Global patterns of diversity and phylogenetic community structure in free-living nematodes: a metabarcoding meta-analysis

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1   Introduction

The field of community ecology seeks to describe and explain the abundance, distribution and interaction of species as well as the ecological and evolutionary consequences thereof (Cavender-Bares et al., 2009; Leibold et al., 2004). An ecological perspective highly relevant to the marine realm, especially in the years following pioneering deep-sea research, is that of the distribution of biodiversity as a function of depth. Consequently, the question whether species richness follows a predictable bathymetric gradient from shallow to abyssal depths (>4 km) has been a topic of interest since the 1980s. A general pattern that has since then emerged from studies which have focused on several animal groups (e.g. nematodes, isopods, gastropods, bivalves, cumaceans, fish) is that of a unimodal relationship of diversity with depth. Specifically, species richness increases with increasing depth, up to a maximum at approximately 2000 m, after which it decreases toward the abyss (Gambi et al., 2010; Rex, 1981; Rex, Crame, et al., 2005; Stuart & Rex, 2009). Thus far this has only been evidenced in regional morphological assessments of metazoan eukaryotes. DNA-based assays on the other hand, examine the entire community rather than a subsample while simultaneously providing phylogenetic information, thereby allowing an evolutionary perspective of community ecology and biodiversity relationships with bathymetry.

Insights gained from community ecology can be used to reveal mechanisms of community assembly, i.e. the processes by which taxa colonise and persist in an assemblage (HilleRisLambers et al., 2012). The assembly of biological communities is thought to be driven by three mechanisms: neutrality-, niche-, and history-based processes (Qian & Jiang, 2014). Neutral dynamics assume ecological equivalence between species, the persistence of which is thus determined by the stochastic element inherent to processes such as offspring production, mortality and dispersal (Hubbel, 2001). Niche-related processes influence community assembly through competitive exclusion and/or local environmental filters (Carvajal-Endara et al., 2017; Diamond, 1975). Finally, when historical factors such as starting conditions, patterns of speciation and dispersal are dominant, they outweigh local conditions for community assembly (Ricklefs, 1987). The integration of phylogenetic data to the study of community ecology in recent years has allowed the investigation of the relatedness of species

comprising a local assemblage, thereby providing an evolutionary perspective into the mechanisms driving their assembly and enabling a better understanding of the relative dominance of neutral, historical and niche-related processes (Webb et al., 2002). From a phylogenetic perspective, the taxa comprising a community can exhibit relatedness that is either higher (clustering), lower (overdispersion), or no different to that of a random community. Clustering and overdispersion are the result of environmental filtering of conserved and convergent traits, respectively. Overdispersion can also occur through competitive exclusion of conserved traits, although it has been suggested this may result in clustering (Mayfield & Levine, 2010; Webb et al., 2002). Random community structure is due to competitive exclusion of convergent traits (Webb et al., 2002), neutral dynamics between species (Hubbel, 2001), the assembly being weakly influenced by phylogeny (Cooper et al., 2008), or multiple factors opposing and nullifying each other (Helmus et al., 2007). The few studies that have explored this phylogenetic dimension at a local scale in deep-sea taxa, have found that community assembly is mainly driven by niche-related processes with the influence of environmental filters dominating over competitive interactions with conspecifics (Judge & Barry, 2016; Macheriotou et al., 2020; Quattrini et al., 2017). It is not yet known if this remains true at a larger geographic scale encompassing diverse environments. At a time when the marine realm is faced with unprecedented loss of biodiversity through an increasing number of anthropogenic activities [e.g., ocean warming, marine pollution, deep-sea mining, ocean acidification, harmful fishing practices, etc; (Brodeur et al., 2019; Dupont & Pörtner, 2013; Niner et al., 2018)] and the potential subsequent reductions to ecosystem functioning (Danovaro, Gambi, Dell'Anno, et al., 2008; Hoegh-Guldberg & Bruno, 2010; Olsgard et al., 2008), it becomes imperative to understand community assembly in order to identify the principal mechanisms maintaining these assemblages.

Free-living nematodes represent one of the most successful metazoan phyla and can be found in nearly every sedimentary environment where they are typically the dominant meiofaunal group (>32 μm) (Bongers & Ferris, 1999; Vanreusel et al., 2010). Despite their small size, nematodes play a major role in maintaining vital ecosystem services such as denitrification, heavy metal removal, and organic matter mineralization as well as providing a high-quality food source to consumers (Bonaglia et al., 2014; Leduc, 2009; Schratzberger & Ingels, 2018). Morphology-based assessments of nematode abundance and diversity have revealed taxon- and region-specific patterns. In Arctic and Mediterranean sediments, for example, diversity decreased linearly with depth from the bathyal to the abyssal zone (Danovaro, Gambi, Lampadariou, et al., 2008; Grzelak et al., 2017) while no clear bathymetric pattern could be discerned at bathyal depths of the Adriatic sea (Danovaro et al., 2013). Genus-

64 specific trends were documented in the Western Indian Ocean from the shelf to the bathyal zone with some

65 families exhibiting concave and others convex abundance curves as a function of depth (Agnes et al., 2011) while

66 indices of diversity increased with depth in the bathyal southwest Atlantic (Dos Santos et al., 2020). In this study

67 we provide the first meta-analysis of publicly available metabarcoding datasets focused on free-living nematodes

68 from intertidal to abyssal depths representing different environments to answer the following questions:

69   (i)   Do DNA-based assessments show a unimodal bathymetric trend of nematode diversity?

70   (ii)   Which are the mechanisms driving nematode community assembly?

71 2   Materials and Methods

72   Raw .fastq data were extracted from published and unpublished sources through the public sequence

73 databases   Sequence   Read   Archive   (SRA,   https://www.ncbi.nlm.nih.gov/sra)   and   Dryad

74 (https://datadryad.org/stash). Both databases were scanned for datasets using the terms "marine sediment

75 metabarcoding". In the event of Roche 454 data (.fasta + .qual), these were converted to .fastq with the command

76 convert_fastaqual_fastq.py in Qiime1 (Caporaso et al., 2010). Only datasets targeting the 18S rRNA V1-V2 region

77 with the primer set SSU_FO4 (5'- GCTTGTCTCAAAGATTAAGCC -3') and SSU_R22 (5'- GCCTGCTGCCTTCCTTGGA

78 -3') or SSU_R22_mod (5'- CCTGCTGCCTTCCTTRGA -3') were retained. The full list of datasets can be found in ESM

79 Table 1. Samples were pooled by geographic location when the 1st decimal place of the latitude and longitude

80 coordinates in decimal notation were the same value (e.g. MTB1 Long: 45.987, Lat: 15.367; MTB3 Long: 45.956,

81 Lat: 15.362 were pooled as a single sample). Samples were categorised by Biogeographic Marine Realms based

82 on species Endemicity (BMRE) (Costello et al., 2017), mean depth, depth zone (Intertidal: 0-3 m, Shelf: 3-200 m,

83 Bathyal: 200-4000 m, Abyssal: 4000-6000 m) and environment type (i.e., hadal trench, canyon, hydrothermal

84 vent, mud volcano, seamount, and plain for common sediment samples which did not originate from any of the

85 aforementioned environments).

86 2.1   Sequence Data Analysis

87   Gene-specific adapter sequences were truncated from the raw reads using Cutadapt (v2.8) with the

88 maximum error rate and minimum overlap set to 5 and 0.1 respectively. Amplicon Sequence Variants (ASVs)

89 were   generated   following   the   DADA2   workflow   for   large   datasets

90 (http://benjjneb.github.io/dada2/bigdata_paired.html) using default parameters with the exception of

91 maxEE=(2,5), truncQ=2 and the truncation of forward and reverse reads at 250 bp or 200 bp based on the read

92 quality profiles. Roche 454 data were processed as single reads and default parameters with the exception of

93 maxEE=5, truncLen=300, truncQ=2. Taxonomy was assigned using the RDP classifier (minBoot=80) in two steps.

94 First, taxonomy was assigned using a large custom Eukaryotic database derived from Silva (123 for QIIME1, 99%

95 OTUs, n=18991). Subsequently, all ASVs assigned to phylum "Nematoda" were extracted and taxonomy was re-

96 assigned to them using a custom marine nematode-specific database containing Silva and UGent barcode

97 sequences (n=971). To ensure equal read coverage across samples, rarefaction was applied to 7985 reads. The

98 final dataset contained 8548 ASVs and 33 samples from five environments (Table 1).

99 2.2   Diversity Analyses

100   Diversity analyses were completed with the "phyloseq" package (McMurdie & Holmes, 2013). Alpha-

101 diversity metrics (Observed, Shannon, Simpson) were calculated for all Nematoda ASVs. Beta diversity was

102 assessed using the weighted and unweighted UniFrac distance (Lozupone & Knight, 2005) and visualised using

103 Principal Coordinates Analysis (PCoA). Shared and unique Nematoda ASVs were visualised using the R package

104 "UpSetR" (Conway et al., 2017). Data normality and homoscedasticity were assessed using the Shapiro and

105 Levene test, respectively. Differences in alpha diversity by environment type and depth zone were assessed by

106 two one-way Analysis of Variance (ANOVA) with factor "environment type" (levels: canyon, hydrothermal vent,

107 mud volcano, seamount, plain) or factor "Depth Zone" (levels: intertidal, shelf, bathyal, abyssal), respectively, at

108 $\alpha=0.05$.

