

## Morphological and molecular identification of four new species of marine nematodes

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### Abstract

Marine nematodes play a vital role in the ecosystem of seas and estuaries as a diverse species, universally rich and often showing sensitive responses to ecological changes. During present study, surveys were conducted at four locations of Sindh coast viz., Kaemari, Korangi Creek, Ibrahim Haidry, Mubarak Village and two of Balochistan coast viz., Gadani and Sonmiani beach. As a result, four new species *Oncholaimus paraoxyuris* n. sp., *Metoncholaimus medispiculatum* n. sp., *Theristus (P) karachiense* n. sp. and *Theristus cylindricus* n. sp. were found. *O. paraoxyuris*, *M. medispiculatum* and *T. karachiense* were molecularly identified on the basis of 18S ribosomal gene with accession numbers KY497017, KY979964 and KY979968, respectively.

**Keywords:** Taxonomy, *Oncholaimus paraoxyuris* n. sp., *Metoncholaimus medispiculatum* n. sp., *Theristus (P) karachiense* n. sp., *Theristus cylindricus* n. sp., marine nematodes.

Marine nematodes play a complex and significant role in the ecology of the marine environment because of their great abundance, adaptation to a wide diversity of habitat's and diverse morphology (Gray & Elliot, 2009). According to Cook *et al.*, (2000) nematode abundance in marine sediments declines with depth and distance from continents and is related to food supply and local productivity. That is one of the main parameters for global estimation of biodiversity patterns. Moreover, the marine fauna is still poorly known and many new species remain undiscovered (Mokievsky & Azovsky, 2002).

To estimate the biodiversity of marine nematodes surveys were conducted during 2015, for the collection of marine nematodes at four localities of Sindh coast and two of Balochistan coast. In this article, detail information has been presented regarding the marine nematode population studies along with

description and illustrations of four new species *Oncholaimus paraoxyuris* n. sp., *Metoncholaimus medispiculatum* n. sp., *Theristus (P) karachiense* n. sp. and *Theristus cylindricus* n. sp. One new species *Aulolaimus mubarakvilli* Salma, Saima, Nasira & Shahina, 2017 has also been isolated during this survey is the part of population studies.

### Materials and Methods

**Sites of sampling:** Water and sediment samples were collected from the four localities of Sindh coast (26.64°N 67.50°E); Kaemari, Korangi Creek, Ibrahim Haidry and Mubarak Village while from two localities of Balochistan coast (30.12°N 67.01°E) Gadani and Sonmiani beach (Fig.1). A total of 100 water samples were collected from these localities during the present study.

**Sample collection:** Five water samples were collected at random from each sampling spot in two liter plastic bottles and sediments in sterile

plastic bags by using hand held cylindrical corer (5 cm long) with internal diameter of 2.4 cm. The samples were taken from a depth of 4-10 cm as in the top 10 cm of the sediment, where most of the meiofauna are found. For further processing these samples were brought to laboratory in cool boxes.

**Extraction of nematode:** Each sample was transferred in clean plastic bucket, for homogeneity of water content. Two liters of fresh water was poured and allowed to settle for few minutes to collect nematodes. They were decanted and sieved through 45 µm mesh sieve then preserved in 4% formaldehyde and stained with Rose Bengal in sea water. The nematodes were handpicked with dropper under binocular microscope and transferred into 1.5% aqueous glycerine which was slowly dehydrated to anhydrous glycerol at 40° C by the method of Hooper (1986).

### Sample analysis

**Quantitative analysis:** Quantitative analysis was done to count the average number of nematodes in 100 ml of suspension. For quantitative analysis 5 ml of extracted nematode suspension was poured in a counting chamber and nematodes were counted by a counter under a stereoscopic microscope. An average number of nematodes in 100 ml of suspension were calculated by taking three readings.

**Qualitative analysis:** For qualitative analysis, observations were made on temporary mounts with the help of stereomicroscope. The nematode suspension was allowed to settle for about 2 hours. The excess supernatant water was poured off by a dropper. Remaining concentrated content was transferred to a cavity block for examination under stereomicroscope. Three small drops of concentrated nematode suspension were placed on a 3x1 inches glass slide. The slide was then placed over a hot plate for killing the nematodes, taking care not to overheat them. Then they were covered with a

cover slip and sealed with Zut. Nematode population was identified up to the species level.

**Diversity index:** The Shannon diversity index ( $H'$ ) is commonly used to characterize species diversity in a community. Shannon's index accounts for both abundance and evenness of the species present. The Shannon Weiner index ( $H'$ ), richness ( $d1$ ) and evenness ( $J$ ) were calculated with the following formulas:

$$H' = -\sum p_i \log p_i$$

Where  $p_i$  is the proportion of total number  $N$  belonging to the  $i$ -th species (Shannon & Wiener, 1949).

**Similarity index:** The similarity between nematode species found during survey is based on the quantitative (presence/absence) which was calculated on the basis of Jacord's coefficient of similarity (Rohlf, 2005). All computations were carried out using the computer application JAVA Runtime Environment (JRE) supported software BioSim 2.0.03 Version B (Pearson *et al.*, 2005).

**Taxonomic identification:** For the morphological studies and identification of marine nematodes systematics provided by Lorenzen, 1981; Tarjan, 1980; Platt & Warwick, 1988 and Warwick *et al.*, 1998 were used. Nematodes were classified according to their feeding groups as proposed by Weiser (1953). Illustrations were prepared by using camera Lucida attached to a compound microscope. Measurements were made with a calibrated ocular micrometer.

### Molecular identification

**DNA extraction:** DNA was extracted from single nematode which was crushed in 1.5 ml Eppendorf tube containing 25 µl of lysis buffer [50mM KCL: 250 µl, 0.05% Gelatin: 250 µl, 100 mM Tris: 50 µl, 2.5 mM: MgCl<sub>2</sub>: 12.5 µl, 0.45 % Tween 20: 22.5 µl; 0.45 % NP40:45µl; proteinase K: 10 mg in 5 ml water] with sterilized micropipette tip.



**Fig.1.** Surveyed localities and collection of water and sediment samples for marine nematodes.

The tubes were frozen at -80 °C for 10 min and followed by rapid incubation at 65 °C for 1 hr 52 min, and then at 95 °C incubation for 10 min to completely lyse the cells, digest the proteins and inactivate proteinase K, respectively. The tubes were cooled at room temperature for a minute and centrifuged at 12,000 rpm for 2 min. The 20 µl supernatant containing the DNA was collected for using in PCR reaction in 1.5 ml Eppendorf tube. The tubes were labeled and DNA was preserved at -80 °C. Quality of DNA was visualized by gel electrophoresis and quantity was analyzed by calculating optical density by spectrophotometer.

**Polymerase chain reaction (PCR):** For amplification PCR tubes were placed on cryo box. Master mix of Promega GoTaq® Green, 2X (M712B) having 400 µM dnTPs and 3mM MgCl<sub>2</sub> were used as a reaction buffer with primer 18S ribosomal RNA gene with a primer set Forward: MN18F [59-CGCGAATRGTCTATTACAACAGC-39] and Reverse: NEM18SR [59-GGGCGGTATCTGATCGCC-39]. The cycling profile for amplification was initial denaturation for 5 min at 94 °C, denaturing for 40 sec at 90 °C, annealing for 40 sec at 53 °C, elongation for 1 min at 72 °C, final elongation for 10 min at 72 °C, cycle termination at 16 °C.

**Gel electrophoresis:** For confirmation of amplification results, samples were visualized on gel electrophoresis by using a 0.5% agarose gel in 1X TBE buffer (54.0 g Tris base + 27.5 g Boric acid + 20 ml 0.5 M EDTA (pH 8.0). Staining dye VisualaNa (10 µl / 100 ml of gel) were used for detection. 5µl of a DNA template was mixed with 1µl blue loading dye (Fermentas) and transferred into the gel well. One well was loaded with 3µl of molecular marker (Fermentas, 100 to 2000 base pairs); amplified DNA was subjected to 80 Volts, 250 mA for 25 minutes. DNA bands were then visualized using UV transilluminator.

**Purification:** Purification of PCR positive samples was done by using ExoSap- IT (P-

78200B). In 10 µl of sample 4 µl of clean up product ExoSap- IT was added and incubates at 37 °C for 15 min followed by 80 °C for 15 min. PCR product was then ready to use and may be stored at -20°C until required.

**Sequencing:** The genomic DNA concentration was measured using a DU 730, Beckman Coulter spectrophotometer. This was followed by a cycle sequencing reaction in a final volume of 20 µl, Big Dye Terminator v3.1, 5x sequencing buffer, containing 100ng/µl purified DNA template single stranded. Cycling reactions were carried out separately for each of 10 µM of forward or reverse template specific primers.

## Results and Discussion

Thirty species of marine nematodes were traced belonging to 29 different genera, only genus *Theristus* identified with two species as given in the list of marine nematodes.

Four species *Oncholaimus paraoxyuris* n. sp., *Metoncholaimus medispiculatum* n. sp., *Theristus (P) karachiense* n. sp. and *Theristus cylindricus* n. sp. are described as new to science in marine nematology. *O. paraoxyuris* n. sp. and *M. medispiculatum* n. sp. were found from Ibrahim Haidery only (Fig. 2) with highest population 8 and 13%, respectively. In Korangi Creek *T. cylindricus* n. sp. and *Theristus (P) karachiense* n. sp. were found at 4 % (Fig. 3). *T. (P) karachiense* n. sp. was traced 7% from Kaemari (Fig. 4.) and 11% from Mubarak Village (Fig. 5) from Sindh coast. *A. mubarakvilli* was found 3% from Mubarak Village, Gadani (Fig. 6) and Sonmiani (Fig. 7), whereas from Kaemari this species was found 5%. At Balochistan coast, the highest population of *T. cylindricus* n. sp. was found as 12 % from Gadani and 10% from Sonmiani beach, whereas, at Kaemari and Mubarak Village it was 7%. In all six localities, eight species richness have been shown (Table 1). Cluster analysis of thirty nematode species population shows two main clades with nine sub clades (Fig. 8). *A. mubarakvilli* Salma *et al.*, 2017 shows clade with *Halichoanolaimus balochiensis*, *Endeolophus minutes* and

*Eurystomina indica*, *M. medispiculatum* n. sp. and *O. paraoxyuris* n. sp. are in a same clade. *T. cylindricus* n. sp. shows population clade with *Diplolaimelloides delyi* and

*Viscosia elegans*. *T. (P) karachiense* n. sp. forms a clade with *Paracomesoma longispiculum* Jacord's coefficient of similarity among species is shown in Table 2.

