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Spatial distribution of nematode assemblages in Cienfuegos Bay (Caribbean Sea), and their relationships with sedimentary environment

José A. Pérez-García*, Maickel Armenteros*,**, Lisbet Díaz-Asencio***,
Misael Díaz-Asencio***, Alexei Ruiz-Abierno*, Raúl Fernández-Garcés***,
Yoelvis Bolaños-Alvarez*** and Carlos Alonso-Hernández***

Abstract

Spatial distribution of nematode assemblages was studied at six subtidal stations in Cienfuegos Bay, Cuba on September, 2005. The processes of re-suspension of sediment and transport of water masses would explain homogeneity of salinity and temperature across stations; as well the relative high levels of some heavy metals at stations far from the main pollution sources (power station, city). Density and diversity of nematode assemblages were negatively correlated with the concentration of Co and Zn in sediments, but the influence of hydrodynamic regime prevent to separate natural and anthropogenic causes of this pattern. The high recorded levels of Ni did not have apparent effects on nematodes; possible explanations were presence of tolerant assemblages and/or low bioavailability of the metal. The structure of assemblages was typical from soft bottoms around the world and dominant genera were *Terschellingia* and *Sabatieria*. The fine grain size and high organic content in sediments enhanced the dominance of deposit feeder nematodes. The presence of dense mats of benthic microalgae at one station, as result of eutrophication, possibly caused the observed increase in the percentage of epistrate feeders. Vertical distribution of nematodes showed a general decrease of density and diversity along depth within sediment; thiobiotic species occurred in all strata. The complex spatial pattern in the vertical distribution suggests migration inside sediment due to physical disturbance and/or predation.

Introduction

In any study about marine sediments, free-living nematodes are of interest due to (Heip et al. 1985, Danovaro et al. 2004): comprise between 60-90 % of total faunal abundance in estuarine systems (up 10^6 animals · m^{-2}), present high diversity of species (reflecting diversification in trophic requirements), contribute to the flux of energy through ecosystems, and spend all their

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* Centro de Investigaciones Marinas, Universidad de La Habana, 16 # 114, CP 11300, Playa, Ciudad Habana, Cuba

** Corresponding author; Marine Biology Section, Department of Biology, Ghent University. Krijgslaan 281/S8, B-9000, Gent, Belgium; e-mail: maickel.armenteros@ugent.be; maickel@cim.uh.cu

*** Centro de Estudios Ambientales de Cienfuegos, Ministerio de Ciencia, Tecnología y Medio Ambiente. AP 5, Ciudad Nuclear, Cienfuegos, Cuba

life cycle in sediments (being good indicators of sedimentary quality). Despite of their importance, comparatively few research papers (e.g. Alongi 1987a,b, 1990, Ndaro & Ólafsson 1999) have been published on ecology of free-living nematodes in tropical systems.

The distribution patterns of free-living nematodes and the causes that determine them are key information in order to understand the ecology of communities and their role in the dynamics of the ecosystems. There is little evidence of the existence of a "superfactor" (in the sense of Snelgrove & Butman 1994) as grain size or organic content of sediment causing these distributional patterns of nematofauna. Instead, nematodes appear to respond to a complex setting of abiotic factors where, in particular sites, some of them could be more important than others; e.g. food availability (Danovaro & Gambi 2002), salinity (Forster 1998, Moens & Vincx 2000), and grain size (Ward 1975, Schratzberger et al. 2004).

The vertical zonation of nematodes within sediment is an important component in the analysis of the spatial distribution of assemblages. Most meiofauna appear in the upper four centimetres of sediment, and density tends to decay along depth (Fleeger et al. 1995, Soltwedel 1997). The vertical distribution of diversity and density of nematodes is related with penetration of dissolved oxygen (Coull 1999), which depends closely on the physical properties of sediment particles (e.g. porosity, grain size). However, other authors (e.g. Moodley et al. 2000, Tita et al. 2001) state that oxygen penetration has a limited direct effect on vertical distribution of nematodes, and they concede more importance to availability of trophic resources.

There is a vast literature that analyze the effects of pollutants on meiofauna communities (e.g. Coull & Chandler 1992, Austen et al. 1994, Fichet et al. 1999, Lee et al. 2001, Lee & Correa 2005, Gyedu-Ababioa & Baird 2006). In general, nematodes appear to be more resistant to heavy metal pollution than other groups of the meiofauna (Heip et al. 1985). They show particular abilities to acumulate Cd, Cu, Pb and Zn present in the sedimentary environment and constitute a significant path for transference of heavy metals from sediment to living resources through the food web (Fichet et al. 1999). Particularly, the nematode genera *Theristus*, *Microlaimus*, *Paramonohysthera* and *Sabatieria* are common inhabitants of sediments polluted with heavy metals (Lampadariou et al. 1997, Gyedu-Ababioa & Baird 2006).

