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Revealing higher than expected meiofaunal diversity in Antarctic sediments: a metabarcoding approach

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Abstract

Although studies show that Antarctic mega- and macrofauna are highly diverse, little is known about meiofaunal biodiversity in sediment communities, which are a vital part of a healthy and functional ecosystem. This is the first study to analyse community DNA (targeting meiofauna) using metabarcoding to investigate biodiversity levels in sediment communities of the Antarctic Peninsula. The results show that almost all of the meiofaunal biodiversity in the benthic habitat has yet to be characterised, levels of biodiversity were higher than expected and similar to temperate regions, albeit with the existence of potentially new and locally adapted species never described before at the molecular level. The Rothera meiofaunal sample sites showed four dominant eukaryotic groups, the nematodes, arthropods, platyhelminthes, and the annelids; some of which could comprise species complexes. Comparisons with deep-sea data from the same region suggest little exchange of Operational Taxonomic Units (OTUs) between depths with the nematodes prevalent at all depths, but sharing the shallow water benthos with the copepods. This study provides a preliminary analysis of benthic Antarctic Peninsula meiofauna using high throughput sequencing which substantiates how little is known on the biodiversity of one of the most diverse, yet underexplored communities of the Antarctic: the benthos.

Introduction

Much recent effort has been expended into characterising Antarctic marine biodiversity and it is clear that it is significantly higher than was thought in previous decades, particularly in relation to marine invertebrates[1,2]. An increasing number of cryptic species are being discovered[3] and in some invertebrate groups, such as pycnogonids and polychaete worms, Antarctica has significantly higher diversity than the global averages[4]. However, even the most recent reviews of marine biodiversity in Antarctica have concentrated on marine mega- and macrofauna with relatively little discussion on endemic meiofauna, particularly those metazoans inhabiting marine sediments[5]. In a recent UN Assessment of the State of the Ocean I (http://www.worldoceanassessment.org/), meiofauna and the poles were highlighted as being of particular importance for future research. There is currently very limited knowledge on polar meiofauna; the extent of their biodiversity and their contribution to polar ecosystem functioning

Marine sediments are some of the most species-rich habitats on Earth. They are one of the main contributors to ocean health and functioning, but one of the least studied habitats in the biosphere[6]. Within marine sediments, the meiofauna (the microscopic taxa generally between 45-500μm) are important members of the benthic ecosystem, playing a critical role in carbon transfer and nutrient cycling[6]. They participate in ecosystem energy flows via the consumption of dissolved organic carbon and from grazing on primary producers and bacteria[7]. In addition they play important roles in the consumption of detritus and predation. They excrete nutrients which can be used by phytobionts, bacteria and associated meiofauna, but they also act as a food source for benthic invertebrates and higher predators[6]. Thus, evaluations of benthic meiofauna biodiversity are of critical importance for understanding ecosystem functioning, sustainability and resilience, as well as understanding carbon cycling in the largest part of the World, the seabed[6]. In addition meiofauna represent useful tools for studying change within an ecosystem and could be particularly useful for understanding the effects of anthropogenic impacts and climate change[6].

One region of particular note with respect to environmental change is the Western Antarctic Peninsula, some areas of which, particularly in the north west, are regarded as experiencing the most rapid rate of climate warming on the Antarctic continent[8]. However, the situation is complex and exacerbated by the lack of high density measurements. Recent analyses suggest that the atmospheric warming along the Peninsula has ceased[9], but there is uncertainty whether this trend will continue, what the drivers are, and whether this cessation of warming is reflected in oceanographic data which is still showing changes in sea ice and retreat of glaciers[10]. What is clear is that this is still a region in transition and highly vulnerable[11]. Surface ocean temperatures rose by more than 1°C in the second half of the 20th Century and the deeper layers have also warmed due to increased upwelling of warm Upper Circumpolar Deep Water. Sea ice duration has reduced significantly in the past few decades (by 100 days since 1978), which impacts not only on primary production and water column stratification, but also on the frequency of iceberg scouring[11,12]. About 80% of glaciers along the Peninsula are in retreat, which has increased the amount of sediment and fresh-water in the system[10]. Given the huge uncertainty concerning climate trends in this region, continued monitoring is vital, as is the evaluation of the potential impact on the endemic fauna. The Southern Ocean fauna have evolved to life in freezing seas in relative isolation for the last 15Myr[13] and as a consequence have evolved a series of physiological and biochemical adaptations to life in the cold, are highly stenothermal and poorly adapted to rapid change[14].

Advances in molecular and sequencing methodologies now enable us to evaluate biodiversity levels from even the most remote habitats, in a way, not previously possible. Large-scale environmental DNA (eDNA) approaches using high throughput sequencing (shortly referred to as metabarcoding) have recently been applied to examine biodiversity levels at the poles. To date polar marker gene studies have mainly focussed on microbial communities within soil, ice cores, microbial mats and melt water[15,16], marine viruses[17], freshwater picoplankton[18] and more recently, microbial

biodiversity, on the shelf and the deep-sea[19,20]. These studies have provided intriguing pilot data on micro- and meiofaunal biodiversity in this largely understudied and extreme environment. Whilst there is a long history of biological sediment analyses at research stations along the Peninsula, these have been based on either taxonomic identification or stable isotope analyses[21-25]. High throughput sequencing of DNA derived from community environmental samples provides a powerful tool with which to complement existing approaches and provides a timely opportunity to gain insight into alpha and beta-diversity of Antarctic meiofauna and start to assess their likely resilience in the context of climate change.

The first aim of this study was to provide a global description of marine Antarctic meiofaunal diversity and community structure in shallow waters, using high throughput sequencing approaches on community DNA. Secondly, to compare Antarctic shallow-water datasets with deep-sea samples taken in the same area (both published and un-published) to identify general diversity trends in freezing habitats and potential depth gradients. A third aspect was to compare the data generated here with those of another metabarcoding study on meiofaunal samples from a mid-temperate region using the same 18S rRNA region to identify relative levels of biodiversity and whether these were markedly reduced in the Antarctic samples.

RESULTS

The total number of reads derived from the 454 FLX sequencing platform from the Antarctic Peninsula sampled sites was 61,057; which was reduced to 49,655 reads after filtering and chimera removal. This level of reduction in read numbers was comparable with previous 454 eDNA studies[26,27]. This particular chemistry introduces higher error rates than the Illumina platform within homopolymer regions due to accumulated light intensity variation, but these reads can be identified and removed *in silico*. Additional reads were removed as they were only present in singletons and

124 through the application of UCHIME, which is known to be a stringent filtering step[27]. Metazoan
125 OTU numbers varied moderately between sample sites with a mean number of 90 OTUs in Hangar
126 Cove (stdv ± 36.09), 48.7 OTUs in Rothera Point (stdv ± 26.05), 87 OTUs in Islands (stdv ± 60.65)
127 and South Cove with mean OTUs number of 47 (stdv ± 24.24). A major proportion of the OTUs
128 from each site (16-31%) were not assigned to any annotated taxa in SILVA database (Table 1). In
129 terms of those taxa with matches in SILVA, the nematodes had the highest OTU numbers among
130 the main phyla, with 92 OTUs followed by the arthropods and platyhelminthes represented by 47
131 and 37 OTUs respectively **(**Figure 1**).** More detailed taxonomy assignments retrieved for each
132 clustered OTU (using a cut-off of 90% to any reference nSSU) showed that the majority (95-98%)
133 of platyhelminth, arthropod and nematod OTUs were not present in the SILVA database (Figure 1).
134 In total this represented 171 OTUs (30% of OTUs comprising 37671 individual sequences) which
135 may represent un-sampled diversity. The annelids and molluscs, however, had 23% and 50%
136 respectively, of their OTUs with a 100% identity to previously sequenced taxa. The Brachiopoda,
137 Echinodermata, Cnidaria, Gastrotricha and Bryozoa were grouped as BECGB with a total of 9
138 OTUs where 11% of which had 100% identity matches to previously annotated sequence data.
139 Sampling saturation profiles showed that the sequencing effort was not sufficient to determine the
140 full extent of the diversity for any of the four sampled sites (Figure 2**)**. The slope of the OTU
141 rarefaction curves did not approach saturation at 97% cut-off for all the meiobenthic phyla and
142 more specifically for the nematodes, arthropods and even for the platyhelminthes which comprised
143 a low abundance phylum where rarefaction curves tend to converge and reach an asymptote[28]
144 (Supplementary Figure S1) and therefore the data described here are underestimates.
145
146 Community composition by number of OTUs did not show significant differences between the
147 sites, with the nematodes totalling ca 30-50 OTUs (Kruskal-Wallis, p=0.189) followed by the
148 arthropods with ca. 20-30 OTUs (Kruskal-Wallis, p=0.901), the platyhelminthes with ca. 10-20
149 (Kruskal-Wallis, p=0.494), OTUs and the annelids with ca. 3-9 OTUS (Kruskal-Wallis, p=0.110),

found in the Antarctic meiobenthic samples (Supplementary Figure S2). In fact, the majority of the samples showed that 90-100% of the OTUs were shared between sites, with the exception of one of the triplicates of the Islands sample that had approximately 30% of unique OTUs (Figure 3). Whilst all sites showed globally very similar communities, cluster analysis for taxonomic patterns of meiofaunal communities based on Sørensen similarities of OTU presence/absence data for the combined sites showed two well-defined groups within the Antarctic Peninsula sampling sites (Supplementary Figure S3). The Islands and Hangar Cove were more similar to each other, sharing approximately 20% more OTUs than with South Cove and Rothera Point (data not shown).

Graphical representation of community composition from all sample sites was visualized with the Krona chart (Figure 4a). Here, the eukaryotic taxonomic composition of all sites combined showed that the nematodes comprised 32% of the total eukaryotic OTUs. Followed by the arthropods, platyhelminthes and annelids with 18%, 12% and 4% representing the total eukaryotic biodiversity, respectively. Within the nematodes two taxonomic classes predominated: the Chromadorea (80% OTUs) and the Enoplea (20% OTUs) (Figure 4b, Supplementary Table S1.1 – S1.3, Supplementary Material S1). Within these two taxa, Monhysterida (37% OTUs) and Enoplida (19% OTUs) comprised the major proportion of the identifications respectively (Figure 4b, Supplementary Table S1.1 – S1.3). Copepoda dominated the arthropods with 87% of the identified OTUs. The Harpacticoida were particularly abundant at 76% of the Copepoda (Figure 4b, Supplementary Table S1.1 – S1.3, Supplementary Material S1). Outside of the crustaceans, the Acari represented 2% of the arthropod OTUs. The platyhelminthes were mainly represented by with the Rhabditophora (97%) with predominance of the orders Rhabdocoela (62%) and Macrostomida (31%) (Figure 4b, Supplementary Table S1.1 – S1.3, Supplementary Material S1). The annelids were mainly composed of the Polychaeta (85%) and the Haplotaxida (15%). The Polychaeta were dominated by the subclass Palpata (31%) and infraclass Scolecida (54%). The Palpata comprised the Phylodocida order (23%) and taxa with uncertain taxonomic position (Incertae Sedis) (8%). The Scolecida

covered five distinct families with the Spionida (15%), Orbibidae (15%), Terebellida (8%), Ophellidae (8%) and the Capitellida (8%) (Figure 4b, Supplementary Table S1.1 – S1.3, Supplementary Material S1), identifications which have been further substantiated by 18s rRNA molecular barcoding of polychaete samples from shallow-water hard and soft sediment communities near Rothera (Clark, unpublished data).

The shallow-water comparisons with deep-water samples taken from along the Antarctic Peninsula showed very different community compositions (Supplementary Figure S4). Although the annelids and nematodes were found at both depths, they were particularly dominant in the deep-water samples. Shallow-water samples had a much higher percentage of arthropods (or more precisely, copepods). The Nemertea and Hemicordata were essentially only found in the deep samples, with the Cnidaria, Echinodermata and Mollusca more common in the shallows. The difference in community composition was further substantiated by pairwise comparisons of the number of shared OTUs between the different deep-water samples with the combined shallow samples, with the shallow-water sites sharing on average ca. 15% of OTUs with the different deep-water sites (Supplementary Figure S5). It should be noted that comparisons of two of the deep-water sites taken at a similar depth (CTD, 515m and Laubeuf, 500m) showed only 20.4% shared OTUs, indicating the patchiness of distributions (similar shallow-water comparisons between the Islands (13m) and Rothera Point (15m) showed 26.7% shared OTUs) (data not shown).

DISCUSSION

This study shows interesting insights into levels of meiofaunal biodiversity in Antarctic sediments, suggesting similar levels of meiobenthic diversity when compared to other marine studies carried out in more temperate regions using the same nSSU gene region[26], which is higher than expected. Such evidence emerges when comparing the incomplete slopes of the rarefaction curves and OTU numbers obtained here with a previous study on a Scottish temperate benthic ecosystem[26] using an

identical 18S rRNA gene region, a 97% identity cut-off and the same number of replicates, showing both sites to be very similar (e.g. 540 Antarctic and 650 Scottish meiofauna total OTUs). This evidence is not in line with paradigms of reducing diversity with latitude[29]. It also suggests that Antarctic meiofaunal biodiversity could be as rich and diverse as that found in temperate areas.