#### List of marine nematode fauna found during survey.

1. *Aulolaimus mubarakvilli* Salma, Saima, Nasira & Shahina, 2017
2. *Camacolaimus tardus* De Man, 1889
3. *Chromadora nudicapitata* Bastian, 1865
4. *Desmodora cliftensis* Turpeenniemi, Nasira & Maqbool, 2001
5. *Diodontolaimus karachiensis* Nasira, Kamran & Shahina, 2005
6. *Diplolaimella divangatensis* Jacobs, Velde, Geraert & Vranken, 1990
7. *Diplolaimelloides delyi* Andrassy, 1958
8. *Eleutherolaimus inglisi* Timm, 1967
9. *Endeolophus minutes* Gerlach, 1967
10. *Eurystomina indica* Yoshimura, 1980
11. *Halichoanolaimus balochiensis* Turpeenniemi, Nasira & Maqbool, 2001
12. *Haliplectus monodelphis* Shahina, Siddiqi & Nasira, 2016
13. *Heterodorus longidens* (Jairajpuri & Loof, 1968) Andrassy, 2009
14. *Leptolaimus luridus* Timm, 1963
15. *Marylynnia musharafii* Nasira, Kamran & Shahina, 2007
16. *Metachromadora remaneii* Gerlach, 1951
17. ***Metoncholaimus medispiculatum* n. sp.**
18. *Microlaimus amphidius* Kamran, Nasira & Shahina, 2007
19. ***Oncholaimus paraoxyuris* n.sp.**
20. *Oxystomina elongata* Butschli, 1874
21. *Paracanthonchus hawaiiensis* Allgen, 1951
22. *Paracomesoma longispiculum* Timm, 1961
23. *Ptycholaimellus sindhicus* Turpeenniemi, Nasira & Maqbool, 2001
24. *Sabatieria microsetosa* Timm, 1967
25. *Synonchium pakistanense* Kamran, Nasira & Shahina, 2009
26. *Terschellingia longicaudata* De Man, 1907
27. ***Theristus (P) karachiense* n. sp.**
28. ***Theristus cylindricus* n. sp.**
29. *Trissonchulus benepapillosus* Schulz, 1935
30. *Viscosia elegans* Kreis, 1924

**Table 1. Diversity index of marine nematodes.**

Diversity index	Ibrahim Haidry	Korangi Creek	Kaemari	Mubarak Village	Gadani	Sonmiani beach
Number of Individuals (N)	8	8	8	8	8	8
Species Richness (S)	2475	3106	3106	3066	1.150	916
Shannon-Wiener Index of Diversity (H)	1.365	1.342	1.342	1.287	0.553	1.301
Species Evenness (d1)	0.656	0.645	0.645	0.619	1.666	0.626

***Oncholaimus paraoxyuris* n. sp.****Morphological characters****(Fig. 9-11; Table 3)**

**Male:** Body cylindrical, ventrally curved upon fixation. Cuticle smooth without discernible striations, about 1.5-2.5  $\mu\text{m}$  thick at mid body. Lip region truncate, continuous with body contour with six small and indistinct inner labial papillae. Six longer outer labial setae and four short cephalic setae almost equal in length, arranged in a single circle at level of anterior region of buccal cavity. Somatic setae scattered sparsely over the body.

Buccal cavity, large barrel shaped with three teeth, one dorsal and two sub ventral, the left sub ventral is longer and massive. Buccal cavity 38-40  $\mu\text{m}$  deep and 20-22  $\mu\text{m}$  wide with heavily cuticularized walls. Amphidial fovea rounded oval 6-8  $\mu\text{m}$  wide at level of dorsal tooth occupying 1/4 of the labial diameter. Pharyngeal collar surrounding the base of buccal cavity. Pharynx cylindroid, muscular.

Body width at pharyngo-intestinal junction 50-55  $\mu\text{m}$ . Nerve ring 180-212  $\mu\text{m}$  from anterior end i.e., 37.8-42.2 of the neck length. Excretory pore situated at a distance 43-50  $\mu\text{m}$  from anterior body end. Pharynx leading into pyriform cardia, enclosed with anterior intestine. Male reproductive system diorchic,

testes outstretched and both situated to left of intestine. Vas deferens well developed. Spicules paired, slender, slightly curved with weakly capitula, 1.11-1.25 times cloacal body diameter long. Gubernaculum absent. Three pairs of sub ventral preanal setae 4-5  $\mu\text{m}$  long. Preanal elevation with single duct opening is present. A large ventral swelling about three fifth of the way down the length of the male tail. Tail short, elongate conical bending ventrally with few small caudal setae along its length. Terminal setae close to tail tip, sub dorsal in position. Three caudal glands present with the caudal duct opening at tail terminus.

**Female:** Similar to male in general morphology except slightly longer body size. Genital system mono-prodelphic. Ovary reflexed, lying on the right side of the intestine. Large oocytes arranged in single row except near tail tip. Uterus accommodating 8-9 large intra uterine eggs of 54  $\times$  96  $\mu\text{m}$  dimension having blunt polar ends. Vulva a transverse slit situated posterior to mid body; vulval lips not sclerotized. Vagina slightly directed upward. Post uterine sac present, conspicuous.

Demanian system well developed. Uvette inconspicuous located 454  $\mu\text{m}$  anterior to anus. Main duct connecting uvette and ductus entericus prominently bifurcating into paired terminal ducts that open through two slit like terminal pores located at distance 30-32  $\mu\text{m}$  anterior and 8-9  $\mu\text{m}$  posterior at 115 and 82.5  $\mu\text{m}$ , respectively from tail terminus. Tail elongate, gradually tapering to rounded terminus, bearing conspicuous spinneret showing the opening of caudal gland duct.

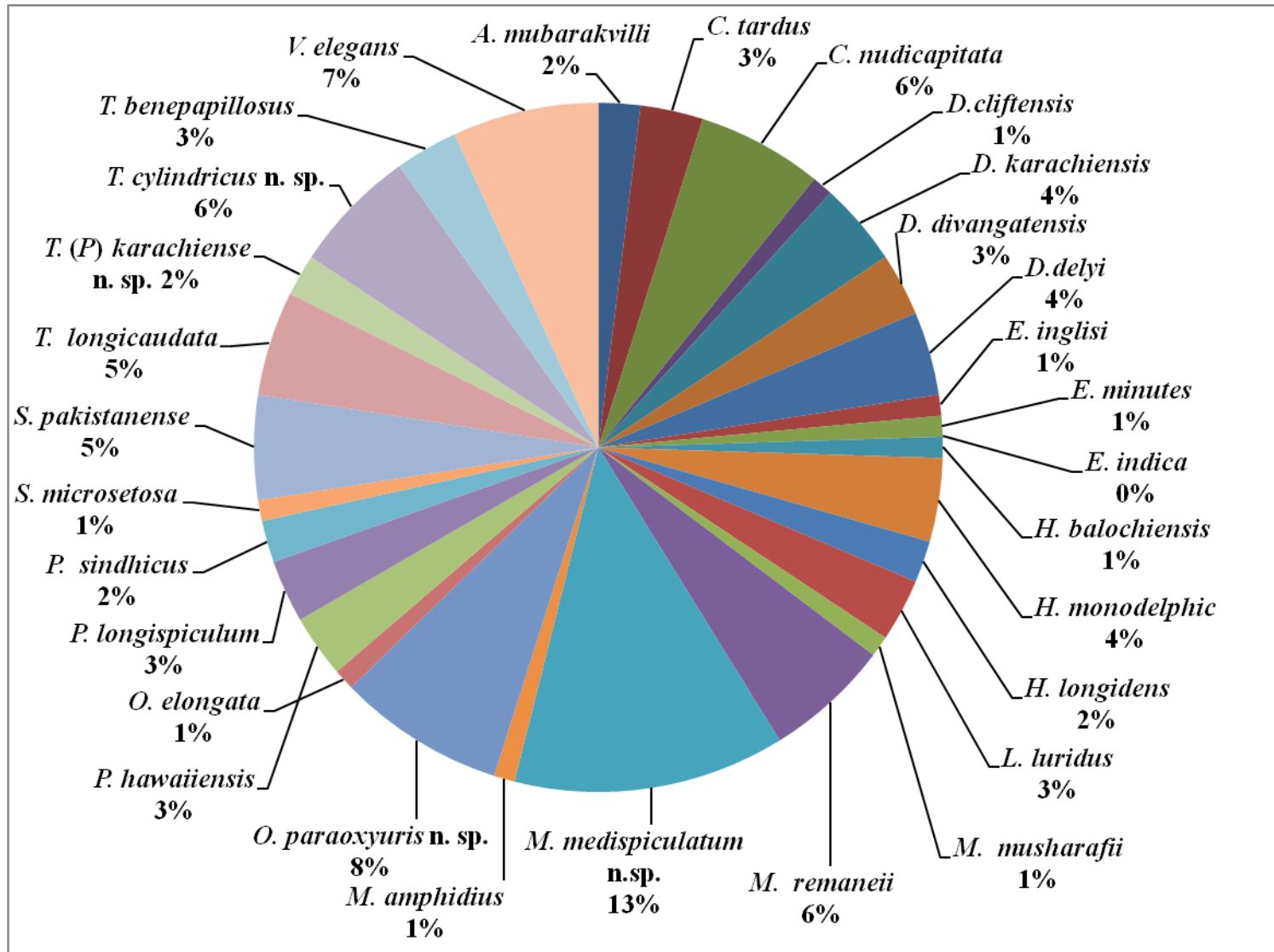


Fig.2. Percentage occurrence of marine nematodes from Ibrahim Haidry, Sindh coast.

