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Meiobenthic community composition and biodiversity along a 5500 km transect of Western Antarctica: a metabarcoding analysis

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ABSTRACT: Meiobenthic organisms, consisting of meiofauna and benthic microeukaryotes, are key components of marine ecosystems and facilitate bentho-pelagic coupling. However, their biogeographic ranges and dispersal abilities are poorly known, especially in Antarctic waters where knowledge is extremely limited. Many Antarctic marine invertebrates are reported to have circumpolar distributions despite lecithotrophy and brooding development being common. Similarly, most meiofauna have developmental stages that are often assumed to have limited dispersal capabilities. To assess Antarctic meiobenthic community distribution patterns and diversity, the hypervariable V9 region of the 18S small subunit ribosomal RNA (SSU rRNA) gene was used to metabarcode shelf sediment samples (water depth 223 to 820 m) across a 5500 km region of the Western Antarctic. We found that some taxa had broad geographic distributions given that 28 operational taxonomic units (OTUs) were present in every core processed, 74 OTUs were found at every sampling event, and 722 OTUs were present in all of the major water basins sampled. Among these broadly distributed OTUs, metazoan taxa from 4 phyla (annelids, arthropods, kinorhynchs, and nematodes) were dominant members. As many of these OTUs relate to taxa expected to have limited dispersal capabilities based on current life history information, these results highlight our limited understanding of how small organisms move around in the sea. We also noted that the Antarctic Peninsula hosts a strikingly different and less diverse community than higher latitude regions, in contrast to benthic macrofauna.

KEY WORDS: 18S rRNA · Antarctic sediment · Illumina · Meiobenthos · Meiofauna · Metabarcoding · Stramenopiles

INTRODUCTION

Biodiversity of Antarctic benthic communities has been of interest for several decades (Dayton et al. 1974, Clarke 2008, Gutt et al. 2011). The Southern Ocean (SO) is characterized by a number of major oceanographic features, including the Antarctic Circumpolar Current (ACC), which is the largest oceanographic current on Earth (Cunningham 2003), and serves as a vector for organismal dispersal around the Antarctic (Thornhill et al. 2008, Galaska et al. 2017b). Another oceanographic feature, the Antarctic Polar Front (APF), is associated with the ACC and acts as a physiological barrier to organisms moving in and out of the SO (Lawley et al. 2004, Thatje et al. 2005, Thornhill et al. 2008, Galaska et al. 2017a). Both of these features have been invoked for explaining the distribution of predominately macro-

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faunal organisms around Antarctica (Thornhill et al. 2008, Griffiths et al. 2009, Leese et al. 2010, Dueñas et al. 2016). However, the traditional view that many SO benthic invertebrate taxa have a circumpolar distribution has come under scrutiny by a number of recent studies (Wilson et al. 2007, Janosik et al. 2011, Harder et al. 2016, Galaska et al. 2017b, Hauquier et al. 2017). Furthermore, Antarctic meiofauna (i.e. benthic invertebrates that are 0.45 µm to 1 mm in size living between sediment grains in aquatic environments; Mare 1942, Giere 2009) and benthic microeukaryotic organisms (herein collectively referred to as the meiobenthic community) have yet to be examined in this context, despite their key roles in the transformation of carbon and nitrogen through food-web interactions (Snelgrove et al. 1997, Schratzberger & Ingels 2018) as well as aid in transport of other nutrients (such as O_2) at the bentho-pelagic interface (Aller & Aller 1992).

Assessment of meiobenthic communities in the SO have been conducted in limited geographic areas. Specifically, the majority of these studies have focused on coastal regions along the Antarctic Peninsula (Vanhove et al. 1998, Peck et al. 1999, de Skowronski & Corbisier 2002, Smale et al. 2008, Gutt et al. 2011, Pasotti et al. 2012, 2014, 2015a,b, Hauquier et al. 2015, 2017, Rose et al. 2015, Sinniger et al. 2016, Fonseca et al. 2017, Veit-Köhler et al. 2018) with a limited number of studies within the Ross Sea (Fabiano & Danovaro 1998, 1999), near the Larsemann Ice Shelf area (Ingole & Singh 2010), or Weddell Sea (Herman & Dahms 1992, Vanhove et al. 1995, 1999, Lee et al. 2001, De Mesel et al. 2006, Sinniger et al. 2016, Hauquier et al. 2017). However, comparisons of meiobenthic community composition between different Antarctic regions are limited (Fontaneto et al. 2015, Hauquier et al. 2017) and mainly rely on literature reviews to compare species distributions. The difficulty of comparing meiobenthic organisms in different Antarctic regions is likely due to the fact that researchers have utilized different collection methods, sample amounts (e.g. core diameter and depth, as well as depth of sample collection), variation in sieve size, and temporal differences. Given this, there are no studies to date of Antarctic meiobenthic communities that have examined multiple regions concurrently, especially over a large geographic range.

Along with the above, the majority of the studies published on Antarctic meiofaunal communities have implemented traditional morphological approaches (Herman & Dahms 1992, Vanhove et al. 1995, 1999, Fabiano & Danovaro 1998, 1999, Peck et al. 1999, Lee et al. 2001, de Skowronski & Corbisier 2002, De Mesel et al. 2006, Smale et al. 2008, Ingole & Singh 2010, Pasotti et al. 2012, 2014, 2015a,b, Gutt et al. 2015, Rose et al. 2015, Stark et al. 2017, Veit-Köhler et al. 2018). Unfortunately, such identification methods rely on taxonomic expertise, are extremely time-consuming, and focus on a limited number of taxonomic groups, thus greatly limiting inferences on, and understanding of, community structure. On the other hand, high-throughput DNA sequencing approaches are a promising tool that allows rapid and more comprehensive examination of meiobenthic community composition than traditional morphological methods (Brannock & Halanych 2015, Leray & Knowlton 2016). To date, only 2 published studies have used such metabarcoding approaches to examine meiobenthic communities in Antarctica (Sinniger et al. 2016, Fonseca et al. 2017). While one sought to fill gaps in deep-sea biodiversity knowledge by examining 2 locations within the SO (Sinniger et al. 2016) and the other examined Antarctic meiofaunal diversity and compared it to other geographic regions (Fonseca et al. 2017), neither of these studies determined how meiobenthic communities within the SO compared or varied over a broad geographic scale and between multiple basins within the SO.

