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## Original article

# Spatial distribution of subtidal Nematoda communities along the salinity gradient in southern European estuaries

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## ABSTRACT

This study investigated the spatial distribution of subtidal nematode communities along the salinity gradients of two Portuguese estuaries exposed to different degrees of anthropogenic stress: the Mira and the Mondego.

The nematode communities were mainly composed of *Sabatieria*, *Metachromadora*, *Daptonema*, *Anoplostoma*, *Sphaerolaimus* and *Terschellingia* species, closely resembling the communities of Northern European estuaries. In both estuaries, nematode density and community composition followed the salinity gradient, naturally establishing three distinct estuarine sections: (i) freshwater and oligohaline – characterised by the presence of freshwater nematodes, low nematode density and diversity; (ii) mesohaline – dominated by *Terschellingia*, *Sabatieria* and *Daptonema*, with low total density and diversity; and (iii) polyhaline and euhaline – where nematodes reached the highest density and diversity, and *Paracomesoma*, *Synonchiella*, and *Odontophora* were dominant.

Despite the similarities in community composition and total nematode density, the proportion of different nematode feeding types were remarkably different in the two estuaries. In Mira, selective deposit feeders were dominant in the oligohaline section, while non-selective deposit feeders were dominant in the other sections. On the contrary, in the Mondego estuary, epigrowth-feeders and omnivores/predators were dominant in the freshwater sections and in the euhaline sector of the southern arm.

Differences observed along each estuarine gradient were much stronger than overall differences between the two estuaries. In the Mondego estuary, the influence of anthropogenic stressors seemed not to be relevant in determining the nematodes' spatial distribution patterns, therefore suggesting that mesoscale variability responded essentially to natural stressors, characteristic of estuarine gradients. Nevertheless, the proportion of the different feeding types was different between the two estuaries, indicating that the response of nematode feeding guilds is able to reflect anthropogenic-induced stress and can be useful in assessing biological quality in transitional waters ecosystems.

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## 1. Introduction

Nematode dominance in marine and estuarine meiobenthic communities has been largely documented, and there are studies illustrating the prime importance of salinity and sediment properties on spatial distribution, abundance and species composition of free-living nematodes (Austen and Warwick, 1989; Vincx et al., 1990; Coull, 1999). Also, it is well known that the salinity regime in estuaries is a key independent factor determining the communities' structure and controlling species composition, abundance and diversity (Soetaert et al., 1995). Total meiobenthic density and species number decrease as one moves away from the sea towards freshwater (Austen and Warwick, 1989). Nevertheless, the available information regarding the influence of salinity gradients on nematode populations' quantitative parameters is rather incomplete and focused on intertidal sediments. Moreover, although a number of studies have been carried out on intertidal nematode communities, these studies were performed within narrow salinity ranges (e.g. Capstick, 1959; Austen and Warwick, 1989; Warwick and Gee, 1984). On the other hand, studies on the spatial distribution of subtidal estuarine nematodes along salinity gradients are very scarce (Soetaert et al., 1994). Salinity is, however, a well-known natural stressor commonly related to the variability of nematode communities' composition and abundance in estuarine sediments. In combination with other natural stressors, salinity creates a set up for communities adapted to particular disturbance-like conditions, giving rise to situations where it is rather difficult to distinguish between natural and human-induced stress on community patterns – a concept coined by Dauvin (2007) as “*Estuarine quality paradox*”. Briefly, this concept states that the features of communities under anthropogenic stress coincide with those caused by natural stress because of the high variability of environmental parameters in estuaries; consequently, species living in these environments adapt to their intrinsic variability and become tolerant to further changes (Elliot and Quintino, 2007).

The spatial and temporal patterns of estuarine nematode communities have been intensively studied along the North Atlantic region, with special emphasis on the intertidal sediments (e.g. Warwick and Gee, 1984; Heip et al., 1985; Li and Vincx, 1993; Smol et al., 1994; Steyaert et al., 2003; Rzeznik-Orignac et al., 2003). There is a notorious imbalance between the northern European systems, relatively well studied, and the southern ones, which are insufficiently described (Soetaert et al., 1995; Adão, 2004). With this study we aim to advance the general understanding of southern European estuaries by comparing the nematode communities in two Portuguese systems: the Mira estuary, located inside a Natural Park – “Costa Vicentina” (SW coast of Portugal), and the Mondego estuary exposed to high anthropogenic stress caused by several industries, a harbour and agricultural run-offs. This study included analysis of: (a) the most important natural environmental variables influencing the structure and distribution of nematode assemblages in the two estuaries, and of (b) spatial distribution patterns of density, genera composition, and feeding types along the salinity gradient. Two main questions were addressed: (a) how did nematode

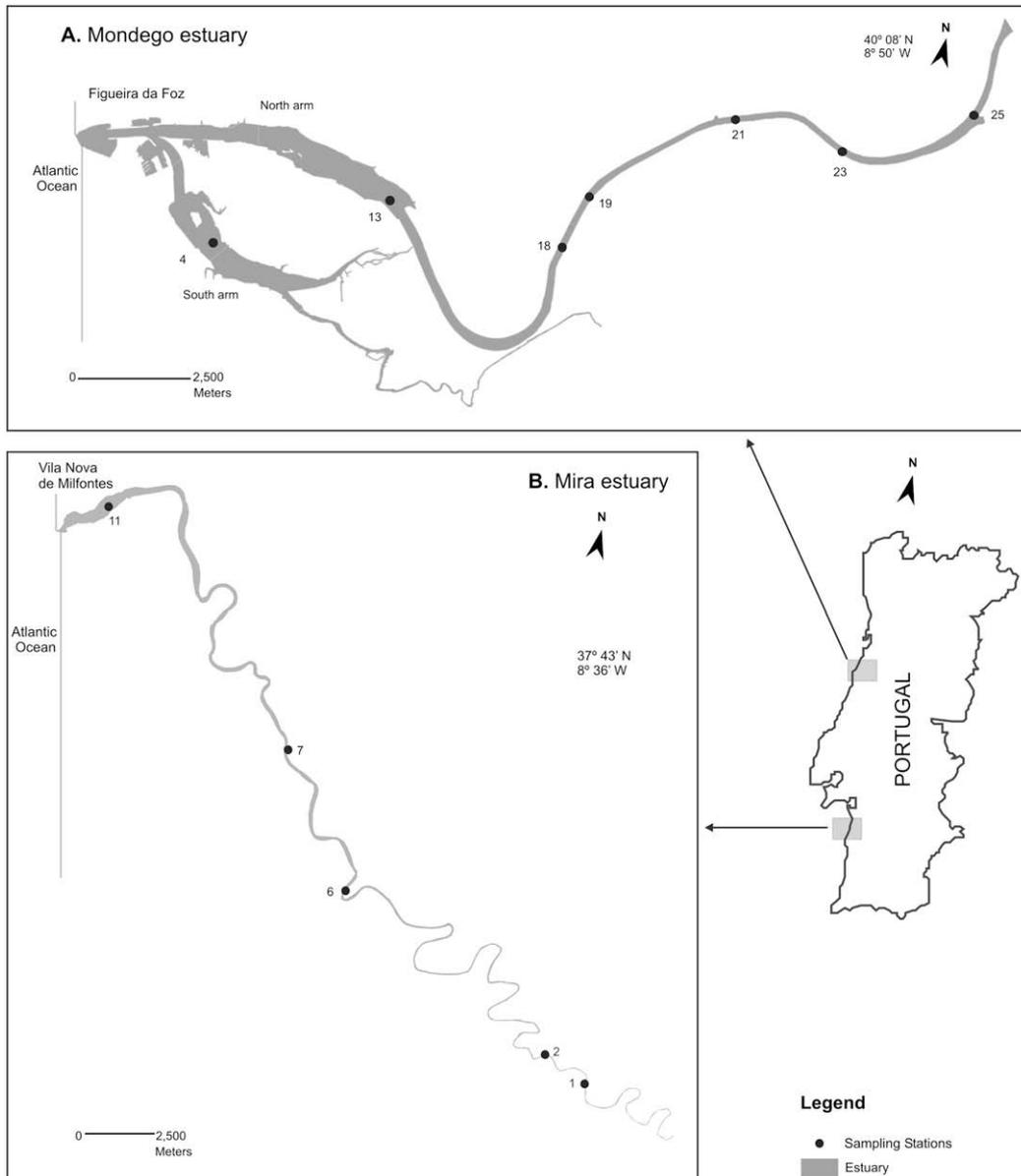
communities living in subtidal sediments vary in response to the salinity gradient? and (b) were there significant differences between the two nematode communities that were imputable to the distinct anthropogenic pressures?

### 1.1. Study areas

This comparative study was carried out concurrently at the Mondego and Mira estuaries, both located on the Western Coast of Portugal.

