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DEPTH-RELATED DIVERSITY PATTERNS OF FREE-LIVING NEMATODE ASSEMBLAGES ON TWO TROPICAL ROCKY SHORES

Patrones de diversidad asociados a la profundidad en asociaciones de nemátodos de vida libre en dos áreas tropicales rocosas

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ABSTRACT

Depth-related spatial patterns of diversity have been described for free-living nematode assemblages at four depth horizons (intertidal, 1 m, 5 m and 10 m) at two subtidal rocky shores on the NW coast of Cuba: Bacunayagua and Miramar. The patterns were described based on species identity and biological traits. Species richness was similar across depth horizons ranging from 12 to 18 species; it was also similar between sites with 30 and 33 species for Bacunayagua and Miramar respectively. Diversity variation was higher between the intertidal and the subtidal horizons suggesting that microhabitat plays a key role. Depth was not an important driver of the diversity of nematodes on the studied rocky shores both in terms of species richness and diversity variation. The type of microhabitat and the hydrodynamics may explain the β-diversity patterns within sites. The site-related diversity variation was larger than the depth-related variation suggesting that a regional pattern in β-diversity may be present. The biological traits constituted a useful complementary approach for analyzing diversity patterns indicating the prevalence of nematodes adapted to an epiphytic life-style and the effects of two putative diversity drivers: microhabitat and hydrodynamics.

KEYWORDS: nematodes, biological traits, species composition, α-diversity, β-diversity, Caribbean Sea, Cuba.

RESUMEN

Se describen los patrones espaciales de diversidad de nemátodos marinos de vida libre en relación con la profundidad; se muestreó en cuatro horizontes de profundidad (intermareal, 1 m, 5 m y 10 m) y dos sitios someros tropicales de la costa NW de Cuba: Bacunayagua y Miramar. Los patrones se describieron en base a la identidad de las especies y los rasgos biológicos. La riqueza de especies fue similar entre los horizontes de profundidad, con un rango de 12 a 18 especies; la riqueza fue también similar entre sitios con 30 y 33 especies en Bacunayagua y Miramar respectivamente. La variación en la diversidad fue más alta entre los horizontes intermareal y los submareales lo que sugiere que el micro-hábitat tiene un papel

importante. La profundidad no tiene un efecto importante sobre la diversidad de nemátodos en las costas estudiadas tanto en términos de riqueza de especies como de variación en la diversidad. El tipo de micro-hábitat y la hidrodinámica pueden explicar los patrones de β-diversidad dentro de los sitios. La variación de la diversidad entre sitios fue mayor que la variación relacionada con la profundidad sugiriendo que un patrón regional en la β-diversidad puede estar presente. Los rasgos biológicos constituyeron un enfoque complementario útil para analizar los patrones de diversidad e indicaron la prevalencia de nemátodos adaptados a un estilo de vida epífito y los efectos de dos potenciales determinantes de la diversidad: el micro-hábitat y la hidrodinámica.

PALABRAS CLAVE: : *rasgos biológicos, composición de especies, α-diversidad, β-diversidad, Mar Caribe, Cuba.*

INTRODUCTION

The study of the biodiversity is a major topic in the context of ecological and evolutionary theory and it is strongly based on patterns of occurrence of the species (Gray, 2000). The description of patterns of species can be separated into two components: (i) the estimation of diversity inventory and (ii) the estimation of distinctness or complementarity of assemblages (Colwell and Coddington, 1994; Whittaker *et al*., 2001). Inventories of species richness in tropical assemblages are necessary for disentangling fundamental issues such as latitudinal patterns of biodiversity and the appropriate selection of protected areas. The diversity variation, also termed β- or δ-diversity depending of the spatial scale, captures an essential component of the diversity but it has received limited attention in the marine realm (Gray, 2000; Terlizzi *et al*., 2009). In a recent synthesis, Anderson *et al*. (2011) suggested the use of univariate and multivariate approaches and the combined analysis of taxonomic, phylogenetic and functional information to reveal the mechanisms driving the β-diversity patterns.

Recent research (*e.g.* Wood *et al*., 2010; Violle *et al*., 2011) has highlighted the role of biological traits (*e.g*. body size, fecundity) in explaining patterns of diversity along environmental gradients. The exploration of patterns based on functional traits can give more generality and predictability than those based on species identities (McGill *et al*., 2006) and they allow direct links between the organisms and their environment to be detected (Bremner *et al*., 2006).

Free-living marine nematode assemblages have been the focus of many studies about diversity patterns in aquatic environments. The main reasons are their overwhelming abundance in marine sediments and the high number of sympatric species commonly found in a single sample. Microhabitat heterogeneity has a main effect on the diversity components in a variety of habitats such as coastal lakes (Flach *et al*., 2012), coral reefs (De Troch *et al*., 2008; Armenteros *et al*., 2012), deep-sea (Danovaro *et al*., 2009; Vanreusel *et al*., 2010), intertidal beaches (Gingold *et al*., 2010) and mangroves (Pinto *et al*., 2013). On the other hand, nematode species richness does not seem to be directly related to geographical latitude (Gobin and Warwick, 2006; Lee and Riveros, 2012) and there is evidence for the cosmopolitan distribution of many species (Mundo-Ocampo *et al*., 2007; Bik *et al*., 2010). Phylogenetic diversity has been also used to reveal patterns in species richness (*e.g*. Pereira *et al*., 2010) because in some groups there is cryptic diversi-

ty or conversely polymorphism.

