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LISTS OF SPECIES

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Checklist of free-living marine nematodes from intertidal sites along the central west coast of India

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Abstract: The present study provides a checklist of free-living marine nematode species from nine intertidal sites located along the central west coast of India covering an area between latitudes 16°33′08.47″ N and 16°38′50.75″ N and longitudes 073°19′30.13″ E and 073°23′34.97″ E. The list includes 33 species of marine nematodes belonging to 20 genera and 13 families. The occurrence of nematode species identified appears to be correlated to character of the sediments across sampled intertidal sites. Ten families were encountered in sandy sites while in muddy sites the number was ten. Three families, namely Camacolaimidae, Ironidae, and Microlaimidae, were exclusive to the sandy sites, while Anoplostomatidae, Comesomatidae, and Linhomoeidae were only found across muddy sites. Some of the dominant free-living marine nematode groups encountered across all sites was represented by the genera *Ptycholaimellus*, *Viscosia*, *Oncholaimus*, *Halalaimus*, and species such as *Sphaerolaimus balticus* and *Metachromadora suecica*.

Key words: intertidal coastal areas; free-living marine nematodes; India

INTRODUCTION

In estuarine and coastal sediments, free-living marine nematodes are usually dominant for abundance and diversity (e.g., Lambshead 2004). Recent estimates have shown that marine nematode species number could be as high as 50,000 with 86% of the existing species waiting to be discovered (e.g., Appletans et al. 2012). They are abundant in particulate shores, reaching their highest number in muddy estuaries and salt-marshes (around 20 million per m^2) and lowest in very exposed sandy shores as well as in deep sea sediments (0.1 million per m^2) (Balsamo et al. 2010).

Free-living marine nematodes play key roles in decomposition of organic matter, recycling of nutrients as well as serving as a food source for higher trophic groups (Austen 2004; Nascimento et al. 2012). Additionally, marine nematodes can be effectively used as bioindicators for environmental stress and pollution across the marine realms (Boyd et al. 2000; Moreno et al. 2011). Despite their ecological importance, studies of marine nematodes are largely neglected because their identification, which is predominantly based on morphological characters, requires taxonomic expertise (e.g., Platt and Warwick 1988; Bhadury et al. 2008). The understanding of an ecosystem depends not only on holistic synthesis of all components, but also on how the individual components work. Therefore, the accuracy of the identification is fundamental to our understanding of ecological attributes of any organism in its environment.

Moreover, most of the studies that have focused on free-living marine nematodes systematic and biogeography are focused on European coastal ecosystems and our understanding of tropical and subtropical coastal environments is very poor (Semprucci and Balsamo 2012). One of the poorly studied tropical coastal settings is the central west coast of India. The central west coast of India represents the continental shelf region and the region facing the Arabian Sea is highly productive (Rajendran and Biddandra 1994) and known to harbour a rich marine biodiversity, including benthic macrofauna (e.g., Ramaiah et al. 1996; Joydas and Damodaran 2009). Several river mouths, creeks, small bays, cliffs and beaches make up the physical attributes of this coastal zone. The region also harbour eco-sensitive habitats, including mangroves and coral reefs (e.g., Ndaro and Ólafsson 1999; Raes et al. 2007; Ansari et al. 2012; Semprucci et al. 2013).

Ratnagiri and Vijaydurga, located along the central west coast of India, represent ecologically sensitive coastal areas based on their rich marine biodiversity (Apte et al. 2012). To date, the benthic community and diversity, including especially the free-living marine nematodes, from these two areas have been poorly studied. A single study although from subtidal environments, reported presence of several nematode species along the central west coast of India (Nanajkar and Ingole 2010). It is important to know the structure of the free-living marine nematode community from these two regions, since these organisms can be used in the future as an "environment proxy" to monitor ecologically sensitive coastal zones located along the central west coast of India.