This preliminary study reveals that almost all of the main meiobenthic biodiversity is yet to be described, particularly with regard to taxonomic identification and development of associated barcodes, since only 1-4% of our taxa had a full taxonomy match against public databases (Figure 1). Such low levels of taxonomy assignments are almost certainly the result of the lack of Antarctic species in eukaryotic sequence databases, limited and patchy sampling regimes and the almost total absence of knowledge of Antarctic meiobenthic biodiversity in many taxa[30]. Studies on the benthos around the Antarctic Peninsula have found more than 20% of new families, genera and species, which emphasizes that these habitats contain not only new species records but previously undescribed taxa[3,31]. For example, more than half of the known gastropods and bivalve mollusc species in the Antarctic have only been found once or twice[30]. Although this level of novelty might seem atypical for such an extensive but harsh environment, it is somehow reasonable that a topographically complex and remote area such as the Antarctic would be bound to contain new species due to the long period of biogeographic isolation via the Antarctic Circumpolar Current, especially if some of these areas have been little or never sampled before[32].

In this study, the phylogenetic analysis and the taxonomic assignments retrieved from the SILVA database produced four dominant taxonomically distinct metazoan groups, the nematodes, arthropods, platyhelminthes and the annelids (Figure 4a and b, Supplemental Material S1). These results are supported by previous studies showing that nematodes and Harpaticoid copepods dominate the Antarctic benthos[33, 34]. Additionally, very few studies describe platyhelminthes living within Antarctic sediments possibly because they are commonly known to live in the sea-ice and

feed on sea ice diatoms[35] but may also be explained by a likely high destruction rate of their soft bodies when sampled for physical taxonomic studies. Most annelids found in this study, were dominated by the polychaetes, which tend to be transient meiofauna associated with Antarctic sediments[36]. These data are supported by a macrofaunal (>1mm) taxonomic study in the same region, which showed a predominance of Arthropods and Annelids (polychaete worms) in the sediments[37]. The more fragile nematode samples were largely identified using molecular techniques, which showed them to be the dominant microtaxa, followed by Arthropods and Platyhelminthes[37]. In our study there were also some identified phyla with very few assigned OTUs (Mollusca, Brachiopoda and Echinodermata). However, given the size fractionation methodology used in this study (<500µm, >45µm), these low abundant OTUs would be either traces of larval or very early post-settlement stages or more likely, gut contents of detritivores, cell debris, faeces, pieces of dermis etc. from adult benthic colonisers. Indeed the macrofaunal study showed that molluscs were highly represented, particularly by *Mysella charcoti* and *Aequiyoldia eightsi*, which would have been largely excluded in meiofaunal fractionation[37]. Taxonomy studies in the Southern Ocean[1,2] have described a greater number of species than presented in this data set here (for example 524 nematode species compared with our estimate of 140 OTUs). However the fact that we identified such a number of OTUs in shallow waters at four sampling sites, some of which are geographically close (rather than the whole of the Southern Ocean for the 524 species[2]) (Supplementary Figure S2) validates the conclusion that there is still much to discover, especially in the sediments.

While, the four meiobenthic phyla described here are the main representatives found in the benthos anywhere in the world, there will be taxonomic differences in community structures at the species level. This is reflected in trophic features and reproductive strategies, which in the case of the shallow-water meiofauna in Antarctica are adjusted to a cold, highly disturbed and food limiting environment. Stable isotope analyses of meiofaunal communities in Potter Cove, Antarctic

Peninsula (latitude -62.235, longitude -58.663) have shown relatively small food webs, based mainly on non-selective deposit feeders, epistrate feeders and a higher proportion of predators[22]. This was substantiated in our study where the taxonomic assignment within the nematodes were dominated by the *Neochromodora*, *Desmolaimus* and *Sabieteria* genera, suggesting that nematode assemblages were mainly composed of deposit feeders and epistrate feeders, which can minimize interspecific competition. There was also a proportion of Enoplea nematodes that are known to be predators/omnivores. Such different feeding strategies will alleviate species competition to available food[38,39]. Molecular analyses, such as metabarcoding used here, allow the identification of previously unknown levels of biodiversity[20] and enable studies that would otherwise not be possible in such detail using other methodologies. In this study, for each of the main meiobenthic phyla (nematodes, arthropods, platyhelminthes and annelids) (Supplemental Material S1) there were some well-supported clades, particularly in the nematodes and nematodes, where OTUs assigned to the same genus, could potentially comprise species complexes. However without further molecular and taxonomic analysis, these would be difficult to define, but would be highly likely[40].

Clustering of sites according to community composition similarity revealed two well-defined groups (Supplementary Figure S3). The first composed of South Cove (8m depth) and Rothera Point (15m depth), represented virtually adjacent sites and thus their clustering confirmed the similarity of their meiobenthic community assemblages. The second cluster was comprised of Hangar Cove (18m depth) and the Islands (13m depth). This is substantiated by the macrofaunal study which showed significant patchiness and differences between different coves[37]. South Cove and Rothera Point are more exposed areas with smaller levels of sediment than Hangar Cove or the Islands and likely subject to different current patterns within Ryder Bay and also increased iceberg scour. Generally, replicates of each ecological location always clustered together and thus the combined replicate meiobenthic samples accurately reflected alpha diversity from the Antarctic Peninsula, as shown previously in similar studies in more temperate areas[41,42]. Meiobenthic

community composition can be extremely variable even within small spatial scales[21,26,43-46]. Local patchiness and structure within these communities is probably a consequence of a combination of several biotic and abiotic factors[41,42]. Similar to global observations, sediment type and grain size play large roles in structuring Antarctic communities[21,23,37], with the additional factors of food supply, which influences species richness and ice disturbance[23]. Glacial retreat, ice shelf collapse and the increasing frequency of iceberg scour are significantly impacting the Antarctic benthos, particularly the more shallow waters[12,21-23,47]. Species return is largely dictated by motility, with the three main methods of return being locomotion, advection by storms and larval re-colonisation[48]. Overall, only the most resilient animals (probably r-selection species) are able to regularly resist such local impacts and prosper in these harsh environments[49]. Studies on Antarctic sediments have shown that nematodes are able to resist and survive in such harsh conditions, namely after ice disturbance nematode communities are very little impacted[33], which again reflects their dominance within the benthos described here.

The shallow-water data were also compared to six deep sea samples from the Peninsula region (Supplementary Figures S4 and S5). There was a clear difference in phyla composition with the deep sea sites dominated by nematodes and the shallow by both nematodes and arthropods (or more specifically copepods). These data confirm existing published information on the differences between shallow and deep meiofauna and fit with previous analyses showing biodiversity patterns associated with sediment type and grain size. The shallow samples comprised coarser grains, which are a more favourable habitat for copepods, whilst the deeper sites comprised more fine sediments (mud) suitable for nematodes, as noted in previous studies[20,23,37]. What was interesting to note was the relatively small overlap in shared OTUs between the shallow and deep samples (Supplementary Figure S5). Because of the way the OTUs were clustered at 97% similarity, “same OTU” in these comparisons may represent the same genus or family, but is unlikely to be the same species in all OTUs[50,51]. However, the 97% cut-off for OTU clustering is a known proxy for most meiofaunal

306 studies. Although the physical processing of the shallow and deep samples was slightly different,
307 the rest of the process was identical (primers used in the initial amplification reactions and
308 processing of the data such as removal of non-metazoan OTUs from the comparisons between the
309 two studies) and contributed to standardising the data comparison. Moreover, the higher sensitivity
310 for extracellular DNA of the methods used in the deeper sediments should have actually increased
311 the amount of overlap between shallow and deep due to sedimentation, yet very limited overlap was
312 observed.
313
314 This lack of overlap between shallow and deep sites is particularly interesting as the deep CTD
315 samples were quite close to all the shallow sites (Figure 5) and the CTD sampling site was at the
316 bottom of the Marguerite Bay trough. One could expect all the OTUs from the shallow sites to
317 passively sink/disperse to the deepest point and this clearly does not happen or the conditions at
318 depth select against shallow dwelling species. This depth zonation has been shown previously[20,34]
319 and as yet, there is not a clear answer as to whether there is true depth zonation of meiofauna or
320 whether the shallow DNAs are simply too diluted or degraded by the time they reach the deep.
321 Further to this, more sampling effort would be needed to clarify meiofauna zonation patterns since
322 the rarefaction curves for the sampled Antarctic areas remained incomplete and thus community
323 composition and diversity levels are yet to be determined. The question of faunal exchange between
324 deep and shallow waters is the subject of much debate and may vary according to species ecology,
325 but is a clear area for further research[23]. Interestingly even after five years, the meiofaunal
326 communities of the innermost embayments of Larsen B (at 242-427m depth) were still much more
327 similar to those from the deep sea (800-4000m), than shallow shelf communities suggesting that
328 perhaps such zonation does exist. In addition these data show that recolonisation and restructuring
329 of meiofaunal communities is not rapid and less likely to be subject to the rapid shifts as seen in
330 motile megabenthic communities[21-23,52]. Because they are less motile, they may be forced to adapt
331 and thus the signals of change may be clearer in these smaller species[6]. However, what is clear in

both shallow and deep-sea Antarctic samples is the high levels of undiscovered taxa and potentially high levels of biodiversity, in what are often described as species-poor regions of the globe.

Conclusions

Our results suggest that meiofaunal biodiversity in the shallow waters of the Antarctic is at least similar to that of temperate regions. The Antarctic comprises ca. 10-11% of the World's continental-shelf-area and the total number of validated marine species (mega- and macrofauna) described for the Southern Ocean exceeds 8,000 species, with at least as many more expected[1,2]. Antarctic meiofaunal descriptions are relatively few to date and have concentrated on taxonomic characterisation. Taxonomically classification of all species is often not practical due to the lack of suitably qualified taxonomists and the sheer volume of work required, thus environmental high throughput sequencing enables faster surveys into understanding biodiversity, albeit providing a slightly different type of data. It also facilitates studies that would otherwise be impossible particularly when applied to bulk environmental samples containing small and easily damaged taxa obtained from inhospitable regions[27]. The study described here showed that much of the Rothera meiofaunal biodiversity is yet to be described, as no plateau was reached from the rarefaction curves and most OTUs could not be annotated with confidence using the public databases. It also shows that the genomic variability of the 18S rRNA gene can effectively be used to reflect the high but also intangible level of biodiversity even in such a relatively small dataset used in this study and that the methodology is highly tractable for more detailed samplings in the future. These will enable us to gain a more accurate understanding of patchiness and adaptation of meiobethic communities to different environments. This approach may be particularly useful for detecting molecular taxonomic signatures of response to climate change not only in terms of gradual sea warming and acidification, but also the emergence of new habitats resulting from anthropogenic change.

MATERIAL AND METHODS

358

359 **Sample collection**

360 Sediment samples were collected in triplicate at different depths in four different sites near Rothera
361 Station, Adelaide Island on the Antarctic Peninsula (Figure 5). Sampled areas comprised the Islands
362 (67º35.6' S, 68º15.1' W, 13m depth), Hangar Cove (67º33.8' S, 68º07.6' W, 18m depth), South
363 Cove (67º34.2' S, 68º7.9' W, 8m depth) and Rothera Point (67º34'19'S, 68º6'44'W, 15m depth).
364 Samples were collected using a standard corer methodology. All samples were immediately fixed in
365 500 ml storage pots containing 300 ml of DESS (20% DMSO and 0.25 M disodium EDTA,
366 saturated with NaCl, pH 8.0)[53]. The meiofaunal size fraction was mechanically separated from the
367 sand and concentrated by decanting five times with filtered tap water through a 45 µm filter.
368 Subsequent separation from fine silt was achieved by repetitive centrifugation in 1.16 specific
369 gravity (sg) LUDOX-TM solution[54]. Following centrifugation, each sample was retained on a
370 distinct mesh sieve which was then folded, sliced and placed in a 15 ml falcon tube and kept at -
371 80°C until DNA extraction. Samples were lysed overnight at 55°C in lysis buffer (100 mM Tris-
372 HCl, pH7.5; 100 mM NaCl; 100 mM EDTA; 1% SDS, 500 µg/ ml proteinase K), assisted by
373 spinning wheel mixing, and DNA extracted with the QIAamp DNA Blood Maxi Kit (Qiagen)
374 following the manufacturer's protocol[26].