The Mondego estuary (40°08'N, 8°50'W) is a 21 km long warm-temperate intertidal system (Fig. 1A). Its terminal part consists of two arms, northern and southern, separated by an alluvium-formed island (Murraceira Island) and joined again near the estuary's mouth. The two arms have very different hydrological characteristics. The southern arm is shallower (2–4 m during high tide, tidal range 1–3 m) and has large areas of intertidal mudflats (almost 75% of the area) exposed during low tide. The northern arm is deeper (5–10 m during high tide, tidal range about 1–3 m) and receives most of the system's freshwater input, therefore being strongly influenced by seasonal fluctuations in water flow (Flindt et al., 1997; Neto et al., 2008). In general, the Mondego estuary is under severe environmental stress, supporting several industries and receiving the agricultural run-offs from rice and corn fields in the Lower River valley (Lillebø et al., 2007). Moreover, the Figueira da Foz harbour is located in the northern arm, where regular dredging is carried out to ensure shipping conditions. During the early 1990s, clear eutrophication symptoms were observed in the South arm, including the occurrence of seasonal blooms of *Ulva* spp. As a result of the competition with macroalgae (Marques et al., 2003), there was a concomitant and severe reduction in total area occupied by *Zostera noltii* beds, previously the richest habitat in terms of productivity and biodiversity (Marques et al., 1997). Several interventions were gradually undertaken since 1998 to decrease eutrophication symptoms and to test for ways of improving the system's ecological condition (Lillebø et al., 2005, 2007; Neto et al., 2008).

The Mira estuary, located on the south-western coast of Portugal (37°40'N, 8°40'W) (Fig. 1B) is a small mesotidal system with a semidiurnal tidal regime. It is formed by a single channel, 5–10 m deep and up to 400 m wide, which allows tidal influence to extend 40 km upstream. Due to the low, seasonal and limited freshwater input, the lower section of the estuary has a dominant marine signature and is characterised by extensive *Z. noltii* meadows, bare sandy areas and muddy substrates, with salt-marshes occurring as far as 20 km upstream (Amaral and Paula, 2007). Together with its surrounding area, the Mira River is included in a protected area, the Natural Park of “Sudoeste Alentejano e Costa Vicentina”. This estuary is considered relatively undisturbed and free from industrial pollution (Costa et al., 2001), and the physical and chemical fluctuations result mainly from: (a) its morphology, since the terminal section of the river is rather regular and facilitates the upstream tidal penetration, and (b) a normally reduced outflow determined by the region's annual rainfall distribution (concentrated between January and March with the rest of the year being usually dry) (Paula et al., 2006).



**Fig. 1 – Station location (black circles) in (A) Mondego estuary and (B) Mira estuary.**

### 1.2. Sampling strategy

Nematodes were sampled at both estuaries in the summer of 2006. Sampling stations were previously allocated to one of the five Venice salinity classes (Anon., 1959) (freshwater < 0.5; oligohaline 0.5–5; mesohaline 5–18; polyhaline 18–30 and euhaline > 30) (Table 1) according to information gathered in earlier studies (Teixeira et al., 2008). Five sampling stations were analysed in the Mira estuary (stations 1, 2, 6, 7 and 11) and 7 stations in the Mondego estuary (stations 4, 13, 18, 19, 21, 23 and 25), covering both the northern (station 13) and southern arm (station 4) subsystems (Fig. 1). At each station, three replicates were collected by forcing a 4.6 cm inner-diameter “Kajak” sediment corer 3 cm into the sediment. All samples were preserved in 4% buffered formaldehyde. Nematodes were later extracted from the sediment fraction

using “Ludox HS40” colloidal silica at a specific gravity  $1.18 \text{ g cm}^{-3}$  and a  $38 \mu\text{m}$  sieve (Heip et al., 1985), and counted under a stereomicroscope. A random set of 120 nematodes, or the total number of individuals in samples with less than 120 nematodes, were picked from each replicate, cleared in glycerol-ethanol solution, stored in anhydrous glycerol and mounted on glycerine slides for identification (Vincx, 1996). Genus identification was done according to Platt and Warwick (1988) and Warwick et al. (1998).

Water salinity, temperature ( $^{\circ}\text{C}$ ), pH and dissolved oxygen (DO) ( $\text{mg l}^{-1}$ ) were measured in situ with a Data Sonde Survey 4. Water nitrate ( $\text{NO}_3^- \text{-N}$ ) ( $\text{mg l}^{-1}$ ) and nitrite ( $\text{NO}_2^- \text{-N}$ ) ( $\text{mg l}^{-1}$ ) concentrations were analysed according to standard methods described in Strickland and Parsons (1972). Ammonium ( $\text{NH}_4^+ \text{-N}$ ) ( $\text{mg l}^{-1}$ ) and phosphate ( $\text{PO}_4^{3-} \text{-P}$ ) ( $\text{mg l}^{-1}$ ) concentrations were analysed following the Limnologisk Metodik (1992)

**Table 1 – Salinity classes from the Venice salinity classification and correspondence with the sampled stations in the Mira and Mondego estuaries.**

Venice classification	Salinity ranges	Mira stations	Mondego stations
Freshwater	<0.5	–	25
Oligohaline	0.5–5	1, 2	23, 21
Mesohaline	5–18	6	19, 18
Polyhaline	18–30	7	–
Euhaline	>30	11	13, 4

methodology. Sediment organic matter (OM) content was quantified by weight difference between the sediment's weight after oven drying at 60 °C for 72 h and the sediment's weight after combustion at 450 °C for 8 h, and then expressed as a percentage of total weight. Grain size analysis was carried out by mechanical separation through a column of sieves with different mesh sizes. Sediment grain size was divided into five classes according to Brown and McLachlan (1990): gravel (>2 mm), coarse sand (0.500–2.000 mm), mean sand (0.250–0.500 mm), fine sand (0.063–0.250 mm) and silt + clay (<0.063 mm), and the different fractions expressed as a percentage of the total sample's weight.

### 1.3. Data analysis

Data were analysed in order to (a) compare the distribution pattern of the environmental factors between estuaries, (b) characterise the nematode communities distribution along the salinity gradient in both estuaries, taking into account their composition, density and feeding groups, (c) find possible differences between estuaries, and (d) associate specific nematode assemblages with different environmental factors.

Total nematode densities from each estuary were compared by means of one-way ANOVA (square root transformation was applied whenever ANOVA assumptions were not met), using the software GMAV5 for Windows (Underwood and Chapman, 1997) and, *a posteriori* Student–Newman–Keuls

(SNK) test was used, whenever significant differences were detected by ANOVA.

Multivariate analysis was applied according to the procedures described by Clarke (1993), using the software PRIMER (Plymouth Marine Laboratory, UK). Physicochemical data were first normalised and submitted to square root transformation (except dissolved oxygen and pH data), and then underwent Principal Component Analysis (PCA). Data on nematode density were transformed by square root and then analysed by non-metric multidimensional scaling (MDS) using the Bray–Curtis similarity index. Formal significance tests for differences between groups of samples were addressed using one-way ANOSIM tests. The contribution of each genus for the dissimilarities between groups of stations was determined by using the similarity percentage analysis procedure (SIMPER). The relationship between environmental variables and the nematodes' community structure was explored by carrying out BIOENV analysis (Clarke and Ainsworth, 1993).

To investigate the trophic structure of the community, nematodes were grouped into four feeding guilds. According to Wieser's (1953) feeding type classification, we distinguished selective (1A) and non-selective (1B) deposit feeders, epigrowth-feeders (2A) and omnivores/predators (2B). The proportions of each feeding group at each station were compared by non-metric multidimensional scaling (MDS) analysis using the Bray–Curtis similarity index and formal significance tests for differences between groups of samples were addressed using one-way ANOSIM tests.

## 2. Results

### 2.1. Abiotic factors

Physicochemical parameters measured along the salinity gradient in both estuaries are provided in Table 2.

In the Mira estuary, the proportion of fine particles in the sediments increased from the upstream section towards the mouth of the estuary. Coarse sediments were predominant in

**Table 2 – Environmental variables measured at each sampling station in the Mira and Mondego estuaries.**

Estuary	St.	Sal	Transp (m)	T (°C)	O <sub>2</sub> (mg/l)	pH	PO <sub>4</sub> <sup>3-</sup> (mg/l)	NO <sub>3</sub> <sup>-</sup> (mg/l)	NO <sub>2</sub> <sup>-</sup> (mg/l)	NH <sub>4</sub> <sup>+</sup> (mg/l)	OM (%)	Gravel (%)	Coarse sand (%)	Mean sand (%)	Fine sand (%)	Silt + Clay (%)
Mira	1	1.1	0.3	23.6	4.5	7.4	0.016	0.407	0.004	0.019	4.0	71.3	17.8	5.3	2.1	3.5
	2	2.0	0.8	26.0	5.0	7.4	0.010	0.771	0.008	0.023	6.2	42.3	27.4	8.7	6.0	15.7
	6	14.6	0.6	27.2	4.0	7.5	0.008	0.538	0.014	0.064	8.8	1.9	4.9	6.9	18.4	67.9
	7	22.4	0.6	24.8	5.0	7.6	0.013	0.195	0.013	0.035	10.5	2.3	0.9	1.4	11.7	83.7
	11	36.6	1.5	21.4	4.8	8.1	0.005	0.000	0.001	0.000	2.3	4.8	22.3	39.3	28.7	5.0
Mondego	25	0.1	0.6	24.0	6.4	7.4	0.096	1.331	0.060	0.184	0.2	35.8	46.0	16.2	1.9	0.2
	23	0.5	0.7	23.6	5.9	7.4	0.093	1.263	0.043	0.130	4.1	8.8	3.1	16.9	64.4	6.7
	21	2.7	0.7	23.3	6.2	7.3	0.067	1.134	0.025	0.101	3.0	38.4	1.7	15.9	39.0	5.1
	19	10.0	1.1	22.8	6.2	7.3	0.067	1.134	0.025	0.101	3.8	0.2	0.9	14.4	74.1	10.4
	18	13.6	1.1	22.8	7.1	7.4	0.054	0.566	0.014	0.092	4.8	1.1	11.4	16.2	59.1	12.2
	13	31.6	2.8	19.0	7.6	7.5	0.045	0.372	0.010	0.066	1.4	29.7	26.3	22.0	17.5	4.5
	4	33.1	3.2	17.6	8.4	7.9	0.030	0.299	0.002	0.042	0.9	1.6	7.9	27.6	60.9	2.0

