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ECOLOGY AND TAXONOMY OF FREE-LIVING MARINE NEMATODES FROM CIENFUEGOS BAY, CARIBBEAN SEA

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SUMMARY

Present thesis focuses on ecology of assemblages and taxonomy of free-living marine nematodes. Most of the data are from Cienfuegos, a semi-enclosed bay in the Caribbean Sea; but, we also provided data on biodiversity from other areas in Cuban marine waters. Four main topics are included: description of biodiversity patterns, a microcosm experiment about effects of organic enrichment on assemblages, a taxonomic revision of the genus *Terschellingia* de Man, 1888, and the description of four new genera for science.

Spatial and temporal biodiversity patterns of free-living marine nematodes were studied in Cienfuegos Bay, a tropical semi-enclosed basin in the Caribbean Sea. Taxonomic (to species level) and functional (biological trait) approaches were applied for describing the assemblage structure and relating it to abiotic environment based on a sampling scheme in six subtidal stations and three months. Biological trait approach added relevant information to species pattern regarding relationships between diversity patterns and the abiotic environment. The most common trait combinations were deposit feeding nematodes, with intermediate colonising abilities of 2–3 (in a scale from 1 to 5), tail conical cylindrical or filiform and body slender; and their abundance were correlated with depth, organic matter and silt/clay fraction. The number of trait combinations and the number of species was highly correlated suggesting that the increase of biodiversity can lead to potential increase of functional diversity. Chemical pollution (organic enrichment and heavy metals) and hydrodynamic regime possibly drove the biodiversity patterns. The spatial distribution of assemblages is in agreement with the previously supposed existence of two well differentiated basins inside the bay, the northern basin more polluted than the southern one. The low hydrodynamic regime would determine a poor dispersion of nematodes resulting in high spatial variance in the assemblage structure; and also the associated hypoxic conditions and pollutants in sediments can explain the dominance of tolerant nematode species such as *Daptonema oxycerca*, *Sabatieria pulchra*, *Terschellingia gourbaultae*, and *Terschellingia longicaudata*. A comparison of spatial–temporal patterns of biodiversity between Cienfuegos Bay and other semi-enclosed bays in temperate regions suggests several similarities: nematode assemblages are strongly influenced by anthropogenic disturbance, temporal trends are weak or overridden by spatial ones, and few cosmopolitan genera/species tolerant to pollution and hypoxic conditions are dominant

Marine nematodes from subtidal tropical sediments in Cienfuegos Bay were subjected to additions of phyto-detritus (microalgae *Spirulina*) in a microcosm experiment. The follow up of the experimental conditions was measured at days 0, 4, 15 and 30. Observed effects on the nematodes were a decrease in abundance and diversity, and changes in the taxonomic and trophic structure due to the organic enrichment. The results suggested that the nematodes were not food limited in the microcosms and probably neither in their natural environment. The main factor affecting the nematodes was probably the byproducts (hydrogen sulphide and ammonia) due to enhanced bacterial development in microcosms. Hypoxic conditions occurred in all experimental units, as well in the field suggesting a nematode assemblage adapted to naturally enriched sediments. However, tolerant (dominant) species showed a grade of sensitivity to reduced conditions, in increasing order: *Spirinia parasitifera, Terschellingia longicaudata, Metalinhomoeus filiformis,* and *Sabatieria pulchra*. We predict that further organic enrichment in sediments from Cienfuegos Bay may cause a phase shift into a strongly depleted benthic fauna and reduced conditions in water and sediments.

The cosmopolitan and often ecologically dominant genus *Terschellingia* (Nematoda: Linhomoeidae), with 39 nominal species, is taxonomically a problematic taxon. Its species show high morphological plasticity, possess few diagnostic characters and identification keys are lacking. A revision of the genus was carried out based on morphological and morphometric data from the literature and from observations of specimens collected in Cienfuegos Bay, Caribbean Sea, Cuba. The diagnosis of the genus *Terschellingia* is amended. Of the current 39 nominal species, 16 are considered as valid species based on morphological characters related to size and position of amphidial fovea; presence and position of cephalic and cervical setae; presence, size, and shape of pharyngeal bulb; shape of spicular apparatus and shape of tail. Tabular and pictorial keys were provided based on these characters. Three sympatric species: *T. communis*, *T. gourbaultae* and *T. longicaudata* were redescribed based on recently collected Cuban specimens. Each of them showed relatively large differences in body size in comparison with the respective type specimens, suggesting possible variation due to local environmental differences. The highest intraspecific variation pertains for the most widely spread cosmopolitan species *T. longicaudata*, suggesting that morphological plasticity enhanced adaptation to different environmental conditions. The notable taxonomic inflation

within the genus (14 species inquirenda, 9 junior synonyms), probably also present in other highly specious genera of marine nematodes, can lead to an overestimation of the alpha-diversity.

Four new free-living marine nematode genera and species are described: *Cienfuegia cachoi* gen. nov., sp. nov. (Xyalidae, Chromadorida), *Guitartia tridentata* gen. nov., sp. nov. (Xyalidae, Chromadorida), *Macrodontium gaspari* gen. nov., sp. nov. (Microlaimidae, Chromadorida), and *Pseudoterschellingia ibarrae* gen. nov., sp. nov. (Linhomoeidae, Monhysterida). For each species, detailed morphological descriptions, drawings and photos are provided, tabular keys were built and relationships with other genera within each family are discussed.

The following general discussion is focused on five topics: scientific novelties of the research, evaluation of techniques for environmental assessment, coupling between distribution patterns in the nature and microcosm experiments, ecological characterization of the four new species and biodiversity of free-living marine nematode in Cuban marine waters. Future research avenues are presented covering both experimental ecology and molecular taxonomy. Two appendixes are included regard to an ecological study of meio- and macrofaunal assemblages in Havana bay; and a taxonomic checklist of nematodes recorded by the author and colleagues from Cuban marine waters.

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CHAPTER 1
GENERAL INTRODUCTION AND AIMS

1.1 Background

1.1.1 Generalities

Free-living nematodes play an important role in the structure and functioning of marine ecosystems (Heip et al., 1985; Snelgrove et al., 1997; Gray and Elliot, 2009) and constitute in terms of abundance a major component of the meiobenthos in soft bottoms (Higgins and Thiel, 1988). The rich populations of nematodes inhabitant in all soft marine sediments on the Earth can substantially affect ecological processes such as regeneration of nutrients, transfer of energy to higher levels in the benthic food webs and bioturbation of sediments (Giere, 2009). Thus, the understanding of the structure and functioning of benthic ecosystems encourages the investigation of the nematode assemblages.

The ecology and taxonomy of free-living marine nematodes cover a huge amount of information mainly related to temperate ecosystems. Recently, very comprehensive reviews on biology of meiobenthos (Giere, 2009) and ecology of soft sediments (Gray and Elliot, 2009) have been published; both works highlight the synthesis of current knowledge in the ecological field and discuss the avenues for future research.

Nematologists now focus on research topics in the mainstream of ecology such as: latitudinal patterns of biodiversity (e.g. Mokievsky and Azovsky, 2002; Gobin and Warwick, 2006), links between taxonomic diversity and functional traits (e.g. Schratzberger et al., 2007), and ecological factors driving the structure of assemblages (e.g. Hua et al., 2009). The more recent techniques in taxonomy (e.g. DNA barcoding, 4D microscopy and video vouchering) have been applied to nematodes to disentangle phylogenetic relationships within the phylum (e.g. Meldal et al., 2007), genetics of populations (e.g. Derycke et al., 2005; 2008) and identification and discrimination of species (e.g. Bhadury et al., 2008; Fonseca et al., 2008). Relatively few research results have been published regarding studies in

tropical areas; the latter are scarce and application of recent and costly techniques is still limited.

1.1.2 Diversity of marine nematode assemblages

Within the marine environment, free-living nematodes are recognized as one of the most abundant and diverse metazoan taxa (Boucher and Lambshead, 1995). They are the most abundant animal group in marine soft bottoms, and often are the only persistent taxon in heavily polluted/stressed habitats (Coull and Chandler, 1992). The diversity of nematodes, assessed as number of species, remains controversial because hundreds of species can coexist in a few square centimeters, but many of them show a global distribution. One of the main handicaps for global estimation of biodiversity patterns is that the fauna is still poorly known (Mokievsky and Azovsky, 2002) and many new species remain undiscovered. Global estimates of number of known and described species of free-living marine nematodes are so far in the range of 4 000 – 5 000 species (Eyualem-Abebe et al., 2008); while global estimated total (unknown + described) species numbers vary between 10 000 to 20 000 species (Mokievsky and Azovsky, 2002), and up to more than 1 x 10^6 species (Lambshead, 1993; Snelgrove et al., 1997). However, the diversity estimates based only on morphological criteria probably provide an underestimation of the real biodiversity of the phylum Nematoda due to existence of cryptic diversity in some specious taxa of nematodes such as *Rhabditis* (*Pellioditis*) (Derycke et al., 2008) and *Terschellingia* (Bhadury et al., 2008).

Currently, three main issues are going on to increase the accuracy of estimates of biodiversity of marine nematodes. First, there is an increase of the sampling effort in habitats previously poorly studied such as deep waters, tropical muddy bottoms and polar areas. Secondly, initiatives for the barcoding of nematodes are taken and promissory results have been published already (e.g. De Ley et al., 2005; Bhadury et al., 2006; 2008). DNA barcoding can add valuable information in order to disentangling the diversity in highly specious nematode genera which are problematic to assess based only on external morphology. However, till now very few information have been published on this topic (e.g. *Rhabditis (Pellioditis) marina* species complex in Derycke et al., 2005; 2008). In the third place, the operative

global databases about biodiversity (e.g. Global Biodiversity Information Facility, GBIF) can enhance the exchange between researchers and access to information necessary for global assessments (Gewin, 2002). For marine nematodes, the NeMys database (http://nemys.ugent.be) is the most comprehensive record of species; it is connected to global biodiversity databases such as GBIF (http://www.gbif.org) and OBIS (http://www.iobis.org). These are on-line open access databases providing data about biodiversity to the international community with the goal of making this information available for scientific and conservation purposes.

The existence of a latitudinal species diversity gradient, with higher species diversity in tropical areas, has been detected for several groups of biota and explanatory models have been proposed with regard to plants, fungi, vertebrates and marine invertebrates (Jablonski et al., 2006). Those latitudinal gradients are rather common in the marine environment, although for benthic small fauna they tend to be weak (Hillebrand, 2004). However, tropical marine regions do not have higher biodiversity of free-living nematodes compared to subtropical or temperate regions (Boucher, 1990; Boucher and Lambshead, 1995; Gobin and Warwick, 2006). The general lack of fossil records of nematodes hampers any evolutionary explanation of this observation.

On the basis of current data on the biodiversity of free-living marine nematodes the type of biotope is the main determinant of the diversity; with deep-sea biotopes (bathyal and abyssal) being the most diverse (Boucher and Lambshead, 1995). Therefore, there is no expectation of higher biodiversity of nematodes in tropical semi-enclosed bays in comparison with temperate counterparts; although this comparison has not been done before. In addition, caution should be exerted since biodiversity data from these ecosystems are related to α- or β-diversity (i.e. in a single point of sampling or a single habitat) instead of γ-diversity that is more appropriate for global comparisons of diversity (Gray and Elliot, 2009). Estimates of α- or β-diversity are mainly affected by the habitat and factors occurring in ecological time scales; these estimates can lead to wrong interpretations of broad patterns of species richness (Gray, 2000). Only with a large number of samples covering a broad spatial scale the real patterns of biodiversity can be estimated and the evolutionary causative mechanisms identified (Gray, 2000).

1.1.3 Distribution patterns of nematode assemblages

The review on the ecology of marine nematodes by Heip et al. (1985) covers much of the general principles of distribution of nematodes, although most of its information comes from temperate regions. The more recent review by Giere (2009) constitutes an update of the current knowledge on patterns of distribution of meiofauna in general. The patterns of distribution of benthic fauna are strongly depending on the scales at which sampling is performed and also on the particular type of habitat studied (Gray and Elliot, 2009). Therefore, extrapolation of general conclusions from studies elsewhere is limited, and convokes high uncertainty in the predictions about biodiversity and distribution patterns at local scales and in poorly studied regions.

Research on the ecology of nematode assemblages in tropical ecosystems has been mostly restricted to environments such as coral reefs and mangroves (see reviews by Alongi, 1989a; 1989b; 1990). In subtidal muddy tropical basins considerably less effort has been made, even when these systems cover extensive areas of seabed and often are subject to highest human impact due to harborage, industrial and urban activities (MacCracken et al., 2009).

The complex interaction between hydrodynamic regime and physical and chemical properties of sediments is the main determinant of distribution of meiofauna in soft bottoms (Snelgrove and Butman, 1994; Giere 2009). These ecological factors can change in spatial scales of few centimeters determining one of the most striking aspects of the distribution of nematodes: their high spatial patchiness occurring at cm-scale. The responses of infauna to environmental disturbance at small scales (< 1 m^2) are particularly variable (Zajac et al., 1998) and thus patterns of distribution and diversity of nematodes are difficult to model (Merckx et al., 2009). Additionally, the high spatial variability in the structure of nematode assemblages determines that standard error and confidence limits of estimators is usually large, leading to a critical balance between precision of estimates (directly depending on the size of the sample) and the cost of the sample. Often, the size of the sample is limited by practical considerations related to the cost of processing.

Going back to the ecological factors that drive the distribution of nematodes, hypoxia appears to be particularly important to fauna distribution in semi-enclosed marine systems because of longer residence time of the water and stronger stratification

(Rabalais and Gilbert, 2009). Additionally, the production of hydrogen sulfide and ammonia from bacterial metabolism is associated often to hypoxic condition sediments and it can be an important limiting factor due to toxicity (Gray et al., 2002). The high solar irradiance and temperature during the entire year in tropical environments enhance the stratification of waters and the low dissolution of oxygen, although the high productivity of these areas (Ouillon et al., 2008) can balance this fact by production of oxygen from photosynthesis.

Pollution has significant effects on distribution and biodiversity of meiobenthos (see revisions by Coull and Chandler, 1992; Giere, 2009). Particularly, semi-enclosed marine systems are impacted by pollution on a wide spatial and temporal scale (MacCracken et al., 2009). Therefore, effects of pollution on biodiversity and distribution patterns of nematodes are expected although the type of response is not easily predictable (Merckx et al., 2009).

The patterns of distribution can be described from a point of view of composition of species, but also analyzing the composition by functional groups (Gray and Elliot, 2009). Recently, the distribution of nematode assemblages based on biological traits has been studied (Schratzberger et al., 2007) and promissory results are expected of the application of this approach to the study of ecological patterns. However, main handicaps hamper the application of functional traits; for instance, the widely used feeding type classification by Wieser (1953) suffers some shortcomings due to high feeding selectivity and flexibility of nematodes (Moens et al., 2004). Additionally, key functional traits as metabolic rate or individual biomass are not easily measurable due to small body size of nematodes.

Currently, the interest about the links between biodiversity and ecosystem functioning is increasing (e.g. reviews by Heip et al., 2009; and Reiss et al., 2009). In this context, some studies stress the importance of the diversity of functional groups in ecosystem functioning more than species diversity; but often also because lack of information about the real species diversity patterns. Examples of benthic processes affected by the diversity of functional marine groups are the organic matter mineralization (Norling et al., 2007) and sediment stability and transport (Snelgrove et al., 1997; Ieno et al., 2006). However, patterns of distribution of individual species are still important in order to understand the relationships between biodiversity patterns and ecosystem functioning (Snelgrove, 1999; Waldbusser et al., 2004). We

consider that quantitative distribution patterns are very important to disentangling the effects of the diversity of the phylum Nematoda on ecosystem process; using both taxonomic (species level) and functional composition.

1.1.4 Experimental ecology with nematode assemblages

Since the review by Coull and Palmer (1984) about the experimental work carried out on meiofauna much more research has been done. Use of microcosm experiments is a natural and very comprehensive step for testing in controlled conditions a hypothesis originating from field patterns (Daehler and Strong, 1996; Oksanen, 2001). In case of ecological studies, this is necessary because correlative patterns of distribution of species do not identify the processes causing them (Underwood et al., 2000; Clarke et al., 2008). The small size of meiofauna and the relatively high resistance of their communities to manipulation of the sediments are useful features when the objective is re-creating a model ecosystem for testing hypotheses (Coull and Palmer, 1984).

The disruption of the interstitial environment within the sediment, the homogenization of the distribution (loss of patchiness) and the mortality of sensitive species to mechanical damage and temporal hypoxia are the main handicaps when sediment is collected from nature. Nematodes from organically enriched soft bottoms probably are less affected by these handicaps since they are dominated by few tolerant species with relatively large size and high abundance. The response of nematode species to these transformations is identified as “microcosm effect”. A key point for the validity of the experimental study is that it should be as homogeneous as possible across experimental units and the effects of treatments must be stronger than the effects of previous manipulation of sediments (Austen and McEvoy, 1997).

In spite of “microcosm effect”, nematode communities have been useful for testing effects of a variety of stressors such as xenobiotic compounds (Austen et al., 1994; Austen and McEvoy, 1997; Schratzberger et al., 2002; Hedfi et al., 2007), organic enrichment (Sandulli and Nicola-Giudici, 1989; Webb, 1996; Schratzberger and Warwick, 1998b; Mahmoudi et al., 2005; Gyedu-Ababio and Baird, 2006), sedimentation (Schratzberger et al., 2000a; 2000b) and physical disturbance

(Schratzberger and Warwick, 1998a). Importantly, microcosm experiments have been successfully applied in the study of ecological processes such as colonization (Ullberg and Ólafsson, 2003; Schratzberger et al., 2004). Validity of extrapolation from small-scale manipulative studies to larger scales can be negatively affected due to scale-dependent processes operating in nature (Carpenter, 1996; Ellis and Schneider, 2008). However, organic pollutants appear to be an exception allowing some kind of prediction based on experimental manipulation in small-scale laboratory experiments (Zajac et al., 1998).

The step after experimental work with nematodes in microcosms is to set up field experiments in order to increase the environmental relevance of the predictions and results (Carpenter, 1996); however, the high diversity and tiny size of nematodes constitute a real challenge to attempt manipulations. Successful experimental work with nematodes has been made under field conditions; however, most of these studies constitute manipulations of external agents (disturbance) such as hypoxia (Van Colen et al., 2009), burial (Whomersley et al., 2009) and colonization (Fonsêca-Genevois et al., 2006). To our knowledge, successful manipulations of the diversity and structure of nematode assemblages in order to set up treatments have done only in microcosm experiments both with marine nematodes (e.g. De Mesel et al. 2006) and soil nematodes (e.g. Mikola, 1998; Postma-Blaauw et al., 2005). For instance, the recently arisen interest about the relationships between functioning of ecosystem and biodiversity have been dominated by manipulations of the diversity of macrofaunal assemblages and further measurement of changes in ecosystem responses (e.g. Bolam et al., 2002; Ieno et al., 2006). No meiofaunal analogue have been made in field marine conditions: the task of manipulation of the diversity of meiofaunal assemblages still presents a challenging issue in the ecology of benthic communities.

1.1.5 Taxonomy of free-living marine nematodes

Taxonomy of free-living marine nematodes is a field where research tools and development are ongoing, for instance, with regards to population genetics (Derycke et al., 2005), barcoding (Bhadury et al., 2006; 2008), and video vouchering (De Ley et al., 2005). A relatively solid phylogenetic framework exists for analyses of

nematodes as phylum (Aleshin et al., 1998; Blaxter et al., 1998) and also particularly devoted to free-living marine taxa (Lorenzen, 1994; Meldal et al., 2007).

Despite or as result of the long standing debate existing about advantages and disadvantages of molecular and morphological approach to taxonomy (e.g. Hebert et al., 2003; Will and Rubinoff, 2004; Hebert and Gregory, 2005) a combined approach is emerging in nematode taxonomy. At least partially, it has been the result of particular features of taxonomy of nematodes such as considerable effort and skills required for traditional morphological-based taxonomy and the plethora of cryptic species or complexes of species stressing the interpretation of sequence-based information. The analysis of sequence-based data are an important source of autoapomorphies and consequently for discovery of new species (Nadler, 2002); but the morphological approach still being largely the first comprehensive step for the documentation of biodiversity (e.g. studies by Derycke et al., 2008; Fonseca et al, 2008).

The discovery and characterization of new species demand quantitative description of morphological features as well as information about habitat requirements and natural history (e.g. abundance, other sympatric species, life cycle). The bulky accumulation of DNA sequences without other reference to natural history of the corresponding species is not enough for creating taxonomic knowledge (Ebach and Holdrege, 2005a; 2005b). DNA barcoding has been proposed as useful tool for ecological studies (e.g. Valentini et al., 2008; Johnson et al., 2009). For nematodes, it has been successfully applied in estimates of diversity in soil (e.g. Floyd et al., 2002) and marine ecosystems (e.g. Bhadury et al., 2006); but a right link to already known species is very desirable in order to a appropriate interpretation of the detected patterns.

Many genera of free-living marine nematodes are regarded as cosmopolitan based on morphology (Warwick et al., 1998); but molecular data are necessary to clarify this conclusion since morphological evidence may be misleading in some taxa (Meldal et al., 2007). For example some genera such as *Sabatieria and Terschellingia* have numerous species but the presence of few diagnostic morphological features hampers taxonomic discrimination and is problematic for ecological purposes (Soetaert et al., 1995). A key point for further advance in ecology and taxonomy is to disentangle the taxonomic status of dominant complex taxa.

Taxonomy as a science has been facing a shortage in expertise and funding, this is particularly sensible in developing countries where much of the biodiversity is unknown (Gewin, 2002). Nematology is no exception; and in particular, taxonomic studies of marine taxa are at a disadvantage for funding compared to studies of parasitic or soil nematodes. Nowadays, several capacity building initiatives are in action looking to overcome the taxonomic impediment (Secretariat of the Convention on Biological Diversity, 2005) and the field of taxonomy of nematodes has benefited from these efforts. The development of widely accessible electronic databases is a key issue since taxonomic literature is often dispersed and/or hard to obtain. The Generic Biological Information System NeMys (http://nemys.ugent.be) hosted in the Marine Biology Section at Ghent University is currently the best online tool for access to a very complete record of taxonomic information on free-living marine nematodes.

Although some reports of new nematode species from the Caribbean Sea exist, only two articles (Andrassy, 1973; López-Cánovas and Pastor de Ward, 2006) are devoted specifically to the inventory of the diversity of free-living nematodes in the region. The first study is devoted only to species living in ponds within caves, and the second to species inhabiting in seagrass meadows. Many other habitats in the Caribbean Sea such as mangroves, streams, coral reefs, and deep sea remain unknown in terms of taxonomy and diversity.

1.1.6 Cienfuegos Bay, the ecosystem under study

The site selected for this study is Cienfuegos Bay, a semi-enclosed shallow tropical bay with connection to the Caribbean Sea. The meiofauna in semi-enclosed bays/harbors have been subject of research mainly related to assessment of environmental quality as these ecosystems are largely used for harborage activities and urban settlements and thus subject to human disturbance. Research carried out in these ecosystems (e.g. Lampadariou et al., 1997; Moreno et al., 2008) shows that pollution and seasonal hypoxia are key environmental processes determining the distribution patterns of meiofauna. However, published information about meiofauna and more in particular nematodes from tropical bays is not available. In temperate semi-enclosed bays the free-living nematode assemblages often are dominated by few genera including species tolerant to hypoxia and pollution (e.g. *Terschellingia*

and *Sabatieria*). The km-scale distribution of benthic fauna depends strongly on the location of main sources of pollutants and physiography of the basin (rivers, connection to open sea). The composition of nematode assemblages probably is related to the regional pool of species, but in general the biodiversity tends to be low. There is no previous information about identity of nematode species in tropical semi-enclosed bays.

Cienfuegos Bay is one of the best studied marine ecosystems in the Cuban archipelago. Research has been carried out on hydrology (Seisdedo, 2006), geochemistry of sediments (Alonso-Hernández et al., 2006), circulation (Muñoz et al., 2008), pollution (Tolosa et al., 2009) and meiofauna (Díaz-Asencio et al., 2009; Pérez-García et al. 2009). The system shows seasonal behaviour with rainy season characterized by stratification of waters mainly due to vertical gradients in salinity. There is eutrophication of the waters in Cienfuegos Bay mainly due to runoff of river and creeks that flow through agricultural areas (Seisdedo and Muñoz, 2004; Seisdedo, 2006). The bay is characterized as well by a high rate of primary productivity (Ouillon et al., 2008); both features determining an organically enriched system. The resulting seasonal hypoxia in bottom waters of the deeper areas within the bay probably reduces the diversity of benthic fauna, but only the mentioned two studies on meiofauna supports this statement.

There are two previous studies about meiobenthos carried out in Cienfuegos Bay. The first one describes the analysis of distribution patterns of meiofauna in 16 stations during four months in 2004 and 2005 (Díaz-Asencio et al., 2009). Nematodes were the most abundant taxon but did not show any clear pattern of distribution maybe as a result of low taxonomic resolution and the heterogeneity of studied bottoms. The second research on meiofauna in Cienfuegos Bay described the structure of nematode assemblages in six subtidal stations in 2005; this time nematodes were identified to species level and a clear spatial pattern emerged (Pérez-García et al., 2009). Namely: (i) a dominance of epigrowth feeding nematodes in the south basin to difference of other stations in north basing where deposit feeders were dominant; (ii) a negative correlation of density and diversity with content of heavy metals in sediments; and (iii) a clear gradient in vertical distribution of nematodes within sediments. The main handicap of this study consisted in that the temporal variability could not be assessed; however, the sampling scheme followed in present thesis (i.e. three sampling events in a year) hardly can describe fully the

temporal variability of nematodes assemblages in Cienfuegos Bay. Temporal variability in tropical waters is strongly influenced by meteorological forces which are hard to predict currently (cold fronts in dry season and hurricanes in wet season); this means that periods of the relatively small seasonal changes can be followed by rapid and strong changes (pulse disturbance in the sense of Underwood, 1997) in environmental conditions and therefore by changes in the benthos.

The above studies provided the basic framework upon which present research was developed. They offered some clues for ecological processes driving the distribution patterns of meiobenthos and nematodes. In addition, the increase of anthropogenic disturbance in Cienfuegos Bay mainly due to petroleum-related industry suggested two key points to be highlighted in current research: the effects of pollution on the biological patterns in the benthos, and the evaluation of nematode assemblages as tools for monitoring the environmental quality of the bay.

1.2 General aim

The general aim of this PhD study is to characterize the fauna of free-living marine nematodes in Cienfuegos Bay, Caribbean Sea from an ecological approach (e.g. description of pattern of biodiversity, abiotic limiting factors, and effects of pollution) as well as from a taxonomical one with the inclusion of the description of species new to science and critical review of already known group of species. For this an extensive field study was performed together with a microcosm experiment.

1.3 Specific objectives

(i) To describe the biodiversity patterns of free-living marine nematode assemblages in space and time and to discuss possible causes determining them.

(ii) To test the effects of organic enrichment (phyto-detritus) on nematode assemblages by means of a microcosm experiment.

(iii) To review the genus of free-living nematode *Terschellingia* de Man, 1888 and to propose keys for species identification.

(iv) To describe new taxa of free-living marine nematodes and to develop keys for their identification based on morphological features.

1.4 Outline of the thesis

The thesis consists of two main parts; the first one deals with the ecology of nematode assemblages and includes two linked topics: a) description of biodiversity patterns in Cienfuegos Bay and b) an experimental study about effects of organic enrichment on assemblages using microcosms. On the basis of patterns of biodiversity described from the field sampling we proposed several alternative models which would explain the results. Then, the experimental approach was used for testing in microcosms the effects of organic enrichment on nematodes, one ecological factor which was identified as a possible driver of the biodiversity patterns in the studied ecosystem.

The second part deals with taxonomy, and is based on the collection of samples used for the ecological part. The taxonomic part consists of a revision of the genus *Terschellingia* de Man, 1888, the most abundant genus in the bay. *Terschellingia* is a taxonomically difficult genus because of the high number of poor descriptions and species synonymies. We have tried to clarify the taxonomic status of the 39 nominal species and to provide pictorial and tabular keys for identification of valid species. Four new genera of free-living marine nematodes are proposed in the present thesis: *Cienfuegia* gen. nov., *Guitartia* gen. nov., *Macrodontium* gen. nov., and *Pseudoterschellingia* gen. nov. For each genus, detailed morphometric and morphological characterization and drawings and photographs are provided in order to facilitate identification of species. In addition, a comparison with other genera within each of the respective families is presented and main differences and similarities are discussed.

Two appendices are included in the thesis. The appendix 1 is a research article in a comparable heavily polluted semi-enclosed bay (Havana) and the appendix 2 is a taxonomic checklist of all nematode species identified from Cuban marine waters by the author in collaboration with colleagues at Centro de Investigaciones Marinas, Universidad de La Habana.

CHAPTER 2

BIODIVERSITY PATTERNS OF FREE-LIVING MARINE NEMATODES IN A TROPICAL BAY: CIENFUEGOS, CARIBBEAN SEA

Modified version from the published article:

Armenteros M, Ruiz-Abierno A, Fernández-Garcés R, Pérez-García J.A., Díaz-Asencio L, Vincx M, Decraemer W. 2009. Biodiversity patterns of free-living marine nematodes in a tropical bay: Cienfuegos, Caribbean Sea. *Estuarine, Coastal and Shelf Science,* **85**: 179-189.

Abstract

Spatial and temporal biodiversity patterns of free-living marine nematodes were studied in Cienfuegos Bay, a tropical semi-enclosed basin in the Caribbean Sea. Taxonomic (to species level) and functional (biological trait) approaches were applied for describing the assemblage structure and relating it to abiotic environment based on a sampling scheme in six subtidal stations and three months. Biological trait approach added relevant information to species pattern regarding relationships between diversity patterns and the abiotic environment. The most common trait combinations were deposit feeding nematodes, with intermediate colonising abilities of 2–3 (in a scale from 1 to 5), tail conical cylindrical or filiform and body slender; and their abundance were correlated with depth, organic matter and silt/clay fraction. The number of trait combinations and the number of species was highly correlated suggesting that the increase of biodiversity can lead to potential increase of functional diversity. Chemical pollution (organic enrichment and heavy metals) and hydrodynamic regime possibly drove the biodiversity patterns. The spatial distribution of assemblages is in agreement with the previously supposed existence of two well differentiated basins inside the bay, the northern basin more polluted than the southern one. The low hydrodynamic regime would determine a poor dispersion of nematodes resulting in high spatial variance in the assemblage structure; and also the associated hypoxic conditions and pollutants in sediments can explain the dominance of tolerant nematode species such as *Daptonema oxycerca*, *Sabatieria pulchra*, *Terschellingia gourbaultae*, and *Terschellingia longicaudata*. A comparison of spatial–temporal patterns of biodiversity between Cienfuegos Bay and other semi-enclosed bays in temperate regions suggests several similarities: nematode assemblages are strongly influenced by anthropogenic disturbance, temporal trends are weak or overridden by spatial ones, and few cosmopolitan genera/species tolerant to pollution and hypoxic conditions are dominant.

2.1 Introduction

The description of distribution patterns is still one of the fundamental starting blocks in the ecology of biological communities (Underwood et al., 2000). Current research is largely based on description of assemblages using a taxonomic approach to species or higher taxa. There is other approach to the ecology of communities which is based on biological traits of organisms; latter are defined as measurable properties at individual level that can be used comparatively across species (McGill et al., 2006). Analyses of biological traits of species and subsequent creation of functional groups have been recently introduced in studies focusing on assemblage structure. This approach allows obtaining insight into the functioning of ecosystems (Norling et al., 2007) and reveals additional relationships in assemblages (Schratzberger et al., 2007). Coupling of taxonomic and functional diversity can be a powerful tool in ecological research, although the relationships between them and with ecosystem functioning is still in an explorative field (Stachowicz et al., 2007; Heip et al., 2009; Reiss et al., 2009).

Schratzberger et al. (2007) in a comprehensive study on functional diversity and nematode assemblage structure revealed effects of individual species rather than of functional groups. They detected a positive relationship between number of species and functional diversity. An additional interesting point addressed in our study is if this relationship is also present at smaller spatial scales (i.e. few kilometers within a bay) and in tropical ecosystems. Further, the relationships between taxonomic and biological trait approaches and environment deserve further exploration using new data.

Very little information on structural aspects of biodiversity has been published for tropical areas regarding free-living nematode assemblages (e.g. Boucher and Gourbault, 1990; Boucher and Lambshead, 1995; Gobin and Warwick, 2006). In addition, the nematode assemblages in semi-enclosed bays have been studied only in temperate regions (e.g. Lampadariou et al., 1997; Liu et al., 2008; Moreno et al., 2008).

Semi-enclosed bays are characterized by relatively shallow waters, low hydrodynamics, and fine and organically enriched sediments; in addition, these

habitats are very often affected by anthropogenic disturbances due to urban settlements, harbour activities and industrial development. The link between anthropogenic and natural processes implies that both should be addressed in surveys of biodiversity patterns in these ecosystems. In the context of relationship pollution–biodiversity, free-living nematodes have been identified as valuable bio-indicators to monitor the anthropogenic impact on biodiversity in coastal ecosystems (Moreno et al., 2008; Vanaverbeke and Vincx, 2008).

Studies in Cienfuegos Bay have been carried out about hydrology (Seisdedo and Muñoz, 2004; Seisdedo, 2006), sediments (Alonso-Hernandez et al., 2006), circulation (Muñoz et al., 2008), and benthic communities (Díaz-Asencio et al., 2009; Pérez-García et al., 2009). They suggest a relatively complex coastal system with high spatial and temporal variance in natural processes and clearly influenced by human activities.

In the present study, we describe the spatial and temporal biodiversity patterns of free-living marine nematode assemblages. The investigation was carried out based on samples from a semi-enclosed tropical bay (i.e. Cienfuegos Bay, Caribbean Sea) and by using both the taxonomic and biological trait approach. Based on the design of a quantitative study of nematode assemblages in six selected stations inside the bay we address to answer the following three questions:

(1) Does the biological trait approach provide new interpretable information in comparison to a ‘‘classical’’ taxonomic approach?

(2) Which natural and/or anthropogenic processes determine the biodiversity patterns of free-living nematodes within the Bay?

(3) How different are the biodiversity patterns in a tropical semi-enclosed bay in comparison with those in similar ecosystems in temperate regions?

2.2 Materials and methods

2.2.1 Study site

Cienfuegos Bay is a semi-enclosed bay in the Caribbean Sea (22º10 N, 80º20 W) with 90 km^2 of area and mean depth of 14 m; it is divided by a submerged ridge of 1.5 m depth in northern and southern basins (Fig. 2.1). Most of the subtidal area of the bay is characterized by muddy bottoms with high content of organic matter; although there are other habitats (e.g. seagrass beds, sand flats, mangroves) covering a more limited extension. An extensive previous study on meiofaunal assemblages (to main taxa) in 16 subtidal stations from this bay can be found in Díaz-Asencio et al. (2009).

The circulation in Cienfuegos Bay is driven mainly by tidal currents and to a minor extent by winds (Muñoz et al., 2008). There is a seasonal change in water column with salinity stratification and hypoxic conditions in bottom waters of deepest areas (>10 m depth) in the wet season (May–October). In the dry season (November–April) the water column remains mostly mixed and bottom water is oxygenated (Seisdedo and Muñoz, 2004). Further, the rate of sedimentation is seasonally variable, but relatively high (0.47–0.50 g cm^{-2} y^{-1}) (Alonso-Hernandez et al., 2006).

The anthropogenic sources of pollution are mostly located in the northern basin (e.g. power plant, refinery of petroleum, Cienfuegos city) with two rivers as main point sources of freshwater and sediments. The southern basin does not have any main source of pollutants along the coast but it also has two main rivers discarding into it (Fig. 2.1). Eutrophication exists derived from diffuse sewage discharges close to the city, other human settlements and agricultural activities located around the basin (Seisdedo, 2006). A rise of economic activity in the bay, mostly related to refinery of petroleum and derived industries, increases the potential disturbance on the ecosystem by dredging for enhancing of navigation, chemical effluents and accidental spills.

2.2.2 Sampling strategy

Samples were taken in six subtidal stations characterized by fine and organically enriched sediments, four of them close to pollution sources: Cienfuegos City (12 and 12a), oil refinery (7a), and power plant (10) and two other stations relatively far from pollution sources i.e. in the northern basin (5) and southern basin (15). The six

stations were located in shallow waters (mean depth 9.3 m, range: 3.3–14.3 m); the use of GPS device and the small variations in depth in each of the stations among the three months suggest a reasonable accuracy in the sampling of the same site (Table 2.1). The spatial scale of sampling (i.e. among stations) was in the order of tens of kilometers (shortest distance between two stations: 6 km; largest: 23 km). Three sampling trips were carried out in February, May, and September 2006 (6 stations x 3 months = 18 sampling events).

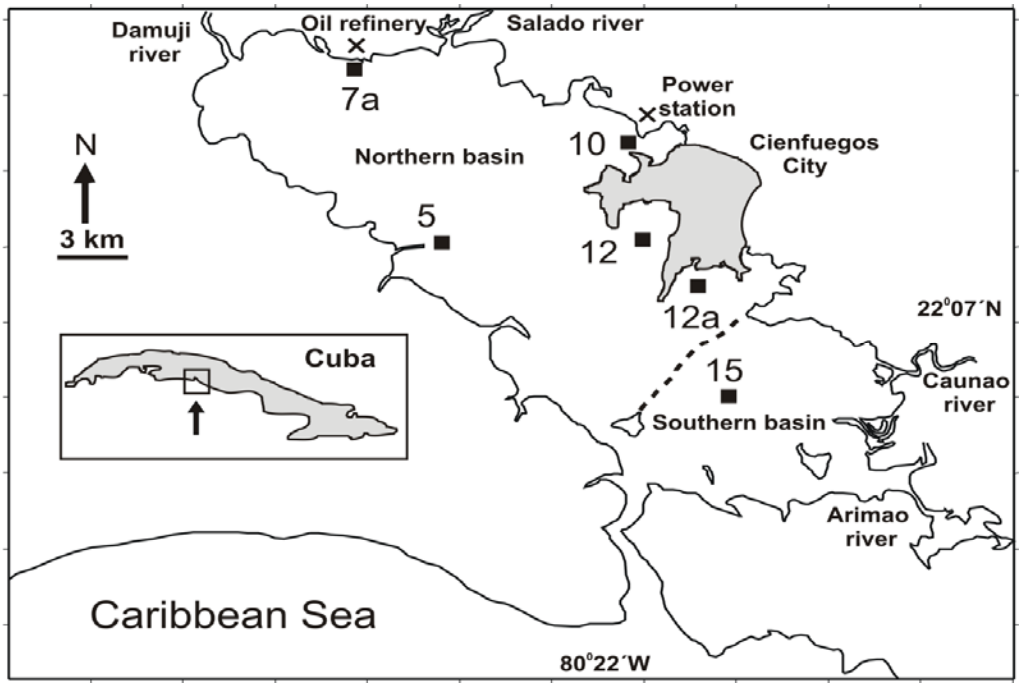

Figure 2.1. Study area in Cienfuegos Bay. The six sampling stations are shown. Submerged ridge between northern and southern basins is indicated with a dashed line. The main point pollution sources and rivers are marked.

Four cores of sediment for meiofauna (2.6 cm inner diameter, surface 5.3 cm^2 and 6 cm deep into the sediment) were carefully taken in each sampling event by SCUBA divers; the four cores were taken at random in plots of 4 m x 4 m approximately. The cores were immediately fixed in buffered 10% formalin. From the four cores taken in February, two were immediately divided in four vertical strata (0–1, 1–2, 2–4, and 4–6 cm) in order to investigate the vertical distribution of meiofauna inside the sediment. In station 12, the strata 2–4 and 4–6 cm could not be sampled due to the fact that sediment below 2 cm was relatively coarse (gravel) therefore coring was not

so effective and we decided not to include these strata. For the February samples, data belonging to strata from a same core were summed to obtain the structure assemblage of the whole column of sediment.

Two cores (3.0 cm i.d. PVC pipes) were taken for determination of abiotic factors in each sampling event. One core was used for determination of salinity in the interstitial water using a portable refractometer Atago (1 unit accuracy); salinity is presented in the text as practical salinity units; the interstitial water was separated from the sediment by centrifugation. The other core was immediately frozen for determination of the content of silt-clay, and concentrations of readily oxidizable organic matter and heavy metals (Cd, Co, Cr, Cu, Ni, Pb, V, Zn). Temperature within sediment (1 cm depth) and depth was determined in situ using a mercury thermometer (0.1 ºC accuracy) and a diving barometer (0.1 m accuracy) respectively.

2.2.3 Sample processing

Samples for meiofauna were sieved through 500 and 45 μm mesh size; material retained on the smallest mesh size was collected. Sorting of meiofauna from sediment was done by flotation technique in high density solution (commercial sugar crystals dissolved in filtered water, 1.17 g cm^{-3}); the efficiency of sorting using this method is around 95% (Armenteros et al., 2008). Sorted meiofauna was stained with alcoholic eosin 1% and preserved in buffered formalin 4%. Meiofauna was observed under stereomicroscope (maximum magnification 36x); the animals were identified to higher taxonomic level (e.g. nematodes, copepods) and counted. The first 100 nematodes (if present) were picked out, mounted in microscope slides (Vincx, 1996) and identified to putative species using a microscope (maximum magnification 1000x). The pictorial keys of Platt and Warwick (1983, 1988), Warwick et al. (1998) and the NeMys Database (Deprez et al., 2007) were used for the identification of species.

The content of silt -clay was determined by ‘‘silt content–dry sediment’’ method after Bale and Kenny (2005). By this method the silt-clay fraction was separated on a 63 mm sieve and the retained sediment was weighted for determination of the loss of sediment. The amount of readily oxidizable organic matter in the sediment was

determined by the modified Walkley–Black wet titration method (Loring and Rantala, 1992) using oxidation with $K_2Cr_20_7$ and H_2SO_4. The concentration of heavy metals was determined, after acid total digestion (nitric and sulphuric acids during 48 h), by flame atomic absorption spectrometry using an Avanta 3000 (GBC) Atomic Absorption Spectrophotometer with air/acetylene flame and autosampler. All used reagents were pure for analysis.

2.2.4 Biological traits of the nematode assemblages

Each nematode species was classified into four different biological traits based on its morphological and functional features:

(1) Feeding types based on morphology of buccal cavity. Wieser (1953) classified the free-living nematodes into four feeding types: selective deposit feeder (1A), non-selective deposit feeder (1B), epigrowth feeder (2A) and omnivore/predator (2B). In addition, ratio of percentage of 1B and 2A types (i.e. 1B/ 2A) was calculated since it can be an indicator of pollution (Lambshead, 1986).

(2) Life strategy. Bongers (1990) and Bongers et al. (1991) proposed a scale (c-p score) to classify the genera of nematodes upon their ability for colonising or persisting in a certain habitat. The scale range is defined from extreme colonisers (c-p score = 1) to extreme persisters (c-p score = 5). Also, a maturity index (MI) can be calculated for each habitat/station based on c-p scores of inhabiting species using the formula (Bongers et al., 1991):

$$MI = \sum_{1}^{s} (v\ x\ f)$$

where s = number of species, v= the c-p value of taxon i and f = the relative frequency of that taxon.

(3) Tail shape. Thistle et al. (1995) proposed a classification (may be linked to locomotion mode) of deep-sea nematodes on basis of tail shape into four types: rounded (1), clavate-conicocylindrical (2), conical (3), and long (4).

(4) Body shape. We used the operative classification by Schratzberger et al. (2007) who recognized three body shape categories: stout (length–width ratio< 18), slender (length–width ratio 18–72), and long/thin (length–width ratio> 72).

2.2.5 Data analysis

Multi- and univariate techniques were used for data analysis using the software PRIMER 6.0.2 (Clarke and Gorley, 2006) and STATISTICA 6.0 from StatSoft. Statistical differences among stations and months were tested by two-way crossed analysis of variance (ANOVA) for the quantitative information of the nematode assemblages: density, number of nematode species, Simpson dominance index (λ´), maturity index and proportion of epigrowth feeding type. We chosen this feeding type for statistical analysis because the classification into selective or non-selective deposit feeder involves a higher uncertainty (i.e. depend of the relative size of buccal cavity); and regarding to predator/omnivore feeding type the abundance was very low. Data were checked for fulfillment of parametric assumptions using diagnostic graphics (mean vs variance, residuals vs mean); when needed, data were transformed and re-checked to ensure that transformation improved the distribution. When the results of ANOVA were significant, post-hoc multiple comparisons of means (Student–Newman–Keuls, SNK) were carried out.

Four univariate measures of assemblages were analyzed in order to test differences in the vertical distribution of nematodes; the variables were density, number of nematode species, maturity index and proportion of epigrowth feeding type. There is a problem of non-independence of the observations because assemblages from a same core probably are related across vertical strata. We applied a split plot partially nested ANOVA in order to minimize the effects of the dependence of the samples. We used as main crossed factors station and strata; and cores were nested within station. Stations 12 and 15 were excluded from analysis because only two strata could be sampled in the station 12 and one replicate was lost on the station 15. Data were checked for fulfillment of parametric assumptions (including sphericity) and when needed, data were transformed and re-checked to ensure that transformation improved the distribution. Post-hoc comparisons were not performed for the analysis

of vertical distribution due to low replication (n = 2). Relationships between measurements of assemblages and abiotic factors were tested by the Pearson's product–moment correlation. Abiotic variables were untransformed due to they did not show positive trends between mean and variance; univariate measurements of assemblages were transformed with the same scale which was used for ANOVA.