375

376 **Primer design and PCR**

377 Due to the extreme sensitivity of this methodology, all PCR and DNA extractions were carried out
378 in separate rooms and recommended eDNA practices were applied to avoid cross-contamination
379 between samples. The primers were SSU_ F04 primer (GCTTGTCTCAAAGATTAAGCC) and
380 SSU_R22mod (5′- CCTGCTGCCTTCCTTRGA -3′) were used to amplify approximately 450 bp of
381 the V1–V2 regions of the nuclear small subunit rDNA (18S rDNA)[20]. Fusion primers, PCR
382 amplification and 454 Roche sequencing were performed as described previously[26,27]. Specifically,
383 PCR amplification of the specified nSSU region was performed using 1 µl of genomic DNA

template (1:500 dilutions) in 3x40 µl independent reactions with Pfu DNA polymerase (Promega). PCR conditions involved a 5 min denaturation at 95 °C, then 35 cycles with 1 min at 95 °C, 45 s 57 °C, 3 min 72 °C and a final extension of 10 min at 72 °C. Negative controls (ultrapure water only) were included for all amplification reactions. Subsequently, triplicates of PCR products were visualized and the expected 450 bp fragment was purified (QIAquick Gel Extraction Kit, Qiagen) in an agarose gel and quantified using the Agilent Bioanalyser 2100. All purified PCR products were diluted to the same concentration, pooled together to create one metagenetic sample/ library and sequenced in one direction (A-Amplicon) on half a plate of a Roche 454 GSFLX platform (2x250 bp) at the Centre for Genomic Research, Liverpool. For full details of replicated PCRs and associated MID tags, see Supplementary Table S2.

Data analysis and generation of OTUs

Raw sequence reads were filtered and denoised using FlowClus[55]. The filtering criteria included truncating reads prior to the first ambiguous base, the reverse primer, or a window of 50bp whose average quality score was less than 25.0. Any reads shorter than 200bp or longer than 600bp were eliminated. For the denoising step, in which pyrosequencing errors were corrected by clustering the flowgrams, a constant value of 0.50 was used for the denoising distance[56]. After denoising, PCR chimeras were removed using UCHIME[57] (Supplementary Table S2). The remaining reads were then analysed using QIIME[58]. They were clustered into OTUs at 97% sequence similarity using UCLUST[59] (pick_otus.py), and taxonomic assignment was performed using the Silva 111 database[60] (assign_taxonomy.py), which uses uclust. The uclust consensus taxonomy assigner retrieves the maximum assigned matches for each query sequence. It then assigns the most specific taxonomic label that is associated with at least min_consensus_fraction of the matches. It is acknowledged that the threshold used for the OTU clustering at 97% similarity might cluster genus or family from the same taxa, as intra-specific variability will differ across many taxa/ species. However, this cut-off is

known as proxy for most meiofauna species[50], but cut-offs such as 99% have also been justified as a proxy for some nematode species in more targeted studies[51]. For direct ecological comparisons among samples with different read numbers, the percentage of reads in each sample was used instead of read counts and downstream analyses targeted main representatives within meiofauna phyla occupying the Antarctic Peninsula sediment habitats[42].

Data Deposition: All sequence reads have been deposited in the European Nucleotide Archive (ENA) with accession number ENA: PRJEB1952.

Diversity and community analysis

Rarefaction curves were generated with EstimateS 8.2.0 software[61] using the Chao1 richness estimator; nonetheless other richness estimators were tested (ACE, Chao1, Jackknife1 and Bootstrap) and yielded similar results. Sørensen's similarity coefficient among samples was computed based on a presence/absence similarity matrix and was used to create cluster dendrograms with 50 random starts, using primer 6[62]. Using the same software, a similarity profile ('SIMPROF') permutation test, was performed on group-average cluster analysis to test whether the meiobenthic samples differ from each other. In order to further test for significant differences in community composition among sampling sites, a permutational multivariate analysis of variance ('PERMANOVA') was performed. Analyses were based on Sørensen's similarity coefficient on untransformed data of an OTU presence/absence matrix over the four sampled sites, with 1000 permutations. Further comparisons between the Antarctic and a Scottish study[26] were performed to illustrate possible differences between the numbers of meiofauna OTUs found per phyla in the two habitats. In order for the two studies to be as comparable as possible, all analysis were performed using triplicated samples, similar 18S gene regions and using the same OTU clustering threshold of 97%. Antarctic eukaryotic OTUs retrieved from the data analysis were used in a Neighbour-Joining (NJ) phylogeny reconstruction to confirm the taxonomic assignments (Supplemental material S6).

Taxonomic contributions using total OTU proportions were visualized using Krona graphs, plotted using the Krona web interface software[63] and a non-parametrical statistical test was performed (Kruskal-Walis) to check if number of OTUs per replicated sample site were significantly different · Taxonomic assignment for this purpose was also performed using SILVAngs 1.5 database at https://www.arb-silva.de/ngs/.

Comparison with deep sea samples

Comparisons of OTUs were made with deep sea meiofaunal data from samples taken along the Antarctic Peninsula[20] and comprise SED 415 (Laubeuf Fjord) (500m) (67°52.583S 68°5.842'W), SED 390 and SED 410 (duplicate CTD samples at the same site and depth) (515m) (67°35'6.57S 68°12'17.38W), SED385 (390m) (67°35'6.16"S 68°8'35.42"W), SED395 (off Anchorage Island) (290m) (67°36'5.23"S 68°13'29.75"W) with an additional sample denoted Adria2 (1120m) (74°29'.00S 104°25'.00W) kindly provided by Holly Bik and Adrian Glover (data unpublished) (Figure 5). Published data were obtained from direct extractions of minimal amounts of frozen sediments[20] while the data from the additional sample "Adria2" was processed using the same methodology (DESS fixed samples, meiofauna isolated from sediments and then DNA extraction) as the shallow-water data presented here.

Acknowledgements

The authors would like to thank both Holly Bik and Adrian Glover for allowing us to use the data from their deep-sea Peninsula sediment sample in our analyses. We would also like to thank the Rothera Dive Team for help in collecting core samples, Susannah Pereira for meiofaunal decantation and molecular technical assistance and Laura Gerrish for producing the maps. The NERC National Facility for Scientific Diving (Oban) provided overall diving support. This paper was financed by NERC core funding to BAS within the Polar Sciences for Planet Earth Programme (MSC and LSP) and a NERC Antarctic Funding Initiative Collaborative Gearing Scheme to SC (No

57). VGF and DMP were supported by CCMAR core funding from the Portuguese Science Foundation (FCT.UID/Multi/04326/2013). VGF would like to thank the Portuguese Foundation for Science and Technology (FCT) for a post-doctoral grant (SFRH/BPD/80447/2014). FS was supported by the European Community FP7 Marie Curie International Incoming Fellowship project MARMEDIV (no. 253251).

Author Contributions

VGF performed most of the shallow-water bioinformatics analyses, interpreted the data and wrote the first draft of the paper. FS was involved in collecting the samples, advised on the bioinformatics analyses, supplied the deep-sea data, performed the deep-sea analyses and contributed to the paper. JMG and CQ both performed some of the shallow-water bioinformatics analyses and provided bioinformatics advice. SC jointly conceived the project with LSP, supervised sample collection and the molecular extractions, provided metagenomics advice and contributed to the paper. DMP supervised the biological interpretations and the production of the manuscript. LSP jointly conceived the project with SC, managed the Antarctic fieldwork, provided advice on Antarctic ecology and contributed to the paper. MSC supervised the project and the analyses and wrote the final draft of the paper.

Competing Financial Interests

The authors state that they have no conflicts of interest.

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Figure Legends

Figure 1 - Percent identity to known sequences and number of OTUs found for the main meiofauna phyla retrieved from the Antarctic Peninsula sampled sites. The red full line represents the total number of OTUs found per phyla and the blue bar represents the percentage identity BLAST match against the SILVA 111 nucleotide database. OTUs percentages of BLAST match identity against SILVA database are shown black (100% BLAST), dark to light grey (100-97% BLAST), light to dark blue (97-93%) and light to dark orange (93-90% BLAST). BECGB: Brachiopoda, Echinodermata, Cnidaria, Gastrotricha, Bryozoa.

Figure 2 - Operational taxonomic unit saturation profiles at 99% sequence similarity level, for the Antarctic samples collected. Hangar Cove (HC), Islands (I), Rothera Point (RP) and South Cove (SC), where 1- 3 represent each sample replicate.

Figure 3 – Venn diagram depicting OTUs that are shared or unique to each of the four sampling sites found in the Antarctica meiofaunal shallow waters. Numbers in the diagram represent the number of total OTUs found in the different samples, South Cove (blue), Islands (Red), Rothera Point (yellow) and Hangar Cove (green).

Figure 4 – Krona graphical representation of the relative taxonomic contributions (OTU

666 percentages) of the main eukaryotic (a) and meiofauna representatives (b) found at Rothera
667 Peninsula sampled sites, using taxonomic assignment from SILVAngs 1.5 database at
668 https://www.arb-silva.de/ngs/. Depicted are also OTU percentages of four of the main meiofauna
669 phyla found, the nematodes, arthropods, platyhelminthes and the annelids.
670
671
672 **Figure 5 –** Map showing the main sampling sites along the Antarctic Peninsula, with finer detail of
673 the deep-water sites in Ryder Bay. SED 385 is closest to Rothera Research Station and the sites of
674 the four shallow-water sediment-sampling sites (not shown at this scale). Maps made in-house at
675 BAS using ArcGIS v10.1 by the Mapping and Geographical Information Centre (MAGIC).

676 **Table 1:** Summary data for the sampled areas Hangar (HC), Rothera point (RP), Islands (I) and
677 South Cove (SC) at Rothera in the Antarctic Peninsula. The number of reads before (No reads) and
678 after denoising (QC/CC): QC: quality score; CC: chimera check) and total OTU numbers are
679 shown. OTUs numbers were taxonomically assigned to the eukaryotes and unknown. The latter
680 samples comprised both sequences with no matches in the SILVA reference database and also
681 matches to unannotated environmental samples.

682

683

Location	**Depth (m)**	**No Reads**	**QC/CC**	**Number of OTUs**		
				Eukaryote	**Unknown**	**Total**
Hangar	18	18391	14445	116	43	159
Rothera Point	15	8110	6898	85	16	101
Islands	13	23882	20109	127	58	185
South cove	8	5740	8203	76	19	95