Wider Caribbean is an extensive tropical area characterized by high biodiversity and a mosaic of marine habitats like muddy flats, mangroves and coral reefs. Very few researches (e.g. Boucher & Gourbault 1990, Gamenick & Giere 1994) have been published on nematofauna of this region. Therefore, basic and descriptive studies (i.e. baseline) are necessary in order to generate hypothesis on particular ecological traits of nematode assemblages and to evaluate of effects of pollutants in the area.

Whence the objectives of this study were: i) to describe the vertical and horizontal distributional patterns of nematode assemblages; and ii) to infer the biotic and abiotic ecological settings that would explain the found patterns in Cienfuegos Bay.

Materials and methods

Cienfuegos Bay is located in the centre-southern portion of Cuba, at 22°09'N and 80°27'W. The bay has 88 km^2 of area and has a shallow subtidal ridge (mean depth 1.5 m) dividing it in two basins: a northern (where main point sources of impact occurred), and the southern (Fig. 1). The bay is subject to a moderate human impact; the main impact sources are: the city of Cienfuegos (around 150 000 inhabitants and concentration of industries), a power station, and an oil refinery. Four rivers (Damují, Salado, Caunao and Arimao) are the main sources of organic matter and suspended solids to the bay.

On the basis of an extensive previous study at 16 subtidal sampling stations in four months, six stations with predominance of mud and temporal stability in abiotic factors (i.e. salinity, organic content and silt/clay fraction) were selected for the present study (Fig. 1). Four cores (60 ml and 6.6 cm^2 surface) of sediment were taken in each station for the description of meiofauna assemblages to main taxa and immediately preserved in 4 % buffered formalin. From each station (except 15), two cores (= replicates) were divided in the following vertical strata: 0-1, 1-2, 2-4 and 4-6 cm; and their nematodes were identified to putative species. For the analysis of horizontal distribution, all vertical strata pertaining to a corer were summed in order to estimate the assemblage inhabiting in a whole column of sediment (i.e. 0-6 cm). As result, estimates of density of main

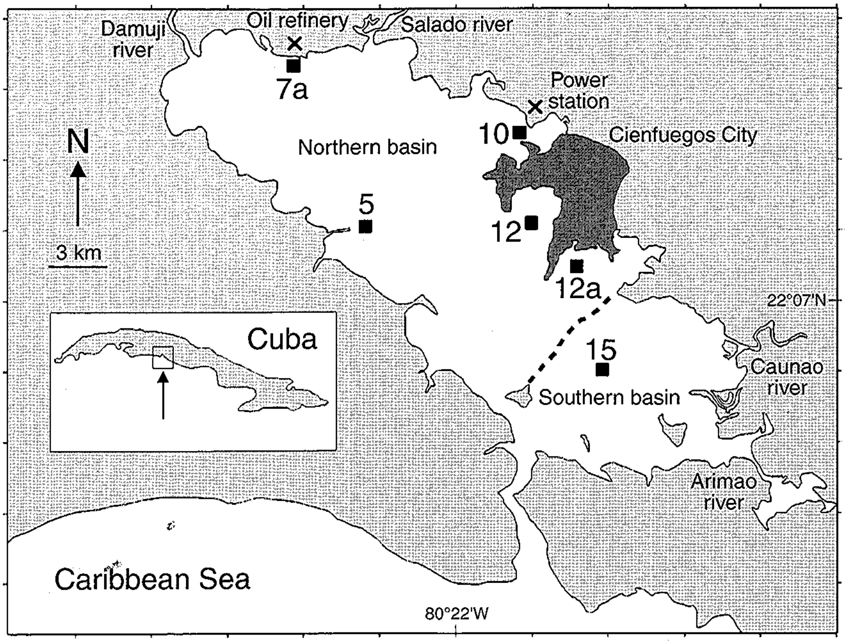

Fig. 1. Map of Cienfuegos Bay. Sampling stations and main sources of human disturbance are indicated. The subtidal ridge dividing the two basins within the bay is showed with a dotted line.

taxa were based on four cores and number of species of nematodes on two cores. Three cores of sediment were taken, in each station, for the determination of grain size (expressed as percentage of silt + clay), content of organic matter and concentration of heavy metals (Co, Ni, Cd, Cu, Hg and Zn) respectively. In situ, measures of interstitial temperature (1 cm depth) and salinity were carried out using a mercury thermometer and a hand-held refractometer respectively.

