *134*, 131–1141. https://doi.org/10.1016/j.csr.2016.12.010

Antich, A., Palacín, C., Cebrian, E., Golo, R., Wangensteen, O. S., & Turon, X. (2021). Marine biomonitoring with eDNA: Can metabarcoding of water samples cut it as a tool for surveying benthic communities? *Molecular Ecology*, *30*(13), 3175–3188. https://doi.org/10.1111/mec.15641

Antich, A., Palacin, C., Wangensteen, O. S., & Turon, X. (2021). To denoise or to cluster, that is not the question: Optimizing pipelines for COI metabarcoding and metaphylogeography. *BMC Bioinformatics*, *22*(1), 177. https://doi.org/10.1186/s12859-021-04115-6

Appeltans, W., Ahyong, S. T., Anderson, G., Angel, M. v., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błażewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C. B., Brandão, S. N., Bray, R. A., Bruce, N. L., Cairns, S. D., Chan, T. Y., … Costello, M. J. (2012). The magnitude of global marine species diversity. *Current Biology*, *22*(23), 2189–2202. https://doi.org/10.1016/j.cub.2012.09.036

Arntz, W. E., Brey, T., & Gallardo, V. A. (1994). Antarctic zoobenthos. *Oceanography and Marine Biology: An Annual Review*, *32*, 241–304.

Arntz, W. E., Gutt, J., & Klages, M. (1997). Antarctic marine biodiversity: An overview. In *Antarctic communities: Species, structure and survival*. Cambridge University Press. https://books.google.es/books?hl=en&lr=&id=syZC19PKpV8C&oi=fnd&pg=PA3&dq=antarctic+marine+biodiversity.+Arntz+1997&ots=JZ7IP1eRKI&sig=L-GlZkcss1p1PkvGIIMfywT62n-8

Atienza, S., Guardiola, M., Præbel, K., Antich, A., Turon, X., & Wangensteen, O. S. (2020). DNA metabarcoding of deep-sea sediment communities using COI: Community assessment, spatio-temporal patterns and comparison with 18S rDNA. *Diversity*, *12*(4). https://doi.org/10.3390/D12040123

Aylagas, E., Borja, Á., Irigoien, X., & Rodríguez-Ezpeleta, N. (2016). Benchmarking DNA metabarcoding for biodiversity-based monitoring and assessment. *Frontiers in Marine Science*, *3*, 1–12. https://doi.org/10.3389/fmars.2016.00096

Baldwin, R. J., & Smith, K. L. (2003). Temporal dynamics of particulate matter fluxes and sediment community response in Port Foster, Deception Island, Antarctica. *Deep Sea Research Part II: Topical Studies in Oceanography*, *50*(10–11), 1707–1725. https://doi.org/10.1016/S0967-0645(03)00089-4

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution*, *29*(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Bourlat, S. J., Borja, A., Gilbert, J., Taylor, M. I., Davies, N., Weisberg, S. B., Griffith, J. F., Lettieri, T., Field, D., Benzie, J., Glöckner, F. O., Rodríguez-Ezpeleta, N., Faith, D. P., Bean, T. P., & Obst, M. (2013). Genomics in marine monitoring: New opportunities for assessing marine health status. *Marine Pollution Bulletin*, *74*(1), 19–31. https://doi.org/10.1016/j.marpolbul.2013.05.042

Boyer, F., Mercier, C., Bonin, A., le Bras, Y., Taberlet, P., & Coissac, E. (2016). Obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176–182. https://doi.org/10.1111/1755-0998.12428

Brannock, P. M., Learman, D. R., Mahon, A. R., Santos, S. R., & Halanych, K. M. (2018). Meiobenthic community composition and biodiversity along a 5500 km transect of Western Antarctica: A metabarcoding analysis. *Marine Ecology Progress Series*, *603*, 47–60. https://doi.org/10.3354/meps12717

Brey, T., & Clarke, A. (1993). Population dynamics of marine benthic invertebrates in Antarctic and subantarctic environments: Are there unique adaptations? *Antarctic Science*, *5*(3), 253–266. https://doi.org/10.1017/S0954102093000343

Brown, K. M., Fraser, K. P. P., Barnes, D. K. A., & Peck, L. S. (2004). Links between the structure of an Antarctic shallow-water community and ice-scour frequency. *Oecologia*, *141*(1), 121–129. https://doi.org/10.1007/s00442-004-1648-6

Bullivant, J. S. (1959). Photographs of the bottom features, fauna of the Ross Sea area, Antarctica. *Geological Society of America Bulletin*, *72*, 787–790.