Statistical differences in multivariate structure of assemblages among samples were tested by two-way crossed analysis of similarity (ANOSIM) using a similarity matrix; the Bray-Curtis coefficient was used as measurement of similarity. Data of density of nematode species were transformed as fourth-root in order to reduce the contribution of dominant species to the similarity matrix. An ordination plot of samples using non-metric multidimensional scaling was performed on the same matrix. Principal component analysis was carried out for the ordination of samples and variables on basis of abiotic factors; the latter were normalized due to different units of measurement. Relationships between multivariate structure of community and subsets of abiotic factors were determined by BIOENV procedure, using Spearman's correlation between similarity matrices.

2.3 Results

2.3.1 Abiotic environment

The values of abiotic factors measured in the study are shown in the table 2.1. Temperature showed a clear temporal change, with the interstitial water in February (dry season) colder than in May and September (wet season). Salinity was stable also across stations, with lowest salinity in February compared with May and September. The content of silt + clay and organic matter was high for all stations, the variation was relatively small (CV = 18 and 23% respectively), and correlation between both variables was low ($r = 0.29$, $p > 0.05$, $n = 18$). Presence of hydrogen sulphide was apparent in sediments of the deepest stations (5, 7a, and 15) as indicated by the strong characteristic smell of gas. The visual inspection of sediment color indicated that the oxidized layer of sediment was only few millimeters thick,

suggesting that the presence of free oxygen was limited to an even narrower sediment horizon.

The levels of cadmium and lead in sediments were below the limits of detection (2.1 and 27.8 mg kg^{-1} DW respectively). The concentration of heavy metals in the sediments was different among stations, with relatively small temporal changes in the same station. Nevertheless, these spatial trends were not the same for all metals (Fig. 2.2): nickel, vanadium and zinc showed a similar trend, with highest values in station 10 (close to thermoelectric plant power), and lowest values in station 12a. Chromium showed a different trend, with highest values in stations 5 and 7a; and cobalt and copper showed the highest values in station 15.

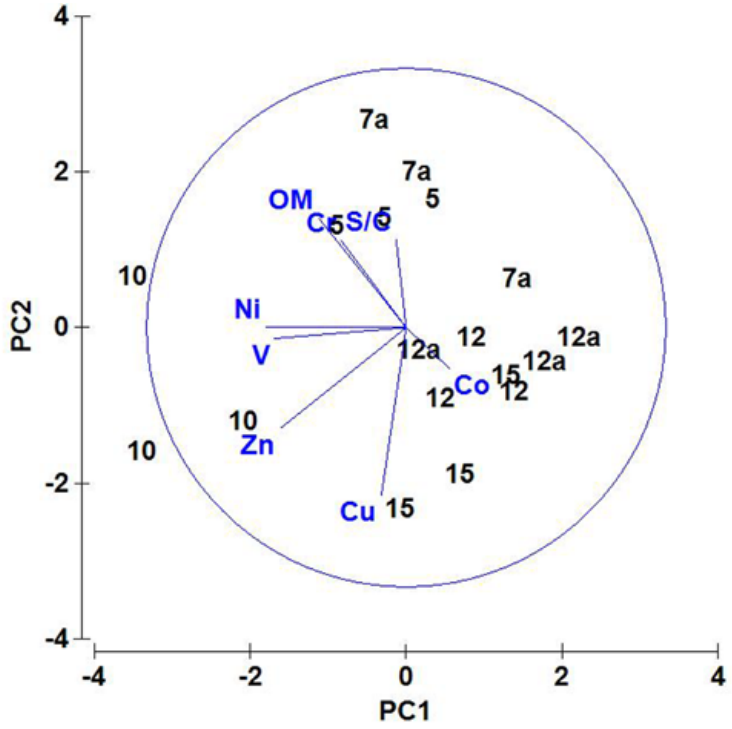

Figure 2.2. Principal component analysis based on sedimentary abiotic factors measured in six stations and three months. The percentage of explained variance by two first PCs: 57 %. OM = organic matter, S/C = silt + clay. The equation of axes: PC1 = - 0.54Ni - 0.51V -0.48Zn – 0.34OM – 0.25Cr + 0.17Co – 0.10Cu -0.04S/C. PC2 = - 0.65Cu + 0.42OM -0.39Zn + 0.34S/C + 0.34Cr – 0.16Co – 0.04V + 0.003Ni. Circle represents the vector unit.

Table 2.1. Abiotic factors measured in six stations and three months in Cienfuegos Bay, Caribbean Sea. D = depth, T = temperature, S = salinity, S/C % = percentage of silt + clay, OM = readily oxidizable organic matter content. Units for OM and heavy metals are mg kg^{-1} DW sediment. T and S were measured in the interstitial water.

Station	Month	D (m)	T (ºC)	S	S/C %	OM	Co	Cr	Cu	Ni	V	Zn
5	February	12.4	24.0	34	70	44.5	15.5	141.8	62.5	97.7	141.9	151.4
	May	11.8	28.0	38	97	48.4	15.0	111.6	66.9	105.2	106.7	108.2
	September	12.8	30.4	39	95	30.9	14.1	108.5	60.0	65.3	114.8	107.4
7a	February	11.3	24.2	33	96	49.5	15.6	110.3	68.9	87.7	124.5	121.6
	May	10.3	28.2	37	95	54.7	13.8	91.8	55.1	90.9	96.0	92.6
	September	12.5	30.4	39	97	30.9	16.1	92.4	61.7	55.0	110.2	100.8
10	February	5.4	24.2	32	96	47.0	9.7	103.0	117.9	135.3	339.2	244.0
	May	3.3	29.0	36	56	35.4	10.0	69.9	90.6	146.6	305.4	199.6
	September	5.7	31.0	38	86	66.9	7.7	64.5	78.7	100.6	733.6	175.8
12	February	10.2	24.2	34	83	31.9	7.0	48.3	95.6	47.6	88.7	114.0
	May	9.0	30.2	31	90	37.0	15.3	76.1	100.2	71.3	103.4	141.0
	September	7.0	30.8	38	58	39.3	12.6	63.0	72.1	41.1	109.5	154.6
12a	February	4.3	24.6	34	57	40.4	14.1	80.3	87.5	78.7	102.6	143.8
	May	5.5	29.6	37	85	33.9	10.3	42.7	83.4	35.7	95.7	106.6
	September	5.9	30.8	38	79	38.3	9.7	32.8	79.3	18.1	60.9	97.8
15	February	14.3	24.8	34	75	28.8	23.2	80.5	115.2	89.7	138.2	184.3
	May	11.7	29.2	39	97	33.9	27.5	81.9	116.7	64.2	147.7	145,0
	September	13.9	30.8	38	98	48.5	25.8	55.4	96.5	43.4	94.8	118.8
Mean all samples		9.3	28,0	36	84	42.1	14.6	80.8	83.8	76.3	167.4	139.3

2.3.2 Density, number of species and dominance

Seven meiofauna taxa were collected in the three sampling events: Nematoda, Copepoda, Polychaeta, Decapoda, Sipuncula, Ostracoda, and Kynorhyncha. The Nematoda counted for 98 % of total density of meiofauna, therefore the main effort was devoted to their ecological patterns. Copepods ranked second in abundance, with low abundance in station 12 (mean ± SD of three months: 22 ± 21 ind. 10 cm^{-2}; range: 2 – 55 ind. 10 cm^{-2}) and practical absence in other stations.

The density of nematodes was significantly different among months, stations and in the interaction after two-way ANOVA indicating that temporal trends are not consistent across stations (Table 2.2 and Fig. 2.3). Post hoc comparison could not detect clear differences between combinations station – month. The mean ± SD values (range) of density of nematodes over all samples were 765 ± 772 (21 – 4596) individuals 10 cm^{-2}.

The number of nematode species was significantly different among stations; however, the factors month and interaction station x month were not statistically significant in their effect on number of species (Table 2.2). Consistently, station 12 had the highest number of species in the three months (Fig. 2.3) (SNK, $p< 0.05$); the post hoc multiple comparisons could not detect differences among the other five stations (SNK, $p > 0.05$). The mean ± SD (range) value of number of species over all samples was 12 ± 5 (4 – 26) species 10 cm^{-2}.

The Simpson index of dominance was significantly different among stations; but factors month and interaction were no significant (Table 2.2). The station 5 showed the highest values of dominance (SNK, $p < 0.05$); other stations did not show differences in the dominance after post hoc comparison (SNK, $p > 0.05$). The mean ± SD (range) value of dominance index over all samples was 0.3 ± 0.1 (0.06 – 0.7).

Table 2.2. Results of analyses of variance on univariate measures of nematode assemblages. Line separates two designs. Upper, two way crossed ANOVA testing differences for station ($F_{5, 53}$), month ($F_{2, 53}$), and interaction ($F_{10, 53}$). Below, split plot partially nested ANOVA testing differences for vertical strata ($F_{3, 12}$), station ($F_{3, 4}$), and their interaction ($F_{9, 12}$); the sources of variation used as error are not shown. MS = mean square, p = probability. Significant results in bold type.

Measurement	Factor	MS	F-ratio	p-value
Density (log-transformed)	**Station**	**12,1**	**23,4**	**< 0,001**
	Month	**2,4**	**4,7**	**0,013**
	Station x month	**2,7**	**5,2**	**< 0,001**
Number of species	**Station**	**252,1**	**29,0**	**< 0,001**
	Month	22,0	2,5	0,089
	Station x month	6,5	0,7	0,68
Simpson dominance	**Station**	**0,15**	**26,2**	**< 0,001**
	Month	0,001	0,01	0,98
	Station x month	0,001	1,6	0,14
% epigrowth feeders (rank transformed)	**Station**	**3490,6**	**37,1**	**< 0,001**
	Month	**638,4**	**6,8**	**0,002**
	Station x month	**589,7**	**6,3**	**< 0,001**
Maturity index	**Station**	**0,4**	**31,7**	**< 0,001**
	Month	0,004	0,4	0,71
	Station x month	**0,05**	**4,2**	**< 0,001**
Density (log-transformed)	Station	0,7	0,9	0,51
	Stratum	**8,9**	**169,1**	**< 0,001**
	Stratum x station	**1,2**	**22,5**	**< 0,001**
Number of species	Station	18,2	0,7	0,59
	Stratum	**55,6**	**14,2**	**< 0,001**
	Stratum x station	6,1	1,6	0,23
% epigrowth feeders (rank transformed)	Station	328,1	5,7	0,063
	Stratum	212,9	1,3	0,32
	Stratum x station	54,7	0,3	0,95
Maturity index	**Station**	**0,3**	**13,2**	**0,015**
	Stratum	**0,1**	**3,8**	**0,04**
	Stratum x station	**0,11**	**3,6**	**0,02**

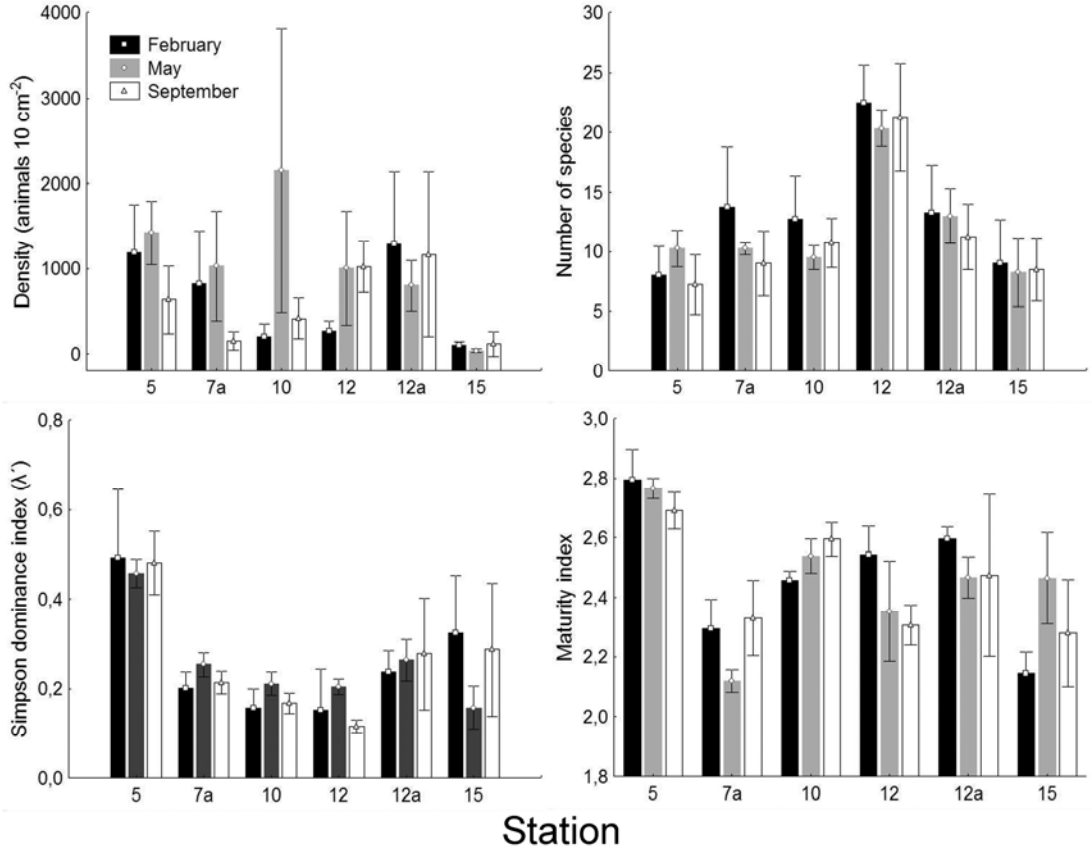

Figure 2.3. Mean ± standard deviation (n = 4) of univariate measures of nematode assemblages in six stations and three months.

2.3.3 Taxonomic composition of nematode assemblages

A list of all recorded nematode species and their mean density in each combination station–month is presented in the table 2.3. A total of 78 nematode species belonging to 18 families were identified. There was a strong dominance with sixty species contributing individually less than 1% to total density (all samples combined). Ten species (belonging to five families) accounted for 80% of nematode total density: *Terschellingia longicaudata* (21 %), *Sabatieria pulchra* (14 %), *Terschellingia communis* (9 %), *Macrodontium gaspari* (8 %), *Terschellingia gourbaultae* (7 %), *Cienfuegia cachoi* (5 %), *Sabatieria breviseta* (5 %), *Pseudoterschellingia ibarrae* (4 %), *Spirinia parasitifera* (3 %), and *Metachromadora pulvinata* (3 %).

The most spatially widespread nematode species was *Terschellingia longicaudata*, followed by *Terschellingia gourbaultae*, *Sabatieria breviseta*, and *Sabatieria pulchra*

(Table 2.3). Several species appears to be characteristic of particular stations: *Macrodontium gaspari* (stations 10 and 12a), *Cienfuegia cachoi* (stations 5 and 7a), *Metacyatholaimus chabaudi* (station 12), and *Dorylaimopsis punctata*, *Hopperia muscatensis* and *Vasostoma* sp. (station 15). In addition, other species showed a strongly patched spatial–temporal distribution, i.e. appearing only in one station and one month in high density; for instance: *Pseudoterschellingia ibarrae* (7a on May) and *Spirinia parasitifera* (12a on February).

A two-way crossed analysis of similarity (ANOSIM) on fourth-root transformed data of density showed clear global differences in structure of assemblages among stations ($R = 0.84$; $p < 0.001$; 999 permutations) and among months ($R = 0.71$; $p < 0.001$; 999 perm.). The pair-wise comparisons showed differences ($p < 0.001$) between each pair of stations, and between each pair of months as well. The ordination of samples by multidimensional scaling (MDS) technique showed clear differences among stations, but not so clear among months (Fig. 2.4). Most of the samples (i.e. cores) belonging to a same station and/or month were clustered together; particularly the stations 12 and 15 have a very distinctive assemblage structure; the latter station is characterized also by higher variability.

2.3.4 Biological traits

We analyzed each trait individually as well as their combination. There was negative and significant correlation among percentage of the three dominant feeding types: selective deposit feeders (1A), non-selective deposit feeders (1B) and epigrowth feeders (2A). Therefore, we tested differences among stations and months just for percentage of type 2A. The results of two-way crossed ANOVA on rank-transformed data showed significant differences in percentage of feeding type 2A for the factors station, month and the interaction (Table 2.2). No significant differences could be detect after post hoc SNK test between pairs of combination station – month.

Table 2.3. Mean values of density (n = 4; individuals 10 cm^{-2}) of nematode species. The five most abundant species in each column are in bold type. Hyphen indicates absence. 1B/2A ratio is presented also.

Species	February						May						September					
	5	7a	10	12	12a	15	5	7a	10	12	12a	15	5	7a	10	12	12a	15
Acanthonchus viviparus	-	-	-	12	-	-	-	2	-	5	-	-	1	-	-	45	2	-
Aegialoalaimus sp.	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Ammostheristus sp.	-	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Amphimonhystrella megastoma	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	3
Aponema torosus	42	-	-	10	13	-	-	-	-	-	-	-	-	-	-	-	5	-
Choniolaimus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Chromadorella macris	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Chromadorita tenuis	-	-	2	13	1	-	-	-	-	13	-	-	-	-	-	7	-	-
Chromaspirinia inglisi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-
Cienfuegia cachoi	**45**	**265**	**37**	-	7	-	18	**175**	3	-	-	1	**52**	**45**	**37**	3	2	-
Comesa warwicki	-	1	-	-	-	-	-	-	-	-	-	**3**	-	-	-	3	-	-
Comesoma sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Cyartonema elegans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Cyartonema germanicum	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Daptonema longicaudatus	-	4	-	9	2	**53**	-	34	3	**59**	-	1	-	-	8	13	-	-
Daptonema oxycerca	1	**59**	5	9	1	2	3	37	2	**57**	-	2	1	4	9	4	-	**5**
Daptonema propius	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	**1**
Desmodora comunis	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Desmolaimus sp.	-	-	-	1	-	-	7	-	-	-	-	-	-	-	-	1	-	-
Dichromadora apapillata	-	-	-	-	1	1	-	-	-	1	2	-	-	-	-	**9**	-	-

Doliolaimus sp.	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Dorylaimida	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Dorylaimopsis punctata	-	6	-	1	-	**4**	-	-	-	9	2	**4**	-	1	-	**68**	-	**7**
Eleutherolaimus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Ethmolaimus sp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Eumorpholaimus sp.	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Gammanema sp.	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Gomphionema tipicum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	18	-
Graphonema sp.	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Guitartia tridentata	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-
Halalaimus floridanus	-	-	-	1	-	-	-	-	-	3	2	-	-	-	-	17	2	-
Halalaimus monstrocaudatus	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Halichoanolaimus sp.	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Hopperia muscatensis	-	-	-	2	-	3	-	-	-	12	-	2	-	-	-	-	10	**66**
Laimella longicaudata	-	-	-	7	-	**5**	-	-	-	7	-	-	-	-	-	-	-	-
Leptolaimus elegans	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Linhystera problematica	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Longicyatholaimus capsulatus	-	-	-	-	-	1	-	-	-	15	-	1	1	-	-	**68**	-	-
Macrodontium gaspari	-	2	17	-	**543**	1	3	5	**329**	-	14	-	-	-	**72**	-	**136**	-
Marilynia johanseni	-	10	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
Megadesmolaimus sp.	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-
Metachromadora pulvinata	-	-	1	-	**107**	-	-	-	60	-	5	-	-	1	8	-	**173**	-
Metacyatholaimus chabaudi	-	1	-	**20**	-	1	-	4	-	**309**	-	1	-	-	-	57	-	**10**
Metacyatholaimus effilatus	**69**	3	-	-	-	-	-	-	-	-	-	-	-	-	-	59	-	2
Metadesmolaimus sp.	-	-	-	1	-	-	-	-	-	15	1	-	-	-	-	7	-	-

Metalinhomoeus filiformis	-	-	-	7	6	-	**146**	4	51	-	-	-	**57**	5	-	1	-	-
Minolaimus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Molgolaimus sp.	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncholaimus sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Paracanthonchus longicaudatus	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Paracomesoma dubium	-	2	-	-	1	-	-	-	-	-	2	-	-	-	-	-	44	-
Paradesmodora campbelli	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Paralinhomoeus lepturus	-	5	-	-	3	-	13	-	4	4	10	1	-	-	-	-	-	-
Paralongicyatholaimus sp.	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	3	-	-
Paramonohystera proteus	-	-	-	2	-	1	-	-	6	-	-	-	-	-	-	68	-	2
Parodontophora xenotricha	23	17	-	**17**	8	2	4	-	-	31	**39**	**4**	-	-	-	14	5	-
Prooncholaimus ornatus	2	19	2	3	-	-	-	-	-	-	-	-	-	-	-	5	-	-
Pomponema sp.	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prochromadora sp.		-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudoterschellingia ibarrae	-	2	4	1	-	-	-	**453**	-	-	5	-	-	**13**	10	1	-	-
Sabatieria breviseta	**98**	**188**	**19**	2	15	1	19	**133**	**142**	3	**16**	-	4	3	9	7	22	-
Sabatieria praedatrix	-	--	10	6	37	-	2	4	10	19	15	-	-	-	-	**150**	2	5
Sabatieria pulchra	1	-	**23**	**14**	**173**	-	**118**	**69**	**751**	**90**	**315**	-	**75**	**21**	**80**	**155**	**69**	-
Setosabatieria hilarula	9	5	2	4	-	-	-	-	-	17	1	-	-	-	-	10	10	-
Sphaerolaimus maeoticus	-	-	7	-	3	-	-	-	-	9	-	2	-	-	-	-	10	1
Spirophorella sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-
Spirinia parasitifera	-	-	11	4	**190**	-	5	1	21	24	14	-	-	3	11	15	84	-
Steineria sterreri	9	3	-	-	-	-	-	-	28	-	-	-	-	-	-	9	-	-
Synonchiella hopperi	**96**	**48**	3	-	-	-	-	-	-	-	3	-	**9**	-	-	5	-	-
Terschellingia communis	**795**	**156**	**27**	8	3	-	**20**	4	54	-	5	-	3	3	**92**	3	15	-

Terschellingia gourbaultae	1	20	**22**	**15**	**71**	-	**83**	41	**227**	9	**102**	-	5	**11**	**43**	62	**269**	-
Terschellingia longicaudata	-	-	10	**83**	67	**8**	**934**	**65**	**484**	**233**	**229**	**7**	**424**	**41**	31	**70**	**220**	**13**
Thalassoalaimus tardus	-	-	2	-	-	**-**	13	-	-	-	-	**-**	-	-	-	-	-	-
Trichotheristus sp.	-	-	-	1	-	**-**	-	-	-	-	-	**-**	-	-	-	-	-	-
Vasostoma sp.	-	-	-	2	-	**9**	-	-	-	-	9	**3**	-	-	-	-	67	1
Viscosia abissorum	-	-	-	2	33	**-**	21	-	-	15	6	**-**	2	-	5	8	2	-
Viscosia glabra	-	-	-	2	1	**-**	3	2	-	10	-	**-**	-	-	-	26	-	-
Total	1192	819	205	265	1294	98	1421	1031	2147	1003	801	38	635	154	415	1024	1167	117
1B/2A ratio	1.8	32.0	5.6	1.1	0.3	8.3	30.0	77.2	2.2	1.4	21.3	1.5	23.0	46.0	1.9	1.9	0.4	1.7

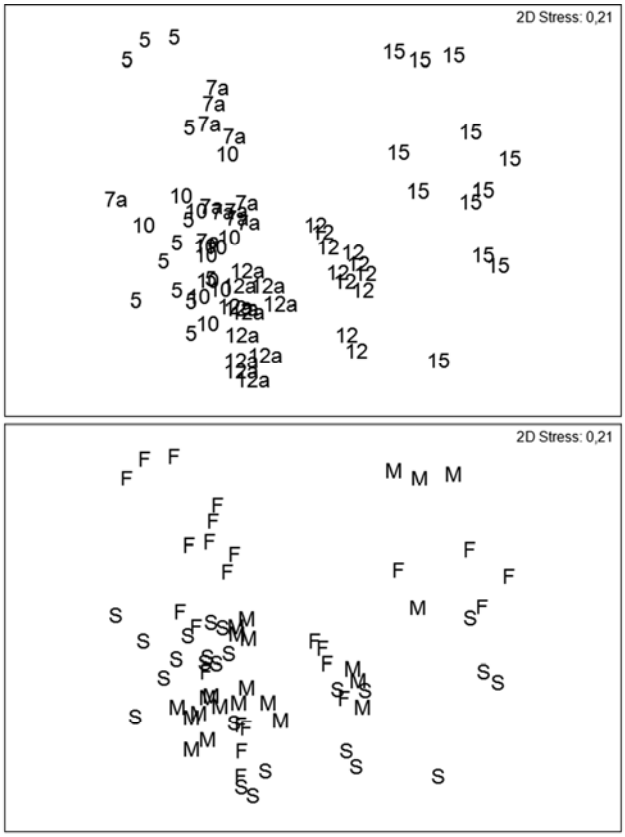

Figure 2.4. Multidimensional scaling plots of nematode assemblages based on fourth-root transformed data of density from six stations and three months. Plots are coded by stations and by months. The labels of the samples: F = February, M = May, and S = September; the number indicates the station.

The feeding type composition was characterized by dominance of deposit feeding nematodes over all samples (mean ± SD): selective deposit feeders (37 ± 21 %), non-selective deposit feeders (37 ± 22 %), epigrowth feeders (15 ± 17 %), and predator/omnivore (11 ± 15 %). The interpretation of the significant interaction station x month in the ANOVA for type 2A holds also for the other feeding types: the temporal changes (among months) are not consistent across stations (Fig. 2.5). For instance, station 12 shows a remarkable temporal stability in the trophic composition, but station 12a shows large fluctuations in the percentage of non-selective and epigrowth feeding types. Station 15 was also characterized by the relative highest percentage of predator/omnivore nematodes in all months. The 1B/2A ratio for each combination of station and month did not show clear spatial or temporal trends, with the exception of the highest values in station 7a in all sampled months (Table 2.3).

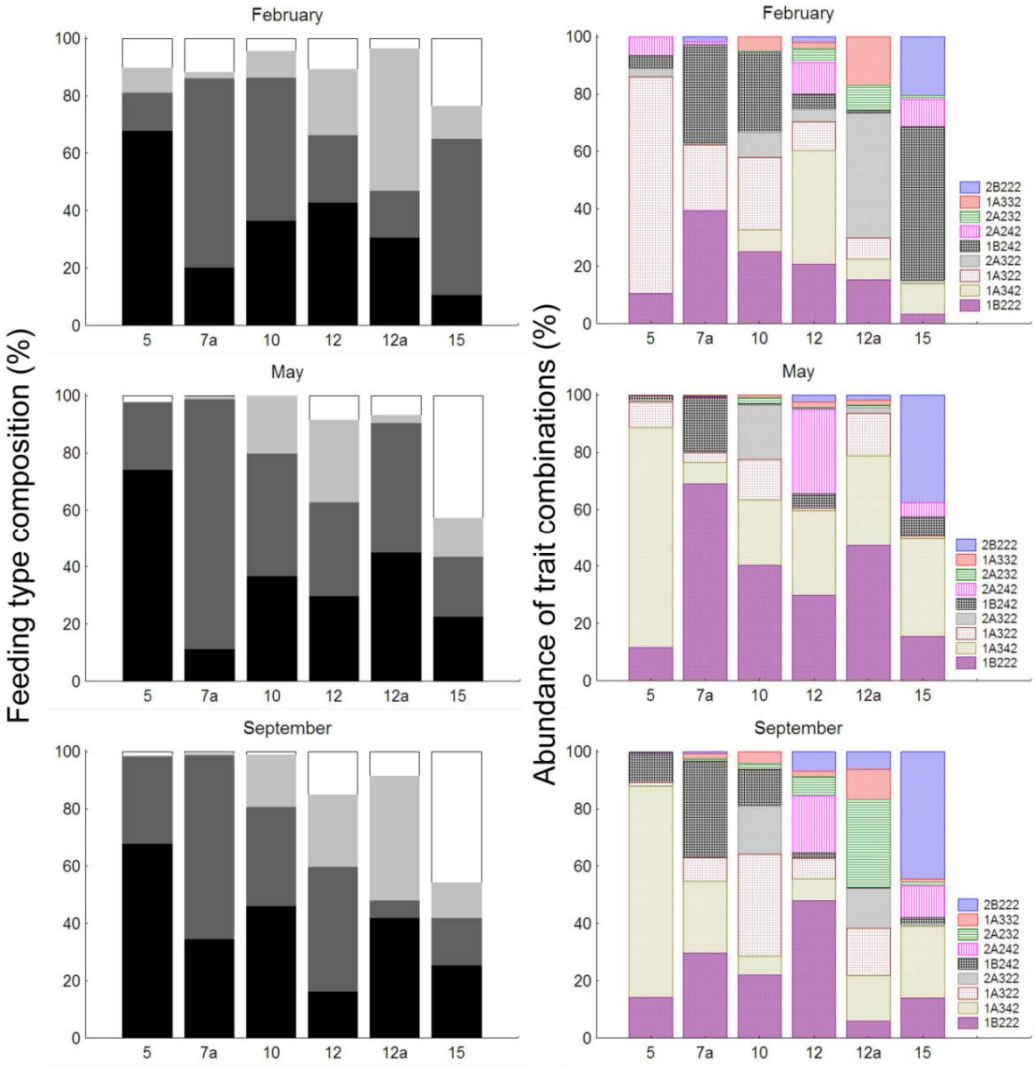

Figure 2.5. Left: Trophic composition of nematode assemblages on basis of average percentages in six stations for three months. Feeding types defined after Wieser (1953): Selective deposit feeder (■); *non selective deposit feeder* (■), *epigrowth feeder* (■), *predator/omnivore* (□). *Right: Percentage of the most abundant trait combinations of nematode assemblages. Combinations based on four traits: feeding type, life strategy, tail shape, body shape.*

The maturity index was calculated for each combination of station – month; and it showed significant differences among stations and in the interaction (Table 2.2). The post hoc comparisons did not indicated any group of means consistently different to the other (Fig. 2.3).

Most of the nematodes (57 %) had a clavate-conicocylindrical tail shape (type 2), the tail shape long/filiform ranked second (35 %). There was no clear trend in the percentage of tail shape groups across stations or months. The body shape of nematodes was mainly slender (97 % of total of nematodes) and in less proportion long/thin; there was absence of nematodes with stout bodies.

The combination of the four biological traits produced a total of 28 trait combinations; the number of combinations was highly correlated to number of species ($r = 0.94$; $p < 0.05$; $n = 71$). Just three trait combinations contributed 65% of total abundance: (1) nonselective deposit feeder/c-p 2/tail conico-cylindrical/body slender: corresponded to species *Sabatieria breviseta*, *Sabatieria praedatrix*, *Sabatieria pulchra*, *Daptonema oxycerca* and *Pseudoterschellingia ibarrae*; (2) selective deposit feeder/c-p 3/tail filiform/body slender: corresponded to *Terschellingia longicaudata*; (3) selective deposit feeder/c-p 3/tail conico-cylindrical/body slender: corresponded to *Terschellingia communis* and *Terschellingia gourbaultae*. In addition, we selected nine trait combinations accounted for 90 % of total abundance and represented the spatial and temporal changes in their relative abundance (Fig. 2.5). There were remarkable spatial and temporal changes in the structure by trait combinations, not only for the three mentioned most abundant trait combinations, as well for the other ones.

2.3.5 Vertical distribution

The samples analyzed for description of vertical distribution included 53 species of nematodes (70 % of total number of species recorded for the three sampling periods). Partially nested two way analyses of variance were applied to the four univariate measures of assemblages; i.e. density, number of species, % epigrowth feeders, and maturity index. There were significant differences in the interaction strata-station in log-transformed density of nematodes (Table 2.2). The figure 2.6 shows that vertical changes in the density are not consistent across stations. The stations 5, 10, and 12a show higher density in surface layers of sediment (0 – 1 and 1 – 2 cm), and less than 100 nematodes 10 cm^{-2} in deeper strata. However, a vertical gradient in density was not so clear in stations 7a and 15.

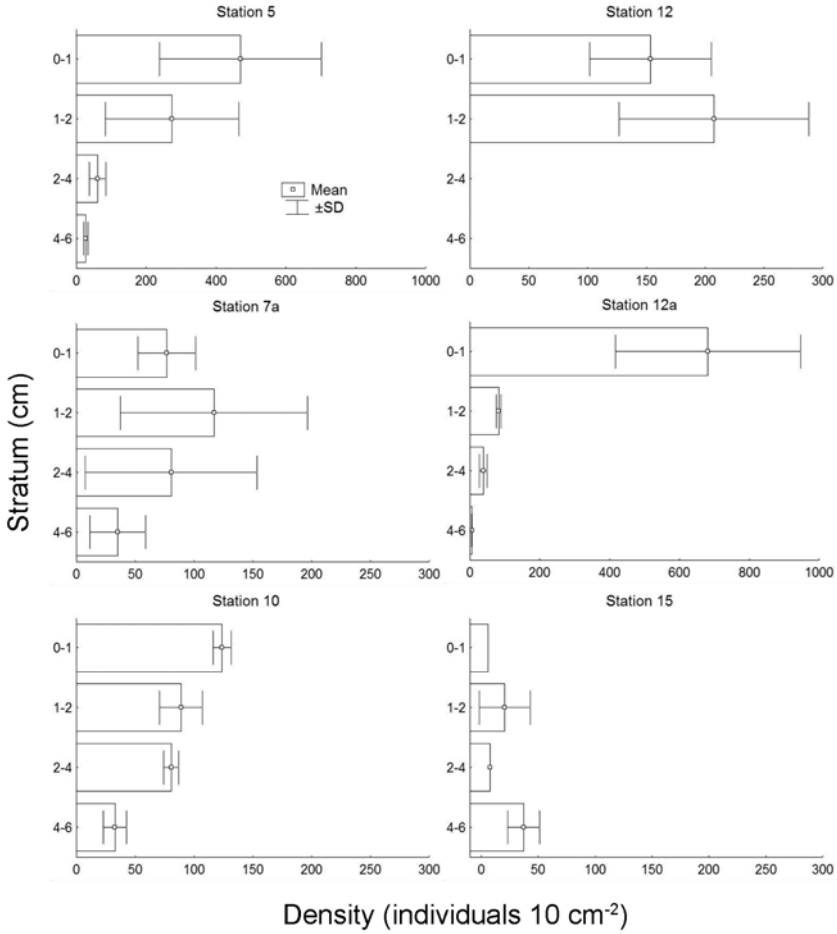

Figure 2.6. Mean ± standard deviation (n = 2) of density of nematode assemblages in four vertical strata within sediment and in six stations in February 2006. Note that scales on x-axis are not the same.

The number of nematode species showed significant differences among vertical strata but no differences among stations neither the interaction (Table 2.2). There was a clear reduction in number of species in deeper strata, with less than seven species in the 4–6 cm stratum (Fig. 2.7). The analysis of variance failed to detect significant differences in the main effects and the interaction for the rank-transformed percentage of epigrowth feeding nematodes (Table 2.2). The maturity index showed significant differences in the interaction, and also for the main factors; no clear vertical trends were evident in both of these latter variables (graphs not show).

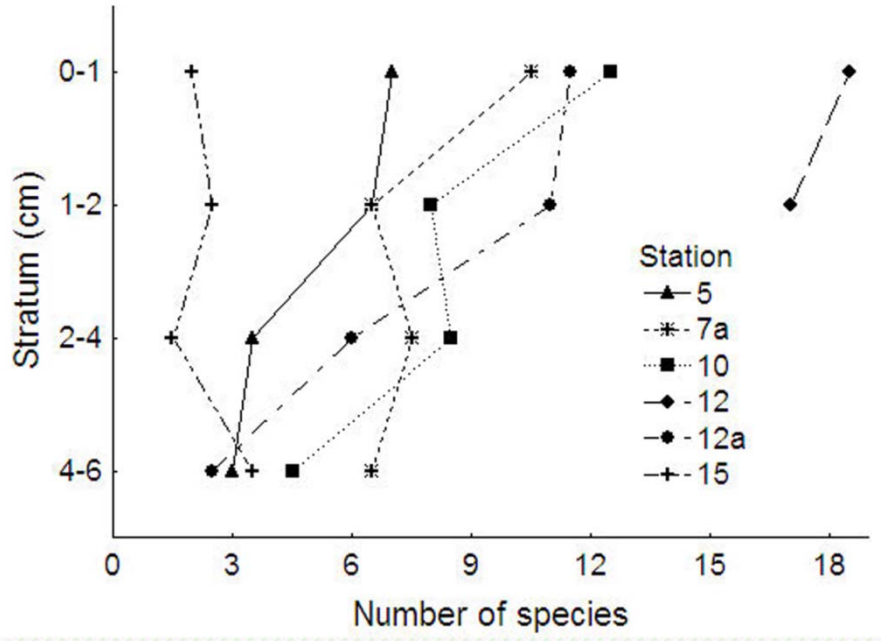

Figure 2.7. Mean values of number of species of nematodes in four vertical strata within sediment and in six stations in February 2006.

A two-way crossed ANOSIM permutation test showed statistical differences in multivariate composition of assemblages among stations (R = 0.70; p <0.001; 999 perm.), but there are no statistical differences among vertical strata within sediment (R = 0.12; p = 0.16; 999 perm.). Further pair-wise comparisons between strata were not interpreted due to the global test was non-significant. The multivariate pattern between stations did not add much more information that it was already analyzed using four replicates (section 2.2.3). The ordination by MDS of samples was characterized by differences among stations, but there is no particular grouping of samples by vertical strata (plot not presented). With regard to species, the following three species appeared in all strata: *Sabatieria breviseta*, *Spirinia parasitifera*, and *Terschellingia longicaudata*. We did not detect species restricted to deep strata; instead, most of them occurred only in the surface strata. There was no clear spatial pattern of biological traits (i.e. feeding types, tail shape, and body shape) in the vertical distribution within sediments.

2.3.6 Relationships nematodes – abiotic environment

Univariate correlations were performed between the four measures of the nematode assemblage (i.e. density, number of species, % epigrowth feeders, and maturity index) and the set of twelve abiotic factors (n = 18 for all correlations performed). The log-transformed density of nematodes was significantly correlated with cobalt (- 0.61), copper (- 0.58), depth (-0.49), and silt-clay percentage (-0.47). The only other univariate measurement of assemblages which had a significant correlation was the rank-transformed percentage of epigrowth feeders with percentage of organic matter (r = - 0.57).

Table 2.4. Results of BIOENV analysis for matrices of similarity derived from data of density of species and biological traits. The combination of abiotic factors with highest value of Spearman rank correlation (ρ) and significance values (p) are showed.

Matrix	ρ	p	Abiotic factors
Species	0.60	0.01	depth, Co, Cr, Cu
Feeding type	0.50	0.03	depth, Co, Cu
Life strategy	0.49	0.01	organic content, Co, Cu
Tail shape	0.43	0.02	depth, Co, Cu
Body shape	0.58	0.01	V, Co
Combined biological traits	0.60	0.01	depth, silt + clay, Co, Cr, Cu

BIOENV procedure was applied on similarity matrices derived from density data for nematode species, individual biological traits (e.g. feeding type, life strategy, tail shape, body shape), and a combination of the four traits (Table 2.4). In general, heavy metals (cobalt, copper) and depth showed the best matching with multivariate biological pattern. Interestingly, matrix of similarity based on life strategy suggests an additional putatively important variable: organic content, while matrix based on combined biological traits matrix suggests the silt/clay content as other variable with explanatory power.

2.4 Discussion

The integration of our results provided a quantitative description of the biodiversity patterns of free-living marine nematodes in Cienfuegos Bay in temporal (month) and spatial (horizontal and vertical) scales, and also a relatively good characterization of the abiotic environment including natural and pollution related variables. In order to address the most relevant topics arising from our results we divided this section following the three main research questions presented in Introduction.

2.4.1 Does the biological trait approach provide new interpretable information in comparison to a ‘‘classical’’ taxonomic approach?

In the present study, the biological trait approach shows a possible influence of two key abiotic factors on nematode assemblages: organic content and silt/clay fraction in sediment. This information could not be clearly deducted from the analysis of the species matrix and it is relevant in the context of factors affecting the structure of assemblages (for discussion see next topic). Despite the different spatial scales used in our study (few kilometers) and in the study by Schratzberger et al. (2007) two features are common for both studies: (1) biological trait approach is not more powerful than taxonomic approach in detecting spatial patterns; but (2) biological trait approach offers more reliable correlative links with environmental factors than the taxonomic one.

The significant correlation between number of trait combinations and number of nematode species supports the hypothesis that the increase of species richness can lead to potential increases of functional diversity (Schratzberger et al., 2007). Evidence suggests that analysis of functional traits of assemblages can reveal more reliable relationships ecosystem functioning – biodiversity than analysis of species composition (Reiss et al., 2009), and it also can apply to marine ecosystems (e.g. Raffaelli et al, 2003). We hypothesize that the high temporal and spatial variability in biological trait combinations of nematode assemblages in Cienfuegos Bay can lead

to significant effects on the functioning of sedimentary ecosystem. However, no measurement of ecosystem processes has been carried out in this bay and further elaboration of this idea has to wait for more data.

The dominance of two trait combinations (deposit feeder/c-p 2 or 3/tail conical cylindrical or filiform/ body slender) across months and stations is consistent with the relatively high homogeneity of the studied muddy bottoms and with the small spatial scale of this study; it means that we are dealing with a habitat species pool instead of a regional pool (Emerson and Gillespie, 2008).

Our findings strongly encourage the analysis of biological traits, in addition to analysis by species, when diversity patterns and related ecological processes are the aims of the investigation. Biological trait approach can overcome two of the weak points/sides of taxonomic approach, namely: (1) ratio response/noise in species data sets can be substantially low due to latter are typically characterized by strong fluctuations in values of abundance, high numbers of zeros and marked skewness in distribution; and (2) problems of misidentification of some species complex with high morphological similarity (e.g. *Terschellingia* and *Sabatieria*) are less critical in the context of biological traits. Additionally, the classification of nematode species into functional traits can reveal more straightforward links between diversity and ecosystem functioning or not. For macrofauna, some studies (e.g. Bolam et al., 2002; Raffaelli et al., 2003) suggest importance of functional traits over species richness in the effects on ecosystem functioning. Other studies (e.g. Ieno et al., 2006; Norling et al., 2007) suggest that identity of particular species plays a key role in determining these effects; however, there is evidence that the short duration of experiments has a sampling effect supporting the existence of the idiosyncratic response (Stachowicz et al., 2008). To our knowledge, very few data (but see De Mesel et al., 2006) exist for marine nematodes regarding the relationship diversity (taxonomic, biological trait, etc.) – ecosystem functioning, being a promising research avenue.

2.4.2 Which natural and/or anthropogenic processes determine the biodiversity patterns of free-living nematodes within the Bay?

The integration of information about environmental quality of Cienfuegos Bay strongly suggests that the ecosystem is subject to organic (e.g. hydrocarbons, Tolosa et al., 2009) and inorganic pollution (e.g. heavy metals). Spatial changes in the multivariate structure of assemblages and abiotic data are in agreement with the existence of two well characterized basins within Cienfuegos Bay, a northern basin, more polluted than the southern basin. Historical records of contamination within sediments using nuclear techniques suggest that relatively high levels of Co, Cr, and Cu in southern basin are of natural origin (C. Alonso, Centro de Estudios Ambientales de Cienfuegos, Cuba, pers. com.). However contamination by other heavy metals persists and a comparison with reference values (Long et al., 1995) indicates concentration higher than threshold values causing biological effects in all stations. The negative correlations between measures of assemblages and content of heavy metals support the existence of these deleterious effects of metals.

Although heavy metals in sediments have harmful effects on nematode assemblages (Lampadariou et al., 1997; Gyedu-Ababio and Baird, 2006; Hedfi et al., 2007) toxicity to nematode populations inhabiting Cienfuegos Bay can be reduced by the high organic content in sediments (Rzeznik-Orignac et al., 2003; Gyedu-Ababio and Baird, 2006) and relatively high salinity (Dauvin, 2008). In addition, nematode species from Cienfuegos Bay probably have developed tolerance to chronically metal-enriched habitats, a process discussed by Millward and Grant (1995) for an estuary.

We suggest that a combination of organic enrichment – hypoxia – hydrodynamic regime drives (in interaction with pollution) the biodiversity patterns of nematode assemblages. Low hydrodynamic regime could explain the existence of a thick flocculent layer (M. Armenteros, pers. observation), high content of silt + clay in sediments, and high sedimentation rate in the bed of the deepest stations (Alonso-Hernandez et al., 2006). These features are mostly associated with a stable near-bed stratification of bottom water (Friedrichs et al., 2000) which can reduce the passive dispersal of small-size organisms (Silvester and Sleigh, 1985) resulting in the highly patchy distribution of nematode assemblages observed in the bay. The low hydrodynamic regime probably enhances the negative effects of organic enrichment

and hypoxia on the benthos (Snelgrove and Butman, 1994; Gray et al., 2002), and this synergistic relationship can be particularly important in semi-enclosed bays (Kröger et al., 2006). Dominant species in Cienfuegos Bay (belonging to genera *Terschellingia, Sabatieria* and *Daptonema*) are typical of poorly oxygenated and organically enriched bottoms around the world, and they appear tolerant to a variety of xenobiotic compounds (Soetaert et al., 1994; Schratzberger et al., 2006; Steyaert et al., 2007). Also, the dominance of the species *T. longicaudata* supports the idea of a relatively undisturbed physical environment (but chemical pollution still existing) because it is sensitive to physical disturbance as was demonstrated in experimental conditions (Schratzberger and Warwick, 1999).