In order to further characterize the composition, diversity, and biogeography of meiobenthic communities in the Antarctic, this study sampled SO shelf sediment (223 to 820 m) in Western Antarctica from the Ross Sea to the Antarctic Peninsula. In the Antarctic, the shelf is depressed. In total, this transect encompassed over 5500 km during 2 research expeditions in 2013. Although sampling occurred in Antarctic waters, we were also interested in exploring the potential ranges of shelf meiobenthos in general. Thus, focus was given to 3 specific objectives: (1) examination of large- and small-scale variation in meiobenthic community composition; (2) determination of whether any taxa, represented as operational taxonomic units (OTUs), were found along the whole geographic range sampled; and (3) determination of how sediment nutrient profiles correlate with meiobenthic community composition within this geographic region. To accomplish these objectives, a high-throughput metabarcoding approach was employed targeting the hypervariable V9 region of the 18S small subunit ribosomal RNA (SSU rRNA) gene to characterize meiobenthic communities (Brannock & Halanych 2015) from this 5500 km transect of the Antarctic.

MATERIALS AND METHODS

Sample collection

Sediment samples (223 to 820 m water depth) were collected from a total of 26 locations during 2 research expeditions to Antarctica in 2013 (Table 1). At each sampled location, 3 to 4 sediment cores were isolated (see below, this section). Specifically, the first research expedition on the RVIB 'Nathaniel B. Palmer' (December 2012 and February 2013, NBP 12-10 expedition) surveyed portions of Western Antarctica including the Bellingshausen, Amundsen, and Ross Seas as well as Wright's Gulf (Fig. 1). Within each of these water basins, 3 separate locations were sampled (Table 1). For the Ross Sea, sampling was separated into 2 water basins, the Near Ross Shelf (NRS) and an open-water area in the Eastern Ross Sea (herein referred to as Ross Sea or RS), for a total of 6 collection locations. The second expedition on the ARSV 'Laurence M. Gould' (November and December 2013, LMG 13-12 expedition) surveyed the Western Antarctic Peninsula and portions of the Eastern Peninsula (all samples are designated with PEN). During NBP 12-10, samples were collected using a megacorer, where the top 2 cm of 4 arbitrarily chosen 10 cm diameter cores was isolated. For LMG 13-12, a box corer was utilized, where water was siphoned off the top and 3 to 4 tubes (10 cm diameter) were arbitrarily placed within the box corer to isolate the top 2 cm of the sediment. All core samples were placed in separate Whirl-Pak® bags, immediately frozen at −80°C, and shipped to Auburn University (Auburn, AL) on dry ice, where they were stored at −80°C until processed. Notably, these samples were collected at the same time as those used in Learman et al. (2016), but represent different cores from the same deployments. Sediment characteristics as well as bacterial and archaeal community structure from these locations have been previously reported (Learman et al. 2016).

Table 1. Sample collection information from the 2013 Antarctic expeditions. Indication of the water basin groupings (with abbreviations used in parentheses) are also provided

Basin, Location	Event	Abbreviation	Latitude	Longitude	Depth (m)
RVIB *Nathaniel B. Palmer* (January and February 2013, NBP 12-10 expedition) — Western Antarctic					
Bellingshausen Sea (BS)					
Bellingshausen Sea	009	BS.009	71.705612° S	91.711318° W	412
Bellingshausen Sea	011	BS.011	71.699500° S	93.803000° W	672
Western Bellingshausen Sea	017	BS.017	70.807893° S	95.326003° W	476
Western Bellingshausen Sea	021	BS.021			
Eastern Amundsen Sea (EAS)					
Eastern Amundsen Sea	021	EAS.021	71.750785° S	102.265610° W	471
Eastern Amundsen Sea	026	EAS.026	72.495735° S	104.484718° W	597
Eastern Amundsen Sea	031	EAS.031	72.766783° S	104.592217° W	572
Wright's Gulf (WG)					
Wright's Gulf	051	WG.051	73.272850° S	129.269700° W	473
Wright's Gulf	057	WG.057	73.492750° S	129.928000° W	510
Wright's Gulf	064	WG.064	73.263133° S	129.448650° W	478
Ross Sea (RS)					
Ross Sea	068	RS.068	75.330138° S	176.987793° W	567
Ross Sea	075	RS.075	76.341225° S	170.850510° W	531
Ross Sea	080	RS.080	76.490017° S	165.688783° W	460
Near Ross Shelf (NRS)					
Near Ross Shelf	098	NRS.098	76.917015° S	170.0007480° E	765
Near Ross Shelf	103	NRS.103	75.831582° S	166.4922330° E	552
Near Ross Shelf	108	NRS.108	74.666648° S	168.5000000° E	528
ARSV *Laurence M. Gould* (November and December 2013, LMG 13-12 expedition) — Antarctic Peninsula					
Fjord					
Flanders Bay	003	PEN_FB	65.015417° S	63.271317° W	623
Andvord Fjord	106	PEN_AF	64.859667° S	62.569717° W	486
Wilhemina Bay	130	PEN_WB	64.643267° S	62.247567° W	728
Charlotte Bay	136	PEN_CB	64.572683° S	61.635517° W	390
Bransfield Strait (BF)					
Trinity Island	012	PEN_TI	63.806483° S	60.479333° W	399
Mid-Bransfield Straight	085	PEN_MB	62.725600° S	59.147267° W	820
Eastern Peninsula (EP)					
Tabarin Peninsula	051	PEN_TB	63.695133° S	56.829767° W	403
Vega Island	045, 046	PEN_VI	63.738267° S	57.392900° W	593, 706
Erebus and Terror Bay	057	PEN_ETB	63.973283° S	56.557383° W	395
James Ross Island	035	PEN_JRI	64.032300° S	56.742950° W	223