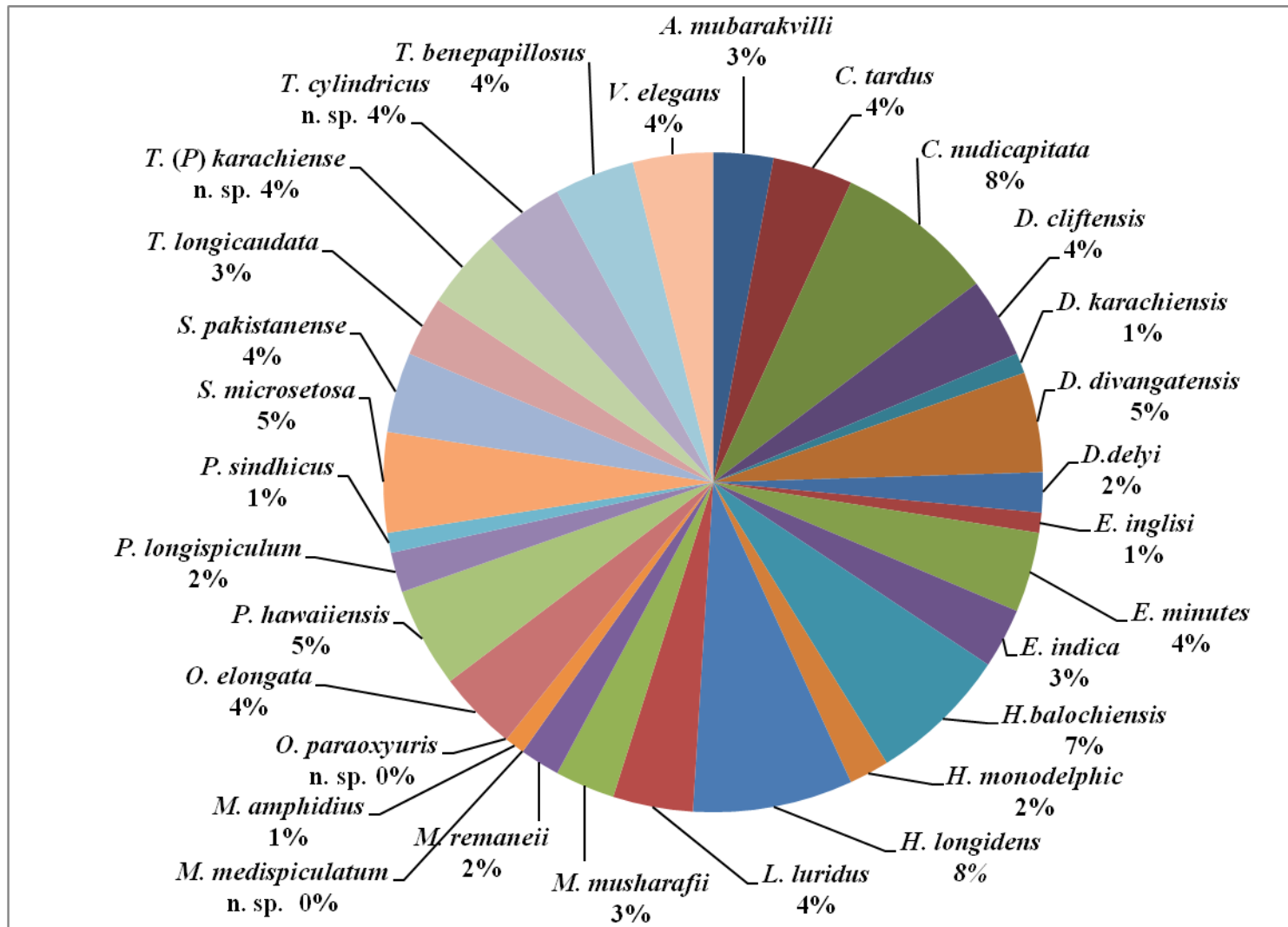


Fig.3. Percentage occurrence of marine nematodes from Korangi Creek, Sindh coast.



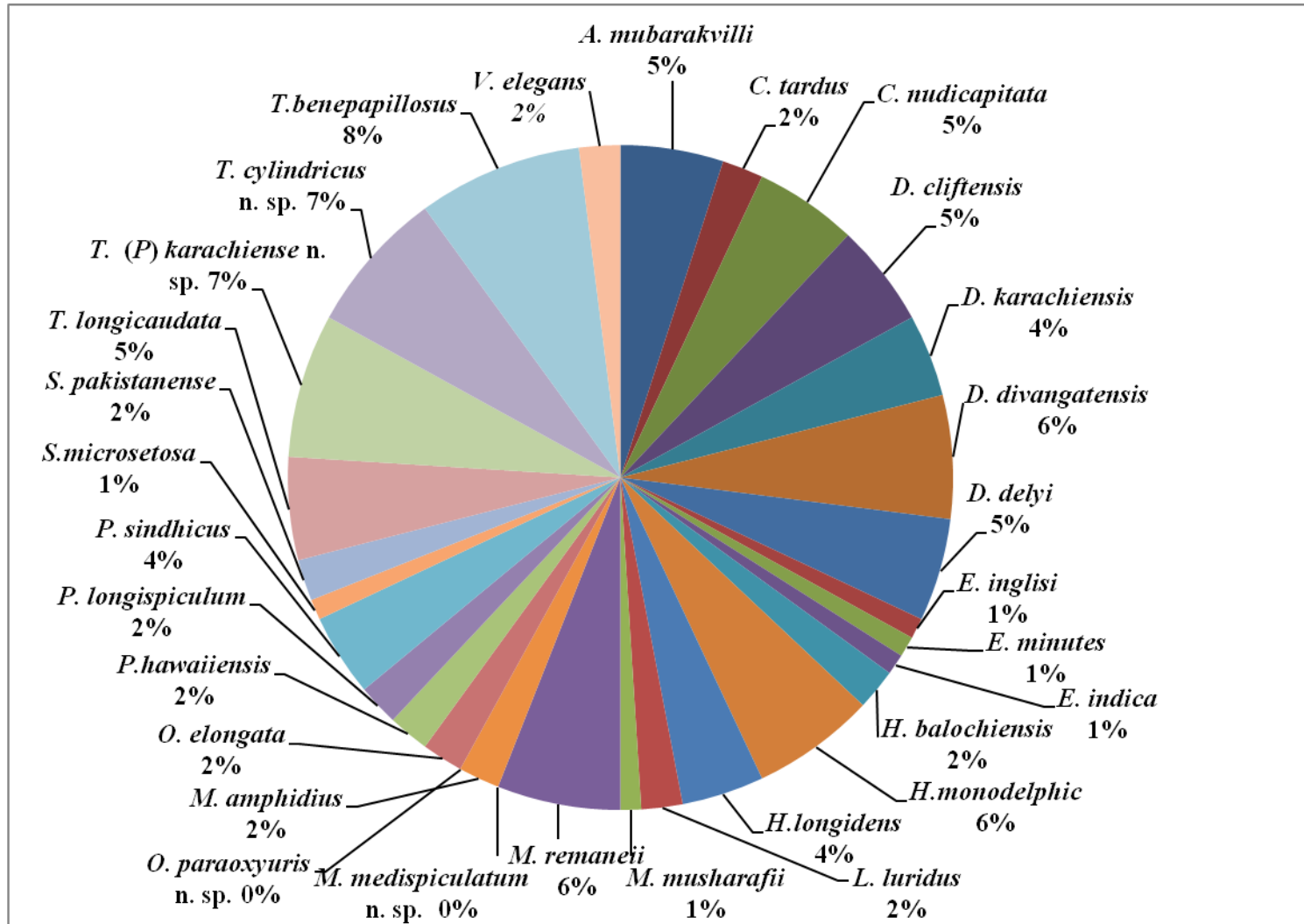


Fig.4. Percentage occurrence of marine nematodes from Kaemari, Sindh coast.

