



Nematode diversity patterns at different spatial scales in bathyal sediments of the Mediterranean Sea

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Abstract. Understanding biodiversity patterns and how they are driven at different spatial scales is a crucial issue in ecological studies. This is particularly evident for the deep sea, the largest biome of the biosphere, where information on the scales of spatial variation is very scant. Here, we investigated deep-sea nematodes species richness, turnover and functional diversity, and life strategies at different spatial scales (from local to macro-regional) to identify the factors that shape regional (γ) and macro-regional (ε) deep-sea diversity. This study was conducted in several deep-sea habitats (canyons, open slopes, deep-water corals, and bathyal plains) over > 2000 km across the whole Mediterranean Basin, at a bathymetric range comprised between ca. 600 and 1300 m. Our results indicate that the patterns of local (α) diversity across the deep Mediterranean follow the gradients of the trophic conditions, which decrease from the western to the eastern basins. For all of the sites and habitats, the α diversity is generally low. Conversely, the turnover diversity changes significantly among habitats (β diversity) and between regions (δ diversity), showing values of dissimilarity (based on species presence/absence matrixes) between 59 and 90 % for β diversity and between 81 and 89 % for δ diversity. This suggests that patterns and values of γ and ε diversities in the deep Mediterranean Sea are related to turnover diversity among habitats and between regions (β and δ diversities), rather than to the local biodiversity (α diversity). These results indicate also that the differences in β and δ diversities are even more important than those in α diversity for the comprehension of the drivers of biodiversity in the deep Mediterranean Sea. We conclude that the presence of different habitats and gradients in environmental conditions, by promoting a high turnover diversity across the Mediterranean

Sea, may play a crucial role in the levels of γ diversity of deep-sea nematodes.

1 Introduction

A comprehensive understanding of the patterns of biodiversity requires the identification of the drivers that generate these patterns and of the biodiversity components that respond to these drivers. High values of regional diversity can arise from a combination of local and turnover diversities, or they can be mostly driven by one single component (Loreau, 2000; Koleff and Gaston, 2002; Witman et al., 2004).

The drivers of local (α) and habitat turnover (β) diversities are likely to be different, as α diversity is generally associated with high abundance and high resource availability, whereas β diversity can be more sensitive to the heterogeneity of the habitat and/or of the resource distribution (Lamshead et al., 2002; Soininen et al., 2008; Levin et al., 2010).

Deep-sea ecosystems represent the most extensive biome on Earth, as they cover ca. 65 % of Earth's surface and account for 95 % of its volume. Together with the rain forests, deep-sea ecosystems host the largest portion of the yet-to-be-discovered biodiversity and have key roles in global biogeochemical cycles (Danovaro et al., 2010). For a long time, deep-sea ecosystems have been believed to be characterised by very low diversity (Grassle, 1989). However, research conducted over the last few decades has changed our perceptions (Rex and Etter, 2010). Despite the typically low abundance of the fauna of deep-sea ecosystems, their evenness and expected species richness are indeed amongst the highest on Earth (Danovaro et al., 2010). It has been also assumed

for centuries that the deep-sea floors are characterised by flat and monotonous desert-like landscapes. Due to the present availability of sophisticated technologies, we know now that the deep-sea floors are far more complex and heterogeneous than what was previously believed (Danovaro et al., 2010; Ramirez-Llodra et al., 2010; Vanreusel et al., 2010; Ingels et al., 2011). However, the patterns of deep-sea biodiversity and the factors that control these patterns remain controversial, yet (Snelgrove and Smith, 2002; Danovaro et al., 2008a, b, 2010).

As observed for terrestrial ecosystems (Gaston, 2000), the presence of different habitats (such as canyons, open slopes, landslides, and bathyal and hadal plains) can certainly influence the distributions of deep-sea species (Rex et al., 2006; Ramirez-Llodra et al., 2010; Vanreusel et al., 2010). Deep-sea canyons, for instance, are characterised by high hydrodynamism, as they are “fast-track” corridors for materials and organisms that are rapidly transported from the land to the deep sea (Canals et al., 2006; Tyler et al., 2009; Pusceddu et al., 2010a, b, 2013; López-Fernández et al., 2013). Some of these systems, such as seamounts, deep-water corals and cold seeps, contain many deep-sea species and have a high level of endemism, which are possibly linked to the peculiar environmental and geomorphological conditions (Raes and Vanreusel, 2006; Roberts et al., 2006; Vanreusel et al., 2009).

The Mediterranean region is a “hot spot” of terrestrial biodiversity, with a high fraction of endemic species (Myers et al., 2000). Despite its small dimensions (0.82 % of the global ocean surface, and 0.32 % of the global ocean volume), the Mediterranean Sea hosts approximately 17,000 marine species (7.5 % of the global marine biodiversity; Coll et al., 2010). This “miniature ocean” can therefore also be considered as a hot-spot of marine biodiversity, which is characterised by the co-occurrence of temperate and subtropical organisms.

In all deep-sea sediments, nematodes are the most abundant metazoan taxon, and they are ubiquitous in all of these habitats, with their dominance increasing with increasing water depth (typically > 80–90 % of the total faunal abundance in the deep sea, Danovaro et al., 2002). Nematodes are also characterised by high species richness, and they have key roles in benthic trophodynamics, which provides an excellent opportunity for the testing of ecological hypotheses (Danovaro et al., 2008a, 2010).

In the present study, we used marine nematodes as a model for comparing the patterns of local (α), regional (γ) and macro-regional (ϵ) diversity, as well as habitat (β) and regional (δ) turnover diversity in different deep-sea habitats (canyons, open slopes, deep-corals and bathyal plains) over > 2000 km of the Mediterranean Basin. We combined different data sets from various habitats characterised by different topographic settings, productivities and physico-chemical conditions from three different deep Mediterranean Sea regions (Danovaro et al., 2009a, 2010; Bongiorno et al., 2010) with unpublished data collected in the eastern Mediterranean

Sea. We tested the hypothesis that the key variable that generates patterns of deep-sea biodiversity is the turnover diversity. We also determined the effects of increasing the spatial scale of observation on the turnover diversity.

2 Methods

2.1 Study area and sampling

Sediment samples were collected from three regions of the Mediterranean Sea: the north-western, central and eastern basins. In each region, samples were collected from different habitats (i.e. canyon, open slope, coral rubble or bathyal plain) at the same bathymetric range, comprised between ca. 600 and 1300 m in depth, from a total of 18 sampling sites (Fig. 1; Table 1).

In the north-western Mediterranean, the samples were collected from two different canyons (i.e. one site in the Cap de Creus and one site in the Lacaze-Duthiers Canyon) and from two open slopes adjacent to the two canyons (one site in the northern and one site in the southern open slope), located in the Gulf of Lions.

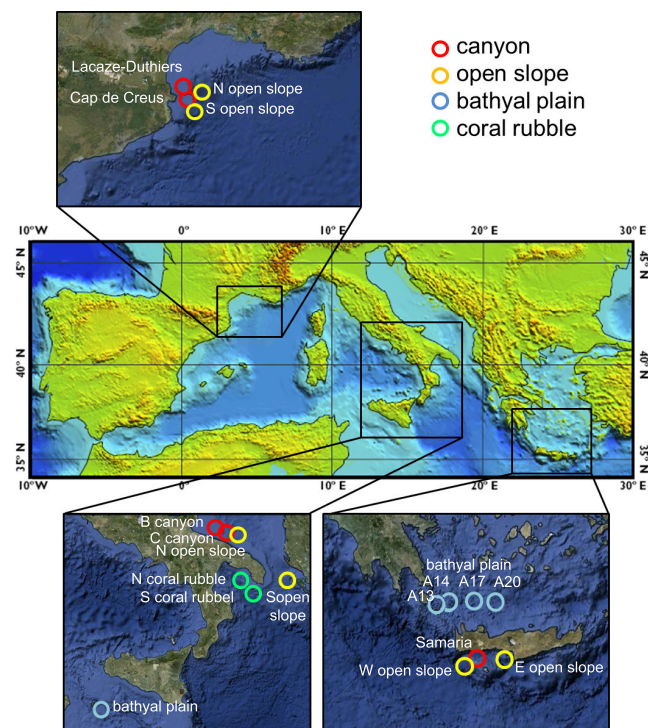
In the central Mediterranean, the samples were collected from two canyons (one site in the B and one site in the C Bari Canyon) and from two open slopes adjacent to the canyons (one site in the northern and one site in the southern open slope), along the south Adriatic margin. Samples were also collected from two sites that are characterised by deep-water coral rubble (northern and southern deep-coral sites) along the Ionian margin, and from one site in a bathyal plain (central Mediterranean bathyal plain).

In the eastern Mediterranean, the samples were collected from one canyon (one site in the Samaria Canyon) and from two adjacent open slopes (one site in the eastern and one site in the western open slope), along the Cretan margin, and from four sites along the bathyal plain. The geographical coordinates and water depths of all of these sampling sites are given in Table 1.

The sampling was carried out during several oceanographic cruises, from September 1989 to May 2006, using different research vessels (R/V *Universitatis* in the western Mediterranean Sea, R/V *Urania* in the central Mediterranean Sea and R/V *Aegaeo* and *Bannock* in the eastern Mediterranean Sea). At all of the sampling sites, replicate sediment samples were collected using a NIOZ-type box corer, except for the north-western Mediterranean Sea, where the sediment samples were collected by means of a multiple corer. Both sampling devices allowed the recovery of undisturbed sediment samples. A total of three cores (internal diameter 3.6 cm) from two or three independent deployments were analysed for nematode species diversity (from 0–1 cm sediment layer). Sediment samples for organic matter analysis (the top 1 cm from three different cores from each site) were preserved at -20°C until analysis in the laboratory.