Water depth is an environmental factor that affects the structure and functioning of shallow ecosystems and could be a major driver of the biodiversity patterns. For example, depth determines the vertical zonation of benthic photoautotrophs because of light limitation (Markager and Sand-Jensen, 1992). Depth also mainly contributes to hydrodynamic regime that affects larval dispersion (Eckman, 1983), the feeding of suspensivores (Aller and Stupakoff, 1996) and the persistence of small-sized benthic fauna (Fegley, 1987). Thus, some kind of relationships between the diversity patterns and the depth in coastal ecosystems is expected.

The species-level taxonomic composition of nematode assemblages constitutes the basis for the assessment of the diversity; that is powered by the usually high number of sympatric species which offer a rich-data framework for the exploration of patterns. In addition to species occurrence, we also analyzed combinations of five biological traits that incorporate morphological and ecological features of nematodes: life history, buccal morphology, tail shape, size and body shape. The use of biological traits has been useful for revealing ecological patterns in free-living nematode assemblages (*e.g.* Schratzberger *et al*., 2007; Armenteros *et al*., 2009; Alves *et al*., 2014). As part of the exploration of environmental drivers of diversity, we also calculated the correlation between the macroalgae biomass and the nematode assemblages as macrophytes constitute a matrix that provides food and shelter for invertebrates (Konar *et al*., 2009).

Therefore, we aim to answer two research questions: (i) How do α- and β-diversity vary in relationship to the depth? and (ii) Does biological trait analysis provide additional information for the interpretation of diversity patterns?

MATERIAL AND METHODS

Study area

The study was carried out at two sites, separated by approximately 80 km, on the northwestern coast of Cuba: Bacunayagua (23º08′40.19′′N, 81º40′27.34′′W) and Miramar (23º07′40.69′′N, 82 º25′21.32′′W). We chose these sites because they are representative of the subtidal rocky shore ecosystems on the NW coast. The hydrodynamic regime at both sites is characterized by strong near-coast currents with direction and magnitude that are highly variable from one day to the next. The tidal regime is semi-diurnal with a tidal amplitude of around 0.2 m causing a rather narrow intertidal fringe, approximately 4 m wide. The intertidal rocky bottom has high substrate heterogeneity (*i.e*. crevices, ridges, plateaus) with associated organisms (*e.g*. encrusting algae, chitons, periwinkles). Subtidal depth horizons are mostly rocky bottom with a flat relief and sparse patches of sand and macroalgae. The subtidal bottoms were covered mostly by turf algae or sessile invertebrates (*e.g.* bryozoans, corals, sponges) with fine sediment that was settled or close to suspension.

Nevertheless, Bacunayagua and Miramar sites differed in some geomorphologic features and therefore they were not treated as replicates within each depth nor as an orthogonal factor to depth (*i.e*. a cross design site x depth). Instead, we nested the depth horizons within each site and then estimated the variation between sites. Bacunayagua is located in a more topographically heterogeneous area, close to an inlet with a small river, a beach, and coralline structures including patch reefs

and spur and groove formations. Miramar has a more homogeneous substrate without patch reefs, a submarine terrace (4–8 m deep) and followed by a steep seaward slope (9–15 m deep). Both sites have been subjected to historical artisanal fisheries leading to a modified trophic web without large fishes. No pollution signals were evident during the sampling but at Miramar though wastewater discharges do occur after intense rainfall and the presence of sea-bathers is usual.

Sampling

Miramar (M) was sampled in July 2006 and Bacunayagua (B) in July 2010. During the surveys the hydrographic regime was similar at both sites: salinity: ~ 35 ppm, water temperature: 27–28 ºC, and air temperature in the littoral area: 31–32 ºC (1 cm above the substrate at around 07:00 h).

We applied the standardized sampling protocol defined by the Natural Geography in Shore Areas (NaGISA) program within the Census of Marine Life (Rigby *et al*., 2007; (http://nagisa.cbm.usb.ve/cms/protocols/). The protocol suggests the division of the intertidal horizon in three vertical heights (*i.e*. high, mid, and low). But, we defined a single fringe littoral (*i.e*. low to high water level) because of the narrow tidal amplitude in our study sites. At each study site, four depth horizons were defined: intertidal, 1, 5 and 10 m deep. At each depth, five replicate samples (sampling points) were taken at randomly selected positions along a 30-m transect parallel to the coastal line. At each sampling point two nested square frames of 50 cm and 25 cm were placed. Therefore, the distances between replicates were in the order of few meters (*i.e*. < 30 m) within a site and at the same depth. The distance between depth horizons varied according the bottom profile. The intertidal and 1 m horizons were relatively close (< 10 m apart), while the distances between 1, 5 and 10 m depth horizons typically varied between 50 and 100 m.