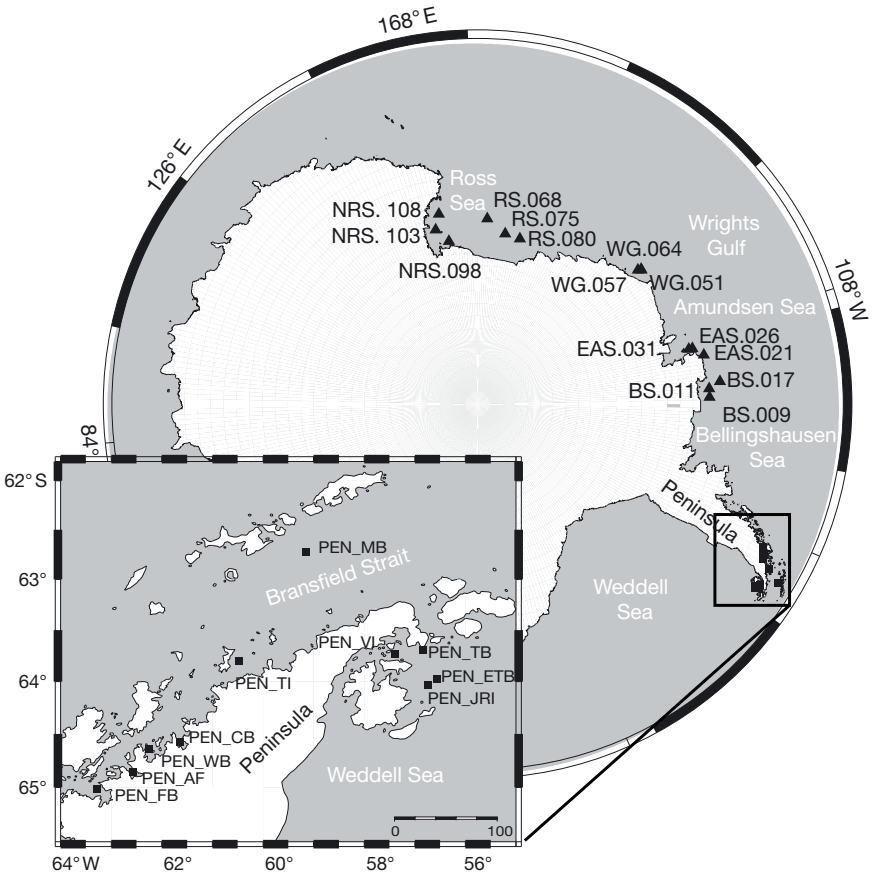

Fig. 1. Geographic representation of all sample locations between both Antarctic research expeditions in 2013. (▲) Sample locations collected on the RVIB 'Nathaniel B. Palmer' (January and February 2013, NBP 12-10 expedition); (■) samples collected in November and December 2013 on the ARSV 'Laurence M. Gould' (November and December 2013, LMG 13-12 expedition). All samples collected in the Peninsula region are designated with 'PEN'. Other abbreviations and detailed information on sample locations can be found in Table 1. (Modified from Learman et al. 2016)

Meiobenthic community isolation, DNA extraction, and sequencing

Two cores from each sampling event were arbitrarily chosen and processed independently, with the meiobenthic community fraction isolated as described in Brannock et al. (2014), except that the decanting protocol was repeated a total of 7 times instead of 10 due to a larger quantity of silt particles that impeded filtration. Genomic DNA was extracted using the MoBio Laboratories PowerSoil® DNA Isolation Kit (Cat. # 12888). Prior to sample addition, 200 µl of the PowerBead tube liquid was removed and discarded on recommendation of the manufacturer due to the potential higher water content in the sample. Decanted material was allowed to thaw on ice, mixed gently by inversion, and 500 µl of slurry was added to the PowerBead tube. The remainder of the manufacturer's supplied protocol was followed except the 10 min vortexing step was replaced with a 2 min Mini Beadbeater (BioSpec) step and the resulting DNA eluted in 55 µl of Milli-Q water. DNA integrity and size was checked by gel electrophoresis, and extracted nucleic acids were stored at −20°C until they were sent for amplification and sequencing.

Total genomic DNA was sent to the Genomics Services Laboratory (GSL) at HudsonAlpha Institute of Biotechnology (Huntsville, AL) for metabarcoding paired-end (PE) sequencing of the hypervariable V9 region of the 18S SSU rRNA gene (Amaral-Zettler et al. 2009) following previously published protocols (Brannock et al. 2014, 2016a, Brannock & Halanych 2015). The V9 gene region was chosen due to sequencing length limitations of the Illumina platform at the time samples were sequenced. Additionally, our previous work (Brannock et al. 2014, 2016a,b, Brannock & Halanych 2015) suggested that this region held taxonomically informative variation, and had been validated against morphological variation (Brannock et al. 2017). All samples from the NBP 12-10 expedition were sequenced on an Illumina HiSeq 2500 as 100 bp PE reads, while those from the LMG 13-12 expedition were sequenced on an Illumina MiSeq as 150 bp PE reads. Two samples from the NBP 12-10 expedition (NRS.E108.C4 and EAS.E26.C2) were sequenced with the LMG 13-12 samples on the Illumina MiSeq due to low read numbers from the initial Illumina HiSeq 2500 run. Although previous studies have illustrated consistency between samples being run on different Illumina platforms (HiSeq vs. MiSeq; Caporaso et al. 2012, Brannock et al. 2014), a hierarchical clustering analysis was performed according to Brannock et al. (2014) to further test for consistency between sequencing platforms and technical replicates. Resulting demultiplexed raw sequence reads were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (BioProject ID PRJNA476328, Accession Numbers SAMN09429846 to SAMN0942871).

Bioinformatic analyses

The workflow to generate OTUs from the raw PE reads was similar to that reported in Brannock et al. (2016a). Briefly, PE reads were overlapped using PandaSeq version 2.5 (Masella et al. 2012). During the overlapping process, primers were removed and sequences containing any uncalled bases or those >200 bp were discarded. Overlapped sequences were quality filtered using UPARSE (Edgar 2013) with a maximum expected error set to 1. Remaining sequences were clustered into OTUs at a 97% similarity using UCLUST in QIIME version 1.9.1 (Caporaso et al. 2010b) 'pick_open_reference_otus.py' workflow with singleton OTUs (herein referred to as singletons, and are OTUs that are represented by a single sequence within the whole data set) being discarded. The most likely explanations of singletons are that they either represent rare taxa or sequencing artefacts. Given that previous metabarcoding studies have demonstrated that a majority of singletons were sequencing errors (Kunin et al. 2010, Tedersoo et al. 2010), some workflows have a common practice to remove singletons (Quince et al. 2009, Edgar 2013) to avoid overestimating the actual diversity measurements. To be more conservative with our diversity estimates we decided to apply these same practices. The SILVA version 123 (Quast et al. 2013) database was employed as a reference set for both OTU clustering and taxonomic assignment. Sequences failing to align with PYNAST (Caporaso et al. 2010a) and/or those that were classified as Bacteria or Archaea were excluded so that the resulting OTU table only contained eukaryotes (eOTU).

