

Yong Huang · Yuqing Guo

Free-living Marine Nematodes from the East China Sea

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Foreword

Free-living marine nematodes are the most dominant and diverse meiofaunal group in marine benthic habitats. They have strong adaptability and wide distribution, and they play a very important role in the material circulation and energy flow of benthic ecosystem. The changes of their abundance and biodiversity have great significance in dynamic monitoring of the benthic environment. Nematodes have already been employed in biomonitoring studies and turned out to be suitable indicators for pollution-induced disturbances of benthic ecosystems. The species identification and taxonomy of free-living marine nematodes are the most basic research work. In recent years, we carried out taxonomical research on free-living marine nematodes from the East China Sea with the support of national natural science foundation of China. More than 300 species of nematodes have been identified. Among them, three new genera, 61 new species have been established. Two hundred and ninety-three species which belong to 2 classes, 7 orders, 33 families and 148 genera have been described in this publication and including 13 new species.

The present described species are the part of species list of free-living marine nematodes in China. We hope that this publication will provide basic data and a valuable source of information for students and scientists who are working in free-living marine nematodes, marine ecosystem management and protection.

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Part I
General Introduction

Chapter 1

Introduction to Marine Nematodes and the East China Sea



1.1 General Introduction to the Ecological Importance and Research Significance of Free-Living Marine Nematodes

Free-living marine nematodes represent the most widely distributed, abundant and diverse metazoan group. In general, they are the most advantage group in the ocean meiofauna; usually there are millions of nematodes in every square metre of the soft-soled sediment. They can occupy different trophic levels and play an important role in energy flow and material cycle in benthic ecosystem. The changes of their abundance and biodiversity have great significance in dynamic monitoring of the benthic environment. Nematodes have already been employed in biomonitoring studies and turned out to be suitable indicators for pollution-induced disturbances of benthic ecosystems.

The whole nematode phylum currently contains some 20,000 nominal species. About 7000 species are free-living marine nematodes (Appeltans et al. 2012). Some 500 species have so far been reported from the Chinese sea areas. The species identification and taxonomy of free-living marine nematodes are the most basic research work. The further research on marine nematode diversity inventory in China will provide scientific data for the study of benthic microfood ring, sustainable utilization of marine biological resources and marine environmental monitoring.

The ecological research on free-living marine nematodes in China began in the 1980s with the background of the joint investigation of sedimentary dynamics in the Yellow River Estuary, the Yangtze River Estuary and their adjacent waters. In recent years, NSFC has given strong support to the research of marine nematodes in the East China Sea, supported by the fund projects such as “classification and diversity of free-living marine nematodes in the East China Sea”, “taxonomic study on marine nematodes in mangrove wetlands in China” and “research on benthic diversity and environmental effect mechanism of mangrove wetland” and other fund projects. A group of Chinese nematode researchers conducted a comprehensive and in-depth

study on the classification and diversity of free-living marine nematodes in the East China Sea. The research scope mainly focused on the Yangtze River Estuary and its adjacent waters and the Taiwan Strait. The research contents mainly involved the abundance and biomass of meiofauna in the subtidal zone and intertidal zone such as mangrove wetland, mudflat and beach, as well as the community of marine nematodes. There are few reports on the deep water area of the continental shelf. More than 300 species of nematodes have been identified in the East China Sea, and most of them are the same as those in the Yellow Sea. Three new genera, 37 new species and 24 new mangrove wetland species have been established. The classification system and species list of free-living marine nematodes in China have been preliminarily established.

1.2 General Introduction to the East China Sea

The East China Sea is located in the east of the middle of China's coastline. It straddles the temperate zone and subtropical zone longitudinally. It is affected by the Asian continental high in winter. It is a marginal sea in the Western Pacific Ocean. The total area of the East China Sea is 77×10^4 km², with an average water depth of 370 m and a maximum depth of 2719 m. The northern boundary of the East China Sea is the line from Qidongzui to the southwest corner of Jeju Island. The northeast is connected with the Sea of Japan through the Korean Strait and the Taiwan Strait, and the dividing line is generally the connecting line between the eastern end of Jeju Island, the five-island archipelago and the Nomosaki Cape of Nagasaki Peninsula. The east border of the East China Sea is the connecting line of kyushu, ryukyu islands and Taiwan island, and adjoining to the Pacific. Its south border is Maobi tip at the southern Taiwan strait.

The continental shelf of the East China Sea is very wide, wide in the north and narrow in the south. It inclines gently from the mainland to the sea. It is one of the widest continental shelves in the world, with an area of two thirds of the East China Sea. There is a broad continental shelf in the west of the East China Sea and a deep-sea trough in the East, which has both shallow and deep-sea characteristics, but the characteristics of shallow sea are more obvious (Feng et al. 1999).