All the algae, invertebrates, rubble, and sediment within the 25 cm frame were carefully removed with a scraper, collected in plastic bags and sealed. The macroalgae (excepting encrusting algae) in the remaining portion of each 50 cm frame were collected in another plastic bag for the biomass estimation. The macroalgae from the 25 cm frame were rinsed over 500 and 45 μm mesh sieves to separate the macro- and meiobenthos respectively. This macroalgae fraction was added to the corresponding 50 cm frame and the whole algal material was weighed (*i.e*. wet biomass), after rinsing, with a precision of 0.1 g.

The invertebrate samples (macro- and meiobenthos) were sieved and the material retained in both sieves was preserved in 8 % formalin. We examined macro- and meiobenthic fractions under a stereomicroscope (80x) and we counted and identified metazoans to higher taxa (*e.g*. Copepoda, Polychaeta). The nematodes from both fractions were sorted and mounted on permanent slides using the method proposed by Vincx (1996). We identified nematodes species to the lowest possible taxonomic level based on the synopses by Platt and Warwick (1983; 1988), Warwick *et al*. (1998) and the online database NeMys (http://www.nemys.ugent.be). We did not measure nematode abundance since the collection method (*i.e*. scraping into a bag) underestimated their abundance. Thus, the diversity of nematode assemblages was analyzed based on species incidence data.

Biological traits

Nematode species were classified for five different biological traits (BT); therefore, each species was characterized by a combination of the five states of the BTs (Table 1). Finally, BT states were coded to generate a matrix of trait combinations x samples.

Ability to colonize a habitat, measured on a scale from best (1) to worse (5) colonizer (scale c-p in Bongers, 1990);

Buccal morphology that describes the feeding strategy divided into four categories, selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), and omnivores/predators (2B) (Wieser, 1953);

Tail shape with four categories, short/ round, elongated/filiform, conical, and clavate (*i.e*. conical-cylindrical) (Thistle *et al*., 1995);

Adult length (mm), < 1, 1-2, 2-4, and > 4 (Schratzberger *et al*., 2007); and

Body shape of the adult based on the ratio of total length/maximum body diameter = de Man ratio a; with three morphotypes (Schratzberger *et al*., 2007): stout (a < 18), slender (18 < a < 72), and long/thin (a > 72).

Data analysis

The sampling design of the study was based on two sites and four depth horizons nested within each site. We had five replicates (*i.e*. sampling units) per combination of site and depth. This resulted in 2 sites x 4 horizons x 5 replicates = 40 sampling units. The diversity statistics were computed using EstimateS 8.2.0 (Colwell, 2006).

The estimation of species richness (SR) was made based on individual-based species accumulation curves (rarefaction curves) using the observed SR (Mau-Tau function, S_{obs}) with 50 randomizations without replacement (Colwell and Coddington, 1994). We assessed the relationship between macroalgae biomass and fauna species richness using the Spearman Rank Order Correlation with both sites pooled (*i.e*. the 40 sampling units).

The diversity variation (β-diversity) was estimated using the Chao-Sørensen similarity index corrected for undetected species and based on incidence data (Chao *et al*., 2005). Note that Chao-Sørensen is originally computed as a similarity index; so, the complementary amount (*i.e*. 1 – Chao-Sørensen similarity index) gives the dissimilarity and by extension a directly proportional measure of distinctiveness or diversity variation. We pooled the five sampling units within each depth for a better estimation of the species composition, but sites were not pooled. The mean pairwise dissimilarity was computed (*i.e*. intertidal *vs*. 1m, 1 m *vs*. 5 m, etc.) using a bootstrap procedure with 200 resampling permutations.

We used a non-metric multidimensional scaling ordination analyses (nmMDS) to evaluate the similarity among samples based on the incidence composition of species with the software PRIMER 6.1.15 (Clarke and Gorley, 2006). We used non-transformed data and the Sørensen index as similarity measure. To address the identity of the species that most contribute to the similarity within- and between-depth horizons we used a SIMPER analysis (Clarke and Warwick, 2001)

The matrix of biological traits x samples was used to independently estimate the functional diversity of the assemblages. We computed the average value (± CI) of the number of trait combinations. Based on the biological traits, a similarity matrix was built using the Sørensen similarity index and graphical output was presented by nmMDS ordination. We assessed the association between the SR and the average number of bio-

Table 1. Biological traits defined for nematodes.

