

# A CHECKLIST OF FREE-LIVING MARINE NEMATODES AT DIFFERENT ECOSYSTEM IN NORTHERN STRAITS OF MALACCA, MALAYSIA

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## ABSTRACT

Nematodes present in all substrates and sediment types with an enormous number of species. The studies on free-living marine nematodes are still limited in Malaysia. The present study provides general information on genus checklist of free-living marine nematodes in the northern part of Peninsular Malaysia in a different ecosystem which are intertidal zones of estuarine, sandy beach and mangrove ecosystems. Samples were randomly collected at three stations in each ecosystem. A total of 34 genera (22 families and ten suborders) were recorded in three ecosystems. Each ecosystem had common genera which also commonly found worldwide. *Viscosia* genus was observed in all three ecosystems. The nematode genera of Estuary and mangrove ecosystems had greater similarities than those in the sandy beach.

KEY WORDS: nematodes checklist, Peninsular Malaysia, sandy beach, estuarine, mangrove

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## INTRODUCTION

Nematodes are wormlike unsegmented invertebrate organisms live in terrestrial, marine, and freshwater ecosystems. The majority of nematode species are free-living in terrestrial soils and aquatic sediments, primarily occupy all soft sediment ecosystems and, even in lower abundances, biofilms on secondary and/or hard substrates (Moens *et al.*, 2013). Marine nematodes usually are of a meiofaunal size, which passes through a 500 µm sieve, but is retained on mesh sieve of 31–42 µm; only some are larger, such as some enoplids and oncholaimids (Tahseen, 2012).

Nematode abundances are higher in the upper centimeters of the sediment (Moens *et al.*, 2013). Its abundance and diversity differ among different ecosystems. Mudflat and sandy ecosystems have different assemblages with unique characteristics. Nematodes from sandy ecosystem are slenderer because they have to move through the interstitial gap while nematodes from mudflat ecosystem are more robust to burrow through the sediment (Tita *et al.*, 1999). In enriched estuarine, muddy sediment is characterized by a high density of nematodes (> 3,000 individuals per 10 cm<sup>2</sup>) (Heip *et al.*, 1985).

Studies on free-living marine nematodes in Malaysia are limited, particularly in regard to taxonomical studies (Rosli *et al.*, 2010). Only a few researchers have been concentrated on free-living marine nematodes in Malaysia (Chen *et al.* 2012a; Chen *et al.* 2012b; Rosli *et al.* 2013). The studies in Malaysia are mostly on the ecology and diversity of meiobenthos (Sasekumar, 1994; Somerfield *et al.* 1998; Ibrahim *et al.* 2006; Long, 2006).

Thus, the major aim of this study was to establish a checklist of free-living marine nematodes in different habitats which were a sandy beach, mangrove, and

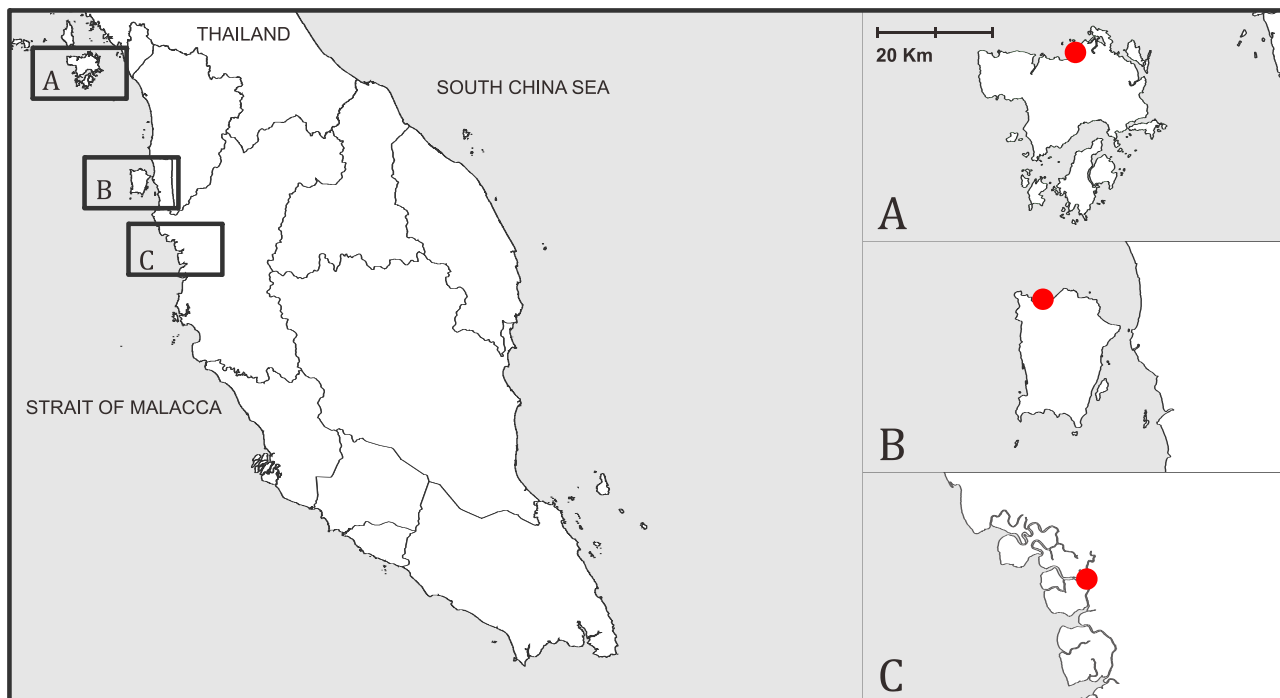
estuarine ecosystems in northern Straits of Malacca. This research can also be used as a baseline for future studies and provide an overview of the nematodes assemblage in different ecosystems along the northern Straits of Malacca.