The East China Sea is the area with the largest number of rivers flowing into the sea in China. The influence of the Yangtze River on the East China Sea is extremely important. In addition, there are Qiantang River and Minjiang River. A large amount of sediment flows into the East China Sea and brings rich sediment. Three zones parallel to the coastline are formed from west to East: nearshore fine-grained sediment zone, intermediate coarse-grained sediment zone and offshore coarse-grained sediment zone. The distribution pattern of the median particle size distribution in the adjacent continental shelf area of the Yangtze River estuary is coarse in the east and fine in the west and coarse in the north and fine in the South (Yang et al. 2014). The grain size of the surface sediments in the southwest shelf of the East China Sea has become coarser from shore to sea, and there is an obvious boundary

between fine and coarse sediments. The main material source of the inland shelf deposition is provided by the Yangtze River, and the marine material mainly affects the outer shelf. The spatial distribution of heavy metals in marine surface sediments is mainly controlled by material sources and hydrodynamic conditions. In the north of the East China Sea, the spatial distribution of six kinds of heavy metal elements, Cu, Cd, Pb, Cr, Zn and Hg, in sediment is characterized by more nearshore and less far from the shore. The content of heavy metals in the sea area east of 123° E was obviously decreased. The spatial distribution pattern of heavy metals is also affected by the grain size and organic carbon content of sediments. The high-value area of heavy metals is consistent with the high-value area of organic carbon content and the fine-grained area, and the low-value area is consistent with the coarse-grained area (Yang and Jin 2019; Zhao 1983).

On the west coast of the East China Sea are the coasts of Fujian, Zhejiang and Taiwan of China. The coastline is tortuous, and there are many ports and bays. The largest bay is Hangzhou Bay. Most of the coastal types are eroded coast in the north and muddy coast in the bay. Mangrove coast appears in the south, mainly regular semidiurnal tide, and irregular semidiurnal tide near Zhoushan Archipelago.

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Chapter 2

Morphology of Free-Living Nematode



2.1 Structure

Free-living marine nematode is a kind of small invertebrate, which lives in marine benthic sediments. It is generally a small, nonsegmented, unisexual animal with thread-like body. The body consists of an external cylinder and an internal cylinder. The external cylinder is the body wall consisting externally of a cuticle layer and internally of a longitudinal muscle layer. The internal cylinder is the digestive system, which is differentiated into buccal cavity, muscular pharynx, intestine, a short rectum and anus. The internal cylinder is the digestive system, which is terminal at the anterior but subterminal posteriorly, that's why nematodes have a tail. The digestive system is differentiated into a buccal cavity, a muscular pharynx, an intestine and a short rectum. The structure between body wall and digestive system is a fluid-filled cavity called a pseudocoelom which contains a number of cells and other organs, such as the reproductive tract (Platt and Warwick 1988; Warwick et al. 1998; Decraemer et al. 2014).

2.2 Size and Shape

Free-living marine nematodes are usually around 1 mm long although some of the larger species such as those found in kelp holdfasts may be several millimetres in length. The smallest species so far recorded was a female of *Hapalonus minutus* (Steiner, 1916) Lorenzen, 1969, only 82 μm long, whereas the longest species was *Cycolaimus magnus* (Villot, 1875) de Man, 1889, up to 34 mm long.

Most of known free-living marine nematodes have long and narrow cylindrical body shapes, tapered towards both ends. However, in families Epsilonematidae and Draconematidae, the bodies are ϵ -shaped or S-shaped, respectively, with swollen pharyngeal and mid-body regions. In genus *Desmoscolex*, the body has a strongly