Species	C-p value	Buccal morphology	Tail shape	Adult length (mm)	Adult shape
Acanthonchus cobbi	2	2A	conical	1-2	slender
Acanthonchus viviparus	2	2A	conical	1-2	slender
Acanthopharynx rigida	3	2A	conical	2-4	slender
Actinonema pachydermatum	4	2A	conical	< 1	slender
Actionema longicaudatum	4	2A	conical	< 1	slender
Anaplostoma viviparum	2	2B	clavate	1-2	slender
Anticoma filicauda	2	1A	elongate/filiforme	2-4	slender
Anticoma insulae	2	1A	elongate/filiforme	2-4	slender
Aponema torosus	3	2A	clavate	< 1	slender
Araeolaimus boomerangifer	3	1A	conical	< 1	slender
Ascolaimus elongatus	2	1B	conical	2-4	long/thin
Axonolaimus drachi	2	1B	clavate	2-4	slender
Belbolla californica	4	2B	clavate	2-4	slender
Cephalanticoma chitwoodi	2	2A	elongate/filiforme	> 4	slender
Choanolaimus sp.	3	2B	short/round	1-2	slender
Chromadora macrolaima	3	2A	conical	< 1	slender
Croconema cinctum	3	2A	conical	2-4	slender
Desmodora extensa	2	2A	conical	1-2	slender
Desmodora pontica	2	2A	conical	1-2	slender
Dichromadora cephalata	2	2A	conical	< 1	slender
Draconema sp.	4	1A	conical	< 1	slender
Enoplus brevis	5	2B	clavate	> 4	slender
Enoplus mammillatus	5	2B	clavate	2-4	slender
Epsilonema crypthamphis	4	1A	conical	< 1	stout
Eubostrichus sp.	3	1A	conical	2-4	long/thin
Euchromadora atypica	3	2A	conical	1-2	slender
Euchromadora gaulica	3	2A	conical	1-2	slender
Euchromadora vulgaris	3	2A	conical	1-2	slender
Eurystomina filicaudatum	4	1B	conical	2-4	slender
Filoncholaimus capensis	4	2B	elongate/filiforme	> 4	slender
Halichoanolaimus sp.	3	2B	conical	2-4	slender
Hypodontolaimus angelae	4	2A	conical	1-2	slender
Linhystera sp.	2	1A	elongate/filiforme	< 1	slender
Longicyatholaimus egregius	2	2A	elongate/filiforme	2-4	slender
Neochromadora appiana	2	2A	conical	1-2	slender
Odontanticoma sp.	2	2B	clavate	2-4	slender
Paracanthonchus austropectabilis	2	2A	conical	1-2	slender
Paracanthonchus perspicus	2	2A	conical	1-2	slender
Paracanthonchus platticus	2	2A	conical	1-2	slender
Parachromadorita sp.	3	2A	conical	1-2	slender
Paracyatholaimus helicellus	2	2A	conical	1-2	slender
Paracyatholaimus sp.	2	2A	conical	1-2	slender
Paramonohystera longicaudata	2	1B	elongate/filiforme	1-2	slender
Parapinnanema harveyi	3	2A	conical	2-4	slender
Perspiria sp.	3	2A	conical	2-4	slender
Phanoderma campbelli	4	1B	conical	2-4	slender
Phanoderma serratum	4	1B	conical	2-4	slender
Pomponema sp.	4	2A	clavate	1-2	slender
Pontonema problematicum	4	2B	short/round	> 4	slender
Ptycholaimellus sp.	3	2A	conical	< 1	slender
Sabatiera sp.	2	1B	clavate	1-2	slender
Sabatieria pulchra	2	1B	clavate	1-2	slender
Sabatieria praedatrix	2	1B	clavate	1-2	slender
Spilophorella candida	2	2A	conical	< 1	slender
Spilophorella paradoxa	2	2A	conical	< 1	slender
Spirinia parasitifera	3	2A	conical	2-4	slender
Symplocostoma sp.	4	2B	conical	> 4	slender
Synonchiella micramphis	3	2B	clavate	2-4	slender
Syringolaimus smarigdus	4	2A	conical	< 1	slender
Viscosia abyssorum	3	2B	clavate	1-2	slender
Viscosia meridionalis	3	2B	clavate	1-2	slender

C-p value after Bongers (1990): scale from good (1) to bad (5) colonizers; buccal morphology after Wieser (1953): 1A = selective deposit feeders, 1B = non-selective deposit feeders, 2A = epistrate feeders, 2B = omnivore/predator.

logical trait combinations (*i.e.* richness of BT combinations) using the Spearman Rank Order Correlation with both sites pooled (*i.e.* the 40 sampling units). This was done to link the taxonomic diversity with the functional diversity.

RESULTS

Species richness (α-diversity)

The free-living nematode assemblages had a total of 638 individuals and 61 species belonging to two classes, three orders and 18 families (Table 2). The dominance was strong with only 11 species contributing to the 75 % of the total abundance and one single species (*Euchromadora atypica*) contributing to the 21 %. There were 29 singleton species (*i.e.* those represented by a single individual) constituting almost the 50 % of the total species richness of the assemblages (Table 2).