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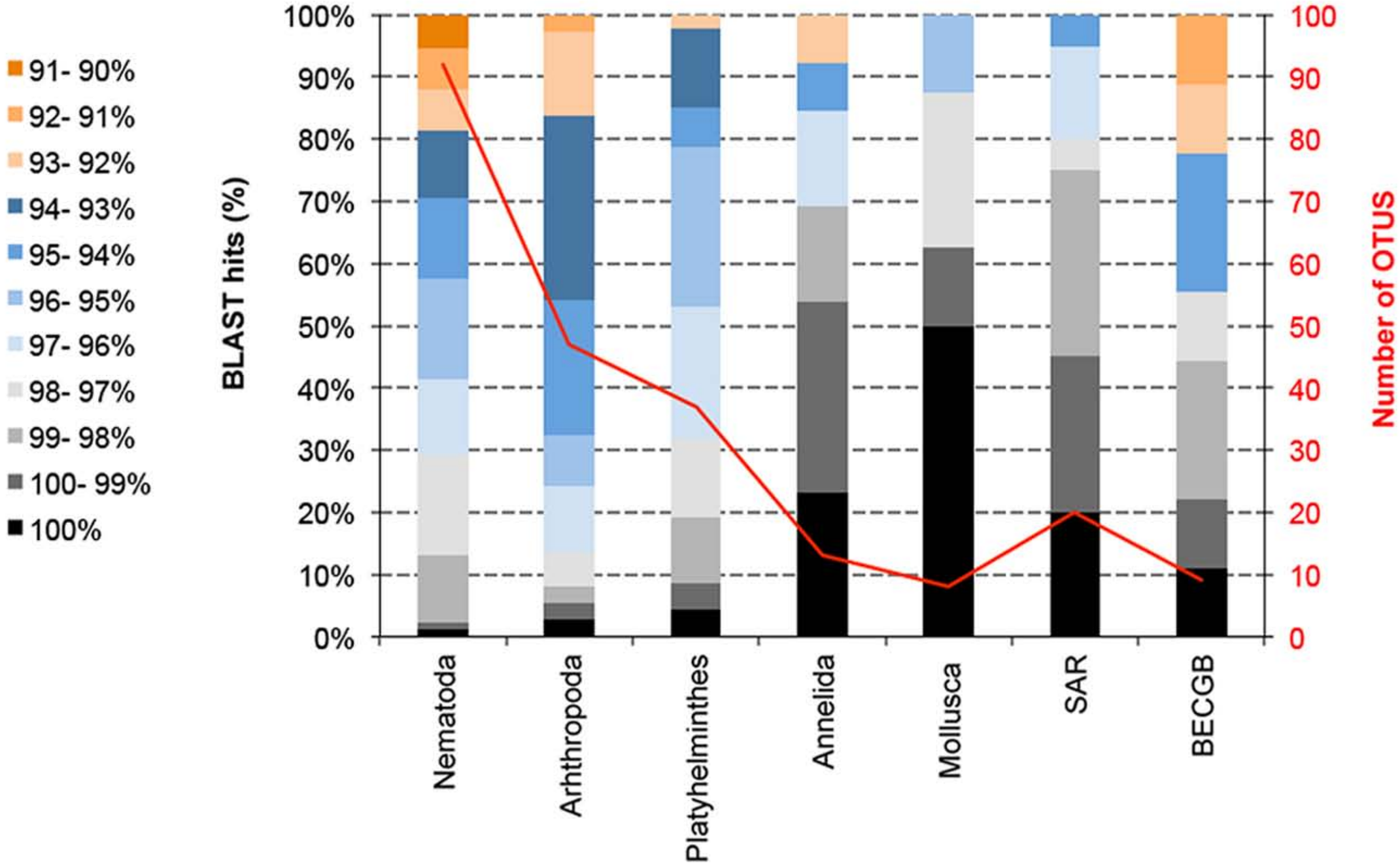
91- 90%
92- 91%
93- 92%
94- 93%
95- 94%
96- 95%
97- 96%
98- 97%
99- 98%
100- 99%
100%
BLAST hits (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
100
90
80
70
60
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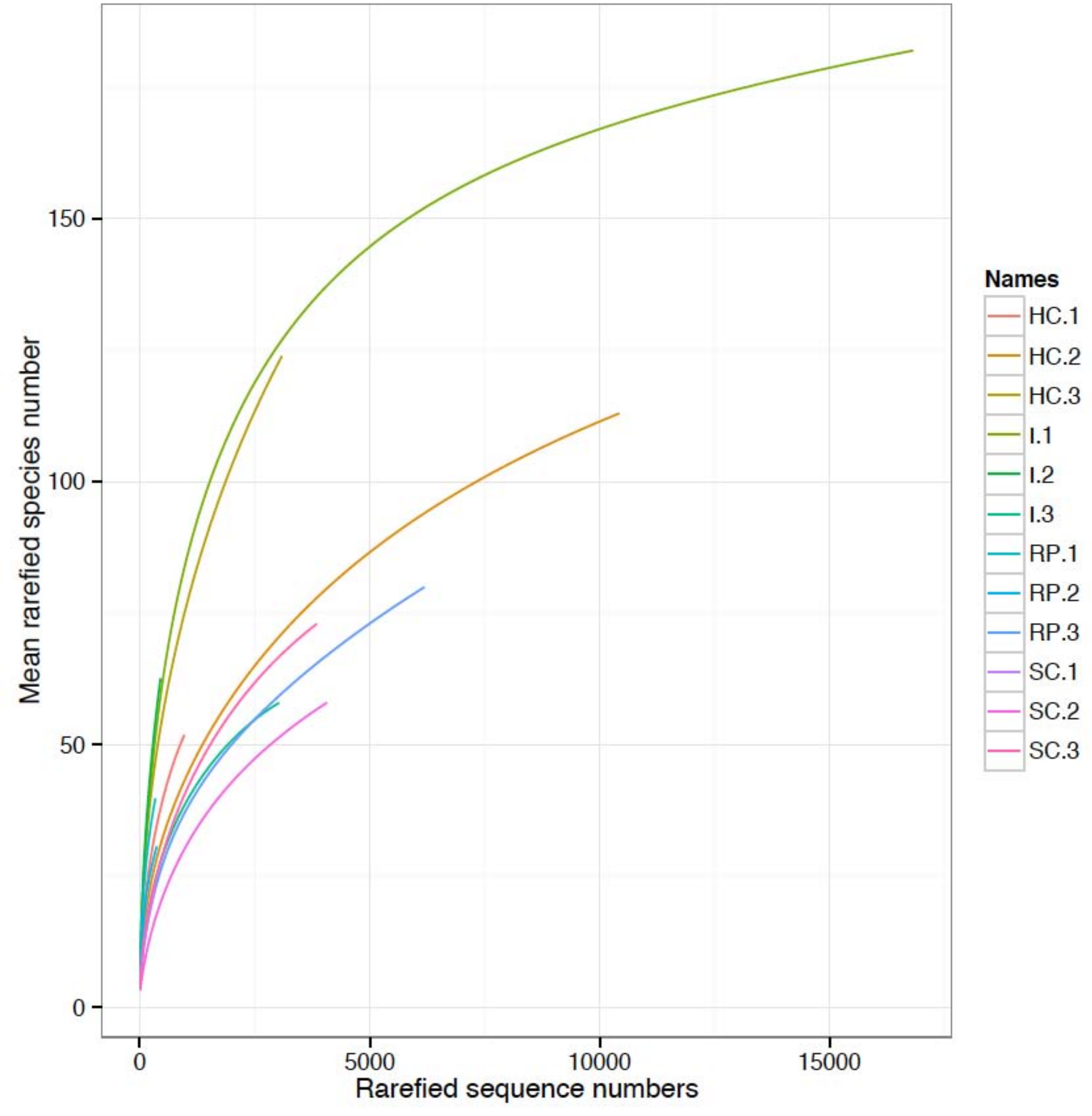
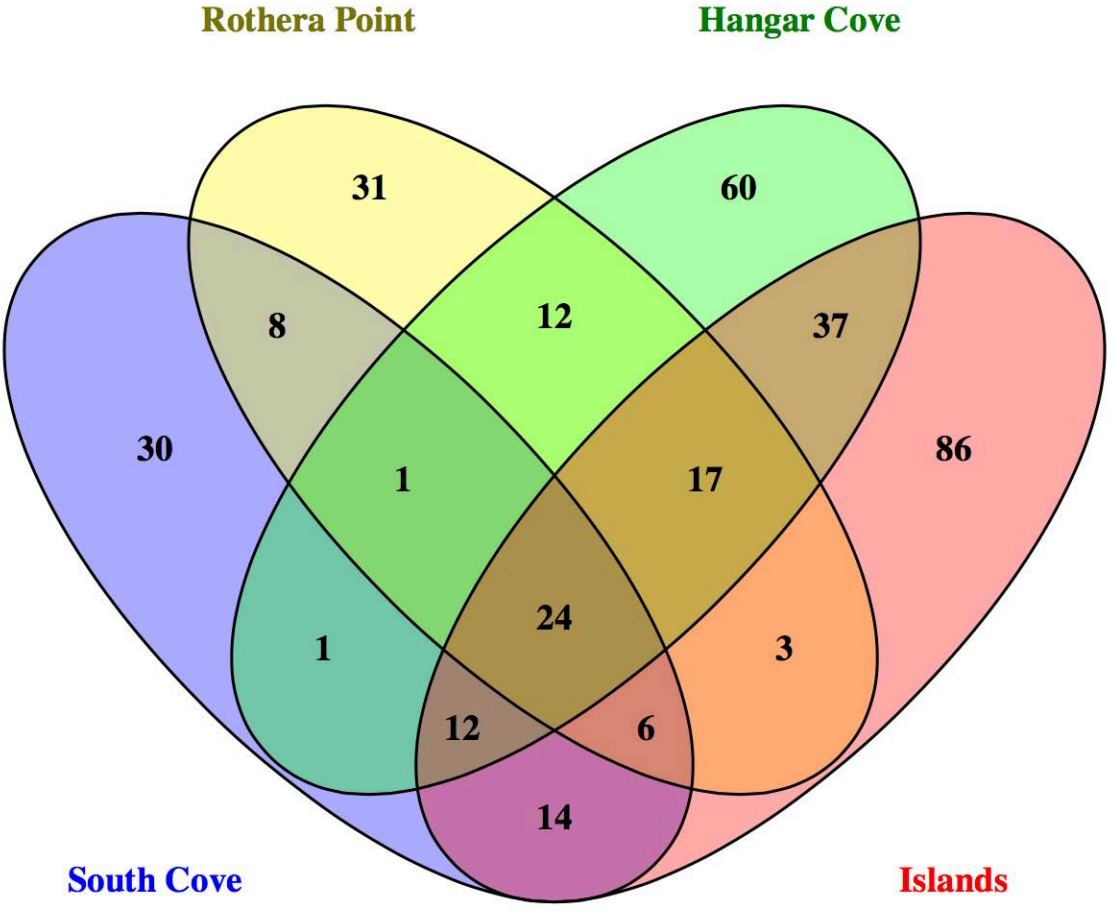
0
Number of OTUS
Nematoda
Arhthropoda
Platyhelminthes
Annelida
Mollusca
SAR
BECGB


Mean rarefied species number
150
100
50
0
0
5000
10000
15000
Rarefied sequence numbers
Names
HC.1
HC.2
HC.3
I.1
I.2
I.3
RP.1
RP.2
RP.3
SC.1
SC.2
SC.3


Rothera Point
Hangar Cove
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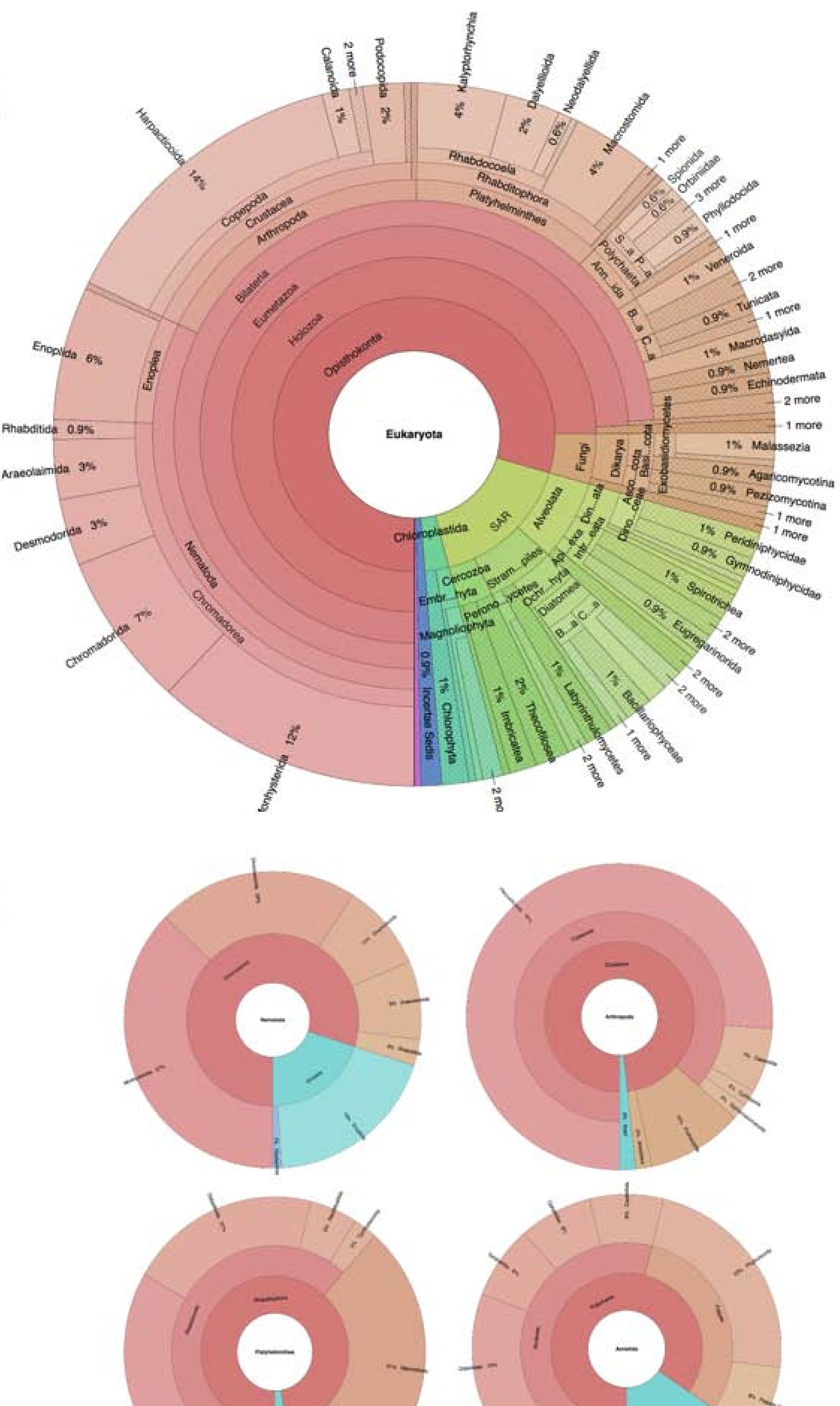
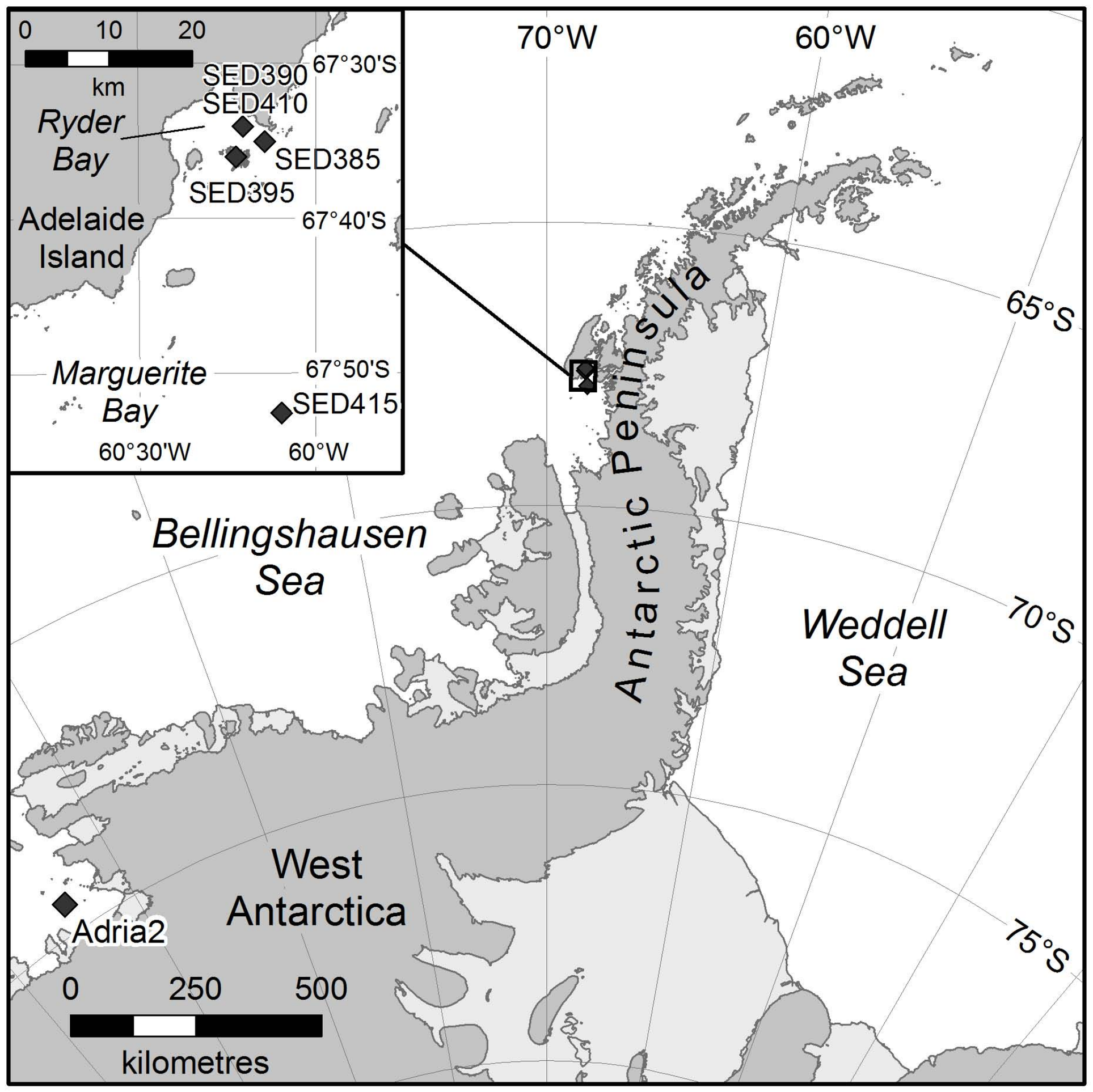
South Cove
Islands