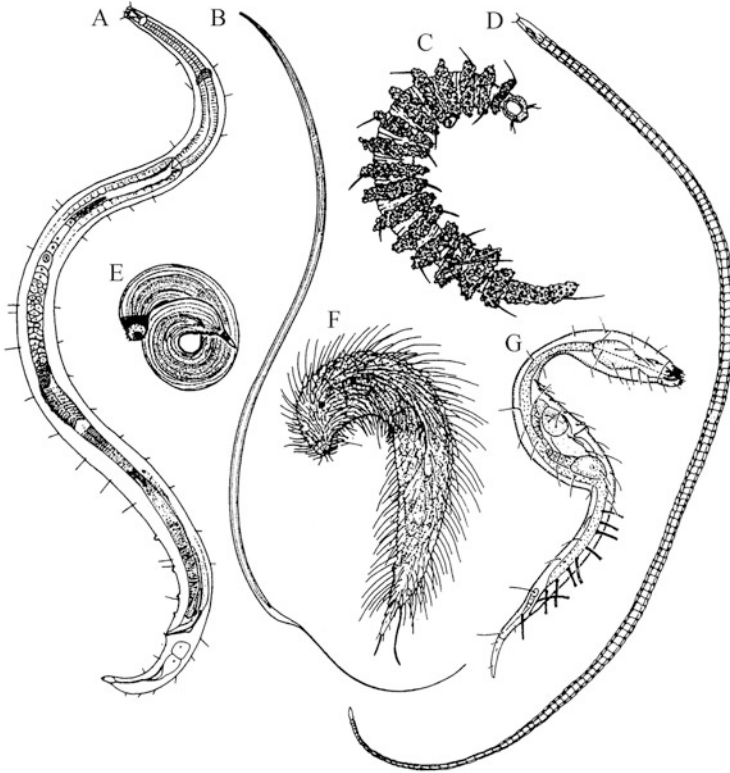


Fig. 2.1 Shapes of free-living marine nematodes (from Higgins and Thiel 1988). (a) *Echinotheristus*; (b) *Halalaimus*; (c) *Desmoscolex*; (d) *Pselionema*; (e) *Richtersta*; (f) *Greeffiella*; (g) *Dracograllus*

annulated appearance (Fig. 2.1). The body is bilaterally symmetrical, more or less circular in cross section (roundworms). The ventral side can be recognized by the position of the secretory-excretory pore, the cloaca in males or the vulva and anus in females (Decraemer et al. 2014).

2.3 Body Wall

The body wall of nematodes is composed of an external non-cellular cuticle, lined inside by the epidermis, and the longitudinal muscles. The cuticle may appear to be smooth or to be transversely annulated (striated cuticle). In many species, there may also be dots (punctations) which are arranged irregularly or in rows. These dots may be confined to the lateral parts of the cuticle only, which is called lateral differentiation. In some species, pore-like canals, alae can be observed through the cuticle.

2.4 Sense Organs

There are a number of sensory structures on the body, also called sensilla (sensillum) or sense organs which may take a variety of forms. Hair-like sensilla are called setae (seta), and nipple-like sensilla are called papillae (papilla). Sensilla on the body surface (somatic sensilla) may be arranged in definite longitudinal rows or irregularly. Sensilla on the tail may be longer or stouter than those on the rest of the body which are called caudal setae. Specialized sensilla at the tail tip are called terminal setae. The region between the head and the mid part of the pharynx is often called cervical region, and the setae here may be called cervical setae.

The anterior sensilla consist of two circles of six labial sensilla each, one circle of four cephalic sensilla and a pair of lateral amphideal fovea. The arrangement of the sensilla on the head is specialized and conforms to a basic pattern. The first circle of six inner labial sensilla (two lateral and four submedian) surround the mouth opening are usually papilliform, or only short setae. The second circle of six outer labial sensilla are usually setiform. The third circle of four (two subventral and two subdorsal) cephalic sensilla are normally setiform, that's why they are called cephalic setae. This basic arrangement of the anterior sensilla can be denoted by the formula $6 + 6 + 4$ (Fig. 2.2b). However, the second circle and the posterior circle setae may be situated at the same level (the same circle), this arrangement denoted by the formula $6 + 10$ (Fig. 2.2d). In some species, the outer labial sensilla may be papilliform, so that there appears to be only four setae (Fig. 2.2c). In several species, additional setae may be present associated with the basic ten cephalic setae. Several nematodes have paired pigment spots or ocelli situated laterally or dorsolaterally on the anterior part of the pharynx.

The amphideal fovea are the main sensilla of nematode which situate laterally on the head. An amphid consists of fovea, duct and fusus. The dendrites enter the amphid at the base of the fusus, which is formed by the amphidial gland. In subclass Enoplia and Dorylaimia, the fovea typically forms an invaginated pocket (Fig. 2.2a)

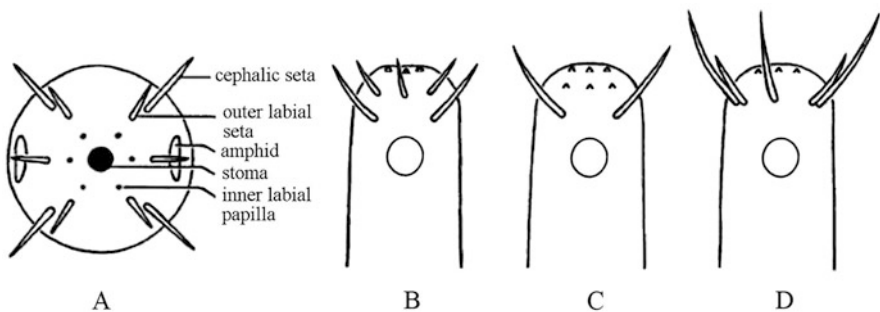


Fig. 2.2 Head sensilla. (a) Apical view showing typical distribution of head sensilla; (b) lateral view of head sensilla in the $6 + 6 + 4$ pattern; (c) lateral view of head sensilla pattern where only the third circle (cephalic seta) are setiform; (d) head sensilla in the $6 + 10$ pattern. (From Warwick et al. 1998)