Table 1. Characteristics of the sampling sites in the present study.

Region	Habitat	Site	Latitude (° N)	Longitude (° E)	Water depth (m)	N. of cores	Sampling date
North-western Mediterranean	Lacaze-Duthiers Canyon	LD2	42.44	3.53	990	3	October 2005
	Cap de Creus Canyon	CC1	42.31	3.61	960	3	October 2005
	Northern open slope	NS2	42.44	3.86	1022	3	October 2005
	Southern open slope	SS2	42.13	3.78	985	3	October 2005
Central Mediterranean	Bari Canyon B	2	41.34	17.18	590	3	May 2006
	Bari Canyon C	9	41.31	17.26	721	3	May 2006
	Northern open slope	11	41.23	17.59	908	3	May 2006
	Southern open slope	77	39.75	19.19	1096	3	May 2006
	Bathyal plain	St 7	36.61	12.25	1290	3	July 1998
	Northern coral rubble	19	39.84	17.63	1084	3	May 2006
	Southern coral rubble	33	39.83	17.61	1276	3	May 2006
Eastern Mediterranean	Samaria Canyon	11	35.19	23.93	1216	3	May 2006
	Western open slope	12	35.01	23.70	1081	3	May 2006
	Eastern open slope	5	34.95	24.59	1176	3	May 2006
	Bathyal plain	A13	36.03	23.30	892	3	September 1989
	Bathyal plain	A20	35.92	24.60	1078	3	September 1989
	Bathyal plain	A14	36.05	23.43	1215	3	September 1989
Bathyal plain	A17	36.03	24.06	1147	3	September 1989	

**Fig. 1.** Location and typology of the sampling sites in the deep Mediterranean Sea.

2.2 Nematode biodiversity

All of the meiofaunal organisms, including the nematodes, had been extracted from the sediment. The sediment samples had been sieved through a 1,000 μm mesh, with a 20 μm mesh then used to retain the smallest organisms. The fraction remaining on the 20 μm sieve was re-suspended and washed three times (800 g, 10 min, room temperature) in Ludox HS40 colloidal silica (density, 1.31 g cm^{-3} ; according to Heip et al., 1985; Higgins and Thiel, 1988; Pfannkuche and Thiel, 1988; and Danovaro, 2010). All of the animals that remained in the supernatant were again sieved through a 20 μm mesh net, washed with tap water, stained with 0.5 g L^{-1} rose bengal solution, and sorted under a stereomicroscope (magnification, 40 \times), according to Danovaro (2010, and citations therein).

For the nematode diversity analysis, 100 randomly-selected nematodes for each of the three replicates (or all of the nematodes when the abundance was lower than 100 specimens per sample, the number of identified individuals at each sampling site was reported in Table 2) were mounted on slides, following the formalin-ethanol-glycerol technique to prevent dehydration (Seinhorst, 1959; Danovaro, 2010). The nematodes were identified to species level according to the presently used manuals (Platt and Warwick, 1983, 1988; Warwick et al., 1988; Deprez et al., 2005) and the recent literature dealing with new nematode genera and species. All of the unknown species were indicated as sp₁, sp₂, sp₃, ..., sp_n.

Table 2. Nematode diversity indices, calculated cumulatively from the individuals retrieved from the three replicates of the investigated sampling sites. SR, species richness; ES(51), expected species number for 51 individuals; H^2 , Shannon's index; J , species evenness; 1-ITD, index of trophic diversity; MI, maturity index.

Region	Habitat	Site	N. identified individuals	SR	ES (51)	H^2	J	1-ITD	MI
North-western Mediterranean	Lacaze-Duthiers Canyon	LD2	304	62	26.98	25.98	0.86	0.69	2.64
	Cap de Creus Canyon	CC1	314	81	31.66	31.45	0.88	0.71	3.11
	Northern open slope	NS2	283	66	30.03	29.36	0.90	0.73	2.91
	Southern open slope	SS2	301	68	28.60	27.64	0.86	0.69	2.75
Central Mediterranean	Bari Canyon B	2	232	56	28.52	26.82	0.89	0.65	2.75
	Bari Canyon C	9	251	81	32.62	32.23	0.89	0.66	2.95
	Northern open slope	11	230	61	26.13	23.46	0.81	0.61	2.70
	Southern open slope	77	101	45	30.48	26.13	0.93	0.67	3.19
	Bathyal plain	St 7	247	57	28.57	27.46	0.90	0.73	2.89
	Northern coral rubble	19	222	73	32.03	30.62	0.89	0.66	3.17
	Southern coral rubble	33	192	61	30.42	27.44	0.88	0.65	2.83
Eastern Mediterranean	Samaria Canyon	11	124	48	28.49	24.70	0.89	0.60	3.10
	Western open slope	12	92	35	25.70	21.42	0.90	0.63	3.23
	Eastern open slope	5	117	22	22.00	17.90	0.95	0.74	3.03
	Bathyal plain	A13	225	30	22.91	21.99	0.96	0.67	2.95
	Bathyal plain	A20	101	11	10.90	10.56	0.94	0.66	3.63
	Bathyal plain	A14	219	16	15.26	15.05	0.97	0.64	2.86
	Bathyal plain	A17	114	12	11.89	12.25	0.98	0.73	3.00

The nematode diversity was estimated using the species richness (SR), as the total number of different species identified at each site. Each replicate sampling from each site was analysed separately; then the biodiversity/other diversity descriptors for the nematode community at each site were determined cumulatively as the total number of species retrieved from the independent samplings. As species richness is strongly affected by sample size, the expected number of species, ES(X), was also considered, which provides a standardisation of the values of the species richness according to the sample size. The expected number of species for a theoretical sample of 51 specimens, ES(51), was chosen to facilitate the comparisons among habitats and regions.

At almost all sampling sites, 100 randomly selected individuals were identified from each replicate. However, at some sites the abundances were less than 100 individuals per replicate, particularly in central and eastern Mediterranean Sea, where the meiofaunal abundances were typically lower than in the western Mediterranean Sea (Bianchelli et al., 2010). For this reason we pooled together the data from different replicates, to have a minimum of 51 identified individuals, in order to calculate the expected species number on > 51 individuals for the investigated habitats. This procedure might lead to possibly biased results, as different proportions of the nematode communities could be extracted from different samples. Nevertheless, we adopted this procedure as it is the standard methodology used in practically all nematode studies in the deep sea (Danovaro et al., 2008a, b; Leduc

et al., 2012; Ingels and Vanreusel, 2013), thus allowing the comparison of our data with previous studies.

The species diversity (H' , using log-base 2, expressed as H^2) was also measured by the Shannon–Wiener index (Shannon and Weaver, 1963), and the evenness was measured by the Pielou index (J ; Pielou, 1975). These indices were calculated from the sum of the individuals of the three replicates of each of the sampling sites, using PRIMER v6.0+ (Plymouth Marine Laboratory, UK; Clarke and Gorley, 2006).

We measured local (α diversity), regional (γ diversity) and macro-regional (ϵ diversity) species richness as the numbers of different nematode species within each site (local diversity, sensu point species richness), region (north-western, central and eastern Mediterranean Sea) and macro-region (i.e. the whole Mediterranean basin; Gray, 2000; Danovaro et al., 2009a).

We also measured the habitat species richness (i.e. the species richness of a defined habitat; Gray, 2000) as the numbers of different nematode species found within each habitat.

To calculate the percentage of exclusive species, we considered the number of species exclusively retrieved from a specific habitat (namely exclusive species). Then the number of exclusive species has been reported as percentage of the total number of species found in each region. However, it must be acknowledged that since we used an uneven number of samples per habitat in the central and eastern

Mediterranean regions, the number of exclusive species may be susceptible to a certain bias.

The γ and ε diversities were also assessed by computing estimates of total species richness using non-parametric estimators (Leduc et al., 2012, and citations therein): in particular, we used both abundance- (Chao1 and ACE) and incidence-based estimators (Chao2 and ICE). Estimates of species richness using these estimators were computed using the EstimateS software v8.2.0 (Colwell, 1997). Results from species data were compared by plotting randomised, cumulative species richness estimates against number of samples.

We also measured the turnover diversity between sites (β diversity; sensu Gray, 2000) and between regions (δ diversity). The β diversity and δ diversity were measured using the similarity percentage analysis (SIMPER) routine that is included in the PRIMER v6.0+ software as the percentage of the dissimilarity between sites and regions, respectively, calculated from resemblance matrices based on Bray–Curtis dissimilarity using presence/absence data.

The trophic composition of the nematode assemblages was defined according to the Wieser classification (Wieser, 1953). Nematodes were divided into four groups: no buccal cavity or a fine tubular one-selective (bacterial) feeder (1A); large but unarmed buccal cavity non-selective deposit feeders (1B); buccal cavity with scraping tooth or teeth, epistrate or epigrowth (diatom) feeders (2A); and buccal cavity with large jaws, predators/omnivores (2B).