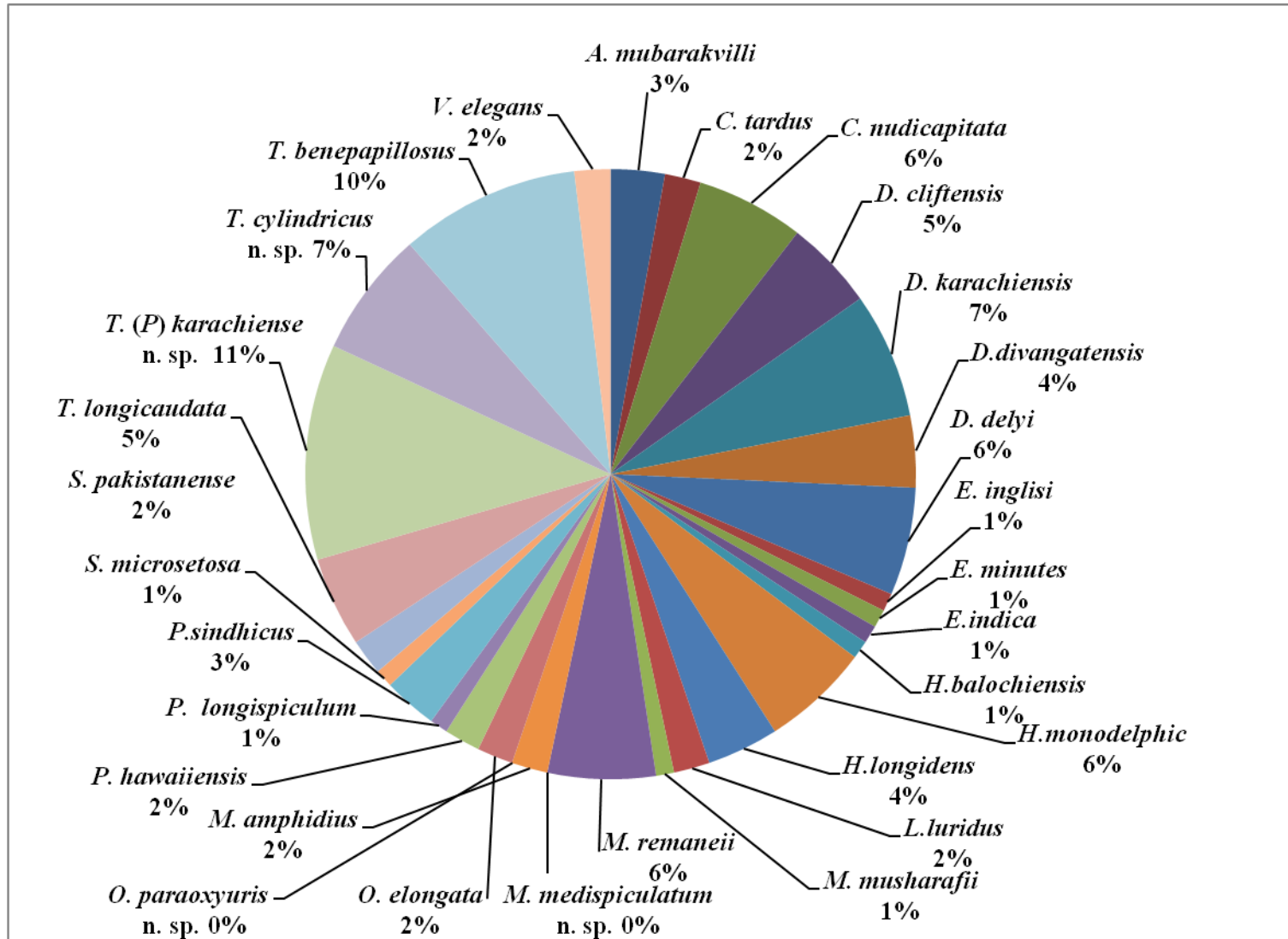
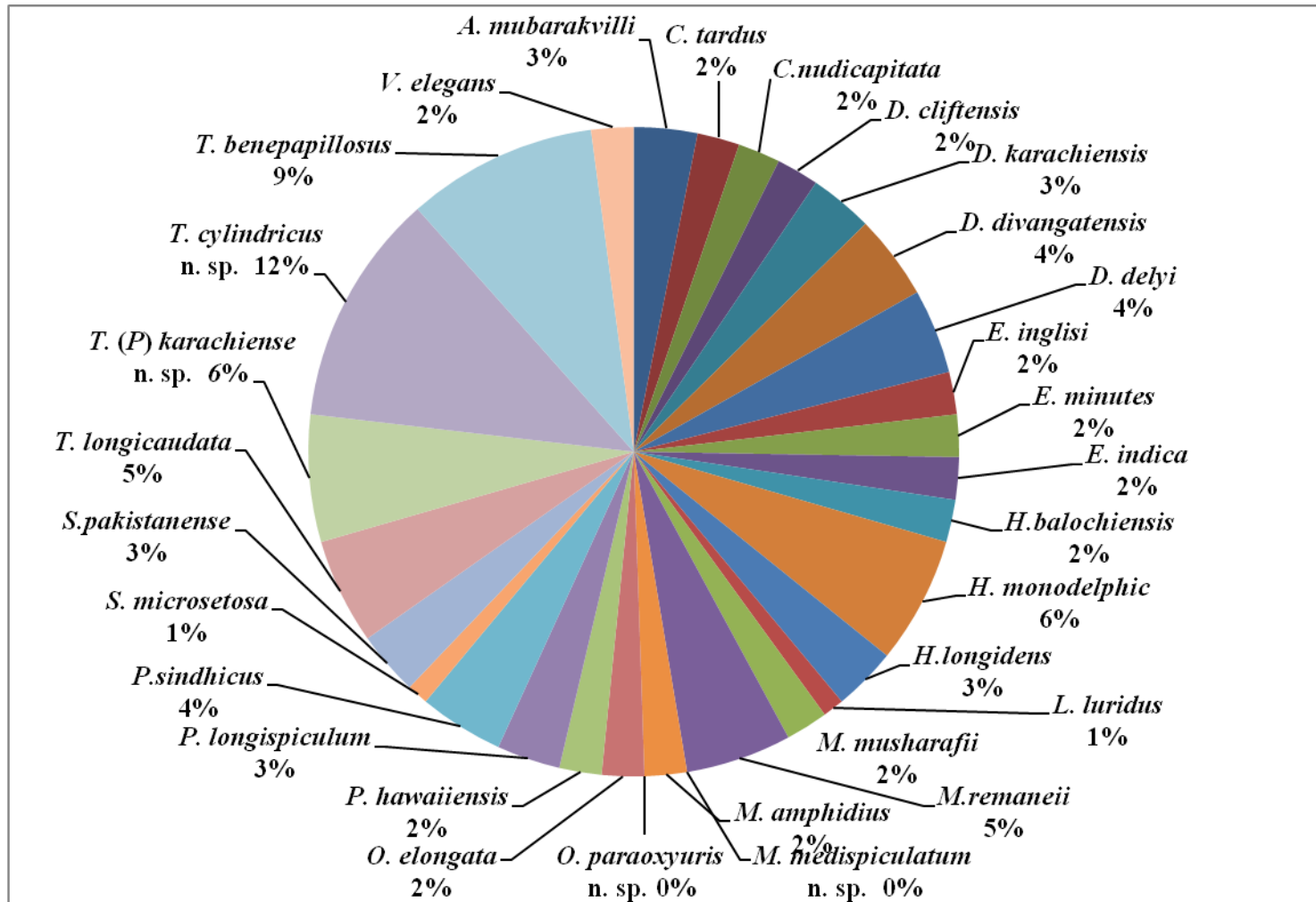


Fig.5. Percentage occurrence of marine nematodes from Mubarak Village, Sindh coast.



**Fig.6.** Percentage occurrence of marine nematodes from Gadani, Balochistan coast.

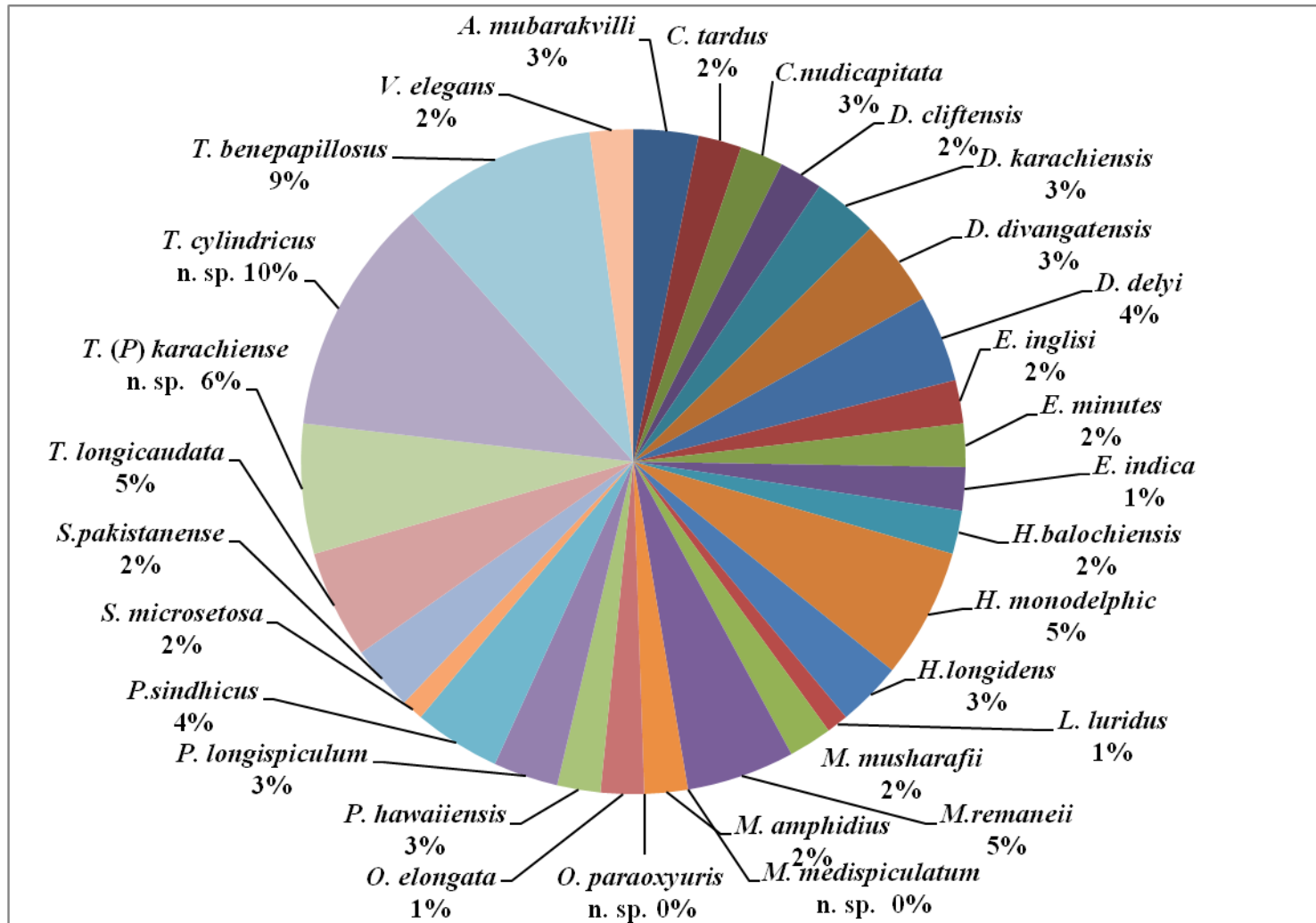
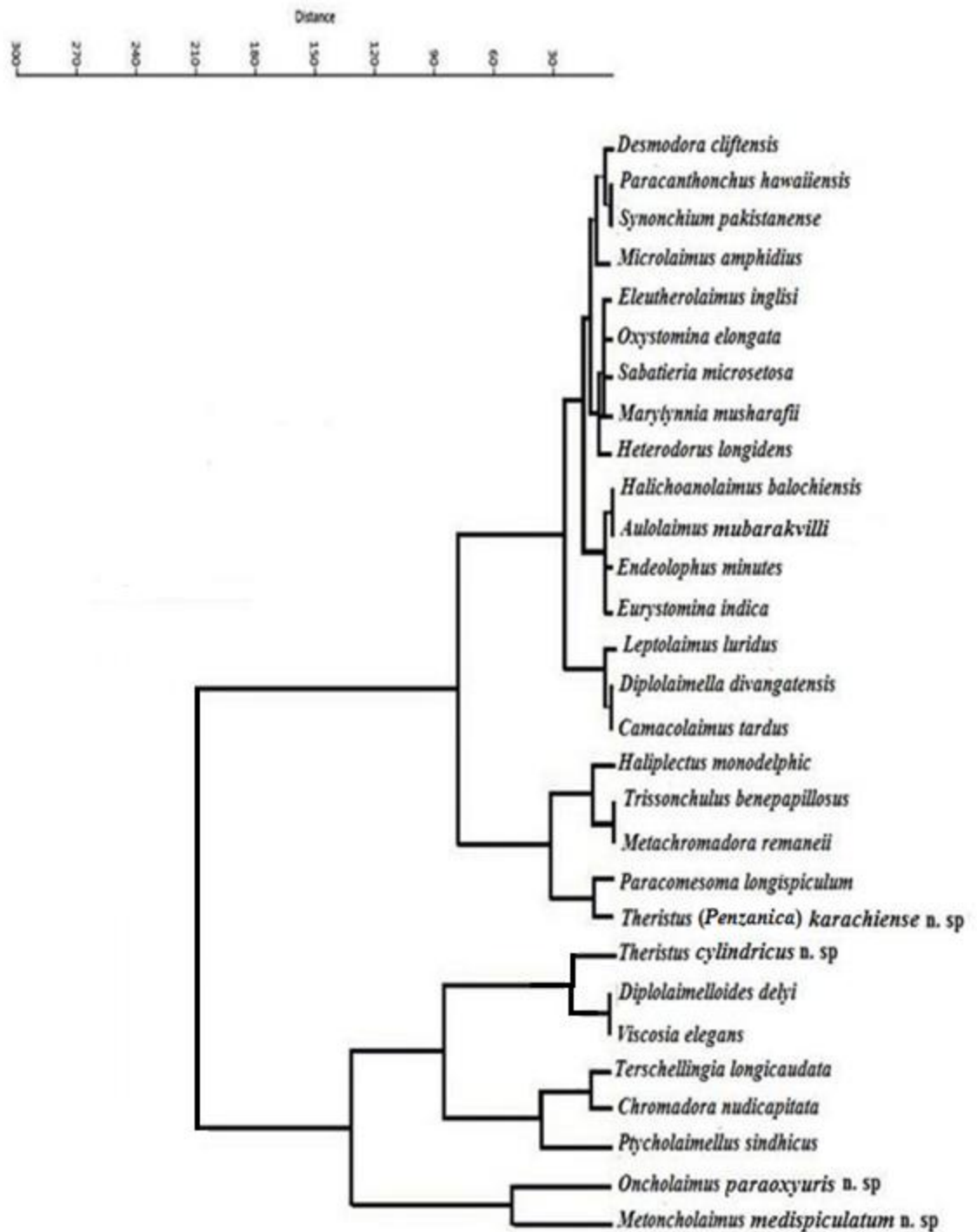