The hydrodynamic regime also can influence the vertical distribution of nematodes in muddy bottoms (Soetaert et al., 1994; Steyaert et al., 2003); however, centimeter - scale distribution patterns of nematodes in Cienfuegos Bay (i.e. vertical distribution) would be determined to a larger extent by biotic interactions (Zajac et al.,1998). For instance, in polluted and very fine sediments, pollution-tolerant deposit feeding species belonging to genera *Sabatieria* and *Terschellingia* are dominant. The dominance of one or another species may be the result of interspecific competition for food or space: In Cienfuegos Bay the larger body size of *Terschellingia longicaudata* compared to *Sabatieria pulchra* might offer an additional advantage (i.e. more pushing force) for inhabiting silty sediments where movements within sediments depend burrowing abilities. Data from another heavily polluted tropical semi-closed bay (Armenteros et al., 2009, Appendix 1) showed a dominance of *S. pulchra* species over *T. longicaudata* linked may be due to coarser sediment in comparison with Cienfuegos Bay. Further research on interactions between these two sympatric and tolerant species should be addressed.

2.4.3 How different are the biodiversity patterns in a tropical semi-enclosed bay in comparison with those in similar ecosystems in temperate regions?

To our knowledge, the present study is the only one published describing biodiversity patterns of nematode assemblages in a tropical semi-closed bay. Our results show

similar outputs with comparable habitats in temperate regions (Table 2.5); unfortunately, there are no data on unpolluted harbors/bays serving as reference. The comparison suggests three general ideas that are discussed below: (1) anthropogenic disturbance has a strong influence on the patterns of meiofaunal distribution within these systems; (2) temporal trends are lacking or are overridden by spatial differences; and (3) there is a notable dominance of a few cosmopolitan genera/species of nematodes tolerant to hypoxic and polluted conditions.

The pollution derived from harbour activities, industrial development, and human settlements is one of the main factors determining distribution patterns in semi-enclosed bays included in table 2.3; in addition, the negative effects are reinforced by reduced exchange of water with adjacent open water. Effects of pollution on benthos are closely dependent on location of sources of pollutants (e.g. cities, factories) and temporal changes in human activities causing pollution are usually of less importance. This can explain the stronger changes in response of meiofauna (and nematodes) to human disturbance in the spatial scale (e.g. among stations) than in the temporal one (e.g. months).

Data sets obtained from small scale studies (dealing with estimates of alpha-diversity) are not suitable for latitudinal comparisons of biodiversity patterns (dealing with gamma-diversity) (Gray and Elliott, 2009). However, we noted the high similarity of nematode assemblage composition between tropical and temperate bays regarding identity and number of genera/species (Table 2.3); this supports the cosmopolitan nature in the assemblage structure of free-living marine nematodes. Recent research (e.g. Bhadury et al., 2008; Derycke et al., 2008) using DNA based techniques indicates the existence of cryptic species complexes for some species-rich and cosmopolitan genera of nematodes; as a result, may be cosmopolitanism as revealed by the molecular taxonomy is not so widely extended in marine nematode's realm.

*Table 2.5. Comparison of main biodiversity features of nematode assemblages in semi-enclosed bays/harbors. MI = maturity index. * Values assessed from graphs.*

Bay/harbor	Density (ind 10 cm^{-2}) Diversity	Dominant genus/ species	Ecological remarks	Reference
Iraklion, Crete (without external station O)	range*: 0 – 2530 ind 115 spp.	*Sabatieria*, *Metalinhomoeus, Daptonema, Terschellingia, Paracomesoma*	clear spatial heterogeneity in 100 m-scale, pollution and physical disturbance shaping the distribution of assemblages	Lampadariou et al. (1997)
Wrangel Bay, Sea of Japan	mean: 538 ± 87 ind 48 spp.	*Dorylaimopsis piculiaris, Viscosia stenostoma, Sabatieria pulchra, S. palmaris*	spatial distribution depended of grain size and heavy metal content in sediments	Pavlyuk et al. (2003)
Genoa-Voltri, Italy	mean: 225 ± 25 ind; 23 genera	*Paracomesoma*, *Terschellingia, Daptonema, Desmodora, Sabatieria*	high dominance, lacking of temporal distribution patterns, clear spatial heterogeneity in 100 m-scale, pollution rather than food supply are the main ecological driven, MI was not sensitive	Moreno et al. (2008)
Victoria, Hong Kong (stations B2 and B3)	range*:13 – 272 ind 127 spp.	*Sabatieria praedatrix, Terschellingia communis, T. longicaudata, Parodontophora sp.*	coarse silt + very fine sand, polluted habitat, No temporal variation in nematode composition	Liu et al. (2008)
Cienfuegos Bay, Caribbean Sea	range: 21 – 4596 ind 78 spp.	*Terschellingia longicaudata, Sabatieria pulchra, T. communis*	high dominance, lacking of temporal patterns, clear spatial heterogeneity in 100 m-scale, pollution and depth linked to structure of assemblages. MI interpretation confused by interaction of anthropogenic and natural factors	Present study

2.5 Conclusions

(1) The biological trait approach added relevant information to the taxonomic approach regarding relationships between diversity patterns of nematodes and the abiotic environment. Two trait combinations were dominant in Cienfuegos Bay, both including deposit feeder nematodes, probably due to the relatively low heterogeneity of the studied soft bottoms but also because a local (habitat) pool of species was analyzed instead of a regional one.

(2) Chemical pollution and hydrodynamic regime possibly drove the biodiversity pattern of nematode assemblages in Cienfuegos Bay. The low hydrodynamic regime could determine a poor dispersion of nematodes resulting in high spatial variance in assemblage structure and also the associated hypoxic conditions and pollutants in sediments could explain the dominance of tolerant nematode species.

(3) The spatial–temporal patterns of biodiversity of nematode assemblages in the studied semi-enclosed bays, both tropical and temperate, are characterized by similar features: strong influence of anthropogenic disturbance, temporal trends being weak or overridden by spatial ones, and dominance of a few genera/species tolerant to pollution and hypoxic conditions.

2.6 Acknowledgements

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CHAPTER 3

EFFECTS OF ORGANIC ENRICHMENT ON NEMATODE ASSEMBLAGES IN A MICROCOSM EXPERIMENT

Modified version from the article accepted for publication:

Armenteros M, Pérez-García J.A., Ruiz-Abierno A, Díaz-Asencio L, Helguera Y, Vincx M, Decraemer W. Effects of organic enrichment on nematode assemblages in a microcosm experiment. *Marine Environmental Research*.

Abstract

Marine nematodes from subtidal tropical sediments of Cienfuegos Bay (Cuba) were subjected to additions of phyto-detritus (microalgae *Spirulina*) in a microcosm experiment. The follow up of the experimental conditions was measured at days 0, 4, 15 and 30. Observed effects on the nematodes were a decrease in abundance and diversity, and changes in the taxonomic and trophic structure due to the organic enrichment. The results suggested that the nematodes were not food limited in the microcosms and probably neither in their natural environment. The main factor affecting the nematodes was probably the byproducts (hydrogen sulphide and ammonia) due to enhanced bacterial development in microcosms. Hypoxic conditions occurred in all experimental units, as well in the field suggesting a nematode assemblage adapted to naturally enriched sediments. However, tolerant (dominant) species showed a grade of sensitivity to reduced conditions, in increasing order: *Spirinia parasitifera, Terschellingia longicaudata, Metalinhomoeus filiformis,* and *Sabatieria pulchra*. We predict that further organic enrichment in sediments from Cienfuegos Bay may cause a phase shift into a strongly depleted benthic fauna and reduced conditions in water and sediments.

3.1 Introduction

Organic enrichment is an important ecological process in marine sediments (Kelly and Nixon, 1984) and it is related to the presence of fine sediment, low hydrodynamic regime and poor content of dissolved oxygen in coastal ecosystems (Snelgrove and Butman, 1994). Benthic assemblages inhabiting those environments are dominated by deposit feeding organisms able to use the pool of particulate organic matter in the bottom and the associated bacterial biomass. Free-living marine nematodes do a main numerical contribution to deposit feeding guild and they play an important role in the process of transformation of organic matter in marine sediments (Gerlach, 1971; Findlay and Tenore, 1982; Li et al., 1997; Piepenburg et al., 1997). The content of organic matter can partially explain the spatial patterns of distribution of free-living nematodes in some habitats (e.g. Ólafsson and Elmgren, 1997; Schratzberger et al., 2006). However, further developments on the type of relationships between organic content and nematodes and the mechanisms underlying them are required. At least several ecological factors such as type of habitat, origin of organic input and intensity of human disturbance affects this relationship (Schratzberger et al., 2008).

Effects of organic enrichment on meiofauna and/or nematodes in experimental microcosms have been analyzed by Sandulli and Nicola-Giudici (1989), Sundbäck et al. (1990), Webb (1996), and Schratzberger and Warwick (1998b); all of these studies were carried out in temperate regions. Extrapolation of these results to tropical regions remains inconclusive because at least two important ecological issues are different: (1) high irradiance throughout the year leads to continuous high primary production, and (2) high temperature of shallow water enhances hypoxic conditions and stratification in the water column (see Alongi, 1990 for review). In addition, a general lack of information on tropical ecosystems prevents across-region synthesis (e.g. reviews by Gray et al., 2002 and Wu, 2002).

Cienfuegos Bay is a semi-enclosed shallow bay in the Caribbean Sea, most of the subtidal area in the basin is constituted by organically enriched muddy bottoms, although mangroves, seagrass meadows and beaches occur within the bay. Additionally, heavy metal and hydrocarbon pollution is widespread in sediments of the bay originated from industrial activities and human settlements in the basin (Díaz-

Asencio et al., 2009). A previous study (Pérez-García et al., 2009) about distribution of nematode assemblages in several sites in the basin indicated a notable spatial heterogeneity at a 100 m-scale and dominance of nematode species tolerant to organic enrichment, such as *Sabatieria pulchra* and *Terschellingia longicaudata*. The spatial patterns were linked with content of organic matter and heavy metals in sediments and water depth; however, these correlations suggest alternative possible ecological mechanisms which determine the distribution, for instance: toxic effects, limitation in benthic primary productivity, and hydrodynamic regime affecting both dispersion/transport of sediment. Since no causative mechanism could be found in the field sampling, we conducted an experiment in order to gain knowledge on the ecological processes that drive the distribution in the nature.

We designed an experimental study to test the effects of organic enrichment on nematode assemblages in microcosm ecosystems using sediment collected from Cienfuegos Bay. The response of assemblages to the addition of organic matter can suggest alternative explanations for patterns detected in the nature. Two main handicaps derived of the experimental constrictions are the absence of recruitment into the sediment enclosed in microcosms and the relatively short term duration of the study.

A possible outcome is the depletion of the abundance and diversity of nematodes and it would be related to toxicity effects due to byproducts from bacterial activity (Gray et al., 2002) and/or exacerbation of inter-specific competition as a response to organic enrichment (Riebesell, 1974). However, some kind of enhancing effects on assemblages can occur such as increase in the abundance due to outbreaks of opportunistic species and it can suggest that these species were food limited in field conditions. Even, if not change can be detected, the resilience of the assemblages can be argued as explanation and this is valuable information in the context of the pollution exposition. Organic pollution of water and sediments is one of the main stressors in marine environment (Diaz and Rosenberg, 2008) and it is often an important disturbance in semi-enclosed basins due to the limited renovation time of these ecosystems (Urban et al., 2009). The recent development of petroleum-related industries in Cienfuegos Bay probably increases the risk of deleterious effects of organic enrichment on this ecosystem.

With the aim to test the effects of organic enrichment on nematode assemblages, we proposed based on both univariate and multivariate measurements of assemblages the following null hypothesis (H0): there is no change in the structure of assemblage in the treated microcosms compared to field and control samples; and an alternative hypothesis (H1): there is change in the structure.

3.2 Materials and methods

3.2.1 Field site

The collection of surface sediment was carried out in a subtidal habitat in Cienfuegos Bay, Cuba, Caribbean Sea (N 22º07.970´ W80º29.824) in May 2007. The site was previously included in two studies of meiofaunal and nematode community structure (station 5 in Díaz-Asencio et al., 2009; and in Pérez-García et al., 2009). The study site is characterized by muddy bottom (87 % silt/clay less than 63 μm), organically enriched sediments (47.3 mg C g^{-1} DW), 12 m depth, and annual range of salinity and temperature, 34 – 37 and 24 – 30 ºC respectively. Historical data (1991 – 2001) of concentration of dissolved oxygen in the bottom water of this station was used for further comparison with values in microcosms; only measurements clearly belonging to wet season (i.e. period April – September) were selected due to there are seasonal fluctuations of dissolved oxygen and because the sediment for the experiment was collected in the wet season.

3.2.2 Collection and manipulation of sediment

For the field control, five plastic cores (2.6 cm internal diameter) were taken to 6 cm depth within sediment for description of the community structure (three cores) and organic content (two cores); the same day and 5 - 10 m apart, approximately 15 l of surface sediment (top 3 - 4 cm) was carefully collected by SCUBA divers using glass flasks. The fresh sediment was transported to the lab, stored in two plastic containers with aeration for approximately 18 h; thereafter, the sediment was gently

homogenized with a plastic spoon and finally transferred to the experimental units. Before transfer, four random aliquots (= small spoons) of sediment were checked for presence of living (= moving) nematodes. Since nematodes appeared well alive, it was assumed that manipulation of the sediment had low impact on the survival of the nematodes.

3.2.3 Microcosms

Microcosms consisted of 500 ml glass flasks, and each microcosm was considered to be an independent experimental unit. Approximately the same amount of homogenized fresh sediment (150 – 180 ml) was added to each microcosm resulting in a layer of sediment of 2.0 – 2.5 cm depth and surface area of ca. 20 cm^2 (Fig. 3.1). Care was taken to avoid the inclusion of large macrofaunal animals (e.g. polychaetes) into the microcosms. However, one week after the start of the experiment, holes and some bioturbation activity was evident in the microcosms. The flasks were carefully filled with 45 µm filtered seawater and sealed with a 45 µm mesh to prevent upward migration of meiofauna. The 72 microcosms used in the experiment were placed into four tanks of 50 l capacity (18 per tank), maintained in darkness to avoid primary production; and left for 48 h for acclimatization before application of treatments. The tanks were completely filled with seawater and connected to the closed seawater circuit of the laboratory; this included a biological filter, the control of water temperature (27 ^{0}C at noon), saturation of dissolved oxygen (average 6.2 ± 0.1 mg O_2 l^{-1}) and constant salinity (36) for the duration of the experiment.

3.2.4 Treatments

Values of readily oxidizable organic matter (hereafter referred to as organic matter) of 50 mg C g^{-1} DW sediment (range: 44.5 – 61.8 mg C g^{-1} DW; n = 8) at the site where sediment was collected indicate organic enrichment. Unfortunately, the rate of natural input of organic matter to sediments is largely unknown for similar tropical

ecosystems. Even in more studied ecosystems (i.e. temperate estuaries) estimates of organic input to sediments are not well documented and often underestimated (Kendall et al., 1995). Other microcosm studies have included as high doses of added organic matter as 200 or 400 g C m^{-2}, sometimes without responses of nematode assemblages (e.g. Austen and Warwick, 1995).

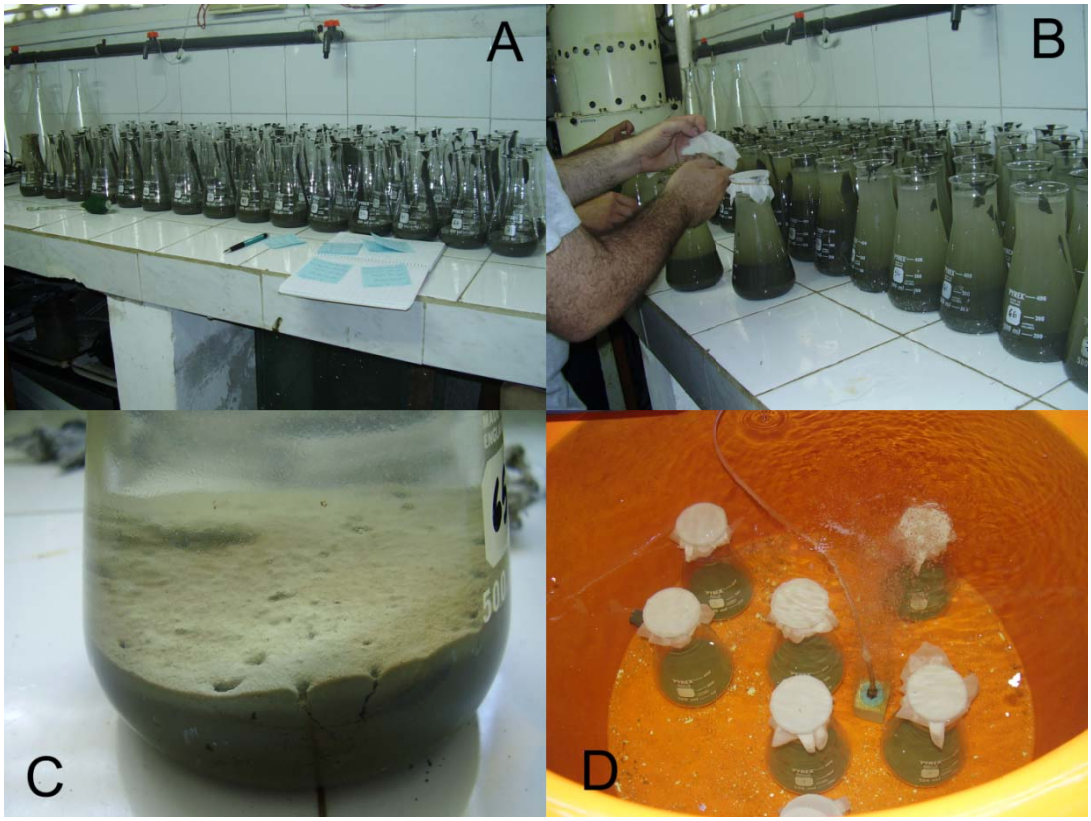

Figure 3.1. Experimental setup. A) Microcosms with sediment added; B) Microcosms with water and while they are sealed with mesh; C) Signals of bioturbation activity within a microcosm; D) Microcosms within the tank.

Therefore, in order to induce a significant disturbance on our microcosms we choose three treatments: 1) control: without addition of organic matter; 2) medium: addition of 25 mg C g^{-1} DW sediment (equivalent to 50 % of natural average organic content in sediment, ca. 400 g C m^{-2} sediment); and 3) high: addition of 50 mg C g^{-1} DW (equivalent to an increase of 100 % of natural average content, ca. 800 g C m^{-2} sediment). The organic matter was added as powder of *Spirulina* microalgae; the content of organic matter by weight of algae was determined (536 mg C g^{-1} *Spirulina*

powder) using the same technique as for other determination of organic content in this study (see below). The average (± SD) dry – weight of sediment in each microcosm was assessed (103 ± 9 g), and the quantity of *Spirulina* powder to add was calculated (treatment Medium: 5 g powder added; treatment High: 10 g added).

3.2.5 Experimental design

We assigned the microcosms to the tanks using a systematic assignation in order to obtain a maximum of interspersion of treatments; i.e. each tank contained controls and treatments. Within each tank the position of the microcosms was assigned at random. At day 0, four microcosms (one per tank) were extracted and processed (control at day 0). After that, treatments were applied: each microcosm was extracted from the tank, the previously weighted aliquot of *Spirulina* powder added, the flask re-sealed, and placed again into the tank.

At days 4, 15 and 30, 18 microcosms (3 treatments x 6 replicates) were extracted at random with the restriction that a balanced number of microcosms per tank should be left; i.e. no more than two microcosms belonging to a same treatment could be removed from the tank. From six replicated microcosms, four were taken for analysis of meiofaunal community structure and two for determination of content of organic matter. At day 30 the content of dissolved oxygen in the water inside the microcosms were measured.

3.2.6 Processing of samples

The content of each microcosm was sieved over a 45 μm mesh sieve using filtered water. Meiofauna was sorted from sediment by flotation in a high density solution of commercial sugar dissolved in water (1.17 g cm^{-3}); the sorting efficiency of meiofauna using this technique is ca. 95 % in Havana´s lab (Armenteros et al., 2008). Sorted meiofauna was counted under stereomicroscope (36x) using a counting dish, and 50 nematodes (when available) from each sample were pick-out for identification. Nematodes were mounted in microscope slides after the procedure by Vincx (1996)

and identified to species level using the pictorial keys by Platt and Warwick (1983, 1988), Warwick et al. (1998) and the NeMys Database (Deprez et al., 2007).

Concentration of readily oxidizable organic matter in sediment was determined by the modified Walkley–Black wet titration method (Loring and Rantala, 1992) using oxidation with $K_2Cr_20_7$ and H_2SO_4. Concentration of dissolved oxygen in the water inside the microcosms (1 – 2 cm above sediment) was measured with a Hanna DO probe (accuracy 0.01 mg O_2 l^{-1}). Additionally, a time series of dissolved oxygen in the bottom water was included for comparison with experimental units; the data come from the same station where sediment was collected and in the same weather season (wet).

3.2.7 Measures of assemblages

Traditional metrics of structure of assemblages (i.e. abundance of nematodes and number of species) were determined. Each nematode species was classified into a scale of coloniser/persister (c-p value) according to several aspects of the life-trait after Bongers (1990) and Bongers et al. (1991). The maturity index of the community was calculated on the basis of the score in the c-p scale. Nematode species were assigned to feeding types according to Wieser´s (1953) classification based on the morphology of the buccal cavity: selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A) and predator/omnivores (2B). The percentage of contribution of each feeding type to total abundance was calculated for each sample. Only the most variable feeding type (non-selective deposit feeding, 1B) was subjected to statistical analysis because putatively it contains more interpretable information. The other feeding types were correlated each other so they were not used in the statistical analysis.

3.2.8 Data analysis

Multi- and univariate techniques were used for data analysis using the software PRIMER 6.0.2 (Clarke and Gorley, 2006) and STATISTICA 6.0 from StatSoft. We

tested the existence of statistical differences in seven variables: organic matter, dissolved oxygen, number of nematodes, number of species, Shannon diversity index, maturity index and percentage of non-selective deposit feeders. Two graphical methods were performed before application of a statistical test in order to know if data fulfil the premises of parametric analysis: scatter plots of mean vs. standard deviation; and residual vs. predicted mean. If needed, data were transformed and re-checked in order to known if the transformation improved their suitability to apply parametric assumptions. Two types of comparisons were performed due to a full crossed design treatment x time could not include the field samples (no time).

(i) Comparison between control groups in order to assess the “microcosm effect” using a one-way ANOVA with five levels: field control, microcosm controls at days 0, 4, 15, and 30. If ANOVA was significant, two planned comparisons using least square means were performed: field vs. day 0 to test differences between experimental conditions and natural environment; and day 0 vs. day 30 to test the temporal changes in the microcosm controls. The organic matter content was not measured at day 0, so ANOVA levels were four and the following planned comparisons were performed: field vs. day 4, and day 4 vs 30. The dissolved oxygen was measured only at day 30, so the comparison was done using one-way ANOVA between treatments with five levels: field, tank, control, treatment medium, treatment high.

(ii) Two-way crossed ANOVA was carried out in order to compare: treatment (main factor, 3 levels: control, medium, high), time (main factor, 3 levels: 4, 15, 30 days) and their interaction.

Non-parametric analysis of similarity (ANOSIM) was employed for test differences in multivariate structure of assemblages. Data were square-root transformed for downweigh the contribution of dominant species to structure and the similarity matrices were built using Bray-Curtis similarity index. The same matrix used for ANOSIM was employed to build an ordination plot by non-metric multidimensional scaling. The procedure SIMPER was applied when looking for species which contribute most to similarity/dissimilarity across treatments and/or time.

Dominance curves were built using the data of abundance of species to compare the level of dominance/evenness in each sample; the curves cumulate the number of individuals according to the rank of species in each replicate/sample.

3.3 Results

3.3.1 Visual description of microcosms

Clear changes in sediment and overlying water were observed in microcosms along the duration of the experiment. Control microcosms were apparently stable during the 32 days of the study: with clear water, a relatively thick oxidized layer of sediment (ca. 5 mm) and presence of mounds and holes, presumably due to activity of small macrofauna. Two to three days after addition of organic matter, treated microcosms showed a gradual trend of change for both sediment and water. At day 30, all treated microcosms showed high turbidity, strong smell of hydrogen sulfide, blackening of sediment and presence of dense mats (presumably bacteria) on the water-sediment interface (Fig. 3.2).

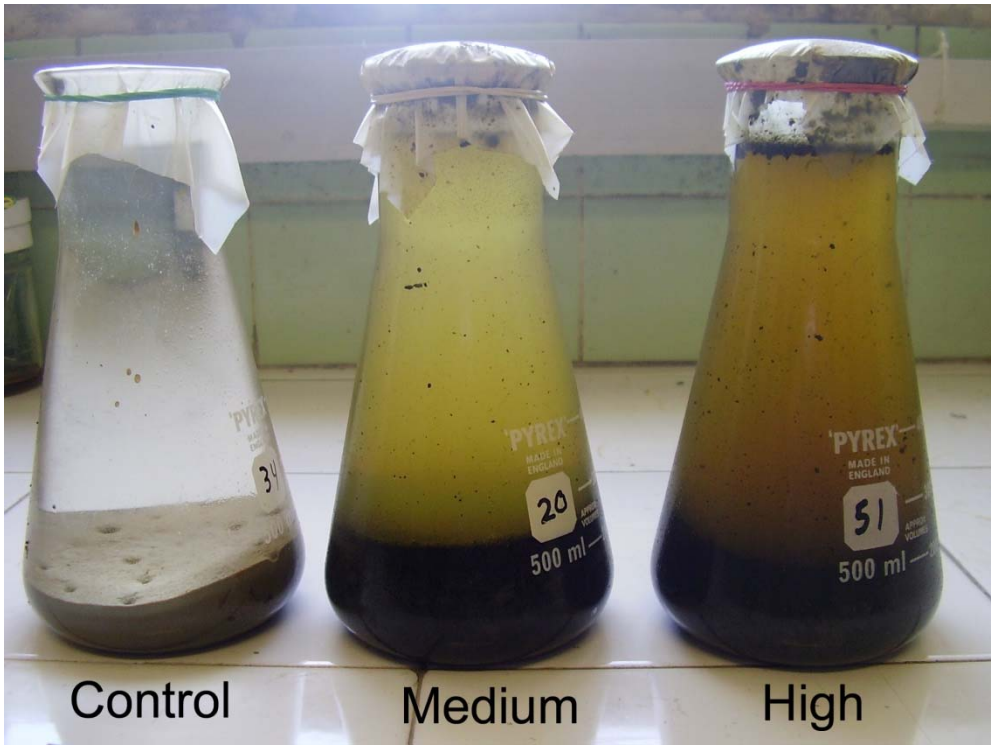

Figure 3.2. Visual differences among experimental units subjected to two levels of addition of organic matter and control at day 30 after started the experiment.

3.3.2 Abiotic factors

There were differences in the log-transformed values of organic matter among field and microcosm controls (one-way ANOVA, $F_{3,4} = 6.0$, $p = 0.06$). Planned contrasts indicated significant differences between field and day 4 ($t_{2,4} = -3.8$, $p = 0.02$) suggesting higher levels of organic matter in the field. No significant differences between controls at day 4 and 30 ($t_{2,4} = -0.74$, $p = 0.5$) were detected; this indicates that levels of organic enrichment were stable in control microcosms (Fig. 3.3). There were differences among treatments in the log-transformed values of organic matter (two-way ANOVA, $F_{2,9} = 235.9$, $p < 0.001$) but not among days ($F_{2,9} = 0.5$, $p = 0.6$) neither in the interaction ($F_{4,9} = 0,9$, $p = 0.6$). This indicated that treatments were effective in increasing the amount of organic matter in treated microcosms and that the organic content was steady during time for each treatment (Fig. 3.3).

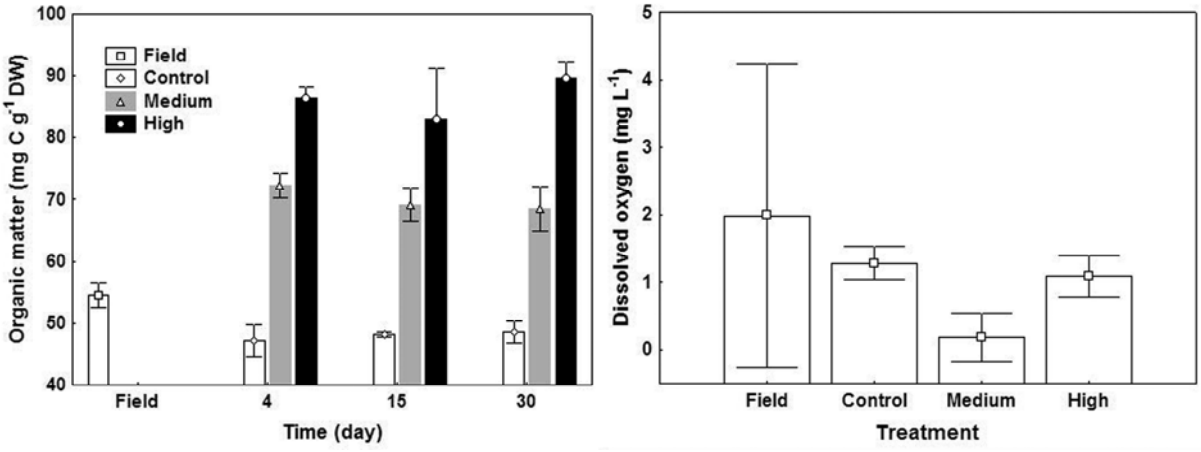

Figure 3.3 Mean values and SD of abiotic factors measured from a field site and from experimental microcosms. Left: Organic content (n = 2) in sediments. Right: Dissolved oxygen from a field site and from microcosms (n = 6) at day 30; field average is a composite of 23 measures taken during wet season (from May to September) in 1991 – 2001.

A comparison of values of dissolved oxygen among treatments (at day 30) and field did not show statistical differences (one-way ANOVA, $F_{3, 36} = 1.64$, $p = 0.20$). Measurements of dissolved oxygen were rather low, with mean values less than 2 mg O_2 l^{-1}; field measurements showed a larger variability (Fig. 3.3).

3.3.3 Univariate metrics of assemblages

We recorded a total of 36 species of free-living marine nematodes in our study of which only 32 species were present in the microcosms (i.e. four were recorded only in the field samples) (Table 3.1). A comparison of univariate metrics of assemblages between field and control samples 0 could indicate how much the assemblage in the experimental conditions mimic the ones in the natural environment (Table 3.2, one-way ANOVA). There were significant differences in the log-transformed number of nematodes; planned comparisons shown no difference between field and control at day 0 ($t_{2, 38}$ = 0.6, p = 0.6), but significant differences between control microcosms at days 0 and 30 ($t_{2, 38}$ = 4.1, p < 0.001) with lesser nematodes in controls at the end of experiment (Fig. 3.4). The number of species shown significant differences; but post hoc planned comparisons did not detect differences between field and controls at day 0 ($t_{2, 38}$ = -1.3, p = 0.2) neither between controls at day 0 and 30 ($t_{2, 38}$ = 1.2, p = 0.2).

Shannon diversity index had significant differences among field and controls (Table 3.2); post hoc planned comparisons indicated no difference between field and control at day 0 ($t_{2, 38}$ = 0.2, p = 0.8), but significant differences in control microcosms between days 0 and 30 ($t_{2, 38}$ = 2.7, p = 0.01). Maturity index showed significant differences among field and controls (Table 3.2); however, post hoc comparisons could not detect significant differences between field and control at day 0 ($t_{2, 38}$ = -1.4, p = 0,16), neither between days 0 and 30 ($t_{2, 38}$ = -1.84, p = 0.07). Percentage of non-selective deposit feeding nematodes showed significant differences between field and control samples (Table 3.2). Planned post hoc comparison indicated no differences between field and control at day 0 ($t_{2, 38}$ = 0.88, p = 0.4), but significant differences between controls at day 0 and 30 ($t_{2, 38}$ = 2.9, p = 0.006). In general, results of comparison among controls suggest that univariate measures of nematode assemblages were similar in field and in control microcosms at day 0; however, several metrics of assemblages changed significantly at the end of experiment in comparison with day 0, indicating that experimental conditions affect the existence of the assemblages in enclosure conditions (i.e. microcosm effect).

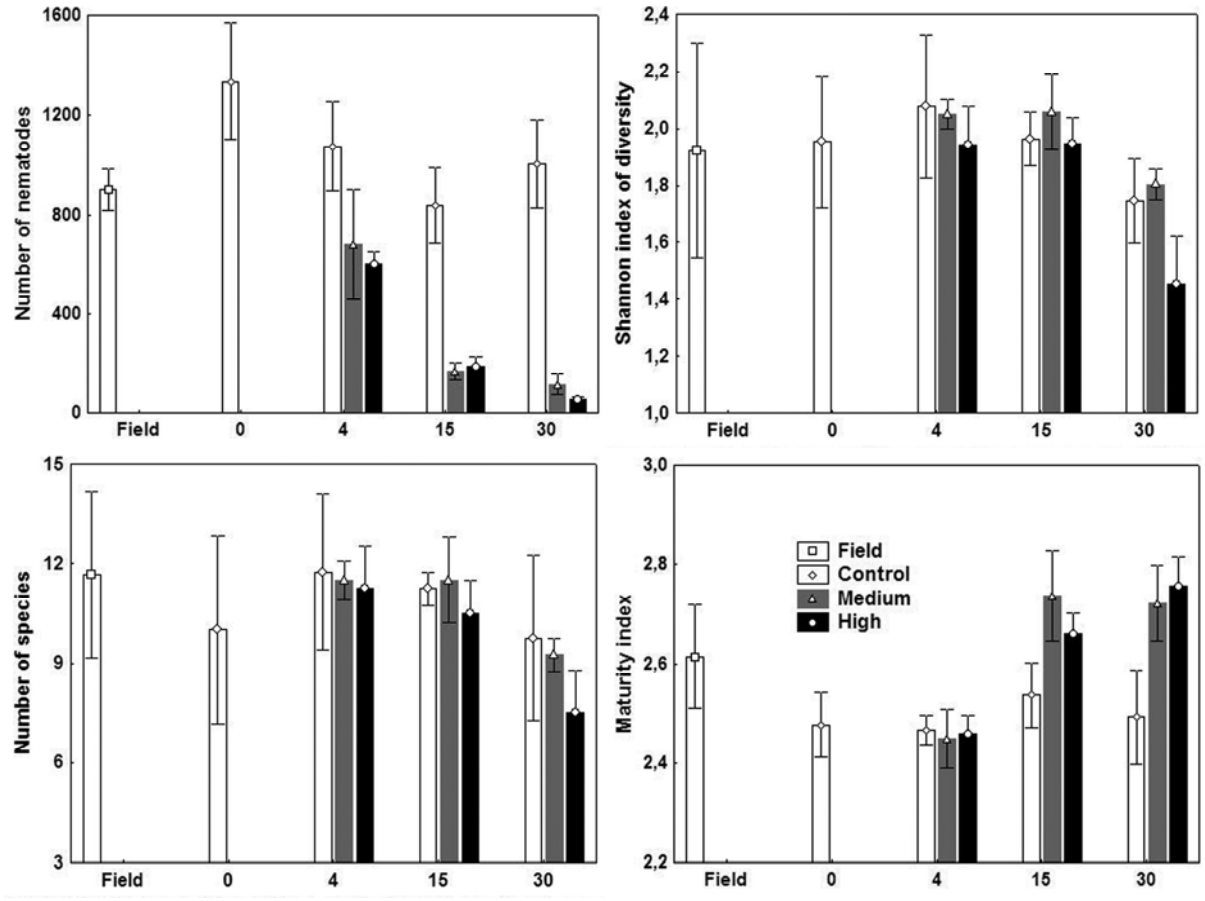

Figure 3.4. Univariate metrics (mean and SD, n = 4; except for field mean, n = 3) of nematode assemblages in sediments from a field site and from experimental microcosms. Day 0 at start of the experiment, and days 4, 15 and 30 after addition of organic matter in two levels (medium and high).

A two-way crossed ANOVA for comparison among treatments (three levels: control, medium and high) and at different times (days 4, 15 and 30) informs about the effects of the treatment itself on univariate metrics of assemblages, but also about possible interaction. The results showed significant differences of log-transformed nematode abundance in factors: treatment, time and treatment x time (Table 3.2). A Tukey´s post-hoc test detected significant differences among three groups of mean values: highest abundance (controls and medium treatment at day 4), intermediate abundance (medium treatment at days 15 and 30, and high at 15), and lowest abundance (treatment high at day 30) (Fig. 3.4).

Table 3.1. Mean abundance of nematode species (n = 4) in microcosms subjected to three treatments (C = control, M = medium, and H = high) at four times (days 0, 4, 15, and 30). Hyphen indicates absence.

Species	0C	4C	4M	4H	15C	15M	15H	30C	30M	30H
Aponema torosus	26	26	25	6	23	18	7	-	2	-
Chromadorita tenuis	-	-	-	3	-	-	-	-	-	-
Cienfuegia cachoi	32	50	15	15	30	5	11	4	-	1
Comesoma arenae	-	-	-	-	-	-	-	4	4	-
Cyartonema germanicum	-	-	2	-	-	-	-	-	-	-
Daptonema sp.	4	22	-	6	-	-	-	-	-	-
Desmodora granulata	-	7	-	-	-	-	-	-	1	-
Desmoscolex longisetosus	-	5	-	-	18	2	-	15	1	1
Diodontolaimus sabulosus	-	-	-	3	4	-	2	7	-	1
Dorylaimopsis punctata	8	33	-	0	5	-	3	17	-	-
Gnomoxyala sp.	-	-	-	-	-	-	-	-	1	-
Halalaimus floridanus	-	-	9	-	-	1	-	-	1	-
Halichoanolaimus sp.	-	-	-	6	-	-	-	-	-	-
Leptolaimus elegans	-	-	4	3	-	7	-	4	4	2
Linhystera problematica	-	5	-	-	4	-	-	11	-	-
Longicyatholaimus capsulatus	15	-	25	4	-	-	1	13	-	-
Megadesmolaimus sp.	4	5	-	3	-	1	1	-	-	-
Metachromadora pulvinata	21	-	-	-	-	7	6	-	7	5
Metalinhomoeus filiformis	216	188	62	47	110	6	10	152	15	1

Paramonohystera proteus	27	21	10	12	21	1	3	9	1	-
Parodontophora xenotricha	32	22	49	29	39	11	11	15	2	4
Pseudoterschellingia ibarrae	-	-	-	-	-	-	-	10	-	-
Sabatieria pulchra	329	217	212	208	175	5	19	280	-	-
Setosabatieria hilarula	-	5	-	-	-	2	1	-	-	-
Sphaerolaimus maeoticus	-	-	-	3	-	-	-	-	-	-
Spirinia parasitifera	133	26	46	25	20	42	28	17	35	25
Synonchiella hopperi	-	-	4	-	-	-	-	-	-	-
Terschellingia communis	55	45	43	31	48	-	-	-	-	-
Terschellingia gourbaultae	137	154	53	58	62	21	21	163	8	-
Terschellingia longicaudata	294	226	116	132	263	37	66	279	24	12
Thalassomonhystera sp	-	-	-	-	14	1	-	-	13	-
Viscosia sp.	-	12	7	6	-	2	-	4	-	-
Total	1335	1075	681	601	838	169	189	1005	116	53

The number of species was different among times, but no significant change was observed among treatments, neither in the interaction (Table 3.2). A Tukey´s post-hoc comparison indicated that samples from day 30 had a significantly lower number of species than at days 4 and 15 (Fig. 3.4). The Shannon´s and maturity indices showed significant differences in factors time and treatment, but not in the interaction (Table 3.2). There was a consistent reduction of diversity at the end of the experiment at day 30 and also in the treatment involving high addition of organic matter. The maturity index was higher in treatments medium and high at day 30, but for the controls no difference was observed (Fig. 3.4).

Table 3.2. Results of statistical comparisons of univariate measures of nematode assemblages. Line separates two different ANOVAs (one way and two way crossed). Values of statistic F, probability are shown. N = number of nematodes, S = number of species, H´ = Shannon diversity index, MI = maturity index, 1B = non-selective deposit feeding nematodes. Significant results in bold type.

Comparison	Log N	S	H´	MI	% 1B
Microcosm effect ($F_{4, 38}$)	**7.3, < 0.01**	**4.9, 0.01**	**6.8, < 0.01**	**4.4, 0.01**	**6.3, < 0.01**
Treatment ($F_{2, 27}$)	**181.3, < 0.01**	2.3, 0.11	**6.3, 0.01**	**4.0, 0.03**	**31.8, < 0.01**
Time ($F_{2, 27}$)	**116.0, < 0.01**	**12.0, < 0.01**	**24.1, < 0.01**	**9.4, < 0.01**	**40.5, < 0.01**
Treatment x time ($F_{4, 27}$)	**29.4, < 0.01**	0.5, 0.72	1.4, 0.24	2.5, 0.07	**13.2, < 0.01**

The dominance curves showed a similar profile among the three field samples and four microcosm control samples at day 0. However, control samples did show a lower variability that field samples probably due to homogenization of sediment carried out before setting the microcosms (Fig. 3.5). There was a temporal trend from day 4 to day 30 in the relative position and slope of curves among treatments. At day 4 profiles of dominance are practically undistinguishable from one another; at day 30, the dominance increased in the assemblages subjected to high addition of organic matter, and to a lesser extent in medium addition.

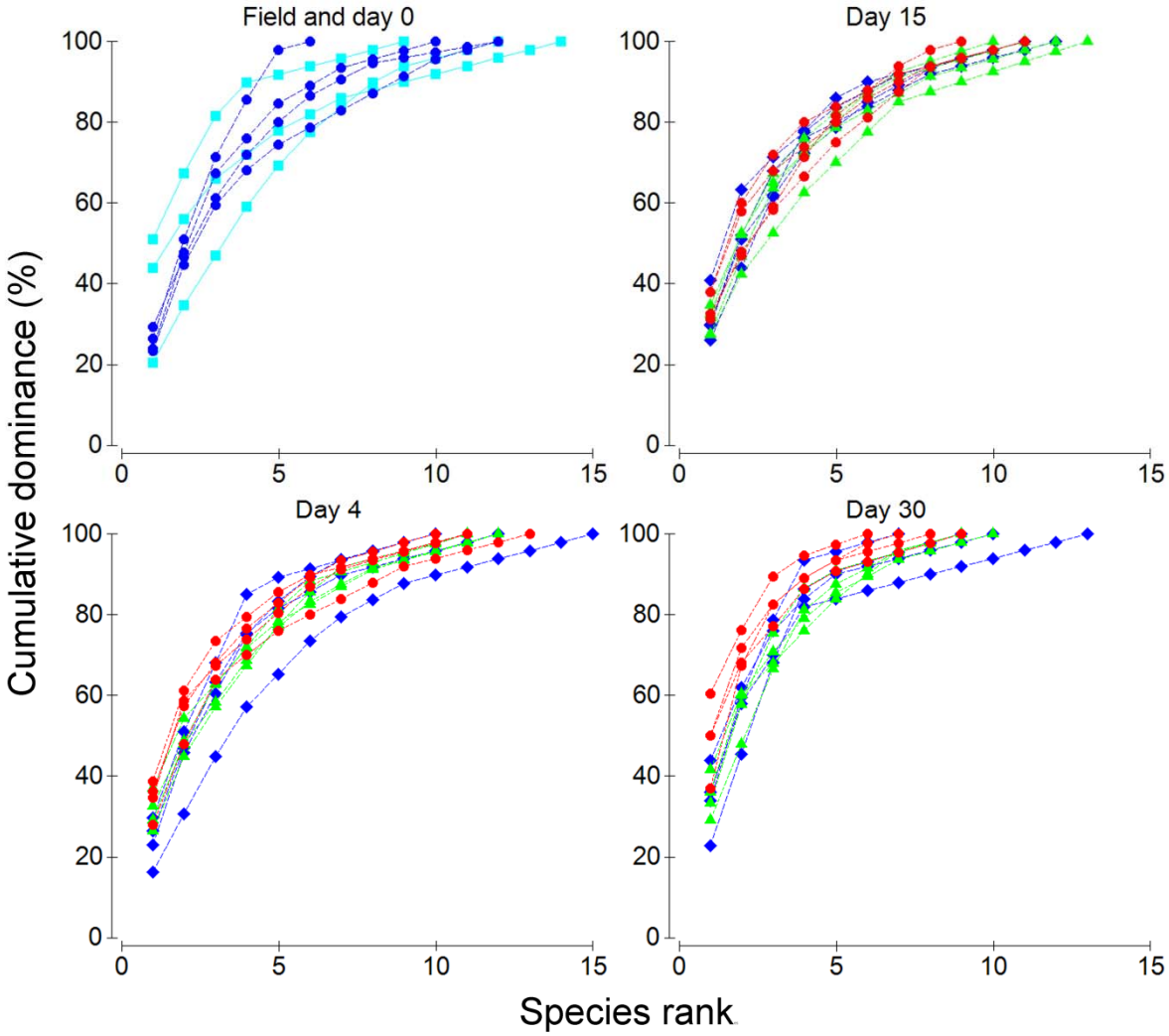

Figure 3.5. Cumulative curves of dominance in nematode assemblages in sediments from a field site and from experimental microcosms. Each curve corresponds to a replicate. Day 0, the start of experiment, and days 4, 15 and 30 after addition of organic matter in two levels (medium and high). Symbols in the curves: field samples (■), control (♦), medium (▲) and high (●).

3.3.4 Multivariate metrics of assemblages

Two different comparisons were carried out on the basis of multivariate composition of assemblages using the ANOSIM procedure: (i) one-way comparison of field versus control samples at several days (five levels: field, 0, 4, 15, 30 days); (ii) two-way crossed comparison of treatments (control, medium, high) and times (4, 15 and 30 days).

The field and control samples showed significant differences (ANOSIM, global test, R = 0.49, p = 0.001; 999 permutations). Most of the pair-wise comparisons between controls showed significant differences in the multivariate structure with R-values higher 0.5; exception was control 0 vs control 4 (R = 0.22, p = 0.17, 35 perm.). This suggests the existence of a microcosm effect changing the structure of nematode assemblages. However, in the ordination, the pattern of dispersion among control samples is not so clear, although field samples showed the highest dispersion in the plot (Fig. 3.6).