109 2.3   Community assembly analyses

110   Sequences were aligned using package "DECIPHER" and a chained guide tree. Approximately Maximum-

111 likelihood phylogenetic trees were constructed using FastTree (v2.1) (Price et al., 2010). Due to computational

112 requirements it was not possible to root the phylogenetic trees. The following phylogenetic metrics were

113 calculated for each environment type with the package "picante" (Kembel et al., 2010): Phylogenetic Distance

114 (PD, (Faith, 1992)), Mean Nearest Taxon Distance (MNTD, (Webb et al., 2002)), Mean Pairwise Distance (MPD,

115 (Webb et al., 2002)). The observed metric (Obs) was compared to that obtained from 999 randomizations of the

116 assemblage (Null) representing a random community obtained under all null models offered by the function; i.e.

117 taxa.labels, richness, frequency, sample.pool, phylogeny.pool, independentswap (Gotelli, 2000), trialswap

118 (Miklós & Podani, 2004). The standard effect size (ses) for each value was obtained using the standard deviation

119 of the null metric (StDevNull) as follows: ses= (Obs-Null)/StDevNull. Positive, negative and zero values for ses

120 indicate phylogenetic overdispersion, clustering and random community structure, respectively. Evolutionary

121 distinctiveness (ED) was calculated for all Nematoda ASVs and summed per depth zone using the equal splits

122 method (Redding & Mooers, 2006) with the package "picante". The equal-splits approach divides the

123 evolutionary time represented by a branch equally among its daughter branches, thus providing an

124 approximation of how genetically distinct a taxon is from the others in the tree. Given a non-normal distribution,

125 differences between zones were statistically evaluated by the non-parametric Kruskal-Wallis test followed by

126 multiple pairwise comparisons between groups with p-values adjusted with the Benjamini-Hochberg method

127 (Benjamini & Hochberg, 1995).

128 3   Results

129 3.1   Nematode taxonomic diversity by depth and environment type

130   The pre-processing dataset consisted of 63 samples and 11034 Nematoda ASVs. A total of 33 samples and

131 8548 Nematoda ASVs representing 263505 reads from 10 studies and 10 BMREs (Figure 1) were included in the

132 final dataset after filtering to the amplicon length (> 350 bp) and applying rarefaction at 7985 reads.

133 Approximately 50% (n=4318) of the Nematoda ASVs were unassigned at genus-level while the remainder

134 comprised 81 nematode genera, representing 35 families and 8 orders (ESM Table 2). The four most abundant

135 genera in terms of reads were *Chromadorita*, *Halalaimus, Desmoscolex* and *Acantholaimus*, representing 27%,

136 25%, 22% and 15% of all genus-assigned reads, respectively. Per depth zone, *Chromadorita* was most abundant

137 at the shelf (28%), rarest at abyssal depths (6%) and represented 18-19% of reads in the intertidal and bathyal

138 (Figure 2, A). Interestingly, these four genera comprised just over 20% of reads in the intertidal, while

139 *Oncholaimus* constituted >35% of reads at this depth. *Halalaimus* and *Desmoscolex* were relatively rare in the

140 intertidal and shelf, and constituted >25% of reads in abyssal and bathyal depths, respectively while

141 *Acantholaimus* was only abundant in the abyss with over 28% of reads. When looking at read abundance by

142 environment, *Chromadorita* was overrepresented in the canyon sample with >40% of reads while quite rare at

143 the mud volcano with just over 1% of reads (Figure 2, B). *Halalaimus* consistently comprised over 10% of reads

144 in all five environments, being most abundant in the canyon (21%) and rarest in hydrothermal vent samples

145 (12%). *Desmoscolex* was highly abundant at the mud volcano samples (41% reads) and generally abundant

146 (>24%) at all environments except plains where it comprised 6% of reads. *Acantholaimus* was more evenly

147 distributed across environments with up to 12% of reads at plain samples and 4% in canyon samples. The

148 majority of the 81 nematode genera were shared between two or more environments or depth zones (63% and

149 72%, respectively, ESM Figure 1). The plains which had the highest number of samples (n=20) had the highest

number of unique genera while environments with the lowest sample number, i.e. canyon (n=1) and mud volcano (n=2), did not have any unique genera. Each depth zone had a comparable number of unique genera; nine in the bathyal and an average of five in the other zones.

3.2   Nematode ASV diversity by depth

The average number of Nematoda ASVs per sample was lowest in the intertidal (128 ± 54) and increased with depth at the shelf (193 ± 138), reaching a maximum in the bathyal (362 ± 185) with a decrease towards the abyssal (303 ± 149) (Figure 3). The one-way ANOVA did not reveal statistically significant differences between depth zones for the number of observed Nematoda ASVs, Shannon and Simpson index (p=0.113, 0.137, 0.529, respectively). A significant linear regression, generalised linear model or generalised additive model could not be found for Nematoda ASV abundance as a function of depth (Figure 4). The majority of Nematoda ASVs were unique to each depth zone and no single ASV was ubiquitous (ESM Figure 2). The highest number of shared ASVs (n=75) were common to the shelf and bathyal, representing 10% of the total diversity of the former.

The unweighted UniFrac PCoA generated a pattern in which all deep-sea samples were dispersed although segregated from the shelf and intertidal (Figure 5, right). The two intertidal samples from the southeast and northwest Atlantic clustered closely together despite the large geographic distance between them (> 7000 km) while the shelf samples were more dispersed. Interestingly, a hydrothermal vent sample from the northwest Atlantic overlapped with a plain sample from the Indo-Pacific. Bathyal mud volcano samples from the northwest Atlantic were closely associated with abyssal plain samples from the east Pacific. For the weighted UniFrac, abyssal samples formed a distinct cluster including one bathyal sample from the Indo-Pacific (Figure 5, left). Certain environment types exhibited close associations such as a mud volcano and seamount from the northwest Atlantic. The remaining samples did not exhibit close clustering by environment and were interspersed with plain samples.

3.3   Phylogenetic community structure

All standard effect size values across all environments for PD and MNTD were negative and significantly different from zero (where possible), indicating a strong propensity for phylogenetic clustering (Figure 6, ESM Table 3). In contrast, only the plain samples remained clustered for MPD; the canyon and mud volcano samples were overdispersed (positive ses values) while the hydrothermal vent and seamount samples did not differ from a randomly structured community. Clustering was the dominant mode of structuring when samples were grouped by depth zone with only the shelf and bathyal samples exhibiting randomness for MNTD and MPD,

179   respectively (ESM Figure 3). Evolutionary distinctiveness was significantly different between depth zones

180   (p=0.0002) and was highest in the intertidal (0.051 ± 0.066), followed by abyssal (0.045 ± 0.065), bathyal (0.042

181   ± 0.098) and shelf (0.040 ± 0.059) samples (ESM Figure 5). Statistically significant differences were not found

182   between abyssal-intertidal and shelf-bathyal, while all the remaining pairwise comparisons did differ (ESM Table

183   4).

184   4   Discussion

185   4.1   A metabarcoding perspective of nematode diversity

186   The composition of nematode assemblages was largely consistent with morphological assessments;

187   typical deep-sea genera (e.g. *Acantholaimus*, *Desmoscolex*) were present in high numbers in the bathyal and

188   abyssal samples yet absent or in low abundance in the intertidal or shelf (Miljutin et al., 2010; Miljutina et al.,

189   2010; Muthumbi et al., 2004). Similarly, the genus *Halomonhystera* was most abundant at the hydrothermal vent

190   and mud volcano samples (Van Gaever et al., 2006). The different environments or depth zones did not harbour

191   exclusive assemblages with over 60% of genera being shared, a pattern consistent with morphology (Vanreusel

192   et al., 2010). However, an increased number of samples from categories underrepresented in our analysis, such

193   as the intertidal depth zone and environments other than plains, could alter this result. Incongruences to

194   morphological investigations were also found. *Desmoscolex* for instance constituted 38% of the hydrothermal

195   vent reads while this genus represented merely 0.24% of the morphological assemblage in Vanreusel et al., 2010.

196   It may be that the real abundance of this genus is underestimated in morphological assessments in which, for

197   sake of feasibility, a maximum of 100-150 nematodes are identified rather than the entire community.

198   Alternatively, individuals from this genus may be carrying more copies of the 18S rRNA gene, leading to an

199   inflated number of reads despite low abundance in the sample (Porazinska et al., 2010). DNA-based genus

200   diversity estimates, which excluded unassigned ASVs, were lower than that based on morphology due to the

201   extremely diverse nature of the Nematoda which remains largely undescribed (Appeltans et al., 2012) and which

202   is consequently insufficiently represented by reference sequences in public databases; this manifested as a large

203   portion of Nematoda ASVs that were unassigned at the genus level in our analysis.

204   A global parabolic bathymetric trend was demonstrated for our DNA-based assessment of nematode

205   diversity with total number of Nematoda ASVs being overall lowest in the intertidal, increasing with depth into

206   the shelf, reaching a maximum in the bathyal and decreasing again in the abyssal zone (Figure 3). Such a non-

207   linear relationship has also been reported for morphological investigations of global nematode fauna (Boucher

208 & Lambshead, 1995). Specifically for the Mediterranean samples, DNA- and morphology-based assessments

209 produced similar shifts in diversity with Nematoda ASV richness decreasing steadily with depth to 2800 m rather

210 than peaking in the bathyal zone, likely due to the dramatic decrease in faunal abundance (Danovaro et al., 2010;

211 Ramirez-Llodra et al., 2010). Although the statistical analysis failed to find significant differences in Nematoda

212 ASV richness between depth zones, this is attributable to the large variation within the depth zones themselves,

213 which exceeded that between them. This was especially true for the bathyal in which all environment types and

214 most biogeographic regions were represented with highly varying Nematoda ASV richness, while only the plains

215 and sometimes just a single biogeographic region were included in the remaining depth zones. The bathyal

216 having the most ASV-rich but also some of the most ASV-poor samples can be explained by the confluence of

217 several factors. It included communities within the depth of oceanic oxygen minimum zones (OMZ, 200-1500 m)

218 which generally tend to have a suppressive effect on diversity, even though some nematode species can be very

219 tolerant of hypoxia (Gooday et al., 2000; Helly & Levin, 2004). Concurrently, it also included samples beyond the

220 OMZ and at depths shallow enough for food to not be a limiting factor as it is in the abyss (Smith et al., 2008).

221 Finally, modelling has shown that unlike shallow and abyssal fauna, the bathyal has been spared from extinctions

222 due to past cycles of hypoxia, anoxia and oxygenation (Rogers, 2000). In combination, these attributes generated

223 a highly diverse bathyal deep-sea setting beyond the OMZ.