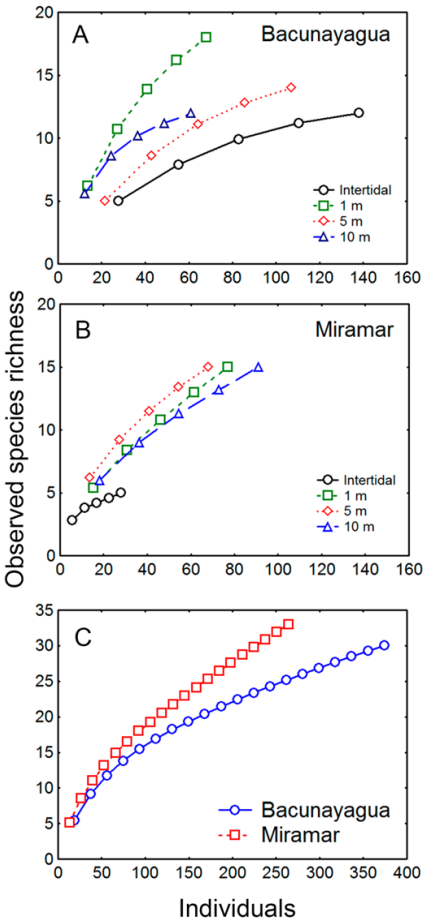

Fig. 1 Species richness (α-diversity) of nematodes. Individual-based species accumulation curves of the observed number of nematode species (Mau-Tau function) at two sites and four depth horizons. (A) Bacunayagua. (B) Miramar. (C) Sites with all depth horizons pooled.

The species accumulation curves did not reach an asymptote in the observed species richness (SR) at any of the sites nor the depth horizons (Fig. 1A and B). This fits with the high number of singletons species that were present in the samples. The shapes of the curves in Bacunayagua were widely divergent across the depth horizons. In contrast, curves for 1, 5 and 10 m horizons in Miramar were relatively similar. The SR of the horizons at both sites were similar ranging from 12 to 18 species (Table 2). However, an exception was the intertidal at Miramar with a marked depletion in richness (5 species). The SR of the sites, *i.e.* all depth horizons pooled, was similar with 30 and 33 species for Bacunayagua and Miramar respectively. The shapes of the accumulation curves for the sites were non-asymptotic suggesting an insufficient sample size (Fig. 1C). The species richness was correlated with the abundance (Spearman $R = 0.60$, $p < 0.001$, $N = 40$).

The macroalgae biomass was poorly correlated with the nematode abundance (Spearman $R = 0.24$, $p = 0.14$, $n = 39$) and was also poorly correlated with the observed SR (Spearman $R = 0.43$, $p = 0.006$, $n = 39$).

Diversity variation (β-diversity)

The diversity variation was higher between the intertidal and the other three subtidal horizons; the lowest values of diversity variation occurred between the deepest horizons (*i.e.* 5 m and 10 m). (Fig. 2A). Miramar

Table 2. Abundance and species richness of nematode at two sites (Bacunayagua and Miramar) and four depth horizons. Each cell represents the sum of five sampling units. IT = intertidal.

	Bacunayagua				Miramar				Total
Species	IT	1 m	5 m	10 m	IT	1 m	5 m	10 m	
Euchromadora atypica	96	9	26	4	0	0	0	0	135
Euchromadora gaulica	0	0	0	0	10	4	18	39	71
Pontonema problematicum	8	5	2	8	10	17	1	0	51
Euchromadora vulgaris	5	3	20	20	0	0	0	0	48
Chromadora macrolaima	0	0	0	0	0	28	7	1	36
Croconema cinctum	0	8	14	7	0	0	0	0	29
Phanoderma serratum	0	9	12	4	0	0	0	0	25
Acanthopharynx rigida	0	3	12	9	0	0	0	0	24
Draconema sp.	0	0	0	0	0	1	13	9	23
Acanthonchus cobbi	0	0	0	0	4	16	0	0	20
Desmodora extensa	0	0	0	0	0	0	2	18	20
Spilophorella candida	3	9	0	3	0	0	1	0	16
Paracanthonchus austropectabilis	0	0	0	0	0	0	12	2	14
Desmodora pontica	0	0	0	0	0	0	5	6	11
Enoplus brevis	10	0	0	0	0	0	0	0	10
Paracanthonchus platticus	0	5	3	2	0	0	0	0	10
Symplocostoma sp.	3	2	3	1	0	0	0	0	9
Anticoma filicauda	0	0	0	0	0	2	1	4	7
Longicyatholaimus egregius	0	5	2	0	0	0	0	0	7
Viscosia abyssorum	1	3	2	0	0	0	0	0	6
Enoplus mammillatus	5	0	0	0	0	0	0	0	5
Halichoanolaimus sp.	0	0	5	0	0	0	0	0	5
Cephalanticoma chitwoodi	0	0	0	0	0	1	0	3	4
Eurystomina filicaudatum	0	0	4	0	0	0	0	0	4
Epsilonema crypthamphis	0	0	0	0	0	0	3	0	3
Paracanthonchus perspicus	3	0	0	0	0	0	0	0	3
Paracyatholaimus helicellus	0	0	0	0	3	0	0	0	3
Anaplostoma viviparum	0	1	0	1	0	0	0	0	2
Belbolla californica	0	0	0	0	0	0	0	2	2
Neochromadora appiana	2	0	0	0	0	0	0	0	2
Spilophorella paradoxa	0	0	0	0	0	1	0	1	2
Viscosia meridionalis	0	0	0	0	0	0	0	2	2
Acanthonchus viviparus	1	0	0	0	0	0	0	0	1
Actinonema pachydermatum	0	0	0	0	0	0	1	0	1
Actionema longicaudatum	0	1	0	0	0	0	0	0	1
Anticoma insulae	0	0	1	0	0	0	0	0	1
Aponema torosus	0	1	0	0	0	0	0	0	1
Araeolaimus boomerangifer	0	0	0	0	0	0	0	1	1
Ascolaimus elongatus	0	0	0	0	0	0	1	0	1
Axonolaimus drachi	0	0	0	0	0	1	0	0	1
Choanolaimus sp.	0	0	0	0	1	0	0	0	1
Dichromadora cephalata	0	0	0	0	0	0	1	0	1
Eubostrichus sp.	0	1	0	0	0	0	0	0	1
Filoncholaimus capensis	0	0	0	0	0	0	1	0	1
Hypodontolaimus angelae	0	0	0	0	0	1	0	0	1
Linhystera sp.	0	0	1	0	0	0	0	0	1
Odontanticoma sp.	0	0	0	1	0	0	0	0	1
Parachromadorita sp.	0	0	0	0	0	0	0	1	1
Paracyatholaimus sp.	0	0	0	1	0	0	0	0	1
Paramonohystera longicaudata	0	0	0	0	0	1	0	0	1
Parapinnanema harveyi	0	0	0	0	0	1	0	0	1
Perspiria sp.	0	0	0	0	0	0	1	0	1
Phanoderma campbelli	0	0	0	0	0	1	0	0	1
Pomponema sp.	1	0	0	0	0	0	0	0	1
Ptycholaimellus sp.	0	0	0	0	0	1	0	0	1
Sabatiera sp.	0	1	0	0	0	0	0	0	1
Sabatieria pulchra	0	0	0	0	0	1	0	0	1
Sabatieria praedatrix	0	1	0	0	0	0	0	0	1
Spirinia parasitifera	0	0	0	0	0	0	0	1	1
Synonchiella micramphis	0	0	0	0	0	0	0	1	1
Syringolaimus smarigdus	0	1	0	0	0	0	0	0	1
Total abundance	138	68	107	61	28	77	68	91	638
Species richness	12	18	14	12	5	15	15	15	61
Species richness per site	30				33				