Sal, salinity; Transp, transparency; T, temperature; O<sub>2</sub>, dissolved oxygen; PO<sub>4</sub><sup>3-</sup>, phosphate; NO<sub>3</sub><sup>-</sup>, nitrate; NO<sub>2</sub><sup>-</sup>, nitrite; NH<sub>4</sub><sup>+</sup>, ammonium; OM, sediment organic matter; gravel, >2 mm; coarse sand, 0.5–2.0 mm; mean sand, 0.25–0.50 mm; fine sand, 0.063–0.250 mm; silt + clay < 0.063 mm.

the upstream section while the remaining areas were characterised by sediments with a diameter less than 0.250 mm and the bottom of the euhaline section was composed mainly of sand. The finest sediments had also the highest percentage of organic matter (OM) content. Sediments in the upstream section of the Mondego estuary consisted mostly of fine sand, with the exception of the freshwater part, where the proportion of gravel + coarse sand was approximately 82% and OM content was the lowest. The North arm had coarse sediment bottoms, while the southern arm bottoms consisted mainly of mean and fine sand. Fine sand bottoms in the estuarine upper sections contained higher OM contents in sediments.

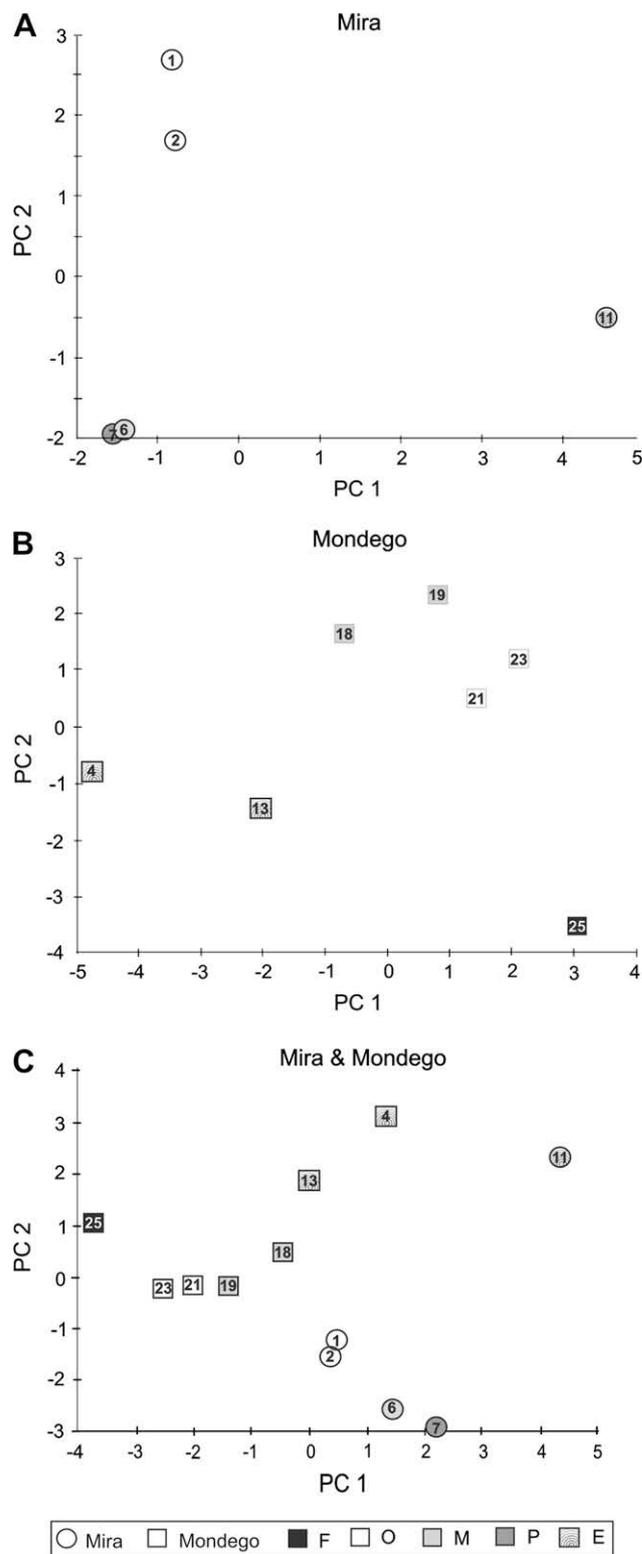
In the Mira estuary, water nitrites and ammonium concentrations were highest in the mesohaline section, with values clearly decreasing towards both the mouth and the uppermost section of the estuary. In the Mondego estuary, the concentration of nitrates and phosphates in the water column showed some spatial heterogeneity but, in general, nutrients concentration ( $PO_4^{3-}$ -P,  $NO_3^-$ -N,  $NO_2^-$ -N and  $NH_4^+$ -N) was higher in the upstream section decreasing towards the mouth of the estuary.

No significant variations in pH values were detected along the Mira estuary, while in Mondego, which had an average pH similar to the Mira, the individual pH values were higher in the southern arm than in the northern one.

Dissolved oxygen (DO) concentrations in the Mira estuary reached maximum values at the oligohaline and polyhaline sections, with a minimum being recorded at the mesohaline section. In Mondego, DO increased from the head to the mouth of the estuary and the average concentration was higher than in Mira. The highest values of water transparency in the Mira estuary were observed in the euhaline section and the corresponding minimum in the freshwater area. In Mondego, water transparency also increased from the upstream section towards the mouth along both arms of the estuary.

PCA of Mira's physicochemical environmental factors provided a clear distinction of three groups of stations (Fig. 2A): Group I, included oligohaline stations, Group II included mesohaline and polyhaline stations and Group III, included the only euhaline station. The first two principal components (PC1 and PC2) explained 85.3% of the total variability. Along PC1, variability was mainly explained by an increase in the proportion of mean sand and a parallel decrease in the concentration of nitrates, nitrites and ammonium. Along PC2, variability was mainly explained by the contrast between stations with higher salinity values and higher proportions of silt + clay in the sediments and located closer to the mouth, and the stations having lower salinities and coarser bottom sediments and located further upstream in the estuary.

Regarding the Mondego estuary, PCA also identified three groups of stations based on the physicochemical variables (Fig. 2B): Group I, included the freshwater station, Group II, included oligohaline and mesohaline stations, and Group III, included euhaline stations. The first two principal components explained 87.7% of the total variability. Variability along PC1 was mainly explained by an increase in the concentration of nitrates, nitrites, ammonium and phosphates from the mouth to the inner stations of the estuary, and a concomitant decrease of salinity values. On the other hand, variability



**Fig. 2 – Principal component analysis (PCA) plot based on the abiotic parameters at each station from (A) Mira estuary (Axis 1 = 51.1%, Axis 2 = 34.2%); (B) Mondego estuary (Axis 1 = 55.5%; Axis 2 = 32.3%); and (C) Mira and Mondego estuaries simultaneously (Axis 1 = 38.3%, Axis 2 = 27.3%). F, Freshwater; O, oligohaline; M, mesohaline; P, polyhaline; and E, euhaline.**

along PC2 was mainly explained by the contrast between stations showing higher proportions of fine sand, silt + clay and OM, and those stations with higher proportion of gravel and coarse sand in the sediments.

PCA of the pooled physicochemical data matrix from both estuaries revealed clear differences between these (Fig. 2C), with the first two principal components explaining 65.5% of the variability. Variability along PC1 mainly corresponded to the estuarine gradient, being explained by the opposition between stations with higher salinity values, closer to the mouth of both estuaries, and the stations with higher concentrations of phosphates, nitrates, nitrites and ammonium, located in inner areas. Differences between the two systems became clear through their separation along PC2. In fact, variability along PC2 was essentially explained by the opposition between higher proportions of OM content and larger fractions of silt + clay observed in bottom sediments in the Mira estuary, and bigger fractions of mean sand observed in Mondego's estuary.

