

DISTRIBUTION AND CLASSIFICATION OF FREE- LIVING MARINE NEMATODES OF PAKISTAN

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ABSTRACT

In Pakistan very little work has been carried out on the free-living marine nematodes of Arabian Sea. During recent marine nematode studies one new genus 13 new and 40 known species were collected and have been reported and described. A list of nematodes with their distribution and classification along the coastline of Pakistan is given.

Key-words: Free living marine nematodes, nematode distribution, classification, Pakistan

INTRODUCTION

Nematodes are the most abundant metazoans in marine sediments, extending from the high water mark into the deepest oceanic trenches (Nicholas 1975). The whole phylum Nematoda currently contain some 20,000 nominal species. About 4,000 species are free-living marine nematodes belonging to some 500 genera. Little is known about the diversity of free-living marine nematodes in Pakistan. Early studies were performed by Timm in 1962 and 1963 from Arabian sea. Since than work was not carried out in Pakistan on the free-living marine nematodes of this area.

The Arabian Sea lies in the north-western section of the Indian Ocean. The Indian Ocean is a vast body of salt water that covers about 75 mil / km² three quarters of the earths surface. Pakistan has a coast line of 527 nautical miles (nm) extending from Sir Creek on the Indian site to Gwatar bay on the Iranian border. A number of new and known species of free-living marine nematodes were reported by Maqbool and Nasira, 1998, 1999, 2000; Maqbool *et al.*, 1999; Nasira *et al.*, 2000; Turpeenniemi *et al.*, 2001; Maqbool and Shahina, 2001; and Nasira & Turpeenniemi, 2002, 2003 from Arabian Sea of Pakistan.

MATERIALS AND METHODS

Collection:

Extensive surveys have been made and several samples were collected by using a cylindrical core sampler from a variety of sources such as surface water, from mangroves, from wild grass, from stagnant water, and from the sediments of intertidal, subtidal and shallow water from the polluted and nonpolluted environments along the coastline of Sindh and Balochistan (Sandspit, Hawksbay, Paradise Point, Clifton, Korangi creek, Ibrahim Haidery, Mubarak Village, Sonmiani and Gidani beaches.)

Preservation:

Samples were bring to laboratory and fixed in 4% formaline in sea water and stained with Rose Bengal. Nematodes were extracted from the sediments by repeated decantation and sieving method.

Identification:

For the morphological studies and identification of free-living marine nematodes in this presentation is based on the systematics provided by Lorenzen 1981, and Tarjan, 1980.

Illustrations:

Illustrations were prepared by using camera lucida attached to a compound microscope.

RESULTS AND DISSCUSSION

SYSTEMATIC ACCOUNT

ORDER ENOPLIDA

Family Thoracostomopsidae:

Only one genus and one new species *Enoplolaimus karachiensis* Maqbool, Nasira & Turpeenniemi, 1999 was reported of the family from Pakistan. Specimens were found in sandy sediments in shallow water from Sandspit beach and Mubarak village. The genus *Enoplolaimus* De Man, 1893 have mandibles which appear as two lateral rods united by an anterior curved bar, the cephalic setae arise from near the base of the cephalic capsule, and the spicules are never very elongate. Useful characters distinguishing the species are the relative length of setae on the head, the number and arrangement of cervical setae in the male, the shape of the tail, the form of the spicules and the position of the precloacal supplement.

Family Ironidae:

The family was represented by one new and one known species of the genus *Trissonchulus* Cobb, 1920 Six specimens of *Trissonchulus lichenii* Nasira & Turpeenniemi, 2002 were collected from lichen at Paradise point and Hawksbay while the male of *Trissonchulus benepapillosus* Schulz, 1935 was collected from Sandspit beach and reported by Nasira and Turpeenniemi (2002). *Trissonchulus* species have four teeth in the buccal cavity, with dorsal pair fused together to varying degrees, often appearing completely separate in the replacement teeth of the juveniles. They have ten cephalic papillae and relatively short tails.

Family Oxystominae:

Two genera with one new and one known species of the family viz., *Halalaimus gidanensis* Nasira & Turpeenniemi, 2002 and *Oxystomina elongata* Butschli, 1874 were collected from Gidani and Sandspit beach respectively. The characteristic feature of the genus *Halalaimus* De Man, 1888 is the long slit-like amphid and the strongly attenuated anterior end. The cuticle of some species can be seen to be faintly striated.