Sediment samples for meiofauna were sieved by 500 and 45 µm; the fraction retained in last sieve was subjected to the extraction process of meiofauna using a high density solution of sugar (1.16 g · cm^{-3}) after Heip et al. (1974). The sediment and the sugar solution were mixed in a vortex apparatus using five-six centrifuge tubes (50 ml) (Burgess 2001); this step was repeated by three times. The supernatant was poured on 45 µm sieve, washed, stored in a flask, stained with 1 % alcoholic eosin and preserved with 4 % buffered formalin.

Meiofaunal specimens were identified under a stereomicroscope Zeiss (56× maximum) to high taxonomic level (i.e. copepods, nematodes). The first 100 nematodes, if possible, were picked and mounted in glycerine for the identification to genus level using the pictorial keys of Platt & Warwick (1983, 1988) and Warwick et al. (1998). The silt/clay fraction was determined by wet sieving through a 63 µm sieve to separate the sandy and fine (silt plus clay) fractions. The organic matter content was determined by the Walkey-Black modified method (Loring & Rantala 1992). Briefly, the sediment samples were oxidized with $K_2Cr_2O_7$ and H_2SO_4, and then chemical titration was carried out in order to determine the quantity of readily labile organic matter (the method excludes compounds such as graphite and coal).

For the total trace metals determination (Co, Ni, Cd, Cu, and Zn), wet sediment samples were sieved with a 63 µm plastic sieve and freeze-dried. Around 0.25 g of dry sediment were weighed and then totally digested in a heat plate using 65 % HNO_3 (p.a. ISO Merck), 40 % HF (p.a. ISO Merck) and 60 % $HClO_4$ (p.a. Analar BDH). Metal concentrations were determined by flame atomic absorption using an Avanta 3000 (GBC) Atomic Absorption Spectrometer with air/acetylene flame and autosampler. The determination of total mercury concentrations was carried out in the Advance Mercury Analyser AMA-254 (AAS technique at 253.65 nm), controlled by WinAMA software (detection limit: 0.01 ng).

Abiotic data were subject to a correlation-based principal component analysis (PCA) in order to look for combinations of abiotic variables that would represent environmental gradients across stations. The coefficient of correlation by ranks of Spearman was calculated between each pair of variables in order to detect association between them. Data of structure of nematode assemblages were subject to univariate and multivariate analysis. One way analyses of variance were applied to univariate measures of assemblages (i.e. density and number of species). Data were transformed in order to fulfill assumptions of parametric statistic; and the results were checked with diagnostic graphics (mean vs variance; predicted vs fixed variable). Tests of hypothesis were done with untransformed and transformed data; in case of agreement of results, untransformed data were presented for an easier interpretation. When analyses of variance showed significant differences, a post-hoc multiple comparison of means (Tukey's HSD test) was carried out to looking for among which stations the differences exist.

The non parametric permutation-based test (ANOSIM) was performed to detect differences in multivariate structure of assemblages. Data of density of nematode species were transformed as square root in order to downweight the contribution of dominant species to similarity matrix. The similarity index of Bray-Curtis was used for construct the similarity matrix among samples. This matrix was used both in a SIMPER test to determinate the percentage of contribution of nematode genera/species to similarity within stations and in the procedure BIOENV (Clarke & Warwick 2001) in order to detect correlations between similarity matrix of samples (based on transformed density data) and similarity matrices derived of subsets of abiotic variables.

Results

Abiotic variables. In general, "natural" abiotic variables showed high homogeneity across the six sampled stations (Table 1). The mean values of heavy metals concentration average by the six sampled stations are reported also in Table 1. Concentrations of Cd were below the detection limit (1.73 µg g^{-1}) of the analytical procedure. The variability of metal concentrations in sediments across stations was high, with coefficients of variation ranging from 20 to 40 %. Measured values of concentrations of the five heavy metals were not correlated among them.