This work aims to provide a list of free-living marine nematode species found in the Ratnagiri and Vijaydurga coastal stretches along the central west coast of India, which will help in expanding our knowledge on marine benthic faunal biodiversity of Indian coastlines.

MATERIALS AND METHODS

Study sites

Nine coastal stretches of Ratnagiri and Vijaydurg along the central west coast of India were selected for analysis of the free-living marine nematode community structure: Ansure, Vijaydurg, Madban, Shindewadi, Waghran, Jaitapur, Ghodepol, Aagarwadi, and Ambolgad. All these sites were intertidal habitats. The sites Ambolgad, Jaitapur, Madban, and Vijayadurg were characterized by sandy beach sediments while the remaining sites are characterized by muddy estuarine sediments and contain mangrove patches. All these coastal stations were highly uneven in nature, usually narrow riverine plains that fringe along the coastline and with extensive lateritic cover. GPS points of the studied sites are detailed in Table 1 and depicted in Figure 1.

Sediment sample collection

Sediment samples were collected by hand held corer (5 cm length and 5.5 cm diameter) from these nine stations during beginning and end of summer 2012 at low tide. *In situ* environmental parameters such as surface water temperature, salinity, and pH were measured using hand-held instruments at the time of sampling.

Immediately after collection, Rose Bengal stain (1g/L) was added to sediment samples, which were subsequently preserved based on established protocol (e.g., Somerfield and Warwick 1996). As part of this study, sediment sub-sample from core weighing 5 g representing top 3 cm were sieved from each station in 63 µm sieve and the meiofauna extraction was undertaken following the method of Somerfield and Warwick (1996). The extracted meiofauna was transferred to a petridish and nematodes were handpicked using a fine needle under a stereomicroscope (Zeiss Stemi DV4). Handpicked meiofauna were transferred to pure glycerin (Seinhorst 1959) and mounted on microscopic glass slides for taxonomic identification. Taxonomic identification of free-living marine nematodes was undertaken based on available pictorial keys (Platt and Warwick 1983, 1988; Warwick et al. 1998) and the NeMys online identification key (Steyaert et al. 2005). Identified marine nematodes were subsequently photographed using an Olympus BX53 microscope equipped with XC50 CCD camera and CellSens Standard software. The voucher nematodes in the form of slides have been submitted to Zoological Survey of India and additionally slide of same specimens have been submitted to Marine Meiofauna Laboratory of Indian Institute of Science Education and Research Kolkata.

Table 1. Geographical coordinates and habitat characteristics of nine sites sampled along the central west coast of India for studying marine nematodes.

No.	Site name	Latitude	Longitude	Habitat
1	Ansure	16°33′08.47″ N	073°21′22.67″ E	Muddy with Mangrove
2	Vijaydurga	16°33′29.72″ N	073°19′58.34″ E	Sandy
3	Madban	16°34′43.94″ N	073°20′28.31″ E	Sandy
4	Shindewadi	16°35′50.54″ N	073°23′34.97″ E	Muddy with Mangrove
5	Waghran	16°36′08.31″ N	073°22′51.45″ E	Muddy with Mangrove
6	Jaitapur	16°36′19.47″ N	073°19′30.13″ E	Sandy
7	Ghodepol	16°36′27.13″ N	073°22′55.65″ E	Muddy with Mangrove
8	Agarwadi	16°36′55.23″ N	073°22′53.25″ E	Muddy with Mangrove
9	Ambolgad	16°38′50.75″ N	073°19′46.80″ E	Sandy