Cárdenas, C. A., & Montiel, A. (2017). Coexistence in cold waters: Animal forests in seaweed-dominated habitats in southern high-latitudes. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas (Eds.), *Marine animal forests*. Springer. https://doi.org/10.1007/978-3-319-21012-4_50

Castro-Fernández, D., Balleste, E., Angulo-Preckler, C., Biggs, J., Avila, C., & García-Aljaro, C. (2023). How does heat stress affect sponge microbiomes? Structure and resilience of microbial communities of marine sponges from different habitats. *Frontiers in Marine Science*, *9*. https://doi.org/10.3389/fmars.2022.1072696

Chen, H. (2018). *VennDiagram: Generate high-resolution Venn and Euler plots*. R package version 1.6. 20. https://cran.R-Project.Org/Package=VennDiagram.

Clark, G. F., Raymond, B., Riddle, M. J., Stark, J. S., & Johnston, E. L. (2015). Vulnerability of Antarctic shallow invertebrate-dominated ecosystems. *Austral Ecology*, *40*(4), 482–491. https://doi.org/10.1111/aec.12237

Clark, G. F., Stark, J. S., Johnston, E. L., Runcie, J. W., Goldsworthy, P. M., Raymond, B., & Riddle, M. J. (2013). Light-driven tipping points in polar ecosystems. *Global Change Biology*, *19*(12), 3749–3761. https://doi.org/10.1111/gcb.12337

Clarke, A. (1990). Temperature and evolution: Southern Ocean cooling and the Antarctic marine fauna. In K. R. Kerry & G. Hempel (Eds.), *Antarctic ecosystems* (pp. 9–22). Springer. https://doi.org/10.1007/978-3-642-84074-6_2

Clarke, A., & Johnston, N. (2003). *Oceanography and marine biology: An annual review: Volume 41*. CRC Press. https://books.google.com/books?hl=en&lr=&id=64crGFXWn5gC&pgis=1

Clarke, A., & Leakey, R. J. G. (1996). The seasonal cycle of phytoplankton, macronutrients, and the microbial community in a nearshore antarctic marine ecosystem. *Limnology and Oceanography*, *41*(6), 1281–1294. https://doi.org/10.4319/lo.1996.41.6.1281

Clarke, L. J., Suter, L., Deagle, B. E., Polanowski, A. M., Terauds, A., Johnstone, G. J., & Stark, J. S. (2021). Environmental DNA metabarcoding for monitoring metazoan biodiversity in Antarctic nearshore ecosystems. *PeerJ*, *9*, e12458. https://doi.org/10.7717/peerj.12458

Conlan, K. E., & Kvitek, R. G. (2005). Recolonization of soft-sediment ice scours on an exposed Arctic coast. *Marine Ecology Progress Series*, *286*, 21–42. https://doi.org/10.3354/meps286021

Cowart, D. A., Murphy, K. R., & Cheng, C. H. C. (2018). Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula. *Marine Genomics*, *37*, 148–160. https://doi.org/10.1016/j.margen.2017.11.003

de Broyer, C., Koubbi, P., Griffiths, H., & Grant, S. A. (2014). *Biogeographic atlas of the Southern Ocean*. Scientific Committee on Antarctic Research.

Descôteaux, R., Ershova, E., Wangensteen, O. S., Præbel, K., Renaud, P. E., Cottier, F., & Bluhm, B. A. (2021). Meroplankton diversity, seasonality and life-history traits across the Barents Sea Polar Front revealed by high-throughput DNA barcoding. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.677732

Downey, R. v., Griffiths, H. J., Linse, K., & Janussen, D. (2012). Diversity and distribution patterns in high southern latitude sponges. *PLoS ONE*, *7*(7). https://doi.org/10.1371/journal.pone.0041672

Dray, A. S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2021). *Package 'adespatial'*. https://doi.org/10.1890/11-1183.1>. Maintainer.