Oxystomina Filipjev, 1921 species have a characteristic oval-shaped amphid, with a curved structure round the anterior margin extending backwards as two projections. There is no buccal cavity. Typically there are six cephalic setae and four cervical setae. Prominent oval cells are usually present, scattered throughout the body cavity. The excretory pore is usually conspicuous and strengthened by a cuticular thickening. The tail is clavate. Species are separated mainly on the size and position of the amphids and the cephalic and cervical setae. *O. elongata* was reported by Maqbool *et al.* (1999).

Family Oncholaimidae:

This family was found to be represented by two genera viz; *Oncholaimus* Dujardin, 1845 and *Viscosia* De Man, 1890. The combination of large left ventrolateral tooth, single ovary and short spicules with no gubernaculum serves to distinguish *Oncholaimus* from other oncholaimid genera. The genus *Oncholaimus oxyuris* Ditlevsen, 1911 obtained from Sandspit beach at Karachi was reported by Maqbool *et al.* (1999).

Ten specimens of the genus *Viscosia elegans* Kreis, 1924 were collected from Hawksbay and Paradise Point and reported by Nasira and Turpeenniemi (2002) as new record of Pakistan. The genus *Viscosia* is distinguished from other oncholaimids by a combination of three subequal teeth in the buccal cavity with the right subventral longer than the others, short spicules, no gubernaculum, male with no copulatory bursa and female with paired ovaries. It is a large and rather difficult genus, the species being separated on the length of the cephalic setae or papillae, the size of the amphids, the form of the dorsal and left subventral length in the buccal cavity, the length of the tail and the male copulatory apparatus. These two genera of the family are reported for the first time from Pakistan.

Family Anoplostomatidae:

The species of the genus *Anoplostoma* Butschli, 1874 were collected from Sandspit beach and are characterized by the large cylindrical buccal cavity which is not surrounded by oesophageal tissue and the presence of a copulatory bursa in the male. The species are distinguished primarily on the length of the cephalic setae, position of the amphids and length of the spicules. *Anoplostoma sunderbanae* Timm, 1967 was reported by Nasira and Turpeenniemi (2002) as a new record of Pakistan.

Family Enchelidiidae:

The only genus *Eurystomina* Filipjev, 1921 of this family was reported from Pakistan by Maqbool & Nasira, 2000. Specimens of the genus *Eurystomina* spp. were collected from Paradise point. *Eurystomina* is the most well known genus of the Enchelidiidae, containing forms with rows of buccal denticles, cuticularised supplements and functional caudal glands.

Family Anticomidae:

Anticoma species have a small conical buccal cavity, characteristic rows of lateral cervical setae, and a tubular precloacal supplement in the male. There are two main groups of species; those with the excretory pore anterior to

or at level with the cervical setae and those with the excretory pore posterior to the cervical setae. Species within these two main groupings are distinguished on rather fine points such as the exact position of the excretory pore, shape of tail, length of cephalic setae, and male copulatory apparatus. The only genus *Anticoma* Bastian, 1865 of the family was reported from Pakistan by Maqbool & Nasira, 2000 . Specimens of the genus *Anticoma* spp. were collected from Hawksbay beach.

SUB-ORDER TRIPYLOIDINA

Family Tripyloididae:

The only genus of the family Tripyloididae is *Bathylaimus* Cobb, 1894 and is distinguished by deeply incised lips and the relatively large buccal cavity. The structure of the spicules and gubernaculum is rather uniform throughout the genus. Characters used to separate the species are the length and form of the cephalic setae, the size and position of the amphids and the shape of the tail. All species have jointed setae. The buccal cavity varies considerably in appearance depending on the angle from which is viewed; this is particularly true of the tooth-like structures in the posterior section. The species of the genus *Bathylaimus australis* Cobb, 1894 was collected from Sandspit beach, Karachi as a new record from Pakistan by Maqbool *et al.* (1999).

ORDER TREFUSIIDA

Family Trefusiidae:

Five specimens of *Trefusia* spp. were collected from Sandspit beach. No other record of any other member of this family from Pakistan is so far available. *Trefusia* De Man 1893 species have jointed cephalic setae, no buccal cavity and two opposed ovaries. Species are distinguished on the relative lengths and position of the head setae, the length of the spicules, the form of the amphids and the length of the tail. It was reported by Maqbool and Nasira (2000).