Fig.7. Percentage occurrence of marine nematodes from Sonmiani, Balochistan coast.



**Fig.8.** Cluster analysis for population of marine nematodes found during survey from Sindh and Balochistan coast.

Nematode species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>M. medispiculatum</i> n. sp.	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>O. paraoxyuris</i> n.sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>T. (P) karachiense</i> n. sp.	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>D. delyi</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>D. cliftensis</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>T. longicaudata</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>P.indhicus</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>E.inglisi</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>H. balochiensis</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>P. hawaiiensis</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>M. musharafi</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>C. nudicapitata</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>V. elegans</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>O. elongata</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>E. minutes</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>H. monodelphis</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>M. amphidius</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. microsetosa</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>E. indica</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>T. benepapillosus</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>P. longispiculum</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>T. cylindricus</i> n. sp.	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. pakistanense</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>A. mubarakvilli</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>H. longidens</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>D. divangatensis</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>D. karachiensis</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>C. tardus</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>M. remanei</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>L. luridus</i>	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table 2. Similarity index of marine nematodes found during the studies.

**Type specimens:** Holotype (male) slide No. NNRC 23/1 and paratype slide No. NNRC 23/2-6 (seven males and one female) are deposited in the Nematodes Collection of the National Nematological Research Centre, University of Karachi, Karachi, Pakistan. One paratype male and one paratype female have been deposited in the nematode collection at Nematode Taxonomy Laboratory, 24 Brantwood Road, Luton England, UK.

**Type habitat and locality:** Samples containing *Oncholaimus paraoxyuris* n. sp. were collected from the marine sediments of low tidal mud of Ibrahim Haidry, Sindh coast, Pakistan.

**Diagnosis and relationships:** The genus *Oncholaimus* Dujardin, 1845 is characterized by a large buccal cavity with three teeth, one dorsal and two sub ventral, the left sub ventral is longer and massive. Tail is short, straight or ventrally curved. Males are diorchic, with short spicules and no gubernaculum. Females are monodolphic with a single anterior ovary and a demanian system (Platt & Warwick, 1983). *O. paraoxyuris* n. sp. belongs to the species group “oxyuris” of the genus *Oncholaimus* in having a large ventro median swelling just posterior to the middle of the tail in male. The new species is characterized by large sized body. Outer labial and cephalic sensilla setose, barrel shaped buccal cavity with thick sclerotized walls having three teeth, one dorsal and two sub ventral, left sub ventral tooth being largest; reproductive system mono-prodelphic with reflexed ovary, uterus with 8-9 large intra-uterine eggs.

Demanian system well developed with un conspicuous uvette. Female have two tubular terminal pores located at distance 30- 32  $\mu\text{m}$  anterior and 8 - 9  $\mu\text{m}$  posterior to anus. The new species is similar to *O. rivalis* Gagarin & Gusakov, 2012 in body, pharynx and tail length, in labial region diameter in male but differs from it in the relatively longer and wider buccal cavity in male (buccal cavity 38-40 vs 35  $\mu\text{m}$

long and 20 vs 18  $\mu\text{m}$  wide). It also differs in ratios a, b, c in female (a= 73-74 vs 48-60; b= 9-10 vs 8.2-8.9; c= 59.2 - 63.1 vs 38.7-46.0) and in smaller tail length (tail= 86-87.5 vs 92-105  $\mu\text{m}$ ).

The new species also resembles *O. brachycercus* de Man, 1889 in most morphometric details but differs from it in having smaller body width in male and female (male= 50-55 vs 70.5-80; female= 70.72 vs 93.3  $\mu\text{m}$ ), longer and slender tail in female (c= 59.2, 63.1 vs 72-85, c'= 2.1, 2.1 vs 1.5) and more anterior located vulva (V= 67.1, 67.7 vs 74).

**Molecular identification of *Oncholaimus paraoxyuris* n. sp.:** *Oncholaimus* n. sp. is characterized genetically by 18S RNA regions. The length of the sequence is 828 bp and its composition is A= 0.267, C= 0.199, G= 0.262 and T= 0.270 with accession no KY497017. In NCBI total 30 sequences of 18S ribosomal RNA gene, partial sequences are found. KY497017 showed 97 % similarity with *Oncholaimus* sp. HM564475, HM564474, HM564402, AY854196 and FJ040493.

#### *Metoncholaimus medispiculatum* n. sp.

#### Morphological characters

#### (Fig. 12-14; Table 4)

**Male:** Body long straight, to slightly ventrally curved upon fixation, tapering at extremities more towards posterior side. Cuticular surface is smooth. Lip region truncate, continuous with body contour. Lip with six small and indistinct inner labial papillae. Cephalic setae 10-12  $\mu\text{m}$  long. Spares somatic setae present throughout the body. Stomata well developed, about twice as long as wide. Buccal cavity widest in the anterior part, narrowing slightly posteriorly with thick sclerotized walls, walls bearing three teeth, left sub ventral largest, right sub ventral and dorsal about equal. Amphidial fovea rounded oval 8-9  $\mu\text{m}$  wide at level of dorsal tooth and 11-12  $\mu\text{m}$  from anterior end.

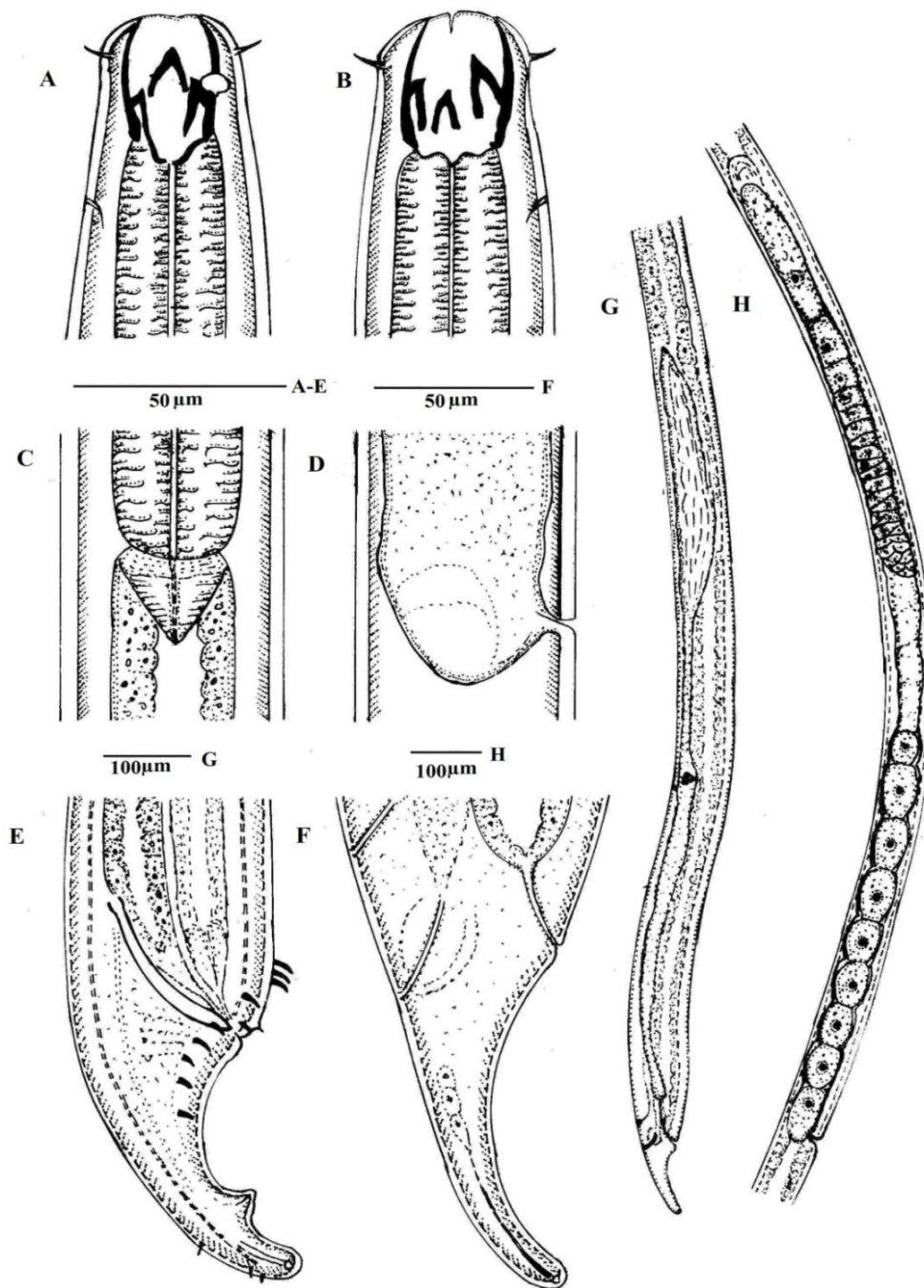
**Table 3. Measurements of *Oncholiamus paraoxyuris* n. sp. in  $\mu\text{m}$  except L.**