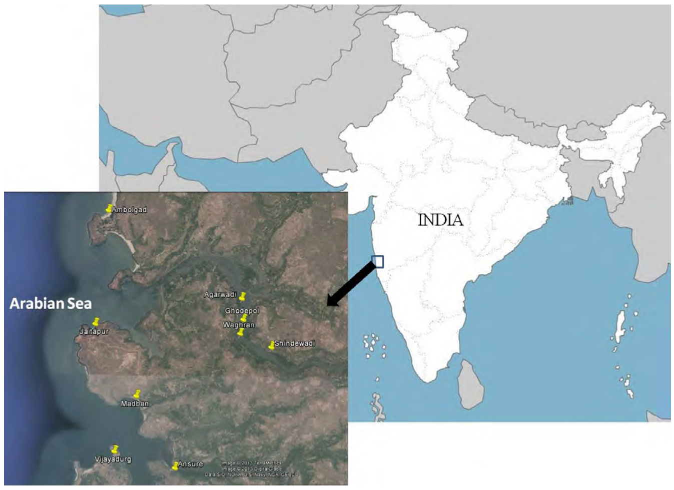

Figure 1. Map showing sampling locations along the Central West Coast of India. Source: Google Maps.

RESULTS

At the time of sediment sampling, average values for surface water temperature, salinity and pH for the sites sampled were 30°C, 35 ppt and 8.4 respectively.

A total of 160 free-living marine nematode specimens were collected in the study area and the specimens belong to 13 families, 20 genera, and 33 species. The list of recorded taxa and their respective sedimentary habitats is given in Table 2. The 13 families based on the number of species encountered during the present study were: Oncholaimidae, Xyalidae, Desmodoridae, Oxystominidae, Sphaerolaimidae, Chromadoridae, Microlaimidae, Linhomoeidae, Comesomatidae, Anoplostomatidae, Ironidae, Camacolaimidae and Neotonchidae. Ten families were encountered in the sandy sedimentary sites,

Table 2. List of free-living marine nematode species recorded from the central west coast of India and their habitat (x denotes present; – denotes absent).

Nematodes	Sandy beach sediment	Muddy mangrove sediment
Camacolaimidae		
Camacolaimus barbatus Warwick, 1970	x	–
Ironidae		
Syringolaimus sp.1	x	–
Anoplostomatidae Gerlach & Riemann, 1974		
Anoplostoma sp. 1	–	x
Chromadoridae Filipjev, 1917		
Ptycholaimellus sp. 1	x	x
Neochromadora poecilosomoides Filipjev, 1918	x	–
Spilophorella candida Gerlach, 1951	x	x
Microlaimidae		
Microlaimus conothelis (Lorenzen, 1973)	x	–
Microlaimus sp. 1	x	–
Comesomatidae Filipjev, 1918		
Sabatieria sp. 1	–	x
Desmodoridae Filipjev, 1922		
Catanema sp. 1	x	x
Desmodora sp. 1	–	x
Metachromadora suecica (Allgén, 1929)	x	x
Pseudonchus sp. 1	–	x
Linhomoeidae Filipjev, 1922		
Terschellingia longicaudata de Man, 1907	–	x
Terschellingia sp. 1	–	x
Neotonchidae Wieser & Hopper, 1966		
Neotonchus sp1	x	x
Oncholaimidae Filipjev, 1916		
Oncholaimus sp1	x	x
Oncholaimus skawensis Ditlevsen, 1921	x	–
Viscosia sp. 1	x	x
Viscosia abyssorum (Allgén, 1933)	x	–
Viscosia elegans (Kreis, 1924)	–	x
Viscosia viscosa (Bastian, 1865)	–	x
Oxystominidae Chitwood, 1935		
Halalaimus sp. 1	x	x
Halalaimus gracilis de Man, 1888	x	–
Halalaimus longicaudatus (Filipjev, 1927)	–	x
Sphaerolaimidae Filipjev, 1918		
Sphaerolaimus sp. 1	–	x
Sphaerolaimus balticus Schneider, 1906	x	x
Sphaerolaimus islandicus Ditlevsen, 1926	x	x
Xyalidae Chitwood, 1951		
Daptonema hirsutum Vitiello, 1967	–	x
Daptonema normandicum de Man, 1890	x	x
Daptonema procerum Gerlach, 1951	x	–
Daptonema sp. 1	x	x
Rhynchonema sp. 1	–	x