had consistently higher values of variation between horizons than Bacunayagua.

The multivariate composition of nematode assemblages showed clear differences between the two sites on the basis of the species composition as shown by the two clusters of samples in the plot (Fig. 2B). The value of diversity variation between the two sites was low (Chao-Sorensen dissimilarity index = 0.89) supporting the high differences between sites. Indeed, there were only two species shared between the two sites (*Pontonema problematicum* and *Spilophorella candida*) (Table 2).

The ordination of the samples showed a moderate clustering of the samples by depth horizons. The extreme horizons in the depth gradient (*i.e.* intertidal and 10 m) had stronger clustering of the samples within each horizon; whereas the intermediate horizons (*i.e.* 1 m and 5 m) were more dispersed in the plot (Fig. 2C). Except for the species *Enoplus brevis* which occurred only in the intertidal zone, there were no species restricted to a particular horizon. Instead, a high species turnover occurred not only across the depth horizons, but also between replicates within each horizon (Table 3).

Biological traits

The assignment of the five biological traits to each nematode species generated a total of 34 trait combinations. The number of trait combinations was similar across the depth horizons (Fig. 3A). There was a strong dominance with more than 75 % of the total of species belonging to only five trait combinations. Most of the nematode species were bad colonizers (c-p values 3 or 4), epigrowth feeders, with conical tails, 1–2 mm length and with a slender body shape. The nmMDS of the samples did not show clear groups suggesting a large variability in the morpho-functional diversity within horizons and sites (Fig. 3B). The number of trait combinations of nematodes was strongly correlated with the species richness (Spearman R = 0.96, p<0.001,n=40).