The index of trophic diversity (ITD) was calculated as the 1-ITD, where $ITD = g_1^2 + g_2^2 + g_n^2$, g is the relative contribution of each trophic group to the total number of individuals, and n is the number of trophic groups (Heip et al., 1985; Gambi et al., 2003). For $n = 4$ (as in the present study), the 1-ITD ranges from 0.00 to 0.75.

To determine the colonisation strategies of the nematodes, the maturity index (MI) was calculated according to the weighted mean of the individual genus scores, as $\sum v(i) f(i)$, where v is the colonisers-persisters (c-p) value of the genus i , as given in the Appendix of Bongers and Bongers (1998), and $f(i)$ is the frequency of that genus.

2.3 Quantity and biochemical composition of sediment organic matter

Chlorophyll a and phaeopigment analyses were carried out according to methods reported in Pusceddu et al. (2009) and Danovaro (2010). Total phytopigments were defined as the sum of chlorophyll a and phaeopigments (reported as mg g DW^{-1} ; Thiel, 1978). The protein, carbohydrate and lipid contents of the sediments were determined spectrophotometrically (Pusceddu et al., 2009, 2010a). A detailed description of the analysis of the sedimentary organic matter is reported by Danovaro (2010). All of the analyses were performed as three replicates, with about 1 g of surface sediment (0–1 cm sediment depth) for each sample. The protein, carbohydrate and lipid sediment contents were converted into

carbon equivalents using the conversion factors 0.49, 0.40 and $0.75 \text{ mg C mg}^{-1}$, respectively, and their sum was defined as the biopolymeric organic carbon (Pusceddu et al., 2009, 2010a). The concentration of biopolymeric carbon (biopolymeric C) was chosen as indicator of the quantity of the sedimentary organic matter.

2.4 Statistical analyses

The differences in the nematode diversity indices among the regions were analysed using one-way analysis of variance (ANOVA). The test used the regions (north-western, central and eastern Mediterranean Sea) as the single sources of variance (with $n = 3$ fixed levels). The differences in the nematode diversity indices among habitats were analysed separately for each region, using one-way ANOVA and the sampling sites as the single source of variation (with $n = 4$ – 7 fixed levels). To meet the ANOVA assumptions, before the analyses, the homogeneity of variances was checked using Cochran's test on appropriately transformed data, whenever necessary. After checking again the homogeneity of variances, for those data sets for which the transformation did not allow obtaining homogeneous variances, a more conservative level of significance was considered (Underwood, 1991). When significant differences were encountered, Student–Newman–Keuls (SNK) post-hoc comparison tests were also carried out (at $\alpha = 0.05$) to determine the patterns of variability among regions or habitats. The ANOVA and SNK tests were carried out using GMAV software (WinGMAV5, University of Sidney, Australia).

Analysis of similarity was performed based on the Bray–Curtis similarity matrices obtained after the presence/absence transformation of the data, to assess the differences in the compositions of the nematode assemblages between sites within the same habitat, between habitats within the same region, and among regions. Analysis of similarity was carried out using the analysis of similarity (ANOSIM) routine included in the PRIMER v6.0+ software (Clarke and Gorley, 2006).

To determine how potential trophic resources and local diversity explained the differences in regional diversity, non-parametric multivariate multiple regression analyses were carried out based on Bray–Curtis distances, using the routine distance-based linear model forward (DISTLM forward) (McArdle and Anderson, 2001; Anderson, 2003). The forward selection of the predictor variables was carried out with tests by permutation; P values were obtained using 4999 permutations of the raw data for the marginal tests (tests of individual variables), while for all of the conditional tests, the routine used 4999 permutations of the residuals under a reduced model. We used the concentrations of the main sedimentary organic matter compounds (phytopigment, protein, carbohydrate and lipid) as indicators of the trophic resources (Pusceddu et al., 2010a), and the α and β diversities as the components of the local biodiversity.

Since the sampling stations were located in a water depth range between ca. 600 and 1300 m, a distance-based multivariate analysis (DISTLM) was also performed to test the statistical significance of the regression of γ versus α and β diversity levels when the effect of water depth, treated as a covariate, is excluded from the model (Anderson, 2001). The results are reported in Appendix S1 of the Supplement.

3 Results

3.1 Nematode diversity for the different deep-sea habitats of the Mediterranean Sea

The different indices of nematode biodiversity, calculated cumulatively from the individuals retrieved from the pooled replicates, are reported in Table 2: SR, ES(51), and H^2 .

The α diversity is expressed as SR within a single site, and this varied from 11 to 81. However, the results of the present study reveal significant differences between sites for the SR and ES(51) only for the north-western Mediterranean Sea (ANOVA, $P < 0.05$; Table 3). The post-hoc comparisons reveal higher values for the Cap de Creus Canyon than for the Lacaze-Duthiers Canyon and the northern and southern open slopes (SNK, $P < 0.05$; Table 3).

Moreover, the results of the present study reveal very weak or no differences in the nematodes' functional diversity, expressed as 1-ITD, and in the nematodes colonisation strategies, expressed as MI (Table 3). Indeed, the 1-ITD shows no differences between different habitats in all the investigated regions (ANOVA ns; Table 3) whereas the MI shows significant differences among habitats in the eastern Mediterranean Sea (ANOVA, $P < 0.05$; Table 3), with the highest value reported from one of the bathyal plains (site A20; SNK, $P < 0.05$; Table 3).

The patterns of the nematode species richness found for each habitat (i.e. habitat species richness) are illustrated in Fig. 2. Within both the western and the eastern Mediterranean Sea, the overall habitat species richness in open slopes, canyons, deep-water corals and the bathyal plains have similar values. In the central Mediterranean sea, however, the nematode overall diversity for the bathyal plains is lower than for the canyons and open slopes.

The average β diversity between the habitats within each region is shown in Fig. 2. The SIMPER analysis reveals that on average the β diversity between the sampling sites and habitats increased when moving from the north-western Mediterranean (ca. 59%) to the central Mediterranean (60–90%) to the eastern Mediterranean Sea (83–95%; Table 4). The ANOSIM analysis reveals significant differences in the composition of the nematode species assemblages among the deep-sea habitats and among the sampling sites belonging to the same habitat in each investigated region (ANOSIM, $P < 0.05$; Table 4).

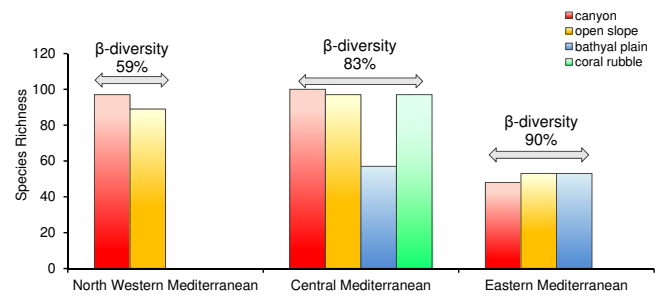


Fig. 2. Species richness of the nematodes in the different habitats in each of the regions investigated, calculated as number of cumulative species retrieved in each habitat. Mean β diversity among the habitats in each investigated region is also shown.

In each region, the species retrieved exclusively from a single specific habitat represented cumulatively more than 50% of the total species (i.e. γ diversity) retrieved from the entire region (Fig. 3), and the complete list of these exclusive species for the north-western, central and eastern Mediterranean Sea is reported in Appendix S2 of the Supplement. In all the investigated regions, the exclusive species in different habitats can be dominant (up to 6.76%, each, in the coral rubble, in the central Mediterranean) or rare. In particular we found that (i) in the western Mediterranean, the exclusive species accounted, each, for 0.16–1.13 and 0.17–1.03% of the entire assemblages, in canyon and slope, respectively; (ii) in the central Mediterranean, the exclusive species accounted, each, for 0.21–2.48, 0.30–1.81, 0.40–4.86 and 0.24–6.76%, in canyon, open slope, bathyal plain and coral rubble, respectively; and (iii) in the eastern Mediterranean, the exclusive species accounted, each, for 0.81–14.52, 0.76–9.92 and 0.57–8.06%, in canyon, open slope and bathyal plain, respectively.

3.2 Regional and macro-regional deep-sea nematode diversity for the Mediterranean Sea

The one-way ANOVA reveals that, on average, all of the diversity indices were significantly higher for the western and central Mediterranean than for the eastern Mediterranean Sea (Table 3, Fig. 4a, b), while the species evenness (the Pielou's index) shows the highest values for the eastern Mediterranean Sea (Fig. 4c). Significant differences are also seen in terms of the functional (trophic) diversity and life strategy among these three regions (Table 3). However, these two variables showed opposite patterns, with the highest values of trophic diversity for the north-western Mediterranean, and highest values of the maturity index for the eastern Mediterranean Sea (ANOVA, $P < 0.05$; Fig. 4d). Comparing these different deep-sea habitats, the maturity index shows the highest values along the bathyal plain (Table 2).

Despite, the generally low α diversity, there is high β diversity both between the different sites belonging to the same habitat, and between the different habitats (Fig. 5a). Such

Table 3. One-way ANOVA. (A) To test the differences in the diversity indices among the Mediterranean regions investigated. (B) Separately for each of the regions investigated, testing for changes among the different deep-sea habitats.