Characters	Holotype male	Paratypes	
		Male (n=8)	Female (n=3)
L (mm)	4.1	3.88 $\pm$ 0.48 (3.0-4.6)	5.28 $\pm$ 105.1 (5.18-5.42)
a	82.8	74.9 $\pm$ 10.5 (60-93)	74.6 $\pm$ 0.63 (73.8-74.9)
b	10.7	9.7 $\pm$ 0.72 (8.4-10.7)	9.93 $\pm$ 0.18 (9.8-10.2)
c	63.7	55 $\pm$ 4.9 (50.1-63.7)	61.0 $\pm$ 1.59 (59.2-63.1)
c'	1.4	1.74 $\pm$ 0.22 (1.4-2.0)	2.42 $\pm$ 0.28 (2.1-2.8)
V	-	-	67.4 $\pm$ 0.29 (67.1-67.6)
Lip width	30	31.5 $\pm$ 1.11 (30-32)	31.6 $\pm$ 1.69 (30-34)
Stoma length	38	38.87 $\pm$ 0.78 (38-40)	39 $\pm$ 0.81 (38-40)
Stoma width	20	20.87 $\pm$ 0.78 (20-22)	21 $\pm$ 0.81 (20-22)
Dorsal tooth length	13.6	13.6 $\pm$ 0.40 (13-14)	17 $\pm$ 0.81 (16-18)
Dorsal tooth width	3.2	3.7 $\pm$ 0.29 (3.2-4.1)	5.5 $\pm$ 0.49 (5-6)
Sub ventral tooth length	12.8	12.6 $\pm$ 0.39 (12-13)	18 $\pm$ 0.81 (17-19)
Sub ventral tooth width	5.6	7.0 $\pm$ 0.89 (5.6-8.0)	6.6 $\pm$ 0.47 (6-7)
Amphid aperture width	8	7.1 $\pm$ 0.64 (6-8)	9.3 $\pm$ 0.47 (9-10)
Amphid aperture from anterior end	14	15.1 $\pm$ 0.79 (14-16)	19.6 $\pm$ 0.47 (19-20)
Cephalic setae length	9	8.7 $\pm$ 0.62 (8-9.6)	9 $\pm$ 0.81 (8-10)
Cephalic setae from anterior end	9.6	8.6 $\pm$ 0.65 (8-9.7)	11.6 $\pm$ 0.47 (11-12)
Cervical setae length	6	5.0 $\pm$ 0.86 (4-6)	4.8 $\pm$ 0.23 (4.5-5)
Excretory pore from anterior end	44	44.8 $\pm$ 2.12 (43-50)	49 $\pm$ 0.81 (48-50)

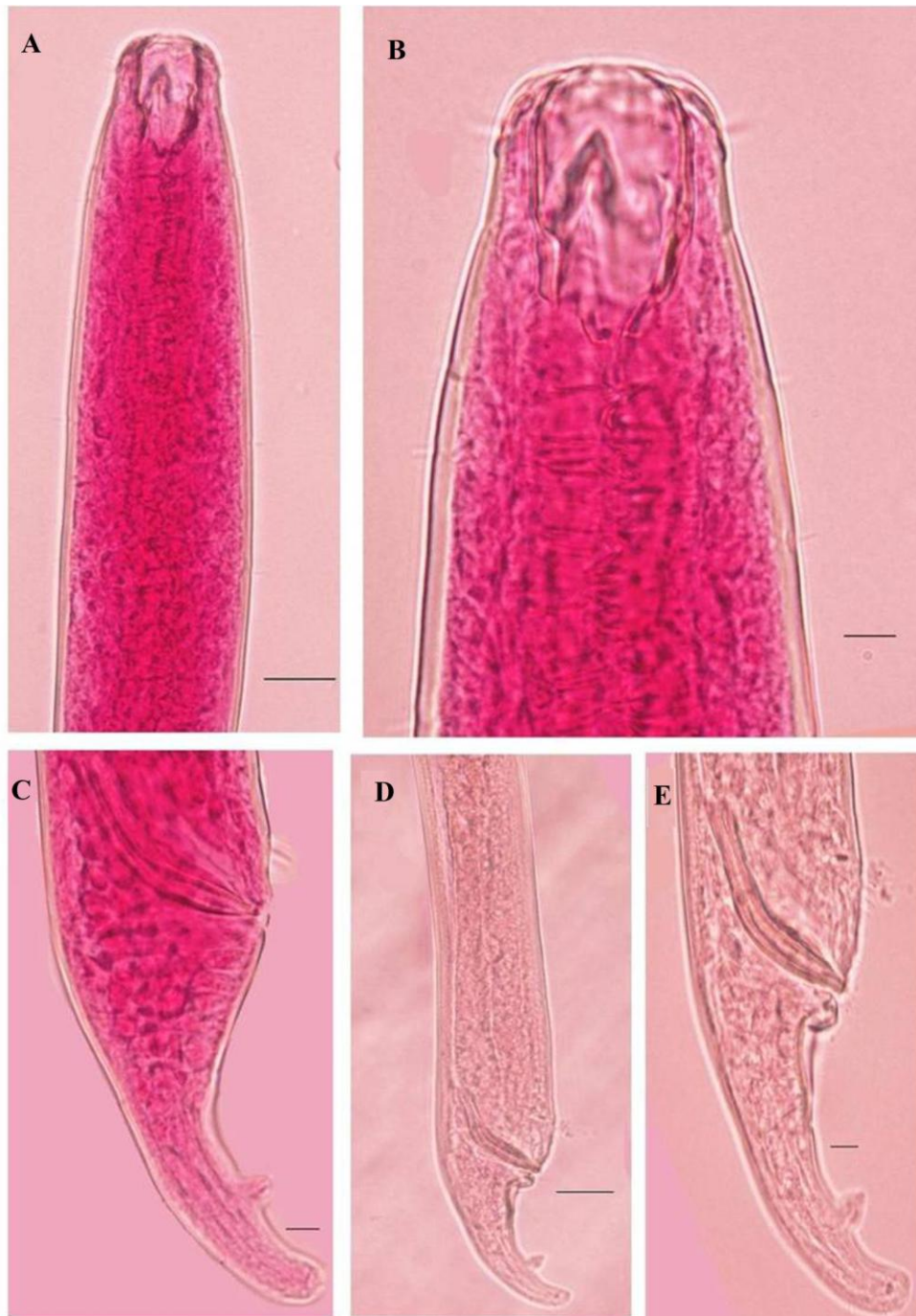


Characters	Holotype male	Paratypes	
		Male (n=8)	Female ( n=3)
Nerve ring anterior end	180	196.3 ± 10.5 (180-212)	173.6 ± 8.17 (166-185)
Nerve ring cbd	50	48.4 ± 1.93 (45-50)	59.3 ± 1.24 (58-61)
Pharynx length	385	398.6 ± 39.0 (356-475)	527.6 ± 2.05 (525-530)
Cardia length	20	21.8 ± 1.5 (20-24)	22 ± 1.63 (20-24)
Body diameter at base of pharynx	52	52.5 ± 1.73 (50-55)	161.6 ± 6.23 (150-170)
Body diameter at mid body	50	51.3 ± 31 (50-58)	70.6 ± 0.94 (70-72)
Body diameter at anus/clocca	45	42.5 ± 2.91 (40-48)	38.6 ± 1.88 (36-40)
Distance from pharynx end to vulva	-	-	3015.6 ± 3.68 (3011-3020)
Distance from pharynx end to cloaca	3200	3176 ± 29.6 (3100-3200)	-
Distance from vulva to anus	-	-	1603 ± 4.96 (1590-1610)
Vaginal depth	-	-	12 ± 1.63 (10-14)
Vulva from anterior end	-	-	3568 ± 57.29 (3510-3646)
Vulva cbd*	-	-	48 ± 2.16 (45-50)
Rectum length	-	-	29.6 ± 0.47 (29-30)
Tail length	65	72.3 ± 6.1 (65-80)	86.5 ± 0.70 (80-87.5)
Body width	50	52.3 ± 3.1 (50-58)	70.66 ± 0.94 (70-72)
Spicule length	50	48.2 ± 2.0 (45-50)	-