and three of them, Camacolaimidae, Ironidae and Microlaimidae, were exclusive to sandy sediments (Table 2). For muddy sedimentary sites, ten families were encountered, and three of them, Anoplostomatidae, Comesomatidae, and Linhomoeidae, were exclusive of this habitat (Table 2). Many of the nematode families were found in both sandy and muddy sites which is also evident in Table 2. Fourteen genera were identified from sites represented by sandy sediments, while 16 genera were encountered in the muddy sites. Twenty-one species were encountered in the sandy sediments, while for muddy sediments, the number of species were 24 (Table 2). Some species such as *Ptycholaimellus* sp. 1, *Catanema* sp. 1, *Metachromadora suecica* (Allgén 1929), *Spilophorella candida* Gerlach, 1951, *Neotonchus* sp., *Oncholaimus* sp. 1, *Viscosia* sp. 1, *Sphaerolaimus balticus* Schneider, 1906, *Sphaerolaimus islandicus* Ditlevsen, 1926, *Halalaimus* sp., *Daptonema normandicum* (de Man, 1890), and *Daptonema* sp. were found in both sandy and muddy sediments. Some of the species such as *Camacolaimus barbatus* Warwick, 1970, *Syringolaimus* sp. 1, *Microlaimus conothelis* (Lorenzen, 1973), *Microlaimus* sp. 1, *Oncholaimus skawensis* Ditlevsen, 1921, *Viscosia abyssorum* (Allgén, 1933), *Halalaimus gracilis* de Mann, 1888, and *Daptonema procerum* (Gerlach, 1951) were found exclusively in the sandy sediments. On the other hand, species such as *Anoplostoma* sp. 1, *Sabatieria* sp. 1, *Desmodora* sp. 1, *Pseudonchus* sp. 1, *Terschellingia longicaudata* de Man, 1907, *Terschellingia* sp. 1, *Viscosia elegans* Filipjev, 1922, *Viscosia viscosa* (Bastian, 1865), *Halalaimus longicaudatus* (Filipjev, 1927), *Sphaerolaimus* sp. 1, *Daptonema hirsutum* (Vitiello, 1967), and *Rhynchonema* sp. 1 were found only in the muddy sites. Morphometric details of six nematode species encountered frequently from study area and accurately identifiable based on available taxonomic keys have been detailed in Table 3. Some of the observable morphological characters for these six species have been shown in Figure 2.

DISCUSSION

During the present study, 33 species were recorded from sampled sites and the dominant genera were *Ptycholaimellus* Cobb, 1920, *Viscosia* de Man, 1890, *Oncholaimus* Dujardin, 1845, *Sphaerolaimus* Bastian 1865, *Halalaimus* de Man, 1888, and *Metachromadora* Filipjev, 1918. In the sandy beach sediment samples as part of this study, *Oncholaimus* and *Viscosia* were found to be dominant genera and the reason for their dominance could be attributed to their large size (Fleeger et al. 2011) and feeding habit (omnivores/predators) (Wieser 1953). It has been shown in various studies that the family Oncholaimidae which includes genera such as *Oncholaimus* and *Viscosia* are usually dominant in sandy environments (e.g., Maria et al. 2013; Steif et al. 2013). Additionally, we found the family Oncholaimidae

Figure 2. Distinguishing morphological features of nematode species from the central west coast of India. **A**: *Viscosia abyssorum*. **B**: *Rhynchonema* sp. 1. **C**: *Microlaimus conothelis.* **D**: *Neochromadora poecilosomoides*. **E**: *Spilophorella candida.* **F**: *Sphaerolaimus islandicus*. Scale bars: A, B, C, E and F = 20 µm; D = 10 µm.