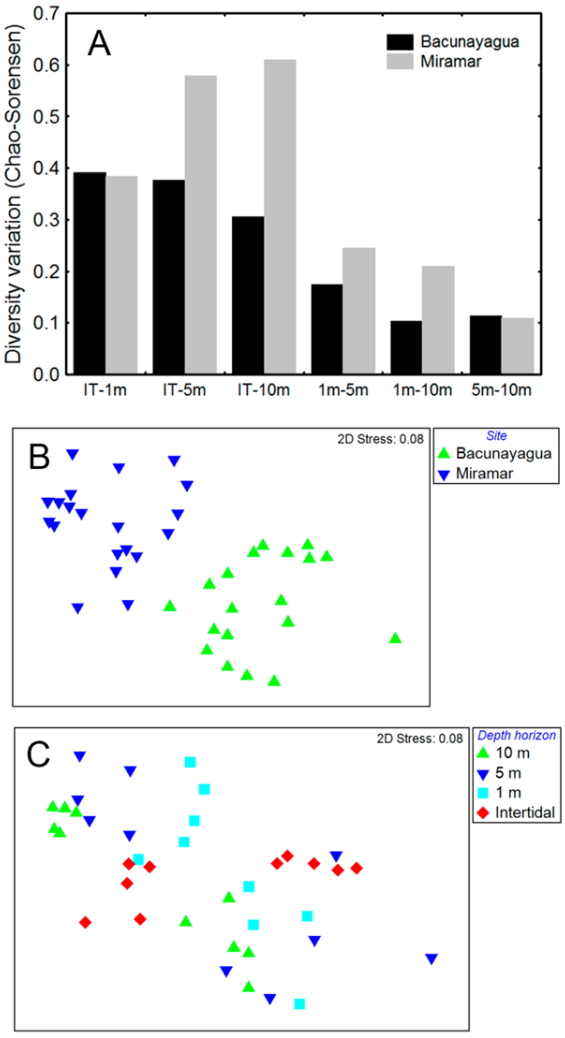

Fig. 2 Pairwise diversity variation between depth horizons. (A) Average of the Chao-Sørensen non parametric estimator of diversity variation at two sites (Bacunayagua and Miramar). Chao-Sørensen is shown as a dissimilarity index (*i.e.* 1–similarity index). (B) Similarity pattern by nmMDS ordination based on species incidence data of nematodes. Samples are coded by sites. (C) The same ordination but samples are coded by depth horizons. IT = intertidal.

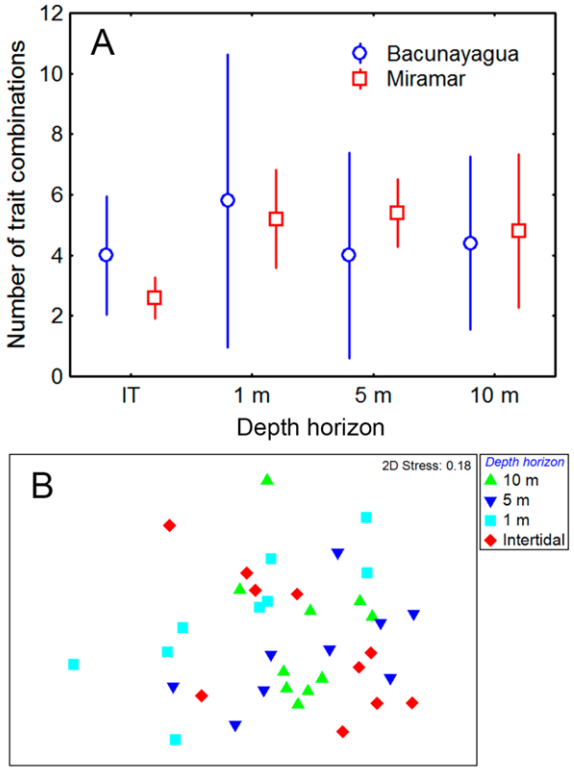

Fig. 3 Biological trait for nematodes at two sites (B = Bacunayagua, M = Miramar) and four depth horizons. (A) Richness of combinations (average ± 0.95 CI). (B) nmMDS ordination based on biological trait data.

DISCUSSION

The temporal differences in sampling years would add an unknown variance that could hamper the interpretation of the spatial patterns. However, we consider that our sampling design is sufficiently robust to answer the posed specific research questions.

How do α- and β-diversity vary in relation to depth?

The species richness (SR) of nematodes in the present study (61 species) was slightly higher than another study specifically investigating the epiphytic nematode assemblages at the Miramar site (41 species, unpublished results). The SR seems to be an underestimate because of the lack of asymptotes in the accumulation curves and the high number of singleton species detected. Free-living nematode assemblages are quite specious (Boucher and Lambshead, 1995) and more individuals should be collected and identified in order to more accurately assess the α-diversity. Nevertheless, the patterns of β-diversity appear to be more robust to the under-sampling probably because most of the information relies on the relative change of species identity (Cardoso *et al*., 2009). Despite the limitation of the sampling size, we think that some insights can be obtained from the comparisons across horizons and sites based on our results.

The α-diversity scarcely varied at both of the studied spatial scales: At the higher scale the SR was similar between the two sites (33 and 30 species); and at lower scale, the SR was also similar across depth horizons (12 to18 species). The latter finding means that the answer to the proposed question is that in terms of α-diversity there are no significant changes in relation to depth.

The β-diversity was higher between the intertidal horizon and the subtidal ones suggesting that desiccation partially drives the species composition. This implies that the microhabitat (*i.e*. intertidal versus subtidal), and not necessarily the depth horizon, is an important driver of the diversity (Gingold *et al*., 2010). Specifically, the highest diversity variation occurred in the intertidal at Miramar probably because of the direct physical disturbance of the sea-bathers walking on the macrophytes in the intertidal. However, alternative explanations may also hold, such as differences in wave exposure and contamination levels between sites. Therefore, in terms of β-diversity, there were consistent changes related with to the microhabitat and they can be explained by the des-

Table 3. Nematode species that most contributed to the diversity variation (measured as Sørensen dissimilarity index) between pairs of depth horizons. Lower triangular matrix refers to Bacunayagua; upper triangular matrix refers to Miramar. Only the species which contributed to up 50 % of accumulative dissimilarity are listed in each cell. IT = intertidal.