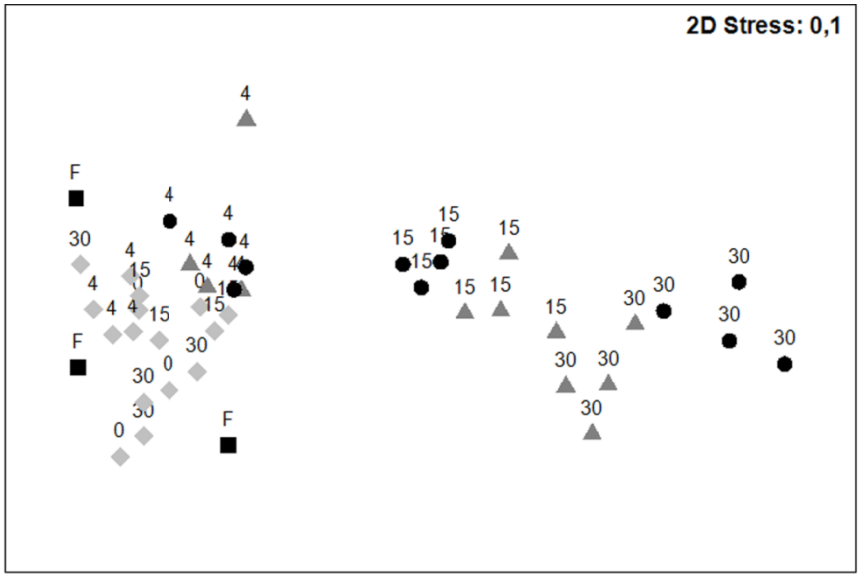

Figure 3.6 Multidimensional scaling ordination of samples based on square root transformed data of density of nematode species in sediment from a field site and from experimental microcosms. Symbols in the plot: field samples (■)*, control* (♦)*, medium* (▲) *and high* (●). *Number upper symbol indicates days after start of the experiment.*

The procedure SIMPER indicated that species contributing to similarity within groups of samples are the same (i.e. *Terschellingia longicaudata*, *Metalinhomoeus filiformis*, *Sabatieria pulchra*, and *Terschellingia gourbaultae*). Differences among groups were accounted by the presence/absence of rare species.

Also significant differences were detected among controls at different times (R = 0.47; p = 0.001; 999 perm), indicating presence of a microcosm effect, i.e. temporal changes of nematode assemblages in the microcosms without any addition of organic matter. However, the differences among controls at day 0 and controls at

subsequent times are mainly related to a decrease of abundance of some species such as *Spirinia parasitifera*, *Metalinhomoeus filiformis*, and *Terschellingia communis* (Table 3.1).

The two-way crossed ANOSIM searching for differences in the multivariate structure of assemblages indicated significant differences among treatments (R = 0.75; p = 0.001; 999 perm) and among times (R = 0.82; p = 0.001; 999 perm). All pair-wise comparisons (using 999 permutations) were significant at 0.05 probability level, and R-values higher than 0.5. However, the ordination of the samples (Fig. 3.6) and the absolute values of R statistic indicate an interaction between treatment and time.

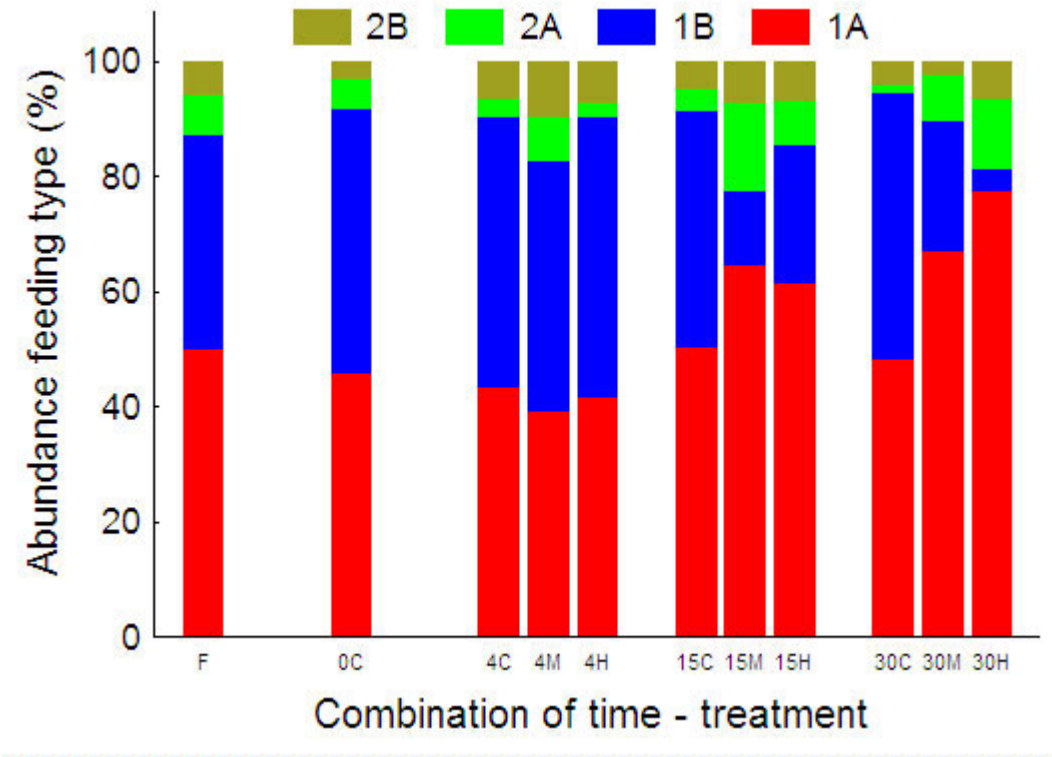

Figure 3.7. Average percentage (n = 4) except for field samples (n = 3) of feeding types of nematode assemblages in sediments from a field site and from experimental microcosms. Feeding types after Wieser (1953): 1A = selective deposit feeder, 1B = non-selective deposit feeder; 2A = epigrowth feeder, 2B = predator/omnivore. Code of samples: F = field samples; numbers are days of the experiment (days 0, 4, 15 and 30); and types indicate the treatments (C = control, M = medium and H = high).

The effects of the treatments became stronger at days 15 and 30; also differences were present between treatments of medium and high organic matter at these times. In general a reduction in the abundance of all species at days 15 and 30 was observed, though more pronounced in the treatment with high doses of organic matter (Table 3.1). The dominant species were the same in the field control as in microcosm controls. However, three of the four dominant species responded differently to the treatment over time (Table 3.1). The abundance of *Spirinia parasitifera* did not change upon addition of organic matter. *Terschellingia longicaudata* and *Sabatieria pulchra* showed a similar dominance in controls (days 0 and 4) and field; however, the depletion was stronger for the latter being nearly absent at day 30. *T. longicaudata* persisted in all microcosms even in high doses of organic matter. Other dominant species such as *Cienfuegia cachoi, Metalinhomoeus filiformis,* and *Terschellingia gourbaultae* were capable of persisting in treated microcosms, although with decreasing abundance relative to controls.

The classification of nematode species into feeding types allowed a searching for effects of organic enrichment on trophic structure of assemblages. The abundance of non-selective deposit feeding nematodes (1B) among field samples and controls at day 0 did not significantly differed. Also the contribution of the representation (%) of 1B among controls at the four times was statistically non-significant (Table 3.2). However, the percentage of 1B type showed significant differences among treatment, time and interaction treatment x time (Table 3.2). There was a clear reduction of nematodes belonging to 1B type in microcosms subjected to medium and high doses of organic matter at days 15 and 30 from the start of the enrichment experiment (Fig. 3.7). This was related with disappearance of *Sabatieria pulchra*, the most abundant species of feeding type 1B at day 30.

3.4 Discussion

The integration of the results leads us to reject the null hypothesis of no changes in the structure of nematode assemblage subjected to experimental treatments. Therefore, we will discuss, in following order, (i) the validity of our experimental setting, (ii) possible models that could explain the obtained results, and (iii) extrapolation of data from the experiment to nature.

3.4.1 Validity of the experimental setting

The changes in the amount of organic matter in the microcosms suggest some loss from the particulate phyto-detritus pool due to mineralization and leaching as dissolved carbon into overlying water (Webb, 1996). The concentration of dissolved oxygen in microcosms towards the end of the experiment was close to recorded data from bottom water in the field; however, higher variability existed in data from the field due to the natural variability of dissolved oxygen and the relatively long period covered by the measures (1991 – 2001). We consider the experimental set-up as successful in recreating an organically enriched environment.

Changes were observed in nematode abundance, and also in the multivariate structure of assemblages between field and control samples, indicating the existence of a "microcosm effect". Such an effect was recorded also by Sandulli and Nicola-Giudici (1989), and Schratzberger and Warwick (1999). The observed changes can be explained by the use of surface sediment where nematodes are concentrated (thus higher abundance in microcosms) and due to the homogenization of sediment prior to setting of microcosms (thus lower variability in assemblage structure). Only four species out of 36 recorded from the field were not recorded in the microcosms, suggesting a good representation in the experimental conditions of the real diversity in nature.

The experimental set-up probably amplified the effects of treatments due to creation of stagnant conditions that avoid the exchange of water and replenishment of sediment (Sundbäck et al., 1990). However, interpretable results can still be obtained from analysis of these laboratory communities, since important features of communities remain the same between field and control samples. In addition, quote from Austen and McEvoy (1997) applied also to our study: "*microcosm effect is uniform across all treatments and most species and it is not so great that it masks experimental differences between treatments and control*".

3.4.2 Possible models explaining the results

The pool of particulate organic matter in sediments was large enough for the maintenance of nematode assemblages in control microcosms along the 32 days of the experiment. Apparently, negative species interactions (competition or predation) were not exacerbated in the control microcosms; although evidence of significant interactions (i.e. predation and interference competition) with macrofauna has been recorded in comparable experiments (e.g. Widbom and Elmgren, 1988).

The dominance of deposit feeding nematodes in the sediments from Cienfuegos Bay (Pérez-García et al., 2009) and the short generation time of some opportunistic species (Heip et al., 1985; Moens and Vincx, 2000) open the possibility of some kind of positive response occurs in the experimental assemblages subjected to the addition of phyto-detritus. No evidence of feeding on the “fresh microbial mats” developed in treated microcosms could be detected in our study in spite of deposit and epigrowth feeding nematodes can feed successfully on the microbial communities (Jensen, 1987b). This suggest that no food limitation occurs in the sediments, in other words, that organic matter is already available to nematodes populations, and then they do not show any enhancing response to the addition of high quality organic matter.

The increase of the maturity index as response to organic load has been documented by Moreno et al. (2008) in organically enriched environment and appeared to be due to development of an assemblage characterized by slow movement and tolerant species (c-p value: 3). An artifact of the experimental microcosm could be the lack of recruitment of opportunistic species (Sandulli and Nicola-Giudici, 1989). The maturity index is strongly dependent of colonizers abilities of species, so in this kind of enclosed system the interpretation of the maturity index as indicator of organic enrichment should be done with caution, since anomalous results can be obtained in comparison with field studies.

Our results are broadly in agreement with previous studies about effects of organic enrichment on nematodes and meiofauna despite the difference in habitats and regimes of primary production (Table 3.3); also results from field sites, reported negative effects of organic enrichment on nematodes (e.g. Mazzola et al., 2000; Sutherland et al., 2007). The explanatory mechanisms of observed patterns in mentioned studies have been the response of nematodes to hypoxic – reduced conditions in sediments and/or the synergistic effects of other stressors (e.g. metals

or physical disturbance). Present study also allows discrimination among these possible causes since hypoxia occurred in control microcosms and in the field site and relatively high levels of heavy metals were present in the field site from which the sediment was collected (Pérez-García et al., 2009).

We postulate as explanation of our results that the products of bacterial metabolism (i.e. hydrogen sulfide and possibly ammonia) are the main cause of depletion of nematode assemblages. Hypoxia, ammonia and hydrogen sulfide interact in a poorly known way and have deleterious effects on benthic assemblages (Gray et al., 2002). Often, confusion on effects of hypoxia and bacterial byproducts occur (Wu, 2002); but in our study, toxic bacterial byproducts appear to be the main cause of observed pattern because hypoxia was present in all microcosms and as well in the field.

The dominant species present in these assemblages, namely *Sabatieria pulchra, Spirinia parasitifera, Terschellingia communis, T. gourbaultae*, and *T. longicaudata* have been recognized extensively as tolerant to a diversity of stressors in soft bottoms (Rzeznik-Orignac et al., 2003; Schratzberger et al., 2006; Steyaert et al., 2007). Physiological and behavioral adaptations of named species to poor-oxygenated environment include a low respiratory rate and slow movements (Warwick and Price, 1979; Warwick and Gee, 1984). Deposition of insoluble metal sulphides in intracellular inclusions in species *Sabatieria wieseri* and *Terschellingia longicaudata* has been suggested as a mechanism of detoxification of sulfide (Nicholas et al., 1987). Our observations of specimens from Cienfuegos Bay suggest existence of granules in the intestine; maybe the mentioned mechanism of detoxification can be present also in these nematodes, but further studies are needed to clarify this. Other strategy for detoxification in nematode inhabiting thiobiotic habitats is to have a slender body shape which increasing the body surface (Jensen, 1987a); however, the body shape does not appear to be related with the tolerance of nematodes to reduced conditions in our experiment. Our results suggest that *S. pulchra* is more sensitive to reduced conditions than *Terschellingia longicaudata* and *Metalinhomoeus filiformis;* the other dominant species, *Spirinia parasitifera*, showed a notable resistance to effects of treatments. The species-specific response of nematode assemblages to organic enrichment has also been noted by Schratzberger et al. (2008); it means that not all species populations deplete at the same rate.

Table 3.3. Outline of studies about effects of organic enrichment on nematode assemblages and/or meiofauna in experimental microcosms.

Treatment	Sampling event	Effects and ecological remarks	Place, habitat	Reference
Sewage sludge, 900, 2700 and 9000 µg C g^{-1} sand, added once time	14, 35, 55, and 84 days after	Depletion proportional to organic load due to oxygen availability, presence of H_2S and pollutants (heavy metals)	UK, unpolluted intertidal beach,	Sandulli and Nicola-Giudici, 1989
Fresh macroalgae, 36 and 71 g C m^{-2} (eq. to 0.9 and 1.8 kg FW m^{-2}) added once time	1, 9, 16, and 23 days after	No effect in low doses of organic load; depletion in high doses probably due to anoxic conditions	Sweden, sandy sediment from a shallow (0.2 m) bay	Sundbäck et al., 1990
Phyto-detritus (several origins), 25, 75, and 200 g C m^{-2}	112 days after	Reduction in meiofaunal abundance, no specific response to different types of phyto-detritus. Deposit feeders were not limited by food availability.	Netherlands and France, intertidal estuaries	Austen and Warwick, 1995
Phyto-detritus, 100 and 200 g C m^{-2}, added once time	3, 12, 28 days after	Positive or no effects to medium dose; depletion in high dose. Input of organic matter possibly limiting factor	Canada, intertidal sandy sediment in salt-mars	Webb, 1996

Phyto-detritus, 100, 200 and 400 g C m^{-2} added in several frequencies	62 days after	Depletion and changes in composition, dominant species: *Terschellingia longicaudata*, and *T. communis.* Reduced sediment leads to few species able to survive	UK, intertidal estuarine sediments	Schratzberger and Warwick, 1998
Phyto-detritus (12.5, 25, 50, 100, 200, and 400 g C m^{-2}, added once time) and physical disturbance	84 days after	Higher diversity in lower levels of both treatments. Highest levels of organic enrichment provoked lower diversity for several levels of physical disturbance	Norway, subtidal muddy sand from a sheltered bay	Austen and Widdicombe, 2006
Powdered cow dung, 20 – 30 mg C g^{-1} (final content in sediment) and several heavy metals	32 days after	Depletion with higher organic load. There were synergistic effects with metals. Dominant genera: *Dorylaimopsis*, *Axonolaimus* and *Theristus*	South Africa, intertidal estuarine sediment	Gyedu-Ababio and Baird , 2006
Phyto-detritus, 25 and 50 mg C g^{-1} DW (eq. to 400 and 800 g C m^{-2}), added once time	4, 15 and 30 days after	Depletion and changes in composition, increase of dominance, dominant species: *T. longicaudata, and Spirinia parasitifera*	Cuba, subtidal (12 m) muddy polluted sediment	Present study

3.4.3 Extrapolation to field conditions

We should be cautious when extrapolating results from laboratory experiments to larger spatial scale in the field since that is one of the main sources of misleading conclusions (Carpenter, 1996). However, the response of infauna to organic enrichment are governed primarily by the adaptations of species to conditions caused by organic load, thus extrapolation of responses from small-scale experiments to larger scale can be accepted (Zajac et al., 1998). We infer that in muddy bottoms in Cienfuegos Bay the nematode assemblages are not food limited; a lack of response of nematode assemblages to food supply in organically enriched sediments has also been recorded by Moreno et al. (2008) in a more or less similar environment. Probably, the nematofauna can use the relatively high pool of organic matter already present in the sediment either as dissolved carbon (Jensen, 1987b) or as particulate carbon with variable quality (Rudnick, 1989).

Presence of mats (presumably cyanobacteria) on sediments has been observed at the deepest sites (i.e. > 10 m) of Cienfuegos Bay (M. Armenteros, pers. obs.); however, this phenomenon appeared to be highly patchy probably involving the spatially variable deposition of detritus from the water column (Levinton and Kelaher, 2004). In addition, the hypoxic conditions do not always lead to production and accumulation of ammonium and hydrogen sulfide by bacterial activity in the water (Gray et al., 2002); in natural conditions the hydrodynamic regime and the photoautotrophic activity probably contribute to a decrease of the reduced conditions of sediments (Snelgrove and Butman, 1994).

Our results suggest that further organic enrichment of sediments in muddy areas of the Cienfuegos Bay can cause drastic phase shift towards strongly reduced sediments with plenty of heterotrophic bacteria and relatively few tolerant species of nematodes in the sediment; strong changes can be expected also in benthic metabolism in response to a putative organic deposition (Kelly and Nixon, 1984).

3.5 Conclusions

(i) Nematode assemblages from naturally enriched sediments suffered deleterious changes when they were exposed to different loads of phyto-detritus in microcosms possibly due to accumulation of byproducts of bacterial metabolism. Hypoxia per se probably was not the direct cause of depletion in assemblages.

(ii) Dominant nematode species showed a different degree of sensitivity to reduced conditions, with *Spirinia parasitifera* as the least sensitive species, followed by *Terschellingia longicaudata*, *Metalinhomoeus filiformis*, and *Sabatieria pulchra*.

(iii) We predict a phase shift in sedimentary environment towards strongly reduced conditions and a depleted nematofauna if further high load of anthropogenic organic matter is added to these muddy bottoms.

3.6 Acknowledgements

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CHAPTER 4

A MORPHOMETRIC ANALYSIS OF THE GENUS *TERSCHELLINGIA* (NEMATODA, LINHOMOEIDAE) WITH REDEFINITION OF THE GENUS AND KEY TO THE SPECIES

Modified version from the published article:

Armenteros M, Ruiz-Abierno A, Vincx M, Decraemer W. 2009. A morphometric analysis of the genus *Terschellingia* (Nematoda, Linhomoeidae) with redefinition of the genus and key to the species. *Journal of the Marine Biological Association of the United Kingdom* 89: 1257-1267.

Abstract

The cosmopolitan and often ecologically dominant genus *Terschellingia* (Nematoda: Linhomoeidae), with 39 nominal species, is taxonomically a problematic taxon. Its species show high morphological plasticity, possess few diagnostic characters and identification keys are lacking. A revision of the genus was carried out based on morphological and morphometric data from the literature and from observations of specimens collected in Cienfuegos Bay, Caribbean Sea, Cuba. The diagnosis of the genus *Terschellingia* is amended. Of the current 39 nominal species, 16 are considered as valid species based on morphological characters related to size and position of amphidial fovea, presence/position of cephalic and cervical setae, presence/size/shape of pharyngeal bulb, shape of spicular apparatus and shape of tail. Tabular and pictorial keys were provided based on these characters. Three sympatric species: *T. communis*, *T. gourbaultae* and *T. longicaudata* were redescribed based on recently collected Cuban specimens. Each of them showed relatively large differences in body size in comparison with the respective type specimens, suggesting possible variation due to local environmental differences. The highest intraspecific variation pertains to the most widely spread cosmopolitan species *T. longicaudata*, suggesting that morphological plasticity enhanced adaptation to different environmental conditions. The notable taxonomic inflation within the genus (14 species inquirenda, 9 junior synonyms), probably also present in other highly specious genera of marine nematodes, can lead to an overestimation of the alpha-diversity.

4.1 Introduction

The genus *Terschellingia* (Nematoda: Linhomoeidae) was erected by de Man (1888) on the basis of the following features: four cephalic setae, buccal cavity small or absent and circular amphidial fovea located far forward on the head region. The etymology of the genus refers to the origin of the type specimens i.e. collected at Terschelling Island in The Netherlands. In general, species pertaining to this genus are cosmopolitan and very often numerically dominant in muddy subtidal bottoms (Heip et al., 1985). Therefore, they play an important ecological role in the sedimentary environment where they inhabit. Despite the notable presence of individuals belonging to the genus *Terschellingia* in samples from benthic studies, currently, identification to species level remains problematic.

The valuable compilation of free-living marine nematodes by Gerlach and Riemann (1973) indicated 28 valid species of *Terschellingia* and six synonymies. The present study describes 39 nominal species and a possible substantial taxonomic inflation (sensu Alroy, 2002). Most of the descriptions of *Terschellingia* species were carried out by pioneers of Nematology (e.g. Cobb, de Man, Filipjev, Gerlach and Timm) dating from more than 50 years ago. This implies the lack of holotypes, the statement of new species on the basis of one or two specimens, often females with relatively few features of taxonomic value. The relatively slow flow of information among researchers in those years and the reduced access to some journals also enhanced the existence of a plethora of synonymies. Three taxonomic keys have been elaborated (Wieser, 1956; Gerlach, 1963; Austen, 1989), however, these keys do not cover all species of the genus and they are not updated.

The problematic assessment of the genus *Terschellingia* fits in the larger gap about the taxonomic status of the family Linhomoeidae. The last revision of this family was published by Gerlach (1963) and no further revision has been carried out since. Lorenzen (1994), in his cladistic phylogenetic outline about free-living nematodes, recognized that more extensive analyses are still needed before relationships can be determined.

The ‘ideal’ taxonomic assessment of any taxon should be based on a phylogenetic approach, combining molecular techniques, like DNA sequence analysis, with

morphological data to constitute an appropriate basis for studies of diversity of nematodes (De Ley, 2000; Nadler, 2002). However, the promising application of molecular techniques for delimitation of species currently rests on a preliminary morphological approach (Derycke et al., 2005). A framework of nearly 40 species of *Terschellingi*a, most of them poorly described and morphologically similar, is not the best scenario for: (i) developing an easier way for identification and classification of relevant taxa in order to reduce the taxonomic impediment (De Ley, 2000); and (ii) applying a molecular approach to the taxonomy of the genus. Currently, the exhaustive revision of any taxon of free-living marine nematodes based exclusively on morphology appears in general not enough for a conclusive statement about taxonomy and relationships though it provides a basis for readdressing future studies on particular morph-species and phylogenetic relationships.

The genus *Terschellingia* possesses relatively few characters of diagnostic value. For example, labial sensilla are reduced (= small), cuticularized structures in buccal cavity as rings or teeth are absent or rarely present, precloacal supplements are rarely present, and the body cuticle lacks ornamentations such as pores or spines. The high morphological plasticity within species of this genus biases to clear identification of morph-species, and is surely related to the cosmopolitan distribution and numerical dominance of the genus in soft bottom habitats. Several appealing features within the genus, such as sperm dimorphism in *T. glabricutis* (Yushin, 2008) and possible presence of cryptic species in *T. longicaudata* (Bhadury et al., 2008), are an incentive for the continuation of the studies about the genus *Terschellingia.* The 'classical' morphological characters used for the diagnosis of species (e.g. relative position of amphidial fovea in the head region, the pattern of somatic setae and tail length) are clearly not sufficient and other morphometric characters were explored in order to refine species diagnoses.

Ecological studies in subtidal muddy bottoms from Cienfuegos Bay, Cuba, Caribbean Sea indicated a notable numerical dominance of the genus *Terschellingia* in the sediments. Three sympatric species are redescribed in the present study. The aims of this research are: (1) to identify the most important diagnostic features of the genus *Terschellingia* de Man 1888 and redefine the genus diagnosis; (2) to provide a comprehensive diagnosis of the valid species within the genus; and (3) to construct a pictorial key to species level. Additional information is provided for known species collected in Cuba.

4.2 Materials and methods

Samples were taken in February 2006 in six subtidal stations from Cienfuegos Bay, Caribbean Sea (22º 07´N, 80º22´W). The bay is a semi-enclosed water body with relatively high organic content in sediment and predominance of muddy bottoms. Samples were collected using hand-held cores and preserved in 8 % buffered formalin. Sediment samples were processed by sieving over two sieves with 500 µm and 45 µm mesh size and specimens were extracted by the flotation technique using a high-density sugar solution (1.17 g cm^{-3}). Sorted animals were transferred to anhydrous glycerol and mounted on glass slides following the standard procedure described in Vincx (1996). The description and measurements of the three identified species (*T. communis, T. gourbaultae, and T. longicaudata*) were done with a microscope Leica DMR (maximum magnification 1000 x) with drawing tube and interference contrast setting.

Most of the data of the species were collected from original descriptions using NeMys database (Deprez et al., 2007). The original type material on slides could not be analyzed because the types were not available to us mainly due to most of the species were described long time ago. From species of which original descriptions lacked relevant morphometric data, measurements were obtained directly from the illustrations. Measuring was carried out by a curvimeter for curvilinear (e.g. body length) and a ruler for straight measurements (e.g. body diameter), the maximum accuracy was 6 µm in 1000x. We used a rule for measurement of cephalic sensilla length in order to obtain the maximum possible accuracy.

The set of morphometric features considered of taxonomic relevance was mainly based on ratios (Table 4.1). Ratios were considered more convenient for comparisons than absolute measurements due to large variability (Fortuner, 1990) and because they were more accurately assessed from original drawings. A set of morphological features was defined for comparison among species: presence of teeth, position and presence of cephalic setae, position and presence of cervical setae, presence of pharyngeal bulb, development of gubernaculum apophysis, and shape of the tail (conical portion less or larger than 50 % of total length).

The great difference in number of described specimens for each species (i.e. replicates) prevented the application of statistical comparisons among species and to

evaluate completely the intraspecific variability. For the species reported from the literature only the measurements correspondent to holotype were used, therefore, statistical significance among species could not be tested. If holotype specimen was not clearly defined we used the measurements of the first male specimen.

4.3 Results and discussion

4.3.1 The genus *Terschellingia* de Man 1888

The genus belongs to the family Linhomoeidae (Monhysterida), a taxon of heterogeneous nature without known holapomorphy (Lorenzen, 1994). Three subfamilies are recognized: Desmolaiminae Schneider, 1926, Eleutherolaiminae Gerlach and Riemann, 1973 and Linhomoeinae Filipjev, 1922. The genus *Terschellingia* belongs to the Desmolaiminae, a subfamily mainly characterized by (modified from Schneider, 1926): cuticle smooth or faintly annulated, second and third circle of anterior sensilla close (6 + 10) or separate (6 + 6 + 4), amphidial fovea circular, buccal cavity conical and presence of cardia between pharynx and intestine.

Terschellingia, amended diagnosis. Desmolaiminae. Cuticle faintly striated. Amphidial fovea rounded. Buccal cavity absent or minute, cuticularized structures rarely present. Pattern of anterior sensilla: 6 + 6 + 4; the labial sensilla hard to detect; the four cephalic sensilla setiform. Spicules curved; gubernaculum with apophyses. Tail conico-cylindrical without terminal setae.

Type species: *Terschellingia communis* de Man 1888.

Table 4.1. Morphometric features defined for the analysis of the genus Terschellingia.

Code	Measurement	Calculation
L	Body length (µm)	
a, b, c	de Man´s ratios	
Amp	Position of amphidial fovea	Distance of end to anterior border of fovea / diameter of fovea
Acbd	Size of amphidial fovea	Diameter of fovea expressed in corresponding body diameter
Nerv %	Position of nerve ring	Expressed as percentage of pharynx length
Excp %	Position of excretory-secretory pore	Expressed as percentage of pharynx length
Bar	Shape of pharyngeal bulb	Length of bulb / width of bulb
T %	Length of male reproductive system	Expressed as percentage of body length
V %	Position of vulva	Distance of anterior end to vulva expressed as percentage of body length
G1 %	Length of anterior genital branch	Length of branch expressed as percentage of body length
G2 %	Length of posterior genital branch	Idem for posterior genital branch
Spicl	Length of spicule along arc	Length of spicule along arc / anal body diameter
Spicar	Shape of spicule	Length of spicule as curve / length of spicule as cord
c´	Tail length	Length of the tail / anal body diameter
Tcon %	Length of conical portion of the tail	Length of conical portion of the tail / total length of the tail

4.3.2 Evaluation of taxonomic diagnostic characters among *Terschellingia* species

The examination of six morphological (= qualitative) and 17 morphometric features allowed the detection of the characters of diagnostic value within the genus. Scatter plots of selected morphometric features were analyzed in order to look for those which discriminate among groups of species (Fig. 4.1). The features related to body size (length and de Man´s ratios a and b) not only showed poor discrimination among species but also tended to show high correlation (Fortuner, 1990). In addition, the significant relationship between body dimensions and food availability (dos Santos et al., 2008) suggested the lesser diagnostic value. Features related to relative position and size of amphidial fovea, length of the tail (relative to anal body diameter) and length and shape of spicules allowed discrimination of groups of species (Fig. 4.1) and were therefore considered of diagnostic value.

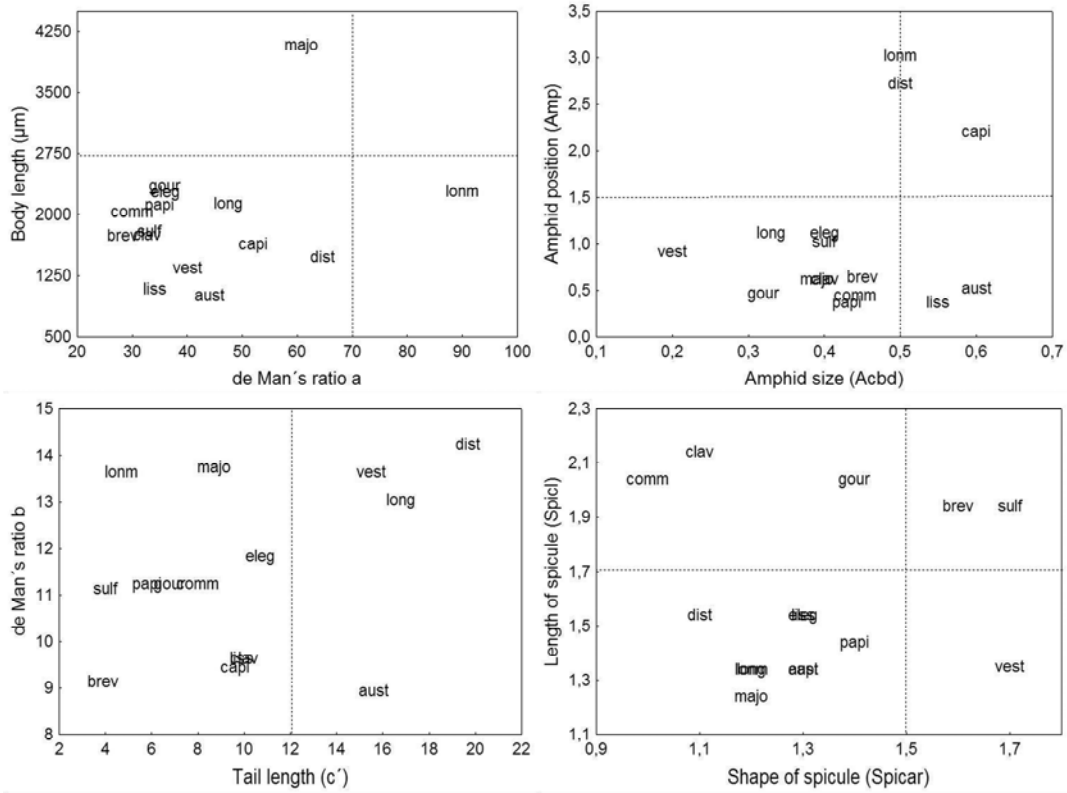

Figure 4.1 Scatter plots of morphometric features of valid species of the genus Terschellingi*a. Measurements correspond to holotype. Labels defined as 3 – 4 first types of the specific names in table 4.3 (except for* T. longisoma = *lonm). Dashed lines indicate possible cut-values for discriminating among species.*

Morphological characters would be highly useful in diagnosis of species. We selected six features: presence/absence of teeth, shape of cephalic setae (papilliform, setiform), presence/position of cervical setae (absent, at level of or posterior to amphidial fovea), presence/absence of pharyngeal bulb, developed/reduced apophysis and shape of the tail (conical portion less or more than 50 % of total length of tail). The number of developed ovaries appears to be a feature with taxonomic value, but it is not always described, mainly in old studies. The presence/pattern of precloacal supplements has been used extensively as taxonomic character; although supplements are present in at least one species (*T. longicaudata*) they are hard to observe with light microscope, and thus less useful as diagnostic character.

4.3.3 Discrimination of species within the genus *Terschellingia*

Within the genus, 39 nominal species have been described. However, according to our results only 16 of them are considered as valid. From the 23 non-valid species, 14 are species inquirenda and nine are junior synonyms of one of the valid former species (see table 4.2 for explanations). A pictorial (Fig. 4.2) and a tabular (Table 4.3) keys summarize the main diagnostic characters for discriminating among valid species of *Terschellingia* and an explanation follows below.

The presence of small teeth in the buccal cavity is the main taxonomic feature that distinguishes *T. elegans* and *T. sulfidrica* from the other species of the genus. Both species can be differentiated from one another by shape and length of spicules (more curved and larger in *T. sulfidrica*), tail shape (> 50 % conical in *T. sulfidrica* vs > 50 % filiform in *T. elegans*), and the presence of a single ovary in *T. sulfidrica*. According to Gagarin and Vu-Thanh (2003), *T. elegans* closely resembles *T. supplementata* (here synonymised with *T. longicaudata*) but mainly differs from it and in extension also from *T. longicaudata* by the presence of a tooth, the shorter cephalic setae and the absence of cervical setae.

Four species of *Terschellingia* have the amphidial fovea located relatively far from the anterior body end: *T. capitata, T. distalamphida, T. longisoma, and T. siphonolaimoides.* Latter species was first described by Wieser (1956) as *Southernia siphonolaimoides* Wieser, 1956 and renamed by Gerlach (1963) as *T.*

siphonolaimoides. The four species can be differentiated by a combination of characters. *T. distalamphida* can be distinguished from the other three by the filiform tail (< 50 % conical and more than 12 anal diameters length). *T. capitata* is characterized by a larger pharyngeal bulb compared to other three species. *T. siphonolaimoides* is very close to *T. longisoma* in the tail shape which is very poorly attenuated to terminus and in the shape of spicules (Gagarin and Vu Thanh, 2006). However *T. longisoma* is characterized by a very long and thin body (total length holotype: 2156 µm, a = 90) and absence of cervical setae; while *T. siphonolaimoides* has a = 41 and presence of cervical setae.

Terschellingia papillata is the only species of the genus with cephalic setae papilliform. The spicules of *T. papillata* are very similar to those in *T. longicaudata* (Fig. 4.2), but the former lacks the cervical setae and the conical portion of the tail is larger than 50 % of the total length; *T. longicaudata* has cervical setae and more than 50 % of the tail is filiform.

On the basis of presence / absence of pharyngeal bulb, two groups of species can be distinguished: a group of ten species with clear set-off pharyngeal bulb (six of them already characterized above). The remaining four species (i.e. *T. communis, T. lissa, T. longicaudata* and *T. vestigia*) differ from each other by a combination of characters (Table 4.3). *Terschellingia lissa* can be differentiated from the other three *Terschellingia* species by a larger size of amphidial fovea (> 0,5 cbd), conical portion of the tail less than 50 % of total length and lack of cervical setae. In addition *T. lissa* has a relatively small body length within the genus (< 1 000 µm).

Main differences between *T. communis* and *T. longicaudata* rest on the length of conical portion of the tail. The length of the tail appears to be useful for differentiating most of the specimens (i.e. *T. communis* c´ < 12; *T. longicaudata* c´> 12). However, we recorded in Cienfuegos Bay some unusually large male specimens of *T. longicaudata* with “short tail” (c´: 5,5 – 8,8); and some females of *T. communis* can have a relatively long tail (c´ 10 – 12). The original description by de Man (1888) of *T. communis* showed a completely conical tail with pointed tip, not found in any other description of the species; the latter feature was discussed by Timm (1962) as “problematic”. Reviewing the literature and based upon our own material, we found that the tail tip is rounded.

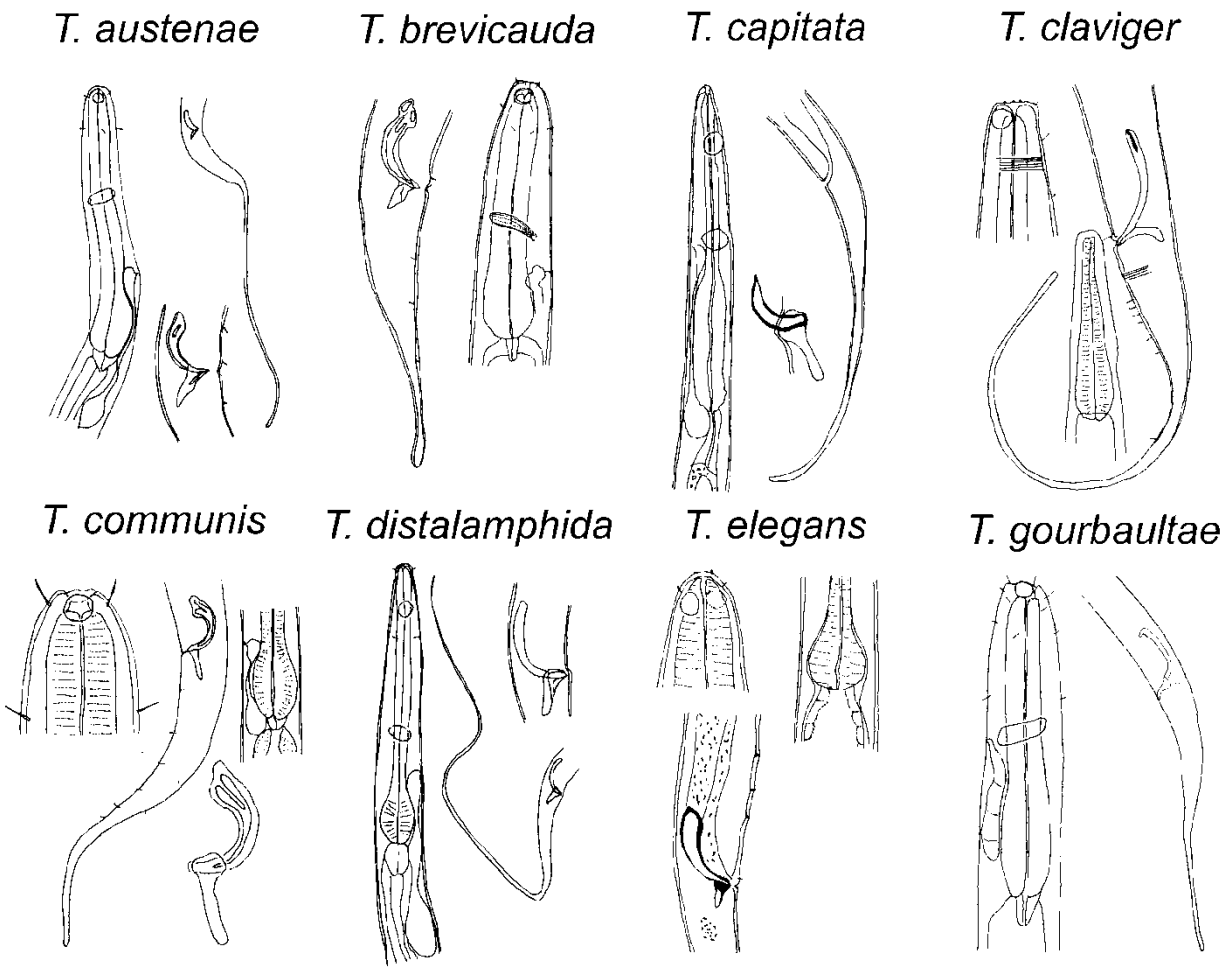

Figure 4.2. Outline of the 16 valid species of the genus Terschellingia *de Man 1888. Redrawing not to same scale and reproduced from original descriptions (except for* T. communis*, reproduced from Gerlach, 1963) (continues in next page).*

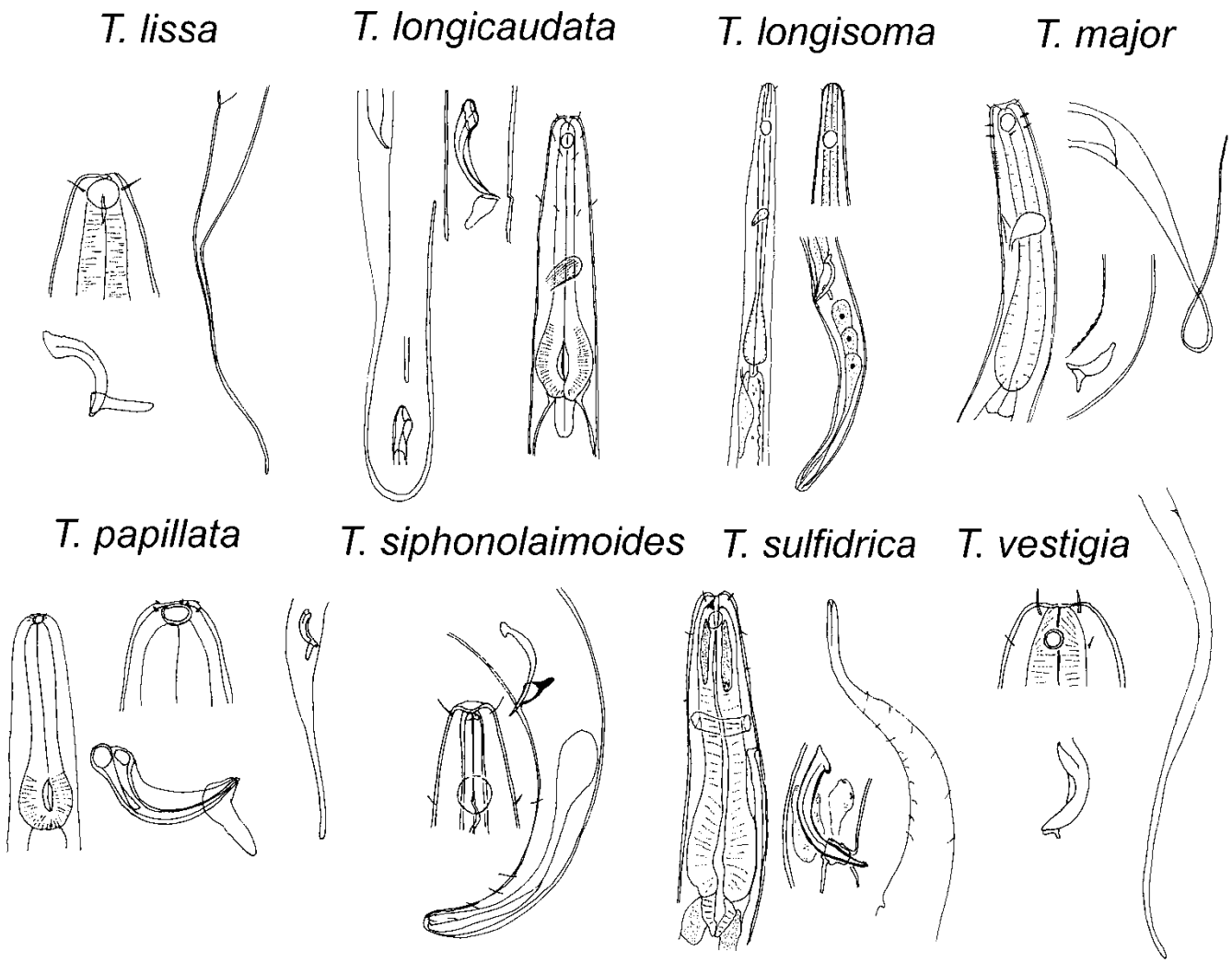

Figure 4.2 Continuation

The length of spicules appears to be an important feature for differentiating between the holotypes of *T. communis* and *T. longicaudata*. However, there are not clear differences between *T. communis* and *T. longicaudata* regarding to spicules length on basis of the few studies including absolute measurements, respectively: 54 – 61 µm (1,6 – 1,9 abd) vs 47 – 48 µm (1,7 abd) in Warwick et al. (1998); 38 – 44 µm (1,2 – 1,4 abd) vs 38 – 113 µm (1,4 – 1,9 abd) in specimens from Cienfuegos Bay. However, since Fig. 4.1 shows a clear cut-value around 1,7 abd for holotypes, we include the spicules length as a useful character for diagnosis.