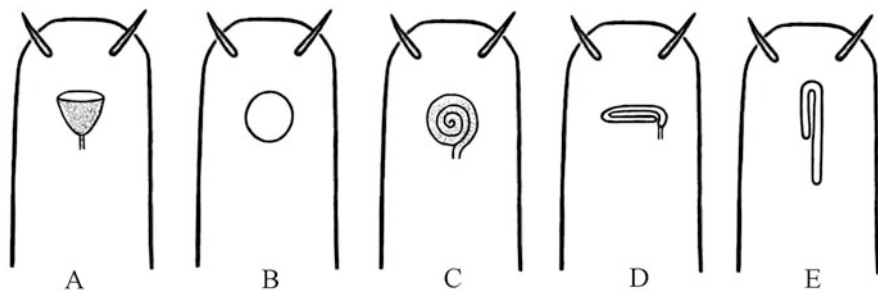


Fig. 2.3 Amphideal fovea. (a) Pocket type; (b) circular type; (c) multi-spiralled type; (d) transverse slit-type; (e) long loop (crook-like) type

where the external opening (aperture) is in the form of transverse slit leading to a cavity (fovea) filled with a gelatinous substance (corpus gelatum). In Chromadoria, the fovea can be circular (Fig. 2.3b), mono- or multispiral (normally turning ventrally although in a few cases may be dorsally wound) (Fig. 2.3c), transverse (Fig. 2.3d), oval, elongated, loop-shaped (crook-like), etc. (Fig. 2.3e). The fovea may also show sexual dimorphism, being larger and more complex in males.

2.5 Nervous System

Nervous system consists of nerve ring, labial papillary cords and ventral and dorsal cords. Nerve ring surrounds the anterior half, mid part or the posterior half of the pharynx. Six labial papillary cords extend anteriorly from the nerve ring. A large ventral, a smaller dorsal and two pair of sublateral cords extend posteriorly from the nerve ring. The ventral cord terminates in the preanal ganglia. Dorsal and sublateral cords terminate in the tail region.

2.6 Digestive System

The digestive system or alimentary canal forms the inner tube of nematode, which consists of three main sections: the buccal cavity (stomodeum), pharynx and intestine. The shape of buccal cavity can be conical, double conical, cylindrical, funnel-shaped, elongated cylindrical, barrel-shaped, several chambers and so on, which reflects the great range in feeding methods (Fig. 2.4a). The buccal cavity may be absent or minute. Some species have buccal cavities unarmed (Fig. 2.4b), but many species have buccal cavities armed with immovable projections of the wall, called teeth (Fig. 2.4c), or moveable structures termed mandibles (Fig. 2.4d). In addition there may be rows of small denticles, or there may be other projections, such as stomatostylet, spear-like structure.

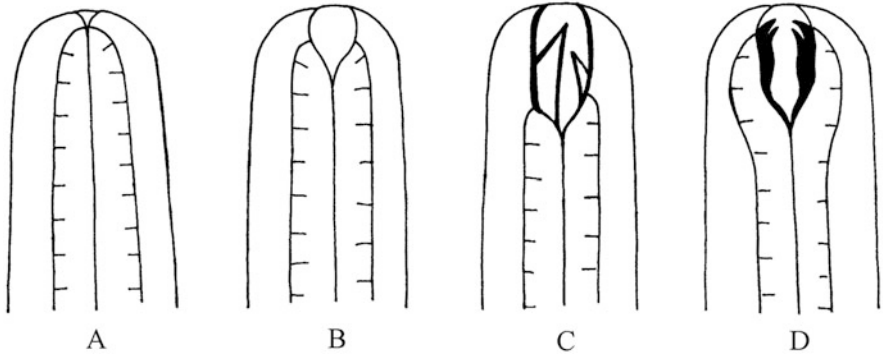


Fig. 2.4 Buccal cavities. (a) Minute form. (b) Unarmed form. (c) Form with fixed teeth. (d) Form with moveable mandibles. (From Warwick et al. 1998)

The buccal cavity connects the pharynx, which is the muscular anterior part of the alimentary canal responsible for pumping food into the intestine. The lumen of the pharynx is tri-radiate in cross section. The pharynx may be cylindrical throughout (Fig. 2.5a) or have posterior swollen or terminal bulb. Many species have only one bulb, but some species have two or many bulbs (Fig. 2.5b). There is a muscular pharyngo-intestinal junction (cardia) in many species, which is a short or elongated conical structure. Typically it is a one-way valve that delivers food to the intestine. The intestine itself is a straight tube formed of a single layer of cells. The rectum connects the intestine to the anus in females or cloaca in males.