An ordination by principal component analysis (PCA) was carried out including variables % S/C; % OM; and the five metals in order to describe possible abiotic gradients in sediments

Table 1. Abiotic variables measured in sediments from Cienfuegos Bay. T, temperature; D, depth; S, salinity; S/C, silt+clay; OM, organic matter; concentrations of heavy metals in µg g^{-1}

Station	T (°C)	D (m)	S (‰)	% S/C	% OM	Co	Ni	Cu	Hg	Zn
5	30.4	12.4	30	63.0	4.2	20.8	89.3	73.2	0.10	154.6
7a	30.2	9.9	33	98.1	4.0	14.5	68.3	68.3	0.11	144.1
10	31.0	3.6	32	96.8	3.8	12.2	125.9	114.6	0.45	144.4
12	30.6	9.8	37	97.9	2.9	17.3	58.8	94.9	0.47	111.4
12a	28.8	3.6	34	79.1	4.5	10.8	36.2	156.9	0.11	87.4
15	30.0	14.4	36	82.4	3.4	25.5	62.4	118.9	0.11	158.3
Mean	30.1	9.0	33.6	86.2	3.8	16.9	73.5	104.5	0.23	133.4

Table 2. Coefficients for each abiotic variable making the first three principal components (PCs). Cumulative explained variance (EV): 85 %; S/C, silt+clay; OM, organic matter.

PC	% EV	% S/C	% OM	Co	Ni	Cu	Hg	Zn
1	35	0.2	0.4	0.3	0.4	−0.4	0.3	0.5
2	33	0.5	−0.3	−0.4	−0.05	0.2	0.6	−0.4
3	18	−0.04	0.6	−0.5	0.6	−0.1	0.03	−0.2

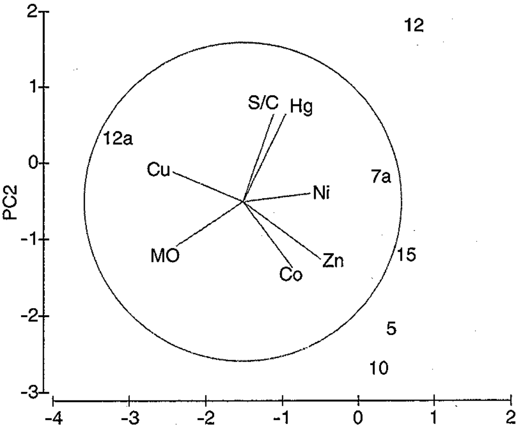

Fig. 2. Ordination plot by principal component analysis (PCA) on basis of abiotic variables measured in six stations in Cienfuegos Bay.

across stations. There was not a clear grouping of stations (= samples) in the plot (Fig. 2). The PCA explained 85 % of cumulative total variance in its three first axis, and the coefficients for each variable suggest that abiotic gradients (PCs) were constituted by a balanced combination of several variables (Table 2). Also, "natural" (e.g. % S/C) and pollution indicator (e.g. Hg) variables often had similar contribution to a same PC (Table 2).

Horizontal distribution of nematodes. Nematodes constituted around the 99 % of total density of meiofauna in any of the samples; therefore we focused on their assemblages. Significant differences (Table 3) were detected in density of nematodes (as much as two orders of magnitude) among stations; for both untransformed and transformed (as logarithm) data results of significance test were equivalent. The highest density (mean ±0.95 confidence intervals) occurred in station 12a (2 429±1 052 animals 10 cm^{-2}) and the lowest in station 15 (24±16 animals 10 cm^{-2}) (Fig. 3). There were significant differences (Table 3) in untransformed number of species of nematodes among stations. The lowest values of number of species was at station 15 (6 species) and the highest at station 12 and 12a (27 and 25 species respectively) (Fig. 3). The low number of replicates (two) prevented to compute reliable confidence intervals for this measure of assemblage.

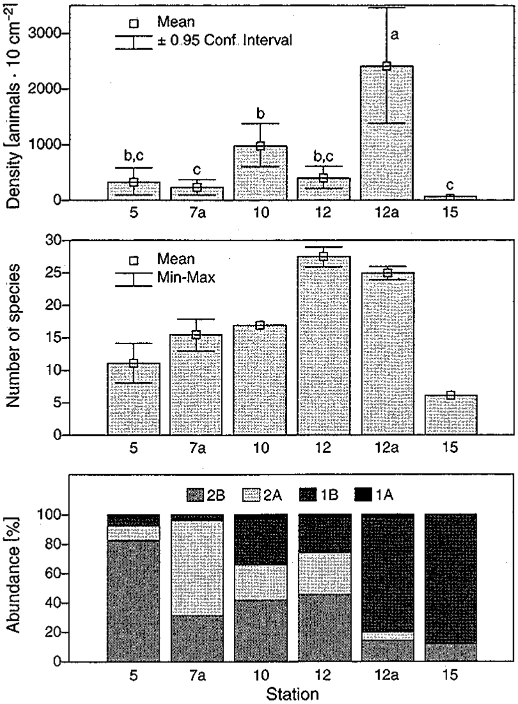

Fig. 3. Density, number of species, and relative abundance by trophic groups of nematode assemblages in six stations in Cienfuegos Bay. Types on the bar show results of post hoc HSD Tukey's test. **1A**, selective deposit feeder; **1B**, non-selective deposit feeder; **2A**, epistrate feeder; **2B**, predator/omnivore.