224 Evolutionary distinctiveness was highest in the intertidal and abyssal samples and lowest in the bathyal

225 and shelf samples. Concurrently, the vast majority of Nematoda ASVs were found in just one depth zone; thus the

226 levels of ED in each zone were due to its unique assemblage. Although the intertidal was underrepresented in

227 this analysis, it is likely that this fluctuating, dynamic environment represents a rich niche landscape to which

228 taxa must specialize to, thus leading to communities composed of highly distinct taxa. Similar levels of ED in the

229 abyssal zone can be explained by the fact that of the four zones, the abyssal represents one of the oldest living

230 spaces and thus a reservoir of accumulated evolution, even if evolution may be decelerated at such great depths

231 (Bik et al., 2012; Rex, McClain, et al., 2005). Moreover, migration of shallow-water taxa to the abyss and vice versa

232 would have also contributed high levels of ED in both zones because the addition of an evolutionarily distinct

233 taxon would result in raising total ED (Bik et al., 2010; Rogers, 2000). The shelf and bathyal zones which are

234 bordered by very shallow and very deep parts of the marine environment, respectively, exhibited comparable

235 lower levels of ED despite the bathyal being the most diverse of all depth categories. These two zones shared the

236 most Nematoda ASVs, with up to 10% of the total shelf diversity shared with the bathyal, demonstrating these to

237   be more closely linked than any other depth zones and less evolutionarily distinct than the intertidal or abyssal.

238   This cannot be attributed to a sample size effect given that the abyssal zone had more Nematoda ASVs than the

239   shelf yet fewer of them were shared with the bathyal (ESM Figure 2).

240   4.2   Mechanisms of community assembly

241   Phylogenetic clustering, i.e. assemblages exhibiting relatedness that is higher than expected at random,

242   was the most prevalent mode of community structuring in the investigated environments. This was especially

243   true for PD and MNTD, which have been shown to provide similar information and are influenced by species

244   richness and assemblage structure in a similar manner (Mazel et al., 2016). Phylogenetic clustering can be

245   attributed to (i) environmental filtering or (ii) competitive exclusion of conserved traits (Mayfield & Levine,

246   2010; Webb et al., 2002). Soft sediment communities in general are assumed to have low rates of competitive

247   exclusion and mortality due to the fact that competitive interactions are relatively uncommon (Wilson, 1990).

248   This holds true also for the deep sea in particular from which most samples of our meta-analysis were derived

249   from. In this setting, competition is likely centred around food availability (Smith et al., 2008), however, it is

250   considered to be relatively weak in structuring the local assemblages (Grant, 2000; Mcclain & Schlacher, 2015).

251   Thus, phylogenetic clustering in this meta-analysis was most likely due to environmental filtering of conserved

252   traits, i.e. the environment poses the strongest selective force, and the taxa that persist are those which are able

253   to tolerate the local conditions. Assuming that ecological similarity is linked to phylogenetic relatedness (Losos,

254   2008; Webb, 2000), the resultant assemblage is composed of ecologically similar taxa that exhibit a higher

255   relatedness than that expected at random. Although this aspect of deep-sea communities has seldom been

256   investigated, environmental filtering has been documented in macroinvertebrates (Judge & Barry, 2016),

257   octocorals (Quattrini et al., 2017) and nematodes (Macheriotou et al., 2020, 2021); our results show that this

258   seems to be a common feature of assemblages inhabiting different soft sediment environments irrespective of

259   depth (Fais et al., 2020; Hauquier et al., 2018).

260   Deviations from clustering were found in all environments except plains for Mean Pairwise Distance

261   (MPD) and these can be interpreted with regard to the attributes of the metrics themselves. Specifically, MPD is

262   characterised as "basal" in that it is most sensitive to deep rooting of a phylogenetic tree and thus more relevant

263   at large evolutionary scales. Contrastingly, Phylogenetic Diversity (PD) and Mean Nearest Taxon Distance

264   (MNTD) are "terminal" metrics influenced mainly by the branching structure closer to the tips of tree, and thus

265   relevant at a finer evolutionary scale (Mazel et al., 2016). The canyon and mud volcano samples were

overdispersed while hydrothermal vents and seamounts were randomly structured. Overdispersion is the result of competitive exclusion of conserved traits and/or environmental filtering of convergent traits (Webb et al., 2002). Convergent evolution has been used to describe similar traits at various levels of relatedness, even within the same genus (Blackledge & Gillespie, 2004). This major process in the development of life lacks a single widely-applied definition, and distinguishing it from parallel evolution is a challenge, as the degree of relatedness separating the two remains arbitrary (Moore & Willmer, 1997; Stayton, 2015). Taking a conservative approach, we estimate intra-phylum relatedness to exceed that which describes convergent traits and thus consider competitive exclusion of conserved traits at distant evolutionary timescales as the likely explanation for phylogenetic overdispersion in the canyon and mud volcano samples. This coincides with the "stability-time" hypothesis in which the modern co-existence of deep-sea species is a reflection of competitive interactions of the past, provided the environment has remained sufficiently stable to allow fine niche partitioning (Mcclain & Schlacher, 2015; R. Hessler & L. Sanders, 1967). Concurrently, we note that the canyon and mud volcano had the lowest sample size; thus the patterns reported herein may be liable to change given additional data.

Random phylogenetic structure results from competitive exclusion of convergent traits, community assembly being unaffected by phylogeny, opposing and nullifying mechanisms and/or species-neutral interactions (Helmus et al., 2007; Hubbel, 2001; Kembel & Hubbell, 2006; Webb et al., 2002). Given that relatedness increases at large evolutionary timescales toward the base of a phylogenetic tree and the basal nature of MPD, we exclude competitive exclusion of convergent traits as an explanation for randomness in the hydrothermal vent and seamount samples. Furthermore, we expect that if phylogeny was ineffectual toward community assembly this would manifest also in PD and/or MNTD and is thus less likely to have caused the observed randomness. Beyond this, (dis)confirming one mechanism for another is challenging with the data at hand. It has been shown that environmental filtering and competitive exclusion can act simultaneously to obscure a phylogenetic signal (Helmus et al., 2007) and that neutral forces could be dominant at areas seepage (Macheriotou et al., 2021). Thus, we expect that either or both mechanisms are contributing to random phylogenetic structure in the hydrothermal vent and seamount samples. Overall, phylogenetic clustering in PD and MNTD suggests that contemporary assemblages are predominantly shaped by environmental filtering of conserved traits, while competitive interactions with conspecifics are of lesser importance. The corollary that follows is one that places a vital importance to the maintenance of the environmental conditions that structure these assemblages to prevent the risk of potentially altering them irreversibly. At a time when most, if not all,

295 marine ecosystems are faced with continuous and unsustainable exploitation of their resources, the protection

296 and preservation of habitats is paramount to prevent permanent loss of diversity.

5   Acknowledgements

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520

521 7   Data accessibility statement

522   The data that support the findings of this study are openly available at the repositories listed in ESM Table

523 1.

524 8   Author contributions

525   LM compiled, analysed, visualised and interpreted the data and wrote the manuscript. SD and AV

526 provided analytical advice as well critical comments throughout.

527   9   Tables and Figures

Figure 1: Map with sampling locations and references used in this study; numbers correspond to biogeographic realms based on species endemicity as listed in Figure 1 of (Costello et al., 2017).

A

Taxon	Intertidal	Shelf	Bathyal	Abyssal
Chromadorita	18.67	29.41	18.21	6.04
Halalaimus	2.59	6.08	17.25	26.65
Desmoscolex	0	1.06	25.45	9.03
Acantholaimus	0	0.11	5.76	28.07
Sabatieria	10.28	25.51	4.81	0.03
Phanodermopsis	0	0.06	3.71	8.01
Syringolaimus	0.02	0	4.32	2.08
Daptonema	8.72	0.62	2.24	1.31
Oncholaimus	35.27	0	0	0
Theristus	0.13	0.01	0.01	8.38
Viscosia	1.61	3.44	1.73	0.47
Molgolaimus	0.9	3.56	1.63	0.11
Bathylaimus	0.35	8.39	0.24	0
Anticoma	0.21	3.86	0.63	1.39
Halomonhystera	0	0.21	1.81	0.19
Leptolaimus	1.6	0.69	1.25	0.06
Oxystomina	0	0.3	0.55	2.29
Cyatholaimus	0.26	5	0.11	0.05
Manganonema	0	0.04	1.37	0.32
Deontolaimus	0.17	0.04	1.24	0.48
Cylindrolaimus	0	0	1.26	0.08
Diplolaimelloides	0	0	1	0.1
Setosabatieria	0.07	1.66	0.49	0
Subsphaerolaimus	0	0.35	0.63	0.42
Bathyeurystomina	0	0	0.22	1.39
Calomicrolaimus	0.06	0.86	0.19	0.53
Xyzzors	0	2.27	0	0
Desmolaimus	0.59	0.44	0.37	0.09
Atrochromadora	5.17	0	0.02	0
Halichoanolaimus	1.13	0.36	0.31	0.1
Sphaerolaimus	0	0.01	0.49	0.1
Remaining taxa (50)	12.22	5.65	2.69	2.21