	IT	1 m	5 m	10 m
IT		*Chromadora macrolaima* *Euchromadora gaulica* *Anticoma filicauda* *Acanthonchus cobbi*	*Draconema* sp. *Acanthonchus cobbi* *Paracanthonchus austropectabilis* *Pontonema problematicum* *Chromadora macrolaima*	*Desmodora extensa* *Pontonema problematicum* *Acanthonchus cobbi* *Anticoma filicauda* *Desmodora pontica*
1 m	*Euchromadora atypica* *Enoplus brevis* *Euchromadora vulgaris* *Spilophorella candida* *Symplocostoma* sp. *Paracanthonchus platticus* *Phanoderma serratum* *Pontonema problematicum*		*Draconema* sp. *Paracanthonchus austropectabilis* *Acanthonchus cobbi* *Pontonema problematicum* *Euchromadora gaulica* *Anticoma filicauda* *Chromadora macrolaima*	*Desmodora extensa* *Pontonema problematicum* *Chromadora macrolaima* *Acanthonchus cobbi* *Desmodora pontica* *Euchromadora gaulica*
5 m	*Euchromadora atypica* *Enoplus brevis* *Spilophorella candida* *Euchromadora vulgaris* *Acanthopharynx rigida* *Croconema cinctum* *Viscosia abyssorum*	*Euchromadora atypica* *Viscosia abyssorum* *Croconema cinctum* *Euchromadora vulgaris* *Longicyatholaimus egregius* *Acanthopharynx rigida* *Paracanthonchus platticus*		*Desmodora extensa* *Anticoma filicauda* *Chromadora macrolaima* *Paracanthonchus austropectabilis* *Desmodora pontica* *Draconema sp.*
10 m	*Acanthopharynx rigida* *Pontonema problematicum* *Croconema cinctum* *Euchromadora atypica* *Phanoderma serratum* *Enoplus brevis*	*Acanthopharynx rigida* *Pontonema problematicum* *Euchromadora vulgaris* *Croconema cinctum* *Phanoderma serratum* *Euchromadora atypica*	*Pontonema problematicum* *Acanthopharynx rigida* *Euchromadora atypica* *Croconema cinctum* *Euchromadora vulgaris* *Phanoderma serratum*	

iccation stress on nematodes in the intertidal horizon.

Depth, on coastal rocky shores, correlates with hydrodynamics due to waves (stronger on shallow bottoms) and currents. Dispersal of nematodes occurs at scales of meters to kilometers because the hydrodynamics (Derycke *et al*., 2013) and can override the effects of the microhabitat in the subtidal horizons. Previous research at Miramar, suggested that the epiphytic nematode assemblage was poorly related to the identity and morphology of host macroalgae and more related to the water transport (unpublished results).

At the larger spatial scale, β-diversity was high between the two sites and it could be related to the restricted dispersal of nematode species living tens of kilometers apart. The high diversity variation of nematode assemblages between sites may respond to a mid-scale spatial pattern. These larger-scale patterns would be more general because they are related to historical processes such as extinction and speciation (Konar *et al*., 2009). On the other hand, the low diversity variation between depth horizons is more contingent on chance ecological phenomena such as dispersion and mortality of individuals in response to the microhabitat (Whittaker *et al*., 2001).

Does biological trait analysis provide additional information for the interpretation

of diversity patterns?

There are two important issues to notice in the biological trait (BT) approach. First, the number and types of selected traits strongly affects the ability of the study to detect patterns. Unfortunately, the paucity of external morphological features of nematodes limits the discovery of new BTs. BT analyses for nematodes essentially rely on five traits as exemplified by the most recent studies on the topic (*e.g*. Armenteros *et al*., 2009; 2012; Alves *et al*., 2014).

Second, the reduction of the list of taxa to a list of trait combinations represents a loss of potentially important ecological information (Bremner *et al*., 2003; 2006). However, it provides higher level of the generalization of the explanations and clues about functional responses of individuals to the environment (McGill *et al*., 2006).

The richness and composition of BTs did not add any additional insight to the diversity patterns. However, the interpretation of the dominant BTs suggests that the physical environment strongly drives the assemblage structure. Most of the nematodes had small-sized bodies, buccal structures adapted for epigrowth feeding and conical tails. These BTs possibly maximize ecological success in a dynamic environment where most of the nematodes are epiphytic. For instance, a small body size would increase the capacity to find refuge within the thallus of the plants, conical tails would enhance the fixation to the substrate because they are often coupled with glands secreting sticky substances and the teeth constitute an adaptation for rasping the biofilm. Thus, depth seems to be poorly related to the dominant BTs. Instead, the diversity of nematodes in a site is probably driven by the combination of the kind of microhabitat (Gingold *et al*., 2010) and the hydrodynamics (Netto *et al*., 1999). Based on the previous discussion, the answer to the posed question is that BT analysis provides valuable information for the interpretation of the diversity patterns.

In summary, depth was not an important driver of the diversity of nematodes on the rocky shores both in terms of species richness and diversity variation. Microhabitat and hydrodynamics may explain the β-diversity patterns within sites. The diversity variation between sites largely overrides the depth-related variation suggesting that a regional pattern in β-diversity may be present. The biological traits constituted a useful complementary approach for analyzing diversity patterns indicating the prevalence of nematodes adapted to an epiphytic life-style and the effects of two putative diversity drivers: microhabitat and hydrodynamics.

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