ORDER MONHYSTERIDA

Family Xyalidae

Very common almost collected from all localities and represented by 8 genera which include one new genus and two new species. Xyalidae typically have the following characters: transversely striated cuticle; ten cephalic setae in a single circle, six always equal to or longer than the other four, often with additional cephalic setae; anterior ovary or testis to the left of the intestine, posterior testis to the right; buccal cavity conical, usually without teeth.

Specimens of the new genus and new species *Arabanema pakistanensis* Turpeenniemi, Nasira & Maqbool, 2001 were collected from Paradise point and Sandspit beach and is characterized by small and more weakly developed equal size spicules and tubular gubernaculum without apophyses.

The new species of the genus *Gonionchus arabica* Nasira & Turpeenniemi, 2003 was collected from Sandspit beach and have anteriorly extended hyaline lips with the labial sensilla appearing as pointed extensions of them, cuticle have longitudinal ridges; buccal cavity spacious, and have two subventral longitudinal elevations; tail long and conical, without terminal setae. The new species is characterized by its smaller body size.

Genus *Theristus* Bastian, 1865 have conical tail, without terminal setae. Two species *T. flevensis* Stekhoven, 1935 and *T. otoplanobius* Gerlach, 1951 are new records from Pakistan and were reported by Nasira & Turpeenniemi, 2003. These two species were collected from Gidani and Sandspit beach respectively.

The species of the genus *Cobbia* De Man, 1907 were collected from Sandspit beach Karachi. *Cobbia* spp. have buccal cavity with three teeth; setiform labial sensilla and filiform tail and reported by Maqbool & Nasira, 2000.

Daptonema Cobb, 1920 species reported by Maqbool & Nasira, 2000 have 10-14 cephalic setae in six groups, unarmed simply conical buccal cavity; conicocylindrical tail with terminal setae; somatic setae not much longer than one corresponding diameter, spicules short, less than 2 anal body diameter. Specimens of *Deptonema* spp. were collected from Sonmiani beach.

Genus *Trichotheristus* Weiser, 1956 is characterized by having very long somatic setae about 4-5 corresponding body diameter. The specimens of *Trichotheristus* spp. were collected from Hawksbay and was reported by Maqbool and Nasira (2000).

Genus *Paramonohystera* Steiner 1916 have elongate spicules more than 2 anal body diameter, slender. The genus was collected from Sonmiani beach and was reported by Maqbool Nasira (2000).

Family Axonolaimidae:

There is only one new species of this family so far has been reported *Odontophora hawksbiensis* by Turpeenniemi, Nasira & Maqbool, 2001. *Odontophora* Butschli, 1874 species have odontia present in the anterior part of the buccal cavity. Ovoid cuticularisations alternate with the tips of the odontia, and more posteriorly a circle of kidney-shaped, often denticulated, plates are found in similar positions. Oesophagus without posterior bulb. Tail conical.

Family Sphaerolaimidae:

Sphaerolaimus gracilis de Man, 1876 was collected from Sandspit beach, reported by Maqbool, Nasira, & Turpeenniemi, 1999 as a new record from Pakistan. *Sphaerolaimus* Bastian, 1865 species have; buccal cavity surrounded by a solid, heavily sclerotised buccal capsule; inner lining of oesophagus strongly cuticularised; groups of subcephalic setae absent in first two juvenile instars, with 4-6 groups in the third stage, and eight in the fourth stage juvenile and adult and have two testes.

Family Linhomoeidae:

Eleutherolaimus inglisi, Timm, 1967 was reported for the first time from Pakistan by Maqbool, Nasira, & Turpeenniemi, 1999. Species have buccal cavity with cylindrical walls and 4+4 cephalic setae.

Species of the genus *Terschellingia longicaudata* Timm, 1967 was collected from Sonmiani beach by Maqbool & Nasira, 2000. *Terschellingia* De Man 1888 species typically have: buccal cavity minute or absent, only four setose cephalic sensilla, four subcephalic setae, amphid usually positioned relatively far forward on the head; tail conico-cylindrical.

ORDER CHROMADORA

Family Desmodoridae:

Desmodora (Pseudochromadora) cliftensis Turpeenniemi, Nasira & Maqbool, 2001 described this species from Clifton beach. *Desmodora* De Man, 1889 species has: unstriated head region of thickened cuticle set off as a conspicuous cephalic capsule; amphids a single loop, lie entirely on cephalic capsule which is wider than long with the amphids occupying most of its length, tubular precloacal supplements; terminal portion of tail smooth, without punctuations.