Other important, but more difficult of standardize, differences between *T. communis* and *T. longicaudata* are regarding to cervical setae, shape of cardia, and shape of spicules and gubernaculum apophysis. So far, the main difference with respect to the pattern of cervical setae is the position (at level of amphidial fovea in *T. longicaudata*; posterior to the fovea in *T. communis*). However, the number of cervical setae and their relative position in the anterior region is variable in specimens of *T. longicaudata* as has been reported by other authors (e.g. Chitwood, 1951; Timm, 1961; Wieser and Hopper, 1967; Bhadury et al., 2008) and from specimens from Cienfuegos Bay. The cardia is larger, rounded and without pericardiac cells in *T. communis* vs cylindrical and rounded by intestinal cells in *T. longicaudata*, nevertheless the shape would be affected by the processes of preservation and mounting for the specimen. In relation to accessorial reproductive structures, *T. communis* has a proximal end of spicules non cephalated and the apophysis of gubernaculum wide and cuticularized in ventral border; *T. longicaudata* has a cephalated spicule with central septum in manubrium and a narrower apophysis of the gubernaculum. However, intraspecific variability in spicule shape and gubernaculum has been observed (i.e. compare *T. communis* in figures 4.2 and 4.3).

The high number of junior synonymies of *T. longicaudata* (in total five, see table 4.2) could be explained by: i) high abundance and cosmopolitan distribution leading to numerous descriptions by different authors; and ii) high morphological plasticity. There are, for instance: large variation in body habitus (de Man's ratio a ranged 29 - 40 after Timm, 1962), sexual dimorphism in size of amphidial fovea (♂ 0,3 cbd; ♀ 0,5 cbd after Wieser, 1956) and in relative position of amphidial fovea (Amp ♂ 0,5; ♀ 0,9 after Vitiello, 1969), different aspect of spicular apparatus (Vitiello 1969) and presence or absence of precloacal supplements.

Table 4.2. Outline of non-valid Terschellingia *species. Abbreviations: sp. inq. = species inquirenda, syn. = synonymy. For additional abbreviations see table 4.1. The numbers of specimens used in the original descriptions are indicated.*

Species	Conclusion	Comment
T. antonovi Filipjev 1922	syn. *T. longicaudata* (by Wieser, 1956)	8 ♂, 9 ♀, 11 j; original description match very well with current diagnosis of *T. longicaudata*.
T. baltica Schulz 1932	sp. inq. (new)	1 ♂ poorly described, Timm (1962) synonymised it to *T. longicaudata*, but original description is not sufficient for determination beyond doubt.
T. baylisi Allgén 1959	sp. inq. (by Gerlach 1963)	1 ♂ poorly described; cephalic sensilla not depicted.
T. exilis Cobb 1898	sp. inq. (new)	1 ♀ poorly described, no illustration included.
T. falklandiae Allgén 1959	sp. inq. (by Gerlach 1963)	2 ♂ poorly described; cephalic sensilla not depicted.
T. gerlachi Inglis 1968	syn. *T. longicaudata* (new)	1 ♂, originally differentiated from *T. longicaudata* just upon length of cephalic setae and presence of precloacal supplements.
T. glabricutis Platonova 1971	sp. inq. (new)	10 ♂, 10 ♀, absence of body setae appears to be a misinterpretation due to mounting techniques used (glycerine/gelatine).
T. heteroseta Schuurmans Stekhoven 1950	syn. *T. longicaudata* (by Gerlach 1963)	6 ♂; species diagnosis based on highly variable characters (length of tail, size of cephalic setae and the amphidial fovea); description matches closely *T. longicaudata.*
T. heterospiculum Allgén 1933	syn. *T. communis* (new)	1 ♂, after Gerlach (1963) syn. of *T. longicaudata*; but position of cervical setae behind amphidial fovea and the shape and length of tail did not support this statement.
T. longispiculata Wieser and Hopper 1967	syn. *T. longicaudata* (new)	The difference with *T. longicaudata* was longer spicules (122 µm) however the shape is closely similar. 2 ♂ from Cienfuegos Bay with long spicules (113 µm) underlined that this character is highly variable.

T. longissimicaudata Timm 1962	sp. inq. (new)	1 ♂; the specimen resembles *T. lissa*; the only difference lies in the 4 cervical setae behind the amphids in *T. longissimicaudata*.
T. magna Timm 1962	sp. inq. (new)	1 ♀. The synonymy of *T. communis* described by Gerlach (1955) is not valid due to differences in the amphidial fovea, cheilostome, cervical setae and tip of the tail.
T. maldivensis Austen, 1989 (nom. nov. pro. *T. claviger* apud Gerlach 1963)	sp. inq. (new)	1 ♂, sp. inq. because of a single male was described, and the only reliable diagnostic character was the absence of pharyngeal bulb.
T. minima Platonova 1971	sp. inq. (new)	6 ♂, 5 ♀, the description does not contain information on pharyngeal bulb and ovaries. Also absence of setae appears to be a misinterpretation due to employed techniques.
T. monohystera Wieser and Hopper 1967	syn. *T. communis* (new)	A pseudo-monodelphic condition is not enough for state a new species since in specimens of *T. communis* we recorded this condition (see Table 4.4). Other characters match to *T. communis.*
T. mora Gerlach 1956	syn. *T. communis* (new)	1 ♂, 1 ♀, specimens resemble to *T. communis* by Timm (1952) but differed from the holotype in the absence of cervical setae; these may be stout and therefore hard to detect.
T. parva Vitiello 1969	syn. *T. communis* (new)	3 ♂, 2 ♀, 3 j. The diagnostic character was the short body length (649 – 873 µm); but the description of *T. communis* by Timm (1962) indicated a similar size (780 µm). The high plasticity of *Terschellingia* suggests the low diagnostic value of the total length of body.
T. paxi Schneider 1939	sp. inq. (new)	1 ♀; poorly described. Species inquirenda because of absence of male specimens, poor description and lacking of discussion.
T. polaris Cobb 1914	sp. inq. (by Wieser 1956)	1 ♂, 1 ♀, poorly described. Amphidial fovea far from anterior end, double wings in the cuticle, small size (730 – 800 µm). Probably those specimens did not belong to the genus *Terschellingia*.
T. pontica Filipjev 1918	sp. inq. (new)	1 ♀, description closely resembles *T. longicaudata*; however the author stated that apparently the ends of ovaries were reflexed.

T. similis Allgén 1933	sp. inq. (new)	1 j, the original description of the juvenile specimen resembles closely *T. longicaudata.*
T. supplementata Tchesunov 1978	syn. *T. longicaudata* (new)	4 ♂, 3 ♀, specimens resemble *T. longicaudata*, only difference is presence of precloacal supplements, but they have been reported by Warwick et al. (1998), Pastor De Ward (1989); and in present study.
T. *viridis* Timm 1961	sp. inq. (by Timm 1962)	1 ♀, after Timm (1962) the specimen probably belongs to another genus due to the far posterior location of the amphidial fovea (1,6 Amp), and a cardia very different other species of *Terschellingia*.

The diagnosis of *T. vestigia* is based on the reduced dorsal apophysis of the gubernaculum. However, since only one male was described, putative intraspecific variability cannot be assessed. We prefer to maintain this species as valid given that it is relatively easy to check this diagnostic character.

Five species of *Terschellingia* lack a set-off pharyngeal bulb; the table elaborated by Austen (1989) and summarizing main differences among these species has been updated by Guo and Zhang (2000) and Huang and Zhang (2005) with addition of one species respectively but without further discussion. We found that some of the proposed diagnostic characters are less useful for species differentiation. Length-related measurements (total body length, de Man´s ratio a and tail length) showed notably overlapping among species. The body length is not a good main diagnostic character, even for species with extreme body sizes (*T. austenae* < 950 µm; *T. major* > 3436 µm) since in some monhysterids, body size is influenced by environmental factors as food availability (dos Santos et al., 2008). In addition, the length of spicules in five specimens of *T. gourbaultae* from Cienfuegos Bay was shorter (59 – 66 µm) than specimens from Tamar Estuary, England reported by Austen (80 - 88 µm), suggesting high variability in this character.

Two species (*T. austenae* and *T. claviger*) have less than 50 % of total tail length conical, with distal third portion filiform. These species can be differentiated from each other by the relative size of amphidial fovea and position of cervical setae; in addition, the length and shape of spicules and apophysis of gubernaculum would be useful diagnostic characters.

Differences among *T. brevicauda*, *T. gourbaultae* and *T. major* are more subtle on basis of taxonomic characters currently proposed. Austen (1989) pointed that the shorter tail in *T. brevicauda* (c´ 3,5 – 4,3) allows its differentiation from other species; the tail of *T. gourbaultae* is effectively larger (c´ 5,5 – 8,0 after Austen, 1989; 5,1 – 9,4 in specimens from Cienfuegos Bay). An additional feature, maybe less variable (and so more useful), is the position of the cervical setae. The spicular apparatus is different in appearance and in absolute measures (spicule length: 47 – 53 µm for *T. brevicauda* vs 80 – 88 µm for *T. gourbaultae*), but other morphometric measures such as relative length and shape of spicules failed to show differences (Fig. 4.1). The single record of *T. brevicauda* in North Caroline, USA (Ott, 1972) does not allow to assess possible intraspecific variability.

Table 4.3. Main diagnostic characters differentiating the valid species of the genus Terschellingia *de Man 1888. Abbreviations in table 4.1; additional: ceph. setae = shape of cephalic setae, cerv. setae = presence / position of cervical setae (first somatic setae) respect to amphidial fovea, phar. bulb = pharyngeal bulb, post. = posterior. States of character: 0 = absence, 1 = presence.* * *No females described.*

Species*	Teeth	Amp	Acbd	Ceph. setae	Cerv setae	Phar bulb	Tcon%	Spicl	Apophysis	Post ovary
T. austenae Guo & Zhang 2000	0	< 1,5	> 0,5	setiform	at level	0	< 50	< 1,7	developed	1
T. brevicauda Ott 1972	0	< 1,5	≤ 0,5	setiform	behind	0	> 50	> 1,7	developed	1
T. capitata Vitiello 1969	0	> 1,5	> 0,5	setiform	absent	1	> 50	< 1,7	developed	*
T. claviger Wieser 1956	0	< 1,5	≤ 0,5	setiform	behind	0	< 50	> 1,7	developed	*
T. communis de Man 1888	0	< 1,5	≤ 0,5	setiform	behind	1	> 50	> 1,7	developed	1
T. distalamphida Juario 1974	0	> 1,5	≤ 0,5	setiform	behind	1	< 50	< 1,7	developed	1
T. elegans Gagarin & Vu- Thanh 2003	1	< 1,5	≤ 0,5	setiform	absent	1	< 50	< 1,7	developed	1
T. gourbaultae Austen 1989	0	< 1,5	≤ 0,5	setiform	at level	0	> 50	> 1,7	developed	1
T. lissa Timm 1962	0	< 1,5	> 0,5	setiform	absent	1	< 50	< 1,7	developed	1
T. longicaudata de Man 1907	0	< 1,5	≤ 0,5	setiform	at level	1	< 50	< 1,7	developed	1
T. longisoma Gagarin & Vu-Thanh 2006	0	> 1,5	> 0,5	setiform	absent	1	> 50	< 1,7	developed	1
T. major Huang & Zhang 2005	0	< 1,5	≤ 0,5	setiform	at level	0	> 50	< 1,7	developed	1
T. papillata Gerlach 1955	0	< 1,5	≤ 0,5	papilliform	absent	1	> 50	< 1,7	developed	1
T. siphonolaimoides Gerlach 1963	0	> 1,5	≤ 0,5	setiform	at level	1	> 50	< 1,7	developed	*
T. sulfidrica Pastor de Ward 1989	1	< 1,5	≤ 0,5	setiform	behind	1	> 50	> 1,7	developed	0
T. vestigia Gerlach 1963	0	< 1,5	> 0,5	setiform	at level	1	< 50	< 1,7	reduced	1

* Some species can not be identified uniquely based on this table, the provided quantitative information and drawings should be used.

Two conspicuous features allow to identify specimens belonging to *T. major;* i.e. large body size (> 3000 µm), and presence of precloacal supplements (> 30). Latter character has been recorded in *T. sulfidrica*; but in specimens described from several geographic regions (e.g. *T. longicaudata*) the presence and number of supplements appeared variable. On the basis of the proposed differential diagnosis for species of *Terschellingia* the discrimination between *T. gourbaultae* and *T. major* would become problematic if high intraspecific variability exists in latter species. The differences in relative length of spicules between holotypes suggest the usefulness of this feature for discrimination.

4.3.4 Description of *Terschellingia* species from Cienfuegos Bay

For most of the features measured on specimens from Cienfuegos Bay they were relatively different from the data for the holotypes. This suggests a continuum in the size of body structures and a high morphological plasticity in the three species analyzed. The high morphological plasticity in some nematode genera with numerical dominance (e.g. *Daptonema*, *Sabatieria, Terschellingia*) could adjust in a more general model relating morphological plasticity and ecological success (Hollander, 2008 and references herein). It should be interesting to assess the level of intraspecific variability (i.e. morphological plasticity) in rarer species of nematodes; and also to test for relationships between morphological plasticity and ecological dominance in free-living marine nematodes.

The morphometric data are presented in table 4.4 and illustrations in figure 4.3. We only include in the text those features with some variation comparing to older descriptions of the species.

***Terschellingia communis* de Man, 1888**

Material measured: 3 ♂; 4 ♀; 2 j

Remarks. Body length of juveniles: 1000 – 1125 µm; differentiation between juvenile stages could not be determined beyond doubt. The degree of development of ovaries was variable either anterior ovary more developed than posterior one, reverse or equally developed. The shape of the spicules and the gubernaculum apophyses show variability in some details among the descriptions by de Man (1888), Timm

(1961; 1962), Gerlach (1963) and our observations. A conspicuous feature is presence of a developed cardia between pharynx and intestine; however, the shape sometimes appears to be affected by preservation or physiological condition of the specimen. This character has not been pointed by the former descriptions of the species but could be a useful character for identification.

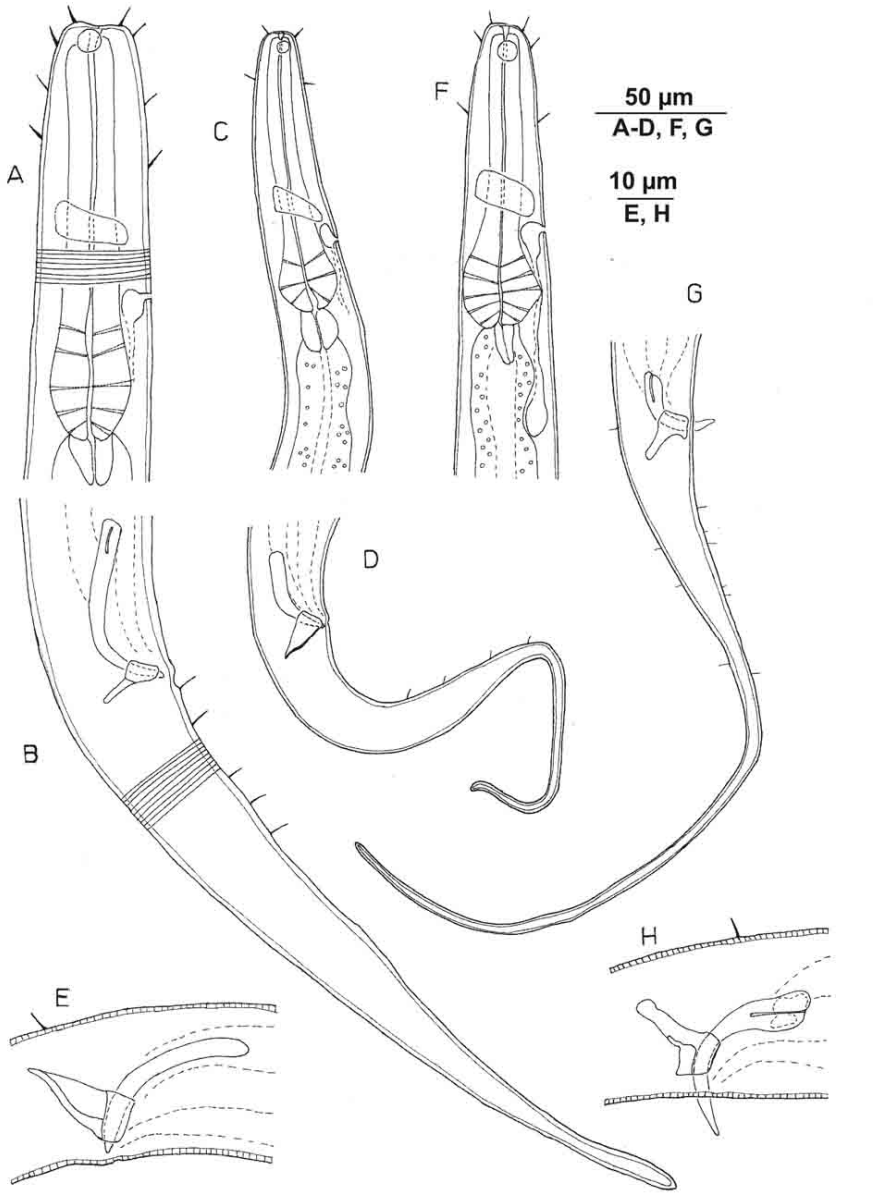

Figure 4.3. Species of the genus Terschellingia *recorded in Cienfuegos Bay, Caribbean Sea.* T. gourbaultae*: A) anterior part, B) tail;* T. communis*: C) anterior part, D) tail, E) spicular apparatus;* T. longicaudata*: F) anterior part, G) tail, H) spicular apparatus.*

Table 4.4. Morphometric data (min – max) for males and females of Cuban specimens of the genus Terschellingia*. Abbreviations in table 4.1; additional: amph Ø = diameter of amphidial fovea, amp.dist = distance of amphidial fovea to anterior end, gubern. = gubernaculum, L = length (µm), spic.arc and spic.cor = length of spicules as arc and cord respectively*.

	T. communis		*T. gourbaultae*		*T. longicaudata*	
Feature	3 ♂	4 ♀	5 ♂	5 ♀	10 ♂	5 ♀
Body L	1313-1400	1125-2000	1563-2063	1563-2500	1367-2438	1267-1750
a	31,1–32,8	22,0–38,9	25,9–31,2	21,6–33,5	25,9–44,9	27,8–45,0
b	12,4 – 13,8	12,0 – 14,4	9,6 – 11,5	9,6 – 3,3	11,5 – 13,9	12,0 –
c	5,0 – 5,6	4,5 – 8,9	5,2 – 7,8	4,6 – 6,0	2,2 – 7,8	2,5 – 3,5
Ceph setae L	4 – 6	4 – 6	4 – 8	4 – 9	2 – 7	5 - 8
Amph Ø (µm)	4 - 5	4 – 5	9 – 12	7 – 10	7 – 9	5 - 10
Acbd	0,3	0,2 – 0,3	0,3	0,2 – 0,3	0,3 – 0,5	0,3 – 0,5
Amp.dist (µm)	4 – 5	2 – 5	5 – 10	4 – 12	3 – 8	2 - 6
Amp	1,0	0,5 – 1,3	0,5 – 1,0	0,6 – 1,6	0,4 – 1,0	0,3 – 0,9
Nerv %	55 – 65	47 – 69	34 – 57	39 – 54	40 – 61	44 - 58
Excp %	78 – 83	69 – 75	50 – 79	61 – 71	57 – 76	65 - 68
Tail L	233 - 275	225 - 263	233 – 333	313 – 433	313 – 488	367 - 688
c´	8,0 – 8,9	6,1 – 11,9	5,1 – 6,5	6,5 – 9,4	5,5 – 18,0	15,5 –
Tcon %	55 –73	50 – 72	58 – 79	44 – 71	17 – 62	18 - 42
Testis L	733 – 767		938 – 1267		533 - 1313	
T %	55 – 65		47 – 68		39 - 51	
Spic.arc L	38 – 44		75 – 88		38 - 113	
Spicl	1,2 – 1,4		1,4 – 1,9		1,4 – 1,9	
Spic.cor L	31 – 33		59 – 66		25 - 86	
Spicar	1,2 – 1,3		1,1 – 1,5		1,4 – 1,6	
Gubernac. L	10 – 11		11 – 13		5 - 21	
Apophysis L	14 – 18		17 – 24		13 - 29	
G1 %		5 – 19		7 – 31		5 - 14
G2 %		5 – 15		5 – 29		5 - 14
V %		44 – 62		45 - 48		38 - 47

***Terschellingia gourbaultae* Austen 1989**

Material measured: 5 ♂; 3 ♀; 4 j.

Remarks. *T. gourbaultae* has been described recently from British and French estuaries. Body length of juveniles: 767 – 2125 µm; differentiation between juvenile stages could not be determined beyond doubt. The pattern of cervical setae described for holotype (i.e. three circles of cervical setae each one with eight setae) was common; but presence of only a single circle also occurred as well as a reduction in number of setae per circle (to 4 – 6). The specimens from Cienfuegos Bay closely resemble the holotype, except for the proximal end of the spicule. This suggests that morphological details of accessory reproductive structures have to be interpreted with caution since they can vary among populations.

***Terschellingia longicaudata* de Man 1907**

Material measured: 10 ♂, 5 ♀, 6 j.

Remarks. The specimens of *T. longicaudata* collected at Cienfuegos Bay closely resemble the original description of the holotype. Total length of juveniles: 733 – 1188 µm. Main differences regard the pattern of cervical setae and shorter length of cephalic setae in juveniles. The intestine often filled with conspicuous green granules all over its length. Precloacal supplements present, visible as small pits (6 - 7 in number) in large specimens using light microscopy; in smaller specimens only visible by scanning electronic microscopy (results not showed). Two large-sized male specimens (4280 and 4800 µm) of *Terschellingia* were described by Murphy (1965) who suggested that they belong to *T. communis*. However, those specimens were similar to *T. longicaudata* in the habitus, pattern of anterior sensilla, and shape and size of spicules; the main difference was the length of the tail (c´: 7). We also collected two large males (2375 and 2438 µm) with tail unusually short (c´ 5,5 and 8,6) and large spicules (113 µm both specimens). A recent study (Bhadury et al., 2008) combining morphological and molecular tools points to presence of cryptic species of *T. longicaudata*. In our study, the exploration of ultrastructure-based characters by SEM (as suggested by Bhadury et al., 2008) did not add any additional character of diagnostic value for discrimination among putatively cryptic species of *T. longicaudata*. Therefore, further refining of molecular techniques on this species (in combination with morphological analysis) is the most promising way for dealing with this taxonomically problematic species.

4.4 Acknowledgements

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CHAPTER 5

CIENFUEGIA GEN. NOV. (XYALIDAE) AND *PSEUDOTERSCHELLINGIA* GEN. NOV. (LINHOMOEIDAE), TWO NEW GENERA OF FREE-LIVING MARINE NEMATODES FROM CARIBBEAN SEA

Modified version from the published article:

Armenteros M, Vincx M, Decraemer W. 2009. *Cienfuegia* gen. nov. (Xyalidae) and *Pseudoterschellingia* gen. nov. (Linhomoeidae), two new genera of free-living marine nematodes from Caribbean Sea. *Journal of Natural History,* **43***: 1067–1081.*

Abstract

Two new genera of nematodes are described from the Caribbean Sea. *Cienfuegia* gen. nov. belongs to the Xyalidae based on the position of the anterior gonad constantly left of the intestine, cuticle clearly striated, second and third circle of anterior sensilla inserted at the same level and buccal cavity surrounded by pharynx. The new genus is differentiated from other genera by the buccal cavity divided in two chambers and by the four cephalic setae being longer than the six outer labial setae. Within the Xyalidae, *Cienfuegia* shows most affinities with the genera *Daptonema* and *Theristus*. *Pseudoterschellingia* gen. nov. is placed within the Linhomoeidae on the basis of the presence of anterior rounded amphidial fovea, unarmed narrow buccal cavity, distinctive cardia and presence of apophysis of gubernaculum. *Pseudoterschellingia* is closely related to the genera *Terschellingia* and *Terschellingioides*, but is differentiated by the crypto-spiral amphidial fovea, and conical buccal cavity surrounded by pharyngeal tissue.

5.1 Introduction

The order Monhysterida (Chromadoria, Nematoda) is characterized by the holapomorphy of outstretched ovaries. It includes three superfamilies and nine families of free-living aquatic nematodes, Monhysteroidea (Monhysteridae, Sphaerolaimidae, Xyalidae), Siphonolaimoidea (Siphonolaimidae, Linhomoeidae) and Axonolaimoidea (Axonolaimidae, Comesomatidae, Coninckiidae, Diplopeltidae) (Lorenzen, 1994), most of them from marine habitats (Warwick et al., 1998). The order also contains some of the most specious and conflicting genera of free-living marine nematodes such as *Theristus* Bastian 1865, *Daptonema* Cobb 1920, and *Monhystera* Bastian 1865. The present study deals with two new genera, belonging respectively to the families Xyalidae (superfamily Monhysteroidea) and Linhomoeidae (superfamily Siphonolaimoidea); both genera were collected from muddy subtidal sediments in Cienfuegos Bay (Cuba, Caribbean Sea).

The family Xyalidae Chitwood 1951 has been reviewed by Lorenzen (1977) and Nicholas and Trueman (2002). The family is characterized by the following taxonomic features according to Nicholas and Trueman (2002): cuticle annulated, six outer labial setae + four cephalic setae arranged in one circle, and female with a single outstretched anterior ovary. In their cladistic analysis, the latter authors recognized 33 genera within the family. The genus *Paramphimonhystrella* Huang and Zhang 2006 was added recently to the family. However, the taxonomy of the Xyalidae remains partially unresolved at species as well as genus level since several highly specious genera (e.g. *Daptonema* and *Theristus*) apparently show taxonomic inflation while other genera such as *Dactylaimus* Cobb 1920 are doubtful.

The family Linhomoeidae Filipjev 1922 was reviewed by Gerlach (1963) and since then no further revision has been carried out till now. According to Lorenzen (1994) the family comprises 22 valid genera belonging to three subfamilies and is characterized by high heterogeneity in morphological features. The following features are characteristic of the family (although exceptions for each of them are present as a rule): cuticle often striated, pattern of anterior sensilla 6 + 6 + 4 with second and third circle separated and rarely at the same level, amphidial fovea rounded (rarely crypto-spiral), buccal cavity frequently with cuticularized tooth-like structures or arches, cardia well developed, females usually with two outstretched ovaries and

males with two testes, with the position of anterior and posterior gonads opposite (i.e. anterior gonad to left and posterior to right side of intestine). The family Linhomoeidae has several conflicting taxonomic issues such as the unclear differentiation between the genera *Linhomoeus* Bastian 1865 and *Paralinhomoeus* de Man 1907, poorly described genera such as *Anticyathus* Cobb 1920 and *Prosphaerolaimus* Filipjev 1918 or genera described upon a single juvenile specimen such as *Perilinhomoeus* Schuurmans Stekhoven 1950.

5.2 Materials and methods

Samples of sediment were collected from Cienfuegos Bay, Cuba, Caribbean Sea (22°9' N and 80°27' W) in February, 2006 using hand-held plastic cores by SCUBA divers. The Bay is characterized by a narrow entrance (i.e. semi-enclosed bay), area of around 90 km^2, with average depth of 14 m, and predominance of muddy bottoms.

Collected samples of sediment were preserved in 8 % hot formalin buffered with borax and processed in the laboratory. Samples were sieved over 500 and 63 µm mesh size sieves and the latter fraction was collected. Sorting of nematodes from the sediment was carried out using a flotation technique with a high density solution (1.16 g cm^{-3}, commercial sugar crystals dissolved in filtered tap water). The supernatant was preserved in 4 % buffered formalin plus 1 % alcoholic eosin for staining the animals. Nematodes were picked out under a stereomicroscope (56 x), transferred to anhydrous glycerol and mounted on glass slides following the standard procedure in Vincx (1996). Species were identified and described using a microscope Leica DMR (maximum magnification 1000 x) and a Reichert Polyvar microscope (highest magnification 1250 x) both with drawing tubes and interference contrast. Measurements of straight and curved features were taken with a ruler and a curvimeter respectively with 1 µm of error at highest magnification and around 60 µm for longer measurements such as body length.

The taxonomic classification by Lorenzen (1994) was followed at taxonomic levels above genus. The abbreviations used hereafter: abd = anal body diameter, amp.fov.d. = distance from anterior border of amphidial fovea to anterior body end, amp.fov. Ø = diameter of amphidial fovea, ant. = anterior, buc.cav. = buccal cavity

diameter, ceph.s. = cephalic setae, V = distance between vulva and anterior body end as proportion of body length, S-E-pore = position of secretory/ excretory pore as proportion of pharynx length from anterior end, gen.branch = length of genital branch in male or female, gubernac. = gubernaculum, nerv.ring = position of nerve ring as proportion of pharynx length from anterior end, o.l.s. = length of outer labial setae, pharyng. = pharyngostom, post = posterior, spic.arc = length of spicules along the arc, spic.cord = length of spicules by the cord. Comparisons with other genera have been made based on literature and original descriptions; i.e. no type material was analyzed.

5.3 Results and discussion

5.3.1 *Cienfuegia* gen. nov.

Order Monhysterida Lorenzen 1981

Superfamily Monhysteroidea de Man 1876

Family Xyalidae Chitwood 1951

Genus *Cienfuegia* gen. nov.

Etymology

Refers to Cienfuegos Bay, Cuba, Caribbean Sea where the specimens were collected.

Diagnosis

Xyalidae. Cuticle coarsely striated. Head with six low lips, buccal cavity conical with two chambers marked by a constriction, without tooth or other cuticularized structures; inner labial sensilla not visible in light microscopy, six outer labial setae shorter than four cephalic setae, second and third circles of anterior sensilla at the same level or very close; amphidial fovea rounded, relatively small and positioned anteriorly in head region, at level of buccal cavity; cardia without pericardial cells. Female reproductive system with one outstretched anterior ovary; male reproductive system monorchic i.e. with one outstretched anterior testis; gonads in both sexes at left of intestine; gubernaculum with well developed dorso-caudal apophyses; tail with three caudal glands, and without terminal setae at tail tip..

Table 5.1. Main shared and differentiating features between Cienfuegia *gen. nov., and related genera within Xyalidae.*

Feature	*Cienfuegia* gen. nov.	*Daptonema* Cobb 1920	*Filipjeva* Ditlevsen 1928	*Paramonohystera* Steiner 1916	*Stylotheristus* Lorenzen 1977	*Theristus* Bastian 1865	*Zygonemella* Cobb 1920
six outer labial sensilla	setiform	setiform	setiform	setiform	setiform	setiform	setiform
cephalic setae	four	four or six	four	four or six	six	four or six	four
additional cephalic setae	no	no	no	no	yes	yes or no	no
buccal cavity shape	unarmed and bipartite	unarmed and one chamber	unarmed and one chamber	unarmed and one chamber	unarmed and one chamber	unarmed and one chamber	unarmed and one chamber
dorso-caudal apophysis	present	mostly absent	absent	absent	absent	present or absent	absent
tail shape	conical cylindrical	conical cylindrical	conical cylindrical	conical cylindrical	conical cylindrical	conical	conical cylindrical
terminal setae	absent	present	present	present	present	absent	present

Type species

Cienfuegia cachoi gen. nov., sp. nov.

Discussion and relationships

Cienfuegia gen. nov. is classified within the family Xyalidae based upon the position of the anterior gonad constantly left of the intestine (holapomorphy for the family), cuticle striated, second and third circle of anterior sensilla inserted at the same level or very close, and buccal cavity surrounded by pharynx. *Cienfuegia* gen. nov. shows some affinities with the genera *Diplolaimella* Allgén 1929 and *Diplolaimelloides* Meyl 1954 (Monhysteridae) due to the presence of a bipartite buccal cavity. However, in *Cienfuegia* gen. nov. the two chambers are indicated only by a slight constriction and change in orientation of their walls compared to two clearly differentiated chambers in the two other genera. *Cienfuegia* gen. nov. is characterized by the following characters: structure of buccal cavity, and four cephalic setae being longer than the six outer labial setae. Nicholas and Trueman (2002) made a cladistic analysis of the family Xyalidae based on maximum parsimony, using eight species of Monhysteridae and four species of *Sphaerolaimus* (Sphaerolaimidae) as outgroups. The data matrix used is not based on any prior judgement as to which character states are primitive and which derived. Transformation series simply define separate observable conditions, only treated as an ordered series where a change implies that the lineage has passed through an intermediate condition. In the parsimony consensus tree the species of the Monhysteridae formed a separate paraphyletic group; the remaining species are divided into 14 groups, a few of them representing clades including the 'out-group' *Sphaerolaimus* (hereafter we use the same labels that these authors used for the clades).

The genus *Cienfuegia* has affinities with the poorly defined groups B and C of Nicholas and Trueman (2002); group B includes several genera: *Daptonema* Cobb 1920 (partim), *Filipjeva* Ditlevsen 1928, *Paramonohystera* Steiner 1916 (partim), *Stylotheristus* Lorenzen 1977 and *Theristus* (*Penzancia*) Bastian 1865. Group C included other species of *Daptonema*, *Paramonohystera* and *Zygonemella* Cobb 1920. The shared features between *Cienfuegia* and the eight mentioned genera are mainly the six outer labial setiform sensilla, absence of additional cephalic setae (although they are present in *Stylotheristus* and in two species of *Theristus*: *T. manicatus* and *T. interstitialis*), a circular amphidial fovea, an unarmed buccal cavity,

a conico-cylindrical tail, and a triangular cardia. An additional feature of *Cienfuegia* relates it to the species belonging to the clade F (includes species of *Theristus* (*Theristus*) Bastian 1865 and the genera *Robustnema* Nicholas 1996 and *Echinotheristus* Thun and Riemann 1967): absence of terminal setae on the tail, and presence of dorso-caudal apophyses of gubernaculum.

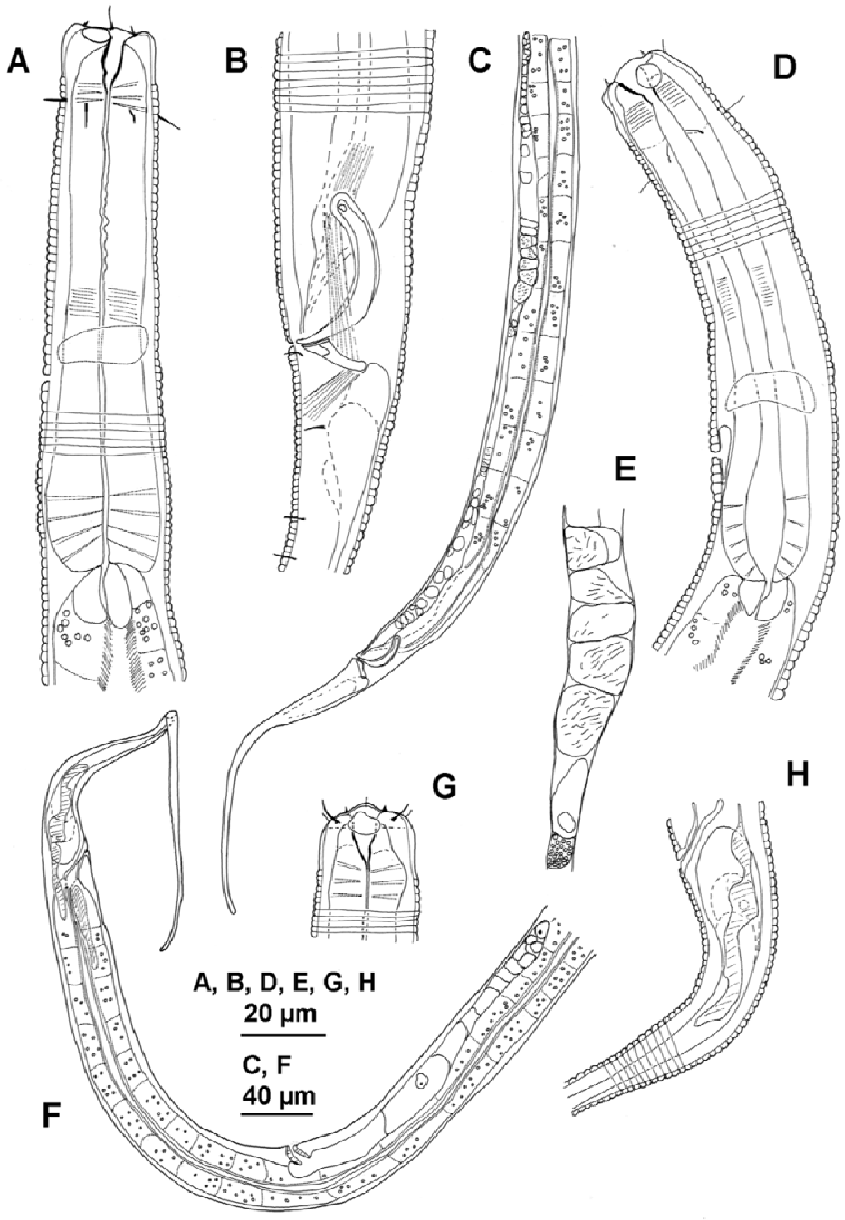

Figure 5.1. Cienfuegia cachoi *gen. nov., sp. nov. A) pharynx, holotype, B) spicular apparatus, C) posterior part, male, D) pharynx, female, E) detail of sperm cells, F) posterior part, female, G) head, female, H) detail of caudal and epidermic glands (stripped area), female. Position of cervical setae were showed in A) and D) even when they are not in the same optical section that internal structures.*

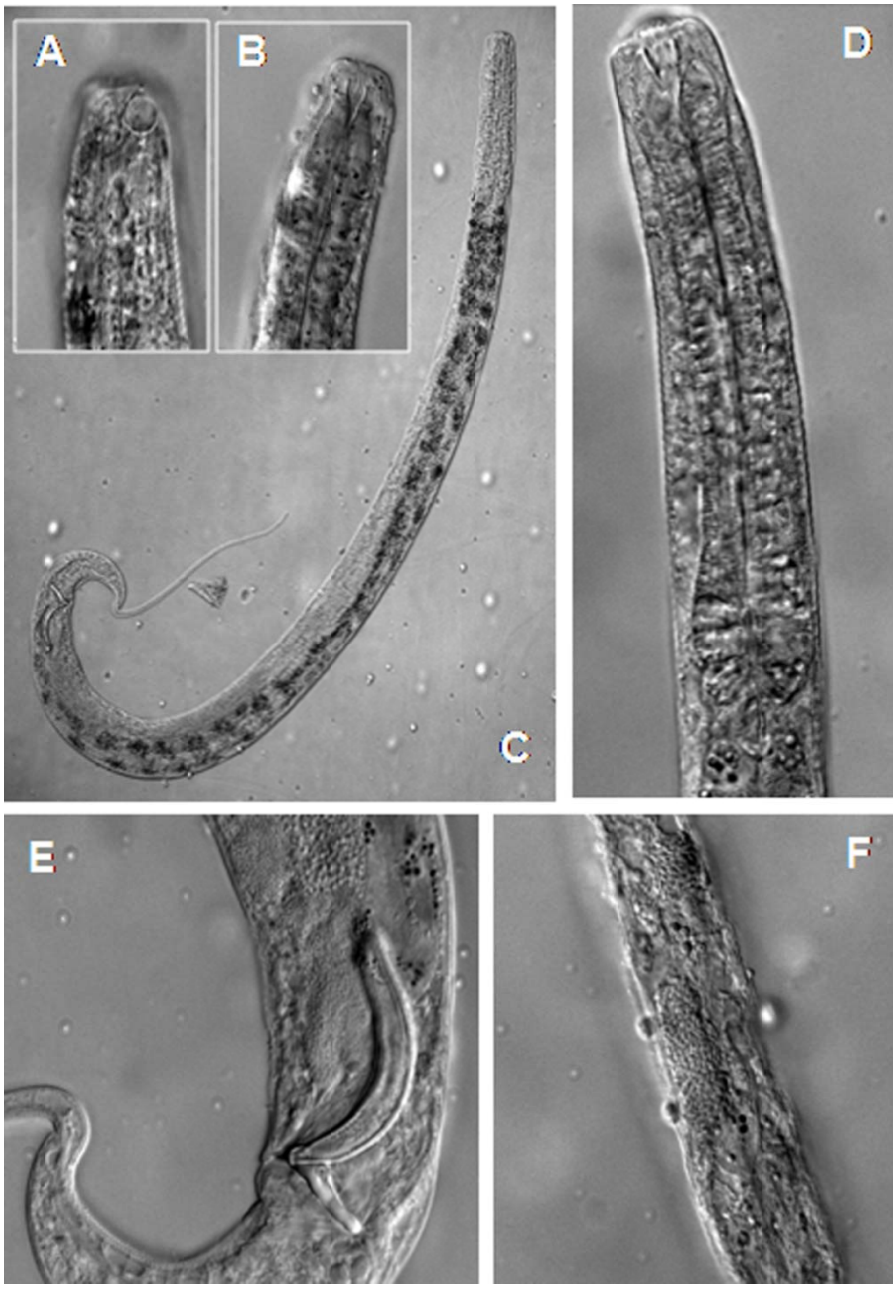

Figure 5.2. Cienfuegia cachoi *gen. nov., sp. nov. A) amphidial fovea, B) buccal cavity, C) habitus, D) pharynx, E) spicular apparatus, F) epidermic glands. All specimens are males.*

The differentiation between the genera *Daptonema* and *Theristus* is problematic and mainly based on tail shape, presence of terminal setae and the structure of the male copulatory apparatus (see Wieser, 1956). *Cienfuegia* gen. nov. possess intermediate tail-related features: conico-cylindrical tail (as in *Daptonema*) without terminal setae (as in *Theristus*); the strong and cuticularized apophysis of the gubernaculum and the shape of spicules is close to several species of the subgenus *Theristus* (*Theristus*)

although a few species of *Daptonema* also possess a well developed gubernaculum apophysis (e.g. in *D. laxus* Wieser 1956, *D. setosum* Bütschli 1874). A summary is presented in table 5.1 about the main shared and differentiating features among *Cienfuegia* gen. nov. and genera related.

Species *C. cachoi* gen. nov., sp. nov.

(Figures 5.1 and 5.2. Measurements in table 5.2.)

Material

1 male holotype, 11 ♂, 8 ♀ and 5 juvenile paratype specimens. Holotype male, slide 412.D.3 deposited in Departmento de Colecciones Marinas, Acuario Nacional de Cuba (ANC). Two male paratypes: slides RIT 746 and RIT 747, and two female paratypes: slide RIT 748; deposited in the nematode collection of the Royal Belgian Institute of Natural Sciences (RBINS). All other paratypes are deposited in ANC, Cuba.

Type locality

Cienfuegos Bay, Cuba; 22°9' N, 80°27' W.

Type habitat

Muddy bottom, depth 4 – 12 m.

Etymology

Species named in honour of Raúl Fernández-Garcés “Cacho”, senior technician of the Centro de Estudios Ambientales de Cienfuegos, Cuba.

Description

Body fusiform and slender, cuticle with coarse, transverse striations approximately 1 µm apart and without lateral differentiation. Six low lips may be retracted resulting in a different relative position of amphidial fovea and shape or anterior profile of head. Anterior sensilla arranged according to pattern 6 + (6 + 4); inner labial sensilla non-detectable under light microscope, six outer labial sensilla setiform but shorter than the four submedian cephalic setae; second and third circle of anterior sensilla at same level or very close. Cervical setae arranged in a circle of eight setae (5 µm long) in the anterior neck region of males and females; other somatic setae absent in

females apart from a row of 5 -10 setae (3 – 5 µm long) in ventral postanal region. Amphidial fovea rounded – flattened, shape influenced by the degree of retraction of anterior end. Buccal cavity narrow and conical with two chambers separated by a slight constriction. Pharynx muscular, surrounding the buccal cavity; anterior part often slightly swollen and posterior part also widened but without clear set off bulb. Cardia narrow, enclosed by intestine and without pericardial cells. Three caudal glands, all located within tail and with common outlet and minute spinneret. Tail shape conical cylindrical, the posterior cylindrical portion of variable length, tip rounded, without terminal setae. Continuous longitudinal bands of epidermic glands lateral, subventral (both sides) and dorsal; the grade of development depends of the specimen. The epidermic glands (where present) are clearly visible in the cardia and tail region, but not visible in the pharyngeal region.

Males monorchic, gonad left of the intestine. Spicules paired, curved, non-cephalated. Gubernaculum with well developed dorso-caudal apophyses. Large sperm cells with fibrilar appearance present, arranged in a single row. Sperm cells were not considered a diagnostic feature in the cladistic analysis of the family Xyalidae but might appear of taxonomic significance in the future. This feature is present also in other monhysterids (e.g. *Terschellingia glabricutis* Platonova 1971).

Females monodelphic, only anterior genital branch developed, at left of intestine and outstretched, vulva at mid-body (54 %).

Diagnosis

Six outer labial setae shorter than four cephalic setae at the same level or very close, head profile can change in shape due to retraction/protrusion of labial region, pharynx without posterior set off bulb, anterior part of the head neck region with only a circle of eight cervical setae, spicules curved and shorter than 2 abd.

Remarks

Specimens show different degree of invagination of lip region and cheilostome, a feature also reported for *Daptonema invagiferous* Platt 1973. The retraction of the anterior end causes relatively high variability in the distance of the amphidial fovea to the anterior end (e.g. often anterior border of fovea located at level of anterior border of body), and change in shape of fovea and of the anterior profile of the head (e.g. from rounded with clear six low lips to flattened squarish). Some specimens were

observed with filiform tail (more than half of total tail length filiform) while in others most of the tail is conical (cylindrical portion (= filiform) 20 %). Apart from differences in tail shape no other morphological differences were observed between these sympatric morphotypes.