B

Taxon	Canyon	HydrothermalVent	MudVolcano	Plain	Seamount
Chromadorita	41.1	6.3	1.01	19.94	15.54
Halalaimus	20.82	12.01	32.18	16.96	14.48
Desmoscolex	28.17	38.12	40.86	6.3	24.56
Acantholaimus	4.42	5.87	9.8	12.03	6.8
Sabatieria	0	0.42	1.79	9.63	6.16
Phanodermopsis	0	0.08	0	4.82	5.74
Syringolaimus	0.65	10.03	0	1.73	3.19
Daptonema	0.18	0.56	0.53	2.6	2.64
Oncholaimus	0	0	0	3.53	0
Theristus	0	0	0	3.38	0
Viscosia	1.49	3.8	0.26	1.57	1.13
Molgolaimus	0	4.73	0.14	1.41	0.4
Bathylaimus	0	0	0	2.35	0
Anticoma	0	0.93	0	1.59	1.09
Halomonhystera	0.03	3.68	5.43	0.19	0.95
Leptolaimus	0	1.39	1.21	0.65	1.44
Oxystomina	0.55	0.13	0	1.26	0.58
Cyatholaimus	0	0.34	0.06	1.3	0.05
Manganonema	0	2.01	0	0.26	2.38
Deontolaimus	0.5	1.52	1.6	0.36	1.62
Cylindrolaimus	0.93	0.13	1.71	0.15	2.57
Diplolaimelloides	0	0.48	0.46	0.05	2.5
Setosabatieria	0	1.31	0	0.42	0.6
Subsphaerolaimus	0	0.92	0.03	0.37	0.9
Bathyeurystomina	0	0.79	0	0.6	0
Calomicrolaimus	0	0.37	0.6	0.45	0.05
Xyzzors	0	0	0	0.57	0
Desmolaimus	0.42	0.22	0.41	0.3	0.46
Atrochromadora	0	0.1	0	0.52	0
Halichoanolaimus	0	0.14	0.5	0.31	0.45
Sphaerolaimus	0.12	0.27	0.23	0.3	0.31
Remaining taxa (50)	0.62	3.35	1.18	4.1	3.38

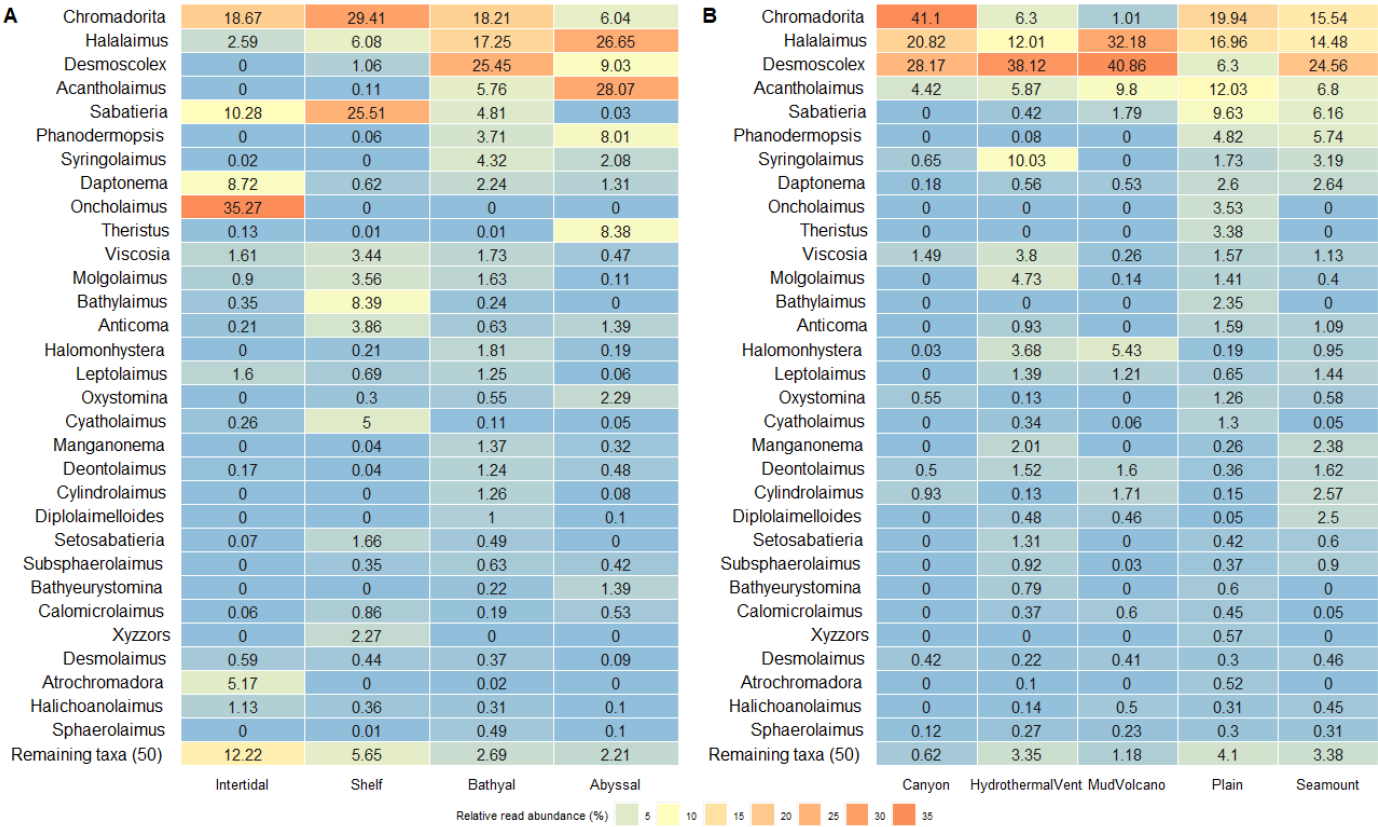Relative read abundance (%) 5 10 15 20 25 30 35

532
533 Figure 2: Taxonomic assignment and relative abundance of reads across the different depth categories (A) and
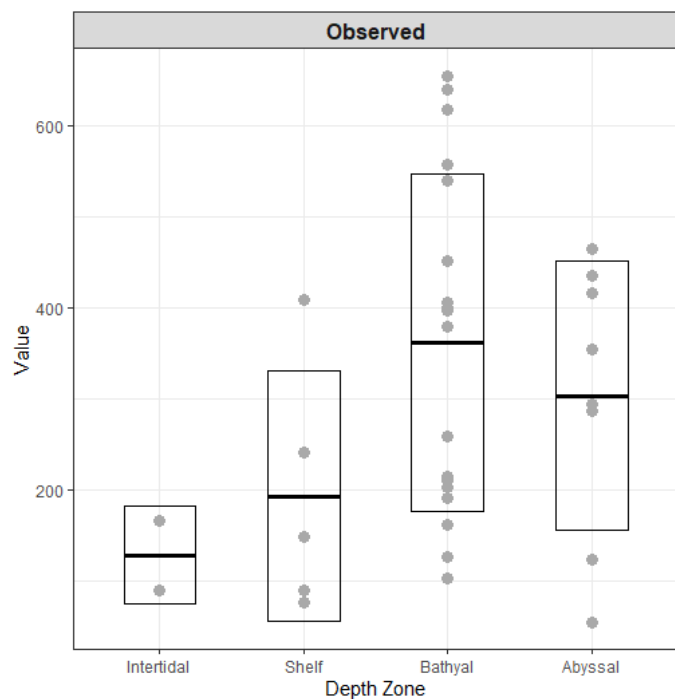534 environments (B). Note: Columns sum to 100% in these plots.