Duarte, C. M., Agusti, S., Barbier, E., Britten, G. L., Castilla, J. C., Gattuso, J. P., Fulweiler, R. W., Hughes, T. P., Knowlton, N., Lovelock, C. E., Lotze, H. K., Predragovic, M., Poloczanska, E., Roberts, C., & Worm, B. (2020). Rebuilding marine life. *Nature*, *580*(7801), 39–51. https://doi.org/10.1038/s41586-020-2146-7

Elbrecht, V., & Leese, F. (2015). Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. *PLoS ONE*, *10*(7), 1–16. https://doi.org/10.1371/journal.pone.0130324

Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., & Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. *Methods in Ecology and Evolution*, *8*(10), 1265–1275. https://doi.org/10.1111/2041-210x.12789

Ershova, E. A., Wangensteen, O. S., Descoteaux, R., Barth-Jensen, C., & Præbel, K. (2021). Metabarcoding as a quantitative tool for estimating biodiversity and relative biomass of marine zooplankton. *ICES Journal of Marine Science*, *78*(9), 3342–3355.

Flaviani, F., Schroeder, D. C., Lebret, K., Balestreri, C., Highfield, A. C., Schroeder, J. L., Thorpe, S. E., Moore, K., Pasckiewicz, K., Pfaff, M. C., & Rybicki, E. P. (2018). Distinct oceanic microbiomes from viruses to protists located near the antarctic circumpolar current. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.01474

Fonseca, V. G., Carvalho, G. R., Sung, W., Johnson, H. F., Power, D. M., Neill, S. P., Packer, M., Blaxter, M. L., Lamdshead, J. D., Thomas, W. K., & Creer, S. (2010). Second-generation environmental sequencing unmasks marine metazoan biodiversity. *Nature Communications*, *1*(1), 98. https://doi.org/10.1038/ncomms1095

Fonseca, V. G., Kirse, A., Giebner, H., Vause, B. J., Drago, T., Power, D. M., Peck, L. S., & Clark, M. S. (2022). Metabarcoding the Antarctic Peninsula biodiversity using a multi-gene approach. *ISME Communications*, *2*(1), 37. https://doi.org/10.1038/s43705-022-00118-3

Fonseca, V. G., Sinniger, F., Gaspar, J. M., Quince, C., Creer, S., Power, D. M., Peck, L. S., & Clark, M. S. (2017). Revealing higher than expected meiofaunal diversity in Antarctic sediments: A metabarcoding approach. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-06687-x

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, *13*(5), 851–861. https://doi.org/10.1111/1755-0998.12138

Grant, R. A., Griffiths, H. J., Steinke, D., Wadley, V., & Linse, K. (2011). Antarctic DNA barcoding; a drop in the ocean? *Polar Biology*, *34*(5), 775–780. https://doi.org/10.1007/s00300-010-0932-7

Griffiths, H. (2010). Antarctic marine biodiversity – What do we know about the distribution of life in the Southern Ocean. *PLoS One*, *5*(8). https://doi.org/10.1371/journal.pone.0011683

Gutt, J. (2007). Antarctic macro-zoobenthic communities: A review and an ecological classification. *Antarctic Science*, *19*(2), 165–182. http://journals.cambridge.org/abstract_S0954102007000247

Gutt, J., Cummings, V., Dayton, P. K., Isla, E., Jentsch, A., & Schiaparelli, S. (2017). Antarctic marine animal forests: Three-dimensional communities in southern ocean ecosystems. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas (Eds.), *Marine animal forests*. Springer. https://doi.org/10.1007/978-3-319-21012-4_8

Gutt, J., & Piepenburg, D. (2003). Scale-dependent impact on diversity of Antarctic benthos caused by grounding of icebergs. *Marine Ecology Progress Series*, *253*, 77–83. https://doi.org/10.3354/meps253077

Gutt, J., Sirenko, B. I., Smirnov, I. S., & Arntz, W. E. (2004). How many macrozoobenthic species might inhabit the Antarctic shelf? *Antarctic Science*, *16*(1), 11–16. https://doi.org/10.1017/S0954102004001750

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G. A. C., & Baird, D. J. (2011). Environmental barcoding: A next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS ONE*, *6*(4), e17497. https://doi.org/10.1371/journal.pone.0017497

Harder, A. M., Halanych, K. M., & Mahon, A. R. (2016). Diversity and distribution within the sea spider genus Pallenopsis (Chelicerata: Pycnogonida) in the Western Antarctic as revealed by mitochondrial DNA. *Polar Biology*, *39*(4), 677–688. https://doi.org/10.1007/s00300-015-1823-8