Table 5.2. Morphometric features of Cienfuegia cachoi *gen. nov. , sp. nov. Mean values (range) are presented. Abbreviations in the text.*

Feature	Males n = 12	Females n = 8	Juveniles n = 5
body length (µm)	1207 (1100–1313)	1219 (1100–1375)	1131 (1033–1233)
a	29,4 (22,7 – 40,3)	27,1 (17,2 – 38,5)	35,0 (25,2 – 40,5)
b	9,1 (7,0 – 10,0)	8,6 (6,8 – 10,9)	9,3 (8,7 – 9,5)
c	4,5 (3,9 – 6,6)	4,3 (3,5 – 5,5)	3,8 (3,5 – 4,1)
c´	8,5 (4,4 – 10,8)	10,5 (6,4 – 15,2)	12,4 (10,5 – 15,1)
head Ø (µm)	20 (13 - 29)	23 (17 – 31)	17 (13 – 20)
o.l.s. (µm)	3 (2 – 4)	3 (2 – 5)	2 (2 – 3)
ceph.s. (µm)	6 (4 – 7)	6 (4 – 7)	4 (3 – 5)
buc.cav. ant. (µm)	5 (3 – 7)	7 (5 – 10)	5 (3 – 6)
buc.cav. post. (µm)	3 (2 – 4)	3 (2 – 5)	2 (2 – 3)
cheilostom (µm)	4 (2 – 8)	5 (3 – 6)	3 (2 – 4)
pharyng. (µm)	11 (9 – 17)	11 (8 – 16)	10 (8 – 13)
amp.fov.d. (µm)	2 (0 – 7)	2 (0 – 5)	2
amp.fov. Ø (µm)	7 (5 – 8)	5 (4 – 6)	6 (5 – 7)
S-E-pore %	63 (60 – 66)	64 (60 – 70)	72 (70 – 74)
nerv.ring %	54 (43 – 61)	52 (45 – 61)	53 (47 – 58)
body Ø (µm)	43 (28 – 55)	49 (32 – 80)	33 (27 – 41)
V %		54 (50 – 62)	
gen.branch (µm)	511 (350 – 633)	251 (75 – 400)	
spic.arc (µm)	57 (50 – 94)		
spic.cord (µm)	43 (37 – 73)		
gubernac. (µm)	8 (7 – 12)		
apophysis (µm)	15 (12 – 21)		
abd (µm)	33 (27 – 45)	29 (23 – 39)	24 (20 – 28)
tail (µm)	274 (200 – 313)	286 (250 – 350)	298 (263 – 338)
tail conical %	48 (32 – 82)	53 (39 – 75)	39 (33 – 52)

5.3.2 *Pseudoterschellingia* gen. nov.

Superfamily Siphonolaimoidea Filipjev 1918

Family Linhomoeidae Filipjev 1922

Subfamily Desmolaiminae Schneider 1926

Genus *Pseudoterschellingia* gen. nov.

Etymology

The genus closely resembles in morphological features the genera *Terschellingia* de Man 1888 and *Terschellingioides* Timm 1967 and therefore we propose the combination of pseudo (= false) and *Terschellingia*.

Diagnosis

Linhomoeidae. Cuticle transversely striated; amphidial fovea crypto-spiral with one loop, located relatively far anteriorly in the head region, buccal cavity narrow and conical, surrounded by pharyngeal tissue and without teeth or other cuticularized structures; pharynx with rounded muscular posterior bulb, cardia small, narrow triangular, surrounded by intestine and pericardial cells; males monorchic, spicules curved and strongly cuticularized; females monodelphic, ovary outstretched to the left of intestine; tail conical-cylindrical with spinneret and without terminal setae.

Type species

Pseudoterschellingia ibarrae gen. nov., sp. nov.

Discussion and relationships

Within the order Monhysterida, Lorenzen (1994) did not find any holapomorphy for the superfamily Siphonolaimoidea. Several features suggest us that the genus *Pseudoterschellingia* belongs to this superfamily: a) the condition monodelphic – prodelphic for females and monorchic for males, b) pharyngeal glands are visible in most of the specimens), c) presence of pharyngeal posterior bulb (difference with Monhysteroidea and Axonolaimoidea) , d) intestinal turgent cells, e) second and third circle of anterior sensilla clearly separated (difference with most of Monhysteroidea), and f) absence of preanal papillae (difference with most of Axonolaimoidea). The superfamily Siphonolaimoidea is composed of the families Siphonolaimidae and Linhomoeidae.

Table 5.3. Main shared and differentiating features between the genera Pseudoterschellingia *gen. nov.,* Terschellingia *de Man 1888 and* Terschellingioides *Timm 1967.*

Feature	*Pseudoterschellingia*	*Terschellingia*	*Terschellingioides*
position of the amphidial fovea	forward	forward	forward
gubernaculum	cuticularized with dorso-caudal apophysis	cuticularized with dorso-caudal apophysis	cuticularized with dorso-caudal apophysis
buccal cavity shape	small and conical	very small and narrow or absent	relatively large and cylindrical walls
pharyngostome	present	present or absent	absent
shape of amphidial fovea	crypto-spiral	circular	crypto-spiral
posterior pharyngeal bulb	enlarged	almost rounded or absent	almost rounded
ventral gland position	far and posterior to cardia	immediately posterior to cardia	immediately posterior to cardia

Pseudoterschellingia gen. nov. can be clearly differentiated from most genera within the Linhomoeidae by buccal cavity narrow and unarmed *vs* a wider buccal cavity with the presence of some kind of sclerotized structures like teeth or arches; the crypto-spiral shape of amphidial fovea *vs* circular; and size and shape of cardia: small and triangular vs well developed and elongated . From the 22 valid genera within Linhomoeidae, *Pseudoterschellingia* gen. nov. shows the highest affinities with the genera *Terschellingia* de Man 1888 and *Terschellingioides* Timm 1967; shared and differentiating features are presented in table 5.3.

Pseudoterschellingia ibarrae **gen. nov., sp. nov.**

(Figures 5.3 and 5.4. Measurements in table 5.4.)

Material

1 male holotype, 7 male paratypes, 11 female paratypes, 6 juveniles. Holotype: Male, 319.I.4, deposited in ANC. 4 paratypes: 2 males, slide RIT 749; 2 females, slide RIT 750; deposited in RBINS. All other paratypes deposited in ANC.

Type locality

Cienfuegos Bay, Cuba; 22°9' N, 80°27' W.

Type habitat

Muddy bottom, depth 4 – 14 m.

Etymology

In honour of the late Prof. Dr. María Elena Ibarra, Emeritus Professor of University of Havana, and tireless driving force of marine research in Cuba.

Description

Shape of body fusiform and slender; cuticle transversely striated; in some specimens transverse striae difficult to observe at mid-body region. Profile of head squarish; labial sensilla not observed in light microscopy, four submedian cephalic setae; four small submedian cervical setae far behind the amphidial fovea. A row of 3 – 6 ventral post-anal somatic setae (3 – 6 µm) present; no other somatic setae could be detected. Amphidial fovea crypto-spiral (0.3 – 0.4 cbd), with one and a half turn; anterior border of fovea located to 0.6 – 1.0 fovea diameters from anterior body end. Cheilostome small (2 µm length), pharyngostome funnel-shaped, weakly cuticularized, without teeth or ring and surrounded by pharyngeal tissue. Small pigmented areas present on both sides of the body (ocelli?) in some specimens at level of cervical region (19 – 25 µm from anterior end). Pharynx relatively long and muscular; posterior pharyngeal bulb with widened lumen clearly set-off and enlarged (aspect ratio explain: 1.4 – 2.2). Small triangular cardia surrounded by intestinal tissue; no pericardial cells. Nerve ring at level of mid-portion of pharynx. Secretory-excretory pore just anterior to pharyngeal bulb. Ventral gland cell along anterior part of intestine, relatively far from cardia. Tail conical in more than 2/3rd of its length; the

last portion relatively cylindrical and “clavated”, with small spinneret. Three caudal glands with a common duct restricted to the tail.

Males monorchic; spicules paired, curved, non-cephalated and relatively simple-structured. Gubernaculum with strongly cuticularized dorso-caudal apophyses.

Females monodelphic with ovary outstretched. Vulva notably rear in the body and conspicuous, very close to the anus.

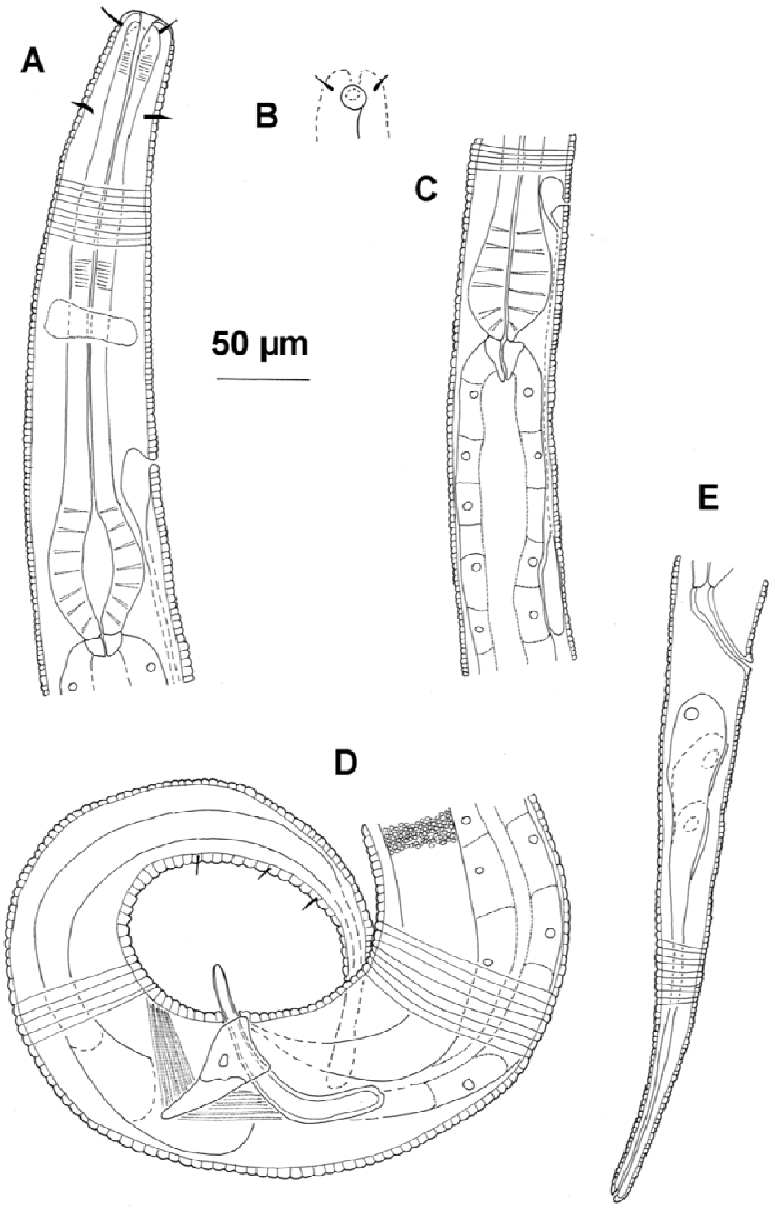

Figure 5.3. Pseudoterschellingia ibarrae *gen. nov., sp. nov. A) pharynx, holotype, B) detail of amphidial fovea, C) pharyngeal bulb, ampulla and ventral gland, D) spicular apparatus, E) tail of a female.*

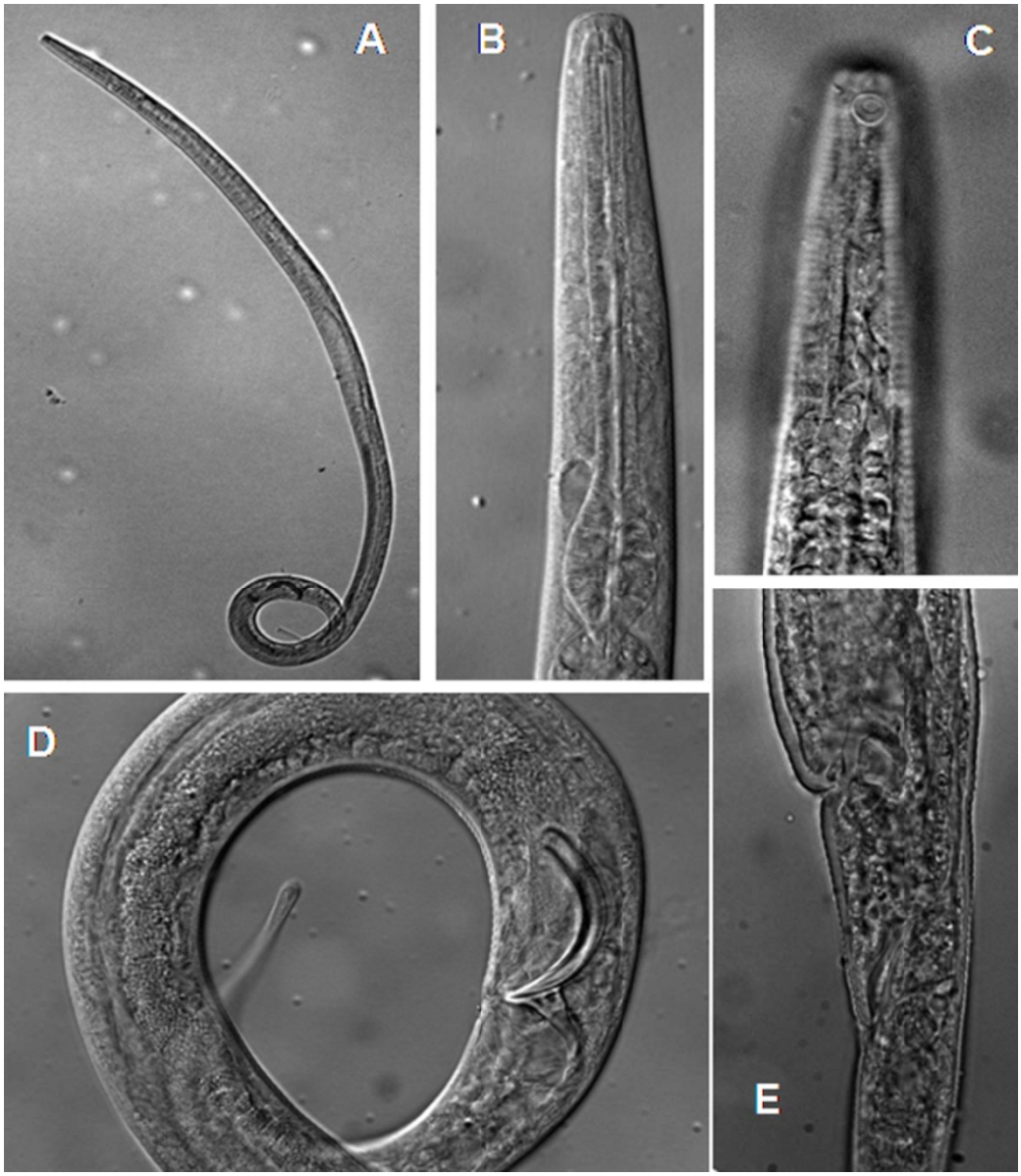

Figure 5.4. Pseudoterschellingia ibarrae *gen. nov., sp. nov. A) habitus, B) buccal cavity and pharynx, C) amphidial fovea and cephalic setae, D) spicular apparatus and tail, E) vulva and anus. All specimens are males except in photo E.*

Diagnosis

In addition to generic features, the features that characterized the new species are the following: only the four submedian cephalic setae (3rd circle of anterior sensilla) visible in light microscopy, four submedian cervical short setae, absence of precloacal supplements, the relatively simple and curved spicules and largest portion of the tail conical.

Table 5.4. Morphometric features of Pseudoterschellingia ibarrae *gen. nov., sp. nov. Mean values (range) are presented. Abbreviations in the text*.

Feature	Males n = 8	Females n = 11	Juveniles n = 6
body length (µm)	1433 (1313 – 1625)	1323 (1125 – 1500)	1116 (933 – 1500)
a	48,1 (42,4 – 52,9)	39,2 (30,5 - 52,6)	44,2 (31,0 – 57,6)
b	11,3 (10,4 – 13,0)	10,7 (9,9 – 11,9)	9,1 (6,5 – 12,0)
c	8,7 (7,5 – 10,0)	9,5 (8,5 – 10,9)	8,8 (6 – 12)
c´	6,6 (5,1 – 8,8)	8,2 (6,6 – 9,4)	7,1 (6,2 – 7,8)
head Ø (µm)	12 (11 – 13)	11 (9 – 13)	11 (10 – 13)
ceph.s. (µm)	3 (2 – 4)	3 (2 – 4)	4 (3 – 4)
amp.fov.d. (µm)	4 (3 – 5)	4 (2 – 5)	3 (2 – 4)
amp.fov. Ø (µm)	4 (4 – 5)	4 (4 – 4)	4 (4 – 4)
S-E-pore %	76 (70 – 81)	74 (70 – 77)	76 (70 – 79)
nerv.ring %	52 (43 – 58)	44 (40 – 53)	53 (46 – 59)
body Ø (µm)	31 (26- 38)	34 (26 – 43)	26 (22 – 30)
V %		86 (83 – 95)	
gen.branch (µm)	814 (688 – 938)	738 (500 – 875)	
spic.arc (µm)	51 (50 – 56)		
spic.cord (µm)	38 (37 – 41)		
gubernac. (µm)	12 (10 – 14)		
apophysis (µm)	19 (14 – 258)		
abd (µm)	27 (23 – 32)	17 (16 – 19)	16 (11 – 21)
tail (µm)	162 (151 – 183)	139 (125 – 151)	116 (69 – 123)

Remarks

Pseudoterschellingia ibarrae is the type species of the monospecific genus *Pseudoterschellingia*. Like other genera of monhysterids (including *Terschellingia* and *Terschellingioides*) the new species appears to be common in muddy hypoxic substrates. The coiling of the posterior part of the body and the presence of the green intestinal globules were common in the specimens studied; mentioned globules were reported also for *Terschellingioides filiformis* Timm 1967 and found by us in several putative deposit-feeder species from Cienfuegos Bay.

5.4 Acknowledgements

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CHAPTER 6

GUITARTIA GEN. NOV. (XYALIDAE) AND *MACRODONTIUM* GEN. NOV. (MICROLAIMIDAE), TWO NEW GENERA OF FREE-LIVING MARINE NEMATODES FROM CARIBBEAN SEA

Modified version from the article in press:

Armenteros M, Vincx M, Decraemer W. *Guitartia tridentata* n. gen., n. sp. (Xyalidae, Monhysterida) and *Macrodontium gaspari* n. gen., n. sp. (Microlaimidae, Chromadorida); two new genera of free-living marine nematodes from Caribbean Sea. *Nematology*.

Abstract

Two new genera of free-living marine nematodes are described from the muddy bottom of Cienfuegos Bay, Caribbean Sea. *Guitartia* gen. nov. (Xyalidae, Monhysterida) is characterised by three long tooth-like structures in the stegostom, second and third circle of anterior sensilla separate and posterior genital branch of the female restricted to a long post-vulvar sac. The type species is *Guitartia tridentata* gen. nov., sp. nov. Within the Xyalidae, *Guitartia* gen. nov. is morphologically close to the genera *Amphimonhystrella*, *Cobbia*, *Elzalia*, *Scaptrella* and *Valvaelaimus*, all of them characterised by sclerotised structures in the stoma and transverse striation of the body cuticle. Main features for discrimination are the type of stoma structure, the shape and relative size of amphidial fovea, the presence of a post-vulvar sac and gubernacular apophyses, and the absence of terminal setae on the tail. *Macrodontium* gen. nov. (Microlaimidae, Chromadorida) is characterised by a heavily sclerotised stoma with one large dorsal tooth and two smaller subventral teeth, sexual dimorphism in size and position of amphidial fovea and males with a single anterior testis. The type species is *Macrodontium gaspari* gen. nov., sp. nov. Within the Microlaimidae, *Macrodontium* gen. nov. is similar to the genera *Acanthomicrolaimus* and *Bolbolaimus* due to sclerotised stoma and presence of large dorsal tooth; the new genus is similar to *Aponema* in presence of sexual dimorphism in size of amphidial fovea, monorchic males, presence of apophysis of gubernaculum and conico-cylindrical tail shape. Morphological characters of diagnostic value within the family are the ornamentation of body cuticle, relative length of cephalic sensilla, stoma sclerotisation and number of testes.

6.1 Introduction

Documenting the biodiversity of free-living marine nematodes is a challenging task because of high biodiversity, the inherent difficulty of the group (i.e., small size) and various taxonomic impediments. Even with relatively modern approaches to the identification of species (barcoding, developmental biology, video vouchering), the classical morphological approach remains the first comprehensive step for recording, describing and classification. In the present study, we use the morphological species concept for delimiting species and propose two new species belonging to two new genera.

The marine nematofauna from coastal areas has been extensively studied in temperate regions, but less effort has been devoted to tropical areas. Since 2005 we have been studying the free-living marine nematodes from a tropical semi-enclosed bay in the Caribbean Sea with the aim of describing ecological patterns and carry out taxonomic research. We detected relatively few species of nematodes (79) in Cienfuegos Bay, most of them belonging to the order Monhysterida due to the dominance of muddy sediments in the basin. Two genera of free-living marine nematodes from this bay have been reported as new for science (*Cienfuegia* Armenteros, Vincx and Decraemer 2009 and *Pseudoterschellingia* Armenteros, Vincx and Decraemer 2009). In this article we present the description of two new genera belonging to the families Xyalidae Chitwood 1951 and Microlaimidae Micoletzky 1922.

The family Xyalidae Chitwood, 1951 appears to be monophyletic after Meldal et al. (2007) and within-family relationships have been revised, according to cladistic principles based on morphological and morphometric features, by Nicholas and Trueman (2002). According to these authors, the family is characterised by an annulated cuticle, six outer labial setae plus four cephalic setae arranged in one circle, and female with a single, outstretched, anterior ovary. From our results, an amended diagnosis of the family is proposed. In their analysis, Nicholas and Trueman (2002) recognised 33 genera within the family. Other genera have been included in the meantime: *Arabanema* Turpeenniemi, Nasira and Maqbool 2001; *Cienfuegia*; *Dactylaimoides* Blome 2002; *Enchonema* Bussau 1993; *Manganonema* Bussau 1993; *Marisalbinema* Tchesunov 1990; *Paragonionchus* Blome 2002;

Paramphimonhystrella Huang and Zhang 2006; *Parelzalia* Tchesunov 1990; *Pseudechinotheristus* Blome 2002; and *Sacrimarinema* Shoshin 2001.

The family Microlaimidae Micoletzky 1922 also appears to be monophyletic according to Meldal et al. (2007). The presence of a 12-fold vestibulum in stoma, teeth in the stegostom and outstretched ovaries are holapomorphies of the family (Lorenzen, 1994). A revision of the Microlaimidae was done by Jensen (1978), who proposed the erection of a new family (Molgolaimidae Jensen 1978) and the subdivision of Microlaimidae into two subfamilies (Bolbolaiminae Jensen 1978 and Microlaiminae Micoletzky 1922). Further arguments by Lorenzen (1994) suggest an absence of support for Jensen´s classification and we therefore follow Lorenzen´s classification concerning the Microlaimidae. After Lorenzen (1994) the family Microlaimidae contains seven valid genera: *Aponema* Jensen 1978; *Bolbolaimus* Cobb 1920; *Calomicrolaimus* Lorenzen 1976; *Cinctonem*a Cobb 1920; *Crassolaimus* Kreis 1929; *Ixonema* Lorenzen 1971; and *Microlaimus* de Man 1880; and one doubtful genus, *Ungulilaimella* Allgén 1958. Another four valid genera have been proposed: *Acanthomicrolaimus* Stewart and Nicholas 1987; *Bathynox* Bussau and Vopel 1999; *Caligocanna* Bussau and Vopel 1999; and *Spirobolbolaimus* Soetaert and Vincx 1988.

6.2 Materials and methods

Samples were taken in subtidal stations from Cienfuegos Bay, Caribbean Sea (22º 07´N, 80º22´W) in February, May and September 2006. The bay is a semi-enclosed basin with relatively high organic content in sediment and a predominantly muddy bottom. Samples were collected using hand-held cores and preserved in 8% buffered formalin. Sediment samples were processed by sieving over two sieves with 500 and 45 µm mesh size and specimens were extracted by the flotation technique using a high-density sugar solution (1.16 g cm^{-3}). Sorted animals were transferred to anhydrous glycerin and mounted on glass slides following the standard procedure described in Vincx (1996).

The description and photos were made with a Leica DMR microscope (maximum magnification 1500x) with drawing tube and interference contrast. Measurements of

straight and curved features were taken with a ruler and a curvimeter, respectively, with 1 µm of error at highest magnification and *ca* 60 µm for longer measurements such as body length. The taxonomic classification of Lorenzen (1994) was followed at taxonomic levels above genus. Comparisons with related genera were performed using literature and original descriptions; i.e. no type material was examined.

6.3 Descriptions

6.3.1 *Guitartia* gen. nov.

Family Xyalidae Chitwood 1951

Diagnosis (amended)

The present diagnosis is based mainly on Lorenzen (1994), Nicholas and Trueman (2002) and on features of the new genus.

Order Monhysterida Filipjev 1929. Body cuticle fine to coarsely annulated; annules smooth or ornamented with longitudinal striae, single or V-shaped ridges. Second and third circles of anterior sensilla inserted at same level or very close, exceptionally in two separate circles (*Guitartia tridentata* gen. nov., sp. nov.). Female with single anterior ovary located to left of intestine (exceptionally right of intestine in *Hofmaenneria niddensis* Gerlach and Meyl 1957 and *Steineria pilosa* (Cobb 1914) Micoletzky 1922), with or without a post-vulvar sac. Males diorchic or monorchic.

Guitartia gen. nov.

Diagnosis

Xyalidae Chitwood 1951. Body cuticle with fine transverse striation. Stoma narrow and cylindrical, with three long teeth-like structures appearing bifid at tip. Pattern of anterior sensilla 6 + 6 + 4, first circle probably papilliform but not visible by light microscopy; second and third circles of setiform anterior sensilla separate and of similar length. Amphidial fovea round, relatively large (more than 50% of corresponding body diameter). Female reproductive system with anterior

outstretched ovary, located to left of intestine, post-vulvar sac present. Male reproductive system with anterior outstretched testis, located to left of intestine. Gubernaculum with short dorso-caudal apophyses. Tail with three caudal glands, terminal setae at tail tip absent.

Etymology

In honor of the late Prof. Dr. Dario Guitart, Emeritus Professor at University of Havana, and professor of several generations of marine biologists in Cuba.

Relationships

Guitartia gen. nov. belongs to the Monhysterida in having an outstretched ovary, a holapomorphy for the order. The new genus is classified within the superfamily Monhysteroidea due to holapomorphy of a single anterior ovary (Lorenzen, 1994). Other features, such as the round amphidial fovea and pharynx lacking a proper posterior bulb also suggest membership of this superfamily. Within Monhysteroidea, *Guitartia* gen. nov. is classified in the Xyalidae due to the anterior gonad (ovary or testis) being located to the left of the intestine. Other features supporting classification within the Xyalidae are the transverse striation of the body cuticle, six outer labial sensilla being about equal or slightly shorter in length than the four cephalic setae of the 3rd circle of anterior sensilla, stegostom well developed and surrounded by pharyngeal tissue but gymnostom reduced or absent, and ventral gland apparently absent.

The Xyalidae is taxonomically a difficult group because of high morphological diversity, relationships within the family not being completely resolved and several genera appearing paraphyletic (Nicholas and Trueman, 2002). Based on the phylogenetic tree obtained, the authors recognised 15 groups, only a few representing clades. An attempt to place the new genus within the cladistic analysis by Nicholas and Trueman (2002) did not show any clade/group where the new genus could be included. In addition, a further subdivision of the family into three subfamilies (Rhynchonematinae de Coninck 1965, Cobbiinae de Coninck 1965, and Corononeminae Nicholas and Stewart 1995) has no clear phylogenetic basis (Nicholas and Trueman, 2002) and so we did not include *Guitartia* gen. nov. in any subfamily.

Two relatively conspicuous morphological features allow an initial discrimination of the new genus within the Xyalidae, *viz*., the sclerotisation of the stoma and the structure and ornamentation of the body cuticle. The presence of sclerotised tooth-like structures is relatively uncommon in the Xyalidae (see genera below) and allows the differentiation of the new genus from the species-rich genera *Daptonema* Cobb 1920 and *Theristus* Bastian 1865. The relatively simple pattern of transverse striation of the body cuticle of *Guitartia* gen. nov. (*i.e*., no longitudinal striae, punctations or ridges) allows further discrimination from other genera like *Gonionchus* Cobb 1920 (only *G. heipi* Vincx 1986 have simple transverse striation),*Omicronema* Cobb 1920, *Rhynchonema* Cobb 1920 and *Xyala* Cobb 1920. Table 6.1 shows the morphologically most similar genera to the new genus within Xyalidae and the most useful features for species differentiation. However, even the genera included in table 6.1 are highly divergent in morphology.

Type species

Guitartia tridentata gen. nov., sp. nov.

Table 6.1. Main morphological features differentiating the genus Guitartia *gen. nov. of the most similar genera within family Xyalidae (cbd = corresponding body diameter).*

Genus	Stoma armature	Amphidial fovea	Post. genital branch	Gubernacular apophyses	Terminal setae
Amphimonhystrella Timm 1961	weakly sclerotised	> 0.5 cbd	poorly developed	absent	present
Cobbia de Man 1907	three teeth	< 0.5 cbd	absent	absent	absent
Elzalia Gerlach 1957	heavily sclerotised	> 0.5 cbd	absent or poorly developed	absent	present
Guitartia gen. nov.	processes teeth-like	> 0.5 cbd	poorly developed	present	absent
Scaptrella Cobb 1917	six odontia	< 0.5 cbd	?	absent	present
Valvaelaimus Lorenzen 1977	processes teeth-like	< 0.5 cbd	poorly developed	absent	absent

***Guitartia tridentata* gen. nov., sp. nov.**

(Figures 6.1 and 6.2. Measurements in table 6.2)

Material examined

One male holotype and two female paratypes.

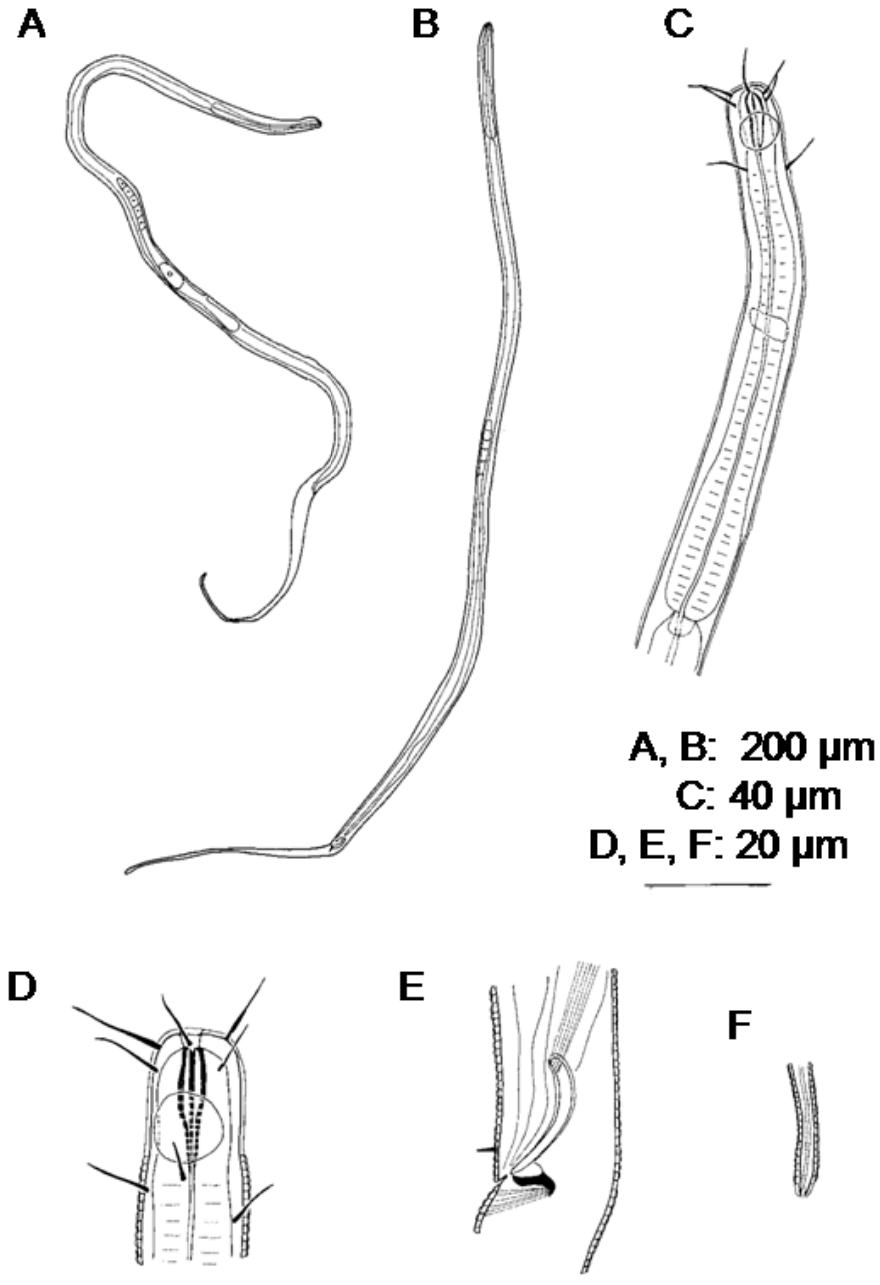

Figure 6.1. Guitartia tridentata *gen. nov., sp. nov. A: Habitus of paratype female; B: Habitus of holotype male; C: Neck region of paratype female; D: Head region of paratype female; E: Spicular apparatus of holotype; F: Tail tip of paratype female*.

Description

Body slender, tail with long filiform portion. Body cuticle with fine transverse striation (striae *ca* 1 µm apart) except for smooth head region. Anterior sensilla pattern 6 + 6 + 4, inner labial sensilla papilliform, not visible with light microscope, outer labial setae and cephalic setae at different level (circles) and of *ca* same length. Four submedian cervical setae arranged in one circle posterior to amphidial fovea, 10-12 µm long, one pair of lateral setae (10-12 µm) immediately posterior to fovea. Somatic setae rare, one precloacal seta present in male. Amphidial fovea large, round, located at level of posterior stoma. Cheilostom present, no apparent gymnostom, stegostom long, tubular, only posteriorly narrowing, surrounded by pharyngeal tissue, walls of lumen well sclerotised, anteriorly provided with three tooth-like structures (13 µm long) with anterior bifid tip (visible as six tips at anterior border stegostom). Pharynx largely cylindrical, gradually enlarging to the posterior end but without a proper pharyngeal bulb, pharyngeal glands apparently with outlet anteriorly in stoma. Cardia weakly developed, variable in shape. Ventral gland, duct and opening apparently absent. Tail conical-cylindrical, filiform, with three caudal glands and small spinneret at tail tip.

Male

Monorchic, testis to left of intestine, *vesicula seminalis* with large cells, *vas deferens* with differentiated granulation. Spicules ventrally curved, no offset capitulum, lamina mostly of equal width, only tapering towards distal tip, velum absent. Gubernaculum with short dorsal cuticularized apophyses. No precloacal supplements, only a single precloacal seta present.

Female

Monodelphic, anterior single genital branch arranged to left of intestine, ovary outstretched, posterior branch not developed except for a post-vulvar sac (spermatheca). Vagina anteriorly directed, with weakly sclerotised lumen wall.

Type habitat and locality

Subtidal muddy bottom at Cienfuegos Bay, Caribbean Sea (22°9' N, 80°27' W). Collected in the wet and dry seasons in 2006 by M. Armenteros.

Type material

Holotype male (specimen 985.I.5) deposited in Departamento de Colecciones Marinas, Acuario Nacional de Cuba (CNCM-ANC). One paratype female deposited in

each of the CNCM-ANC (specimen 1029.D.5) and in the nematode collection of the Royal Belgian Institute of Natural Sciences (specimen RIT764).

Table 6.2 Measurements of Guitartia tridentata *gen. nov., sp. nov. Abbreviations in the text. Other abbreviations: n. o. = not observable, * specimen damaged.*

Feature	Male 1	Female 1	Female 2
body length (µm)	1750	1750	1813
a	70.0	62.5	32.4
b	10.0	9.3	9.6
c	5.0	4.7	5.0
c´	19.4	18.8	14
head Ø (µm)	16	15	22
o.l.s. length (µm)	16	16	18
ceph.s. length (µm)	14	15	16
stego. Ø (µm)	4	4	7
amp.fov.d. (µm)	6	8	11
amp.fov. Ø (µm)	16	12	15
nerv.ring %	54	50	n. o.
body Ø (µm)	25	28	56
V %		59	*
ant.g.branch (µm)	833	132	*
pos.g.branch (µm)		69	*
spic.arc (µm)	25		
spic.cord (µm)	20		
gubernac. length (µm)	4		
apo. length (µm)	5		
abd (µm)	18	20	26
tail length (µm)	350	375	363
tail conical %	36	43	41

Diagnosis

Guitartia tridentata gen. nov., sp. nov. is characterised by the relatively long outer labial and cephalic setae which are of similar length (15-18 µm), large, round, amphidial fovea (more than 50% of corresponding body diameter), spicules 1.4 anal body diameters long, ventrally curved and without offset capitulum, precloacal supplements absent and tail with long, filiform, posterior portion.

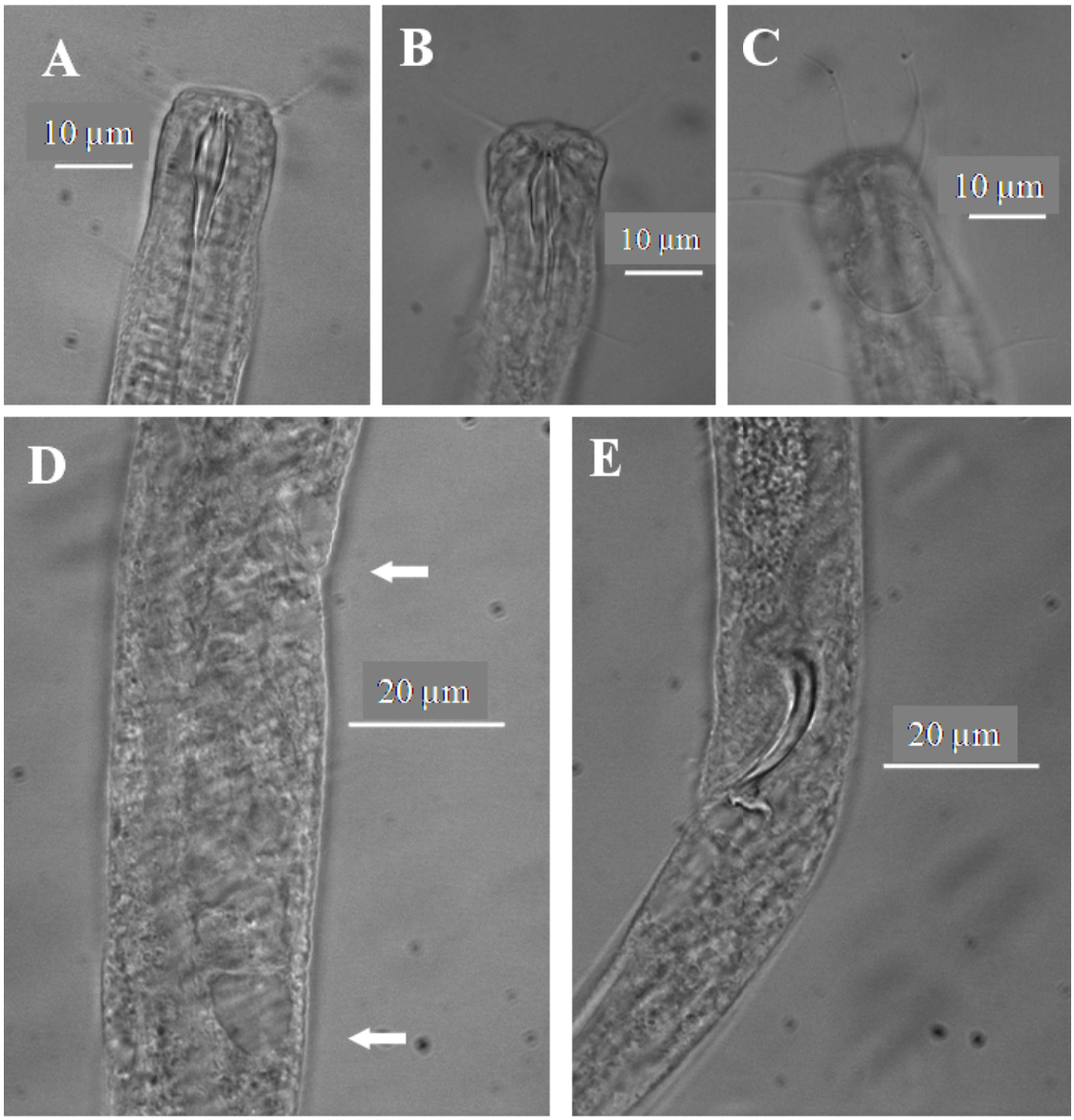

Figure 6.2. Guitartia tridentata *gen. nov., sp. nov. Light microphotographs. A: and B: Stoma of two different specimens; C: Amphidial fovea + cephalic sensilla; D: Posterior genital branch of a female paratype, arrows indicate position of vulva and end of the branch; E: Spicular apparatus (holotype).*

Ecological remarks

Specimens of *Guitartia tridentata* gen. nov., sp. nov. were collected from muddy and organically enriched bottom sediments. They probably show some tolerance to hypoxic conditions and chemical pollution as the concentration of some metals in these sediments (chromium, copper, nickel, vanadium) was high.

6.3.2 *Macrodontium* gen. nov.

Macrodontium gen. nov.

Diagnosis

Microlaimidae Micoletzky 1922. Body cuticle transversely striated. Four cephalic sensilla, larger than six outer labial sensilla. Amphidial fovea cryptospiral without protruding corpus gelatum, larger in males (sexual dimorphism). Stoma rather large, sclerotised, armed with a large dorsal tooth, two minute consecutive subventral teeth. Pharynx without differentiated anterior part but with terminal bulb. Males monorchic, gubernaculum with one well developed dorso-caudal apophysis. Females didelphic-amphidelphic with outstretched ovaries. Tail conico-cylindrical.

Etimology

The generic name refers to the large tooth.

Relationships

The main morphological features differentiating the new genus from other genera within Microlaimidae are summarised in Table 6.3. The most conspicuous feature of *Macrodontium* gen. nov. is the presence of a well sclerotised stoma with one large dorsal tooth and two consecutive subventral teeth, a combination suggesting *Acanthomicrolaimus* Stewart and Nicholas 1987 and *Bolbolaimus* Cobb 1920 as similar genera. *Macrodontium* gen. nov. differs from the former genus in having the body cuticle simply transversely striated *vs* covered with spine-like ornamentations and from the latter genus by cephalic sensilla longer than outer labial sensilla *vs* shorter.

Within the family Microlaimidae, most genera possess males with two testes and only two genera, *Bathynox* Bussau and Vopel 1999 and *Crassolaimus* Kreis 1929, have males with a single testis as observed in the new genus (note that in *Acanthomicrolaimus* the posterior testis is reduced). *Aponema* Jensen 1978 is the only genus in the family that shows a mixture of the monorchic and diorchic condition with four monorchic species and two diorchic species, namely *A. decraemerae* Muthumbi and Vincx 1999 and *A. mnazi* Muthumbi and Vincx 1999. The new genus differs from *Bathynox* by the form of the stoma, non-pedunculate somatic setae and anterior position of the amphidial fovea without protruding corpus gelatum (*vs* weakly

sclerotised stoma, somatic setae on peduncles, amphidial fovea located near mid-pharynx, amphid opening small and protruding corpus gelatum). *Macrodontium* gen. nov. differs from *Crassolaimus* by stoma armature with sclerotised walls and large dorsal tooth, presence of a gubernacular apophysis, and absence of precloacal supplements *vs.* small dorsal indentation in stoma, absence of gubernaculum apophysis, and presence of precloacal supplements in *Crassolaimus*.

The new genus shows some similarities with *Aponema* in body cuticle arrangement, sexual dimorphism in size of amphidial fovea (larger in males), presence of a gubernacular apophysis and conical-cylindrical tail shape. It differs from *Aponema* mainly by the stoma sclerotisation, the monorchic condition (partim in *Aponema*) and the number of gubernacular apophysis.