## 2.2. Nematodes community general characteristics

A total of 48 and 45 genera of nematodes, belonging to 19 families, were identified at the Mondego and the Mira estuaries, respectively. In both estuaries the dominant families were Comesomatidae, Desmodoridae, Chromadoridae, and Xyalidae. In the Mira estuary, the genera *Sabatieria* (24.5%), *Ptycholaimellus* (13.8%), *Metachromadora* (13.2%), *Terschellingia* (12.8%), *Daptonema* (9.2%), *Anoplostoma* (6.3%) and *Sphaerolaimus* (4.5%) represented 84.3% of the total nematode densities, while at Mondego, *Metachromadora*, (19.3%), *Anoplostoma* (13.6%), *Daptonema* (9.8%), *Sabatieria* (9.8%), *Microlaimus* (8.1%), *Sphaerolaimus* (4.3%), *Axonolaimus* (3.8%), *Dorylaimus* (3.4%), *Prochromadorella* (2.8%), *Dichromadora* (2.8%) and *Viscosia* (2.6%) all together, represented 80.3% of the total nematode densities. A complete list of the genera identified and their densities at each sampling station in both estuaries is provided in Tables 3 and 4.

In Mira, the mean nematode density varied between  $109.0 \pm 26.7$  ind.  $10\text{ cm}^{-2}$  at the oligohaline section (station 2) and  $2234.0 \pm 400.2$  ind.  $10\text{ cm}^{-2}$  at the polyhaline section (station 7). Significant differences in nematodes density found between stations ( $F = 30.62, p < 0.05$ ) (Fig. 3A) were essentially due to high values at station 7. In Mondego, the mean nematode density was much lower, varying between  $38.9 \pm 5.3$  ind.  $10\text{ cm}^{-2}$  at the freshwater section (station 25) and  $1323.1 \pm 398.5$  ind.  $10\text{ cm}^{-2}$  at the euhaline zone (station 4). Like in Mira, significant differences in nematodes density recorded between stations ( $F = 12.03, p < 0.05$ ) (Fig. 3B) were due to the occurrence of high values in a single station (station 4).

In general, although the average nematode density was higher in the Mira estuary ( $603.3$  ind.  $10\text{ cm}^{-2}$ ) compared to the Mondego ( $311.0$  ind.  $10\text{ cm}^{-2}$ ), the number of genera present in each salinity range was higher in the Mondego estuary.

In the Mira estuary, the genera *Anoplostoma*, *Daptonema*, *Sabatieria*, *Terschellingia* and *Viscosia* were present in all sampling stations. The lowest diversity was detected in the oligohaline section of the estuary: 10 genera, of which *Oxy-stomina* and *Prochromadorella* were exclusively in this section. The highest diversity was registered in the euhaline station:

33 genera, of which 17 were also exclusive of this section. In the Mondego estuary, the only genus present in all sampling stations was *Daptonema*. The lowest diversity was detected in the freshwater station: 10 genera, with *Monhystera*, *Stygodesmodora* and *Syringolaimus* were found only in this section. The highest diversity was obtained in the southern arm: 29 genera, including 8 exclusive of this section.

## 2.3. Nematodes' community distribution patterns

The MDS analysis, with stress values of 0.08 in Mira and 0.1 in Mondego estuaries, corresponded to a good ordination with no real prospect of a misleading interpretation of the nematodes data used. Therefore, the two-dimensional solution is enough to appreciate the overall structure of these communities (Clarke and Warwick, 2001). The MDS plots clearly reflected the spatial distribution of nematodes along the Mira and Mondego salinity gradients (Fig. 4A, B).

At the Mira estuary, the MDS analysis revealed distinct assemblages corresponding to the different salinity stretches and the ANOSIM analysis showed significant differences between salinity stretches (Global  $R = 0.942, p = 0.1\%$ ). At the Mondego estuary, the ANOSIM analysis showed significant differences between the stretches (Global  $R = 0.777, p = 0.1\%$ ), revealing distinct assemblages corresponding to the different salinity sectors. The only pair without significant differences was the oligohaline/mesohaline stretches pair ( $R = 0.37, p = 0.6\%$ ).

The SIMPER analysis based on Mira data showed a maximum dissimilarity between assemblages from the oligohaline and polyhaline sections (93.8%) due to the presence of *Dorylaimus*, a freshwater nematode, in the oligohaline zone. The dissimilarity between the euhaline and polyhaline sections (93.1%) resulted from the presence of *Paracomosoma*, *Synonchiella* and *Odontophora* in the euhaline section, as well as the highest density of *Sabatieria*, *Ptycholaimellus*, *Metachromadora* and *Daptonema* in the polyhaline section. The mesohaline section was characterised by the presence of *Terschellingia*, *Sabatieria*, *Daptonema* and *Anoplostoma* (Table 5). The SIMPER analysis from Mondego data showed maximum dissimilarity between assemblages from the freshwater section and those from both the euhaline sections of the southern arm (98.9%) and northern arm (98.6%). The freshwater estuarine section was mostly characterised by freshwater nematodes. The southern arm exhibited the highest density of *Metachromadora*, *Anoplostoma* and *Microlaimus*, while in the northern arm *Sabatieria*, *Leptolaimus* and *Dichromadora* reached the highest density (Table 6).

The MDS plot resulting from the analysis of data from both estuaries also allowed the identification of distinct assemblages, according to salinity ranges (Fig. 4C). (i) Freshwater and oligohaline sections, characterised by the presence of freshwater nematodes, with the lowest density ( $38.9\text{--}109.0$  ind.  $10\text{ cm}^{-2}$ ) and diversity (10–15 genera); (ii) mesohaline sections, characterised by communities with low density ( $117.4\text{--}228.8$  ind.  $10\text{ cm}^{-2}$ ) and relatively low diversity (15–24 genera) and (iii) polyhaline and euhaline sections, characterised by the highest density ( $204.0\text{--}2234.0$  ind.  $10\text{ cm}^{-2}$ ) and diversity (14–33 genera). *Paracomosoma*, *Synonchiella*, *Odontophora*, *Sabatieria*, *Metachromadora*, *Daptonema* and *Ptycholaimellus* attained the

**Table 3 – Mean density (number of individuals 10 cm<sup>-2</sup>) and standard error (± SE), of each nematode genera in the sampled stations in the Mira estuary, and respective trophic group (TG).**

Genera	Total	TG	1		2		6		7		11	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Sabatieria</i>	738.7	1B	1.2	1.2	0.5	0.5	76.9	55.9	636.6	237.7	23.5	14.8
<i>Ptycholaimellus</i>	417.1	2A			0.5	0.5	2.4	2.4	405.9	134.1	8.4	4.5
<i>Metachromadora</i>	397.3	2B					2.4	1.2	388.5	216.2	6.3	3.2
<i>Terschellingia</i>	384.6	1A	130.2	75.7	48.8	16.2	29.4	7.5	166.8	81.9	9.3	4.6
<i>Daptonema</i>	277.4	1B	3.3	0.7	1.0	0.5	22.2	6.9	222.6	99.3	28.2	7.6
<i>Anoplostoma</i>	189.6	1B	3.4	1.8	7.3	3.7	26.4	12.5	152.2	58.7	0.3	0.3
<i>Sphaerolaimus</i>	137.0	2B			0.5	0.5	22.4	10.1	113.5	54.7	0.6	0.6
<i>Thalassironus</i>	88.3	2B							86.5	39.9	1.9	1.9
<i>Paracyatholaimus</i>	33.9	2A	15.3	4.4	9.7	6.7	2.9	0.6			6.1	4.8
<i>Viscosia</i>	26.2	2B	2.5	2.5	0.6	0.3	10.4	2.7	10.1	5.4	2.7	1.5
<i>Linhomoeus</i>	25.4	2A					0.6	0.6			24.8	19.6
<i>Axonolaimus</i>	24.3	1B	3.9	2.3	8.1	5.8			12.3	12.3		
<i>Paracomesoma</i>	22.1	1B									22.1	7.7
<i>Odontophora</i>	21.2	1B									21.2	12.6
<i>Dichromadora</i>	18.4	2A							16.6	16.6	1.8	0.9
<i>Synonchiella</i>	16.7	2B									16.7	6.3
<i>Metacomesoma</i>	11.4	1B									11.4	10.9
<i>Aegialolaimus</i>	8.3	1A							8.3	8.3		
<i>Spilophorella</i>	7.9	2A							7.9	7.9		
<i>Ascolaimus</i>	6.5	1B			0.3	0.3			6.2	6.2		
<i>Comesoma</i>	3.5	1B									3.5	3.5
<i>Chromadorella</i>	2.4	2A									2.4	1.5
<i>Metalinhomoeus</i>	2.4	1B	1.2	1.2			0.3	0.3			0.9	0.9
<i>Diodontolaimus</i>	2.1	2A			0.6	0.6					1.6	0.8
<i>Prochromadorella</i>	2.1	2A	2.1	2.1								
<i>Halalaimus</i>	1.8	1A			1.1	0.5	0.8	0.8				
<i>Hypodontolaimus</i>	1.8	2A					0.3	0.3			1.6	0.8
<i>Spirinia</i>	1.8	2A									1.8	0.9
<i>Antomicron</i>	1.5	1A					1.5	1.1				
<i>Leptolaimus</i>	1.3	1A			1.3	0.9						
<i>Camacolaimus</i>	1.2	2A					1.2	1.2				
<i>Eurystomina</i>	1.2	2B					1.2	1.2				
<i>Oxystomina</i>	1.2	1A	1.2	1.2								
<i>Monhystera</i>	0.9	1B									0.9	0.9
<i>Odontanticoma</i>	0.9	1A									0.9	0.9
<i>Anticoma</i>	0.9	1A									0.9	0.9
<i>Oncholaimellus</i>	0.9	2B									0.9	0.9
<i>Oncholaimus</i>	0.9	2B									0.9	0.9
<i>Prochromadora</i>	0.9	2A									0.9	0.9
<i>Chromadorita</i>	0.6	2A			0.3	0.3					0.3	0.3
<i>Aponema</i>	0.3	1A									0.3	0.3
<i>Chomadorina</i>	0.3	2A									0.3	0.3
<i>Chromadora</i>	0.3	2A									0.3	0.3
<i>Cyatholaimus</i>	0.3	2A									0.3	0.3
<i>Wieseria</i>	0.3	1A			0.3	0.3						
Freshwater nematodes	132.4	–	103.3	30.0	28.4	11.8	0.8	0.8				