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Figure 3: Number of observed Nematoda ASVs (Y-axis) in each depth category (intertidal, shelf, bathyal, abyssal). Gray circles, horizontal bar and extent of the box indicate the individual data points, mean and standard deviation, respectively.
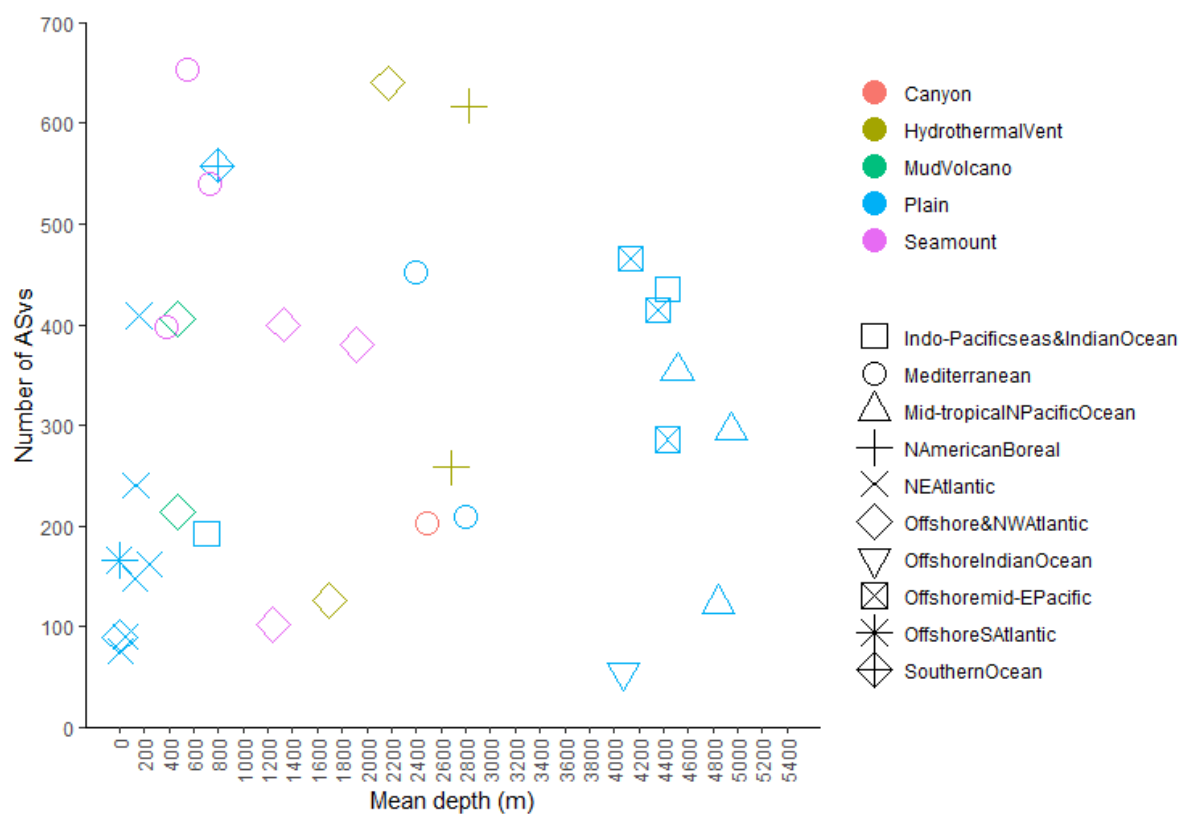
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Figure 4: Number of Nematoda ASVs as a function of depth; colour and shape represents different environments and biogeographic realms, respectively.
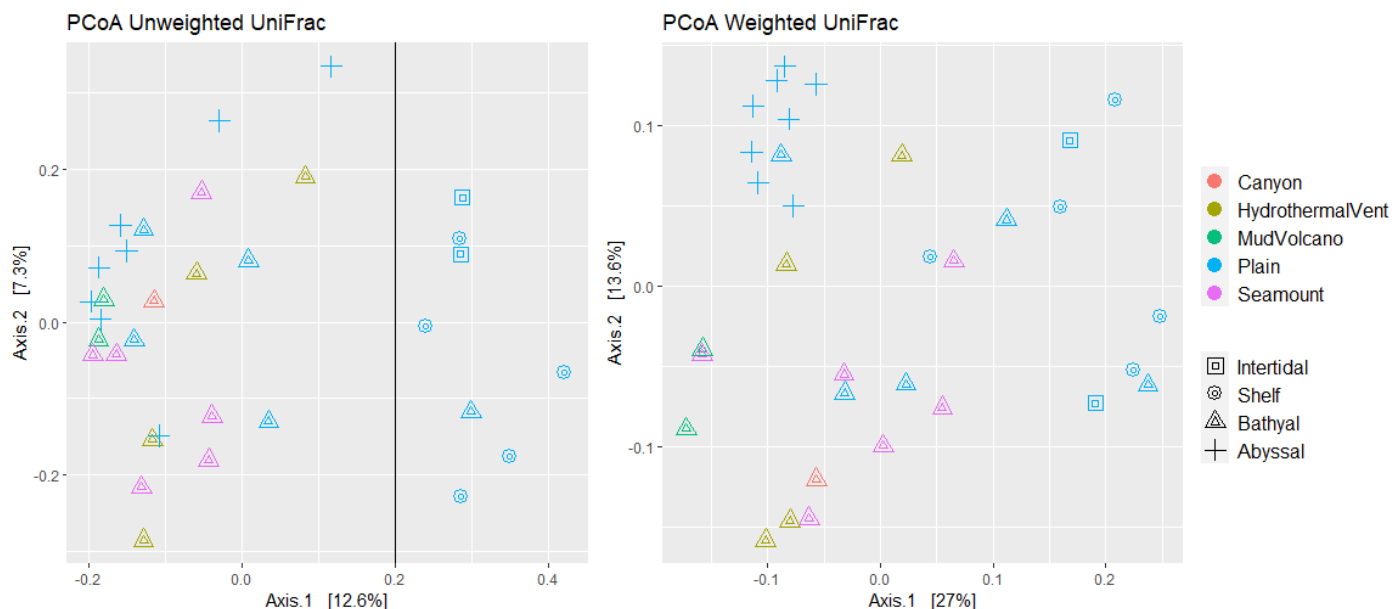
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Figure 5: Principal Coordinates Analysis (PCoA) based on unweighted (left) and weighted (right) Unifrac
distances for all nematode ASVs from the different environments (colours) and depth zones (shapes). Red:
canyon, yellow: hydrothermal vent, green: mud volcano, blue: plain, pink: seamount. Squares: intertidal, circles:
shelf, triangle: bathyal, cross: abyssal; vertical line indicates separation between shallow and deep-sea samples.

550
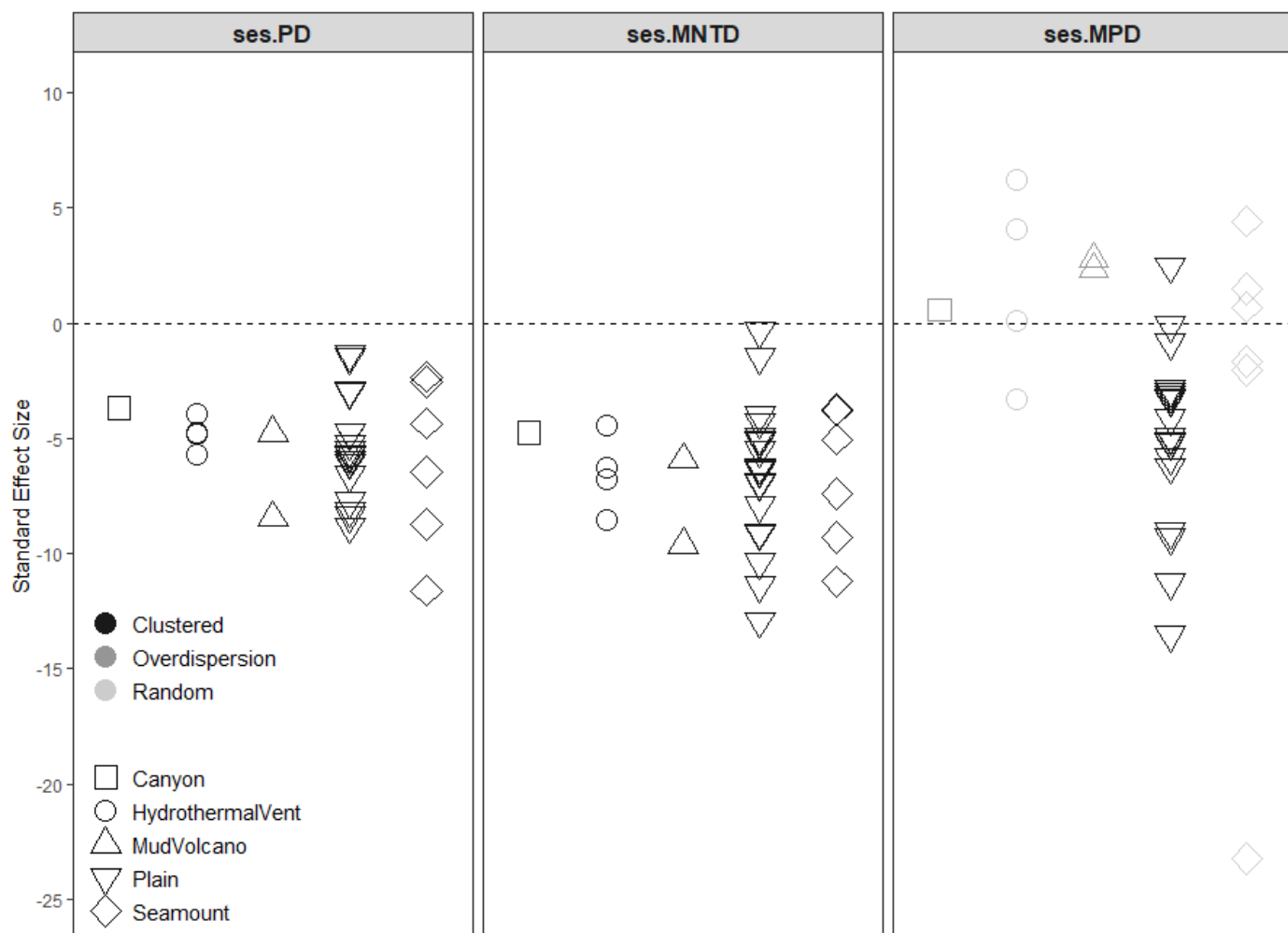
Figure 6: Standard effect size (ses) of phylogenetic diversity (ses.PD), mean nearest taxon distance (ses.MNTD) and mean pairwise distance (ses.MPD) of ASVs in the canyon (square), hydrothermal vent (circle), mud volcano (upward triangle), plain (downward triangle), and seamount (diamond) samples. Gray scale represents outcome of the relevant t-test: black, dark grey and light grey indicate statistically significant clustering, overdispersion and random phylogenetic structure, respectively.

558 Table 1: Name, biogeographic realm, environment, depth zone, mean depth (m) decimal latitude and longitude
559 for the 33 samples used in this study.