The genus *Microlaimus* de Man, 1880 is the most rich-species in the family, showing interspecific morphological variability in shape and size of amphidial fovea and tail shape. However, the heavy sclerotisation of the stoma and presence of an apophysis are important diagnostic features for discrimination between *Macrodontium* gen. nov. and *Microlaimus.*

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*Table 6.3. Main morphological features differentiating the genus Macrodontium gen. nov. of the other genera within Microlaimidae. cbd = corresponding body diameter; * length relative to outer labial sensilla.*

Genus	Striation of cuticle	Somatic setae	Cephalic sensilla*	Amphidial fovea	Sclerotisation of stoma armature	Testis	Apophyses of gubernaculum	Tail
Acanthomicrolaimus Stewart and Nicholas 1987	coarse	like spines	longer	cryptospiral, < 0.5 cbd	heavy, 1 larger dorsal tooth, 2 ventral	posterior reduced	one, dorsal	conical
Aponema Jensen 1978	fine	scarce	longer	cryptospiral, sexual dimorphism	weak, unarmed or 1 minute dorsal and 2 sublateral teeth	single anterior or two	two, dorsocaudal	conico-cylindrical or conical
Bathynox Bussau and Vopel 1999	coarse	on peduncles	longer	< 0.5 cbd, corpus protruded	weak, 1 small dorsal and 1 or 2 small subventral	single anterior	reduced	conical
Bolbolaimus Cobb 1920	fine	scarce	longer	cryptospiral, < 0.5 cbd	heavy, 1 dorsal tooth, 2 pairs of smaller sublateral teeth or denticles	two	?	conical
Caligocanna Bussau and Vopel 1999	coarse with longitudinal bars	scarce	shorter	cryptospiral,	2 dorsal anterior, 2 posterior teeth	two	absent	conical
Calomicrolaimus, Lorenzen 1976	coarse	scarce	longer	cryptospiral, sexual dimorphism,	weak, 1 small dorsal and two minute subventral teeth	two, posterior can be reduced	absent	conical

				corpus protruded				
Cinctonema Cobb 1920	coarse	scarce	longer	round or cryptospiral, < 0.5 cbd	weak, 1 small dorsal and 2 small ventrosublateral teeth	?	?	conical
Crassolaimus Kreis 1929	fine	scarce	longer	cryptospiral, < 0.5 cbd	weak; a single ventral tooth opposite indentation dorsal wall	single anterior	absent	conical
Ixonema Lorenzen 1971	absent	rows of long setae	longer	< 0.5 cbd , corpus protruded	weak; 1 dorsal and 2 smaller consecutive subventral teeth	two	absent	conical
Macrodontium gen. nov.	fine	scarce	longer	cryptospiral, sexual dimorphism	heavy, 1 dorsal tooth, 2 minute consecutive subventral teeth	single anterior	one, dorsocaudal	conico-cylindrical
Microlaimus de Man 1880	coarse	scarce	longer	round or cryptospiral, > or < 0.5 cbd.	weak; 1 small dorsal and 2 minute subventral teeth	two	absent	conical or conico-cylindrical
Spirobolbolaimus Soetaert and Vincx 1988	coarse	scarce	shorter	multispiral	weak; 1 small dorsal tooth, a pair of posterior sublateral teeth and a pair of tooth-like projections	two	absent	conical

Type species

Macrodontium gaspari gen. nov., sp. nov.

Macrodontium gaspari gen. nov., sp. nov.

(Figures 6.3, 6.4)

Etymology

Named in honour of the Prof. Dr Gaspar González-Sansón, Professor of Marine Ecology at the University of Havana, Cuba, and leader of marine research in Cuba.

Material examined

Ten males, ten females and ten juveniles

Measurements in table 6.4

Description

Body slender, anterior half of the body slightly broader than posterior half. Body cuticle transversely striated, striae *ca* 1 µm apart, head region and tail terminus smooth, cuticle showing lateral differentiation visible as a groove from cardia level until cloacal aperture (not seen in all specimens, probably dependent of preservation). Head region about as broad as long, slightly marked by depression at level of amphidial fovea. Pattern anterior sensilla: 6 + 6 + 4, six inner labial sensilla not visible, six outer labial sensilla minute, rarely visible, four submedian cephalic setae at level of (female and juveniles), or anterior to (male), anterior border of amphidial fovea. Four submedian cervical setae, in male located immediately posterior to fovea. Somatic setae rare; short somatic setae scattered in tail region. Stegostom lumen weakly sclerotised, provided with large dorsal tooth and two minute, consecutive, subventral teeth, posterior one slightly larger than anterior one. Amphidial fovea crypto-spiral, external circle interrupted in posterior wall, fovea *ca* twice as large in male as female. Pharynx muscular, with developed posterior bulb. Ventral gland immediately posterior of pharyngeal bulb, not easily seen in majority of specimens, secretory-excretory pore and ampulla not observed. Cardia rather small, surrounded by intestine. Three caudal glands within tail, difficult to observe in most specimens, with common outlet. Tail mostly conical with slightly swollen tip.

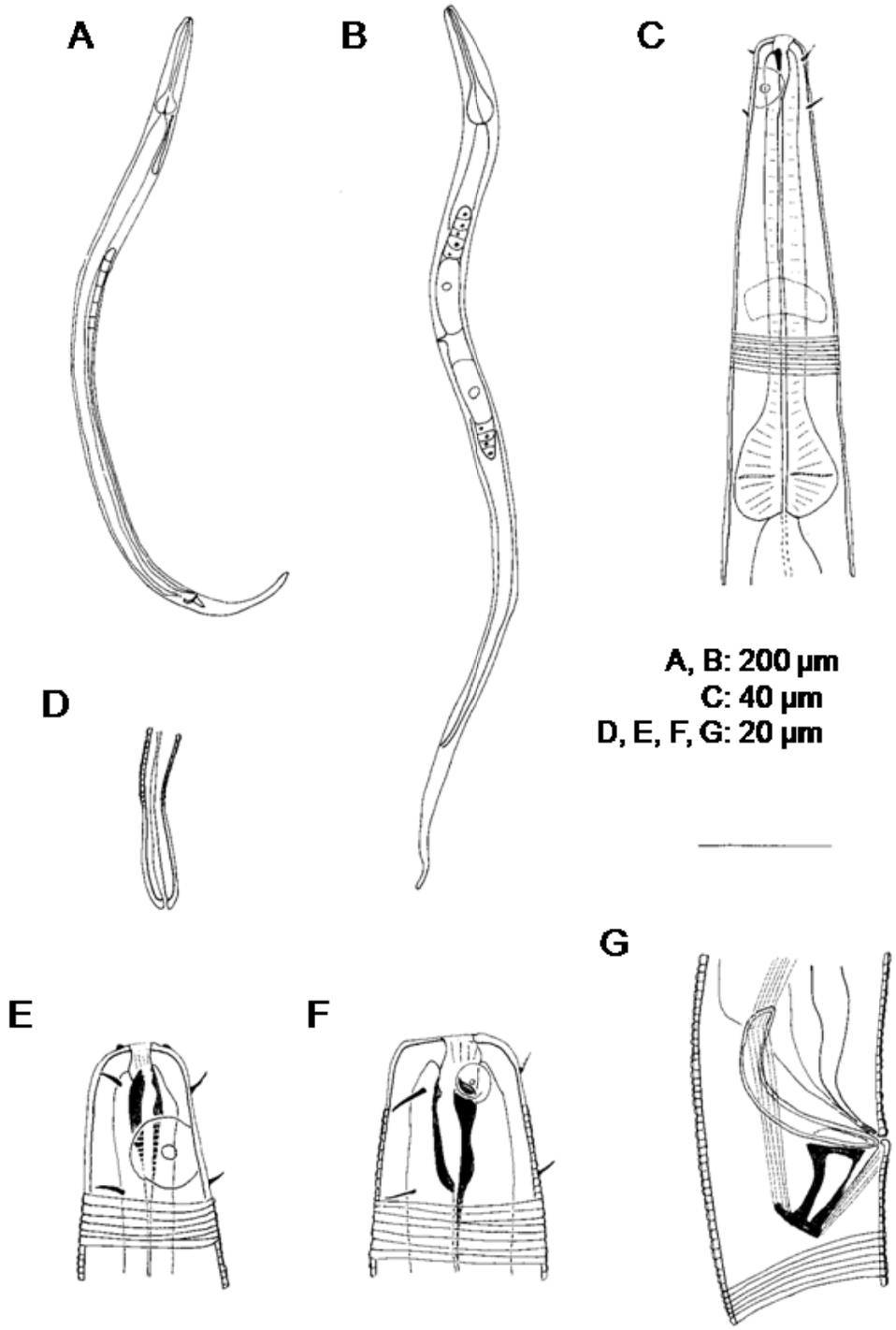

Figure 6.3. Macrodontium gaspari gen. nov., sp. nov. *A: Habitus of holotype male; B: Habitus of a paratype female; C: Neck region of holotype; D: Tail tip of holotype; E: Head region of holotype; F: Head region of a paratype female; G: Spicular apparatus of holotype.*

Table 6.4 Measurements of Macrodontium gaspari gen. nov., sp. nov*. Mean (range) values are presented. Abbreviations in the text.*

Feature	Males	Females	Juveniles
body length (µm)	1137 (1065 – 1300)	1242 (1125 – 1375)	963 (730 – 1125)
a	26,7 (21,0 – 31,4)	25,6 (19,4 – 31,3)	24,2 (19,3 – 34,4)
b	7,7 (7,0 – 8,7)	7,6 (6,4 – 8,3)	7,0 (6,3 – 7,8)
c	6,9 (6,1 – 7,8)	6,2 (5,3 – 6,9)	5,9 (4,9 – 6,8)
c´	5,5 (4,4 – 6,3)	8,3 (5,5 – 9,7)	7,4 (6,3 – 9,6)
head Ø (µm)	16 (13 – 19)	19 (16 – 24)	15 (11 – 20)
ceph.s. length (µm)	3 (2 – 3)	4 (2 – 4)	3 (2 – 4)
stego. (µm)	4 (3 – 5)	5 (2 – 6)	4 (2 – 5)
amp.fov.d. (µm)	8 (6 – 10)	5 (3 – 8)	5 (2 – 7)
amp.fov. Ø (µm)	11 (10 – 12)	4 (4 – 5)	4 (3 – 4)
nerv.ring %	47 (46 – 51)	43 (38 – 47)	45 (36 – 50)
body Ø (µm)	44 (34 – 56)	51 (40 – 62)	41 (29 – 57)
V %		40 (31 – 44)	
ant.g.branch (µm)	581 (467 – 667)	171 (100 – 213)	
pos.g.branch (µm)		159 (88 – 200)	
spic.arc (µm)	35 (31 – 44)		
spic.cord (µm)	25 (22 – 29)		
gubernac. length (µm)	7 (6 – 10)		
apophysis (µm)	17 (13 – 19)		
abd (µm)	30 (26 – 35)	25 (22 – 32)	22 (17 – 28)
tail length (µm)	165 (148 – 188)	204 (175 – 213)	163 (125 – 200)
tail conical %	89 (85 – 93)	87 (82 – 94)	86 (77 – 93)

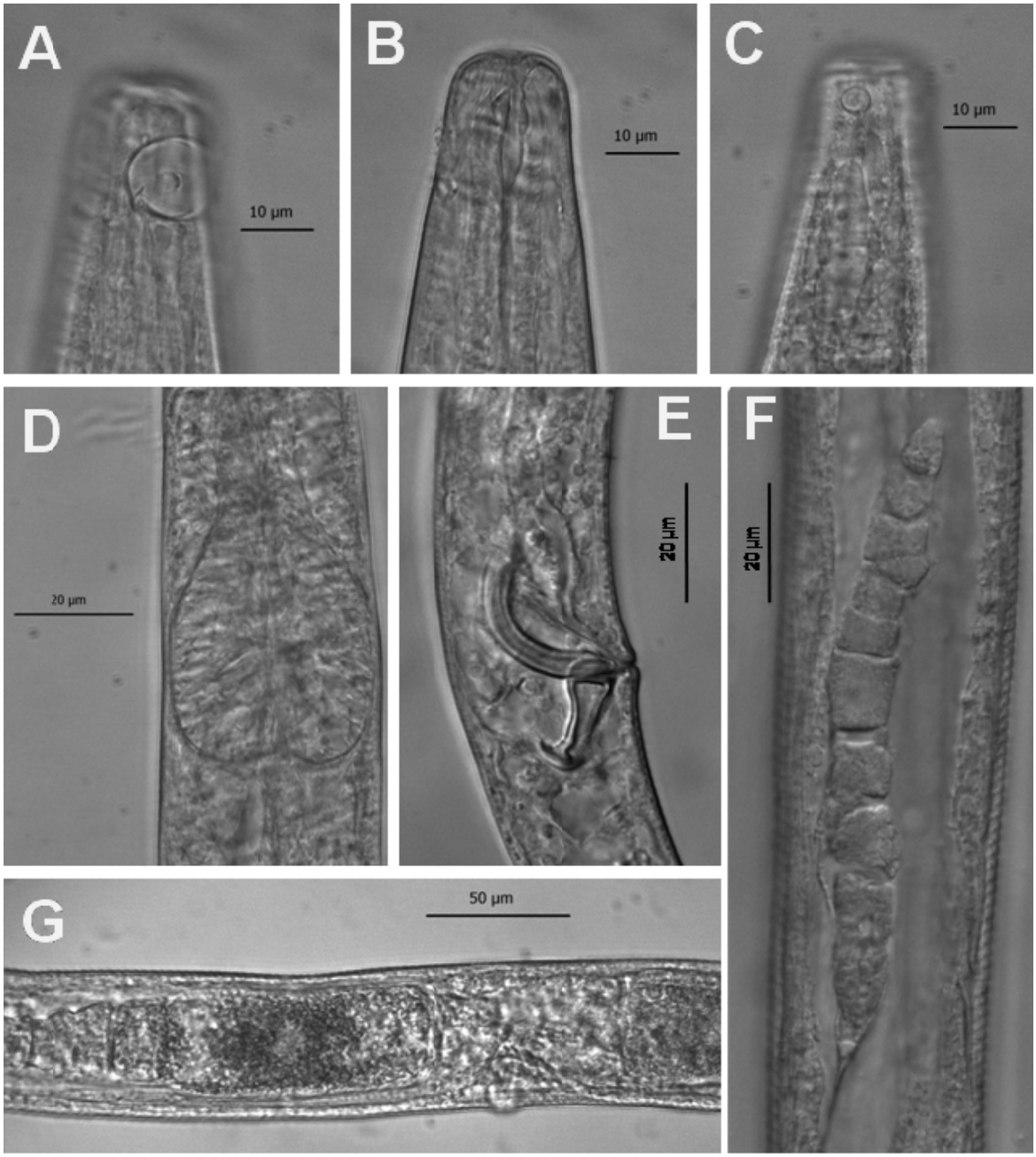

Figure 6.4. Macrodontium gaspari gen. nov., sp. nov. *Light microphotographs. A: Amphidial fovea of a male paratype; B: Stoma of the same specimen; C: Amphidial fovea of a female paratype; D: Pharyngeal bulb; E: Spicular apparatus; F: Single testis; G: Outstretched ovaries.*

Male

Single anterior testis located to right of intestine, *vas deferens* long, narrow. Spicules ventrally curved, non-cephalated. Gubernaculum with small corpus but one well developed dorso-caudal apophysis with sclerotised borders and hammer-like terminus (proximal end for attachment of protractor muscles).

Female

Genital system didelphic, amphidelphic. Both ovaries outstretched, often with one egg in each uterus. Position of genital branches regarding intestine variable, either to left or right of intestine. Vulva relatively anterior in position, lumen wall relatively sclerotised.

Type habitat and locality

Subtidal muddy bottom at Cienfuegos Bay, Caribbean Sea (22°9' N, 80°27' W). Collected in wet and dry seasons in 2006 by M. Armenteros.

Type material

Holotype male (specimen 550.D.1) deposited in CNCM-ANC. Three paratypes (two males and one female; specimens 550.D.3, 550.I.5, and 576.I.4 respectively) deposited in CNCM-ANC; three paratypes (two males and one female, specimens RIT764) deposited in RBINS, Belgium. Other paratype specimens (adults and juveniles) are deposited at Centro de Investigaciones Marinas..

Diagnosis

The new species is characterised by sexual dimorphism in size and shape of the amphidial fovea, size and shape of spicules (31-44 µm, non cephalated) and gubernaculum (6-10 µm with one apophysis), and absence of precloacal supplements.

Ecological remarks

Individuals of *Macrodontium gaspari* gen. nov., sp. nov. were common in muddy, organically enriched, bottom sediments subjected to pollution by heavy metals and hydrocarbons in Cienfuegos Bay. Specimens were always collected in the upper 2 cm of the sediment and were absent below this depth. The species appears to be tolerant to chemical pollution since it was relatively abundant in sediment with high concentrations of heavy metals.

6.4 Acknowledgements

We thank the International Foundation of Science (research grant A-4004/1 to M. Armenteros) for financial support and Ghent University (doctoral scholarship BOF 01W01607 to M. Armenteros). The Belgian Focal Point to the Global Taxonomy Initiative (project 2406JVG2) assisted with the taxonomic expertise and equipment necessary for this research. We acknowledge two anonymous referees for improving the manuscript with their comments and corrections.

CHAPTER 7

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Outline of this chapter: within this chapter we present a general discussion about five relevant topics arisen from previous chapters and future research perspectives derived from this work. The first section (7.1) presents the main novelties for science of the thesis with a brief characterization of each. Section 7.2 deals with an additional discussion of the usefulness of nematode assemblages for assessment of environmental quality. This topic is relevant due to increase of anthropogenic disturbance in Cienfuegos Bay and because it could not be extensively treated in the research article due to constrictions in length of the manuscript. The third section (7.3) links the results and insights from the field work and from further experimentation in the laboratory. Each of the four new species descriptions includes ecological remarks; therefore section 7.4 covers the differences and/or similarities in the ecological information of the four new species and generalizations about their habitat and ecological requirements. Since biodiversity of nematodes in tropical areas is not so widely covered in the scientific literature, a discussion about relevance of this research is done in section 7.5. Section 7.6 covers the further research perspectives of the author and Cuban colleagues working in collaboration with Belgian colleagues and partner institutions. A brief evaluation of the past collaboration and the specific topics that are expected to be developed in a near future are presented in this section as well.

7.1 Novelties of the thesis

This thesis includes three results that constitute novelties for science:

1. A detailed description of ecological patterns of free-living marine nematode assemblages in a tropical semi-enclosed bay using taxonomic data as well as biological traits. To our knowledge, only two research articles have been published with regard to nematode assemblages in this habitat; but other tropical habitats have been studied before such as: beach (Gourbault et al., 1995), coral reef (Raes et al., 2007), continental shelf and slope (Muthumbi, 1997), and mangrove (Tietjen and

Alongi, 1990). The first study about free-living marine nematodes in a semi-enclosed bay was by Pérez-García et al. published in 2009 and described the work prior to present research; it was also carried out in Cienfuegos Bay but did not include analyses of temporal variability of assemblages. So far, analyses of biological traits of nematode assemblages in tropical habitats have not been made before. Our results constitute an additional support of the usefulness of this approach in the study of structure and functioning of nematode assemblages.

- A review devoted exclusively to the taxonomically problematic genus *Terschellingia* de Man 1888. The intraspecific variability of one species of the genus (*T. longicaudata*) has been subject to barcoding recently, but no research provided an overview of the taxonomic status of the genus with regard to the number of species, the intraspecific variability based on morphological features, and the main diagnostic characters for species identification. Even for the family Linhomoeidae, to which *Terschellingia* belongs, the information is dispersed, and the only existing taxonomic review is outdated (Gerlach, 1963).

- The taxonomic descriptions of four new genera of free-living marine nematodes: *Cienfuegia*, *Guitartia, Macrodontium,* and *Pseudoterschellingia* is given. This result is a contribution to the assessment of the biodiversity of free-living nematodes, adding 4 new genera to the already known 450 marine genera described so far. The discussion of relationships between each of the new genera and other genera within the respective families provided further insight on their taxonomic status.

7.2 Evaluation of techniques for detection of environmental quality

Two polluted semi-enclosed bays have been analyzed in present thesis: Cienfuegos Bay (chapter 2) and Havana Bay (Armenteros et al., 2009; it will be referenced in the thesis as appendix 1). Chemical pollution by organic enrichment and heavy metals is present in Cienfuegos Bay; and additional xenobiotic compounds (like pesticides and petroleum) have been recorded in Havana Bay. However, correlative measurements did not suggest strong effects of heavy metals on nematodes, possibly, nematodes have developed tolerance to high levels of heavy metals (Austen et al., 1994; Millward and Grant, 1995) and bioavailability is reduced by salinity regime (Dauvin,

2008) and organic enrichment (Bryan and Langston, 1992). No less important is that total content of heavy metals is not always a good indicator of bioavailable metals to sediment biota (Rainbow, 1995).

The topic of nematode assemblages as environmental bioindicators has been covered in many research articles and also in some reviews (Coull and Chandler, 1992; Giere, 2009). However, an evaluation of performance of several techniques for detecting environmental impact using our data of nematode structure assemblage appears to be relevant in this context.

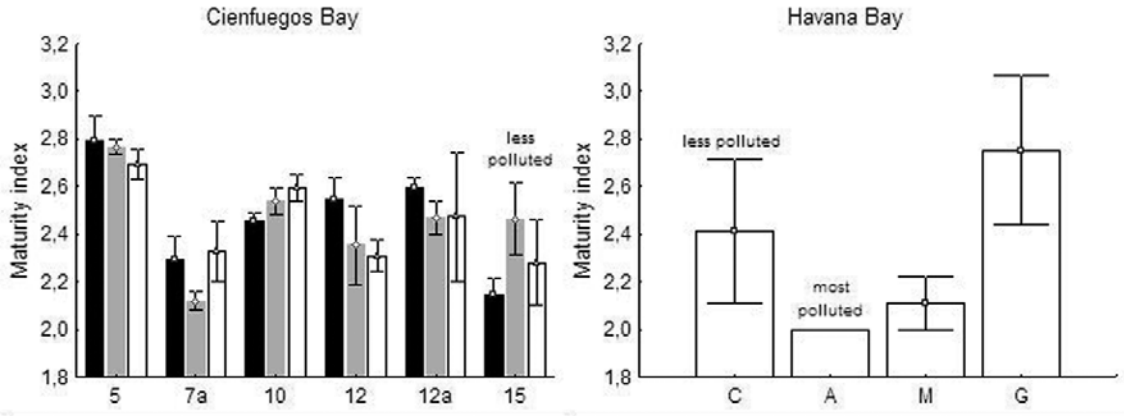

Figure 7.1 Mean (and standard deviation) of maturity index in Cienfuegos Bay (six stations in three months) and Havana Bay (four stations). Less and most polluted stations within each bay are indicated.

Univariate indices of environmental quality (maturity index and 1B/2A ratio) based on structure of assemblages were not sensitive enough for discrimination among stations both in Cienfuegos and Havana bays. Maturity index (MI) would indicate the presence of eutrophication and pollution by xenobiotic compounds in sediments, with a low value in polluted sites (Bongers, 1990; Bongers et al., 1991) but, in similar habitats (i.e. semi-enclosed bay) the MI was not sensitive enough for the detection of a response to environmental conditions (Moreno et al., 2008). The figure 3.4 in present thesis indicates an opposite pattern compared to the expected; i.e. increased of the maturity index as response of organic enrichment. Main cause could be that MI

is strongly based in the colonising abilities of the species, and in the experimental microcosms there was not recruitment into the populations.

Figure 7.1 illustrates that for both ecosystems of Cienfuegos and Havana bays the maturity index in the less polluted stations is overlapped with other stations, doing it less useful as indicator of impact. The interpretation of the maturity index in these ecosystems is hampered by the natural heterogeneity of sediment milieu, the widespread pollution, and for the high variability and patchiness of nematode assemblages. The existence of a clear reference site (i.e. non-polluted) is necessary in order of an appropriate interpretation of the maturity index; in addition, reference sites should be in the same type of habitat and preferably two reference sites should exist in order to evaluate the natural variability in the studied system (Underwood, 1997).

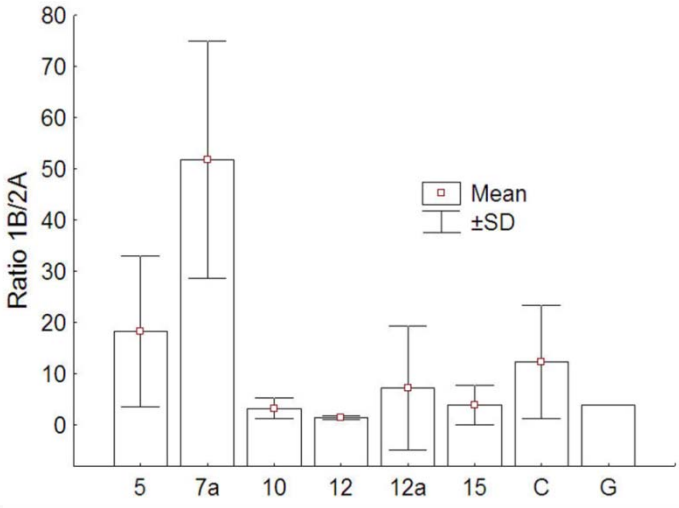

Figure 7.2 Ratio of percentage of non-selective deposit feeder nematodes (1B) to epigrowth feeder (2A) in stations from Cienfuegos (numbers) and Havana (C and G) bays.

Lambshead (1986) suggested that the ratio of non-selective deposit feeder nematodes to epigrowth feeder (1B/2A) can be useful for comparing contaminated and reference sites within a same habitat. However, in Cienfuegos and Havana bays the ratio could not show any clear trend (Fig. 7.2), and additionally it could not be calculated in some stations in Havana Bay where there were no epigrowth feeders. The ratio 1A/2B can reflect the effects of pollution but the interpretation is not always straightforward since variations in the habitat also has a major effect on trophic

composition of nematode (Warwick and Clarke, 1998). Importantly, in absence of a clear non-polluted site, the index appears to be of less usefulness.

We have built dominance curves (Clarke and Warwick, 2001) in order to compare the diversity profiles of nematode assemblages among stations in Cienfuegos and Havana bays (Fig. 7.3). However, curves intersect each other resulting in the fact that the diversity profiles are hard to compare (Platt et al., 1984). The dominance profiles change notably in the time, probably reflecting the temporal responses of nematode assemblages to seasonal factors rather than pollution (e.g. seasonal hypoxia or hydrodynamic regime); similar loss of sensitivity of the profiles of diversity was reported by Hodda and Nicholas (1986). In addition, in those systems like Havana Bay with very low diversity due to heavy pollution the curves show an abrupt slope with poor discrimination between them.

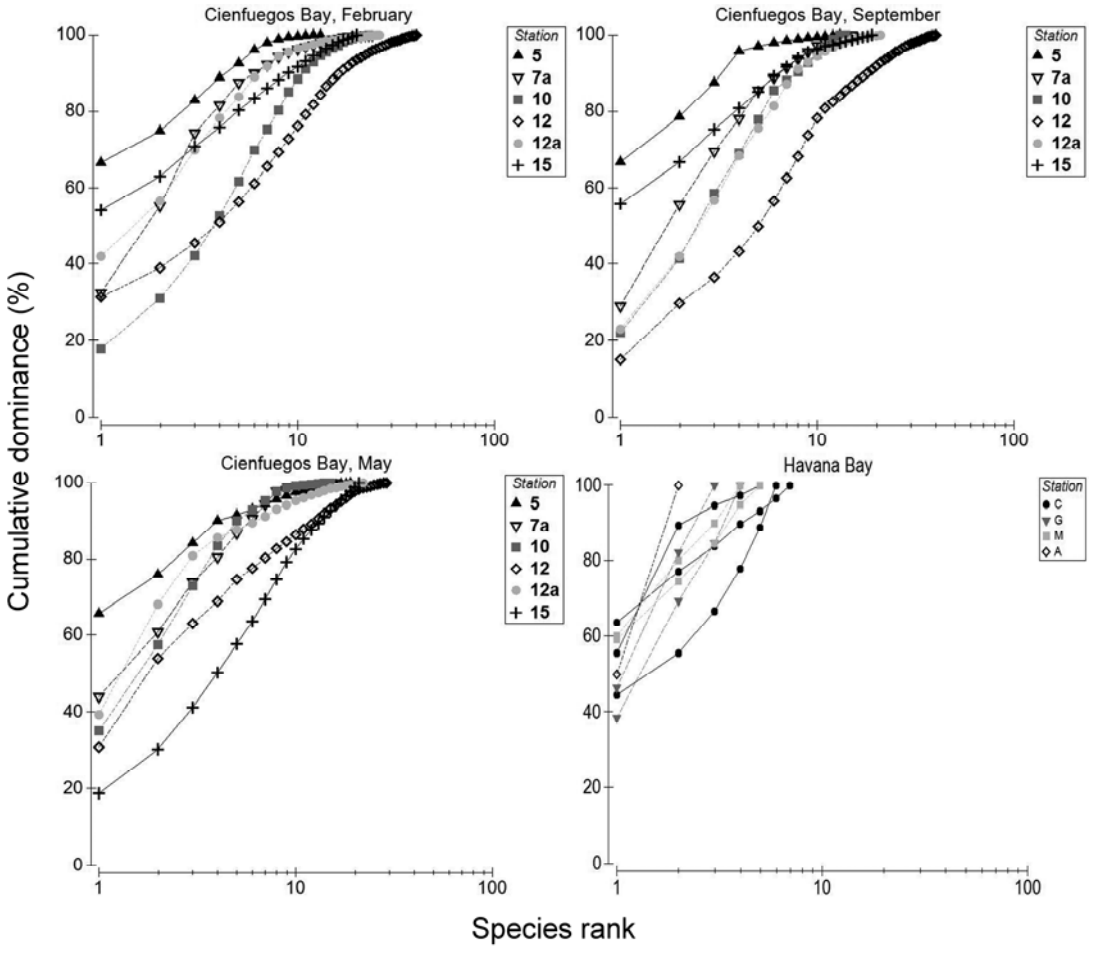

Figure 7.3 Dominance curves of nematode assemblages in the bays Cienfuegos (six stations indicated with numbers) and Havana (four stations indicated with letters).

The multivariate techniques dealing with the structure of meiobenthic communities (or specifically with nematode assemblages) have been useful in a broad range of studies of pollution such as field studies, toxicity bioassays, and microcosm experiments (Giere, 2009). In our study, multivariate techniques based on similarity measurements (outlined in Clarke and Warwick, 2001) were the most effective ways for the interpretation of abiotic and biotic data and the assessment of environmental quality. This array of techniques (including ordination, numerical classification, permutations tests, etc.) constitutes an essential step in the interpretation of patterns of diversity subjected to putative environmental disturbance.

The use of species identity methods for detecting pollution-induced changes is widely adopted; however, it has mainly focused on macrofaunal communities (see Gray and Pearson, 1982; and Pearson et al., 1983 for a rationale and interpretation of results). Although marine meiobenthos have been proposed as very useful indicators of pollution effects (Coull and Chandler, 1992; Kennedy and Jacoby, 1999), in comparison with macrofauna, there are not clearly nematode species identified as indicator species. Additionally, the selection of reliable indicator species (or group of species) is methodologically a complex issue, involving extensive sampling and experimental work (Goodsell et al., 2009). The high diversity and patchiness of nematode distribution appear to be a main handicap for identifying one or few indicator species; although analysis of assemblages generally yields a robust and interpretable pattern of species (Clarke and Warwick, 2001). However, the identity of species can offer valuable information about effects of pollution, mostly when cosmopolitan and tolerant species such as *Sabatieria pulchra* (Jensen, 1984; Hendelberg and Jensen, 1993; Schratzberger et al., 2000b; 2006; Steyaert et al., 2007) and *Terschellingia longicaudata* (Schratzberger et al., 1998b; Buchholz and Lampadariou, 2002; Rzeznik-Orignac et al., 2003; Liu et al., 2008; Moreno et al., 2008) become dominant in the assemblage.

In summary, mainly based on multivariate patterns of nematode assemblages and identity of species we concluded that Havana Bay is strongly polluted (mainly one of the most polluted semi-enclosed basin in the world) but Cienfuegos Bay is also in lesser extent. The heterogeneous nature of the pollution (xenobiotics, organic enrichment, and physical disturbance) and clear effects on biota demand the application of strategies of mitigation in both bays.

7.3 Coupling distribution patterns with microcosm experiment

Correlative measurements from field sampling suggested the influence of depth and heavy metals on nematode biodiversity patterns in Cienfuegos Bay; however, correlative patterns of distribution of species do not identify the processes causing the pattern (Underwood et al., 2000; Clarke et al., 2008). For instance, two of the abiotic factors correlated with multivariate pattern of assemblages suggested different processes influencing the assemblage structure: depth could be a correlate of primary production and/or hydrodynamic regime (Hall, 1994); and cobalt and chromium suggest land influence through runoff (Carlos Alonso, Centro de Estudios Ambientales de Cienfuegos, com. pers.). In order to add insight about response of assemblages to organic enrichment, which can be indirectly related to food availability in the field, we performed a microcosm experiment testing the hypothesis that structure of nematode assemblages does not change when an increasing of organic matter occurs.

Effects of organic enrichment in microcosms can be assessed despite of “microcosm effect” (Sandulli and Nicola-Giudici, 1989; Webb, 1996; Schratzberger and Warwick, 1998b; Mahmoudi et al., 2005; Gyedu-Ababio and Baird, 2006). Our results from the experiment of organic enrichment in microcosms suggested that food availability is not a limiting factor for nematode assemblages. The notable resistance of nematodes in the microcosms to hypoxia and bacterial byproducts (ammonia and hydrogen sulfide) suggested existence of assemblages adapted to inhabiting similar conditions in the field. Validity of extrapolation from small-scale manipulative studies to larger scales can be negatively affected due to scale-dependent processes operating in nature (Carpenter, 1996; Ellis and Schneider, 2008). However, organic pollutants appear to form an exception since they allow some kind of prediction based on experimental manipulation in small-scale laboratory experiments (Zajac et al., 1998).

Results from a study in a heavily polluted semi-enclosed bay (Havana Bay, appendix 1) added valuable information on the interpretation of biodiversity patterns in the field. The more plausible explanation for depletion of benthic assemblages in Havana Bay is the toxicity of sediments due to accumulation of chemical stressors like heavy metals, pesticides and petroleum. No clear correlation could be detected in this ecosystem between key sedimentary variables like silt/clay content or organic matter

and assemblage structure. However, there is limited evidence of positive influence of influx of waters from open sea (i.e. hydrodynamic regime) in one station (Centro). This contrasts with results from Cienfuegos Bay adding indirect evidence that in the latter ecosystem the level of pollution is less than in Havana Bay; and hydrodynamic regime has a strong influence on benthic assemblages (Kröger et al., 2006).

Therefore, the microcosm experiment (chapter 3) reinforced the hypothesized explanations provided in research about causes of biodiversity patterns (chapter 2 and appendix 1). Namely, hypoxic conditions (closely linked to organic content in sediments) and hydrodynamic regime are the main ecological processes driving the structure of assemblages in the semi-enclosed studied bays. When heavy pollution becomes the main factor affecting the assemblages, correlative measurements are weak or absent and nematode populations are depleted.

7.4 Ecological characterization of the four new species

Four new species were described in the present thesis: *Cienfuegia cachoi* n. gen., n. sp., *Guitartia tridentata* n. gen., n. sp., *Macrodontium gaspari* n. gen., n. sp., and *Pseudoterschellingia ibarrae* n. gen, n. sp. With exception of the rare species *G. tridentata* (only three specimens were collected) the other three species showed a marked spatial-temporal patchiness (Table 2.3). The lack of a clear distribution pattern of their populations across stations (km- scale) suggested biological interactions as main drivers of the cm- scale distribution within sediments (Zajac et al., 1998). Our sampling design was designed for description of patterns in km-scales (i.e. between sampling stations) probably associated with other ecological processes such as hydrodynamic regime or pollution level.

The fine particle size and the organic enrichment in sediments explained the dominance of deposit feeder nematodes in all samples. However, the species *G. tridentata*, and *M. gaspari*, present sclerotized structures in the buccal cavity like teeth suggesting predation on other small organisms (e.g. microphytobenthos or other meiobenthos). Species *C. cachoi* was initially classified as non-selective deposit feeder (group 1B after Wieser, 1953) due to presence of a relatively wide buccal cavity and absence of teeth or other sclerotized structures; however, we

documented the ingestion of one nematode belonging to family Linhomoeidae by *C. cachoi* (Fig. 7.4). This supports the statement that the classification of free-living nematodes in four feeding groups (Wieser, 1953) does not represent the real complexity of feeding habits of nematodes (Jensen, 1987b; Moens and Vincx, 1997) and trophic plasticity exists for most feeding types i.e. many are omnivores (Moens et al, 2004; Schratzberger et al., 2008).

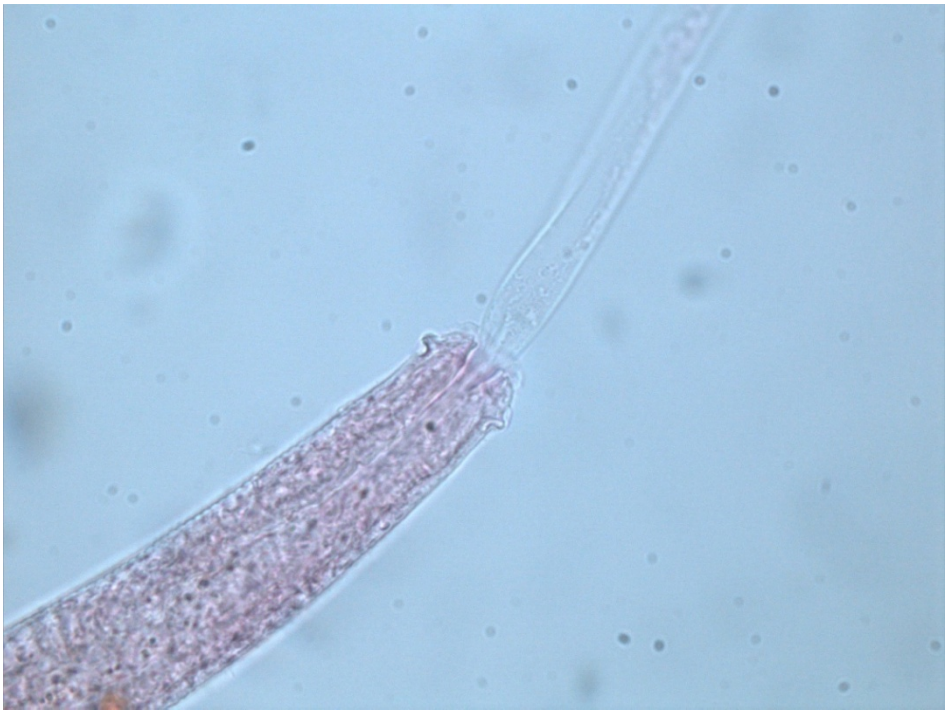

Figure 7.4. Ingestion of a nematode belonging to family Linhomoeidae by Cienfuegia cachoi *gen. nov., sp. nov.*

7.5 Biodiversity of free-living marine nematodes

Free-living marine nematodes are considered a highly diverse group, with global estimates as high as 1 x 10^8 species for the deep sea (Lambshead, 1993); however, these high numbers calculated from “direct multiplication method” can overestimate the real diversity (Mokievsky and Azovsky, 2002). More conservative estimates give approximately 10 000 – 20 000 species of marine nematodes (Mokievsky and Azovsky, 2002); and records of species richness from well studied regions (e.g. British Islands, 450 species belonging to 170 genera, Warwick et al., 1998) are

coherent with this picture. However, any estimates of diversity purely based on morphology should underestimate the real diversity of free-living marine nematodes. Studies of diversity of free-living marine nematodes based on DNA sequences are not so many till date, but first results suggest existence of cryptic species complex which are notably underestimated when solely morphological identification is applied (e.g. Bhadury et al., 2008; Derycke et al., 2008; Fonseca et al., 2008).

A total of 102 nematode species belonging to 79 genera and 26 families have been recorded from Cuban marine waters during this PhD study (appendix 2). Another 61 genera have been recorded, but identification to species level was not possible because well preserved males providing the main identification features were absent. Of the Cuban marine ecosystems, only one list of nematode species has been published so far (López-Cánovas and Pastor de Ward, 2006), reporting a total of 48 nematode species and other 46 species unidentified (only identified to genus level) in seagrass meadows of central-northern shelf of Cuba Island.

Our data from a subtidal tropical semi-enclosed bay did not suggest an especially higher diversity than other comparable sites in temperate regions in terms of number of species in a habitat. Our results are in accordance with results from Boucher and Lambshead (1995) and Gobin and Warwick (2006) showing no evidence of higher diversity of nematodes in tropical samples. The paucity of macrofauna (i.e. low physical disturbance and predation) and the relatively stable environment in muddy bottoms of Cienfuegos Bay can lead to competitive exclusion as a probable cause of low diversity of nematode assemblages (Warwick and Gee, 1984). Muddy bottoms occur under low hydrodynamic regime, and are characterized by relatively high homogeneity in chemical-physical environment; this low habitat heterogeneity provides lesser amount of niches and causes the existence of low number of nematode species (Heip et al., 1985; Soetaert et al., 1994; Steyaert et al., 2003). The assemblages inhabitant in these environments are characterized by dominance of species with low respiration rates and long generation times such as *Metachromadora vivipara*, *Terschellingia spp.*, and *Sabatieria pulchra* (Warwick and Gee, 1984; Steyaert et al., 2007).

With regard to species identified from Cuban marine waters (appendix 2), most of them have been recorded from temperate and boreal waters supporting the statement about cosmopolitan nature of marine nematodes. However, molecular data

are necessary to clarify this point since morphological evidence may be misleading in some nematode taxa (Meldal et al., 2007). For instance, genera *Sabatieria* and *Terschellingia* have numerous species but few morphological features of taxonomic value hampering the discrimination between species (Soetaert et al., 1995).

Species are preferably delimited based on one or more fixed molecular or morphological autoapomorphies, although DNA sequence data are the most promising source of characters (Nadler, 2002). Our study of the relatively species-rich genus *Terschellingia* (chapter 4) indicated that morphological characters alone are insufficient to resolve relationships and delimit species with reasonable accuracy (Coomans, 2000; 2002). Existence of cryptic speciation, even in a small geographic range (Derycke et al., 2005), suggest that assessment of biodiversity based solely on morph-species of free-living marine nematodes can lead to taxonomic errors of type I or II (Adams, 1998), over- or underestimating the real biodiversity of a group. However, use of morphological criterion for delimiting species still being a feasible and common approach when fauna from poorly studied sites (e.g. tropical shallow waters) is under research. To strengthen the morphological approach for delimiting species as many characters as possible should be addressed; in addition, a priori knowledge of evolutionary history of the group is desirable (Adams, 2001).

7.6 Future perspectives and bilateral collaboration between Belgium (Ghent University and Royal Museum) and Cuba

Taxonomic impediment constitutes a serious handicap in the evaluation of biodiversity (Rodman and Cody, 2003; Wheeler et al., 2004) and this holds also for free-living marine nematodes (Coomans, 2000; 2002). Present thesis is a result of a fruitful combination of international funding (International Foundation for Science, Ghent University), personal expertise (e.g. Wilfrida Decraemer, Magda Vincx), trainings on taxonomy (supported by Belgian Focal Point to Global Taxonomy Initiative) and application of web-based tools (e.g. NeMys database at University of Ghent). Now our research team at Universidad de La Habana has the very basic tools (e.g. compound microscope, specialized literature) for further research in ecology and taxonomy of free-living marine nematodes.

We are addressing efforts in experimental ecology both in the field and in the laboratory in order to increase the environmental relevance of our research. Since we have a basic knowledge of the structure of benthic communities in Cienfuegos Bay, we will start the study of processes in benthic ecology mainly related to the relationships between functioning of the ecosystem and the diversity of benthic communities and trophic webs where nematodes are present. Both research topics are in the focus of our short-term research actions.

Coral reefs are distinctive ecosystems from tropical areas, particularly in the Caribbean Sea; and dynamics of nematode assemblages are poorly studied in this habitat. We have started (pilot study running by July 2009) a sampling program in coral reef biotopes from southern coast of Cuba Archipelago in order to describe the patterns of distribution, and the biodiversity of assemblages; additionally, further experimental manipulation is planned in the 2 – 3 years forthcoming.

The immediate logical next step in the taxonomic research is the application of DNA barcoding to the species identified through morphological approach (De Ley, 2000). The continuation of the capacity building in taxonomy is expected; for instance, a specific practical training about barcoding with nematodes will be held in the Royal Belgian Institute for Natural Science with the attendance of a colleague of the Centro de Investigaciones Marinas. Also, financial resources for molecular taxonomy can be obtained in the near future in order to process DNA sequences.

There is a debate about advantages and disadvantages of DNA barcoding (e.g. Hebert et al. 2003; Will and Rubinoff, 2004; Ebach and Holdrege, 2005a; 2005b). We consider that DNA barcoding can allow a more complete and accurate definition of already known species and the discovery of new ones (Hebert and Gregory, 2005), but it must be coupled with essential morphological, physiological, and ecological knowledge. Other advantage in the use of barcoding is that sequences can be properly ordered in electronic datasets widely accessible in the World Wide Web.

However, for barcoding of nematodes, morphologically derived species name for calibration remains essential since physiological and ecological information of particular species is hard to obtain. We intend to apply in short-term a combined approach using molecular and morphological, as well as image vouchering in order to obtain an integrated framework with application to the phylogeny (De Ley et al., 2005; Fonseca et al., 2008).

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APPENDIX 1

EFFECTS OF CHRONIC AND HEAVY POLLUTION ON MACRO- AND MEIOBENTHOS OF HAVANA BAY, CUBA

Modified version from the article in press:

Armenteros M, Pérez-Angulo A, Regadera R, Beltrán J, Vincx M, Decraemer, W. 2009. Effects of chronic and heavy pollution on macro- and meiobenthos of Havana Bay, Cuba. *Revista de Investigaciones Marinas. Universidad de La Habana,* **30,** xx-xx.

Abstract

Infaunal communities (macro- and meiobenthos) and abiotic environmental factors were sampled at four stations in Havana Bay, Cuba in June, 2006. A comparison of concentration of pollutants with reference values indicated that the bay is heavily polluted. Several kinds of pollutants: hydrocarbons from petroleum, heavy metals, organic matter, and organochlorine pesticides were recorded from sediment and eutrophication was present in water. There were differences in the nature of contamination between the inlets: the most polluted were Marimelena, fundamentally by metals of industrial origin (Cr, Co, Mn, Ni, V), and Atarés mainly by metals of urban origin (Pb and Zn) and organic enrichment. The infaunal communities were strongly depleted in number of taxa and density; and defaunation occurred in the most polluted inlet of the bay. Only pollution-tolerant species of free-living marine nematodes (*Sabatieria pulchra*, *Terschellingia longicaudata*, and *Parodontophora xenotricha*) were present in very low densities in the sediments. The most plausible explanation of these features was the deleterious effects of pollution on infauna. Hydrodynamic regime apparently moderates the effects of pollution on nematode assemblages in entrance channel of the Bay by increasing the available microhabitat and/or enhancing process of colonization.