## METHODS

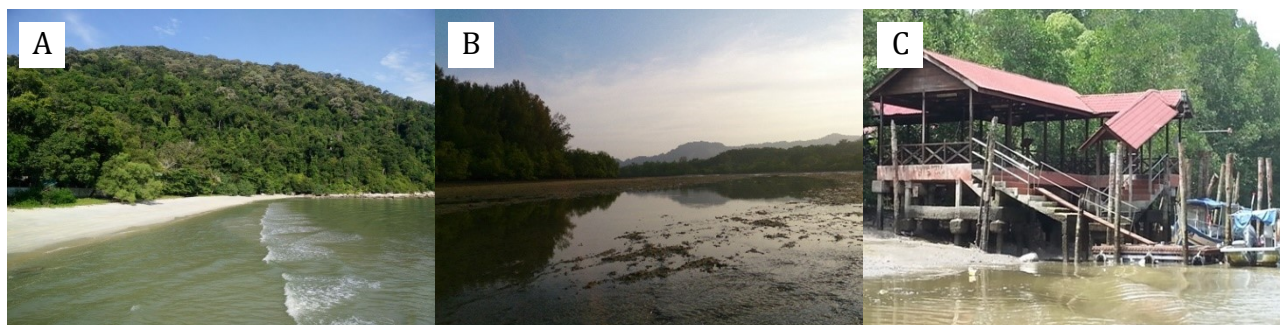
Tanjung Rhu is situated on the northeast coast of Pulau Langkawi, Kedah (about 6°27'3.10"N, 99.49°18.80"E) (Figure 1). It is an estuary area leading towards the open sea, and relatively undisturbed even though there are several developments within the region. Tanjung Rhu is suitable for this study due to its relatively pristine condition. There are few natural ecosystems in this study which include coral reefs, mudflats, mangrove, sandflats and an estuarine-riverine system. This study focused on estuarine-riverine ecosystems which consist of mudflat surrounded by mangroves and a sandy brackish zone whereby the estuarine water flows towards the open sea.

Teluk Aling is located at the northwest tip of Penang (Figure 1), at 5°28'03.7" N 100°11'59.3" E. Universiti Sains Malaysia (USM) research center, CEMACS (Centre for Marine and Coastal Studies) has been established in Teluk Aling since the middle of the 1970s. It consists of a small area of sand flat supported by a hill (average slope of 30°) of *Dipterocarpus* forest with no freshwater input except through seepage and surface runoff. The tidal cycle is semi-diurnal with extreme ranges of 0.2–2.9 m above chart datum (Ong & Krishnan, 1995). Litters and campfires from the research center can be seen along the Teluk Aling. The samples were taken along the sandy shores in front of CEMACS.

Kuala Sepetang is on the west coast of peninsular Malaysia in the state of Perak (Figure 1), and situated at 4°50'37.7" N 100°38'00.5" E. The mangroves are the dominant feature of the coastline, which is almost entirely flat for miles around. The Sepetang River brings down the main riverine discharge from upstream, and there were few fish cages located near the river mouth (Ghaderpour *et al.*, 2014).