highest density in these last sections. ANOSIM analysis showed that although there were no significant differences between the assemblages from both estuaries (Global  $R = 0.136$ ,  $p = 1.7\%$ ), it was possible to observe significant differences between the salinity stretches (Global  $R = 0.658$ ,  $p = 0.1\%$ ), revealing distinct assemblages corresponding to the different salinity sectors.

The BIOENV analysis showed that a combination of four variables (salinity, % of gravel, % of coarse sand, and sediment's organic matter) explained 89% of the variability found in the nematode community at Mira estuary. In the Mondego, the four variables explaining 96% of the variability within the

nematode community were the % fine sand,  $\text{PO}_4^{3-}$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ .

#### 2.4. Nematode feeding groups

The pattern of spatial distribution of the different feeding types was analysed along the salinity gradient in both systems. It was possible to define distinct feeding types' assemblages, according to the salinity ranges (Fig. 5).

In the Mira estuary there was a clear dominance of non-selective deposit feeders (1B: 45%, 11 genera) followed by, in decreasing order of abundance, predators (2B: 23.2%, 8 genera),

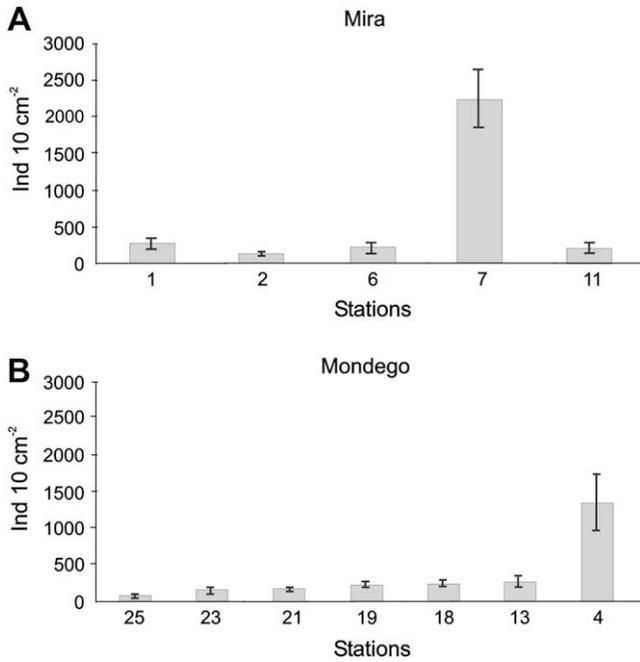
**Table 4 – Mean density (number of individuals 10 cm<sup>-2</sup>) and standard error ( $\pm$ SE), of each nematode genera in the sampled stations in the Mondego estuary, and respective trophic group (TG).**

Genera	Total	TG	25		23		21		19		18		13		4	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Metachromadora</i>	419.4	2B											0.6	0.6	418.8	140.3
<i>Anoplostoma</i>	296.8	1B			0.8	0.4	19.8	6.0	23.1	7.8	118.7	42.5	1.9	1.0	132.3	37.9
<i>Daptonema</i>	213.3	1B	0.8	0.2	27.3	18.6	20.8	3.6	115.9	37.8	10.6	2.9	25.0	11.5	12.9	3.4
<i>Sabatieria</i>	212.6	1B					0.8	0.9	0.7	0.7	1.6	1.6	116.9	52.4	92.6	37.3
<i>Microloaimus</i>	176.1	2A	0.2	0.2			0.3	0.3							175.6	73.8
<i>Sphaerolaimus</i>	92.9	2B					0.7	0.7	0.5	0.5	5.2	2.3	6.7	2.3	79.8	33.7
<i>Axonolaimus</i>	82.1	1B			0.4	0.4	9.9	5.4	2.8	0.3	6.1	2.5			62.9	38.6
<i>Prochromadorella</i>	61.30	2A					0.3	0.3					0.8	0.8	60.2	16.6
<i>Dichromadora</i>	59.9	2A			3.7	2.8	10.2	5.9	7.2	3.8	4.1	1.2	20.9	7.6	13.8	10.8
<i>Viscosia</i>	56.6	2B	0.4	0.2			0.7	0.7	2.9	2.9	21.2	9.2	9.3	3.4	22.1	3.8
<i>Paracyatholaimus</i>	55.4	2A	2.7	1.1	5.1	3.5	29.4	4.8	2.9	1.7	7.2	4.6			7.9	5.1
<i>Terschellingia</i>	45.2	1A					0.4	0.4	4.1	2.1	0.5	0.5	14.5	6.6	25.6	17.6
<i>Leptolaimus</i>	43.3	1A			0.2	0.2	4.8	2.4	4.1	2.4	6.5	6.5	22.8	9.9	4.9	4.9
<i>Calyptonema</i>	34.9	2B													34.9	34.9
<i>Chromadora</i>	30.5	2A			0.4	0.4							0.6	0.6	29.5	13.3
<i>Paralinhomoeus</i>	30.4	1B							0.7	0.7					29.7	29.7
<i>Aegialoalaimus</i>	26.5	1A													26.5	13.5
<i>Linhomoeus</i>	23.9	2A											3.0	3.1	20.8	7.1
<i>Halalaimus</i>	19.1	1A													19.1	13.4
<i>Southerniella</i>	12.9	1A	0.2	0.2					1.2	0.6	1.1	1.1	0.6	0.6	9.9	9.9
<i>Ptycholaimellus</i>	11.3	2A			3.5	2.2	0.3	0.3			0.4	0.4			7.1	4.3
<i>Praeacanthochus</i>	9.9	2A													9.9	9.9
<i>Hypodontolaimus</i>	8.9	2A					1.4	0.9	7.6	4.4						
<i>Camacolaimus</i>	8.7	2A	0.2	0.2	0.8	0.4							1.8	1.4	5.8	5.8
<i>Chromadorita</i>	8.7	2A	0.2	0.2					3.0	2.2			0.3	0.3	4.9	4.9
<i>Ascolaimus</i>	6.5	1B					5.6	2.3	0.9	0.4						
<i>Desmolaimus</i>	4.9	1B													4.9	4.9
<i>Chromadorina</i>	4.5	2A			0.8	0.4	2.7	1.3	0.7	0.7			0.3	0.3		
<i>Oncholaimellus</i>	3.5	2B			0.4	0.4			0.5	0.5	0.4	0.4			2.1	2.1
<i>Cobbia</i>	2.4	2A			1.0	0.6	1.3	1.3								
<i>Aponema</i>	2.1	1A													2.1	2.1
<i>Araeolaimus</i>	2.1	1A													2.1	2.1
<i>Eumorpholaimus</i>	2.1	1B													2.1	2.1
<i>Paracomesoma</i>	1.4	1B			0.4	0.4			0.7	0.7			0.3	0.3		
<i>Cromadorella</i>	1.2	2A											1.2	1.2		
<i>Doliolaimus</i>	1.1	2B					0.3	0.3	0.7	0.7						
<i>Paramonhystera</i>	1.0	1B					0.4	0.4					0.6	0.6		
<i>Stygodesmodora</i>	1.0	2B	1.0	0.5												
<i>Spilophorella</i>	1.0	2A					1.0	1.0								
<i>Tripyloides</i>	0.8	1B					0.3	0.3			0.4	0.4				
<i>Marylynnia</i>	0.7	2A							0.7	0.7						
<i>Paracanthochus</i>	0.6	2A					0.3	0.3					0.3	0.3		
<i>Monhystera</i>	0.6	1B	0.6	0.6												
<i>Valvaelaimus</i>	0.4	2A					0.4	0.4								
<i>Odontophora</i>	0.4	1B									0.4	0.4				
<i>Comesoma</i>	0.3	1B					0.3	0.3								
<i>Diplolaimella</i>	0.3	1B											0.3	0.3		
<i>Syringolaimus</i>	0.2	2B	0.2	0.2												
Freshwater nematodes	97.2	–	32.3	2.9	56.1	33.1	4.7	0.4	1.5	1.5	0.5	0.5			2.1	2.1