Hauquier, F., Leliaert, F., Rigaux, A., Derycke, S., & Vanreusel, A. (2017). Distinct genetic differentiation and species diversification within two marine nematodes with different habitat preference in Antarctic sediments. *BMC Evolutionary Biology*, *17*(1), 1–14.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Janosik, A. M., Mahon, A. R., & Halanych, K. M. (2011). Evolutionary history of Southern Ocean Odontaster Sea star species (Odontasteridae; Asteroidea). *Polar Biology*, *34*(4), 575–586. https://doi.org/10.1007/s00300-010-0916-7

Kvist, S. (2013). Barcoding in the dark?: A critical view of the sufficiency of zoological DNA barcoding databases and a plea for broader integration of taxonomic knowledge. *Molecular Phylogenetics and Evolution*, *69*(1), 39–45. https://doi.org/10.1016/j.ympev.2013.05.012

Lanzén, A., Lekang, K., Jonassen, I., Thompson, E. M., & Troedsson, C. (2016). High-throughput metabarcoding of eukaryotic diversity for environmental monitoring of offshore oil-drilling activities. *Molecular Ecology*, *25*(17), 4392–4406. https://doi.org/10.1111/mec.13761

Legendre, P. (2019). A temporal beta-diversity index to identify sites that have changed in exceptional ways in space–time surveys. *Ecology and Evolution*, *9*(6), 3500–3514. https://doi.org/10.1002/ece3.4984

Legendre, P., Bocard, D., & Peres-Neto, P. R. (2005). Analyzing beta diversity: Partitioning the spatial variation of community composition data. *Ecological Monographs*, *75*(4), 435–450.

Leray, M., & Knowlton, N. (2015). DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(7), 2076–2081. https://doi.org/10.1073/pnas.1424997112

Leray, M., & Knowlton, N. (2016). Censusing marine eukaryotic diversity in the twenty-first century. *Philosophical transactions of the Royal Society B: Biological Sciences*, *112*(7), 2076–2081. https://doi.org/10.1098/rstb.2015.0331

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*(1), 34. https://doi.org/10.1186/1742-9994-10-34

Luria, C. M., Ducklow, H. W., & Amaral-Zettler, L. A. (2014). Marine bacterial, archaeal and eukaryotic diversity and community structure on the continental shelf of the western Antarctic Peninsula. *Aquatic Microbial Ecology*, *73*(2), 107–121. https://doi.org/10.3354/ame01703

Luypaert, T., Hagan, J. G., McCarthy, M. L., & Poti, M. (2020). Status of marine biodiversity in the Anthropocene. In S. Jungblut, V. Liebich, & M. Bode-Dalby (Eds.), *YOUMARES 9 - The oceans:*

Our research, our future (pp. 57–82). Springer. https://doi.org/10.1007/978-3-030-20389-4_4

Magni, P. (2003). Biological benthic tools as indicators of coastal marine ecosystems health. *Chemistry and Ecology*, *19*(5), 363–372. https://doi.org/10.1080/02757540310001603718

Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., & Worm, B. (2011). How many species are there on earth and in the ocean? *PLoS Biology*, *9*(8), 1–8. https://doi.org/10.1371/journal.pbio.1001127

Ogaki, M. B., Câmara, P. E. A. S., Pinto, O. H. B., Lirio, J. M., Coria, S. H., Vieira, R., Carvalho-Silva, M., Convey, P., Rosa, C. A., & Rosa, L. H. (2021). Diversity of fungal DNA in lake sediments on Vega Island, north-east Antarctic Peninsula assessed using DNA metabarcoding. *Extremophiles*, *25*(3), 257–265. https://doi.org/10.1007/s00792-021-01226-z

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., & Stevens, M. H. H. (2019). *vegan: Community ecology package (R package Version 2.5–5)*. https://CRAN.R-project.org/package=vegan

Peck, L. S. (2018). Antarctic marine biodiversity: Adaptations, environments and responses to change. *Oceanography and Marine Biology: An Annual Review*, *56*, 2–133.