The hypothesis test (ANOSIM) showed significant differences among stations in multivariate assemblage structure (R=0.83, p<0.001, 999 permutations) using square root transformed data. The nmMDS ordination plot (Fig. 4), on the same similarity matrix, suggested that station 15 had

Table 3. Results of analyses of variance among stations for univariate measures of nematode assemblages. Values of statistic F, probability (p) and degrees of freedom (df) are showed.

variable	F	p	df effect	df error
Untransformed density	34.8	<0.001	5	18
Logarithm-transformed density	73.0	<0.001	5	18
Untransformed number of species	21.6	<0.001	5	6

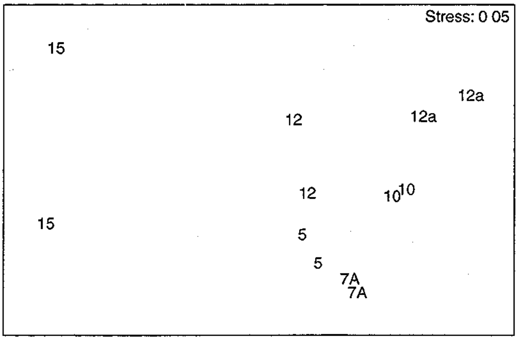

Fig. 4. Non-metric multidimensional scaling ordination plot of samples from six stations based on nematode species density using square root transformed data.

a very different nematode assemblage respect to the others. A remarkable distinctiveness appeared to exist among stations on basis of multivariate structure of assemblage; with replicates from a station clearly separated of the other ones.

The average similarity (measured as Bray-Curtis index) between replicates was relatively high for stations 5, 7a, 10 and 12a (Table 4). The analysis of the contribution of nematode species to similarity within each station suggested that there were broadly distributed species such as *Terschellingia longicaudata* de Man 1907; *Sabatieria pulchra* de Man, 1907; *Spirinia parasitifera* Bastian, 1865; and *Aponema* sp. 1. The station 15 showed different species composition with dominance of *Aponema* sp. 1, *Vasostoma* sp. and *Hopperia* sp. (Table 4). The dissimilarity among stations was given by differences in the density of the aforementioned abundant species and by the presence of other rare species as *Dorylaimopsis* sp., *Synonchiella* sp., *Paramonohystera* sp., *Viscosia* sp., *Metachromadora pulvinata* Wieser & Hopper, 1967, and *Metacyatholaimus chabaudi* Gourbault, 1980 (Table 4).

Vertical distribution of nematodes. The absolute values of density integrating the whole column of sediment showed strong differences among stations (e.g. station 7a vs. 12a) (Fig. 3). Therefore, in order to do not add the across-station variance to variability among vertical strata (i.e. two-way comparison) the signification tests were performed independently for each station. There were differences among vertical strata in density data (transformed as fourth root) after ANOVA (probabilities, in brackets) for stations 5 (0.05), 10 (0.004) and 12a (0.05); however the non – significant results (i.e. for stations 7a and 12) were strongly determined by a low statistical power (<0.6 for any of the two comparisons) due to low number of observations (n=2) in each stratum. Results of significant tests on non-transformed data were only slightly different, but statistical power was less than 0.6 again. The figure 5 shows a clear pattern (except in station 7a), with lower density of nematodes in deep layers (>2 cm depth) in comparison with surficial ones.

Significant differences (p<0.01) in number of

Table 4. Percentage of contribution of nematode genus/species to similarity within stations on basis on SIMPER procedure. The average total similarity (in percentage) between replicates for each station is showed in the heading of the table.

	5	7a	10	12	12a	15
Species	72.1	77.2	79.4	53.3	67.2	36.5
Terschellingia longicaudata	48	20	14	23	6	–
Sabatieria pulchra	15	32	15	9	–	–
Spirinia parasitifera	9	–	10	9	14	–
Metalinhomoeus sp.	–	7	–	5	–	–
Terschellingia sp. 1	–	6	–	–	5	–
Aponema sp. 1	–	–	14	7	29	33
Terschellingia gourbaultae	–	–	13	–	7	–
Metachromadora pulvinata	–	–	8	–	6	–
Metacyatholaimus chabaudi	–	–	–	8	–	–
Terschellingia communis	–	–	–	7	–	–
Aponema torosus	–	–	–	5	–	–
Terschellingia sp. 2	–	–	–	–	4	–
Vasostoma sp.	–	–	–	–	–	33
Hopperia sp.	–	–	–	–	–	33