	SR			ES (51)			H ²			J'			I-ITD			MI			
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	
(A) Region	2	17.5	***	2	17.4	*	2	9.7	***	2	4.1	*	2	3.4	*	2.0	3.5	*	W Med, C Med < E Med
(B) North-western Mediterranean	3	4.1	*	3	3.7	*	3	0.3	ns	3	2.0	ns	3	0.6	ns	3.0	1.8	ns	CCreus > LacDuth, N slope, S slope
Central Mediterranean	6	2.2	ns	6	2.2	ns	6	1.4	ns	6	1.8	ns	6	2.2	ns	6.0	0.7	ns	S slope > N slope, B; N coral > N slope
Eastern Mediterranean	6	2.4	ns	6	2.3	ns	6	3.6	ns	6	1.4	ns	6	0.3	ns	6.0	1.6	*	Basin A20 > Samaria, E slope, W slope, basin A13, A14, A17

W Med: north-western Mediterranean; C Med: central Mediterranean; E Med: eastern Mediterranean.
 CCreus: Cap de Creus Canyon; LacDuth; Lacaze-Duthiers Canyon; N slope: northern open slope; S slope: southern open slope; N coral: northern coral rubble; W slope: western open slope; E slope: eastern open slope.
 SR: species richness; ES(51): expected species number for 51 individuals; H²: Shannon's index; J': Shannon's index; I-ITD: index of trophic diversity; MI: maturity index; df: degree of freedom; F: ANOVA F statistic; P: P statistic level; SNK: Student-Newman-Keuls test; *** P < 0.001; * P < 0.05; ns: not significant.

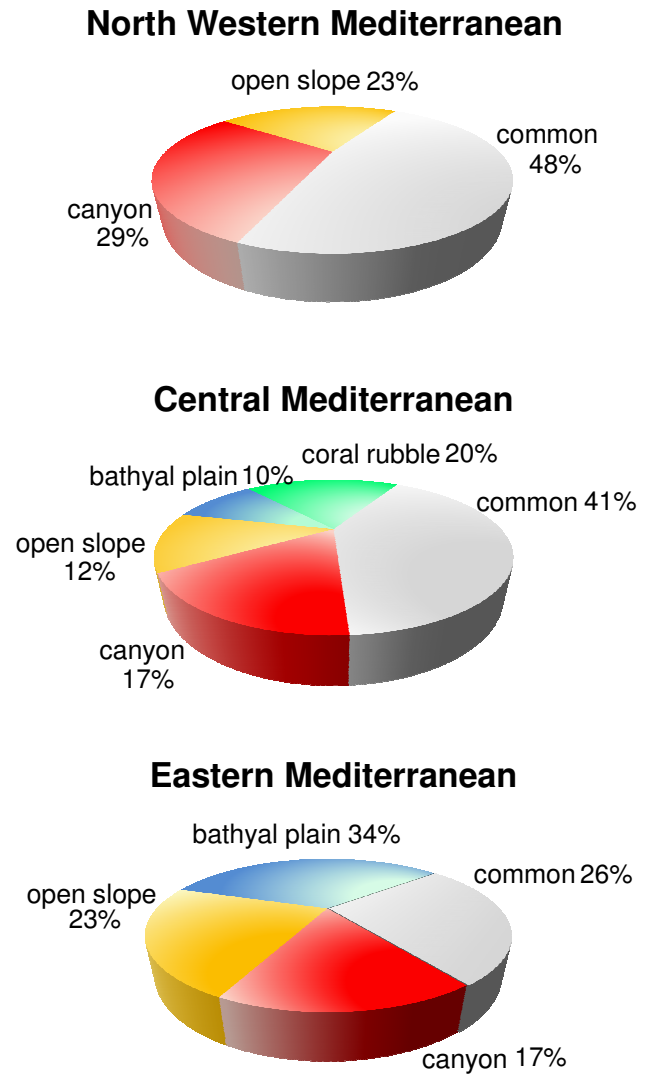


Fig. 3. Pie charts showing the relative importance of the exclusive species (as percentage of exclusive species on the total number of species retrieved in the region) in each of the habitats investigated, along with the species in common to all of these habitats in the north-western, central and eastern Mediterranean regions.

high values are responsible for the high γ diversity (i.e. regional diversity; Fig. 5b), which shows the highest values (210 species) in the central Mediterranean and the lowest (120 species) in the eastern Mediterranean Sea, as illustrated in Fig. 6. Even when the γ and ϵ diversities were calculated by means of estimators (Chao1 and Chao2), the same patterns were observed, with the highest level recorded in the central Mediterranean Sea. Plots of randomised, cumulative Chao2 estimates against number of samples show that species curves for all the investigated regions (except for the eastern Mediterranean Sea), and for regions combined, approached an asymptote (Fig. 7).

The turnover of nematode species among the different regions (the δ diversity) is always $> 80\%$, with the largest difference (89%) in species compositions between the central and eastern Mediterranean Sea, and lowest (81%) between the north-western and the central Mediterranean Sea. The resulting overall species richness (ε diversity) of the deep Mediterranean Sea at 600–1000 m depth was 280 species (Fig. 6).

Concentrations of all sedimentary organic matter compounds are reported in the Appendix S3 (Supplement). The results of the multivariate multiple regression analyses (DISTLM forward) carried out using the sedimentary organic matter compounds (phytopigment, protein, carbohydrate and lipid concentration) and the α diversity and β diversity show that most of the variance seen for the regional diversity can be significantly explained by the α diversity and β diversity (28 and 33%, respectively; Table 6).

4 Discussion

The present study allowed for the first time to analyse the patterns of α , γ and ε diversities, turnover β and δ diversities and functional diversity patterns of deep-sea nematodes across the Mediterranean Sea, comparing different kinds of habitats, at different spatial scales. In previous studies, indeed, investigations on deep-sea nematode diversity at different spatial scales were conducted at genus level (Vanreusel et al., 2010), compared typically not more than two habitats (i.e. canyons vs. slope, Danovaro et al., 2009; coral rubble vs. open slope; Bongiorno et al., 2010) or took into account only few components of diversity (e.g. structural diversity; Danovaro et al., 2010).

4.1 The α diversity and β diversity of nematodes in the deep Mediterranean Sea

The data from the present study indicate that three main features characterise the α biodiversity in the sediments of the Mediterranean Sea at a bathymetric range comprised between ca. 600 and 1300 m: (i) low diversity values (compared to north-eastern Atlantic or south-western Pacific Ocean deep-sea sediments; Danovaro et al., 2009; Leduc et al., 2012), either expressed as species richness or expected species number; (ii) very limited differences in biodiversity within each sampling site or habitat, expressed either in terms of the species richness or ES(51); and (iii) minor differences among habitats within the same region. One single main exception was the north-western Mediterranean Sea, where the sediments of the Cap de Creus Canyon displayed a significantly higher level of α diversity than the adjacent open slope. However, this can be considered an exception that is related to the specific environmental characteristics of this active and dynamic canyon (Canals et al., 2006; Lastras et al., 2007).

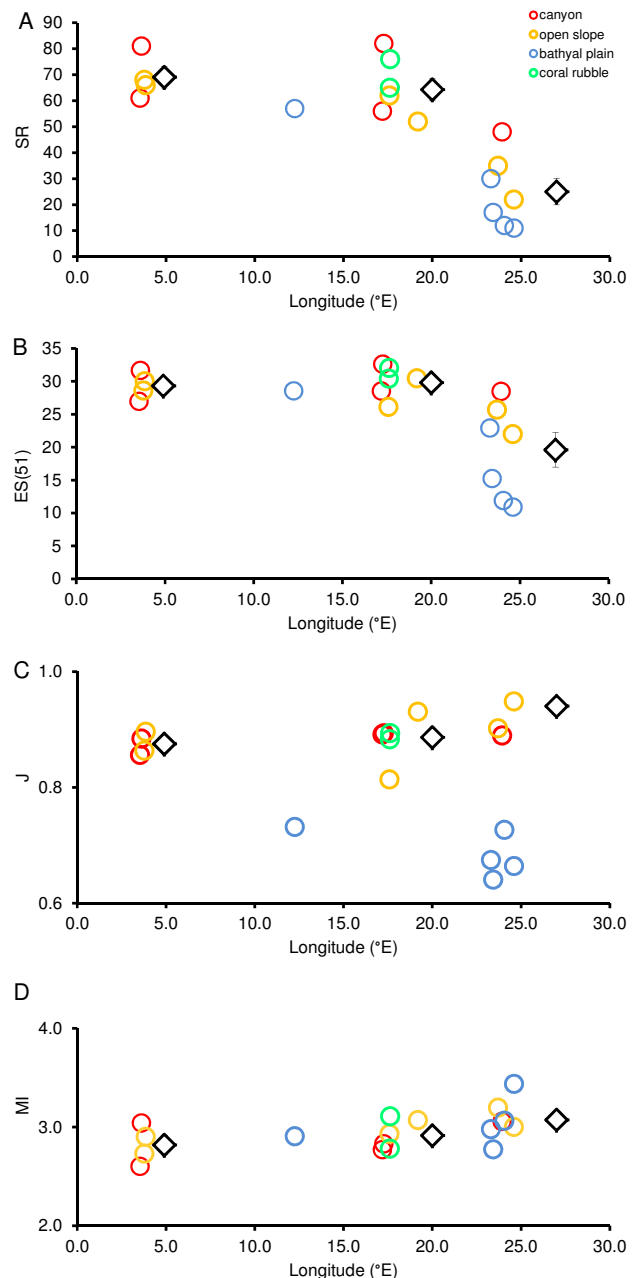


Fig. 4. α diversity in the different deep Mediterranean Sea regions: **(A)** Nematode SR; **(B)** ES(51); **(C)** species evenness J (the Pielou index.); **(D)** maturity index. Black diamonds, mean data (\pm SE) for the individual regions investigated: the north-western (ca. 5° longitude), central (ca. 20° longitude) and eastern (ca. 27° longitude) Mediterranean regions.