Sample name	Realm	Environment	Depth zone	Depth	Latitude	Longitude
Realm3.Sample1.Plain	NEAtlantic	Plain	Shelf	53	58.270	11.440
Realm3.Sample2.Plain	NEAtlantic	Plain	Shelf	7	58.330	11.200
Realm18.Sample3.HydrothermalVent	Offshore&NWAtlantic	Hydrothermal Vent	Bathyal	2170	36.232	-33.874
Realm3.Sample4.Plain	NEAtlantic	Plain	Bathyal	243.5	67.532	15.387
Realm3.Sample6.Plain	NEAtlantic	Plain	Shelf	133	62.137	5.331
Realm3.Sample7.Plain	NEAtlantic	Plain	Shelf	158.8	62.805	6.975
Realm3.Sample8.Plain	NEAtlantic	Plain	Shelf	125.6	62.779	6.930
Realm5.Sample9.Canyon	Mediterranean	Canyon	Bathyal	2490	42.717	6.133
Realm5.Sample10.Plain	Mediterranean	Plain	Bathyal	2400	42.942	6.742
Realm5.Sample11.Seamount	Mediterranean	Seamount	Bathyal	729	36.481	-2.895
Realm5.Sample12.Seamount	Mediterranean	Seamount	Bathyal	381	36.546	-2.814
Realm5.Sample13.Seamount	Mediterranean	Seamount	Bathyal	554	36.516	-2.794
Realm5.Sample14.Plain	Mediterranean	Plain	Bathyal	2800	37.947	2.917
Realm8.Sample15.HydrothermalVent	NAmericanBoreal	Hydrothermal Vent	Bathyal	2683	73.464	7.198
Realm8.Sample17.HydrothermalVent	NAmericanBoreal	Hydrothermal Vent	Bathyal	2826	73.460	7.218
Realm18.Sample18.Seamount	Offshore&NWAtlantic	Seamount	Bathyal	1325	37.340	-24.755
Realm18.Sample19.MudVolcano	Offshore&NWAtlantic	Mud Volcano	Bathyal	470	36.560	-6.949
Realm18.Sample20.MudVolcano	Offshore&NWAtlantic	Mud Volcano	Bathyal	470	36.561	-6.950
Realm18.Sample21.Seamount	Offshore&NWAtlantic	Seamount	Bathyal	1920	36.844	-11.303
Realm18.Sample22.Seamount	Offshore&NWAtlantic	Seamount	Bathyal	1245	37.284	-24.787
Realm18.Sample23.HydrothermalVent	Offshore&NWAtlantic	Hydrothermal Vent	Bathyal	1700	37.170	-32.160
Realm21.Sample24.Plain	OffshoreSAtlantic	Plain	Intertidal	0	-23.817	-45.400
Realm30.Sample46.Plain	SouthernOcean	Plain	Bathyal	790	-63.711	-57.735
Realm9.Sample47.Plain	Mid-tropicalNPacificOcean	Plain	Abyssal	4513	13.850	-123.250
Realm9.Sample48.Plain	Mid-tropicalNPacificOcean	Plain	Abyssal	4949	14.050	-130.133
Realm9.Sample49.Plain	Mid-tropicalNPacificOcean	Plain	Abyssal	4839	18.783	-128.350
Realm22.Sample50.Plain	Offshoremid-EPacific	Plain	Abyssal	4132	11.833	-117.050
Realm22.Sample51.Plain	Offshoremid-EPacific	Plain	Abyssal	4351	11.817	-117.533
Realm22.Sample52.Plain	Offshoremid-EPacific	Plain	Abyssal	4437	11.067	-119.650
Realm13.Sample53.Plain	Indo-Pacificseas&IndianOcean	Plain	Abyssal	4437	-15.403	45.905
Realm13.Sample54.Plain	Indo-Pacificseas&IndianOcean	Plain	Bathyal	704.25	-21.525	41.604
Realm19.Sample55.Plain	OffshoreIndianOcean	Plain	Abyssal	4076	-25.427	41.595
Realm18.Sample57.Plain	Offshore&NWAtlantic	Plain	Intertidal	0	42.162	-9.322

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561   10   Supplementary material

562   ESM Table 1: Reference citation, data repository and accession code for the datasets included in this study. SRA:
563   Sequence Read Archive, NA: Not available.

	Reference citation	Repository	Accession
1	Bik, Holly M., et al. "Metagenetic Community Analysis of Microbial Eukaryotes Illuminates Biogeographic Patterns in Deep-Sea and Shallow Water Sediments." *Molecular Ecology*, vol. 21, no. 5, 2012, pp. 1048–59, doi:10.1111/j.1365-294X.2011.05297.x	Dryad	dryad.vd094
2	Brandt, Miriam I., et al. "Bioinformatic Pipelines Combining Denoising and Clustering Tools Allow for More Comprehensive Prokaryotic and Eukaryotic Metabarcoding." *Molecular Ecology Resources*, no. December 2020, 2021, pp. 1–18, doi:10.1111/1755-0998.13398	SRA	PRJEB33873
3	Cowart, Dominique A., Marjolaine Matabos, et al. "Exploring Environmental DNA (EDNA) to Assess Biodiversity of Hard Substratum Faunal Communities on the Lucky Strike Vent Field (Mid-Atlantic Ridge) and Investigate Recolonization Dynamics After an Induced Disturbance." *Frontiers in Marine Science*, vol. 6, no. January, 2020, pp. 1–21, doi:10.3389/fmars.2019.00783	SRA	PRJNA540908
4	Cowart, Dominique A., Miguel Pinheiro, et al. "Metabarcoding Is Powerful yet Still Blind: A Comparative Analysis of Morphological and Molecular Surveys of Seagrass Communities." *PLoS ONE*, vol. 10, no. 2, 2015, pp. 1–26, doi:10.1371/journal.pone.0117562	NA	Private comm.
5	Fais, Maria, et al. "Small-Scale Spatial Variation of Meiofaunal Communities in Lima Estuary (NW Portugal) Assessed through Metabarcoding." *Estuarine, Coastal and Shelf Science*, vol. 238, no. March, Elsevier Ltd, 2020, p. 106683, doi:10.1016/j.ecss.2020.106683	SRA	PRJNA611064
6	Faria, Laiza Cabral de, et al. "The Use of Metabarcoding for Meiofauna Ecological Patterns Assessment." *Marine Environmental Research*, vol. 140, no. June, Elsevier, 2018, pp. 160–68, doi:10.1016/j.marenvres.2018.06.013	NA	Private comm.
7	Fonseca, V. G., et al. "Revealing Higher than Expected Meiofaunal Diversity in Antarctic Sediments: A Metabarcoding Approach." *Scientific Reports*, vol. 7, no. 1, Springer US, 2017, pp. 1–11, doi:10.1038/s41598-017-06687-x	NA	Private comm.
8	Haenel, Quiterie, et al. "NGS-Based Biodiversity and Community Structure Analysis of Meiofaunal Eukaryotes in Shell Sand from Hållö Island, Smögen, and Soft Mud from Gullmarn Fjord, Sweden." *Biodiversity Data Journal*, vol. 5, 2017, p. e12731, doi:10.3897/BDJ.5.e12731	SRA	PRJNA388326
9	Klunder, Lise, et al. "A Molecular Approach to Explore the Background Benthic Fauna Around a Hydrothermal Vent and Their Larvae: Implications for Future Mining of Deep-Sea SMS Deposits." *Frontiers in Marine Science*, vol. 7, no. March, 2020, pp. 1–12, doi:10.3389/fmars.2020.00134	SRA	PRJEB36829
10	Macheriotou, Lara, Annelien Rigaux, Karine Olu, et al. "Deep-Sea Nematodes of the Mozambique Channel : Evidence of Random Community Assembly Dynamics in Seep Sediments." *Frontiers in Marine Science*, vol. 8, 2021, pp. 1–15, doi:10.3389/fmars.2021.549834	SRA	PRJNA623689
11	Macheriotou, Lara, Annelien Rigaux, Sofie Derycke, et al. "Phylogenetic Clustering and Rarity Imply Risk of Local Species Extinction in Prospective Deep-Sea Mining Areas of the Clarion-Clipperton Fracture Zone." *Proceedings of the Royal Society B: Biological Sciences*, vol. 287, 2020	SRA	PRJNA544999
12	Sinniger, Frédéric, et al. "Worldwide Analysis of Sedimentary DNA Reveals Major Gaps in Taxonomic Knowledge of Deep-Sea Benthos." *Frontiers in Marine Science*, vol. 3, no. June, 2016, pp. 1–14, doi:10.3389/fmars.2016.00092	SRA	PRJEB13170
13	Pantó, Gabriella, et al. "Combining Traditional Taxonomy and Metabarcoding: Assemblage Structure of Nematodes in the Shelf Sediments of the Eastern Antarctic Peninsula." *Frontiers in Marine Science*, vol. 8, no. July, 2021, pp. 1–19, doi:10.3389/fmars.2021.629706	Dryad	dryad.n8pk0p2tr
14	Unpublished	SRA	PRJEB23641

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566 ESM Table 2: Genus-level taxonomic assignment of
567 ASVs.