A

B



Eukaryota
Opisthokonta
Holozoa
Eumetazoa
Bilateria
Arthropoda
Crustacea
Copepoda
Harpacticoida 14%
Calanoida 1%
2 more
Podocopida 2%
Kalyptorhynchia 4%
Dalyellioida 2%
Neodalyellida 0.6%
Macrostomida 4%
Rhabdocoela
Rhabditophora
Platyhelminthes
1 more
Spionida
Orbiniidae
0.6%
0.6%
3 more
Phyllodocida
0.9%
1 more
Polychaeta
Ann...ida
1% Veneroida
2 more
0.9% Tunicata
1 more
1% Macrodasyida
0.9% Nemertea
0.9% Echinodermata
2 more
1 more
1% Malassezia
0.9% Agaricomycotina
0.9% Pezizomycotina
1 more
1 more
Fungi
Dikarya
Exobasidiomycetes
SAR
Alveolata
1% Peridiniphycidae
0.9% Gymnodiniphycidae
1% Spirotrichea
0.9% Eugregarinorida
2 more
2 more
2 more
Cercozoa
Stram...piles
Perono...ycetes
Ochr...hyta
Diatomea
Labyrinthulomycetes
Bacillariophyceae
1% Imbricatea
2% Thecofilosea
1 more
2 more
Chloroplastida
Embr...hyta
Magnoliophyta
0.9% Incertae Sedis
1% Chlorophyta
2 mo
Enoplea
Enoplida 6%
Rhabditida 0.9%
Araeolaimida 3%
Desmodorida 3%
Nematoda
Chromadorea
Chromadorida 7%
Monhysterida 12%


0 10 20
km
SED390
SED410
67°30'S
Ryder Bay
SED385
SED395
Adelaide Island
67°40'S
Marguerite Bay
67°50'S
SED415
60°30'W
60°W
70°W
60°W
65°S
Antarctic Peninsula
Bellingshausen Sea
Weddell Sea
70°S
West Antarctica
Adria2
75°S
0 250 500
kilometres

Revealing higher than expected meiofaunal diversity in Antarctic sediments: a metabarcoding approach

Supplementary Information

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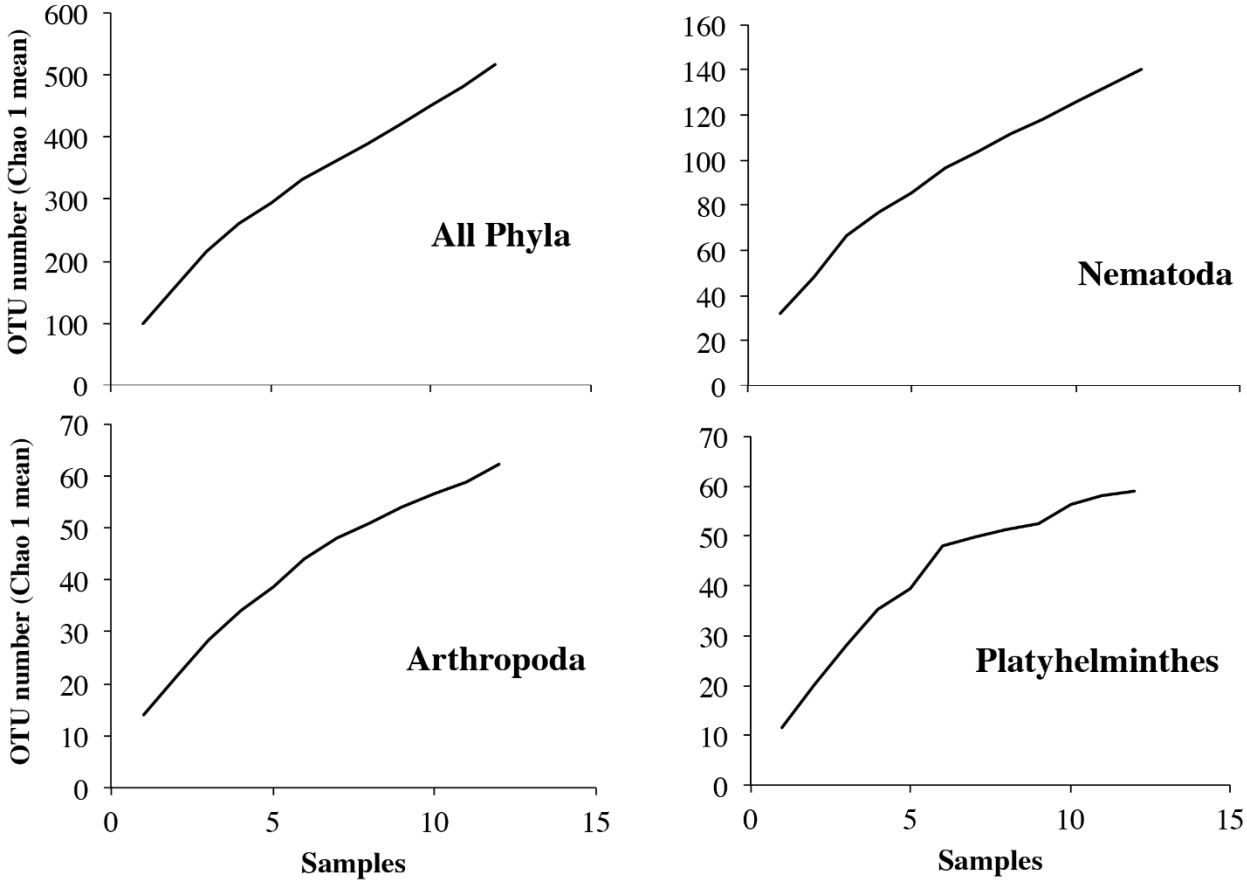
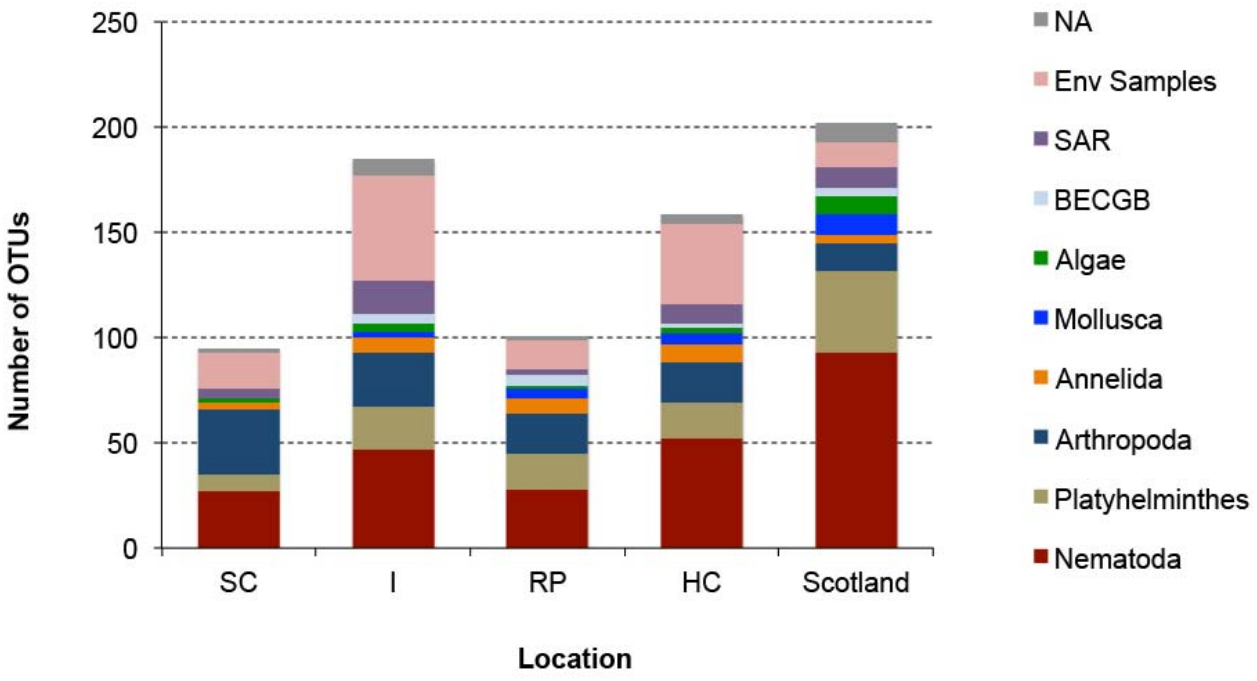
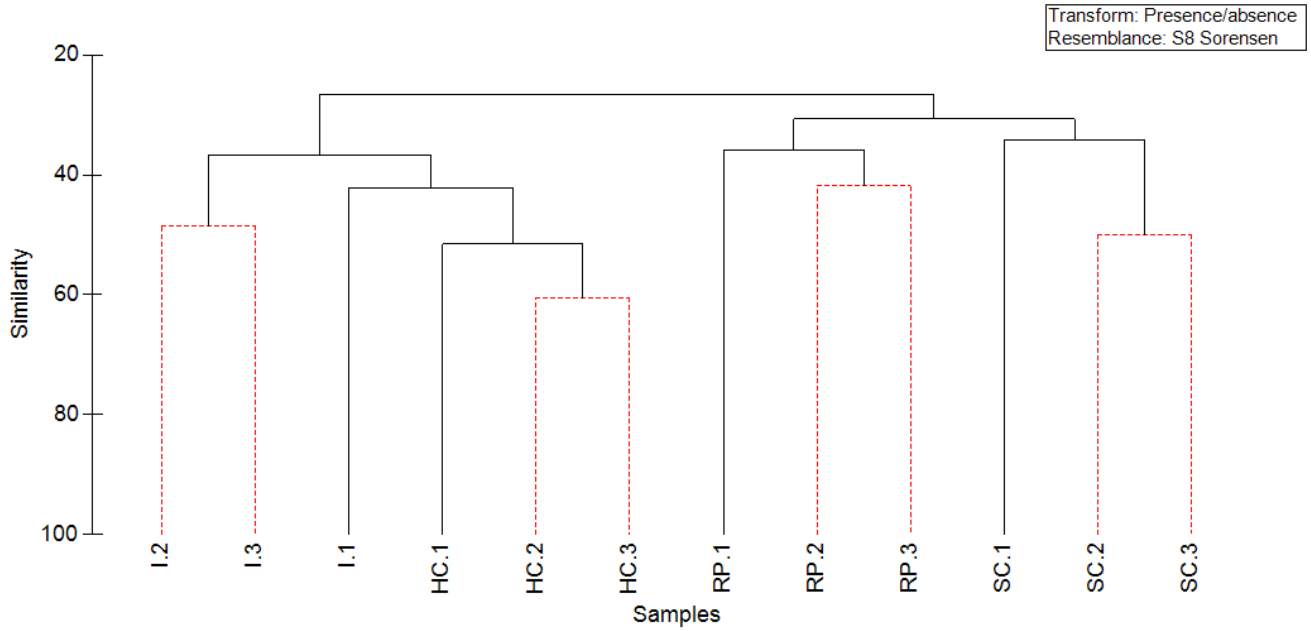