**Figure 1.** The map of Peninsular Malaysia showing the sampling locations which were Pulau Langkawi, Kedah (A); Penang (B), and Perak (C). Map of Tanjung Rhu (estuary), Langkawi; Teluk Aling (sandy beach), Penang; and Kuala Sepetang (mangrove), Perak respectively



**Figure 2.** Photos of three sampling sites, (A) Teluk Aling (sandy beach); (B) Tanjung Rhu (estuarine); (C) Kuala Sepetang (mangrove)

It supports a variety of aquaculture products as the warm tropical weather, and healthy mangroves provide nursery grounds for marine organisms (Ghaderpour, 2013). The sampling was taken near the jetty of Kuala Sepetang.

Sampling was done in three different ecosystems which were sandy beach (Teluk Aling), mangrove (Kuala Sepetang) and estuarine (Tanjung Rhu) as shown in Figure 2, to determine the composition of marine nematodes in different ecosystems. Sediment samples ( $n = 4$ ) were collected at 5 cm depth from the surface by using PVC core tubes (50 mm height x 35 mm diameter) from three ecosystems. There were three sites in each ecosystem with three replicates in each site. The sediment collected was sieved through using two mesh sieves. The pore size of the top sieve was 450  $\mu\text{m}$ , and the pore of the bottom sieve was 32  $\mu\text{m}$ . The sample trapped in bottom sieve was transferred into 50 mL of the plastic jar. The samples were preserved in 70% alcohol and stained with 2.5 mL of Rose Bengal solution.

Extraction of free-living marine nematodes was done using the modified method of Heip *et al.* (1985) which was Ludox centrifugation technique. Samples (20 mL) were transferred into 50mL-centrifuge tubes and 20 mL of Ludox solution was added to each of the tubes. The mixture was

shaken to allow proper mixing of sample and Ludox solution. The sample in centrifuge tube was left for 10 minutes to let the sediment to settle. The supernatant containing nematodes was drained into a 32 $\mu\text{m}$ -sieve and washed. This step was repeated for three times to ensure nematodes were entirely extracted from the sediment. The nematodes were sorted by using stereomicroscope and kept in a 2mL-centrifuge tube that contained glycerol-ethanol (ratio 4:1) mixture with 1% of Rose Bengal before the microscope examination.

Permanent microscope slides were made using the method suggested by Ryss (2003). The slides were observed under the light microscope (Olympus BX41, Xcam Alpha, Image Processing software, version 5.1) 1000X magnifications. The photos were taken for the identification of nematodes (Figure 3). The nematodes were identified to the highest possible taxon using Warwick *et al.* (1998).

## RESULTS AND DISCUSSIONS

A total of 34 genera had been recorded and sorted into four Orders (Enoplida, Trefusiida, Chromadorida, and Monhysterida) in three ecosystems where estuary



ecosystem (Tanjung Rhu) composed of 16 genera, sandy beach ecosystem (Teluk Aling) was eight genera, and mangrove ecosystem (Kuala Sepetang) included 15 genera.

Eleven families of marine nematode from the suborder Chromadorina, Desmodorina, Ironina, Desmoscolecina, Oncholaimina, Monhysterina, and Ceramonematina were recorded in the estuary. A total of eight families under the order of Araeolaimida, Chromadorida, Desmodorida, Enoplida, Monhysterida, and Plectida had been identified in the sandy beach while 14 families from seven suborders (Chromadorina, Desmodorina, Enoplina, Ironina, Oncholaimina, Trefusiina, and Monhysterina) had been recorded in the mangrove (Table 1).

In an estuarine ecosystem with gravelly sand type sediment, *Paradesmodora* and *Spilophorella* were the dominant genera. While in sandy beach ecosystem with sand type sediment, *Microlaimus*, *Odontophora*, and *Onyx* dominated. In mangrove ecosystem with sandy mud type sediment, *Sphaerolaimus* and *Daptonema* were dominant (Figure 4). Furthermore, *Viscosia* has been observed in all three ecosystems. There were four genera (*Spilophorella*, *Daptonema*, *Parodontophora*, and *Viscosia*) presented in both estuary and mangrove, whereas only two genera (*Onyx* and *Viscosia*) lived in both sandy beach and estuary. There were no similar genera observed in both mangrove and sandy beach.