Technical replicates were combined and treated as one sample for the remainder of analyses, referred to herein as the combined OTU (cOTU) table. These samples were combined because hierarchical clustering analysis based on the proportional eOTU table found technical replicates to be more similar in OTU composition in reference to each other than to any other sample (see Fig. S1 in Supplement 1 at www.int-res.com/articles/suppl/m603p047_supp1.pdf; similar results shown in Brannock et al. 2014 and Brannock & Halanych 2015).

In order to directly compare diversity measures between samples, the cOTU table was normalized to 156 940 sequences per sample, representing the lowest number of sequences obtained from any sample, for 100 replicates. For each normalized OTU table, 4 alpha diversity measurements (Chao1, phylogenetic distance [PD], Shannon diversity, and observed number of OTUs) as well as 4 beta diversity measurements (Binary Jaccard dissimilarity, Bray-Curtis dissimilarity, unweighted Unifrac, and weighted Unifrac) were calculated in QIIME (Caporaso et al. 2010b).

The average of each alpha- and beta-diversity metric was determined from the 100 replicates. A oneway analysis of variance (ANOVA) was performed in R version 3.0 (R Development Core Team 2013) to compare alpha-diversities between oceanographic basins. Tukey's honestly significant difference (HSD) test was conducted in R to examine all possible pairwise comparisons for any significant differences ($\alpha \leq 0.05$). For all beta-diversity matrices, a permutational multivariate analysis of variance (PERMANOVA) was performed in PRIMER version 7 (Primer-E) to compare community composition between water basins or regions (Table 1). Given that PERMANOVA significance can be due to differences in samples or dispersion of the data, PERMDISP was performed for each factor to determine whether the multivariate dispersion was homogenous (Anderson et al. 2008). Non-metric multidimensional scaling (nMDS) ordinations based on the beta-diversity matrices were made using PRIMER to observe sample clustering. A significance value of $\alpha \leq 0.05$ was used for all statistical tests.

Community composition was examined using the 'summarize_taxa_through_plots.py' workflow in QIIME and followed Brannock et al. (2016a). Briefly, phyla with a representation within the overall OTU table at an average of <0.5% abundance were combined either into an Other Metazoa or Other Eukaryotes (non-metazoan) classification. Phyla with an average abundance ≥0.5% were examined down to their family level classifications. Family taxonomic assignments were handled similarly. Families <0.5% average abundance were grouped together by class-level assignments. In the present study, we did not explore taxonomic assignments below the family level.

To directly compare OTUs between different sampling events, replicate cores within the cOTU table from the same sampling location were collapsed and combined into one sample using QIIME. The resulting OTU table from combined cores (ccOTU) was normalized to 364 490 sequences per sample, the lowest number of sequences within one sampling event. For each normalized ccOTU table replicate (100 in total), the number of shared OTUs between each sampling event was calculated using QIIME. The average number of shared OTUs per comparison for the 100 replicates was calculated, as well as the number of shared OTUs between sampling events found within the unrarefied ccOTU table. To compare OTUs between different water basins, samples

that were from the same basin (Table 1) were collapsed and combined into one sample as stated above. The resulting water basin combined OTU (wbOTU) table was normalized to 950 030 sequences per sample, the lowest number of sequences within a single water basin. Comparisons of the number of OTUs shared between each water basin were conducted in the same fashion as described above for the normalized ccOTUs.

Sediment analyses

A BIOENV analysis (Clarke & Ainsworth 1993) was performed in PRIMER on environmental data originally reported by Learman et al. (2016). This analysis utilized a dissimilarity-based method to identify the combination of sediment characteristics (e.g. pH, percent nitrogen, percent organic carbon, total organic carbon, total nitrogen, phosphorus, and sulfur) that best explained differences in community composition observed between samples and locations. Prior to performing the BIOENV analysis, environmental variables were normalized in PRIMER using the 'normalize variables' option.

RESULTS

A total of 21 991 788 raw PE reads were obtained from the GSL (see Table S1). Following filtering, 15 785 074 sequences (71.8% of original reads) were clustered (Table S1) and produced 19 732 OTUs. A total of 18 474 OTUs (93.6% of the original) remained in the eOTU table after OTU table filtering.

Notably, samples collected along the Antarctic Peninsula had significantly lower numbers of OTUs and Chao1 diversity ($p \leq 0.02$) compared to those collected in other Western Antarctic locations (Fig. 2 and Table 2). Overall, we identified 1393 to 2824 eukaryotic OTUs ($\bar{x}$ = 2229 OTUs) present within individual sediment cores from non-Peninsula locations, and only 745 to 2263 eukaryotic OTUs ($\bar{x}$ = 1202 OTUs) from the Peninsula region.