Peck, L. S., Brockington, S., Vanhove, S., & Beghyn, M. (1999). Community recovery following catastrophic iceberg impacts in a soft-sediment shallow-water site at Signy Island, Antarctica. *Marine Ecology Progress Series*, *186*, 1–8. https://doi.org/10.3354/meps186001

Pertierra, L. R., Santos-Martin, F., Hughes, K. A., Avila, C., Caceres, J. O., de Filippo, D., Gonzalez, S., Grant, S. M., Lynch, H., Marina-Montes, C., Quesada, A., Tejedo, P., Tin, T., & Benayas, J. (2021). Ecosystem services in Antarctica: Global assessment of the current state, future challenges and managing opportunities. *Ecosystem Services*, *49*, 101299. https://doi.org/10.1016/j.ecoser.2021.101299

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System: Barcoding. *Molecular Ecology Notes*, *7*(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rey, A., Basurko, O. C., & Rodriguez-Ezpeleta, N. (2020). Considerations for metabarcoding-based port biological baseline surveys aimed at marine nonindigenous species monitoring and risk assessments. *Ecology and Evolution*, *10*(5), 2452–2465. https://doi.org/10.1002/ece3.6071

Rodriguez-Ezpeleta, N., Morissette, O., Bean, C. W., Manu, S., Banerjee, P., Lacoursière-Roussel, A., Beng, K. C., Alter, S. E., Roger, F., Holman, L. E., Stewart, K. A., Monaghan, M. T., Mauvisseau, Q., Mirimin, L., Wangensteen, O. S., Antognazza, C. M., Helyar, S. J., de Boer, H., Monchamp, M. E., … Deiner, K. (2021). Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: Comment on "environmental DNA: What's behind the term?" by Pawlowski et al., (2020). *Molecular Ecology*, *December*, *30*, 4601–4605. https://doi.org/10.1111/mec.15942

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *2016*(10), e2584. https://doi.org/10.7717/peerj.2584

Rossi, S. (2013). The destruction of the "animal forests" in the oceans: Towards an over-simplification of the benthic ecosystems. *Ocean and Coastal Management*, *84*, 77–85. https://doi.org/10.1016/j.ocecoaman.2013.07.004

Rossi, S., Bramanti, L., Gori, A., & Orejas, C. (2017). An overview of the animal forests of the world an overview of the animal forests of the world. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas (Eds.), *Marine animal forests* (pp. 1–25). Springer. https://doi.org/10.1007/978-3-319-17001-5_1-1

Sacristán-Soriano, O., Pérez Criado, N., & Avila, C. (2020). Host species determines symbiotic community composition in Antarctic sponges (Porifera: Demospongiae). *Frontiers in Marine Science*, *7*, 2020.03.09.983221. https://doi.org/10.3389/fmars.2020.00474

Sayers, E. W., Cavanaugh, M., Clark, K., Ostell, J., Pruitt, K. D., & Karsch-Mizrachi, I. (2020). GenBank. *Nucleic Acids Research*, *48*(D1), D84–D86.

Schratzberger, M., & Ingels, J. (2018). Meiofauna matters: The roles of meiofauna in benthic ecosystems. *Journal of Experimental Marine Biology and Ecology*, *502*, 12–25. https://doi.org/10.1016/j.jembe.2017.01.007

Smale, D. A. (2007). Ice disturbance intensity structures benthic communities in nearshore Antarctic waters. *Marine ecology Progress Series*, *349*, 89–102.

Smale, D. A. (2008). Continuous benthic community change along a depth gradient in Antarctic shallows: Evidence of patchiness but not zonation. *Polar Biology*, *31*(2), 189–198. https://doi.org/10.1007/s00300-007-0346-3

Smale, D. A., & Barnes, D. K. A. (2008). Likely responses of the Antarctic benthos to climate-related changes in physical disturbance during the 21st century, based primarily on evidence from the West Antarctic Peninsula region. *Ecography*, *31*(3), 289–305. https://doi.org/10.1111/j.0906-7590.2008.05456.x

Smale, D. A., Barnes, D. K. A., & Fraser, K. P. P. (2007). The influence of ice scour on benthic communities at three contrasting sites at Adelaide Island. *Antarctica. Austral Ecology*, *32*(8), 878–888. https://doi.org/10.1111/j.1442-9993.2007.01776.x

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Teixidó, N., Garrabou, J., Gutt, J., & Arntz, W. (2004). Recovery in Antarctic benthos after iceberg disturbance: Trends in benthic composition, abundance and growth forms. *Marine Ecology Progress Series*, *278*, 1–16.