The excretory system consists of a single renette cell, a duct extending anteriorly to ampulla or open and a ventral excretory pore somewhere in the pharyngeal region (Fig. 2.5a).

2.7 Reproductive System

Reproduction in free-living nematodes is sexual, involving males and females, cross-fertilizing, oviparity, or ovoviviparity in the rare species. The male reproductive system consists of testis, gonoduct (*vas deferens*), cloaca and the copulatory apparatus. There is typically two testes (diorchic) which may be opposed or in tandem, where only one testis (monorchic) is present after degeneration of the posterior. The testis is an epithelial tube with germ cells. The terminal part of gonoduct is differentiated into a strongly muscular ejaculatory duct and opens into the cloaca. There is a pair of cuticularized spicules and a guiding piece called a gubernaculum which lie in a sac opening into the dorsal side of the cloaca. The various kinds of precloacal supplements, such as papilliform, setiform, tubular or cup-shaped, are present or absent.

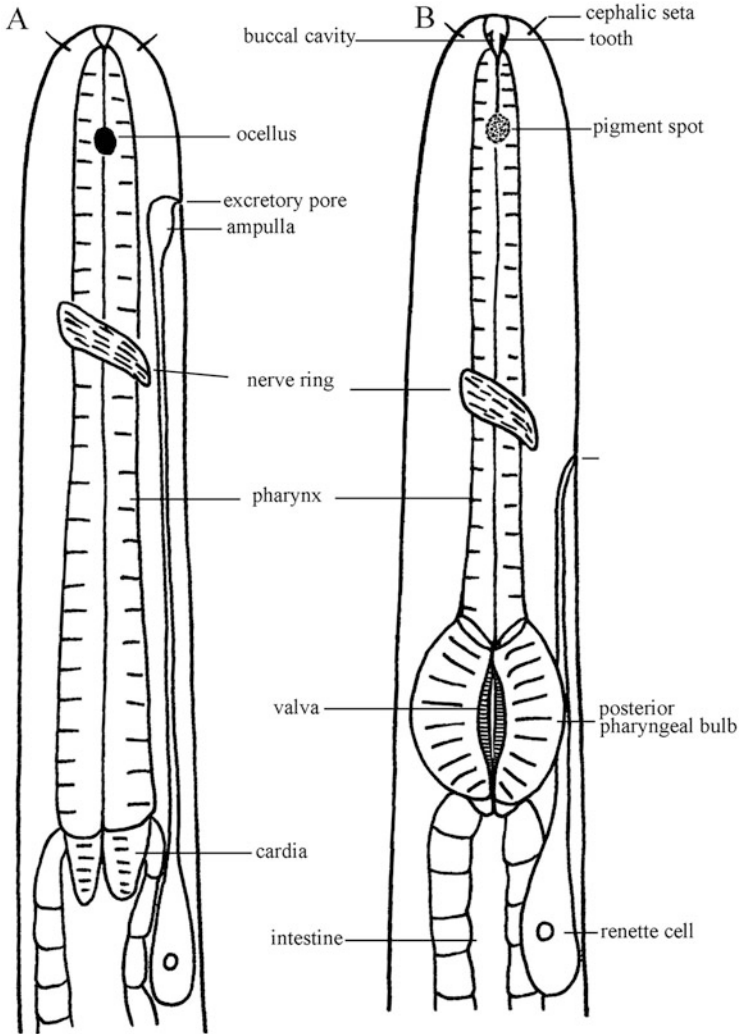


Fig. 2.5 Features of the anterior region. (a) Showing cylindrical pharynx, excretory system and an ocellus. (b) Showing buccal cavity, pharynx with a well-developed posterior bulb and a pigment spot. (From Warwick et al. 1998)

The female reproductive system is didelphic, amphidelphic or monodelphic. Each genital branch consists of a gonad (ovary) which outstretched or reflexed (Fig. 2.6a, b) and a gonoduct, with the uteri connected to a single vagina, which opens by a ventral pore (vulva), located often at mid-body length in didelphic nematodes but can be closer to the anus in monodelphic ones; its position from the anterior is given as a percentage of the total body length ($V\%$).