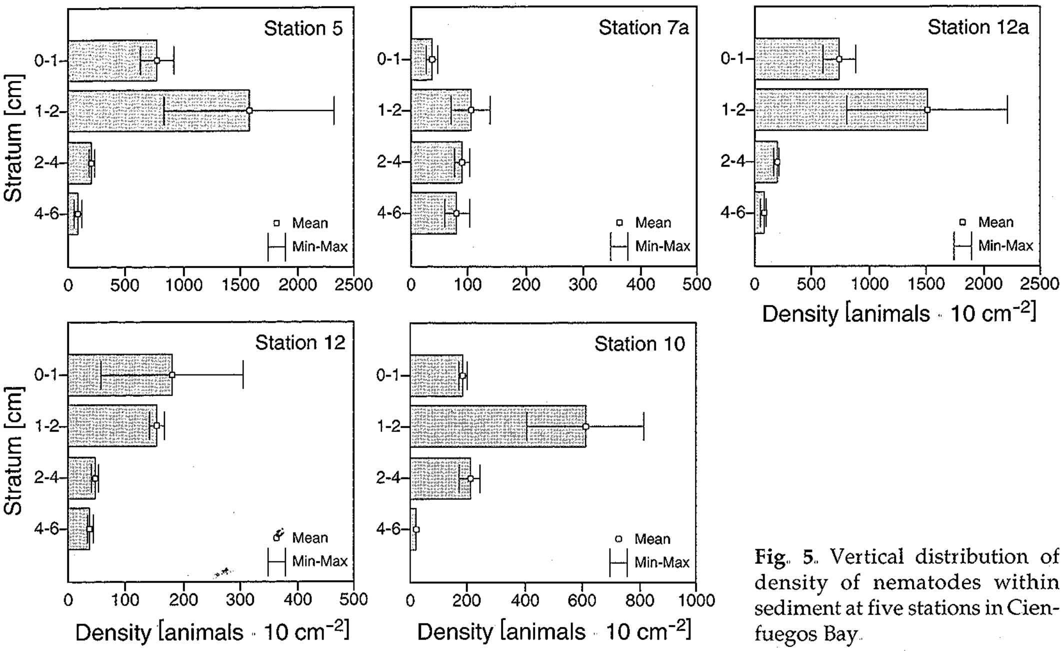

Fig. 5. Vertical distribution of density of nematodes within sediment at five stations in Cienfuegos Bay.

species after ANOVA (probabilities, in brackets) were found for all the stations, except 5 ($p=0.29$). A reduction of the number of species appeared to exist in deeper strata in comparison with the superficial ones for all stations except the station 5 (Fig. 6).

ANOSIM hypothesis tests were applied to the multivariate structure of nematode assemblages for each station independently in order to test differences among strata with square root transformed data. There were significant differences among strata only for stations 5, 7a, and 10 (Table 5). An nmMOS ordination plot (Fig. 7) showed that main differences in assemblage struc-

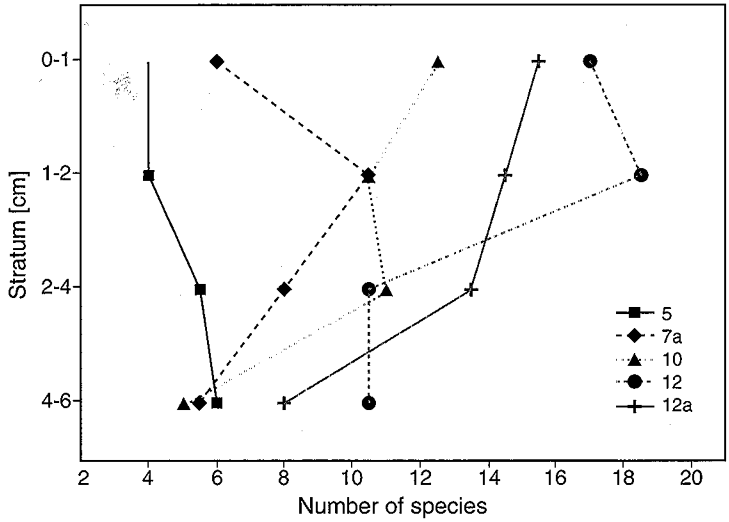

Fig. 6. Mean values ($n=2$) of number of species of nematodes by stratum within sediment at five stations in Cienfuegos Bay.