epigrowth-feeders (2A: 17.9%, 16 genera) and selective deposit feeders (1A: 13.9%, 10 genera) (Fig. 5A). In the oligohaline section, the selective deposit feeders (1A) were dominant, followed by non-selective deposit feeders (1B); in the mesohaline and polyhaline sections, non-selective deposit feeders (1B) were dominant, followed by predators (2B), while in the euhaline section, non-selective deposit feeders (1B) constituted the most representative feeding type, followed by epigrowth-feeders (2A). MDS analysis based on the proportions of each feeding type isolated the oligohaline section's trophic structure

from the other estuarine sections (Fig. 6A). The ANOSIM analysis showed significant differences between the stretches (Global  $R = 0.628$ ,  $p = 0.2\%$ ), revealing distinct assemblages corresponding to the different salinity sectors: assemblages from the oligohaline stretch (stations 1 and 2) were significantly different from the assemblages present in the mesohaline, polyhaline and euhaline stretches ( $R = 0.593$ ,  $p = 3.6\%$ ;  $R = 0.981$ ,  $p = 1.2\%$ ;  $R = 0.981$ ,  $p = 1.2\%$ , respectively).

In the Mondego estuary, similarly to Mira, the non-selective deposit feeders (1B: 41.0%, 15 genera) and predators



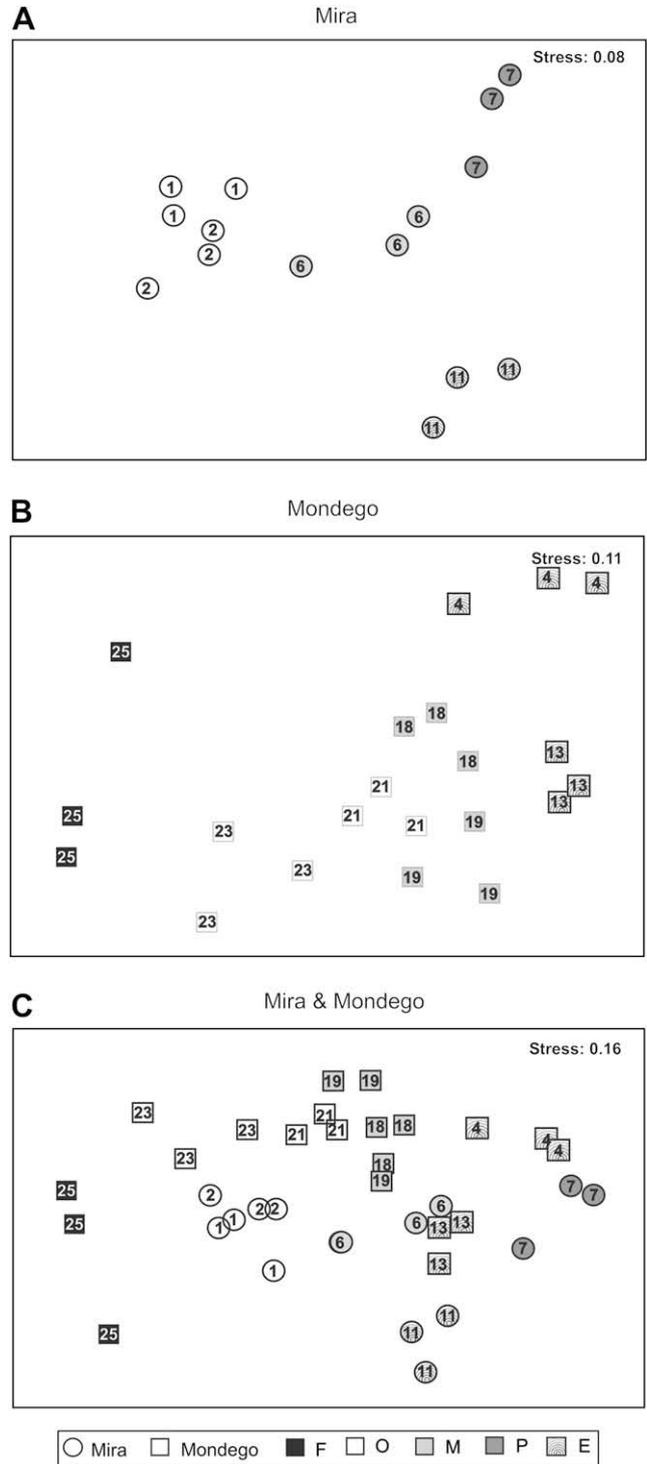
**Fig. 3 – Mean density ± SE of nematodes (ind. 10 cm<sup>-2</sup>) in each one of the sampling station in (A) Mira estuary and (B) Mondego estuary.**

(2B: 29.3%, 8 genera) were the most abundant feeding types, followed by epigrowth-feeders (2A: 22.4%, 18 genera), and selective deposit feeders (1A: 7.3%, 7 genera) (Fig. 5B). Nevertheless, the feeding types' proportions differed between estuaries according to the salinity ranges. In the upstream section of the Mondego estuary, the epigrowth-feeders (2A) were clearly dominant while non-selective deposit feeders (1B) were the most abundant feeding group in other estuarine sections, with the exception of the southern arm euhaline section where predators were dominant. The MDS analysis identified three distinct groups (Fig. 6B). There were also significant differences between stretches (Global  $R = 0.582$ ,  $p = 0.1\%$ ) and between the trophic composition of the South and North arms of the estuary ( $R = 0.963$ ,  $p = 10\%$ ).

Overall no significant differences were found between the trophic compositions of the Mira and the Mondego estuaries (Global  $R = 0.201$ ,  $p = 0.2\%$ ). Nevertheless, the MDS plot resulting from the analysis of feeding types data from both estuaries suggested the separation of the oligohaline and the mesohaline stations from Mira (Fig. 6C).

### 3. Discussion and conclusions

Gradients of salinity and sediment particles size were clearly detected at both estuaries. In Mira, the salinity gradient was mostly dependent on the morphology of the estuary – a single river channel and an almost complete absence of irregularities in its terminal section – allowing the tidal influence to extend about 40 km inland (Paula et al., 2006). On the contrary, in Mondego, two gradients were clearly recognisable along the northern and southern arms due to the different hydrological characteristics of each arm. The northern arm is deeper and



**Fig. 4 – Non-metric multidimensional scaling (MDS) plot based on the nematode density and composition from sampling stations in (A) Mira estuary, (B) Mondego estuary and (C) Mira and Mondego estuaries, simultaneously.**

has been heavily modified, namely in the last two decades, by the construction of stonewalls along the river banks and of small water reservoirs for aquaculture, which has caused changes in hydrodynamics and has had a strong anthropogenic impact (Marques et al., 2003). The tidal penetration is

**Table 5 – Genera determined by SIMPER analysis as those most responsible for contributing for the similarity within salinity stretches for Nematode assemblages in the Mira estuary, in summer 2006.**

Mira estuary	Oligohaline st 1 and 2	Mesohaline st 6	Polyhaline st 7	Euhaline st 11
Oligohaline st 1 and 2	<b>47.3%</b> <i>Terschellingia</i> <i>Dorylaimus</i> <i>Paracyatholaimus</i>			
Mesohaline st 6	<b>76.1%</b> <i>Sabatieria</i> <i>Dorylaimus</i> <i>Terschellingia</i> <i>Anoplostoma</i>	<b>44.5%</b> <i>Terschellingia</i> <i>Sabatieria</i> <i>Daptonema</i> <i>Anoplostoma</i>		
Polyhaline st 7	<b>93.8%</b> <i>Sabatieria</i> <i>Ptycholaimellus</i> <i>Metachromadora</i> <i>Daptonema</i>	<b>84.4%</b> <i>Sabatieria</i> <i>Ptycholaimellus</i> <i>Metachromadora</i> <i>Daptonema</i>	<b>49.5%</b> <i>Sabatieria</i> <i>Ptycholaimellus</i> <i>Metachromadora</i> <i>Daptonema</i>	
Euhaline st 11	<b>91.2%</b> <i>Terschellingia</i> <i>Dorylaimus</i> <i>Daptonema</i> <i>Paracomesoma</i> <i>Sabatieria</i> <i>Linhomoeus</i> <i>Odontophora</i>	<b>72.7%</b> <i>Sabatieria</i> <i>Terschellingia</i> <i>Anoplostoma</i> <i>Paracomesoma</i> <i>Linhomoeus</i> <i>Sphaerolaimus</i> <i>Odontophora</i> <i>Synonchiella</i>	<b>93.1%</b> <i>Sabatieria</i> <i>Ptycholaimellus</i> <i>Metachromadora</i> <i>Daptonema</i>	<b>38.0%</b> <i>Daptonema</i> <i>Paracomesoma</i> <i>Synonchiella</i> <i>Sabatieria</i> <i>Odontophora</i>

Shaded boxes: percentage of similarity (bold) and genera that contributed for similarity in each group. Non-shaded box, percentage of dissimilarity (bold) between salinity stretches and species that contributed for the total dissimilarity (cut-off percentage: 90%).

therefore faster along the northern arm and salinity tends to be higher than in the southern arm during high-water periods. On the contrary, the southern arm is shallower, with a smaller freshwater discharge, which causes the water circulation to depend greatly on tides (Teixeira et al., 2008).