Conversely to our results, previous studies conducted in other oceanic regions (e.g. the Portuguese margin, north-eastern Atlantic Ocean) find relevant differences in the α diversity between sampling sites (García et al., 2007; Ingels et al., 2009). This finding suggests that in the Mediterranean Sea, whatever the habitat and region considered, the level of

Table 4. Results of SIMPER and ANOSIM analyses for the dissimilarities in the nematode species compositions between the different deep-sea habitats and sampling sites in all the regions investigated in the present study.

		SIMPER % Dissimilarity	ANOSIM <i>P</i>
North-western Mediterranean	Canyon vs. open slope	58.7	*
	Cap de Creus vs. Lacaze-Duthiers Canyon	59.3	*
	Northern vs. Southern open slope	59.4	*
Central Mediterranean	Canyon vs. open slope	76.9	**
	Canyon vs. coral rubble	86.0	**
	Canyon vs. bathyal plain	78.2	**
	Open slope vs. coral rubble	87.1	**
	Open slope vs. bathyal plain	86.7	***
	Coral rubble vs. bathyal plain	84.3	**
	B vs. C canyon	59.9	ns
	Northern vs. Southern open slope	90.1	***
Eastern Mediterranean	Northern vs. Southern coral rubble	79.0	**
	Canyon vs. open slope	83.3	***
	Canyon vs. bathyal plain	92.8	***
	Open slope vs. bathyal plain	93.1	***
	Western vs. Eastern open slope	95.0	***
	A13 vs. A14 vs. A17 vs. A20 bathyal plain	88.0	***

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns: not significant.

Table 5. Number of sites, samples, individuals and species identified in the western, central and eastern Mediterranean and overall in the Mediterranean Sea. Chao1 and ACE are abundance-based estimators; Chao2 and ICE are incident-based estimators.

	Western Mediterranean	Central Mediterranean	Eastern Mediterranean	Mediterranean Sea
N. sampling sites	4	7	7	18
N. samples	12	21	21	54
N. individuals identified	1202	814	558	2574
Recorded species richness	124	210	120	280
Chao 1	142	296	147	343
Chao 2	153	301	169	359
ACE	148	271	147	330
ICE	164	300	149	353

α diversity is generally very low, especially in the eastern Mediterranean Sea.

The present study also shows that whichever index is considered (species richness or expected species number), the nematode species diversity decreases significantly when moving eastwards, thus suggesting that the patterns observed are independent of the number of sampling sites within each region. However, the evenness (the Pielou's index) shows opposite patterns, with the highest values observed in the eastern Mediterranean Sea. Previous studies that were conducted from the deep north-eastern Atlantic to the central and eastern Mediterranean Sea have revealed that the nematode species richness decreases eastwards (Danovaro et al., 2008b, 2009a, b; Vanreusel et al., 2010). Although these

investigations were conducted at greater water depths (i.e. 3000–4000 m; Danovaro et al., 2008b), our results here indicate that such a decreasing longitudinal pattern in the nematode α diversity is a particular feature of the whole deep Mediterranean Sea also along the continental margin.

In previous studies several factors have been invoked to explain patterns in benthic biodiversity in deep-sea systems, including the quantity and availability of trophic resources, the hydrodynamic conditions, and topographic features (García et al., 2007; Danovaro et al., 2009a; Bianchelli et al., 2010). As the deep-sea sediments of the Mediterranean Sea are characterised by very low organic matter concentrations (García et al., 2008; Pusceddu et al., 2009), and as the sites investigated generally have very low amounts of bioavailable

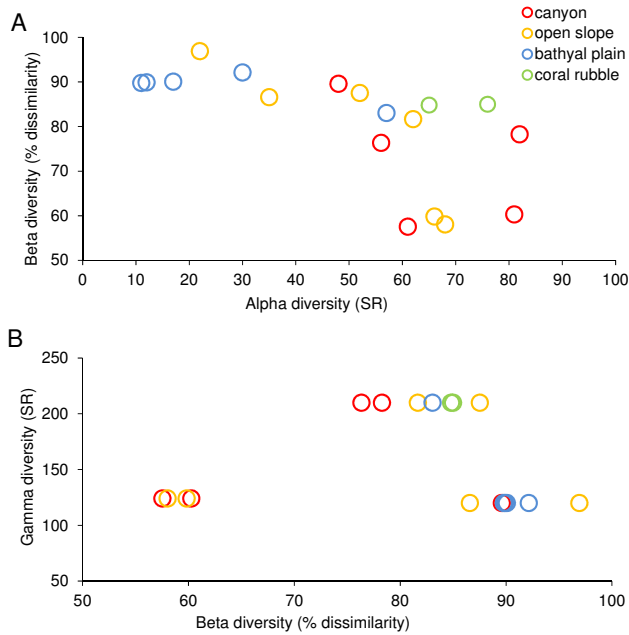


Fig. 5. Relation between: **(A)** α diversity and β diversity; and **(B)** β diversity and γ diversity, across the habitats (as indicated) of the deep-sea Mediterranean sites investigated.

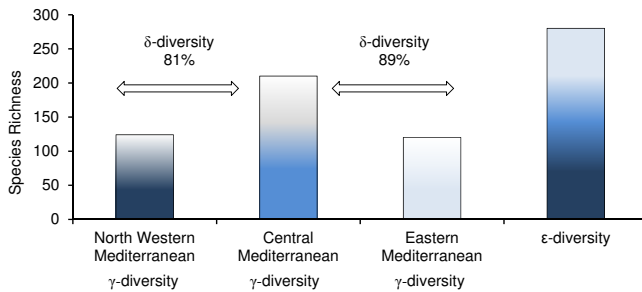


Fig. 6. Nematode species richness at regional and macro-regional spatial scale: γ diversity (as total number of species retrieved from each region), δ diversity between regions and the ϵ diversity (as total species richness in the Mediterranean Sea).

organic matter (Pusceddu et al., 2010b; Dell'Anno et al., 2012), the results of this study would confirm that the low α diversity of the deep Mediterranean Sea is primarily the result of the scarcity of available food resources (Danovaro et al., 2009a). This is consistent with the high meiofaunal abundance and α diversity of the Cap de Creus Canyon at 1000 m water depth, which is characterised by favourable trophic and environmental conditions that have probably promoted colonisation by a higher number of nematode species (Canals et al., 2006; Pusceddu et al., 2010b).

Conversely, the comparison of the α diversity at larger spatial scales (i.e. amongst basins, instead of among habitats or sites), shows the presence of clear differences between the sites of the western, central and eastern Mediterranean Sea,

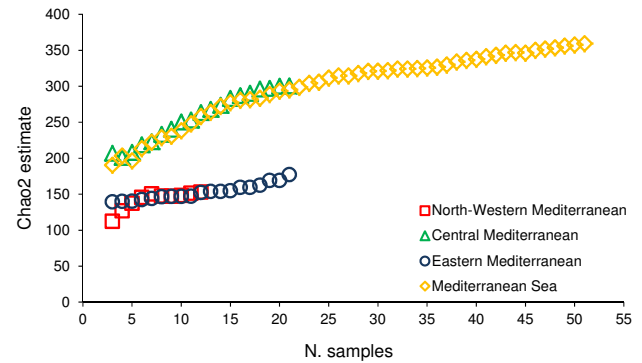


Fig. 7. Plot of randomised, cumulative Chao2 species richness against number of samples of the north-western, central and eastern Mediterranean Sea and of samples of the whole Mediterranean Sea.

with the α diversity in the western Mediterranean up to eight-fold higher than in the eastern Mediterranean Sea (Fig. 5). These differences may be related to the decreasing gradient of food availability from the western to the eastern basin.

Conversely to what is seen for the α diversity, the turnover (β) diversity both between the sites and habitats is consistently very high, ranging from 59 (in the north-western Mediterranean Sea) to 93 % (in the eastern Mediterranean Sea). These data indicate the presence of major differences in the compositions of the nematode species assemblages when either comparing different deep-sea sites belonging to the same habitat, or between different habitats. These data are consistent with recent observations on specific deep-sea habitats, such as seamounts or coral banks versus the adjacent bathyal plains or open slopes (Pusceddu et al., 2009, 2010b, 2013; Bongiorno et al., 2010). However, while high β diversity is expected when we compare deep-sea hot spots of biodiversity with the surrounding sediments (i.e. following the concept of different species in different habitats), our data suggest that high levels of β diversity can be ranked as a rule in the deep-sea sediments of the Mediterranean Sea. This has important implications, because an average β diversity of 80 % based on the presence/absence matrix means that when comparing two samples, eight out of ten of the species in one sample will be absent in the second sample, leading to much higher species richness at larger spatial scales. In addition, a high fraction of the species are exclusively found in each habitat investigated. The canyons of the western Mediterranean have the highest levels of exclusive species (ca. 30 %), although the deep-water corals in the central Mediterranean also have higher percentages of exclusive species than the canyons, open slopes and bathyal plains (20, 17, 13 and 10 %, respectively). In the eastern Mediterranean, the bathyal plain had a higher percentage of exclusive species than the open slope and canyon (34, 23 and 17 %, respectively).