to be most species rich as part of this study. Although some free-living marine nematode species are common to muddy and sandy habitats, many of them are usually mud- or sand-preferring, and occur with different dominance in either sediment type (Heip et al. 1985; Semprucci et al. 2010). Some of the families (Comesomatidae, Linhomoeidae, and Sphaerolaimidae) and genera such as *Sabatieria* Rouville, 1903, *Sphaerolaimus* Bastian 1865, *Terschellingia* de Man, 1888, and *Daptonema* Cobb, 1920 are typically dominant in muddy sediments (Boucher 1972; Vitiello 1974; Travizi 2010), and confirmed in this study. Some of them, such as *Sabatieria* sp. and *Sphaerolaimus* sp., are known to inhabit stressed and anoxic sediments (Vincx et al. 1990; Vanreusel 1990; Gyedu-Ababio et al. 1999; Boyd et al. 2000; Mirto et al. 2002). The genus *Ptycholaimellus* was also encountered frequently in sandy and muddy sediments as part of this study with high abundance in particular in muddy sedimentary sites, indicating that food resources might be plentiful in the study area or that there is an anoxic sediment layer relatively close to the surface layers of the sediment. Recently, the presence of genus *Ptycholaimellus* in high abundance has been also confirmed in intertidal regions of central west coast of India based on 18S rRNA clone library and sequencing approach (Kumar et al. 2014). It is already well established in published literature that the genus *Ptycholaimellus* which is an epigrowth feeder, is present in surface layers of the sediment (e.g., Commito and Tita 2002) and its abundance has been correlated strongly with the depth of anoxic sediment layer (e.g., Eskin and Hopper 1985). According to previous observations (e.g., Platt and Warwick 1983, 1988), many of the species encountered in this study are typically found in intertidal sedimentary habitats.

Nanajkar and Ingole (2010) reported 94 species of free-living marine nematodes in subtidal environments located along the central west coast of India. Some of the species reported by them, e.g., *Anoplostoma* sp., *Daptonema* sp., *Sabatieria* sp., *Terschellingia longicaudata*, *Viscosia abyssorum*, *Sphaerolaimus* sp., and *Microlaimus* sp., were also encountered in this study across intertidal study

Table 3. Morphometric details of selected nematode species from central west coast of India (all measurements in µm; except ratios)

Characteristics	*Viscosia abyssorum*	*Rhynchonema* sp.	*Microlaimus conothelis*	*Neochromadora poecilosomoides*	*Spilophorella candida*	*Sphaerolaimus islandicus*
Total length	1986–2114	507–913	962–1233	565–956	918–1132	1185–1894
Cuticle	Smooth	Distinctly annulated	Transverse striations without lateral differentiation	Complex ornamentation with rows and dots	Complex cuticle with lateral differentiation and large dots	Transversely striated
Head diameter	28–32	4–6	11–15	8–12	9–12	12–16
Cephalic setae	6 long (4.1–4.7) and 4 short (2.7–3.2)	N.V.	6 short (5.2–5.5) + 4 long (7–7.2)	6 short (6–6.5) + 4 long (9.2–9.9)	4 (7–8)	4 longer (8–10)+ 6 shorter (4–6)
Buccal cavity	Large with one larger subventral tooth and two smaller dorsal teeth	N.V.	Large with one larger dorsal and two smaller subventral teeth	Small and narrow with one pointed dorsal tooth and two smaller subventral teeth	Small with hollow dorsal tooth	Large and covered by sclerotised buccal capsule
Amphids	Pocket–like	Circular	Circular	Transverse slit	Transverse slit	Circular
Amphid from anterior end	9–11	1.81–2.1	9–14	5–8	5–9	10–13
Amphid diameter	8–9	0.4–0.9	5–8	5–9	6–10	5–7
Pharynx length	386–412	45–59	121–154	111–134	186–212	287–313
m.b.d.	35–42	18–23	22–30	20–27	38–44	73–119
Vulva from anterior end	1059–1078	N.A.	452–641	N.A.	N.A.	616–1042
Vulva %	51–54	N.A.	47–52	N.A.	N.A.	52–55
Anus from anterior end	1929–2050	417–799	858–1110	475–774	856–1054	1028–1675
a.b.d.	31–39	16–21	20–27	19–23	21–29	38–49
Spicule length	31–34	24–29	32–39	24–29	37–44	42–51
Gubernaculum	Absent	12–14	23–27	20–23	16–20	6–14
Tail length	57 – 64	90–114	104–123	91–182	62–78	157–219
a	42–57	28–40	41–44	28–35	24–26	16–19
b	5–6	11–15	7.95–8	5–7	5.33–5.97	4–6
c	30–34	6–8	9–10	3–5	14.51–14.8	7–9
c′	10–12	5–6	4.5–5	5–8	2.68–2.95	4.13–4.47