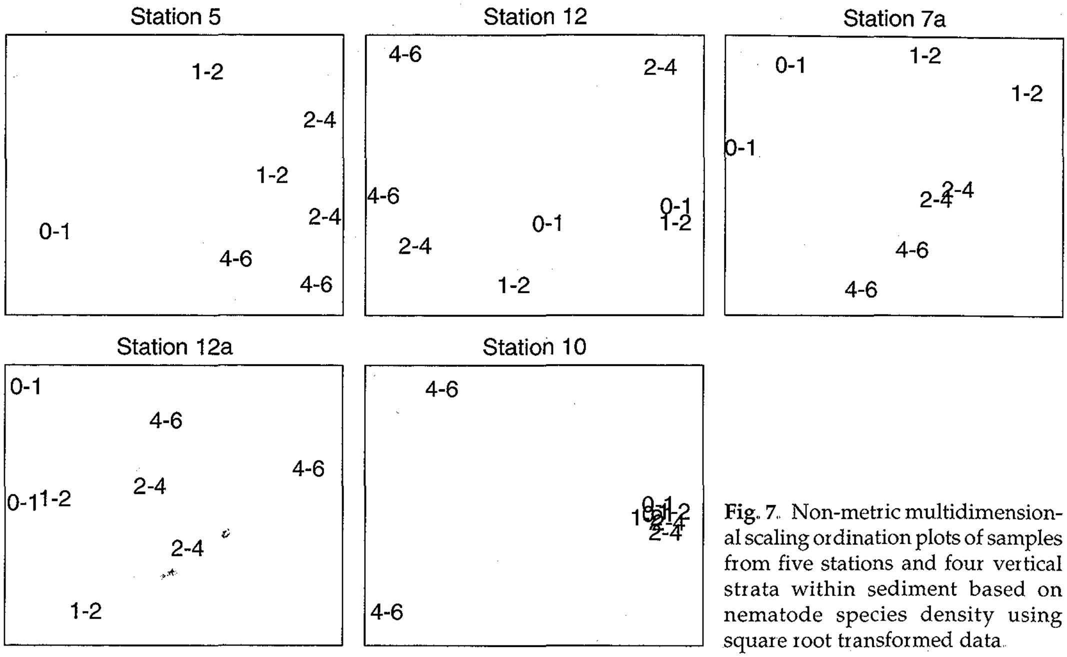

Fig. 7. Non-metric multidimensional scaling ordination plots of samples from five stations and four vertical strata within sediment based on nematode species density using square root transformed data.

ture from stations 5, 7a and 10 occurred among surficial strata (0-1, 1-2 cm) and the deeper ones (2-4, 4-6 cm).

Linking environment to biotic patterns. The exploration of relationships between abiotic and biotic variables on basis of the absolute values of coefficient by ranks of Spearman should be more reliable than formal test of statistical significance due to low number of observations. Heavy metals Co and Zn presented high negative correlation with density and number of species (Table 6); also Cu presented high positive correlation with density. Multivariate procedure (BIOENV) for linking abiotic to biotic patterns showed the highest values of Spearman rank correlation (in brackets) with the combination of the variables Co and Zn (0.48).

Table 5. Results of the global analysis of similarity (ANOSIM) testing for differences among vertical strata in sediments for five stations on basis of multivariate structure of nematodes assemblages.

	5	7a	10	12	12a
R values	0.59	0.77	0.42	–0.21	0.38
Probability	0.02	0.01	0.02	0.71	0.09

Discussion

Abiotic variables. A considerable mixture of water masses within the bay appears to exist due to tidal currents and waving; this result in a basin with low spatial variability of salinity (variation <7 ‰) and temperature (variation <2 °C). However, there are evidences of abrupt decrease of salinity after rainfalls (resulting in

Table 6. Values of the coefficient of correlation by ranks of Spearman between mean values of univariate measures of nematode assemblages and abiotic variables. S/C, silt+clay; OM, organic matter. Number of observations = 6.

	% S/C	% OM	Co	Ni	Cu	Hg	Zn
Density	–0.15	0.6	–0.73	–0.31	0.77	–0.07	–0.79
Number of species	0.40	–0.1	–0.70	–0.33	0.30	0.52	–0.89

stratification of water column); also permanent changes of salinity occurred in areas close to mouth of rivers and creeks (Seisdedo & Muñoz 2004, Seisdedo 2006).