Overall, our findings related to β diversity and the percentage of exclusive species may be affected by the temporal

Table 6. Multivariate multiple regression analysis carried out on the γ diversity. ** $P < 0.01$; * $P < 0.05$; ns: not significant.

(A) Conditional test						
Variable	SS	<i>F</i>	<i>P</i>	Explained variance (%)	Cumulative explained variance (%)	
β diversity	995.33	12.43	**	32.52	32.52	
α diversity	864.23	6.30	*	28.24	60.76	
Phytopigment	156.98	2.08	ns	5.13	65.89	
Lipid	61.56	0.76	ns	2.01	67.90	
Carbohydrate	102.70	1.37	ns	3.36	71.26	
Protein	57.62	0.75	ns	1.88	73.14	

(B) Marginal test					
Variable	SS	<i>F</i>	<i>P</i>	prop	
Phytopigment	2.77	0.01	0.9032	0.001	
Protein	37.62	0.20	0.6657	0.012	
Carbohydrate	339.95	2.00	0.1742	0.111	
Lipid	1.60	0.01	0.9456	0.001	
α diversity	864.23	6.30	0.0242	0.282	
β diversity	22.47	0.12	0.7532	0.007	

(C) Correlations among variables						
	Phytopigment	Protein	Carbohydrate	Lipid	α diversity	β diversity
Phytopigment	1.0000					
Protein	0.4028	1.0000				
Carbohydrate	0.3445	0.6099	1.0000			
Lipid	0.5240	0.6340	0.2619	1.0000		
α diversity	0.0725	0.5703	0.0466	0.4561	1.0000	
β diversity	-0.2118	-0.7188	-4620.0000	-0.4860	-0.6538	1.0000

shift occurred between the collection of samples from different regions (from 1989 to 2006, Table 1; Steiner and Leibold, 2004; Pusceddu et al., 2013). Indeed, temporal variability – even at intra-annual scale – has been recognised as a major driver influencing the deep-sea benthic biodiversity (Pusceddu et al., 2013, and citations therein). However, it is worthy of notice that in the present study, only samples from the bathyal plain in the central and eastern Mediterranean Sea were collected in 1998 and 1989, respectively, and that even excluding such sites/times the average levels of β (among sites) and δ (among regions) diversities remain almost the same, i.e. higher than 80% between sites both in the central and eastern Mediterranean, and between the two regions (Appendix S4, Supplementary Information). This suggests that each Mediterranean region is characterised by high levels of β diversity among habitats, resulting in high levels of regional γ and δ diversities between regions.

Altogether, the data obtained in the present study are supportive of the hypothesis that different habitats, such as deep

canyons, open slopes, basins and deep-water corals, host particular assemblages, and that the higher the number of habitats in a region the higher is the number of exclusive (and potentially endemic) species. Given the low α diversity, it is the high β diversity (both between different sites and habitats) that is the main driver of the high γ diversity at the regional level. This is observed in all of the three regions investigated here, although it is less evident in the western Mediterranean Sea, which has the highest α diversity. Conversely, the central-eastern Mediterranean Sea has a much higher β diversity, and this can explain why the ultra-oligotrophic eastern Mediterranean Sea shows a γ diversity that is identical to that of the much richer western Mediterranean, while that of the central Mediterranean Sea is the highest of the γ diversities.

4.2 Functional diversity across deep-sea habitats

Analysis of the functional traits and diversities is essential to better understand the effects of species richness

and composition on the functioning of deep-sea ecosystems (Danovaro et al., 2008b).

In order to compare the investigated regions and habitats within each region in terms of functional diversity, we analysed the changes in the trophic diversity, recognised as a simple indicator of the functional diversity.

The analysis of variance shows limited differences among the sites and habitats within a given region, although there is a clear decrease in the functional diversity moving from the western to the eastern Mediterranean Sea. A skewed trophic diversity in the eastern Mediterranean might be the result of the lower amounts of food sources and/or of the dominance of specific trophic groups. Indeed, an in-depth analysis also revealed the presence of a decreasing gradient in the relative abundance of predators when moving from the western Mediterranean to the eastern Mediterranean Sea (see Appendix S5, Supplement). A higher abundance of predators can be explained by the larger availability of prey, as evident from a comparison of the samples coming from the western Mediterranean versus the eastern Mediterranean Sea.

The analysis of the life strategies of the nematode assemblages in these three regions shows the highest values of the maturity index in the eastern Mediterranean, which suggests that this region is colonised by a larger fraction of persistent (K strategists) species. The presence of low functional diversity levels in the eastern Mediterranean coupled with a high fraction of persistent species deserves further investigation, to explore potential links between the nematode structural, functional diversity and their life strategies. Indeed, moving from the western to the eastern Mediterranean Sea, we observed a decreasing gradient in both structural and functional diversity, and an opposite pattern in turnover diversity between regions/habitats, assemblages equitability (Pielou's index), percentage of exclusive species in each habitat and fraction of persistent species (maturity index). This suggests that the deep western Mediterranean Sea is inhabited by a higher number of functionally diverse but opportunistic nematode species, a lower percentage of exclusive species in each habitat and a lower rate of species substitution among habitats.

4.3 The nematode γ diversity, δ diversity and ϵ diversity in the deep Mediterranean Sea

The Mediterranean Basin is considered as a hot spot of biodiversity with a uniquely high percentage of endemic species (Danovaro et al., 2010). However, this information is almost completely confined to coastal ecosystems, while data on deep-sea assemblages are still limited. Indeed, the deep Mediterranean Sea has been considered for decades as diversity-depleted (Danovaro et al., 2010 and citations therein).

The results of the present study confirmed that, in the Mediterranean deep sea, the high levels of β diversity are responsible for unexpected high levels of γ diversity, even if

α diversity is low. Conversely to our results, previous studies reported that whilst deep-sea nematode diversity may be very high at the local scale, diversity at the regional scale may result relatively limited (Lamshead and Boucher, 2003; Leduc et al., 2012, and citations therein). Moreover, these studies suggested that low levels of regional diversity in deep-sea environments (despite the greater local diversity) may be related to the lack of dispersal barriers and/or relatively low macro-habitat heterogeneity (Lamshead and Boucher, 2003; Leduc et al., 2012).

The number of species recorded during the present study from the different Mediterranean Sea regions (north-western, central and eastern) appears to be relatively high. Though comparisons with other deep-sea regions result difficult (due to different bathymetric ranges and sampling efforts; Leduc et al., 2012), the biodiversity levels found in the investigated Mediterranean deep-sea regions result similar to those retrieved in other deep-sea regions, as in the north-eastern Atlantic Ocean (Portuguese margin, Danovaro et al., 2009) or in Polar regions (Gallucci et al., 2008; Fonseca and Soltwedel, 2009). Conversely, the number of species recorded in the Mediterranean regions were lower than the regions in the south-western Pacific Ocean (Leduc et al., 2012), but this difference probably reflects differences in water depth range, sediment depth or the utilised methods (e.g. the mesh size, Leduc et al., 2010).

The total number of species retrieved from the central Mediterranean region (210 species) was higher than that in the western and eastern Mediterranean. Despite that such difference could be imputable to the higher number of investigated habitats in the central Mediterranean, it is worth noting that such pattern was consistent regardless of the biodiversity index (total number of species, ES(51)) or species richness estimator (Chao1, Chao2) used.

We also reported significant differences in nematode species composition between the different deep-sea Mediterranean regions. Such differences are highlighted by the δ diversity which was always $> 80\%$. This suggests that each deep-sea region is characterised by a specific nematode assemblage and species composition, thus letting us hypothesising a high habitat heterogeneity, possibly related to low dispersal potential (Leduc et al., 2012).

Several studies have shown that both physico-chemical variables and trophic resources (i.e. temperature, bottom salinity, grain size, and a combination of phytopigments, protein and biopolymeric C concentration) can have key roles in the structuring of the deep-sea nematode biodiversity (Danovaro et al., 2009a; Bongiorno et al., 2010; Danovaro et al., 2013). Indeed, high δ diversity amongst different oceanic regions (e.g. the Mediterranean Sea vs. the Atlantic Ocean; Danovaro et al., 2009) is expected, due to the significant differences in deep-water temperatures (ca. 10°C warmer in the Mediterranean Sea at 1000 m in depth) and trophic conditions (Danovaro et al., 2009a). Conversely, for the western, central and eastern Mediterranean deep basins, such

high δ diversity cannot be explained only by differences in temperature, which are typically close to 0.1–3.0 °C, or in trophic resources (Danovaro et al., 2010). Indeed, the multivariate, multiple regression analyses indicate that the observed variability in regional diversity was mostly driven by different components of local diversity (i.e. α and β diversities), rather than environmental variables. The significant regression of γ versus α and β diversities remains statistically significant also when the effect of water depth, treated as a covariate, is excluded from the model (Appendix S1 of the Supplement).

Moreover, it is worthy of notice that a fraction of variance remains unexplained (ca. 20 %), which leaves unsolved what other factors – not included in this study – could explain regarding the remaining fraction of observed variability in regional diversity. In this regard, it is remarkable that the Mediterranean Sea is characterised by an extremely complex geological history, which led to the identification of ten biogeographic regions (Bianchi and Morri, 2000). As the three regions here have been characterised by different evolutionary histories in relation to the Messinian crisis, the role of the different geological histories and events that characterised the sea-floor at 600–1000 m depth over the last 5 million years might be another key factor in such different species composition amongst these different basins.