Family Chromadoridae:

Family Chromadoridae is represented by four genera *Ptycholaimellus* Cobb, 1920; *Chromadora* Bastian, 1865 *Hypodontolaimus* De Man, 1886 and *Spilophorella* Filipjev, 1917. The new species of the genus *Ptycholaimellus* has an elongated double posterior oesophageal bulb, homogeneous cuticular ornamentation with lateral differentiation of two longitudinal files of dots. The shape of the hollow dorsal tooth is large S-shaped and has a dorsal apophysis. New species *Ptycholaimellus sindhicus* described by Turpeenniemi, Nasira & Maqbool, 2001 from Sandspit beach.

The species of the genus *Chromadora nudicapitata* Bastian, 1865 have transverse slit-like amphids. The lateral cuticle pattern has four longitudinal rows of dots the first punctuation of the transverse rows is frequently enlarged. The two main lateral files of dots are quite separate from the remainder, and are linked by transverse bars. The genus was collected from Paradise Point and reported by Turpeenniemi *et al.* (2001)

Endeolophus minutus Gerlach, 1967 is redescribed by Turpeenniemi, Nasira & Maqbool, 2001, collected from Mubarak village. Elongate chromadorids with 6+4 cephalic setae. Cuticle is homogenous behind head. Buccal cavity sclerotized. Dorsal tooth is more pointed than sub-ventral teeth which are almost as large as dorsal tooth. Proximal end of the gubernaculum with slightly posteriorly pointed. Lateral field extending near tail end.

Hypodontolaimus spp., collected from Sonmiani beach, have homogeneous cuticular ornamentation with lateral differentiation of two longitudinal files of dots. The hollow dorsal tooth is large S-shaped and has a dorsal apophysis. This species was reported by Maqbool & Nasira, 2000.

Genus *Spilophorella* has complex heterogeneous cuticle with lateral differentiation; deep buccal cavity containing long hollow dorsal tooth; elongated double posterior oesophageal bulb perhaps the most distinctive feature, a long pointed unstriated spinneret were set-off from the rest of the tail. *Spilophorella* spp. was collected from Hawksbay beach and reported by Maqbool and Nasira (2000).

Family Microlaimidae:

Two new and one known species of the genus *Microlaimus* have been described and redescribed. The species described is *Microlaimus sonmianensis* Nasira *et al.*, 2000 from Sonmiani beach while another new species *M.*

amphidius n.sp. collected from Sandspit beach. *M. arenicola* Schulz, 1938 was redescribed and collected from Sonmiani beach, reported by Nasira *et al.*, 2000. The main features of importance for separating the species are: degree of development of the buccal cavity; relative lengths of the cephalic setae structure, position and size of the amphids; and form of the copulatory structures.

Family Cyatholaimidae:

Two new and one known species of two genera belongs to the family Cyatholaimidae. *Maryllynnia musharafii* n.sp. collected from Sandspit beach belongs to the genus *Maryllynnia* Hopper, 1977 which has: cuticle with transverse rows of dots; lateral differentiation of larger, more widely spaced dots, i.e. about half as many rows laterally as medially; cuticle pores of two types, simple rounded and longitudinally oval, the latter situated between two dots and referred to as lateral modified punctuations (LMPs) and extending onto the conical part of the tail; buccal cavity with prominent dorsal tooth and two pairs of subventral teeth; gubernaculum expended slightly distally with several small teeth, paired; precloacal supplements cup-shaped, not cuticularised; tail conico-cylindrical.

Genus *Paracanthochus* Micoletzky, 1924 has: cuticle with transverse rows of fine dots which may be slightly larger in the lateral fields and in some species may be irregularly arranged; gubernaculum distally expended and dentate, proximally paired; tubular precloacal supplements. *Paracanthochus sandspitensis* n.sp. is collected from Sandspit beach. *Paracanthochus hawaiiensis* Allgen, 1951 is reported for the first time from Pakistan by Turpeenniemi *et al.* (2001) from Paradise point beach.

Family Selachinematidae:

This family is found to be represented by two genera *Halichoanolaimus* and *Choanolaimus*. One new species *Halichoanolaimus balochiensis* described by Turpeenniemi, Nasira & Maqbool, 2001 was collected from Mubarak village and has setose precloacal supplements, 'teeth' between the two sections of the buccal cavity appear as rows of squarish blocks; tail always elongated with a distal cylindrical section.