Some free-living marine nematode species are common to mudflat and sandy ecosystem and most of them are usually mud- or sand-preferring (Heip *et al.* 1985; Semprucci *et al.* 2010). *Microlaimus* and *Odontophora* were the dominant genera in sandy beach ecosystem. It has been reported in several studies that, in clean sand, genera that are commonly encountered are *Bathylaimus*, *Nudora*, *Odontophora*, *Paracanthochus*, *Paracyatholaimus* and *Microlaimus* (Lee & Riveros, 2011; Fonseca & Fehrlauer-Ale, 2012). The genus *Microlaimus* also dominate sandy beach according to studied by Liu *et al.* (2008) in China.

Families such as Comesomatidae, Linhomoeidae and Sphaerolaimidae and some genera (*Sabatiera*, *Sphaerolaimus*, *Terschellingia*, and *Daptonema*) usually dominate mudflat sediments (Travizi, 2010). A recent study by Bhadury *et al.* (2015) on nematode assemblages in the central west coast of India had similar findings. In this study, *Sphaerolaimus* and *Daptonema* had dominated mangrove habitat comprising of mudflat sediments.

In the present study, *Viscosia* was the only genus found in all three ecosystems as it was listed as one of the common genera to all environments (Venekey *et al.*, 2010). A study done by Long *et al.* (2010) in Sarawak, Malaysia on the distribution of nematodes in the intertidal area found that *Daptonema* genus was more abundant in mudflat sediment compared to sandy sediment. The *Daptonema* genus which was found in both estuary and mangrove ecosystems had dominated mangrove ecosystem that had a high

percentage (90%) of mud sediment. The presence of *Daptonema* genus in mudflat sediment was due to the type of food sources and its feeding type (Long *et al.*, 2010). Food sources such as bacteria or microalgae were abundant in mudflat sediment, and *Daptonema* which has been characterized as 1B (non-selective feeder) mostly consumed bacteria and microalgae.