cbd\*= corresponding body diameter

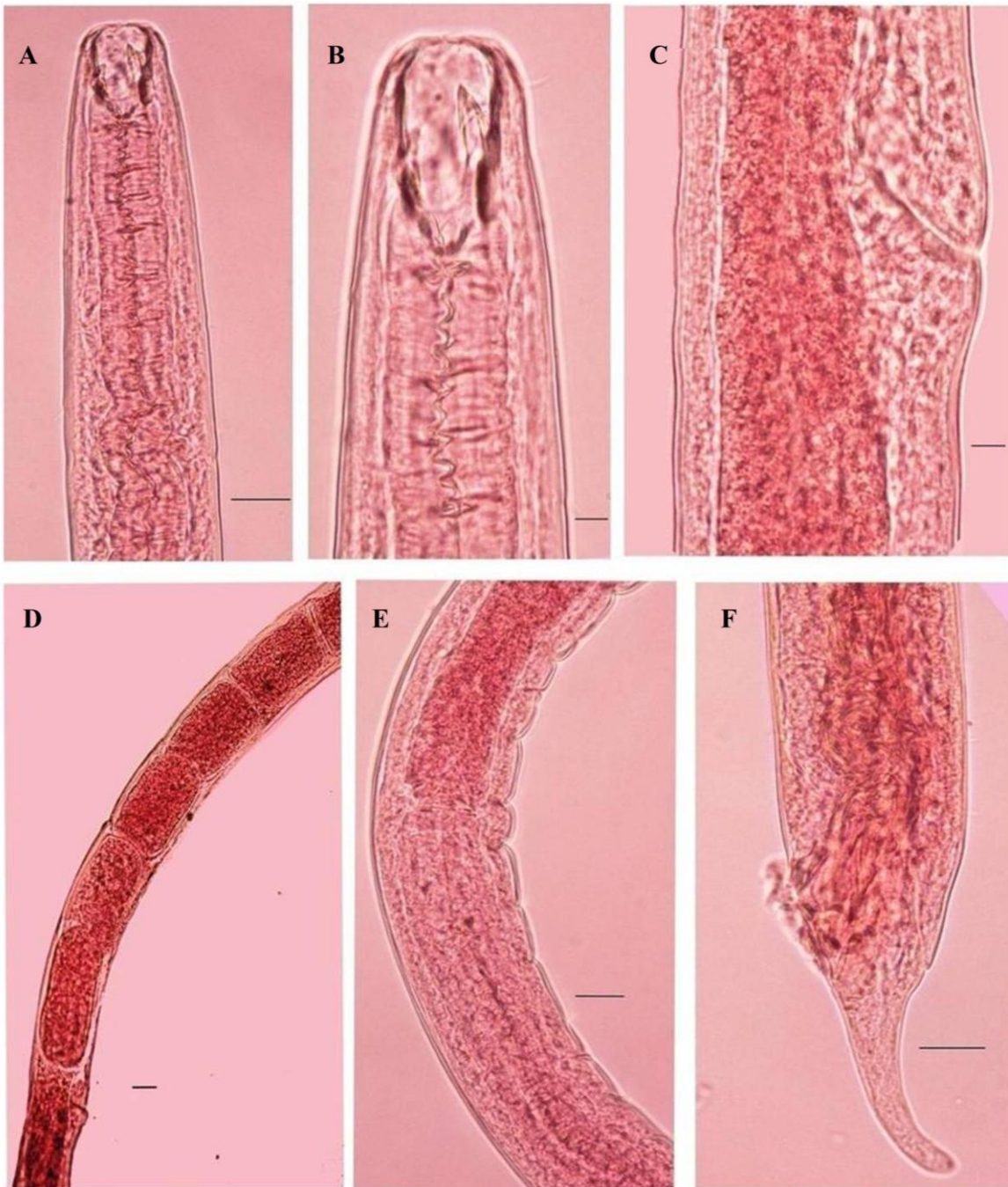


**Fig. 9 (A-H).** *Oncholaimus paraoxyuris* n. sp. A. Anterior region of male; B. Anterior region of female; C. Pharyngo-intestinal junction of male; D. Vulval region; E. Male tail; F. Female tail; G. Demanian system; H. Female genital system.



**Fig. 10 (A-E).** *Oncholaimus paraoxyuris* n. sp. Male A. Pharyngeal region; B. Anterior region; C-E. Tail region showing spicules (Scale: A, D= 40  $\mu$ m; B, C, E= 100  $\mu$ m).





**Fig. 11 (A-F).** *Oncholaimus paraoxyuris* n. sp. Female: A. Pharyngeal region; B. Anterior region; C. Vulval region; D. Female gonads, E. Pharyngeal junction; F: Tail region (Scale: A, E= 40  $\mu$ m; B, C, F= 100  $\mu$ m; D= 10  $\mu$ m).

**Female:** Similar to male in most respect. Reproductive system monodelphic, prodelphic with reflexed ovary. Ovary with a single row of compactly arranged large oocytes. Uterus containing four to six large intra-uterin eggs  $92-96 \times 82-84 \mu\text{m}$  in diameter. Vulval lips sclerotized, inclined anteriorly, post uterine sac absent. Demanian system well developed with uvette located  $360 \mu\text{m}$  anterior to anus and a single, dorsal slit like exit pore anterior to anus. Tail elongate gradually tapers to rounded terminus with spinneret showing the opening of caudal gland duct.

**Type specimens:** Holotype (male) slide No. NNRC 24/ 1 and paratype slide No. NNRC 24/2-4 (five males and three females) are deposited in the Nematodes Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan. One paratype male and one paratype female have been deposited in the nematode collection at Nematode Taxonomy Laboratory, 24 Brantwood Road, Luton England, UK.

**Type habitat and locality:** Samples containing *Metoncholaimus medispiculatum* n. sp. were collected from the marine sediments of low tidal mud of Ibrahim Haidry, Sindh coast, Pakistan.

**Diagnosis and relationships:** *Metoncholaimus medispiculatum* n. sp. belongs to that group of species of *Metoncholaimus* in which a gubernaculum is present in male. *M. medispiculatum* n. sp. is characterized by large sized body; barrel shaped buccal cavity with thick sclerotized walls having relatively wider anterior half and narrower posterior half; left sub ventral tooth large, right sub ventral and dorsal tooth of equal size; reproductive system mono-prodelphic with reflexed ovary; demanian system well developed with conspicuous uvette. Male with long and slender spicules and small gubernaculum. The new species is most closely related to *M. siddiqii*, Shahina *et al.*, 2015 in most morphopmatric detail but differs from it in

having longer spicules and shorter and simple gubernaculum (spicules =  $164-185$  vs  $90-105 \mu\text{m}$ ; gubernaculum =  $20-24$  vs  $25-30 \mu\text{m}$  with a sharp outwardly directed projection distally) and a shorter pharynx ( $333-336$  vs  $400-480 \mu\text{m}$ ).

**Molecular identification of *M. medispiculatum* n. sp.:** The length of 18S RNA gene of *Metoncholaimus* n. sp. was amplified using primer NEM18 and MN18S and the sequence was deposited to Genbank under accession no. KY979964. The nucleotide sequence compositions are A= 0.255, C = 0.204, G= 0.271 and T= 0.68 with 666 bp. New species showed 96 % similarity with *Oncholaimidae* species HM564620 and HM565620. In NCBI much more data with specific names of species are not available for comparisons.

***Theristus (Penzancia) karachiense* n. sp.**

### Morphological characters

(Fig. 15-17; Table 5)

**Male:** Body elongate cylindrical tapering on both ends. Cuticle distinctly annulated; annules  $1.2-1.4 \mu\text{m}$  wide on the mid body region. Labial region  $22-24 \mu\text{m}$  wide, amphid  $12-14 \mu\text{m}$  from anterior end. Six long cephalic setae  $11-12 \mu\text{m}$ , six short cephalic setae  $8-9 \mu\text{m}$  single circle, inner labial sensilla in the form of small papillae. Somatic setae rare and short. Stoma funnel shaped  $6-7 \mu\text{m}$  deep, with poorly cuticularized walls. Amphidial fovea round occupying 41-47% of corresponding body diameter. Pharynx slender, muscular, slightly swollen proximally. Body at posterior end of pharynx 1.5 times as wide as head. Cardia spherical muscular, surrounded with intestinal tissue. Gonads diorchic, testes two, opposite and outstretched. Anterior testis situated to left side of intestine, posterior testis situated to right. Spicules with twisted lateral edges, and well developed capitulum, 1.0-1.2 times as long as the cloacal body diameter. Gubernaculum  $10-12 \mu\text{m}$  long,