A typical gradient of estuarine sediments was observed at Mira, with fractions of silt and clay increasing from the upstream sections towards the mouth of the estuary. The only exception was sampling station 11, located on the top of a sand flat (bottom sediments consisting of 90% sand), very close to the mouth. On the contrary, the gradient of estuarine sediments was much less evident in the Mondego estuary, where subtidal bottoms contain, in general, larger fine sand fractions and much smaller silt + clay fractions than in Mira. Moreover, the sediments distribution was found to be distinct in the two arms (Teixeira et al., 2008). Thus, particles size increases from the mouth to upstream sections along the northern arm, where bottoms consist mainly of coarse sand, while in southern arm particles size decreases from the mouth to the inner most sections.

In Mira, nutrients concentration did not show any spatial pattern of variation during the sampling period, remaining more or less constant along the estuary, which may be explained by the absence of significant inputs from anthropogenic activities. On the contrary, in Mondego, nutrient concentrations (ammonium, oxidised forms of nitrogen, and phosphates) were higher in the northern arm than in the southern one, decreasing seawards in both arms. A previous study in the Mondego estuary also indicated a strong

dependency of dissolved nitrogen oxidised forms on the freshwater inputs from diffuse and/or point sources, which may include precipitation and the consequent freshwater flow with agricultural lands drainage (Lillebø et al., 2007).

Nematode density was higher in the Mira estuary, although the number of genera found was higher in each analogous salinity range in the Mondego estuary. Nematode density values were similar to those found in communities living in subtidal sediments of northern European estuaries, but the number of genera was relatively lower (Smol et al., 1994; Soetaert et al., 1994). On the other hand, nematode density was lower than in intertidal sediments which are often more diverse (Soetaert et al., 1994, 1995; Steyaert et al., 2003).

In both estuaries, the spatial distribution of nematode density, composition, and feeding types appeared clearly related to the salinity gradient. As a whole, nematode assemblage composition in the two systems closely resembled that of northern European estuaries. These communities were mainly composed of *Sabatieria*, *Metachromadora*, *Daptonema*, *Anoplostoma*, *Sphaerolaimus*, and *Terschellingia*, which are also the most common genera in tidal estuarine mudflats, and showed few dominant species as observed in other systems (Austen and Warwick, 1989; Li and Vincx, 1993; Soetaert et al., 1995; Steyaert et al., 2003; Rzeznik-Orignac et al., 2003).

In both systems, the nematode density and composition appeared alike and followed closely the salinity gradients. (i) Freshwater and oligohaline sections, characterised by the presence of freshwater taxa, low total nematode density (39–109 ind. 10 cm<sup>-2</sup>) and diversity (10–15 genera).

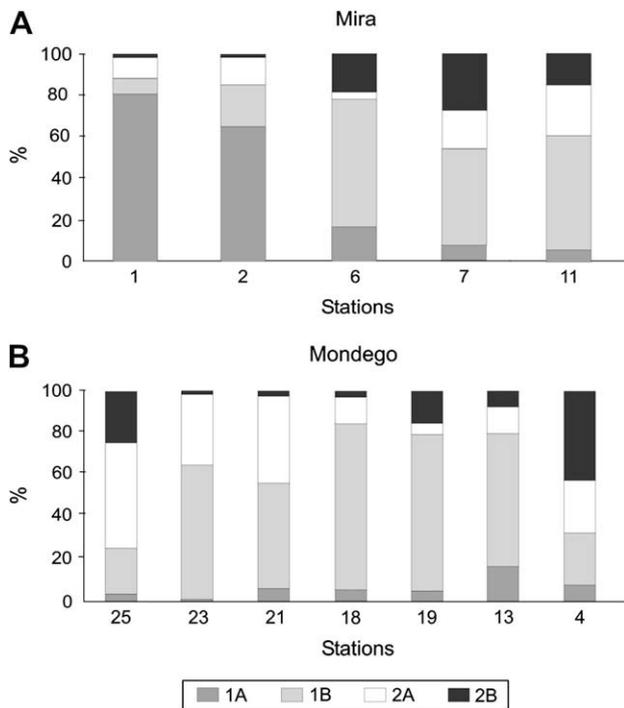
**Table 6 – Species determined by SIMPER analysis as those most responsible for contributing for the similarity within salinity stretches for Nematode assemblages in the Mondego estuary, in summer 2006.**

Mondego estuary	Euhaline NA st 4	Euhaline SA st 13	Mesohaline st 18 and 19	Oligohaline st 21 and 23	Freshwater st 25
Euhaline NA st 4	<b>48.8%</b> <i>Sabatieria</i> <i>Leptolaimus</i> <i>Dichromadora</i> <i>Daptonema</i>				
Euhaline SA st 13	<b>84.8%</b> <i>Metachromadora</i> <i>Microlaimus</i> <i>Anoplostoma</i> <i>Sabatieria</i> <i>Prochromadorella</i> <i>Sphaerolaimus</i> <i>Axonolaimus</i> <i>Paralinhomoeus</i> <i>Terschellingia</i> <i>Chromadora</i> <i>Calyptronema</i>	<b>50.9%</b> <i>Metachromadora</i> <i>Anoplostoma</i> <i>Microlaimus</i> <i>Sabatieria</i> <i>Prochromadorella</i> <i>Sphaerolaimus</i>			
Mesohaline st 18 and 19	<b>79.9%</b> <i>Sabatieria</i> <i>Anoplostoma</i> <i>Daptonema</i> <i>Leptolaimus</i> <i>Dichromadora</i>	<b>85.2%</b> <i>Metachromadora</i> <i>Microlaimus</i> <i>Anoplostoma</i> <i>Sabatieria</i> <i>Prochromadorella</i> <i>Sphaerolaimus</i> <i>Daptonema</i> <i>Axonolaimus</i> <i>Paralinhomoeus</i> <i>Chromadora</i> <i>Calyptronema</i>	<b>37.5%</b> <i>Anoplostoma</i> <i>Daptonema</i> <i>Viscosia</i>		
Oligohaline st 21 and 23	<b>84.6%</b> <i>Sabatieria</i> <i>Dorylaimus</i> <i>Daptonema</i> <i>Leptolaimus</i> <i>Paracyatholaimus</i> <i>Dichromadora</i> <i>Terschellingia</i> <i>Viscosia</i>	<b>93.4%</b> <i>Metachromadora</i> <i>Microlaimus</i> <i>Anoplostoma</i> <i>Sabatieria</i> <i>Sphaerolaimus</i> <i>Prochromadorella</i> <i>Axonolaimus</i> <i>Dorylaimus</i> <i>Paralinhomoeus</i> <i>Viscosia</i> <i>Chromadora</i> <i>Calyptronema</i>	<b>74.4%</b> <i>Anoplostoma</i> <i>Daptonema</i> <i>Dorylaimus</i> <i>Paracyatholaimus</i> <i>Viscosia</i> <i>Dichromadora</i> <i>Leptolaimus</i>	<b>31.8%</b> <i>Paracyatholaimus</i> <i>Dorylaimus</i> <i>Anoplostoma</i>	
Freshwater st25	<b>98.6%</b> <i>Sabatieria</i> <i>Leptolaimus</i> <i>Daptonema</i> <i>Dichromadora</i> <i>Dorylaimus</i> <i>Terschellingia</i> <i>Viscosia</i>	<b>98.9%</b> <i>Metachromadora</i> <i>Microlaimus</i> <i>Anoplostoma</i> <i>Sabatieria</i> <i>Prochromadorella</i> <i>Sphaerolaimus</i> <i>Axonolaimus</i> <i>Paralinhomoeus</i> <i>Viscosia</i> <i>Chromadora</i>	<b>96.8%</b> <i>Anoplostoma</i> <i>Daptonema</i> <i>Dorylaimus</i> <i>Family Dorylaimidae</i> <i>Viscosia</i> <i>Order Mononchida</i> <i>Dichromadora</i>	<b>82.9%</b> <i>Dorylaimus</i> <i>Daptonema</i> <i>Paracyatholaimus</i> <i>Family Dorylaimidae</i> <i>Anoplostoma</i> <i>Order Mononchida</i> <i>Dichromadora</i> <i>Axonolaimus</i>	<b>36.7%</b> <i>Dorylaimus</i> <i>Order Mononchida</i>

Shaded boxes: percentage of similarity (bold) and genera that contributed for similarity in each group. Non-shaded box, percentage of dissimilarity (bold) between salinity stretches and species that contributed for the total dissimilarity (cut-off percentage: 90%).