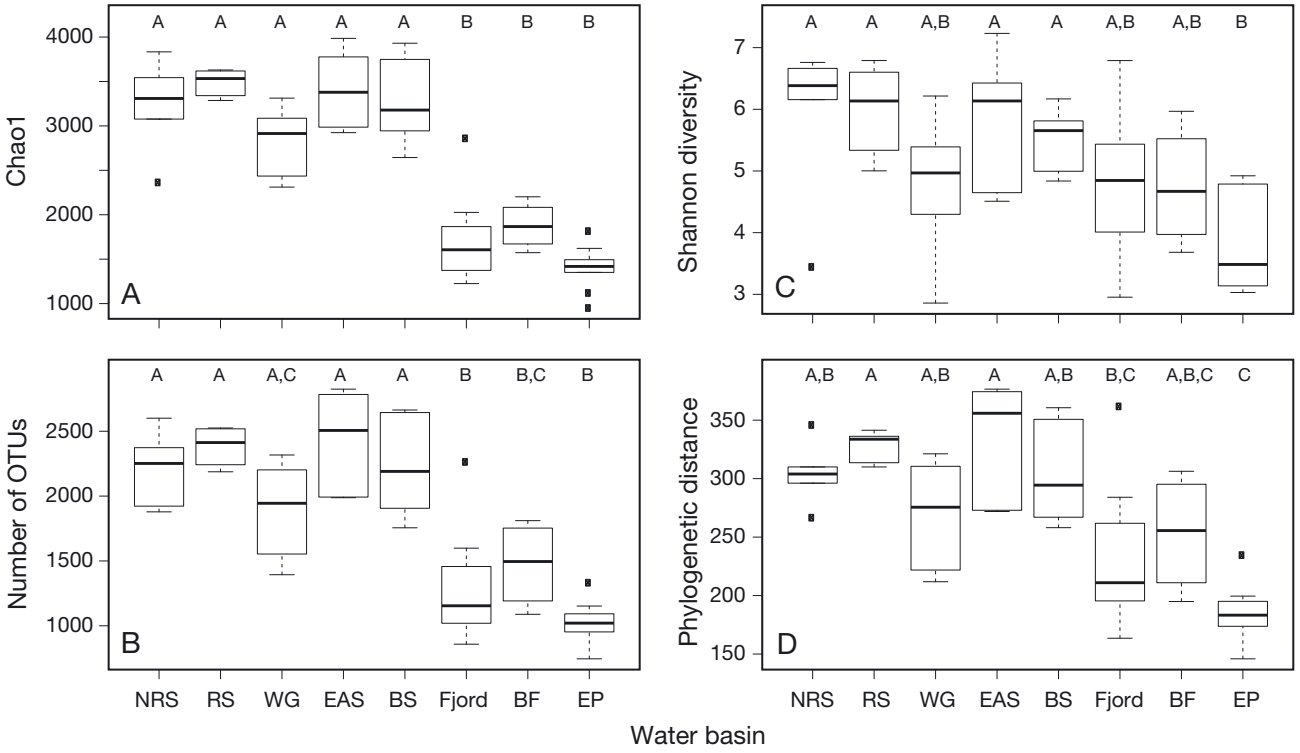

Fig. 2. Alpha-diversity measurements for samples in each water basin. Results represent the mean and standard deviation of the 100 rarefied (156 940 sequences) combined operational taxonomic unit (cOTU) tables for each of the diversity metrics examined. (A) Chao1; (B) number of OTUs; (C) Shannon diversity; (D) phylogenetic distance. Letters indicate significant differences based on Tukey's HSD test following ANOVA. Boxplots—midline: median; top and bottom of box: 25–75% interquartile range; whiskers: non-outlier range; black squares: outliers. NRS: Near Ross Shelf; RS: Ross Sea; WG: Wright's Gulf; EAS: Eastern Amundsen Sea; BS: Bellingshausen Sea; BF: Bransfield Strait; EP: Eastern Peninsula. Information pertaining to the sample locations within each water basin can be found in Table 1

Table 2. Number of operational taxonomic units (OTUs) present in each water basin. Data from both the unrarefied OTU table and an average number of OTUs from the 100 replicate rarefied (950 030 sequences per basin) tables

Water basin	Number of OTUs	
	Unrarefied	Rarefied
Near Ross Shelf (NRS)	7535	6112
Ross Sea (RS)	8560	7030
Wright's Gulf (WG)	6708	5449
Eastern Amundsen Sea (EAS)	7765	6742
Bellingshausen Sea (BS)	7332	6330
Fjord	5067	4149
Bransfield Strait (BF)	3847	3847
Eastern Peninsula (EP)	4097	3008

When examining only the meiofaunal taxa, non-Peninsula locations had 733 to 1584 OTUs ($\bar{x}$ = 1180 OTUs) present in a single core, while those in the Peninsula had 261 to 665 OTUs ($\bar{x}$ = 509 OTUs). Along with this, there was higher similarity between all water basins or the 2 larger regions (Western Antarctic and Antarctic Peninsula; Table 1) in reference to both Shannon Diversity and PD (Fig. 2). Samples collected in the Antarctic Peninsula water basins (Fjord, Bransfield Strait, and Eastern Peninsula) had a significantly different community composition relative to the Western Antarctic water basins (Bellingshausen Sea, Eastern Amundsen Sea, Wright's Gulf, RS, and NRS) (pseudo-$F \geq 3.172$, $p_{PERM} = 0.001$; Fig. 3, Table S2). Pairwise PERMANOVA analyses indicated all water basins differed significantly from each other in reference to the presence of OTUs, while the main difference in relation to abundance of OTUs was between the Eastern Peninsula (EP) or Fjord and other water basins (Table S2). The PERMDISP analysis identified no significant difference in multivariate dispersion ($p_{PERM} \geq 0.133$), implying differences were due to community composition and not community variability. Community composition varied significantly between the 2 larger regions (i.e. Western Antarctic and Antarctic Peninsula) for all beta-diversity metrics (pseudo-$F \geq 8.002$, $p_{PERM} = 0.001$) and the PERMDISP analysis showed no significant difference in multivariate dispersion ($p_{PERM} \geq 0.058$). nMDS ordination plots (Binary Jaccard, Bray-Curtis, and unweighted Unifrac) illustrated clear separation between the 2 regions and some of the water basins (Fig. 3).