(m.b.d. = maximum body diameter; a.b.d.=anal body diameter; a = total body length divided by maximum body diameter; b = total body length divided by pharyngeal length; c = total body length divided by tail length; c′ = tail length divided by anal body diameter; N.A. = not applicable; N.V. = not visible)

Notes
Morphologically nematode specimens are coming under three important ratios. These ratios are collectively called as De Man ratio. Those are a, b and c, while recently nematologist suggest that ratio of c′ also consider for the importance of nematode morphology measurement. The detail of De Man ratios and c′ are given below (see the footnote of table).
Abbreviation of a.b.d. is anal body diameter and we modified spicules to specule length.

sites. Some of the encountered genera from this study have been also reported in other geographical areas of the Indian Ocean region. For example, Semprucci et al. (2013) while studying meiofauna associated with coral sediments in the Maldivian subtidal habitats reported the presence of sediment dwelling nematode taxa such as *Richtersia*, *Ptycholaimellus* and *Molgolaimus* from the study sites. The authors also reported the presence of epifaunal nematode taxa belonging to Epsilonematidae and Draconematidae (Semprucci et al. 2013).

In a very recent paper, Semprucci et al. (2014) studied the nematode assemblages from a lagoon in Maldives of Indian Ocean region and found that the most abundant and richest families were represented by Desmodoridae, Chromadoridae and Xyalidae. In our study we also report the presence of several nematode genera belonging to these families. In a different study, Ansari et al. (2012) reported the presence of 192 species of marine nematodes represented by members belonging to families Xyalidae, Desmodoridae, Comesomatidae and Linhomoeidae from southeast continental shelf region of India (Bay of Bengal sector) in the Indian Ocean. Most common species encountered by them included *Viscosia* spp., *Halalaimus* spp. and *Sabatieria* spp. As part of the present study, many of these genera were encountered by us from sampled sites. Therefore, the similarities in nematode assemblages, in terms of dominant families and genera between geographically distant areas of Indian Ocean region indicate the existence of iso-communities as observed in other studies (Ansari et al. 2012; Semprucci et al. 2014). For example, Semprucci et al. (2013) have reported the presence of sediment dwelling taxa (e.g., *Richtersia*, *Ptycholaimellus* and *Molgolaimus)* in Maldivian subtidal habitats and in our study area (central west coast of India), we encountered some of these sediment dwelling taxa across several sites, although both the areas are geographically separated from each other within the Indian Ocean region.

Some of the sites selected as part of this study are known to harbor mangrove patches and many of the encountered nematode genera such as *Metachromadora*, *Viscosia* and *Sphaerolaimus* have been also reported extensively from other mangrove sedimentary environments in the Indian subcontinent (e.g., Ansari et al. 2014).

The results of this checklist of marine nematodes from intertidal regions of central west coast of India provide a framework for undertaking future studies on biogeography of free-living marine nematodes and comparative studies of their distribution trends with respect to temperate intertidal ecosystems. In addition, data provided in this checklist will can serve as a baseline which can be used in future to monitor natural and anthropogenic changes along the central west coast of India.

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