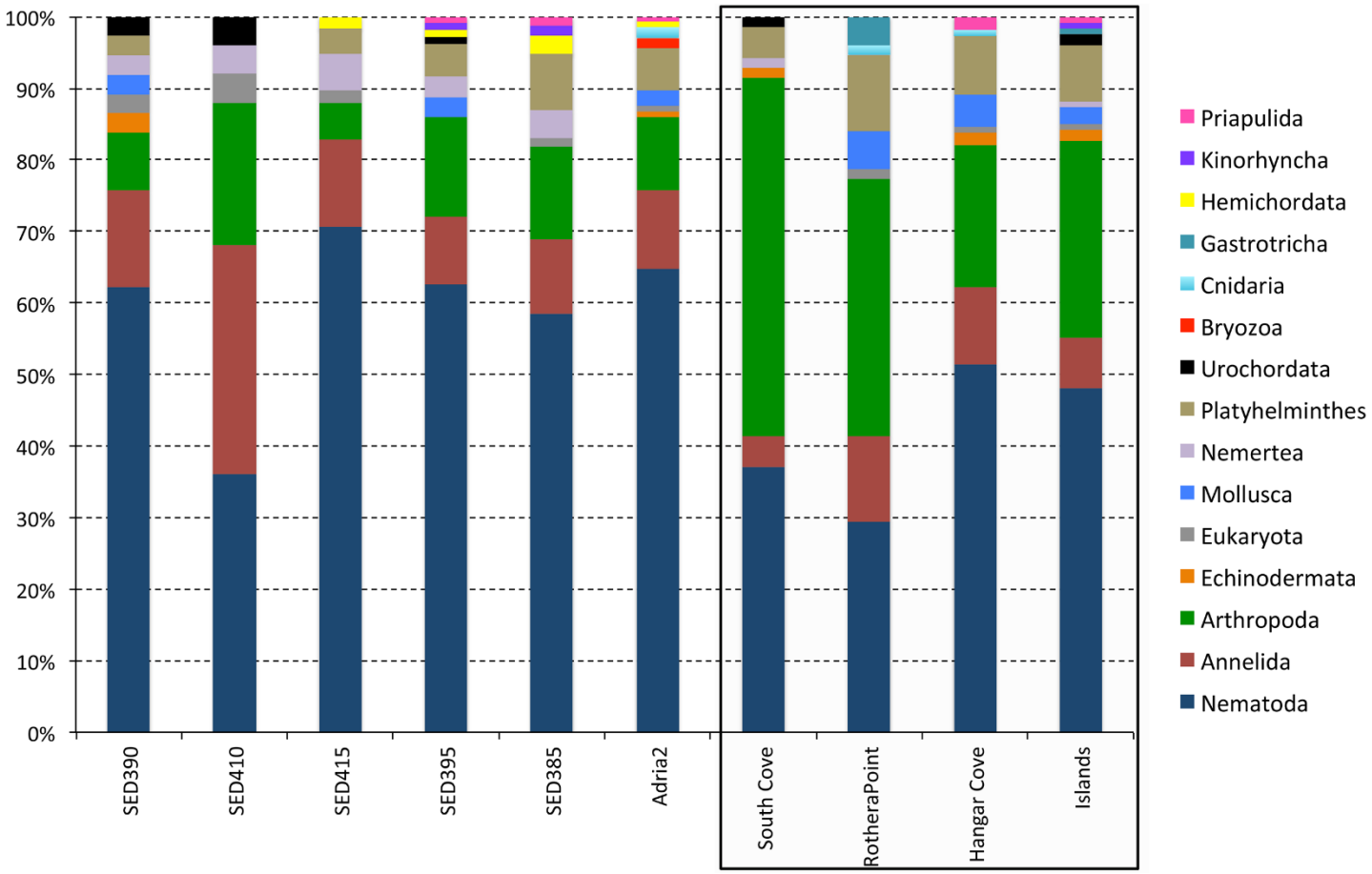
Supplementary Figure S1: Rarefaction curves of the Chao 1 diversity estimator. Plots are shown for all phyla, Nematoda, Arthropoda and Platyhelminthes at 97% identity OTU cut-off for all the Antarctic Peninsula sampled sites samples. Curves were estimated from 100 randomizations, without replacement, using EstimateS, version 8.2.0.



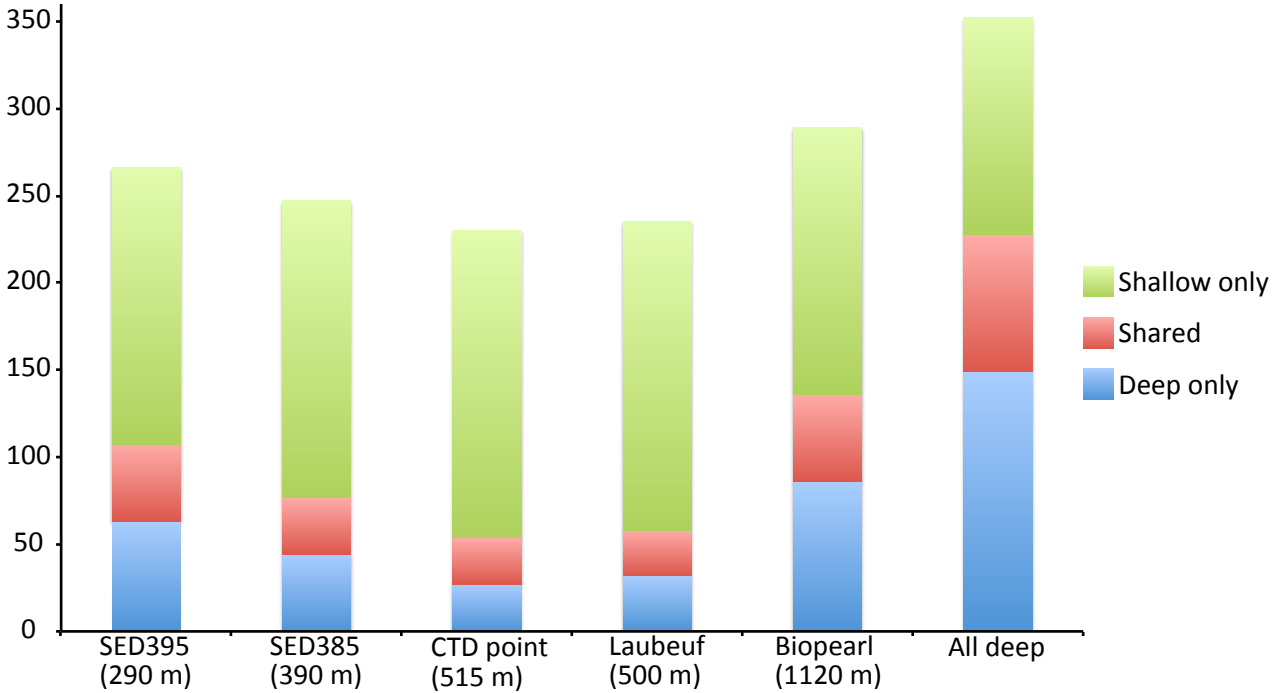
Supplementary Figure S2: Community composition for the Antarctic sampled areas Hangar Cove (HC), Rothera Point (RP), Islands (I), South Cove (SC) and also for the Scottish sampled site[26]. Taxonomy assignment was performed using the SILVA database and the number of total OTUs for each sample site is shown (triplicates were merged per sample site).



Supplementary_Figure_S3: Cluster analysis for taxonomic patterns of meiofaunal communities based on Sørensen similarities of OTU presence/absence data for the combined sites. In the dendrogram, black solid lines represent samples sharing a significant similarity profile with a SIMPROF analysis.



Supplementary Figure S4: Community composition for the shallow and deep-water samples. The shallow-water samples are highlighted within a border. Taxonomy assignment was performed using the SILVA database with the percentage of OTUs per phyla shown in all sample sites.



Supplementary Figure S5: Overlap of metazoan OTUs between merged shallow samples and deep samples (individual and merged). Values are based on presence/absence data, with a total of 203 distinct metazoan OTUs found in all shallow samples and between 54 and 136 distinct OTUs in each of the deep samples for a total of 228 different deep metazoan OTUs.

Supplementary Material S1:

Fonseca et al. "**Revealing higher than expected meiofaunal diversity in Antarctic sediments: a metabarcoding approach**"

Supplementary analysis

Method

All Eukaryotic OTUs retrieved from the data analysis were used to confirm the taxonomic position and community composition within the main eukaryotic metazoan found, using a Neighbour-Joining (NJ) phylogeny reconstruction, 500 bootstrap replications and the Kimura 2-parameter pairwise distance model. The analysis was performed using the software Mega7 (Kumar *et al.*, 2016) and illustrated via a phylogenetic tree produced using the Interactive Tree of Life iTOL tool (Letunic & Bork, 2007).

Result

Phylogenetic analysis of the total Eukaryotic OTUs further confirmed the presence of five taxonomically distinct phyla groups, the Nematoda, Arthropoda, Platyhelminthes, Annelida and the SAR supergroup (Starmenopiles, Alveolata and Rhizaria) and all phylogenetic clusters were supported by strong to moderate bootstrap values (Figure S1). OTUs assigned to Fungi were removed from the analysis and the Chloroplastida OTUS (ALGAE) were used as an out-group (Figure S1). The Arthropoda cluster had a strong bootstrap support but it also showed a smaller independent cluster comprised mainly of the Ostracoda class (Figure S1). Here, three Echinodermata OTUs, two Kynorincha OTUs and one Mollusca OTU also sub-clustered. The Mollusca (7 OTUs) and Gastrotricha (4 OTUs) clustered inside the Annelida phyla. Within the SAR supergroup the Rhizaria (Cercozoa) also showed an independent phylogenetic sub-cluster, whereas the Stramenopiles and Alveolata clustered concurrently (Figure S1).