A majority of the Western Antarctic taxa was dominated by metazoans and primarily composed of

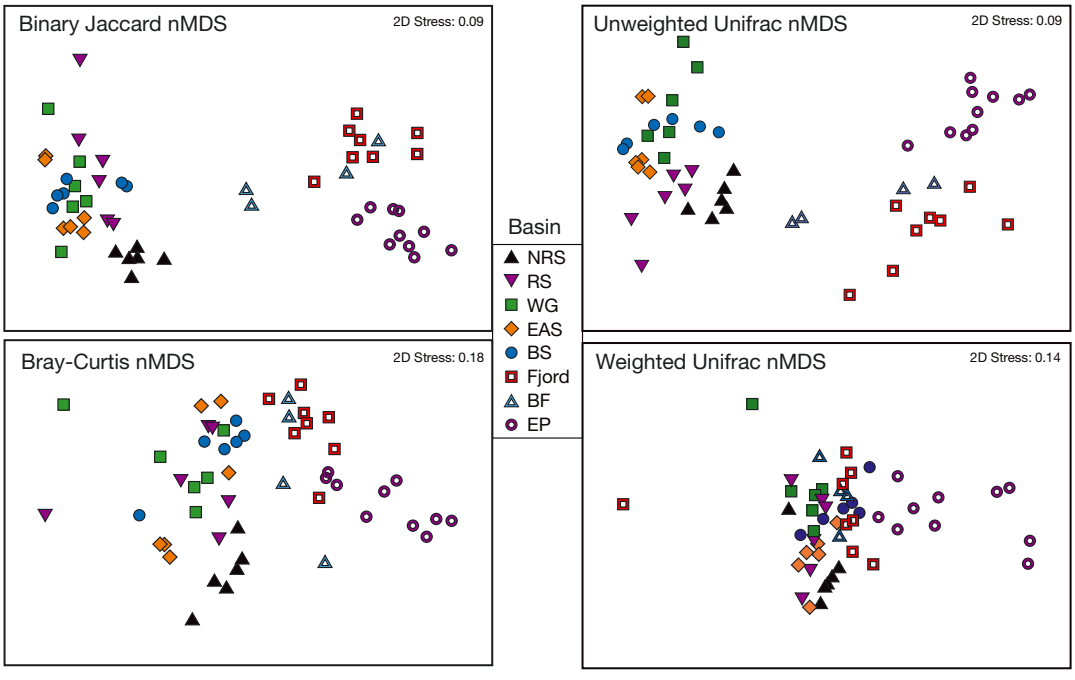

Fig. 3. Non-metric multidimensional scaling (nMDS) ordination based on beta-diversity measurements for samples in each water basin. Results represent average distance of the 100 rarefied (156 940 sequences) combined operational taxonomic unit (cOTU) tables for each of the diversity metrics examined. NRS: Near Ross Shelf; RS: Ross Sea; WG: Wright's Gulf; EAS: Eastern Amundsen Sea; BS: Bellingshausen Sea; BF: Bransfield Strait; EP: Eastern Peninsula. Information pertaining to sample locations within each water basin can be found in Table 1

Table 3. Total number of Eukaryota sequences and operational taxonomic units (OTUs) found in the different water basins. Data is divided into the 2 main contributors (Metazoa and the Stramenopiles, Aveolates, and Rhizaria [SAR] supergroup) and the proportion of each was calculated. Numbers are based on the unrarefied OTU table. Cores included in each water basin are represented in Table 1. Geographical representation of water basins is illustrated in Fig. 1

Water basin	Eukaryota		Metazoa		SAR		Metazoa proportion (%)		SAR proportion (%)	
	Sequences	OTUs	Sequences	OTUs	Sequences	OTUs	Sequences	OTUs	Sequences	OTUs
Near Ross Shelf	2 016 627	7316	1 698 764	3226	288 629	2854	84.24	44.10	14.31	39.01
Ross Sea	2 012 380	8364	1 819 472	3827	162 640	3200	90.41	45.76	8.08	38.26
Wrights Gulf	1 909 642	6547	1 842 133	3215	53 384	2260	96.46	49.11	2.80	34.52
Eastern Amundsen Sea	1 641 738	7590	1 398 045	3098	206 328	3128	85.16	40.82	12.57	41.21
Bellingshausen Sea	1 645 756	7165	1 338 895	2935	269 198	3032	81.35	40.96	16.36	42.32
Fjord	2 166 641	5000	1 876 267	1552	269 250	2482	86.60	31.04	12.43	49.64
Bransfield Strait	948 931	3795	848 629	1501	93 582	1640	89.43	39.55	9.86	43.21
Eastern Peninsula	3 031 681	4047	1 578 367	1412	1 442 873	1921	52.06	34.89	47.59	47.47

either annelid or nematode taxa (Table 3, Fig. 4). The EP significantly differed from the Western Antarctic by possessing a noticeably higher proportion of Stramenopiles, which are found within the Stramenopiles, Aveolates, and Rhizaria (SAR) supergroup (Table 3, Fig. 4).

A number of taxa were found to be common along the examined 5500 km region of Antarctica when comparison of OTUs present in a given core (cOTU) or sets of cores (ccOTU or wbOTU) are considered. Specifically, the unrarefied OTU table as well as the 100 replicated normalized OTU tables found the same number (Table 4) and the same OTUs as shared within the different hierarchical levels examined. Overall, a total of 28 OTUs were present in every processed core, 74 OTUs were present at every sampling event, and 722 OTUs were found in every water basin (Table 4 & S3 in Supplement 2 at www.int-res.com/articles/suppl/m603p047_supp2.xlsx). A majority of these shared OTUs were either members of the SAR supergroup or particular Metazoa taxa (Tables 5 & S3). Within metazoan OTUs, Annelida, Arthropoda, and Nematoda were specific phyla that were shared between every sampling event or water basin (Table 6). Kinorhyncha OTUs were also present in every core sampled (Table 6).