(ii) Mesohaline section, also with low total nematode density (117–229 ind. 10 cm<sup>-2</sup>) and diversity (15–24 genera), and having *Terschellingia*, *Sabatieria*, *Daptonema* and *Anoplostoma* as the dominant genera, and (iii) polyhaline and euhaline

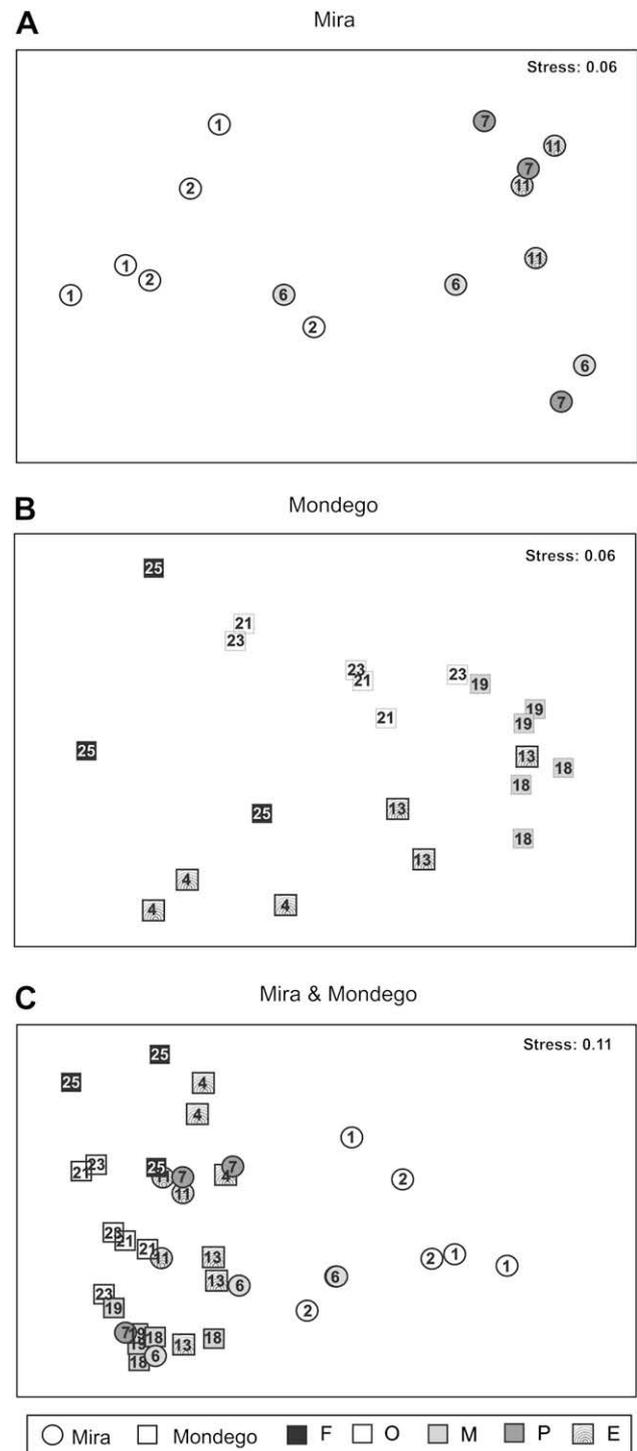
sections, where nematodes reached the highest density (204–2234 ind. 10 cm<sup>-2</sup>) and *Paracomesoma*, *Synonchiella*, *Odontophora*, *Sabatieria*, *Metachromadora* *Daptonema* and *Ptycholaimellus* were particularly abundant.



**Fig. 5 – Percentage of contribution of each feeding group in each of the sampled stations in the (A) Mira and (B) Mondego estuaries. 1A, selective deposit feeders; 1B, non-selective deposit feeders; 2A, epistrate feeders; 2B, predators (Wieser, 1953).**

In the two systems, the density of nematodes decreased towards the inner sections in agreement with the general notion that estuarine nematodes tend to decrease in abundance and number of species as one moves from the sea towards freshwater (Austen and Warwick, 1989). As the preponderance of species in estuaries is marine, there is a decrease in species richness as one moves towards freshwater (Coull, 1999). We could therefore confirm the effect of salinity on estuarine nematode communities, namely in determining changes in the total density and diversity (Coull, 1985; Soetaert et al., 1995; Vincx et al., 1990; Li and Vincx, 1993; Yamamuro, 2000). Nevertheless, our results also illustrate how other environmental factors, such as granulometry, nutrient concentration and sediment organic matter content influenced both density and diversity of nematodes in both estuaries. The higher sediment organic matter content in the Mira estuary could be responsible for the highest nematode density observed, although the number of genera was higher in each analogous salinity range of the Mondego estuary. Nematodes can utilize organic matter in different forms and their density and distribution have been related to the food availability and to the organic matter at the bottom of the sediments (Montagna, 1995; Moens et al., 1999). The predominance of sandy sediments in the Mondego estuary contributed to a decrease in density and an increase in diversity because of the wider range of microhabitats available for nematodes in these sediments when compared to muddy ones (Steyaert et al., 2003).

The relative proportion of each of the four nematode feeding guilds in a community tends to depend on the nature



**Fig. 6 – Non-metric multidimensional scaling (MDS) plot based on the percentage of contributing of each feeding groups from sampling stations in (A) Mira estuary, (B) Mondego estuary and (C) Mira and Mondego estuaries simultaneously.**

of available food, which in turn is dependent on sediment composition (Moens and Vincx, 1997; Danovaro and Gambi, 2002). Accordingly, the nematode feeding types' composition and patterns of spatial distribution were remarkably different in the two estuaries. (i) The oligohaline section at the Mira was

characterised by the dominance of selective deposit feeders, while non-selective deposit feeders were dominant in the rest of the system. (ii) At the Mondego, epigrowth-feeders and omnivores/predators were dominant in the freshwater section and in the euhaline section of the South arm, while non-selective deposit feeders were dominant in the oligohaline, mesohaline and euhaline sections along the North arm. This illustrates how nematode feeding types responded to food availability as a function of differences in physico-chemical conditions between the northern and southern arms of the Mondego, as well as between the upstream and downstream sections in both estuaries. In fact, homogeneous mud habitats are known to be dominated by selective and non-selective deposit feeders. This happens because bacterial feeders, microvore ciliate feeders, bacteria and protozoa living in sediment particles constitute their major food sources (Giere, 1993; Michiels and Traunspurger, 2004).

In general, the spatial distribution of subtidal nematode density and composition reflects both the sediment composition and the hydrodynamic conditions. Our results illustrate that differences in nematodes community observed along each estuarine gradient were much stronger than between the two estuaries. This indicates that mesoscale variability within estuaries, at the scale of km, namely due the estuarine gradients as salinity changes and grain size differences, is more important than variability at the scale of hundreds of km or variability between systems (Soetaert et al., 1995; Li et al., 1997).

On the other hand, in the Mondego estuary, the influence of anthropogenic stressors on the nematode's spatial distribution appeared not to be relevant, suggesting that mesoscale variability essentially responded to natural stressors characteristic of estuarine gradients. Since the responses to anthropogenic and natural stress are apparently difficult to differentiate, there is an obvious problem when attempting to determine the effects of human activities on communities. This problem is defined within the context of the "Estuarine Quality Paradox" (Elliot and Quintino, 2007), which briefly states that the features of communities under anthropogenic stress coincide with those under natural stress as a consequence of the high variability of environmental parameters in estuaries (e.g. salinity, dissolved oxygen, temperature) and that species living in such environments adapt to this variability and become tolerant to further changes. The high natural variability of the system may confer an ability to the community to withstand stress, both natural and anthropogenic, and thus a resilience created by "Environmental Homeostasis" (Elliot and Quintino, 2007). Unless the anthropogenic stressor action is severe, estuaries still need further study in order to define and quantify the way in which communities respond to human activities.

In this study, the proportion of the different feeding types was considerably different across the two estuaries. The nematode's response to food availability and quality as well as to other environmental factors are highly species-specific (Santos et al., 2008) and the food availability and quality are important factors driving the strong heterogeneous small-scale spatial distribution observed in nematode communities (Moens et al., 1999, 2002; Santos et al., 2008). The density, biomass, diversity, species life history and composition

patterns can vary under different conditions of food availability (Santos et al., 2008). For instance, the source of organic matter determines assemblages composition, decaying organic matter attracts some nematode species and repels others (Ólafsson et al., 1999), and the labile organic carbon derived from microphytobenthos and selected phytoplankton constitutes an important carbon source to nematodes; however, the organic matter from terrestrial or riverine origin is a poor contribution to the benthic food webs (Moens et al., 2002).

From a management perspective, the results presented here suggest that the response of nematode's feeding guilds might reflect anthropogenic-induced stress on the system and may become useful in assessing biological quality in transitional water ecosystems.

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