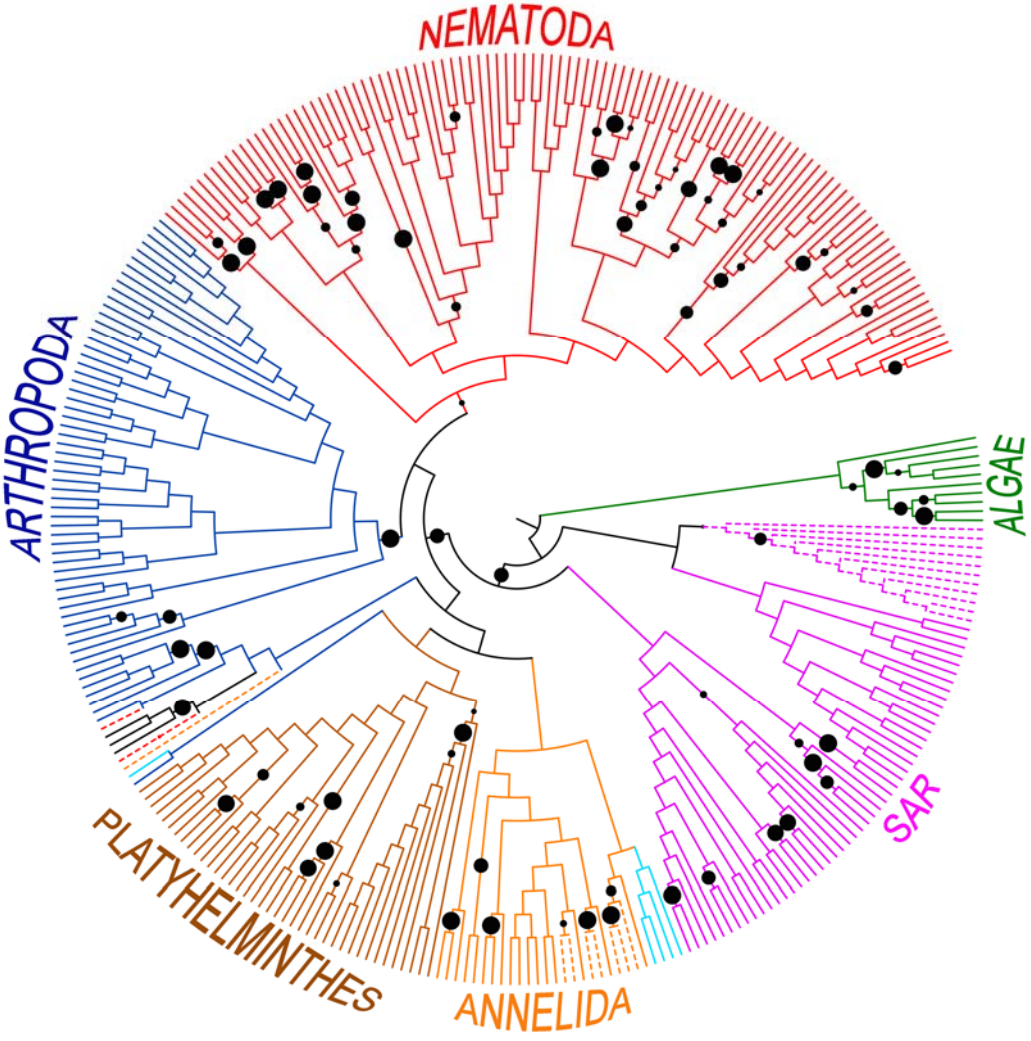


Figure S1- Phylogenetic tree of all Eukaryotic OTUs using a Neighbour-Joining analysis based on the Kimura 2-parameter model. Black symbols at nodes represent the corresponding range of bootstrap support values, from the smallest (75% support) to the largest (100% support). Five main distinct phylogenetic groups were formed the Nematoda, Arthropoda, Platyhelminthes, Annelida and the SAR supergroup (Starmenopiles, Alveolata and Rhizaria). The Rhizaria from the SAR supergroup is depicted in dash-purple. Other phyla are also clustered, the Mollusca (dash-orange), Kynorincha (dash-red), Gastrotricha (light blue) and Echinodermata (solid black). The outgroup is the ALGAE green cluster. SILVA database was used for OTU taxonomy classification.

Supplementary Table S1.1- Closest BLAST matches of Operational Taxonomic Units (OTUs) retrieved from Rothera sample sites, assigned to Nematoda, up to genus or species levels (Description) using SILVA 1.11 database. Depicted are the public accession numbers (AcNumber), BLAST identity percentage against SILVA (BLAST % ID), Phylum and other Taxa ranking.

OTU#	AcNumber	BLAST % ID	Phylum	Taxa Rank	Description
denovo208	gb|AY593940.1|	94,5	phylum:Nematoda	class:Chromadorea	Achromadora cf terricola
denovo219	emb|AJ966473.1|	91,17	phylum:Nematoda	class:Chromadorea	Anaplectus sp.
denovo150	gb|HM564638.1|	98,53	phylum:Nematoda	class:Enoplea	Anticoma sp.
denovo169	gb|HM564638.1|	96,19	phylum:Nematoda	class:Enoplea	Anticoma sp.
denovo46	gb|JN968252.1|	100	phylum:Nematoda	class:Enoplea	Aporcelaimellus sp.
denovo165	gb|KF935309.1|	96,99	phylum:Nemertea	class:Enopla	Argonemertes australiensis
denovo166	gb|FJ040461.1|	96,03	phylum:Nematoda	class:Chromadorea	Axonolaimus sp.
denovo310	gb|FJ040461.1|	92,36	phylum:Nematoda	class:Chromadorea	Axonolaimus sp.
denovo88	emb|AJ966476.1|	92,68	phylum:Nematoda	class:Enoplea	Bathylaimus assimilis
denovo159	gb|AY854218.1|	94,44	phylum:Nematoda	class:Chromadorea	Calomicrolaimus parahonestus
denovo160	gb|AY854218.1|	94,97	phylum:Nematoda	class:Chromadorea	Calomicrolaimus parahonestus
denovo294	gb|AY854218.1|	98,14	phylum:Nematoda	class:Chromadorea	Calomicrolaimus parahonestus
denovo281	gb|JN968284.1|	90	phylum:Nematoda	class:Chromadorea	Calomicrolaimus sp.
denovo336	gb|JX678599.1|	95,62	phylum:Nematoda	class:Chromadorea	Camacolaimus sp.
denovo93	gb|EF591327.1|	97,94	phylum:Nematoda	class:Chromadorea	Camacolaimus sp.
denovo170	gb|HM564544.1|	98,83	phylum:Nematoda	class:Enoplea	Chaetonema sp.
denovo38	gb|JN968217.1|	91,88	phylum:Nematoda	class:Chromadorea	Daptonema sp.
denovo298	gb|JN968217.1|	91,91	phylum:Nematoda	class:Chromadorea	Daptonema sp.
denovo304	gb|EF591333.1|	95,53	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo275	gb|EF591333.1|	97,63	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo138	gb|EF591333.1|	95,79	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo65	gb|EF591333.1|	96,59	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo184	gb|EF591333.1|	97,63	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo267	gb|EF591333.1|	94,23	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo75	gb|EF591333.1|	97,63	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo8	gb|EF591333.1|	94,74	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo195	gb|EF591333.1|	94,47	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo178	gb|EF591333.1|	95,01	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo321	gb|EF591333.1|	93,79	phylum:Nematoda	class:Chromadorea	Desmolaimus sp.
denovo76	gb|FJ182217.1|	97,63	phylum:Nematoda	class:Chromadorea	Draconema japonicum
denovo168	gb|AY854193.1|	98,66	phylum:Nematoda	class:Enoplea	Enoploides brunettii
denovo42	gb|HM564545.1|	98,49	phylum:Nematoda	class:Enoplea	Halalaimus sp.
denovo330	gb|HM564479.1|	98,5	phylum:Nematoda	class:Enoplea	Halalaimus sp.
denovo84	gb|FJ040458.1|	93,18	phylum:Nematoda	class:Chromadorea	Leptolaimus sp.
denovo209	gb|FJ040458.1|	93,18	phylum:Nematoda	class:Chromadorea	Leptolaimus sp.
denovo97	gb|FJ040458.1|	93,07	phylum:Nematoda	class:Chromadorea	Leptolaimus sp.
denovo81	gb|FJ040458.1|	90,84	phylum:Nematoda	class:Chromadorea	Leptolaimus sp.
denovo124	gb|FJ040458.1|	94	phylum:Nematoda	class:Chromadorea	Leptolaimus sp.
denovo149	gb|JF293035.1|	98,45	phylum:Nemertea	class:Anopla	Lineus torquatus
denovo231	gb|EF591337.1|	92,86	phylum:Nematoda	class:Chromadorea	Linhomoeidae sp.
denovo314	gb|JN968218.1|	93,88	phylum:Nematoda	class:Chromadorea	Metadesmolaimus sp.
denovo110	gb|AY854210.1|	97,89	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo299	gb|AY854210.1|	97,36	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo328	gb|AY854210.1|	96,31	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo198	gb|AY854210.1|	96,57	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo252	gb|AY854210.1|	98,29	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo133	gb|AY854210.1|	95,51	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo60	gb|AY854210.1|	97,63	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo10	gb|AY854210.1|	95,78	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo193	gb|AY854210.1|	95,51	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo48	gb|AY854210.1|	93,95	phylum:Nematoda	class:Chromadorea	Neochromadora
denovo333	gb|JN968246.1|	94,72	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo207	gb|JN968246.1|	93,14	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo78	gb|JN968246.1|	96,31	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo197	gb|JN968246.1|	92,61	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo154	gb|JN968246.1|	95,78	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo194	gb|JN968246.1|	94,74	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo261	gb|JN968246.1|	93,44	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo25	gb|JN968215.1|	95,25	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo96	gb|JN968215.1|	91,6	phylum:Nematoda	class:Chromadorea	Neochromadora sp.
denovo66	gb|FJ040459.1|	97,6	phylum:Nematoda	class:Chromadorea	Odontophora sp.
denovo139	gb|FJ040459.1|	94,43	phylum:Nematoda	class:Chromadorea	Odontophora sp.
denovo289	gb|AY854196.1|	96,32	phylum:Nematoda	class:Enoplea	Odontophora sp.
denovo300	gb|FJ040499.1|	96,55	phylum:Nematoda	class:Enoplea	Oxystomina sp.
denovo277	gb|FJ040499.1|	95,78	phylum:Nematoda	class:Enoplea	Oxystomina sp.
denovo274	gb|FJ040499.1|	96,55	phylum:Nematoda	class:Enoplea	Oxystomina sp.
denovo57	gb|KJ638035.1|	95,89	phylum:Nematoda	class:Chromadorea	Paracanthonchus sp.
denovo258	gb|KF591743.1|	92,73	phylum:Nematoda	class:Chromadorea	Pomponema sp.
denovo0	gb|JF293023.1|	98,73	phylum:Nemertea	class:Enopla	Prosorhochmus americanus
denovo316	gb|JN968227.1|	90,81	phylum:Nematoda	class:Chromadorea	Punctodora ratzeburgensis
denovo117	gb|JN968228.1|	98,43	phylum:Nematoda	class:Chromadorea	Sabatieria pulchra
denovo19	gb|JN968228.1|	91,95	phylum:Nematoda	class:Chromadorea	Sabatieria pulchra
denovo141	gb|JN968228.1|	97,45	phylum:Nematoda	class:Chromadorea	Sabatieria pulchra
denovo183	gb|JN968221.1|	97,97	phylum:Nematoda	class:Chromadorea	Sabatieria sp.
denovo43	gb|JN968221.1|	92,15	phylum:Nematoda	class:Chromadorea	Sabatieria sp.
denovo101	gb|JN968221.1|	97,38	phylum:Nematoda	class:Chromadorea	Sabatieria sp.
denovo113	gb|JN968221.1|	94,5	phylum:Nematoda	class:Chromadorea	Sabatieria sp.
denovo68	gb|EF591321.1|	95,26	phylum:Nematoda	class:Chromadorea	Setostephanolaimus spartinae
denovo29	gb|JN968264.1|	95,36	phylum:Nematoda	class:Chromadorea	Sphaerolaimus hirsutus
denovo180	gb|JN968239.1|	91,6	phylum:Nematoda	class:Chromadorea	Sphaerolaimus hirsutus
denovo21	gb|JN968216.1|	99,44	phylum:Nematoda	class:Chromadorea	Spirinia parasitifera
denovo31	gb|JN968216.1|	95,24	phylum:Nematoda	class:Chromadorea	Spirinia parasitifera isolate
denovo54	gb|FJ040468.1|	97,87	phylum:Nematoda	class:Chromadorea	Synonchiella sp.
denovo23	gb|AY284683.1|	90,89	phylum:Nematoda	class:Chromadorea	Teratocephalus terrestris
denovo69	gb|JN968231.1|	93,99	phylum:Nematoda	class:Chromadorea	Theristus sp.
denovo89	gb|JN968231.1|	97,14	phylum:Nematoda	class:Chromadorea	Theristus sp.
denovo128	gb|JN968231.1|	95,56	phylum:Nematoda	class:Chromadorea	Theristus sp.
denovo129	gb|AY763130.1|	96,89	phylum:Nematoda	environmental samples	Uncultured nematode
denovo115	gb|AY854198.1|	97,62	phylum:Nematoda	class:Enoplea	Viscosia viscosa
denovo100	gb|AY854198.1|	94,97	phylum:Nematoda	class:Enoplea	Viscosia viscosa
denovo9	gb|KC920423.1|	93,97	phylum:Nematoda	class:Chromadorea	Zygonemella striata
denovo272	gb|KC920423.1|	90,62	phylum:Nematoda	class:Chromadorea	Zygonemella striata

Supplementary Table S1.2- Closest BLAST matches of Operational Taxonomic Units (OTUs) retrieved from Rothera sample sites, assigned to Arthropoda, Annelida and Mollusca up to genus or species levels (Description) using SILVA 1.11 database. Depicted are the public accession numbers (AcNumber), BLAST identity percentage against SILVA (BLAST % ID), Phylum and other Taxa ranking.