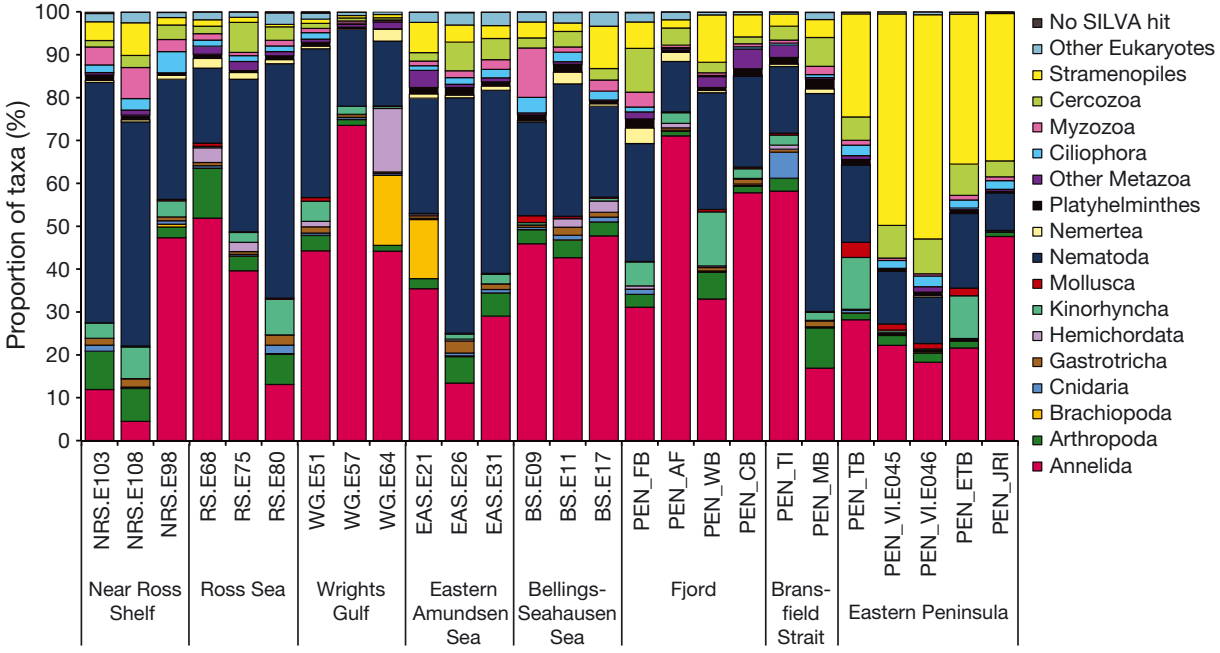

Fig. 4. Meiobenthic community composition along the 5500 km Western Antarctic region surveyed. Proportion of taxa based on unrarefied operational taxonomic unit (OTU) table and average of the 2 cores processed at one sample location/event. Geographic location data are included in Fig. 1 and Table 1

Table 4. Number of core operational taxonomic units (OTUs) shared between different levels. For the 100 rarefied OTU tables the minimum, maximum, average, and combined total unique OTUs shared were examined

OTUs	All processed cores		All sample locations/events		All water basins	
	Rarefied	Unrarefied	Rarefied	Unrarefied	Rarefied	Unrarefied
Average	16	28	59	74	544	722
Minimum	12		53		523	
Maximum	21		67		564	
Total unique	28	28	74	74	722	722

Table 5. Taxonomic breakdown of the shared operational taxonomic units (OTUs) within different examination levels. Both the number of OTUs and the percentages of the total shared OTUs are provided. SAR: Stramenopiles, Aveolates, and Rhizaria supergroup. No SILVA hit: OTUs that were not taxonomically assigned when BLASTing to the SILVA database

Taxonomic group	Every processed core		Every sample location/event		Every water basin	
	No. of OTUs	%	No. of OTUs	%	No. of OTUs	%
Metazoans	6	21.4	20	27.0	261	36.1
SAR	17	60.8	45	60.8	343	47.5
Other microeukaryotes	5	17.9	9	12.2	114	15.8
No SILVA hit	0	0	0	0	4	<1
Total	28	100	74	100	722	100

BIOENV analyses indicated that the combination of pH, percent nitrogen (% N), silicon (Si), manganese (Mn), and total organic delta 13 carbon (i.e. TOC-δ^{13}C) were all correlated with the presence of microeukaryotic OTUs (Rho = 0.579, p = 0.001). On the other hand, the combination of pH, Si, Mn, TOC-δ^{13}C, carbon to nitrogen ratio (C:N), and percent total organic carbon (% TOC) were correlated with the abundance of OTUs (Rho = 0.421, p = 0.001).

Table 6. Metazoan taxonomic breakdown of shared operational taxonomic units (OTUs) within different examination levels. Both the number of OTUs and the percentages are provided

Metazoan phylum	All processed cores	All sample locations/events	All water basins
	No. of OTUs	No. of OTUs	No. of OTUs
Annelida	1	3	81
Arthropoda	2	3	38
Chordata	0	1	3
Cnidaria	0	1	5
Echinodermata	0	0	1
Gastrotricha	0	0	5
Hemichordata	0	0	1
Kinorhyncha	2	2	6
Mollusca	0	1	7
Nematoda	1	7	95
Nemertea	0	2	5
Platyhelminthes	0	0	5
Priapulida	0	0	2
Rotifera	0	0	1
Xenacoelomorpha	0	0	6
Total	6	20	261

DISCUSSION

Here, we report that Antarctic meiobenthic communities possess significantly higher OTU richness along the more southwestern portion of the Antarctic continent relative to the Antarctic Peninsula region (Fig. 2), a similar trend to that of benthic bacterial and archaeal communities sampled during the same expeditions (Learman et al. 2016). The finding of higher meiofauna OTU richness within southwestern Antarctic regions contradicts Fabiano & Danovaro's (1999) report of the Ross Sea having a lower number of taxa in comparison to the Weddell Sea (specifically, Halley Bay Station; Herman & Dahms 1992). Although the specific locations that were sampled within the Weddell Sea (Herman & Dahms 1992) were not examined here, our sampled location in the Ross Sea (both RS and NRS) had some of the highest numbers of OTUs present in our sampling (Fig. 2, Table 2). Furthermore, a large number (722 of all eukaryotic OTUs and 261 of metazoan OTUs) of meiobenthos were present throughout the entire 5500 km surveyed region, suggesting dispersal by meio-fauna may not be as limited as previously assumed.

Possible explanation for differences between regions

A possible driver for the observed differences in meiobenthic community compositions between the Antarctic Peninsula and Western Antarctica is heterogeneity in sediment and nutrient composition between the sampling locations. For example, Learman et al. (2016) reported higher levels of TOC, total nitrogen (TN), ammonium (NH_4^+), and significantly greater TOC-δ^{13}C in the Antarctic Peninsula relative to the Western

Antarctic. In this case, the increase in organic matter found within the Peninsula region was attributed to increased phytoplankton or nutrient levels (Learman et al. 2016) resulting from the area's normally higher temperatures in comparison to the Western Antarctic region (Barnes et al. 2006). Such organic matter deposition to the ocean floor is thought to be important to benthic communities, but previous studies have shown that overabundance or enrichment of organic material (especially of organic carbon) can have a negative impact, causing a decrease in measures such as species (OTU) richness and abundance of benthic fauna communities (Gee et al. 1985, Hyland et al. 2005).