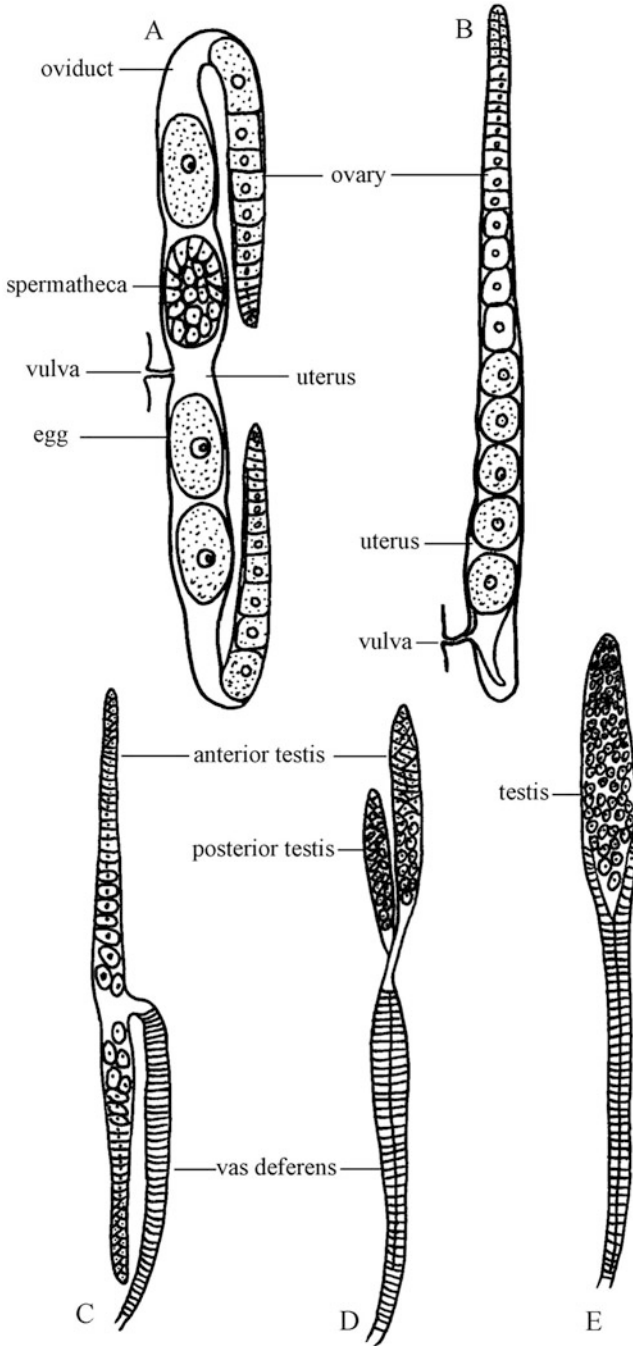


Fig. 2.6 Reproductive systems. (a) Female with two opposed, reflexed ovaries. (b) Female with a single anterior outstretched ovary. (c) Male with two opposed testes. (d) Male with two testes arranged in tandem. (e) Male with a single testis. (From Warwick et al. 1998)

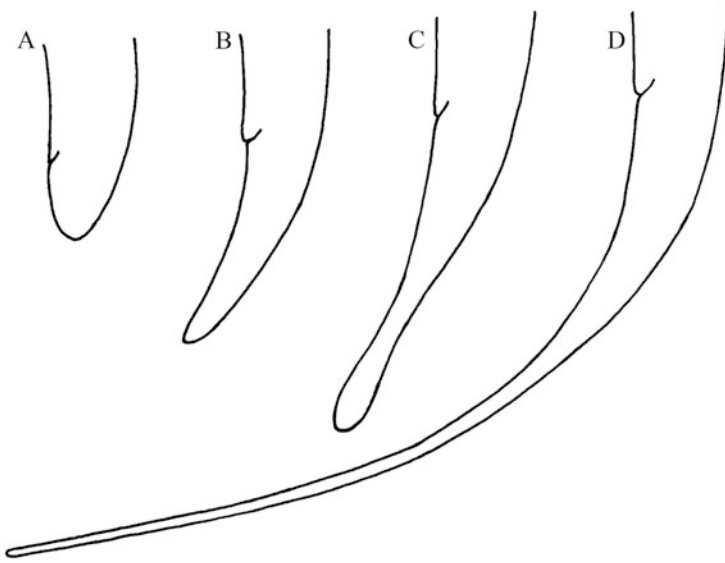


Fig. 2.7 Tail shapes. (a) Short and round; (b) conical; (c) conico-cylindrical with swollen tip (clavate); (d) elongated, filiform. (From Warwick et al. 1998)