OTU#	AcNumber	BLAST % ID	Phylum	Taxa Rank	Description
denovo224	dbj|AB076626.1|	99,74	phylum:Arthropoda	superfamily:Cytheroidea	Howeina sp.
denovo64	dbj|AB076628.1|	95,61	phylum:Arthropoda	superfamily:Cytheroidea	Cytheropteron subuchioi
denovo295	dbj|AB076628.1|	96,38	phylum:Arthropoda	superfamily:Cytheroidea	Cytheropteron subuchioi
denovo36	dbj|AB076644.1|	98,71	phylum:Arthropoda	superfamily:Cytheroidea	Robustaurila salebrosa
denovo200	gb|DQ538499.1|	94,78	phylum:Arthropoda	order:Siphonostomatoida	Kroyeria sp.
denovo322	gb|DQ538499.1|	93,77	phylum:Arthropoda	order:Siphonostomatoida	Kroyeria sp.
denovo238	gb|EU380295.1|	99,22	phylum:Arthropoda	order:Harpacticoida	Dactylopusia sp.
denovo326	gb|AY627016.1|	96,08	phylum:Arthropoda	order:Harpacticoida	Bradya sp.
denovo297	gb|EU380302.1|	93,83	phylum:Arthropoda	order:Harpacticoida	Parastenhelia sp.
denovo103	gb|EU380309.1|	96,87	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo257	gb|AY627016.1|	97,39	phylum:Arthropoda	order:Harpacticoida	Bradya sp.
denovo53	gb|KC815328.1|	96,86	phylum:Arthropoda	order:Harpacticoida	Amphiascoides atopus
denovo162	gb|AY627015.1|	93,23	phylum:Arthropoda	order:Harpacticoida	Bryocamptus pygmaeus
denovo334	gb|AY627016.1|	98,44	phylum:Arthropoda	order:Harpacticoida	Bradya sp.
denovo233	gb|EU380309.1|	95,3	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo105	gb|AY627016.1|	97,13	phylum:Arthropoda	order:Harpacticoida	Bradya sp.
denovo201	gb|EU380309.1|	97,65	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo303	gb|EU380306.1|	98,17	phylum:Arthropoda	order:Harpacticoida	Argestigens sp.
denovo163	gb|EU380285.1|	98,69	phylum:Arthropoda	order:Harpacticoida	Harpacticus sp.
denovo172	gb|AY692343.1|	96,86	phylum:Arthropoda	order:Harpacticoida	Tisbe furcata
denovo273	gb|EU380309.1|	95,05	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo338	gb|EU380309.1|	95,06	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo176	gb|EU380309.1|	93,01	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo265	gb|KC815328.1|	97,38	phylum:Arthropoda	order:Harpacticoida	Amphiascoides atopus
denovo210	gb|AY627016.1|	96,43	phylum:Arthropoda	order:Harpacticoida	Bradya sp.
denovo144	gb|EU380309.1|	95,83	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo228	gb|EU380306.1|	97,38	phylum:Arthropoda	order:Harpacticoida	Argestigens sp.
denovo119	gb|EU380300.1|	95,05	phylum:Arthropoda	order:Harpacticoida	Paramenophia sp.
denovo234	gb|EU380309.1|	96,87	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo332	gb|EU380297.1|	95,4	phylum:Arthropoda	order:Harpacticoida	Diarthrodes sp.
denovo324	gb|EU380309.1|	97,14	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo1	gb|EU380309.1|	93,75	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo11	gb|EU380309.1|	94,27	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo132	gb|EU380309.1|	93,99	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo12	gb|EU380303.1|	98,69	phylum:Arthropoda	order:Harpacticoida	Ameira scotti
denovo77	gb|EU380299.1|	96,08	phylum:Arthropoda	order:Harpacticoida	Sewellia tropica
denovo121	gb|EU380306.1|	96,82	phylum:Arthropoda	order:Harpacticoida	Argestigens sp.
denovo135	gb|EU380295.1|	95,04	phylum:Arthropoda	order:Harpacticoida	Dactylopusia sp.
denovo190	gb|EU380309.1|	96,72	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo212	gb|EU380309.1|	94,26	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo226	gb|EU380309.1|	92,72	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo229	gb|EU380295.1|	95,34	phylum:Arthropoda	order:Harpacticoida	Dactylopusia sp.
denovo291	gb|EU380295.1|	95,48	phylum:Arthropoda	order:Harpacticoida	Dactylopusia sp.
denovo305	gb|EU380297.1|	95,09	phylum:Arthropoda	order:Harpacticoida	Diarthrodes sp.
denovo307	gb|EU380309.1|	95,6	phylum:Arthropoda	order:Harpacticoida	Itunella muelleri
denovo280	gb|AY118078.2|	100	phylum:Arthropoda	order:Calanoida	Ctenocalanus citer
denovo99	gb|FJ372639.1|	93,75	phylum:Arthropoda	infraclass:Paraneoptera	Saldula sp.
denovo2	emb|AJ238061.1|	100	phylum:Arthropoda	genus:Artemia	Artemia franciscana
denovo283	gb|JQ000095.1|	99,22	phylum:Arthropoda	family:Glycyphagidae	Marsupialichus brasiliensis
denovo179	gb|GU902153.1|	100	phylum:Annelida	Clitellata	Grania sp.
denovo206	gb|AF411887.1|	99,74	phylum:Annelida	Clitellata	Heronidrilus gravidus
denovo157	gb|JN936459.1|	99,23	phylum:Annelida	class:Polychaeta	Tharyx sp.
denovo309	gb|AF448150.1|	98,73	phylum:Annelida	class:Polychaeta	Apistobranchus typicus
denovo6	gb|JN852836.1|	100	phylum:Annelida	class:Polychaeta	Neopolynoe paradoxa
denovo329	gb|GU179368.1|	100	phylum:Annelida	class:Polychaeta	Aglaophamus trissophyllus
denovo158	gb|EU418858.1|	98,74	phylum:Annelida	class:Polychaeta	Polycirrus sp.
denovo331	gb|JF509728.1|	96,34	phylum:Annelida	class:Polychaeta	Capitella teleta
denovo182	gb|AY525627.1|	94,85	phylum:Annelida	class:Polychaeta	Eulalia viridis
denovo192	gb|AF508126.1|	96,15	phylum:Annelida	class:Polychaeta	Scoloplos johnstonei
denovo104	gb|DQ153064.1|	99,74	phylum:Annelida	class:Polychaeta	Polygordius jouinae
denovo259	gb|AY532362.1|	92,33	phylum:Annelida	class:Polychaeta	Phylo michaelseni
denovo145	gb|KF511823.1|	99,74	phylum:Annelida	class:Polychaeta	Ophelina sp.
denovo73	gb|KC984696.1|	100	phylum:Mollusca	class:Bivalvia	Yoldia eightsi
denovo127	gb|KC429382.1|	100	phylum:Mollusca	class:Bivalvia	Cyamiomactra laminifera
denovo164	gb|JQ611498.1|	100	phylum:Mollusca	class:Bivalvia	Pecten jacobaeus
denovo111	dbj|AB714767.1|	97,69	phylum:Mollusca	class:Bivalvia	Nipponomontacuta actinariophila
denovo312	gb|KC429372.1|	99,74	phylum:Mollusca	class:Bivalvia	Mysella charcoti
denovo56	gb|KC429331.1|	100	phylum:Mollusca	class:Bivalvia	Mytilus edulis
denovo40	gb|KC984695.1|	95,66	phylum:Mollusca	class:Bivalvia	Neilonella whoii
denovo221	gb|KC429382.1|	97,49	phylum:Mollusca	class:Bivalvia	Cyamiomactra laminifera

Supplementary Table S1.3- Closest BLAST matches of Operational Taxonomic Units (OTUs) retrieved from Rothera sample sites, assigned to Platyhelminthes, up to genus or species levels (Description) using SILVA 1.11 database. Depicted are the public accession numbers (AcNumber), BLAST identity percentage against SILVA (BLAST % ID), Phylum and other Taxa ranking.

OTU#	AcNumber	BLAST % ID	Phylum	Taxa Rank	Description
denovo47	emb|AJ012531.1|	95,84	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo230	emb|AJ012531.1|	93,54	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo34	emb|AJ012531.1|	93,75	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo74	emb|AJ012531.1|	93,51	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo262	emb|AJ012531.1|	92,45	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo260	emb|AJ012531.1|	93,51	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo14	gb|KC869790.1|	94,59	phylum:Platyhelminthes	order:Macrostomida	Macrostomum sp.
denovo79	emb|AJ012531.1|	92,99	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo94	emb|AJ012531.1|	93,77	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo175	emb|AJ012531.1|	94,06	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo243	emb|AJ012531.1|	93,01	phylum:Platyhelminthes	order:Macrostomida	Paromalostomum fusculum
denovo218	gb|KC529506.1|	96,13	phylum:Platyhelminthes	suborder:Dalyellioida	Pogaina sp.
denovo50	gb|KC602396.1|	94,85	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Acrorhynchides robustus
denovo67	gb|KJ887470.1|	95,03	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Uncinorhynchus flavidus v
denovo33	gb|KC529411.1|	96,34	phylum:Platyhelminthes	suborder:Neodalyellida	Proxenetes puccinellicola
denovo16	gb|KC529435.1|	93,93	phylum:Platyhelminthes	suborder:Neodalyellida	Byrsophlebs delamarei
denovo186	gb|AY775738.1|	97,91	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Stradorhynchus sp.
denovo203	gb|AY775741.1|	94,04	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Mesorhynchus terminostylus
denovo340	gb|KJ887440.1|	98,95	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Odontorhynchus aculeatus
denovo7	gb|KJ887470.1|	97,9	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Uncinorhynchus flavidus
denovo17	emb|AJ012507.1|	91,67	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Cheliplana cf. orthocirra
denovo61	gb|KJ887445.1|	94,52	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Opisthocystis goettei
denovo282	gb|KC529506.1|	94,07	phylum:Platyhelminthes	suborder:Dalyellioida	Pogaina sp. 3
denovo98	gb|KC529523.1|	95,63	phylum:Platyhelminthes	suborder:Dalyellioida	Dalyellioida sp.
denovo279	gb|GU936108.1|	93,19	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Schizorhynchidae sp.
denovo239	gb|KJ887448.1|	94,79	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Thylacorhynchus conglobatus
denovo320	gb|KC602396.1|	93,56	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Acrorhynchides robustus
denovo41	gb|KC602396.1|	93,04	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Acrorhynchides robustus
denovo63	gb|AY775746.1|	100	phylum:Platyhelminthes	suborder:Kalyptorhynchia	Schizochilus choriurus
denovo107	gb|KC529518.1|	93,79	phylum:Platyhelminthes	suborder:Dalyellioida	Wahlia macrostylifera
denovo146	gb|KC529521.1|	92,98	phylum:Platyhelminthes	suborder:Typhloplanoida	Austradenopharynx sp.
denovo185	gb|KC529506.1|	96,66	phylum:Platyhelminthes	suborder:Dalyellioida	Pogaina sp.
denovo271	gb|KC869833.1|	92,54	phylum:Platyhelminthes	suborder:Dalyellioida	Baicalellia canadensis
denovo290	gb|KC869833.1|	96,39	phylum:Platyhelminthes	suborder:Dalyellioida	Baicalellia canadensis
denovo313	gb|U70077.1|ARU70	92,23	phylum:Platyhelminthes	order:Proseriata	Archiloa rivularis
denovo268	gb|AY775733.1|	99,74	phylum:Platyhelminthes	order:Proseriata	Cirrifera sopottehlersae
denovo199	gb|AY222124.1|	94,72	phylum:Platyhelminthes	order:Plagiorchiida	Enenterum aureum

Supplementary Table S2: Overview of the Antarctic sampled sites *in silico* statistics for the NGS of 18S rRNA gene region used. Each replicated sampled site had a 8 nucleotide multiplex-identification tag (MID), depth in meters (m), abbreviated description of the sample, post-quality control and chimera checked number of reads and total number of OTUs at the 97% threshold.

Location	MIDTag	Depth (m)	Description	No Reads	QC/ Chimera check reads	Total OTUs
Hangar_1	TCGTCTAC	18	HC.1	1224	970	49
Hangar_2	AGACAGAC	18	HC.2	13007	10399	104
Hangar_3	CTGTTCAC	18	HC.3	4160	3076	117
Rothera Point_1	AGTCAGAG	15	RP.1	402	341	37
Rothera Point_2	TCAGCTCT	15	RP.2	478	376	30
Rothera Point3	ACTCAGAC	15	RP.3	7230	6181	79
Islands_1	CTAGTCCT	13	I.1	19716	16730	157
Islands_2	CAGTTGAC	13	I.2	549	455	54
Islands_3	TAGGTTGC	13	I.3	3617	2924	50
South cove_1	TCTGCTCA	8	SC.1	424	337	21
South cove_2	ATCGTAGC	8	SC.2	4767	4034	51
South cove_3	CATGTGCA	8	SC.3	549	3832	69