Genus	# ASVs
NA	4318
Desmoscolex	1446
Halalaimus	617
Acantholaimus	524
Chromadorita	169
Deontolaimus	119
Sabatieria	96
Molgolaimus	92
Cylindrolaimus	82
Viscosia	70
Desmolaimus	68
Leptolaimus	65
Syringolaimus	58
Daptonema	54
Subsphaerolaimus	52
Oxystomina	47
Manganonema	46
Phanodermopsis	45
Halichoanolaimus	44
Halomonhystera	40
Diplolaimelloides	38
Anticoma	33
Thalassoalaimus	30
Sphaerolaimus	27
Calomicrolaimus	26
Doliolaimus	26
Rhabdocoma	22
Cyatholaimus	20
Odontophora	18
Aegialoalaimus	15
Bathyeurystomina	15
Ceramonema	15
Bathylaimus	13
Theristus	13
Alaimella	11
Epacanthion	10
Punctodora	10
Astomonema	9
Diplopeltula	9

Litinium	9
Spirinia	9
Hirschmanniella	7
Pareurystomina	7
Setosabatieria	7
Eumonhystera	6
Onchium	6
Paracyatholaimus	6
Chromadorina	5
Paramphidelus	5
Ascolaimus	4
Trissonchulus	4
Zygonemella	4
Atrochromadora	3
Enoploides	3
Enoplolaimus	3
Setostephanolaimus	3
Spilophorella	3
Symplocostoma	3
Tripyloides	3
Anoplostoma	2
Axonolaimus	2
Dolicholaimus	2
Domorganus	2
Leptonemella	2
Metoncholaimus	2
Oncholaimus	2
Pseudocella	2
Siphonolaimus	2
Tarvaia	2
Terschellingia	2
Trefusia	2
Wieseria	2
Amphimonhystrella	1
Calyptronema	1
Chaetonema	1
Chromadoropsis	1
Linhystera	1
Paramphimonhystrella	1
Pellioditis	1
Ptycholaimellus	1
Thalassomonhystera	1
Xyzzors	1

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570   ESM Table 3: Standard effect size for PD, MNTD, MPD for the canyon, hydrothermal vent, mud
571   volcano, plain and seamount samples. Statistically significant values indicated in bold (α=0.05).

	Metric	Shapiro	t.test	SW
Canyon	ses.MNTD			
Canyon	ses.MPD			
Canyon	ses.PD			
HydrothermalVent	ses.MNTD	0.945	**0.005**	
HydrothermalVent	ses.MPD	0.828	0.466	
HydrothermalVent	ses.PD	0.839	**0.001**	
MudVolcano	ses.MNTD			
MudVolcano	ses.MPD			
MudVolcano	ses.PD			
Plain	ses.MNTD	0.873	**0.000**	
Plain	ses.MPD	0.426	**0.000**	
Plain	ses.PD	0.349	**0.000**	
Seamount	ses.MNTD	0.398	**0.003**	
Seamount	ses.MPD	**0.010**		0.688
Seamount	ses.PD	0.540	**0.010**	

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574 ESM Table 4: Pairwise post-hoc p-values adjusted with the Benjamini-Hochberg method for
575 evolutionary distinctiveness between depth zones. Statistically significant values indicated in
576 bold (α=0.05).

| Comparison	p-value
Abyssal - Bathyal	**0.0147**
Abyssal - Intertidal	0.1598
Bathyal - Intertidal	**0.0151**
Abyssal - Shelf	**0.0035**
Bathyal - Shelf	0.0549
Intertidal - Shelf	**0.0025**

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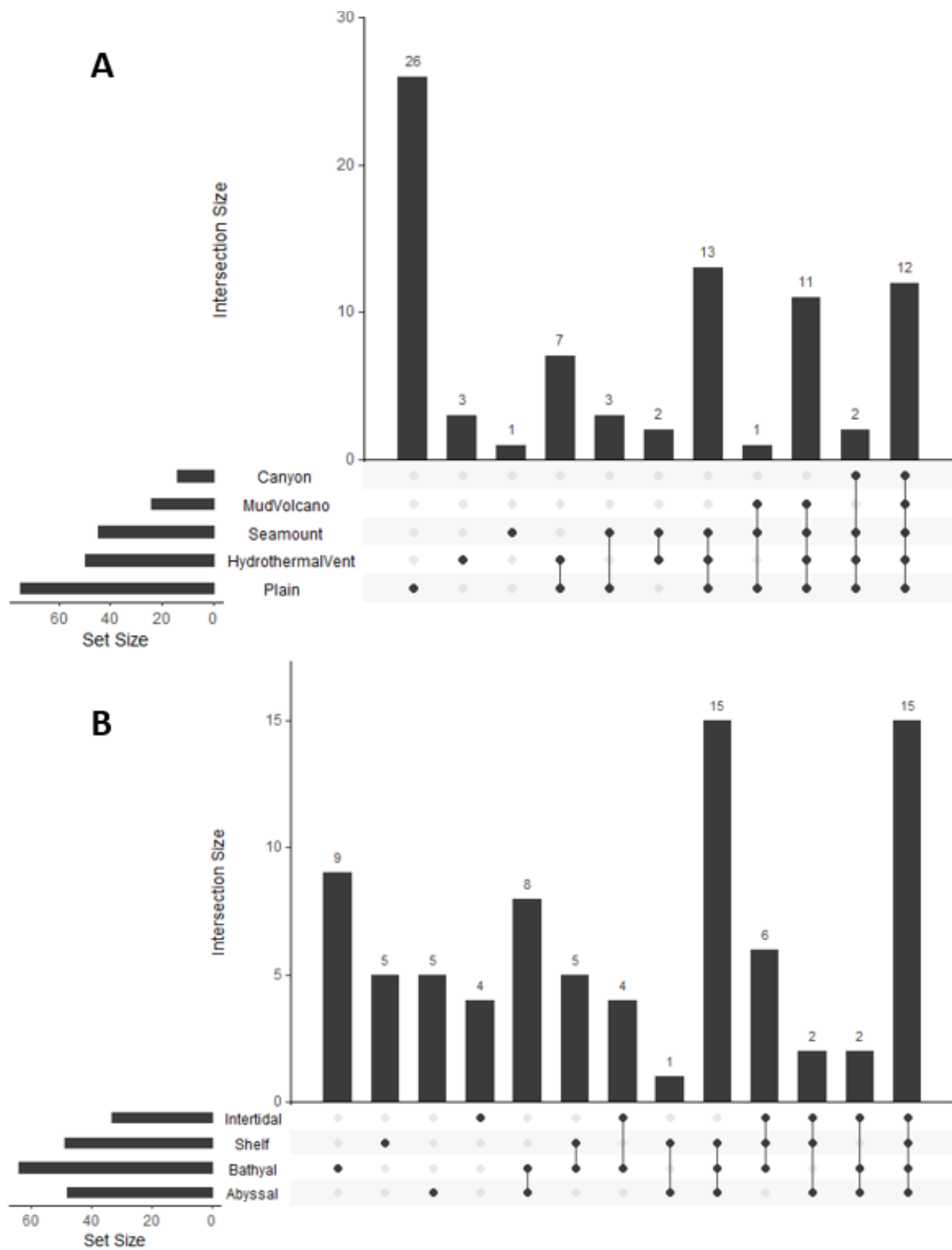
581 ESM Figure 1: Shared and unique nematode genera by environment type (A) and depth zone (B).

582
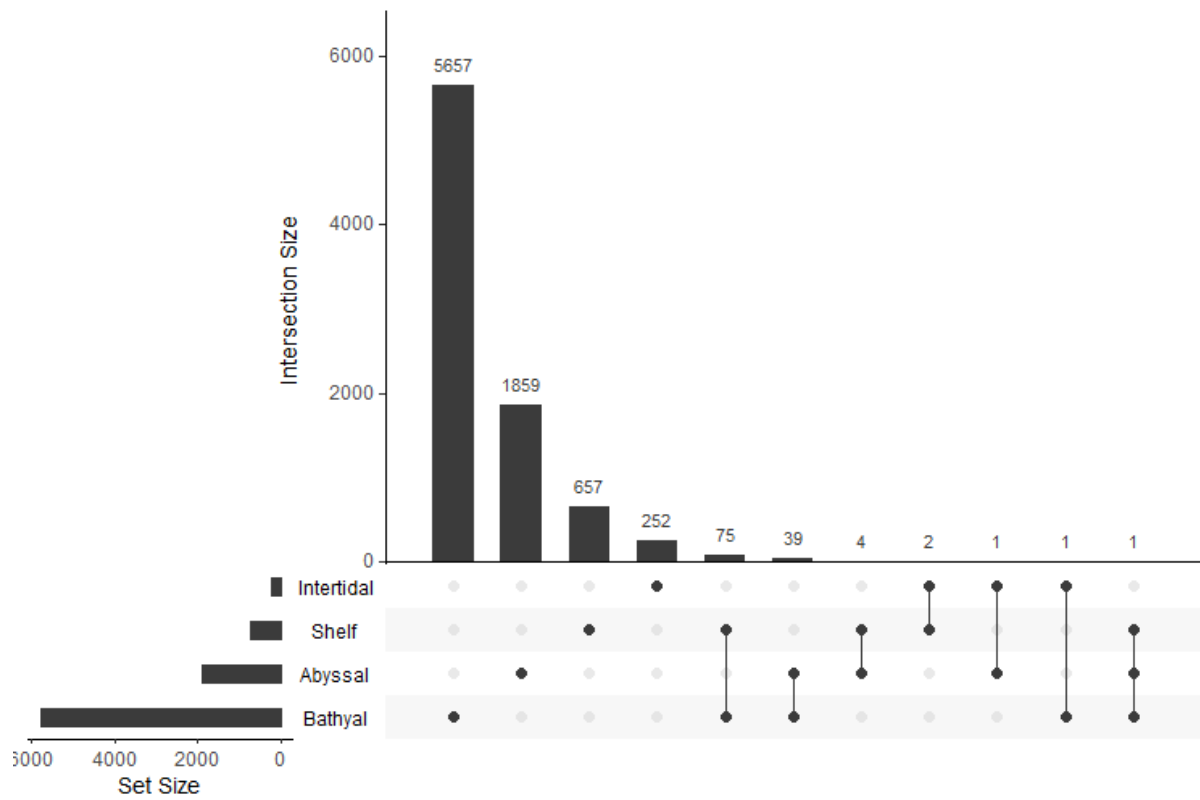
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584
585   ESM Figure 2: Unique and shared nematode ASVs by depth zone.