In the genus *Choanolaimus* the buccal cavity is divided into an anterior cup-shaped section supported by strongly developed buccal rugae and a narrower posterior tubular section supported by longitudinal cuticular rods. 'Teeth' of various kinds are present between these two sections of the buccal cavity. Species in this group are thought to be predators, particularly on other nematodes. *Choanolaimus* is distinguished from other genera in this group in that: none of the cephalic sensilla are setose; cuticle is laterally differentiated with larger more wider spaced punctuations; precloacal supplements are papillae pierced by fine pores. *Choanolaimus* spp. is collected from Mubarak village.

Family Comesomatidae:

The family is represented by two genera *Paracomesoma* Hope & Murphy, 1972 and *Sabatieria* Rouville, 1903. Genus *Paracomesoma* has conical buccal cavity with three small pointed triangular teeth at the anterior end; cephalic setae in two circles; cuticle with fine transverse rows of punctuations, fine transverse striations or unmarked, without lateral differentiation; spicule long and slender, unjointed; gubernaculum small without apophyses. Species of the genus *Paracomesoma longispiculum* Timm, 1961 was reported by Turpeenniemi *et al.* (2001) and collected from Sandspit beach.

Sabatieria microsetosa, Timm, 1967 has punctated cuticle without longitudinal lateral files of dots; cephalic setae in two separate circles posterior ones longer than anterior; amphids immediately posterior to the posterior cephalic setae; buccal cavity cup-shaped without teeth; Spicules short. *Sabatieria* species are typical in habitants of muddy intertidal and subtidal sediments, and are often the dominant members of the nematode fauna in such habitats, collected from Clifton and Sandspit beaches.

Family Ethmolaimidae:

The species of the genus *Paraethmolaimus appendixocaudatus* Jensen, 1994 was collected from Sandspit beach and reported by Maqbool & Nasia, 1999 as a new record from Pakistan. The family have annulated cuticle with transverse rows of punctuations, amphids spiral, buccal cavity with vestibule divided into twelve parts; dorsal tooth in buccal cavity; oesophagus with muscular posterior bulb; ventral precloacal spine; conspicuous cup-shaped precloacal supplements. Each with an external articulated flange; to opposed out stretched testes normally lying on opposites of gut, the posterior one usually being smaller than the anterior; to opposed, reflexed, equally sized ovaries; vulva at about mid point body of length, tail conical, usually with a rounded tip.

Family Monoposthidae:

The only genus *Monoposthia* spp., collected from Mubarak village was reported by Maqbool & Nasira, 2000. Monoposthiids have the following characters: strongly striated cuticle with longitudinal ornamentation usually in the form of several files of V-shaped markings; Labial sensilla short ; 6+4 cephalic sensilla in two separate circles, the posterior four always longer; amphid circular; buccal cavity with strongly developed dorsal tooth; tail conical; testis paired ; opposed single anterior reflexed ovary. *Monoposthia* species lack spicules and the major copulatory structure is the large unpaired heavily cuticularised gubernaculum.

Family Leptolaimidae:

This family is represented by three genera *Leptolaimus*, De Man, 1876 *Halaphanolaimus* Southern, 1914 and *Leptolaimus* Ditlevsen, 1914. *Leptolaimus* spp. has buccal cavity elongated, tubular and cuticularised; amphids circular, each often with a posterior break where the amphid nerve exits, situated well posterior to cephalic setae. Males always have tubular cuticularised precloacal supplements; occasionally one or more supplements *Stephanof* similar structure are present in females, but this type of supplement nerve extends forward into the oesophageal region. Some species have additional anterior cup-shaped supplements which may extend into the oesophageal region. *Leptolaimus* spp., collected from Paradise point.

Genus *Halaphanolaimus* spp. collected from Paradise Point and differs from *Leptolaimus* only in that the tubular supplements extend into the oesophageal region.

Genus *Stephanolaimus* spp. has: buccal cavity conical; amphids kidney shaped, situated between longer cephalic setae but often difficult to distinguish; tail cylindrical with a conical tip and collected from Paradise Point.

Family Haliplectidae :

Only genus *Haliplectus* represents the family Haliplectidae which has the following characters: Oesophagus weakly muscular in anterior part, but with a small bulb in the middle and a large posterior bulb with a well cuticularised lining; cuticle striated without lateral differentiation; amphids circular ; buccal cavity narrow, tubular; males with precloacal papillae; testes paired and opposed, reflexed; tail conical. Genus *Haliplectus* was collected from Mubarak village and reported by Maqbool and Nasira (2000).

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