2.8 Tail

The tail shape is various from short and broadly rounded to long filiform. The basic shape is usually conico-cylindrical (Fig. 2.7). The tail plays an important role in locomotion and may help in anchoring the body or as a tool during eclosion. The length of the tail is often quoted in terms of the anal (or cloacal) body diameter (a.b. d.). The tail usually contains three unicellular caudal glands, which may be confined entirely to the tail or extend anterior to the anus or cloaca in males. The caudal glands secrete adhesive which excrete via a specialized structure (called spinneret) in the tail tip. Certain species may lack caudal glands entirely. Many species have caudal setae and terminal setae in the tail tip.

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Chapter 3

Materials and Methods



3.1 Collection and Preservation

Intertidal sediment samples were collected in multiple locations along the coast of the East China Sea using a sawn-off syringe with a 2.6 cm inner diameter from 2010 to 2019. The samples were taken to a depth of 8 cm and divided into three sections (i.e. 0–2, 2–5 and 5–8 cm) and then fixed with 10% formalin in seawater for long-term preservation. Sublittoral sediment samples were obtained using a 0.1 m² improved Gray-O’Hara box during the Open Research Cruise of National Natural Science Foundation of China by the R/V Kexue 3 in 2012 and 2013 and Dongfanghong 2 in 2015 and 2017.

3.2 Extraction and Mounting

Before sorting, samples will be stained with 0.1% rose Bengal for 2–12 h and let the organic matter in the samples coloured. The stained samples were poured into two layers of sieves (500 and 31 µm mesh sizes, respectively) and washed with tap water to remove silt and separate macrofauna from meiofauna. Heavier sediment particles trapped in the 31 µm mesh are removed using centrifugation in Ludox-™ with a specific gravity of 1.15 g/ml (Jonge and Bouwman 1977). Each sample is washed into a lined Petri dish, respectively, with distilled water, and meiofauna is sorted under a stereoscopic microscope. Nematodes were transferred into a solution containing 5% glycerol, 5% pure ethanol and 90% freshwater by volume in a cavity block to be transparent and then stored in the drying oven letting ethanol and water slowly evaporate (McIntyre and Warwick 1984). After ethanol and water are evaporated for 1–2 weeks, the nematodes are mounted in glycerin on permanent slides.

3.3 Examination

The observation and descriptions are made from glycerin mounts using a differential interference contrast microscope. Line drawings of each species are made with the aid of a camera lucida. All measurements are obtained using measuring software equipped with microscope, and all curved structures are measured along the curved median line.

The voucher specimens have been deposited in the Meiofauna Laboratory of Liaocheng University, Shandong, China.

3.4 Description

Diagnostic characters of the genera have been given mainly with morphological characterization of the type species. The described characters of species are body size, shape, cuticle structure, head sensilla, amphid, buccal cavity, pharynx, cardia, secretory-excretory system, reproductive organs of male and female, tail and habitat. The drawings were made from described nematode specimens or from literatures of original descriptions of some species.

References

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Chapter 4

Systematics and Taxonomy



4.1 Phylogenetic Outline of Free-Living Marine Nematode

Classification and identification to species are mainly on the basis of the pictorial key proposed by Warwick et al. (1998) and a lot of literatures of the nematode taxonomists. Classification of free-living marine nematodes was made according to the systematic proposed by De Ley and Blaxter (2004), Schmidt-Rhaesa (2014) and Bezerra et al. (2020).

The phylum Nematoda consists of two classes, Chromadorea and Enoplea (De Ley and Blaxter 2004), and three subclasses, Chromadoria, Enoplia and Dorylaimia. Among these, some 662 genera, 86 families and 8 orders are free-living marine nematodes. The classificatory outline of free-living marine nematodes is listed in Table 4.1.

4.2 Species List from the East China Sea

Phylum Nematoda Genenbaur, 1859

Class **Chromadorea** Inglis, 1983

Subclass **Chromadorea** Pearse, 1942

Order **Araeolaimida** Filipjev, 1929

Superfamily **Axonolaimoidea** Filipjev, 1918

Family **Axonolaimidae** Filipjev, 1918

Genus **Ascolaimus** Ditlevsen, 1919

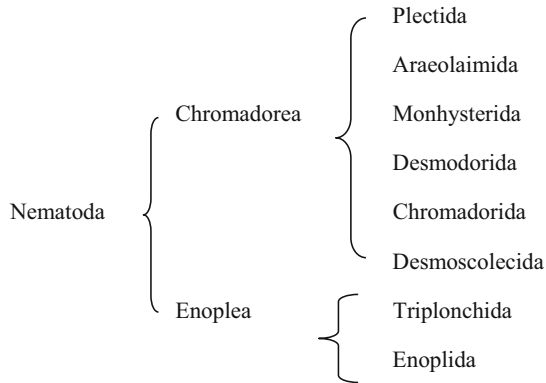
Ascolaimus elongatus (Bütschli, 1874)