The concentration of metals in sediment were within the range reported at the bay in 2001 (S. Pérez, unpublished data) and in the range proposed by Long et al. (1995) as causing occasional or possible effects on fauna. The spatial distribution of heavy metals did not show a clear pattern across stations neither association among them. This suggests the presence of point and diffuses sources of certain heavy metals (e.g. Ni and Hg) in the bay; and the existence of a complex pattern of transport and sedimentation of bounded-sediment pollutants.

Horizontal distribution of nematodes. Nematode assemblages, in Cienfuegos Bay, were mainly composed by few dominant species belonging to the families Microlaimidae, Linhomoeidae, Comesomatidae and Desmodoridae; and more than 10-12 rare species. This assemblage structure is typical from muddy bottoms around the world (Heip et al. 1985, Pallo et al. 1998). Organic content in sediment was relatively high in all stations suggesting an organically enriched environment with high quantity of available food for nematodes; unfortunately, quality of detritus could not be assessed. The dominance of detritivore nematodes in all stations, except in 15, point out that soft-bottoms in the bay are detritus-based systems.

The station 12a had the highest values of organic content in sediment caused possibly by productivity of a dense layer of cyanophytes on the bottom (Armenteros, pers. observation). A process of eutrophication, as response to the input of nutrients from a highly impacted coastal lagoon, appears to support a high standing stock of benthic microalgae. The higher food availability represented by surface mats of cyanophytes would support the high abundance of epistrate feeder nematodes in this station, since the pools of organic matter in sediment is the main source of energy for trophic web of meiofauna (Tita et al. 2001). However, a relative increase of epistrate feeder nematodes (genera *Hopperia* and *Vasostoma*) occurred also at station 15 without an evident organic enrichment suggesting more than one trophic mechanism in nematode assemblages.

No any correlation could be found between any measure of meiofaunal community and the Ni concentration; in addition, high concentrations of Ni in sediments (with no detected negative effect on meiofauna) have been reported (Armenteros, unpublished) in areas adjacent to mines of this metal. Two main causes could explain this pattern: i) that fauna is adapted to them because high levels of Ni occur many time ago (at least for copper, tolerance of some species of nematodes has been demonstrate by Millward & Grant (1995)) and; ii) Ni has a low potential bioavailability due to strong association with silicate matrix of sediment (Hatje et al. 2006).

The negative correlation between densities of organism, number of species and the concentration of Zn and Co is not a surprise. Previous field (Somerfield et al. 1994) and laboratory studies (Gyedu-Ababioa & Baird 2006) has reported than nematodes assemblages have a high sensibility to those metals.

Vertical distribution of nematodes

A concentration of nematodes in the top four centimetres in subtidal sediments has been reported by several authors (e.g. Fleeger et al. 1995, Moodley et al. 2000, Tita et al. 2001). In Cienfuegos Bay, there were a depletion of density and number of species of nematodes into depth inside sediments; however, their relationship with vertical gradients in oxygenation or food availability is hard to state in our study. Presence of H_2S (personal observation) and high quantity of organic matter in sediments suggest the existence of hypoxic/anoxic environment; and therefore, a nematode assemblage characterized by thiobiotic species. The vertical distribution of these assemblages of nematodes probably is responding not only to classical vertical gradients (i.e. oxygen and H_2S) but to the migration by physical disturbance or predation. This results in a complex spatial pattern of the assemblages across the column of sediment.

After Soetaert et al. (1994), the differences among strata in nematode assemblages are related to horizontal distribution patterns; i.e. broadly-distributed species are inhabitants from surface strata and they possess enhanced dispersion. In opposite, species from deeper strata have a limited horizontal dispersion and they are characteristics from particular sites/stations. However, the distributional patterns in our study did not support this statement. The most wide-distributed species across stations were *Terschellingia longicaudata* and *Sabatieria pulchra* and they occurred in all strata. Also, rare species from particular stations (e.g. *Halalaimus* sp., *Longicyatholaimus capsulatus*

Vitiello, 1970, *Neotonchus* sp., and *Neochromadora* sp.) did not show restriction to deeper strata; instead they tended to appear in surface layers.

In summary, the presence of heavy metals (Co and Zn) could have a toxic effect on nematodes, reducing the density and the number of species. The structure of assemblages was typical from soft bottom around the world and it was dominated by species *Terschellingia longicaudata* and *Sabatieria pulchra*. Deposit feeder nematodes were dominant in almost stations, but presence of dense mats of benthic microalgae would be related to an increase of percentage of epistrate feeders in assemblages. Vertical distribution of nematodes showed a general decrease of density and diversity into depth with thiobiotic species present in all strata; the complex spatial pattern in the vertical distribution suggest migration inside sediment due to physical disturbance and predation.

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