Tin, T., Fleming, Z. L., Hughes, K. A., Ainley, D. G., Convey, P., Moreno, C. A., Pfeiffer, S., Scott, J., & Snape, I. (2009). Impacts of local human activities on the Antarctic environment. *Antarctic Science*, *21*(1), 3–33. https://doi.org/10.1017/S0954102009001722

Tittensor, D. P., Mora, C., Jetz, W., Lotze, H. K., Ricard, D., vanden Berghe, E., & Worm, B. (2010). Global patterns and predictors of marine biodiversity across taxa. *Nature*, *466*(7310), 1098–1101. https://doi.org/10.1038/nature09329

Turner, J., Colwell, S. R., Marshall, G. J., Lachlan-Cope, T. A., Carleton, A. M., Jones, P. D., Lagun, V., Reid, P. A., & Iagovkina, S. (2005). Antarctic climate change during the last 50 years. *International Journal of Climatology*, *25*(3), 279–294. https://doi.org/10.1002/joc.1130

van Wessem, J. M., Reijmer, C. H., van de Berg, W. J., van den Broeke, M. R., Cook, A. J., van Ulft, L. H., & van Meijgaard, E. (2015). Temperature and wind climate of the Antarctic Peninsula as simulated by a high-resolution regional atmospheric climate model. *Journal of Climate*, *28*(18), 7306–7326. https://doi.org/10.1175/JCLI-D-15-0060.1

Vause, B. J., Morley, S. A., Fonseca, V. G., Jażdżewska, A., Ashton, G. V., Barnes, D. K., Giebner, H., Clark, M. S., & Peck, L. S. (2019). Spatial and temporal dynamics of Antarctic shallow soft-bottom benthic communities: Ecological drivers under climate change. *BMC Ecology*, *19*(1), 1–14. https://doi.org/10.1186/s12898-019-0244-x

Wangensteen, O. S., Palacín, C., Guardiola, M., & Turon, X. (2018). DNA metabarcoding of littoral hardbottom communities: High diversity and database gaps revealed by two molecular markers. *PeerJ*, *2018*(5), 1–30. https://doi.org/10.7717/peerj.4705

Wangensteen, O. S., & Turon, X. (2017). Metabarcoding techniques for assessing biodiversity of marine animal forests. In S. Rossi, L. Bramanti, A. Gori, & C. Orejas (Eds.), *Marine animal forests*. Springer. https://doi.org/10.1007/978-3-319-21012-4_53

Wickham, H., Chang, W., & Wickham, M. H. (2016). *Package 'ggplot2'*. Create Elegant Data Visualisations Using the Grammar of Graphics. Version, 2(1), 1–189.

Wilson, N. G., Hunter, R. L., Lockhart, S. J., & Halanych, K. M. (2007). Multiple lineages and absence of panmixia in the "circumpolar" crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. *Marine Biology*, *152*, 895–904. https://doi.org/10.1007/s00227-007-0742-9

Wood, S. A., Smith, K. F., Banks, J. C., Tremblay, L. A., Rhodes, L., Mountfort, D., Cary, S. C., & Pochon, X. (2013). Molecular genetic tools for environmental monitoring of New Zealand's aquatic habitats, past, present and the future. *New Zealand Journal of Marine and Freshwater Research*, *47*(1), 90–119. https://doi.org/10.1080/00288330.2012.745885

BIOSKETCH

Carlos Angulo-Preckler is a research scientist at the King Abdullah University of Science and Technology (KAUST). He studies marine life on the seabed of polar environments, mainly in Antarctic shallow waters. He primarily uses SCUBA diving from remote research stations but also polar research vessels. He is particularly interested in benthic ecosystems and how they are affected by climate change and other human impacts.

Author contributions: Carlos Angulo-Preckler involved in conceptualization, sample collection, lab work, data analysis and writing—original draft. Marta Turon involved in lab work, data analysis, review and editing. Kim Præbel involved in funding acquisition, review and editing. Conxita Avila involved in sample collection, funding acquisition, review and editing. Owen Wangensteen involved in lab work, bioinformatic analysis and editing.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Angulo-Preckler, C., Turon, M., Præbel, K., Avila, C., & Wangensteen, O. S. (2023). Spatio-temporal patterns of eukaryotic biodiversity in shallow hard-bottom communities from the West Antarctic Peninsula revealed by DNA metabarcoding. *Diversity and Distributions*, *29*, 892–911. https://doi.org/10.1111/ddi.13703