As a result of the large differences in the species composition of the nematode assemblages observed among the north-western, central and eastern Mediterranean regions, the overall species richness (ϵ diversity) of the deep Mediterranean Sea (280 species at 600–1000 m depth) is very high.

The data from the present study indicate that the differences in β diversity and δ diversity are even more important than those in the α diversity for a better comprehension of the drivers of biodiversity in the deep Mediterranean Sea. These data also allow us to conclude that the presence of different types of habitats and gradients in environmental conditions, together with the other factors listed above and uncontrolled in our study, may also be crucial players controlling the nematode diversity levels.

Supplementary material related to this article is available online at: <http://www.biogeosciences.net/10/5465/2013/bg-10-5465-2013-supplement.pdf>.

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References

- Anderson, M. J.: Permutation tests for univariate or multivariate analysis of variance and regression, *Can. J. Fish. Aquat. Sci.*, 58, 626–639, 2001.
- Anderson, M. J.: DISTLM forward: a FORTRAN computer program to calculate a distance-based multivariate analysis for a linear model using forward selection. Department of Statistics, University of Auckland, New Zealand, 2003.
- Bianchelli, S., Gambi, C., Zeppilli, D., and Danovaro, R.: Metazoan meiofauna in deep-sea canyons and adjacent open slopes: a large-scale comparison with focus on the rare taxa, *Deep-Sea Res. I* 57, 420–433, 2010.
- Bianchi, C. N. and Morri, C.: Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research, *Mar. Poll. Bull.*, 40, 367–376, 2000.
- Bongers, T. and Bongers, M.: Functional diversity of nematodes, *Appl. Soil Ecol.*, 10, 239–251, 1998.
- Bongiorni, L., Mea, M., Gambi, C., Pusceddu, A., Taviani, M., and Danovaro, R.: Deep-water scleractinian corals promote higher biodiversity in deep-sea meiofaunal assemblages along continental margins, *Biol. Conserv.*, 143, 1687–1700, 2010.
- Canals, M., Puig, P., Durrieu de Madron, X., Heussner, S., Palanques, A., and Fabres, J.: Flushing submarine canyons, *Nature*, 444, 354–357, 2006.
- Clarke, K. R. and Gorley, R. N.: PRIMER v6: User Manual/Tutorial, PRIMER-E, Plymouth, 2006.
- Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F. B., Aguzzi, J., Ballesteros, E., Bianchi, C. N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Frogliola, C., Galil, B. S., Gasol, J. M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., Kitsos, M. S., Koukouras, A., Lampadariou, N., Laxamana, E., De La Cuadra, C. M. L. F., Lotze, H. K., Martin, D., Mouillot, D., Oro, D., Raicevich, S., Rius-Barile, J., Saiz-Salinas, J. I., San Vicente, C., Somot, S., Templado, J., Turon, X., Vafidis, D., Villanueva, R., and Voultsiadou, E.: The biodiversity of the Mediterranean Sea: estimates, patterns, and threats, *PLoS One*, 5, e11842, doi:10.1371/journal.pone.0011842, 2010.
- Colwell, R. K.: EstiMateS: statistical estimation of species richness and shared species from samples, Version 8.2.0, University of Connecticut, Storrs, CT, available at: <http://viceroy.eeb.uconn.edu/estimates>, 1997.
- Danovaro, R.: Methods for the study of deep-sea sediments, their functioning and biodiversity, CRC Press, Taylor & Francis Group, 428 pp., Boca Raton, 2010.
- Danovaro, R., Gambi, C., and Della Croce, N.: Meiofauna hotspot in the Atacama Trench, eastern South Pacific Ocean, *Deep-Sea Res. I*, 49, 843–857, 2002.
- Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Frascchetti, S., Vanreusel, A., Vincx, M., and Gooday, A. J.: Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss, *Curr. Biol.*, 18, 1–8, 2008a.
- Danovaro, R., Gambi, C., Lampadariou, N., and Tselepidis, A.: Deep-sea nematode biodiversity in the Mediterranean basin: testing for longitudinal, bathymetric and energetic gradients, *Ecography*, 31, 231–244, 2008b.
- Danovaro, R., Bianchelli, S., Gambi, C., Mea, M., and Zeppilli, D.: α -, β -, γ -, δ - and ϵ -diversity of deep-sea nematodes in canyons and open slopes of northeast Atlantic and Mediterranean margins, *Mar. Ecol.-Prog. Ser.*, 396, 197–209, 2009a.

- Danovaro, R., Canals, M., Gambi, C., Heussner, S., Lampadariou, N., and Vanreusel, A.: Exploring benthic biodiversity patterns and hot-spots on European margin slopes, *Oceanography*, 22, 16–25, 2009b.
- Danovaro, R., Company, J. B., Corinaldesi, C., D'onghia, G., Galil, B., Gambi, C., Gooday, A. J., Lampadariou, N., Luna, G. M., Morigi, C., Olu, K., Polymenakou, P., Ramirez-Llodra, E., Sabbatini, A., Sarda, F., Sibuet, M., and Tselepidis, A.: Deep-sea biodiversity in the Mediterranean Sea: the known, the unknown, and the unknowable, *PLoS One* 5, e11832, doi:10.1371/journal.pone.0011832, 2010.
- Danovaro, R., Carugati, L., Corinaldesi, C., Gambi, C., Guilini, K., Pusceddu, A., and Vanreusel, A.: Multiple spatial scale analyses provide new clues on patterns and drivers of deep-sea nematode diversity, *Deep-Sea Res. II*, 92, 97–106, 2013.
- Dell'Anno, A., Pusceddu, A., Corinaldesi, C., Canals, M., Heussner, S., Thomsen, L., and Danovaro, R.: Trophic state of sediments from two deep continental margins off Iberia: a biomimetic approach, *Biogeosciences Discuss.*, 9, 17619–17650, doi:10.5194/bgd-9-17619-2012, 2012.
- Deprez, T., Steyaert, M., Vanaverbeke, J., Speybroeck, J., Raes, M., Derycke, S., De Smet, G., Ingels, J., Vanreusel, A., Van Gaever, S., and Vincx, M.: NeMys, World Wide Web electronic publication, www.nemys.ugent.be, version (10/2011), 2005.
- Fonseca, G. and Soltwedel, T.: Regional patterns of nematode assemblages in the Arctic deep seas, *Polar Biol.*, 32, 1345–1357, 2009.
- Gallucci, F., Fonseca, G., and Soltwedel, T.: Effects of megafauna exclusion on nematode assemblages at a deep-sea site, *Deep-Sea Res. I*, 55, 332–349, 2008.
- Gambi, C., Vanreusel, A., and Danovaro, R.: Biodiversity of nematode assemblages from deep-sea sediments of the Atacama Slope and Trench (South Pacific Ocean), *Deep-Sea Res. I*, 50, 103–117, 2003.
- García, K. A., Koho, H. C., de Stigter, H. C., Epping, E., Koning, L., and Thomsen, L.: Distribution of meiobenthos in the Nazare canyon and adjacent slope (western Iberian Margin) in relation to sedimentary composition, *Mar. Ecol.-Prog. Ser.*, 340, 207–220, 2007.
- García, R., van Oevelen, D., Soetaert, K., Thomsen, L., De Stigter, H. C., and Epping, E.: Deposition rates, mixing intensity and organic content in two contrasting submarine canyons, *Prog. Oceanogr.*, 76, 192–215, 2008.
- Gaston, K. J.: Global patterns in biodiversity, *Nature*, 405, 220–227, 2000.
- Grassle, J. F.: Species diversity in deep-sea communities, *Trends Ecol. Evol.*, 4, 12–15, 1989.
- Gray, J. S.: The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf, *J. Exp. Mar. Biol. Ecol.*, 250, 23–49, 2000.
- Heip, C., Vincx, M., and Vranken, G.: The ecology of marine nematodes, *Oceanogr. Mar. Biol.*, 23, 399–489, 1985.
- Higgins, R. P. and Thiel, H.: Introduction to the study of meiofauna. Washington DC, Smithsonian Institution Press, 1988.
- Ingels, J. and Vanreusel, A.: The importance of different spatial scales in determining structural and functional characteristics of deep-sea infauna communities, *Biogeosciences*, 10, 4547–4563, doi:10.5194/bg-10-4547-2013, 2013.
- Ingels, J., Kiriakoulakis, K., Wolff, G. A., and Vanreusel, A.: Nematode diversity and its relation to the quantity and quality of sedimentary organic matter in the deep Nazaré Canyon, Western Iberian Margin, *Deep-Sea Res. I*, 56, 1521–1539, 2009.
- Ingels, J., Billett, D. S. M., Kiriakoulakis, K., Wolff, G. A., and Vanreusel, A.: Structural and functional diversity of Nematoda in relation with environmental variables in the Setúbal and Cascais canyons, Western Iberian Margin, *Deep-Sea Res. II*, 58, 2354–2368, 2011.
- Koleff, P. and Gaston, K.: The relationships between local and regional species richness and spatial turnover, *Glob. Ecol. Biogeogr.*, 11, 363–375, 2002.
- Lambshead, P. J. D. and Boucher, G.: Marine nematode deep-sea biodiversity: hyperdiverse or hype?, *J. Biogeogr.*, 30, 475–485, 2003.
- Lambshead, P. J. D., Brown, C. J., Ferrero, T. J., Mitchell, N. J., Smith, C. R., Hawkins, L. E., and Tietjen, J.: Latitudinal diversity patterns of deep-sea marine nematodes and organic fluxes: a test from the central equatorial Pacific, *Mar. Ecol.-Prog. Ser.*, 236, 129–135, 2002.
- Lastras, G., Canals, M., Urgeles, R., Amblas, D., Ivanov, M., Droz, L., Dennielou, B., Fabrès, J., Schoolmeester, T., Akhmetzhanov, A., Orange, D., and García-García, A.: A walk down the Cap de Creus canyon, northwestern Mediterranean Sea: recent processes inferred from morphology and sediment bedforms, *Mar. Geol.*, 246, 176–192, 2007.
- Leduc, D., Probert, P. K., and Nodder, S. D.: Influence of mesh size and core penetration on estimates of deep-sea nematode abundance, biomass, and diversity, *Deep-Sea Res. I*, 57, 1354–1362, 2010.
- Leduc, D., Rowden, A. A., Bowden, D. A., Nodder, S. D., Probert, P. K., Pilditch, C. A., Duineveld, G. C. A., and Witbaard, R.: Nematode beta diversity on the continental slope of New Zealand: spatial patterns and environmental drivers, *Mar. Ecol.-Prog. Ser.*, 454, 37–52, 2012.
- Levin, L. A., Sibuet, M., Gooday, A. J., Smith, C. R., and Vanreusel, A.: The roles of habitat heterogeneity in generating and maintaining biodiversity on continental margins: an introduction, *Mar. Ecol.*, 31, 1–5, 2010.
- López-Fernández, P., Bianchelli, S., Pusceddu, A., Calafat, A., Sanchez-Vidal, A., and Danovaro, R.: Bioavailability of sinking organic matter in the Blanes canyon and the adjacent open slope (NW Mediterranean Sea), *Biogeosciences*, 10, 3405–3420, doi:10.5194/bg-10-3405-2013, 2013.
- Loreau, M.: Are communities saturated? On the relationship between α , β and γ diversity, *Ecol. Lett.*, 3, 73–76, 2000.
- McArdle, B. H. and Anderson, M. J.: Fitting multivariate models to community data: a comment on distance-based redundancy analysis, *Ecology*, 82, 290–297, 2001.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., and Kent, J.: Biodiversity hot-spots for conservation priorities, *Nature*, 403, 853–858, 2000.
- Pfannkuche, O. and Thiel, H.: Sample processing, in: Introduction to the study of meiofauna, edited by: Higgins, R. P. and Thiel, H., London, Smithsonian Institution Press, 134–145, 1988.
- Pielou, E. C.: Ecological diversity, J. Wiley, New York, 1975.
- Platt, H. M. and Warwick, R. M.: A synopsis of the free-living marine nematodes. Part I: British enoplids, C.U. Press, Cambridge, 1983.