1 Introduction

Marine pollution is a highly concerning phenomenon threatening health and habitat of humankind (Worm *et al.*, 2006) and causing significant economical losses (Ofiara and Seneca, 2006). Bays and harbours associated to coastal cities are particularly exposed to pollution because of concentration of people, industries and harbour operations. Often, the effects of pollution are reinforced by natural features of bays as the fine-grained sediments that tend to bind organic and inorganic pollutants, the collection of run-off from land, the limited circulation of water and the reduced tidal flux (Paggi *et al.*, 2006).

The Havana Bay (Cuba Island), surrounded by Havana City with about 800 000 inhabitants, receives contaminants from a variety of sources: point land-based (oil refinery and a power station), diffuse inputs (drainage from city, discharges from ships), riverine/stream discharges and atmospheric fallout (Colantonio and Potter, 2006). During the 1980´s the basin had been collecting a huge quantity of wastewaters and disposal from previously mentioned industrial and domestic activities, transforming the bay into a heavily polluted marine system (UNDP, 2002). At the start of 1990´s, a strong national economical crisis reduced notably the input of contaminants from industrial origin (Maal-Bared, 2006). From 2000 onwards, an economical recovery has been occurring and it was coupled with mitigation initiatives directly related to the bay; those initiatives should show positive environmental impact after year 2007 (UNDP, 2002).

The initiatives on restrain of pollution and the evaluation of their possible success demand the implementation of a program for monitoring pollution. Recently, several research papers have been published concerning to contamination in estuaries and bays by heavy metals (see Fernandez *et al.*, 2007); organic pollutants and hydrocarbons (Muniz *et al.*, 2004) in estuaries or bays. However, only benthic infaunal community studies, together with sediment chemistry and toxicity tests, can be used to determine if contaminants cause pollution, i.e. impacts on resident populations (Chapman, 2007). Present study does not carry out toxicity tests; but the inclusion of both macrobenthic and meiobenthic communities, in addition to sediment chemistry, would be particularly useful for analysing of the effects of disturbance.

After Fernandez *et al.* (2007), there are only three reports in peer review international journals on distribution of metals in Cuba; two of them (Gonzalez and Torres, 1990; Gonzalez *et al.*, 1999) reported information from sites of discharges or outfalls near Havana Bay, but not properly inside the bay. An additional study by Gelen *et al.* (2005) but not referred by Fernandez *et al.* (2007), reported heavy metal levels in Havana Bay using nuclear techniques. There are no studies published in widely accessible journals, on organochlorine pesticides, polychlorinated biphenyls, and hydrocarbons in Cuban sediments (Fernandez *et al.*, 2007). In addition, the most recent study (Herrera-Moreno and Amador-Pérez, 1983) on benthic communities in Havana Bay was done more than 20 years ago. The evaluation and publication of levels of contamination in Havana Bay and effects of pollution on biota is required for three main reasons: i) the lack of information on pollution with respect to Cuba available for the scientific community; ii) Cuban sites have been reported with the highest values of concentration of metals in sediment in the Caribbean basin (Fernandez *et al.*, 2007); and iii) the recovery of industrial and harbour activities would again increase the pressure on the urban ecosystem (Colantonio and Potter, 2006), including the Havana Bay.

Two main components of the ecosystem were included in our study: the abiotic environment and the benthic faunal communities. The overlying water should be the first indicator of recovery of environmental quality and is a key matrix for the primary production of phytoplankton. The sedimentary matrix retains the contaminants in pore water and as binding to particles and thus constitutes an accuracy target for long term monitoring of contamination. The infaunal communities have been historically used as indicators of environmental quality. For nematode assemblages, the numerically dominant taxon in sediments, the identification to putative species/genus level was considered suitable for assessment of environmental quality of marine sediments (Somerfield and Clarke, 1995).

In present contribution, we report the levels of key contaminants and also the status of benthic communities using high taxonomic resolution for nematodes in the Havana Bay. A comparison with biotic and abiotic databases from 20 years ago allows us to estimate the level of recovery of the bay and the usefulness of mitigation/constrain strategies on pollution for the bay. Hereby, we describe the spatial distribution of the contamination and benthic communities in four stations that characterize the bay; and discuss the deleterious effects of pollution on studied biota.

2 Materials and Methods

2.1 Study area and sampling design

The Havana Bay (N23´08´´ and W82´20´´), in the north-western part of the Cuba Island (Gulf of Mexico), is a semi-enclosed bay with 5,2 km^2 area, mean depth of 9 m, and mean tidal range of 0.3 m. The bay has three main inlets (Atarés, Guasabacoa and Marimelena) and several creeks drain to the basin (Fig. 1). The type of sediment varies from fine sand in the entrance channel to fluid mud in the inner of inlets; the colour of sediment was black with strong smell to hydrogen sulfide and petroleum, presence of petroleum drops and disposals was common. The main exchange of water of the bay with oceanic waters is due to tidal currents and the residence time is around 5 - 7 days. However, short-duration weather events as tropical storms in summer or cold-fronts in winter can provoke a very high hydro-dynamism with mixture of waters and transport of sediment inside the bay and exchange with oceanic waters (pers. observation).

Four sampling stations were located inside the bay and sampled on June 2006; three of them within the inlets and a fourth at the central area (Centro) (Fig. 1). At each station, three sampling units (SU) were taken at random for determination of the structure of communities of macro- and meiobenthos. A SU consisted of an extraction of sediment using a Petersen grab (Rigosha Co. Japan); this grab is recognized as a suitable device for quantitative sampling in muddy bottoms in shallow waters (Eleftheriou and Moore, 2005). The main features of the used grab were the followings: surface area of 0.067 m^2; depth of penetration into sediment around 10 cm; and two 40 µm gauze-covered windows in the upper part.

A single measurement of abiotic variables was carried out at each station. A Van Veen grab (surface area: 0.005 m^2; depth of penetration into sediment around 6 cm) was used for taking samples of sediments for the analysis of grain size, organic content and heavy metals concentration. An oceanographic bottle Van Dorn of 4 L was used to collect samples of water in surface for analyses of dissolved oxygen and nutrients; the sampling of water was done during ebb tide to minimize the influence of the oceanic waters on the measures.

Figure 1. Study zone (the inset shows the localization of the Bay in larger scale); the sampling stations are indicated with types. Labels of stations: C = Centro, A = Atarés, M = Marimelena, and G = Guasabacoa.

2.2 Processing of samples

Previous information about depleted density of benthic fauna in Havana Bay led us to process of the whole samples (i.e. no sub-sampling was carried-out) in order to obtain the maximum of animals possible per SU. The samples of sediment for faunal communities were fixed with 8 % formalin within plastic bags. In the laboratory, samples were sieved using 500 and 45 µm mesh sieves; the material retained on the sieves was considered as macro- and meiobenthos respectively. Separation of macrobenthos from sediment was done by manual pickup of animals under stereomicroscope (Zeiss MB-9, 16 - 25x). Extraction of meiobenthos from sediment was done in high density sugar solution (crystals of commercial sugar dissolved in water, 1.16 g cm^{-3}); the efficiency of sorting of this method in our laboratory is higher than 90 % (Armenteros et al., 2008). Sorted organisms were preserved in vials with 4 % formalin and stained with 1 % alcoholic eosin. After one week, at least, organisms

(both macrobenthic and meiobenthic) were identified to high taxonomic level (e.g. polychaetes, copepods) and counted. Nematodes were identified to species/genus following standard protocols. Namely, they were picked out, and subjected to three successive steps of inclusion in mixtures of ethanol - glycerol until they remained in pure glycerol. They were mounted inside paraffin rings on glass slides, covered with glass cover slides and paraffin slowly melted in order to seal the montage. The identification to putative genus/species was carried out with an Olympus CX 31 microscope at maximum magnification (1 000 X) with the use of the pictorial keys of Platt and Warwick (1983; 1988), Warwick *et al.* (1998) and NeMys database (Deprez *et al.*, 2007).

The dissolved oxygen was measured by the Winkler's method (accuracy: 0.01 mg l^{-1}) and the salinity using a digital salinometer (Tsrumi Seiki; accuracy: 0.1 psu). The determination of the concentration of the different types of nutrients in water: nitrate ($NO_{3)}$, nitrite (NO_2), ammonium (NH_4), phosphate (PO_4), total phosphorus (Pt) and silicate (SiO_4) was carried out after the guidelines of Grasshoff *et al.* (1999). Total suspended solids were determined by the gravimetric method. The concentration of total hydrocarbons in sediment was measured with gas chromatography (ATI Mattison Genesis Series FTIR; lower detection limit: 0.001 mg l^{-1}) after APHA´s guideline (APHA, 1998). The samples for heavy metals in sediments were digested with HNO_3/HCl and concentration was determined by inductively-coupled plasma mass spectrometer (ICP-MS). The content of organic matter in sediment was measured by difference of weight after ignition at 550 ºC in a furnace for 3 hours. The percentage of silt plus clay of sediment was determined by the gravimetric method using a shaker (Retsch AS 200) and 63 µm test sieves as limit between sand and silt/clay fraction. All used reagents were pure for analysis.

2.3 Data analysis

The very low number of organisms of macrobenthos and meiobenthos recorded from the samples induced us to express the densities as animals 0.1 m^{-1} (a close approximation to real area of sampling device: 0.067 m^{-2}). Nematodes were grouped by trophic guilds after the classification of Wieser (1953). The maturity index of nematode assemblages (Bongers *et al.*, 1991) was calculated for each sample, as

well as the average per station. Where the c-p value was unknown for the genus/species, we assigned a value based on morphology of buccal cavity.

Data were analyzed by univariate and multivariate techniques, using software PRIMER 5.2.9 (Clarke and Warwick, 2001). Analyses of variance were performed on number and density of taxa. The presence of outlier values and the homogeneity of variance were checked using diagnosis graphics: variance versus mean, and residuals versus mean); when data did not fulfil assumptions of parametric statistic they were transformed as logarithm (x + 1). Coefficients of correlation product-moment of Pearson were calculated between variables for describing trends across stations. Permutation-based tests (ANOSIM) were applied to test differences in multivariate community structure across stations. Numerical ordinations of the samples, on basis of similarity matrices, were represented in 2-d plots using non-metrical multidimensional scaling. The similarity matrices were calculated using the index of similarity of Bray-Curtis; data were not transformed due to relative low number of animals per sample and due to dominance never exceeded two orders of magnitude.

3 Results

3.1 Abiotic variables

The concentration of nutrients in the water column showed a large variability across stations, with coefficients of variation (CV) between 29 and 95 %. The values of dissolved oxygen, salinity and total suspended solids had a lower spatial variation (CV = 10, 5 and 12 % respectively). An intermediate value of the coefficient of variation was 22 % for hydrocarbons in the water. The values of dissolved oxygen were higher than 5 mg l^{-1} at any station; indicating well oxygenated surface waters (Table 1). The station Centro showed lowest values for all nutrients; though presented the highest values of total suspended solids. Peak values of ammonium were recorded in stations Atarés and Marimelena (14 and 16 µmol L^{-1} respectively). There were no clear trends between nutrients after an analysis of correlation, nor for other abiotic variables in the water column.

Table 1. Abiotic variables measured in water column (surface level) at four stations in Havana Bay. DO = dissolved oxygen, S = salinity, TP = total phosphorus, TSS = total suspended solids, TH = total hydrocarbons. DO, TSS, and TH in mg l^{-1}, nutrients in µmol l^{-1}.

Station	DO	S	NO_3	NO_2	NH_4	PO_4	TP	SiO_4	TSS	TH
Centro	6.9	30.4	2.14	0.27	1.44	0.07	1.02	7.2	80	0.18
Atarés	5.8	31.9	0.19	0.51	13.5	0.83	1.82	14.9	72	0.21
Marimelena	7.5	29.5	1.81	0.62	16.4	0.32	1.14	13.4	77	0.27
Guasabacoa	6.8	28.4	0.78	0.48	6.35	1.58	1.75	21.1	61	0.17

The distribution across stations of total hydrocarbon in sediment was relatively homogeneous (CV = 19 %). The other variables showed marked variations across stations, with silt + clay content and concentration of Ni having larger fluctuations (CV = 72 and 79 % respectively) (Table 2). Marimelena station showed the highest concentration of some heavy metals (Co, Cr, Mn, Ni, V and Fe) in the sediments; Atarés had the highest concentration for the metals Pb and Zn.

Trends in abiotic data should be interpreted with caution due to the low number of observations (n = 4) for each variable. However, several apparent trends hatch out from the data; firstly, a high correlation ($r > 0.9$) was found between the percent of silt + clay in the sediment and the organic matter content. The heavy metals, in general, showed a high correlation among them ($r > 0.9$); the metals: Co, Cr, Mn, Ni and Fe had a positive relationship between them. The other two metals (Pb and Zn) showed high correlation between them, but negative correlation with the previously mentioned group.

Table 2. Abiotic variables measured in the sediments at four stations in Havana Bay. OM = organic matter; S/C = silt + clay; TH = total hydrocarbons. OM, S/C, and Fe in %, heavy metals in µg g^{-1} dry weight sediment.

Station	OM	S/C	TH	Co	Cr	Cu	Mn	Ni	Pb	V	Zn	Fe
Centro	16.1	18	1025	6.1	71	181	273	73	244	67	617	2.24
Atarés	32.0	51	1446	7.1	62	138	263	73	271	87	765	1.85
Marimelena	7.9	15	1434	20.0	151	119	578	229	55	116	212	3.79
Guasabacoa	30.3	66	1234	11.0	62	107	413	58	174	87	382	2.78

3.2 Macrobenthic communities

Six taxa were collected from the macrobenthic samples in Havana Bay: Polychaeta, Nematoda, Amphipoda, Nemertinea, Oligochaeta and Copepoda. The mean number of taxa (± SD) was 2.0 ± 1.6; and the range: 0 – 5 taxa (Fig. 2). There were no significant differences in log-transformed number of taxa among stations upon ANOVA test (p = 0.22); however, the statistical power of ANOVA test was low (0.3) after a post hoc analysis.

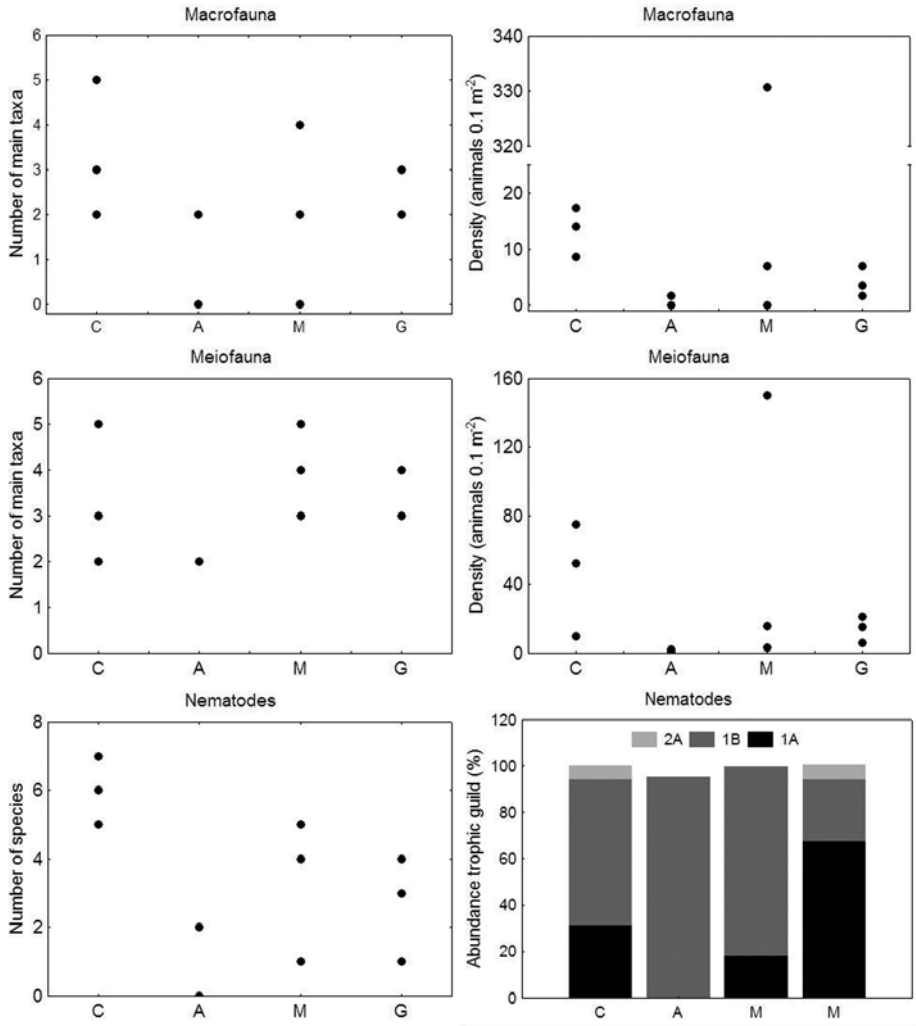

Figure 2. Univariate measures of macro-; meiobenthic and nematode assemblages at four stations from Havana Bay (n = 3 for each station). Labels of stations: C = Centro, A = Atarés, M = Marimelena, and G = Guasabacoa. Trophic guilds: 1A = selective deposit feeder, 1B = non-selective deposit feeder, and 2A = epigrowth feeder.

The mean density (± SD) of macrobenthos was 32.6 ± 94.0 animals 0.1 m^{-2}; with a range of: 0 – 330.6 animals 0.1 m^{-2} (Fig. 2). Polychaetes were the most abundant taxon (46 ± 45 % of total density), followed by oligochaetes and nemertines. The two latter taxa showed high spatial variation, i.e. oligochaetes appeared in only one (153 animals 0.1 m^{-2}) from the 12 replicates taken in the entire bay. This huge variability prevented the detection of significant differences in density (statistical power: 0.3) among stations after ANOVA test ($p = 0.46$).

A multivariate permutation test (ANOSIM) showed significant differences among multivariate structure of macrobenthic communities ($R = 0.46$; $p = 0.006$; 999 permutations); pairwise test between pair of stations could not be applied due to the low number of replicates. The ordination plot showed that Centro and Guasabacoa stations seem to have different community structure and relative high similarity among replicates from a single station (Fig. 3). Macrobenthic communities from Atarés and Marimelena had very high dispersion in the plot; i.e. high variability among replicates from the same station.

3.3 Meiobenthic communities

Five taxa were recorded from the meiobenthic samples in Havana Bay: Nematoda, Polychaeta, Amphipoda, Copepoda and Oligochaeta. The mean number of taxa (± SD) was 3.1 ± 1.1, and the range: 3 – 5 taxa (Fig. 2). There were no differences in the mean number of taxa among stations after ANOVA ($p = 0.15$). A post hoc analysis indicated very low statistical power (0.3) in the ANOVA; obviously the very high variability across stations and the relatively low number of replicates ($n = 3$ by station) prevented the detection of differences.

The density of meiobenthos ranged from 1 to 150 animals 0.1 m^{-2}; with a mean value (± SD) of 29.4 ± 44.2 animals 0.1 m^{-2} for whole bay (Fig. 2). Nematodes were the most abundant group (59 ± 39 % of total density), followed by copepods (16 ± 22 %) and polychaetes (9 ± 24 %). The rank of dominance of taxa was different in Guasabacoa, with copepods reaching 45 % of the total meiobenthos. No differences were detected in untransformed density of meiobenthos among stations after ANOVA test ($p = 0.43$). An analysis of power indicated very low statistical power (0.2) in ANOVA test, due to previously mentioned features of the data. A huge variability was associated with the Marimelena station due to aggregation of 128 juveniles of polychaetes in one of the replicates.

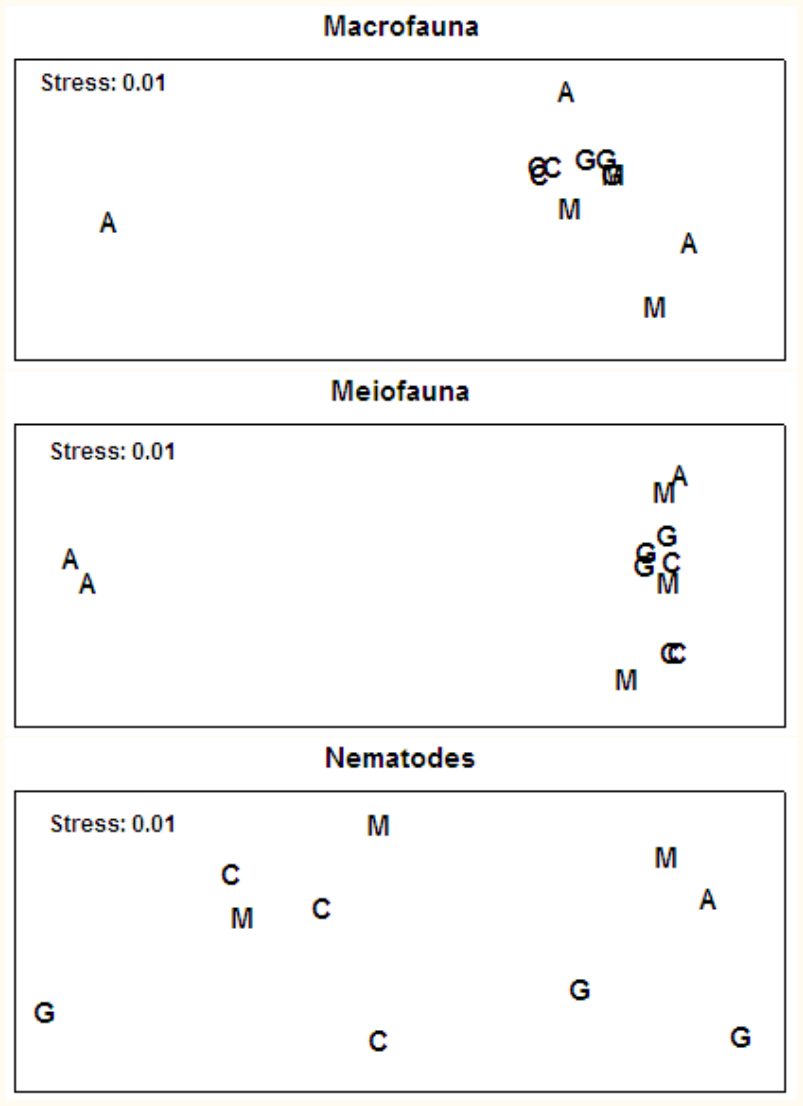

Figure 3. Multidimensional ordination plots of samples from Havana Bay on untransformed data of macro-, meiobenthic and nematode assemblages. Labels of stations: C = Centro, A = Atarés, M = Marimelena, and G = Guasabacoa.

The permutation test on multivariate structure of meiobenthic communities showed significant differences among stations (R = 0.34; p = 0.01; 999 permutations). Interpretation of ordination plot based on meiobenthos data resembled those for macrobenthos (Fig. 3). The stations Centro and Guasabacoa had relatively high similarity among replicates and different composition of meiobenthic communities; the stations Atarés and Marimelena showed very high dispersion in the plot, indicating high variability in the structure of their communities.

Table 3. Number of nematodes in the samples (three replicates per station; grab's area = 0.067 m^2) in Havana Bay. Hyphen indicate absence.

Specie/genus	Centro			Atarés			Marimelena			Guasabacoa			Total	%
Sabatieria pulchra	43	18	1	1	-	-	2	8	15	-	3	2	93	48
Terschellingia longicaudata	2	29	4	-	-	-	-	-	3	-	-	-	38	20
Parodontophora xenotricha	9	-	1	1	-	-	-	-	-	-	-	-	11	6
Terschellingia communis	-	1	-	-	-	-	-	-	-	6	2	-	10	5
Sabatieria breviseta	5	-	-	-	-	-	-	3	1	-	-	-	9	5
Theristus sp.	-	-	-	-	-	-		1	4	-	-	-	5	3
Daptonema sp.	4	-	-	-	-	-	-	-	-	-	1	-	5	3
Pseudoterschellingia ibarrae	-	-	-	-	-	-	-	-	2	-	-	3	5	3
Dorylaimopsis sp.	-	3	1	-	-	-	-	-	-	-	-	-	4	2
Terschellingia gourbaultae	-	-	-	-	-	-	-	1	-	-	-	1	2	1
Acanthonchus cobbi	2	-	-	-	-	-	-	-	-	-	-	-	2	1
Trichotheristus sp	2	-	-	-	-	-	-	-	-	-	-	-	2	1
Metachromadora sp	-	-	1	-	-	-	-	-	-	-	1	-	2	1
Nannolaimoides sp	-	1	-	-	-	-	-	-	-	-	-	-	1	1
Sigmophoranema sp	-	-	1	-	-	-	-	-	-	-	-	-	1	1

3.4 Nematode assemblages

A total of 15 species and 12 genera of free living marine nematodes were collected from sediments from Havana Bay (Table 3). There was a strong ecological dominance, with 68 % of the total abundance constitute by only two species *Sabatieria pulchra* de Man 1907 and *Terschellingia longicaudata* de Man 1906. An analysis of variance showed significant differences among stations ($p = 0.02$) in the number of species of nematodes. The highest average number of species occurred at Centro (mean ± SD: 6 ± 1), the lowest at Atarés (1 ± 1) (Fig. 2).

The relative abundance of trophic guilds showed a dominance of deposit feeder nematodes (groups 1A and 1B); the guild epigrowth feeder (2A) only appears in low abundance in stations Centro and Guasabacoa (Fig. 2). The guild omnivores/predators (2B) were not recorded from the Havana bay. The ranking of the average maturity index (MI) of nematode assemblages for each station indicated a gradient (in brackets the MI values): Atarés (2.00) < Marimelena (2.11) < Centro (2.41) < Guasabacoa (2.75).

A permutation test showed significant differences among stations ($R = 0.41$; $p = 0.001$; 999 permutations) based on multivariate structure of nematode assemblages. The ordination plot did not show any clear trend across samples (Fig. 3). However, two replicates with zero nematodes from station Atarés were eliminated from the plot since upon their inclusion, the remainder samples collapsed in a same point. An analysis of the contribution of each species to the total similarity of its station indicated that presence of *Terschellingia longicaudata* and *T. communis* accounted for most of the similarity at stations Centro and Guasabacoa respectively; *Sabatieria pulchra* and *Theristus sp.* explained > 90 % of similarity at station Marimelena. The absence of nematodes in two out of three replicates at Atarés prevented the calculation of the average similarity among replicates in this station. These samples without nematodes, appeared almost completely defaunated with respect to macrobenthos and meiobenthos; only two and single amphipods respectively occurred.

The very high variability in biotic data and the low number of observations for abiotic variables did not allow obtaining reliable trends (correlations) across stations between community measures and abiotic variables.

4 Discussion

The comparison with reference values (Long *et al.*, 1995; Buchman, 1999) of the levels of contaminants recorded in present study indicated that sediments in Havana Bay are strongly polluted; and that there are high probabilities that they cause adverse effects on biota. Comparisons with regional reports from Latin America and Wider Caribbean (Muniz *et al.*, 2004; Fernandez *et al.*, 2007 and references herein) rank the Havana Bay as one of the most heavily polluted sites in the region. Recent records of organochlorines pesticides (e.g. DDT, dieldrin, endrin, heptachlor) in the bay (unpublished data) reinforce those conditions of heavy pollution in sediments and imply serious risks to human health.

The levels of nutrients in overlying water were high; particularly the phosphorus in overlying water could cause chronic effects on biota (Buchman, 1999). However, the relative high concentration of dissolved oxygen (> 6 mg l^{-1}) in surface water, in comparison with historical records in the Bay (unpublished data), suggests a recovery of environmental quality in overlying water. This would be the first indication that the program of mitigation of contamination in Havana Bay is yielding successful results. However, Atarés still showed low values (< 3 mg l^{-1}) in bottom water suggesting that it is the most organically enriched site in the bay. The large differences in concentration of nutrients in underlying water among stations (e.g. among inlets) should be explain by: i) the main sources of nutrients being land-based (e.g. creeks and industries) and ii) the reduced circulation of mass of water within the bay. The scarce circulation of water appears to reinforce the effects of pollution inside the inlets by avoiding the dilution of contaminants with oceanic waters through entrance channel.

Marked differences in levels of contamination exist among the three inlets. The sediments from Marimelena station had the highest levels of contaminants from industrial origin (hydrocarbons, Co, Cr, Mn, Ni, V, and Fe) due to presence of two

main industries in its basin: a power station and an oil refinery (Gelen *et al.*, 2005). The Atarés station showed the highest contamination from urban origin (e.g. phosphorus in water, Zn and Pb in sediments) due to the presence of dense settlements of human populations in its basin. The high correlation between concentrations of Zn and Pb has been reported for urbanised and polluted areas (Muniz *et al.*, 2004). Hydrodynamic regime (mainly the tidal currents) largely determines the sedimentary characteristics of an area (Hall, 1994); and it is the probable cause of the highest content of suspended solids in water and lowest values of silt/clay in sediments at Centro station. The aforementioned station is located in front of the entrance channel of the bay, where the strongest tidal flows occur between inner bay and oceanic waters (unpublished data).

All univariate measures of benthic community, except number of nematode species, failed for the detection of differences among stations due to low statistical power. This reinforces the importance of the multivariate approach for description of heavily stressed communities characterized by high variability in their structure and low number of counted individuals. The increased variability of community structure, particularly in Atarés and Marimelena stations, would be a symptom per se of environmental stress on biota (Warwick and Clarke, 1993).

The infaunal communities in Havana Bay were strongly depleted both in density of individuals and in number of taxa; the simplest explanation is the deleterious effects of pollution. Only pollution-tolerant taxa as polychaetes and nematodes (Paggi *et al.*, 2006) occurred in relative high frequency in the samples. A comparison of values of density of macrobenthos with data from other bays subjected to several types of pollution (e.g. Flemer *et al.*, 1999; Guerra-García *et al.*, 2003; Hatje *et al.*, 2008) indicates almost defaunation of these communities in Havana Bay.

Regarding to meiobenthos, two main features characterized their communities in Havana Bay: the lowest values of density in comparison with studies carried out in comparable bays (e.g. Dalto *et al.*, 2006; Moreno *et al.*, 2008); and the dominance of two nematode species (*Sabatieria pulchra* and *Terschellingia longicaudata*) recognized as tolerant to pollution in the literature (e.g. Millward and Grant, 1995; Lampadariou *et al.*, 1997; Schratzberger *et al.*, 2000b; 2006; Buchholz and Lampadariou, 2002; Gyedu-Ababioa and Baird, 2006; Steyaert *et al.*, 2007). These

features suggest the existence of chronic and heavy pollution with strong deleterious effects on benthic communities.

Effects of pollution occurred on benthic communities at four studied stations; however, there were differences in the intensity of anthropogenic impact among stations. The interpretation of the univariate and multivariate analysis of community structure suggested that Atarés and Marimelena showed the stronger effects of pollution on their benthic communities. The benthic communities from Centro and Guasabacoa appeared to be slightly less impacted. The significant increase of number of species of nematodes at Centro could be explained by a more intense hydrodynamic regime that enhances: i) a coarser grain size and more availability of microhabitat (Ndaro and Ólafsson, 1999; Steyaert *et al.*, 1999); and ii) the recruitment into sediments (Bell and Sherman, 1980; Palmer, 1988; Commito and Tita, 2002). Unfortunately, the determination of specific importance of each of aforementioned process in shaped nematodes assemblages is not possible in present study. Anyway, we remarked that hydrodynamic regime, mainly the boundary layer flow and the sediment transport (Hall, 1994; Snelgrove and Butman, 1994), plays a key role driving the structure of community even in conditions of heavy pollution.

A comparison of our data with a study carried out in Havana Bay more than 20 years ago (Herrera-Moreno and Amador-Pérez, 1983) should be interpreted with caution since different sampling devices and mesh size of sieves were used in both studies. The general results from these authors indicated a marked depletion of the infaunal communities in the whole bay with high frequency of defaunated samples; particularly Atarés inlet had the worse environmental conditions and almost total absence of infaunal communities. Estimates of our study, in comparison with 20 years ago, showed a slightly higher number and density of taxa in the bay in reference to macrobenthos. However, it is not possible to state if the cause is a response of infauna from polluted sites to the increase in concentration of dissolved oxygen in water (Guerra-García and García-Gómez, 2005) or the use of different mesh sieves in the processing of samples. As we hope, a recovery of the diversity of infaunal communities should cause notable and direct effects on the functioning of the ecosystem (Ieno *et al.*, 2006; Norling *et al.*, 2007), including significant effects on oxygen dynamics at wide scale in the basin (Waldbusser *et al.*, 2004). Anyway, taking in account the very high levels of contaminants accumulated currently in

sediments of Havana Bay, a reduction of the level of contamination would take over 1 to 2 decades after contaminant sources have been reduced (Bothner *et al.*, 1998).

In summary, the Havana Bay maintains high levels of pollution with deleterious effects on benthic communities; the most obvious were the almost defaunation of sediments and the dominance of pollution-tolerant species of free-living marine nematodes. Atarés and Marimelena inlets had the worse environmental quality; and hydrodynamic regime would enhance the nematode assemblages at Centro station.

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APPENDIX 2

TAXONOMIC CHECKLIST OF FREE-LIVING MARINE NEMATODE SPECIES IDENTIFIED FROM CUBAN WATERS

Authors: Maickel Armenteros, Alexei Ruiz, and José Andrés Pérez-García

Class ADENOPHOREA
- Subclass ENOPLIA Pearse, 1942
  - Order ENOPLIDA Filipjev, 1929
    - Suborder ENOPLINA Chitwood and Chitwood, 1937
      - Superfamily ENOPLOIDEA Dujardin, 1845
        - Family ANTICOMIDAE Filipjev, 1918
          - *Anticoma* Bastian, 1865
            - *A. filicauda* Allgén, 1959
            - *A. trichura* Cobb, 1898
          - *Cephalanticoma* Platonova, 1976
            - *C. chitwoodi*, Platonova, 1976
        - Family PHANODERMATIDAE Filipjev, 1927
          - Subfamily PHANODERMATINAE Filipjev, 1927
            - *Phanoderma* Bastian, 1865
              - *P. campbelli* Allgén, 1928
          - Subfamily CRENOPHARYNGINAE Platonova, 1976
            - *Phanodermopsis* Ditlevsen, 1926
              - *P. longisetae* Chitwood, 1936
        - Family THORACOSTOMOPSIDAE Filipjev, 1927
          - Subfamily ENOPLOLAIMINAE de Coninck, 1965
            - *Enoploides* Ssaweljev, 1912
              - *E. bisulcus* Wieser and Hooper, 1967
      - Superfamily IRONOIDEA de Man, 1876
        - Family LEPTOSOMATIDAE Filipjev, 1916
          - Subfamily CYLICOLAIMINAE Platonova, 1970
            - *Cylicolaimus* de Man, 1889
              - *C. magnus* (Villot, 1875) de Man, 1889
        - Family OXYSTOMINIDAE Chitwood, 1935
          - Subfamily HALALAIMINAE de Coninck, 1965
            - *Halalaimus* de Man, 1888

H. floridanus Keppner, 1992
H. gracilis de Man, 1888
H. monstrocaudatus Vitiello, 1970
Subfamily OXYSTOMINIDAE Chitwood, 1935
Thalassoalaimus de Man, 1893
T. tardus de Man, 1893
Superfamily ONCHOLAIMOIDEA Filipjev, 1916
Family ENCHELIDIIDAE Filipjev, 1918
Belbolla Andrassy, 1973
B. californica Allgén, 1951
Family ONCHOLAIMIDAE Filipjev, 1916
Subfamily ADONCHOLAIMINAE Gerlach and Riemann, 1974
Meyersia Hopper, 1967
M. major Hopper, 1967
Subfamily ONCHOLAIMELLINAE de Coninck, 1965
Viscosia de Man, 1890
V. abyssorum (Allgén, 1933)
V. glabra (Bastian, 1865) de Man, 1890
V. meridionalis (Kreis, 1932)
Subfamily ONCHOLAIMINAE Filipjev, 1916
Prooncholaimus Micoletzky, 1924
P. ornatus Kreis, 1932
Subfamily PONTONEMATINAE Gerlach and Riemann, 1974
Filoncholaimus Filipjev, 1927
F. capensis Coles, 1977
Pontonema Leidy, 1855
P. problematicum Chitwood, 1960
Subclass CHROMADORIA Pearse, 1942
Order CHROMADORIDA Filipjev, 1929
Suborder CHROMADORINA Filipjev, 1929
Superfamily CHROMADOROIDEA Filipjev, 1917
Family CHROMADORIDAE Filipjev, 1917
Subfamily CHROMADORINAE Filipjev, 1917
Chromadora Bastian, 1865
C. macrolaima de Man, 1889
Chromadorella Filipjev, 1918

C. macris (Gerlach, 1956) Lorenzen, 1972
Prochromadorella Micoletzky, 1924
P. ditlevseni (de Man, 1922) Wieser, 1954
Subfamily EUCHROMADORINAE Gerlach and Riemann, 1973
Actinonema Cobb, 1920
A. pachydermatum Cobb, 1920
Euchromadora de Man, 1886
E. gaulica Inglis, 1962
Parapinnanema Inglis, 1969
P. harveyi Warwick and Coles, 1975
Subfamily HYPODONTOLAIMINAE de Coninck, 1965
Chromadorita Filipjev, 1922
C. tenuis (G. Schneider, 1906) Filipjev, 1922
Dichromadora Kreis, 1929
D. apapillata Timm, 1961
D. cephalata (Steiner, 1916) Kreis, 1929
Hypodontolaimus de Man, 1886
H. angelae Inglis, 1961
Spilophorella Filipjev, 1917
S. candida Gerlach, 1951
S. paradoxa (de Man, 1888) Filipjev, 1917
Family CYATHOLAIMIDAE Filipjev, 1918
Subfamily CYATHOLAIMINAE Filipjev, 1918
Longicyatholaimus Micoletzky, 1924
L. capsulatus Vitiello, 1970
L. egregius Hopper, 1972
Marilynia Hooper, 1972
M. johanseni Jensen, 1985
Metacyatholaimus Stekhoven, 1942
M. chabaudi Gourbault, 1980
M. effilatus de Bovee, 1973
Subfamily POMPONEMATINAE Gerlach and Riemann, 1973
Minolaimus Vitiello, 1970
M. lineatus Vitiello, 1970
Nannolaimoides Ott, 1972
N. effilatus Boucher, 1976

Nannolaimus Cobb, 1920

N. phaleratus Platt, 1982

Subfamily PARACANTHONCHINAE de Coninck, 1965

Acanthonchus Cobb, 1920

A. cobbi Chitwood, 1951

A. viviparus Cobb, 1920

Paracanthonchus Micoletzky, 1924

P. austropectabilis Wieser, 1954

P. longicaudatus Warwick, 1971

Paracyatholaimoides Gerlach, 1953

P. multispiralis Gerlach, 1953

Paracyatholaimus Micoletzky, 1922

P. helicellus Wieser, 1954

Family NEOTONCHIDAE Wieser and Hooper, 1966

Comesa Gerlach, 1956

C. warwicki Platt, 1982

Gomphionema Wieser and Hooper, 1966

G. typicum Wieser and Hooper, 1966

Neotonchus Cobb, 1933

N. corcundus (Gerlach, 1956) Wieser and Hopper, 1966

Family SELACHINEMATIDAE Cobb, 1915

Synonchiella Cobb, 1933

S. hopperi Ott, 1972

S. micramphis (Stekhoven, 1950) Gerlach, 1964

Superfamily DESMODOROIDEA Filipjev, 1922

Family DESMODORIDAE Filipjev, 1922

Subfamily DESMODORINAE Filipjev, 1922

Desmodora de Man, 1889

D. communis (Bütschli, 1874)

D. extensa Wieser, 1974

D. pontica Filipjev, 1922

Desmodorella Cobb, 1933

D. tenuispiculum Allgén, 1928

Paradesmodora Stekhoven, 1950

P. campbelli (Allgén, 1932) Gerlach, 1963

Subfamily SPIRIINAE (Chitwood, 1936) Gerlach and Murphy, 1965

Chromaspirina Filipjev, 1918
C. inglisi Warwick, 1970
Metachromadora Filipjev, 1918
M. pulvinata Wieser and Hooper, 1967
Spirinia Gerlach, 1953
S. parasitifera (Bastian, 1865)
Subfamily STILBONEMATINAE Cobb, 1936
Eubostrichus Greeff, 1869
E. cobbi (Inglis, 1968)
Family EPSILONEMATIDAE Steiner, 1927
Subfamily EPSILONEMATINAE Steiner, 1927
Epsilonema Steiner, 1927
E. cryptamphis Decraemer and Gourbault, 1987
Superfamily MICROLAIMOIDEA Micoletzky, 1922
Family MICROLAIMIDAE, Micoletzky, 1922
Aponema Jensen, 1978
A. torosus Jensen, 1978
Macrodontium Armenteros, Vincx and Decraemer, 2009
M. gaspari Armenteros, Vincx and Decraemer, 2009
Family MONOPOSTHIIDAE Filipjev, 1934
Rhinema Cobb, 1920
R. retrorsum Cobb, 1920
Suborder LEPTOLAIMINA Lorenzen, 1981
Family AEGIALOALAIMIDAE Lorenzen, 1981
Cyartonema Cobb, 1920
C. elegans Jayasree and Warwick, 1970
C. germanicum Juario, 1972
Family CERAMONEMATIDAE Cobb, 1933
Subfamily CERAMONEMATINAE Cobb, 1933
Ceramonema Cobb, 1920
C. reticulatum Chitwood, 1936
Family LEPTOLAIMIDAE Örley, 1880
Subfamily LEPTOLAIMINAE Örley, 1880
Leptolaimus de Man, 1876
L. elegans (Stekhoven and de Coninck, 1933) Gerlach, 1958
Order MONHYSTERIDA Filipjev, 1929

Superfamily MONHYSTEROIDEA de Man, 1876
Family MONHYSTERIDAE de Man, 1876
Amphimonhystrella Timm, 1961
A. megastoma Timm, 1961
Family SPHAEROLAIMIDAE Filipjev, 1918
Subfamily SPHAEROLAIMINAE Filipjev, 1918
Sphaerolaimus Bastian, 1865
S. maeoticus Filipjev, 1922
Family XYALIDAE Chitwood, 1951
Cienfuegia Armenteros, Vincx and Decraemer, 2009
C. cachoi Armenteros, Vincx and Decraemer, 2009
Daptonema Cobb, 1920
D. longicaudatus Filipjev, 1922
D. oxycerca de Man, 1888
D. propius Lorenzen, 1972
Elzalia Gerlach, 1957
E. floresi Gerlach, 1957
Guitartia Armenteros, Vincx and Decraemer, 2009
G. tridentata Armenteros, Vincx and Decraemer, 2009
Linhystera Juario, 1974
L. problematica Juario, 1974
Paramonohystera Steiner, 1916
P. longicaudata Timm, 1963
P. proteus Wieser, 1956
Steineria Micoletzky, 1922
S. sterreri Ott, 1977
Superfamily SIPHONOLAIMOIDEA Filipjev, 1918
Family LINHOMOEIDAE Filipjev, 1922
Subfamily DESMOLAIMINAE G. Schneider, 1926
Metalinhomoeus de Man, 1907
M. filiformis (de Man, 1907) Stekhoven, 1935
Pseudoterschellingia Armenteros, Vincx and Decraemer, 2009
P. ibarrae Armenteros, Vincx and Decraemer, 2009
Terschellingia de Man, 1888
T. communis de Man, 1888
T. gourbaultae Austen, 1989

T. longicaudata de Man, 1907

T. sulfidrica Pastor de Ward, 1989

Subfamily ELEUTHEROLAIMINAE Gerlach and Riemann, 1973

Eleutherolaimus Filipjev, 1922

E. stenosoma (de Man, 1907) Filipjev, 1922

Subfamily LINHOMOEINAE Filipjev, 1922

Paralinhomoeus de Man, 1907

P. lepturus (de Man, 1907)

Family SIPHONOLAIMIDAE Filipjev, 1918

Astomonema Ott, Rieger, Rieger and Enderes, 1982

A. southwardorum Austen, Warwick and Ryan, 1993

Superfamily AXONOLAIMOIDEA Filipjev, 1918

Family AXONOLAIMIDAE Filipjev, 1918

Ascolaimus Ditlevsen, 1919

A. elongatus (Bütschli, 1874) De Coninck and Stekhoven, 1932

Axonolaimus de Man, 1889

A. drachi Luc and De Coninck, 1959

Parodontophora Timm, 1963

P. xenotricha Boucher, 1973

Family COMESOMATIDAE Filipjev, 1918

Subfamily COMESOMATINAE Filipjev, 1918

Comesoma Bastian, 1865

C. vulgare Bastian, 1865

Paracomesoma Hope and Murphy, 1972

P. dubium (Filipjev, 1918) Stekhoven, 1950

Subfamily DORYLAIMOPSINAE de Coninck, 1965

Dorylaimopsis Ditlevsen, 1918

D. punctata Ditlevsen, 1918

Hopperia Vitiello, 1969

H. muscatensis Warwick, 1973

Laimella Cobb, 1920

L. longicaudata Cobb, 1920

Subfamily SABATIERIINAE Filipjev, 1934

Sabatieria Rouville, 1903

S. breviseta Stekhoven, 1935

S. praedatrix de Man, 1907

S. pulchra (G. Schneider, 1906) Riemann, 1970

Setosabatieria Platt, 1985

S. hilarula (de Man, 1922)

Family DIPLOPELTIDAE Filipjev, 1918

Subfamily DIPLOPELTINAE Filipjev, 1918

Araeolaimus de Man, 1888

A. boomerangifer Wieser, 1959

Diplopeltula Gerlach, 1950

D. asetosa Juario, 1974