Genus **Axonolaimus** de Man, 1889

Axonolaimus seticaudatus Platonova, 1971

Genus **Parodontophora** Timm, 1963

Parodontophora aequiramus Li & Guo, 2016

Table 4.1 Phylogenetic outline of free-living marine nematode (after Schmidt-Rhaesa 2014)

Parodontophora danka Belogurov & Kartavtseva, 1975

Parodontophora deltensis Zhang, 2005

Parodontophora huoshanensis Li & Guo, 2016

Parodontophora irregularis Li & Guo, 2016

Parodontophora longiamphidata Wang & Huang, 2016

Parodontophora marina Zhang, 1991

Parodontophora microseta Li & Guo, 2016

Parodontophora paramicroseta Li & Guo, 2016

Parodontophora wuleidaowanensis Zhang, 2005

Genus *Pseudolella* Cobb, 1920

Pseudolella donghaiensis Wang & Huang, 2016

Family *Comesomatidae* Filipjev, 1918

Subfamily *Comesomatinae* Filipjev, 1918

Genus *Paracomesoma* Schuurmans Stekhoven, 1950

Paracomesoma dubium Filipjev, 1918

Paracomesoma heterosetosum Zhang, 1991

Paracomesoma zhangii Huang & Huang, 2018

Paracomesoma xiamenense Zou, 2001

Subfamily *Dorylaimopsinae* De Coninck, 1965

Genus *Dorylaimopsis* Ditlevsen, 1918

Dorylaimopsis heteroapophysis Huang, Sun & Huang, 2018

Dorylaimopsis rabalaisi Zhang, 1991

Dorylaimopsis turneri Zhang, 1992

Dorylaimopsis papilla Guo et al., 2018

Genus *Hopperia* Vitiello, 1969

Hopperia hexadentata Hope & Zhang, 1995

Hopperia sinensis Guo, Chang & Chen, 2015

Hopperia macramphida Sun & Huang, 2018

- Genus *Metacomesoma* Wieser, 1954
Metacomesoma macramphida Huang & Huang, 2018
- Genus *Vasostoma* Wieser, 1954
Vasostoma brevispicula Huang & Wu, 2011
Vasostoma longispicula Huang & Wu, 2010
Vasostoma longicaudata Huang & Wu, 2011
Vasostoma spiratum Wieser, 1954
- Subfamily *Sabatieriinae* Filipjev, 1934
- Genus *Cervonema* Wieser, 1954
Cervonema donghaensis Hong, Tchesunov & Lee, 2016
Cervonema longispicula Huang, Sun & Huang, 2018
Cervonema tenuicauda Stekhoven, 1950
- Genus *Laimella* Cobb, 1920
Laimella annae Chen & Vincx, 2000
Laimella filipjevi Jensen, 1979
Laimella longicaudata Cobb, 1920
Laimella subterminata Chen & Vincx, 2000
- Genus *Minolaimus* Vitiello, 1970
Minolaimus multisupplementatus Sun, Huang & Huang, 2020
Minolaimus proximamphidum Sun, Huang & Huang, 2020
- Genus *Sabatieria* Rouville, 1903
Sabatieria alata Warwick, 1973
Sabatieria celtica Southern, 1914
Sabatieria conicoseta Guo et al., 2018
Sabatieria minuta sp. nov.
Sabatieria paradoxa Wieser & Hopper, 1967
Sabatieria pisinna Vitiello, 1970
Sabatieria praedatrix de Man, 1907
Sabatieria pulchra Schneider, 1906
Sabatieria punctata Kreis, 1924
Sabatieria stenocephalus Huang & Zhang, 2006
- Genus *Setosabatieria* Platt, 1985
Setosabatieria coomansi Huang & Zhang, 2006
Setosabatieria longiapophysys Guo, Huang, Chen, Wang & Lin, 2015
- Family *Diplopeltidae* Filipjev, 1918
- Subfamily *Diplopeltinae* Filipjev, 1918
- Genus *Araeolaimus* de Man, 1888
Araeolaimus elegans de Man, 1888
- Genus *Campylaimus* Cobb, 1920
Campylaimus gerlachi Timm, 1961
Campylaimus orientalis Fadeeva, Mordukhovich & Zograf, 2016