Although sediment characteristics (Coull 1999, Baguley et al. 2006, Giere 2009) and organic matter input (de Skowronski & Corbisier 2002, Giere 2009) are known to impact the composition of meiobenthic communities, other potential sources driving the observed differences cannot be overlooked. Firstly, samples collected within Western Antarctica and the Peninsula employed two slightly different collection methods (e.g. megacorer and box corer), which can potentially influence the identities and densities of fauna obtained (Blomqvist 1991, Bett et al. 1994). Unfortunately, this discrepancy in equipment was due to the unavailability of the megacorer during the LMG 13-12 expedition. Secondly, the two research expeditions took place within separate austral summers and the intraseasonal amount of organic material present within these regions can be highly variable (Ducklow et al. 2006, Smith et al. 2006, Fragoso & Smith 2012). However, while equipment and temporal environmental heterogeneity might contribute variation to the community differences observed between regions, these appear to be relatively minor, particularly given that both the benthic microbial and meiobenthic communities demonstrated the same overall trend (i.e. consistency between Learman et al. 2016 and the data presented here).

Broad geographic distributions

In spite of appreciable community differences between regions, substantial overlap (722 OTUs; Tables 3 & 4) in organismal membership was observed between basins. While drawing conclusions that differing basins possess similar meiobenthic communities is tempting, we only know which organisms are present and are less certain about abundances or potential interactions between these organisms. We were intrigued to find 2 kinorhynch (mud dragon) OTUs in every core examined, as they are typically thought of as common, but not dominant or highly abundant, meiofauna constituents. However, a kinorhynch species belonging to *Echinoderes* has been found in both the eastern and western portions of the Gulf of Mexico (Sørensen & Landers 2014) and *E. tchefouensis* has been reported in geographic regions from Singapore to the Korean Peninsula to Saipan Islands (Sørensen et al. 2012, 2016). Given this, kinorhynchs appear to have the potential to be found over a broad range, and these taxa are clearly relatively abundant if they are being recovered from every core over a geographic range of 5500 km.

Of the 722 OTUs present throughout the 5500 km region from the NRS to the Eastern Antarctic Peninsula, 74 OTUs were found at every sample location and 28 OTUs were found in every processed core despite variations in granulometry, organic content, and overlying water mass. Thus, although meiofauna have shown large variability within polar regions (Vanhove et al. 1995), some meiofaunal groups have been reported to have broad spatial distributions (nematodes: Vanreusel et al. 2010, Zeppilli et al. 2011; harpacticoid copepods: Menzel et al. 2011). Taken together, these data imply that some meiobenthic taxa (Tables 5, 6, & S3) have the ability to disperse (i.e. maintain genetic continuity) over very broad ranges in spite of life history traits (i.e. production of a limited number of lecithotrophic eggs) that would suggest otherwise.

Comparisons to previous Antarctic metabarcoding studies

Relative to previous Antarctic meiobenthic community metabarcoding studies (Sinniger et al. 2016, Fonseca et al. 2017) that reported either a maximum of 722 or 127 eukaryotic (meiofauna and protist) OTUs within samples from the Antarctic Peninsula, respectively, the current work recovered a higher diversity of microeukaryotic taxa. Specifically, within the Peninsula region, we identified from 745 to 2263 eukaryote OTUs present within single cores, and report an average of 1202 OTUs. Notably, this is at least twice the amount of diversity than previously reported. One possible source for this discrepancy could be the read depth. The previous studies had a substantially lower number of sequences (97 456 sequences in Sinniger et al. 2016 and 49 655 sequences in Fonseca et al. 2017) in Antarctic Peninsula samples in comparison to even our rarefied numbers

for a single core (156 940 sequences). Other attributes contributing to the differences between the 2 studies could be the utilized sequencing platforms (Roche 454 and Illumina HiSeq/MiSeq) and region amplified (V1-V2 region for both Sinniger et al. 2016 and Fonseca et al. 2017; V9 for the current study). Primer choice and region amplified can also potentially influence results in a substantial manner. The V1-V2 region is an ~450 bp fragment at the beginning of the 18S SSU rRNA gene (Fonseca et al. 2010), while the V9 region is ~87 to 187 bp fragment (Amaral-Zettler et al. 2009) at the end of the gene region. From just size alone, V1-V2 provides a longer fragment of DNA that could potentially discriminate between more closely related species than V9. However, for some taxa (i.e. Copepoda), the V9 region can be used to group individuals down to the genus level (Wu et al. 2015). Moreover, the V1-V2 region has been found to target higher proportions of non-metazoan taxa in comparison to either the V4-V5 or V5-V7 region (Capra et al. 2016), while other researchers suggest the 18S region recovers lower meiofaunal diversity than mitochondrial regions (Tang et al. 2012). Therefore, as these protocols are often targeting a wide and diverse group of taxa, more studies need to be conducted pertaining to efficiency of genes and primers to examine diversity patterns.

In addition, all 3 studies used different quality filtering methods, which is important given that Brannock & Halanych (2015) previously demonstrated large discrepancies in the number of OTUs obtained from variation in filtering and clustering methods. All 3 studies were clustered using a 97% similarity cutoff in QIIME (different versions); however, different clustering methods (de novo vs. reference-based or a combination) were used. In spite of these differences, the proportion of metazoan OTUs and metazoan sequences in the Antarctic Peninsula region were similar between Sinniger et al. (2016) (34.76 and 69.35%, respectively) and the current results (35.16 and 76.03%, respectively).

CONCLUSIONS

Knowledge of large geographic distributions for Antarctic meiobenthic communities is limited since studies from the region specifically focus on macro- or megafauna diversity. In contrast, this study provides the first glimpse into the distribution of Antarctic meiobenthos along a 5500 km portion of the continent. Our results suggest Antarctic meiobenthic diversity is higher than previously reported, even when compared to other metabarcoding studies. Furthermore, the current study shows significant overlap in community members (722 OTUs) across all surveyed basins. This finding implies the meiobenthic community of the region may have higher dispersal ability than previously assumed and some may have a circumpolar distribution around Antarctica. Future studies should continue to survey larger regions of Antarctica, including more eastern locations, in order to develop a better understanding of meiobenthic community distributions in the region.

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