586
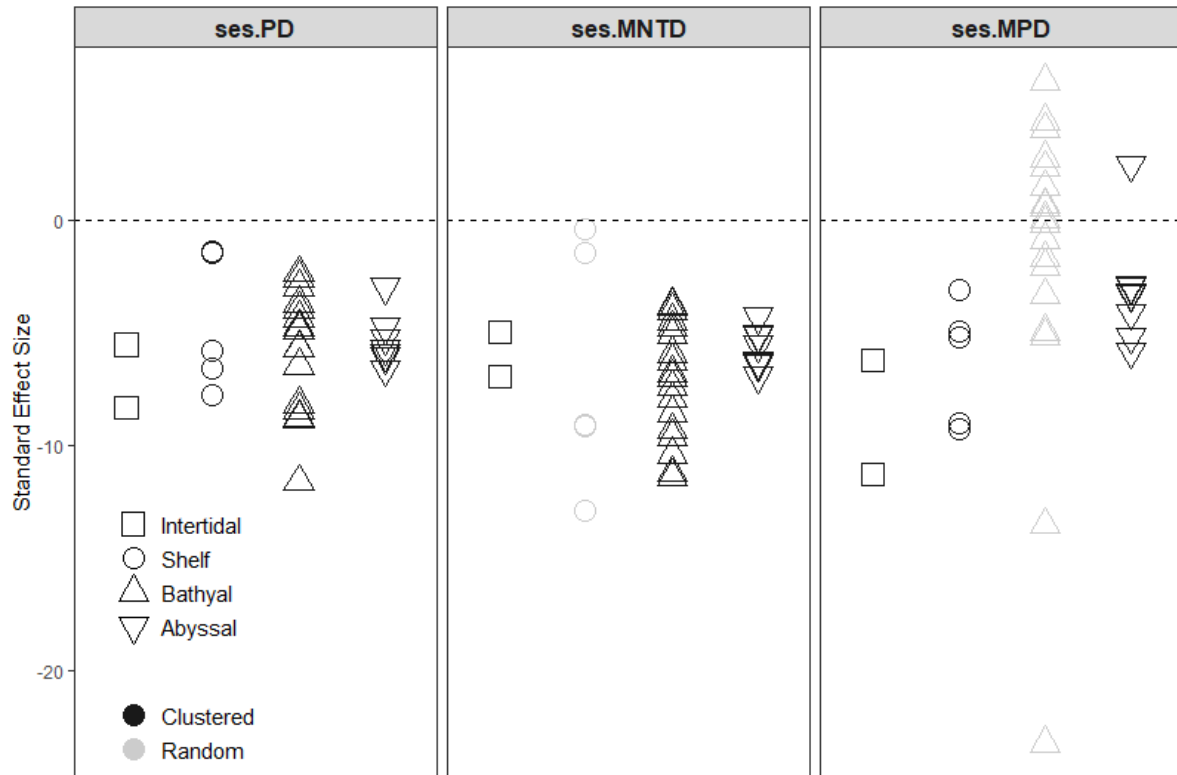
ESM Figure 3: Standard effect size (ses) of phylogenetic diversity (ses.PD), mean nearest taxon distance (ses.MNTD) and mean pairwise distance (ses.MPD) of ASVs in the intertidal (square), shelf (circle), bathyal (upward triangle) and abyssal (downward triangle) samples. Colour represents outcome of the relevant t-test: black and grey indicate statistically significant clustering and random phylogenetic structure, respectively.

595
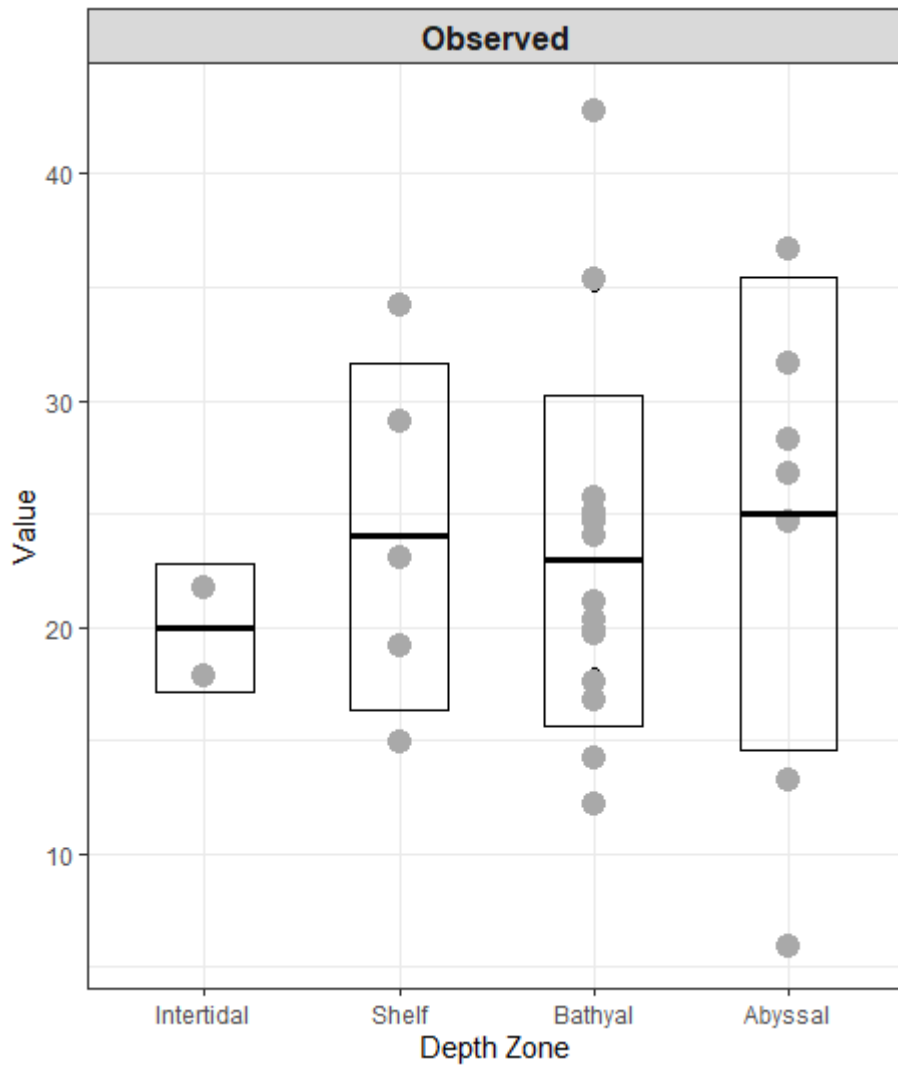
ESM Figure 4: Number of nematode genera (Y-axis) in each depth category (intertidal, shelf, bathyal, abyssal). Gray circles, horizontal bar and extent of the box indicate the individual data points, mean and standard deviation, respectively.
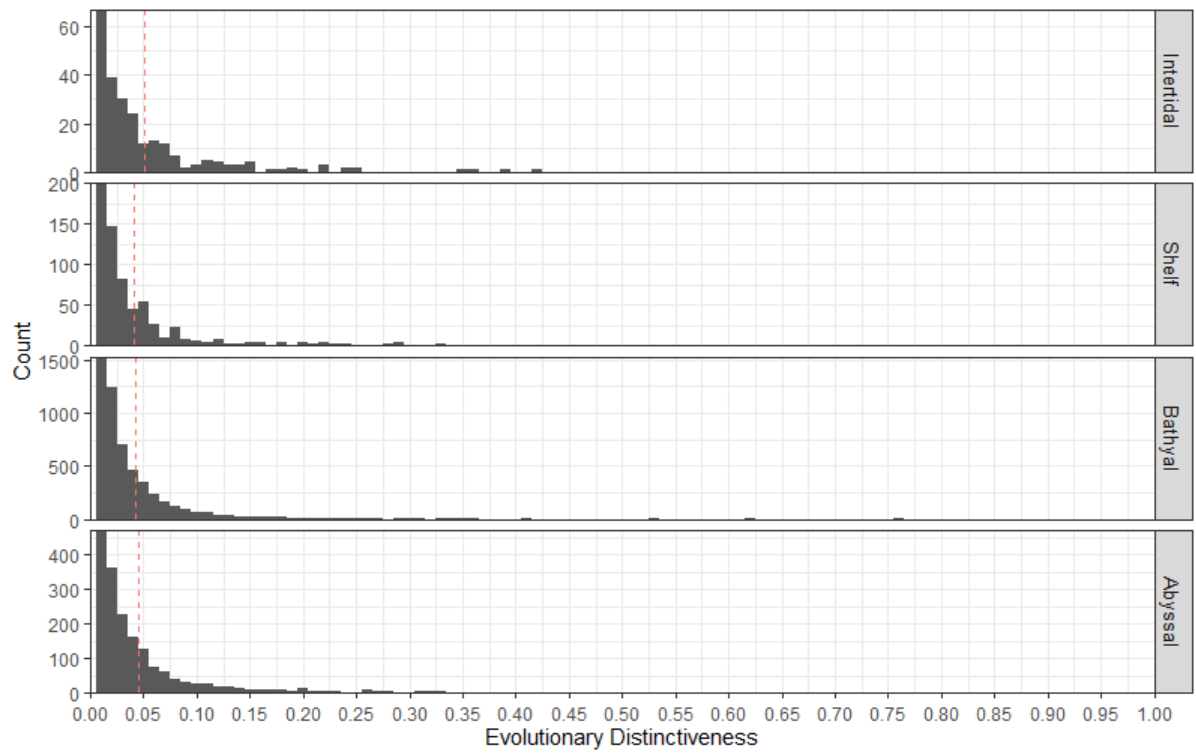
600
601 ESM Figure 5: Histogram of evolutionary distinctiveness (ED) values across depth zones in the
602 intertidal, shelf, bathyal and abyssal samples. Dashed red line indicates mean ED.