- Platt, H. M. and Warwick, R. M.: A synopsis of the free-living marine nematodes. Part II: British chromadorids, C.U. Press, Cambridge, 1988.
- Pusceddu, A., Gambi, C., Zeppilli, D., Bianchelli, S., and Danovaro, R.: Organic matter composition, metazoan meiofauna and nematode biodiversity in Mediterranean deep-sea sediments, *Deep-Sea Res. II*, 56, 755–762, 2009.
- Pusceddu, A., Bianchelli, S., Canals, M., Sanchez-Vidal, A., Durrieu De Madron, X., Heussner, S., Lykousis, V., De Stigter, H., Trincardi, F., and Danovaro, R.: Organic matter in sediments of canyons and open slopes of the Portuguese, Catalan, Southern Adriatic and Cretan Sea margins, *Deep-Sea Res. I*, 57, 441–457, 2010a.
- Pusceddu, A., Gambi, C., Bianchelli, S., Canals, M., Sanchez-Vidal, A., Calafat, A., Heussner, S., Durrieu De Madron, X., Avril, J., Thomsen, L., García, R., and Danovaro, R.: Ecosystem effects of dense water formation on deep Mediterranean Sea ecosystems: an overview, *Adv. Oceanogr. Limnol.*, 1, 51–62, 2010b.
- Pusceddu, A., Mea, M., Canals, M., Heussner, S., Durrieu de Madron, X., Sanchez-Vidal, A., Bianchelli, S., Corinaldesi, C., Dell'Anno, A., Thomsen, L., and Danovaro, R.: Major consequences of an intense dense shelf water cascading event on deep-sea benthic trophic conditions and meiofaunal biodiversity, *Biogeosciences*, 10, 2659–2670, doi:10.5194/bg-10-2659-2013, 2013.
- Raes, M. and Vanreusel, A.: Microhabitat type determines the composition of nematode communities associated with sediment-clogged cold-water coral framework in the Porcupine Seabight (NE Atlantic), *Deep-Sea Res. I*, 53, 1880–1894, 2006.
- Ramirez-Llodra, E., Brandt, A., Danovaro, R., De Mol, B., Escobar, E., German, C. R., Levin, L. A., Martinez Arbizu, P., Menot, L., Buhl-Mortensen, P., Narayanaswamy, B. E., Smith, C. R., Tittensor, D. P., Tyler, P. A., Vanreusel, A., and Vecchione, M.: Deep, diverse and definitely different: unique attributes of the world's largest ecosystem, *Biogeosciences*, 7, 2851–2899, doi:10.5194/bg-7-2851-2010, 2010.
- Rex, M. A. and Etter, R. J.: Deep-sea biodiversity: pattern and scale, Harvard University Press, 654 pp., 2010.
- Rex, M. A., Etter, R. J., Morris, J. S., Crouse, J., McClain, C. R., Johnson, N. A., Stuart, C. T., Deming, J. W., Thies, R., and Avery, R.: Global bathymetric patterns of standing stock and body size in the deep-sea benthos, *Mar. Ecol.-Prog. Ser.*, 317, 1–8, 2006.
- Roberts, J. M., Wheeler, A. J., and Freiwald, A.: Reefs of the deep: The biology and geology of cold-water coral ecosystems, *Science*, 312, 543–547, 2006.
- Seinhorst, J. W.: A rapid method for the transfer of nematodes from fixative to anhydrous glycerine, *Nematologica*, 4, 67–69, 1959.
- Shannon, C. E. and Weaver, W.: The mathematical theory of communication. University of Illinois Press, Urbana, Illinois, 111 pp., 1963.
- Snelgrove, P. V. R. and Smith, C. R.: A riot of species in an environmental calm: the paradox of the species-rich deep-sea floor, *Oceanogr. Mar. Biol.*, 40, 311–342, 2002.
- Soininen, J., Lennon, J. J., and Hillebrand, H.: A multivariate analysis of beta diversity across organisms and environments, *Ecology*, 88, 2830–2838, 2008.
- Steiner, C. F. and Leibold, M. A.: Cyclic assembly trajectories and scale-dependent productivity-diversity relationships, *Ecology* 85, 107–113, 2004.
- Thiel, H.: Benthos in upwelling regions, in: *Upwelling ecosystems*, edited by: Boje, R. and Torniczak, M., Springer Verlag, Berlin, Heidelberg, 124–138, 1978.
- Tyler, P., Amaro, T., Arzola, R., Cunha, M. R., De Stigter, H., Gooday, A., Huvenne, V., Ingels, J., Kiriakoulakis, K., Lastras, G., Masson, D., Oliveira, A., Pattenden, A., Vanreusel, A., Van Weering, T., Vitorino, J., Witte, U., and Wolff, G.: Europe's Grand Canyon Nazare submarine canyon, *Oceanography*, 22, 46–57, 2009.
- Underwood, A. J.: Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations, *Aust. J. Mar. Freshwat. Res.*, 42, 569–587, 1991.
- Vanreusel, A., Andersen, A. C., Boetius, A., Connelly, D., Cunha, M. R., Decker, C., Hilario, A., Kormas, K. A., Maignien, L., Olu, K., Pachiadaki, M., Ritt, B., Rodrigues, C., Sarrazin, J., Tyler, P., Van Gaever, S., and Vanneste, H.: Biodiversity of cold seep ecosystems along the European margins, *Oceanography*, 22, 110–127, 2009.
- Vanreusel, A., Fonseca, G., Danovaro, R., Da Silva, M. C., Esteves, A. M., Ferrero, T., Gad, G., Galtsova, V., Gambi, C., Genevois, V. D., Ingels, J., Ingole, B., Lampadariou, N., Merckx, B., Miljutin, D., Miljutina, M., Muthumbi, A., Netto, S., Portnova, D., Radziejewska, T., Raes, M., Tchesunov, A., Vanaverbeke, J., Van Gaever, S., Venekey, V., Bezerra, T. N., Flint, H., Copley, J., Pape, E., Zeppilli, D., Martinez, P. A., and Galeron, J.: The contribution of deep-sea macrohabitat heterogeneity to global nematode diversity, *Mar. Ecol.*, 31, 6–20, 2010.
- Warwick, R. M., Howard, H. M., and Somerfield, P. J.: A synopsis of the free-living marine nematodes, Part III: monhysterids, Field Studies Council, Shrewsbury, 1988.
- Wieser, W.: Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden, *Ark. Zool.*, 2-4, 439–484, 1953.
- Witman, J. D., Etter, R. J., and Smith, F.: The relationship between regional and local species diversity in marine benthic communities: a global perspective, *P. Natl. Acad. Sci.*, 101, 15664–15669, 2004.