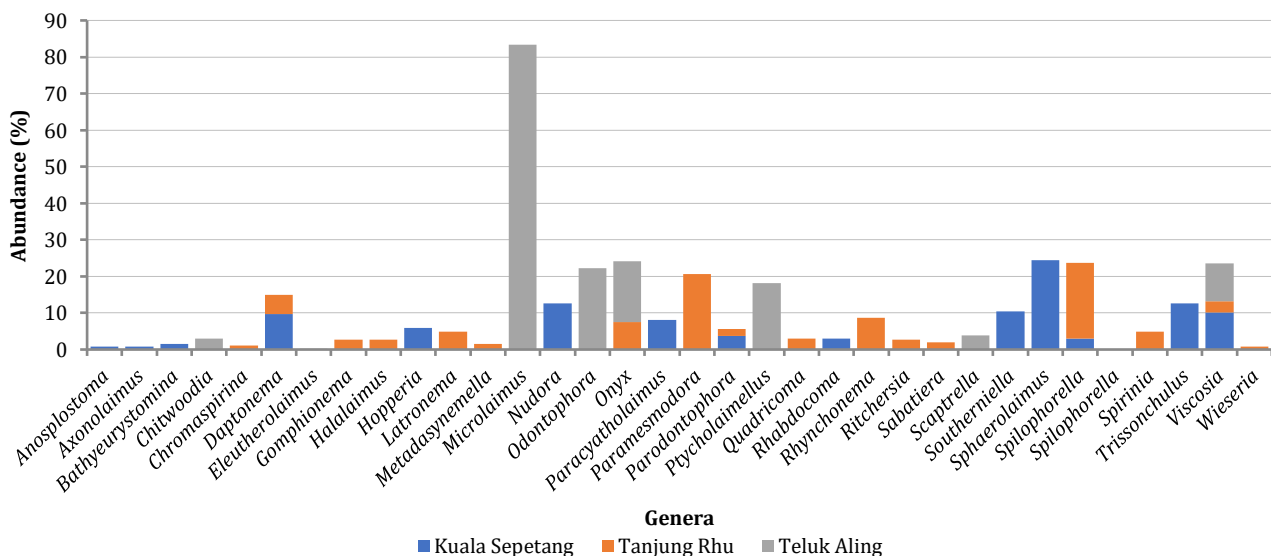


**Figure 3.** Photos of marine nematodes, (A) *Viscosia*; (B) *Spilophorella*; (C) *Onyx*; (D) *Sphaerolaimus*

**Table 1.** Order, suborder, family, and genus of free-living marine nematodes recorded in three different ecosystems

Order	Suborder	Family	Genus	Sandy Beach	Mangrove	Estuarine			
				TelukAling	Kuala Sepetang	Tanjung Rhu			
Araeolaimida		Axonolaimidae	<i>Axonolaimus</i> De Man, 1889	-	+	-			
			<i>Parodontophora</i> Timm, 1963	-	+	+			
		Comesomatidae	<i>Odontophora</i> Bütschli, 1874	+	-	-			
			<i>Hopperia</i> Vitiello, 1969	-	+	-			
Chromadorida	Chromadorina	Diplopeltidae	<i>Sabatiera</i> Rouville, 1903	-	-	+			
			<i>Southerniella</i> Allgén, 1932	-	+	-			
		Chromadoridae	<i>Gomphonema</i> Wieser & Hopper, 1996	-	-	+			
			<i>Latronema</i> Wieser, 1954	-	-	+			
			<i>Ritchersia</i> Steiner, 1916	-	-	+			
			<i>Spilophorella</i> Filipjev, 1917	-	+	+			
			<i>Ptycholaimellus</i> Cobb, 1920	+	-	-			
			<i>Paracytholaimus</i> Micoletzky, 1922	-	+	-			
Desmodorida	Desmodorina	Desmodoridae	<i>Chromaspirina</i> Filipjev, 1922	-	-	+			
			<i>Onyx</i> Cobb, 1891	+	-	+			
		Microaimidae	<i>Paradesmodora</i> Stekhoven, 1950	-	-	+			
			<i>Spirinia</i> Gerlach, 1963	-	-	+			
			<i>Microlaimus</i> De Man, 1880	+	-	-			
			<i>Nudora</i> Cobb, 1920	-	+	-			
			Desmoscolecida	Desmoscolecina	Desmoscolecidae	<i>Quadricoma</i> Filipjev, 1922	-	-	+
						<i>Anoplostoma</i> Bütschli, 1874	-	+	-
			Enoplida	Enoplina	Anoplostomatidae	<i>Trissonchulus</i> Cobb, 1920	-	+	-
						<i>Halalaimus</i> De Man, 1888	-	-	+
Monhysterida	Oncholaimina	Oncholaimidae	<i>Wieseria</i> Gerlach, 1956	-	+	-			
			<i>Viscosia</i> De Man, 1890	+	+	+			
	Trefusiina	Trefusiidae	<i>Bathyeurystomina</i> Lamshead & Platt, 1979	-	+	+			
			<i>Rhabdocoma</i> Cobb, 1920	-	+	-			
	Linhomoeina	Linhomoeidae	<i>Eleutherolaimus</i> Schulz, 1932	+	-	-			
			<i>Sphaerolaimus</i> Bastian, 1865	-	+	+			
	Monhysterina	Sphaerolaimidae	<i>Daptonema</i> Cobb, 1920	-	+	+			
			<i>Rhynchonema</i> Cobb, 1920	-	-	+			
			<i>Scaptrella</i> Cobb, 1917	+	-	-			
			<i>Metadasynemella</i> De Connick, 1942	-	-	+			
Plectida	Ceramonematina	Ceramonematidae	<i>Chitwoodia</i> Gerlach, 1965	+	-	-			
		Tubolaimoididae							
Total				8	15	16			

Notes: (+) present, (-) absent

**Figure 4.** The abundance (%) of genera in each ecosystem

A review by Heip *et al.* (1985) states that several nematode genera common in many estuarine ecosystems were *Adoncholaimus*, *Anoplostoma*, *Axonolaimus*, *Daptonema*, *Leptolaimus*, *Microlaimus*, *Monhysteria*, *Metachromadora*, *Ptycholaimellus*, *Sabatiera*, *Theristus*, *Tripyloides*, and *Viscosia*. In a different study, Quang *et al.* (2010) found other genera that were also present in high abundance in

the Mekong estuarine ecosystem, i.e., *Halalaimus*, *Rhynchonema*, *Parodontophora*, *Terschellingia*, *Desmodora*, *Onyx*, *Leptolaimoides*, *Oncholaimellus*, *Omicronema*, *Rhinema*, and *Halipectus*. However, in our study, *Spilophorella*, *Onyx*, *Rhynchonema*, *Viscosia*, and *Paradesmodora* were present in high abundance (Figure 2) in the estuarine ecosystem.

## CONCLUSION

A total of 34 genera of free-living marine nematodes had been recorded in three different ecosystems along the northern Straits of Malacca. This free-living marine nematodes checklist provides an outline for undertaking future studies on the biogeography of marine nematodes and studies of their relative distribution trends. This checklist is the baseline for future observation of anthropogenic changes in Peninsular Malaysia.

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