**Table 4. Measurements of *Metoncholaimus medispiculatum* n. sp. in  $\mu\text{m}$  except L.**

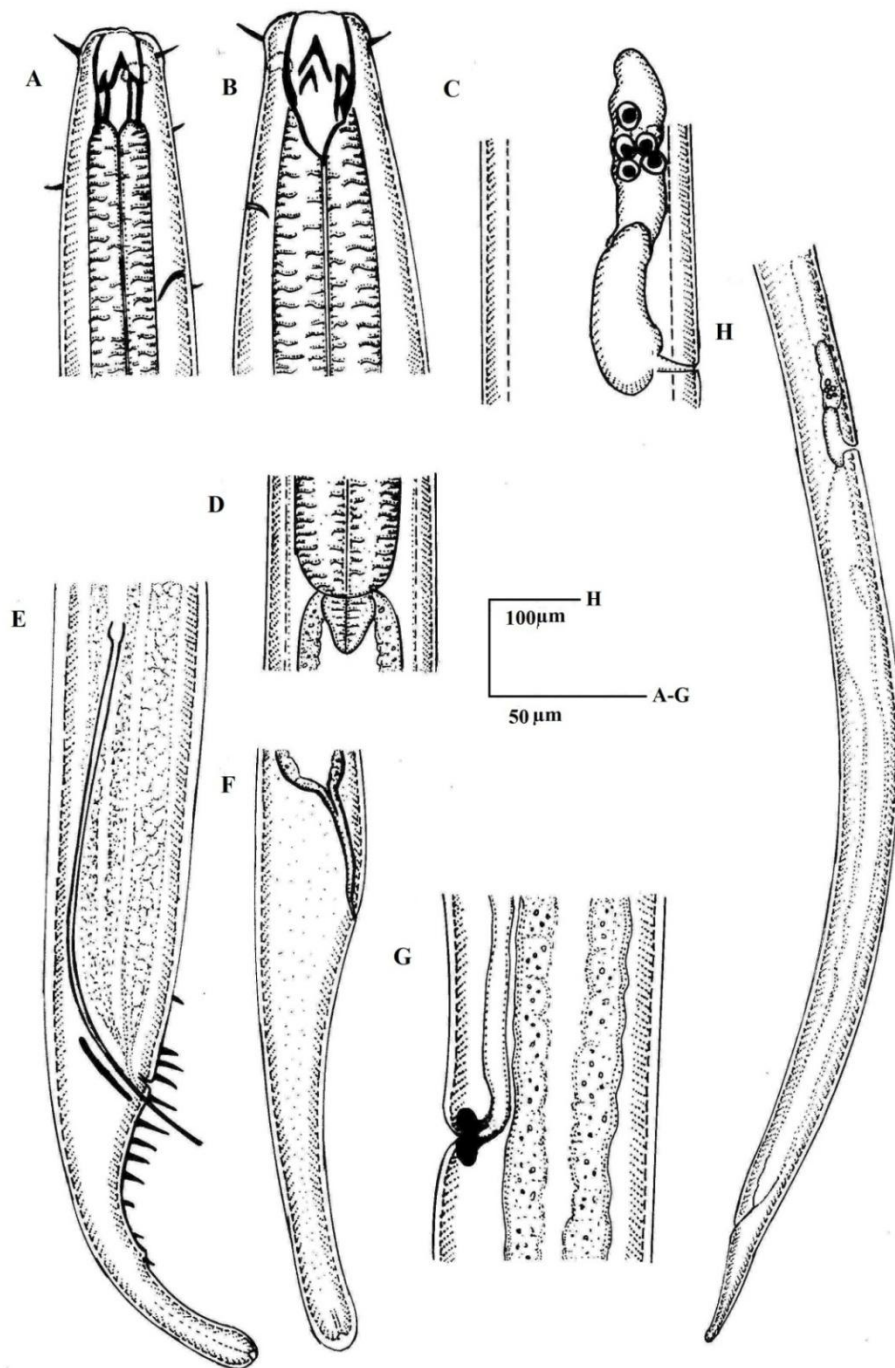
Characters	Holotype male	Paratypes	
		Male (n=4)	Female (n=4)
L (mm)	3.97	3.7 $\pm$ 0.11 (3.6-3.9)	4.2 $\pm$ 0.29 (3.9-4.7)
a	56.8	58.9 $\pm$ 1.52 (56.9-60.7)	64.5 $\pm$ 2.57 (61.2-67.8)
b	11.9	10.9 $\pm$ 0.63 (10-11.9)	12.6 $\pm$ 0.76 (12.1-14.7)
c	39.3	39.4 $\pm$ 0.36 (39.3-40)	32.7 $\pm$ 1.75 (31.0-35.6)
c'	3.2	3.0 $\pm$ 0.08 (3.0-3.2)	4.0 $\pm$ 0.14 (3.8-4.2)
V	-	-	58.8 $\pm$ 3.55 (53-61.6)
Lip width	22	23.5 $\pm$ 1.11 (22-25)	32.7 $\pm$ 0.82 (32-34)
Stoma length	30	30.25 $\pm$ 0.43 (30-31)	33.75 $\pm$ 2.38 (31-37)
Stoma width	14	13.75 $\pm$ 0.43 (13-14)	19.25 $\pm$ 0.82 (18-20)
Left sub ventral tooth length	13	13 $\pm$ 1.0 (12-14)	16 $\pm$ 1.41 (14-18)
Left sub ventral tooth width	6.2	6.25 $\pm$ 1.0 (5-8)	8.5 $\pm$ 1.65 (6-10)
Amphid aperture width	8.75	8.75 $\pm$ 0.43 (8-9)	8.5 $\pm$ 0.5 (8-9)
Amphid aperture from anterior end	11	11 $\pm$ 0.70 (10-12)	11.75 $\pm$ 1.47 (10-14)
Cephalic setae length	10.5	10.5 $\pm$ 1.65 9.8-12	8.75 $\pm$ 0.82 (8-10)
Cephalic setae from anterior end	6	7.12 $\pm$ 0.89 (6-8)	7.25 $\pm$ 0.82 (6-8)
Nerve ring	163	166.5 $\pm$ 2.69 (163-170)	169.75 $\pm$ 3.34 (166-175)
Nerve ring cbd	48	48 $\pm$ 1.41 (46-50)	52.5 $\pm$ 1.65 (50-54)
Pharynx length	333	334.75 $\pm$ 1.29 (333-336)	363.75 $\pm$ 18.4 (340-390)

Morphological and molecular identification of four new species of marine nematodes

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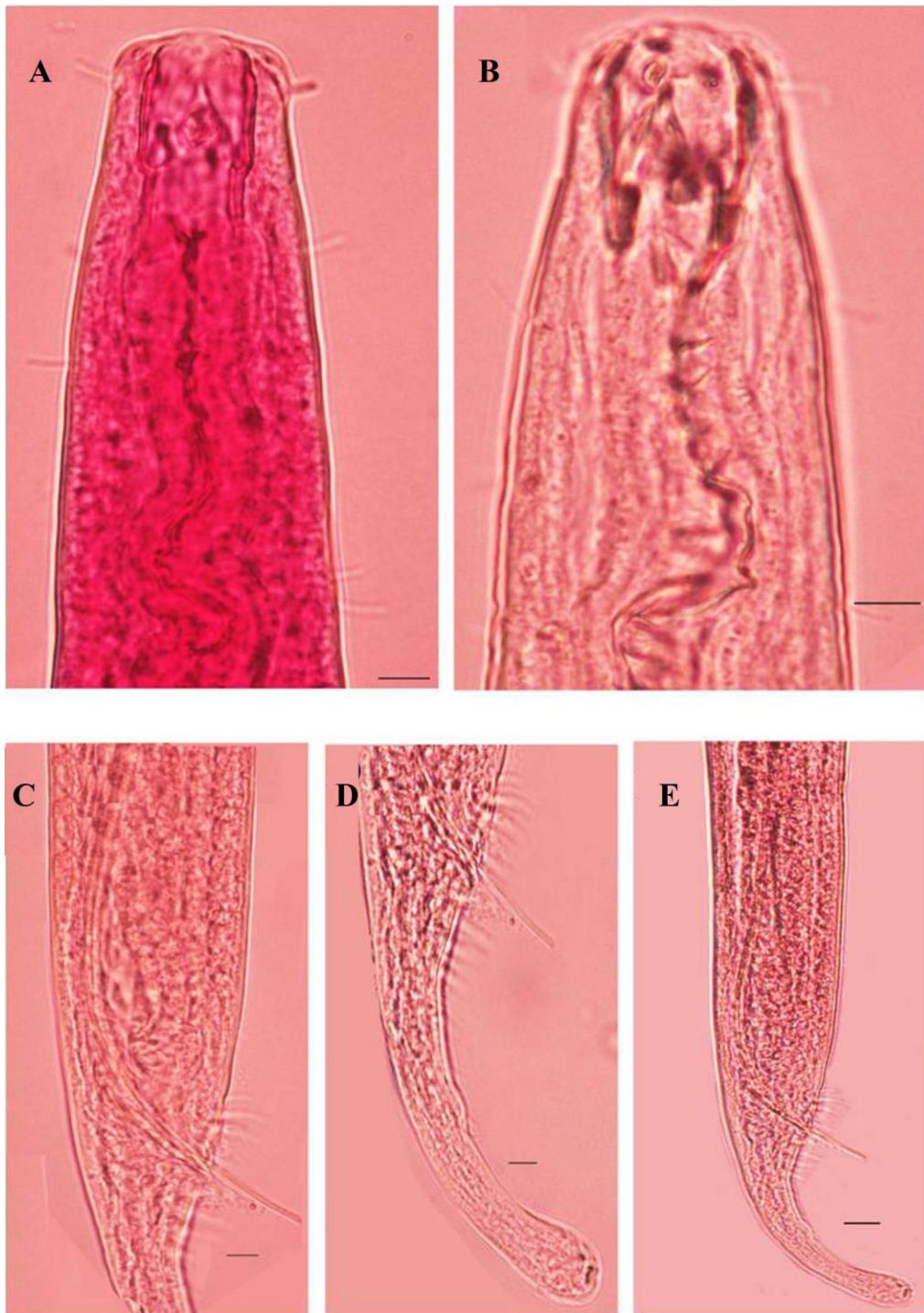
Cardia length	14	13 ± 1.0 (12-14)	17 ± 2.54 (14-20)
Body width	64	62.25 ± 1.47 (60-64)	70.25 ± 0.43 (70-71)
Body diameter at base of pharynx	53	52.75 ± 0.82 (52-54)	53.7 ± 4.14 (50-60)
Body diameter at mid body	64	62.25 ± 1.47 (60-64)	70.25 ± 0.43 (70-71)
Body diameter at anus/cloaca	31	30.5 ± 0.5 (30-31)	34.25 ± 0.82 (33-35)
Excretory pore from anterior end	76	74.7 ± 0.82 (74-76)	68.7 ± 1.29 (68-70)
Distance from pharynx end to vulva	-	-	2179 ± 50.72 (2116-2260)
Distance from pharynx end to cloaca	3542	3378.5 ± 117.20 (3218-3547)	-
Distance from vulva to anus	-	-	2385.75 ± 59.51 (2300-2465)
Vaginal depth	-	-	21.5 ± 1.68 (20-24)
Vulva from anterior end	-	-	2565.5 ± 38.21 (2506-2600)
Vulva cbd	-	-	67 ± 4.94 (60-73)
Rectum length	-	-	34.7 ± 2.94 (30-38)
Tail length	101	97.75 ± 3.4 (92-101)	134.25 ± 0.82 (133-135)
Spicule length	185	176 ± 7.77 (164-185)	-
Gubernaculum length	20	21.75 ± 1.47 (20-24)	-

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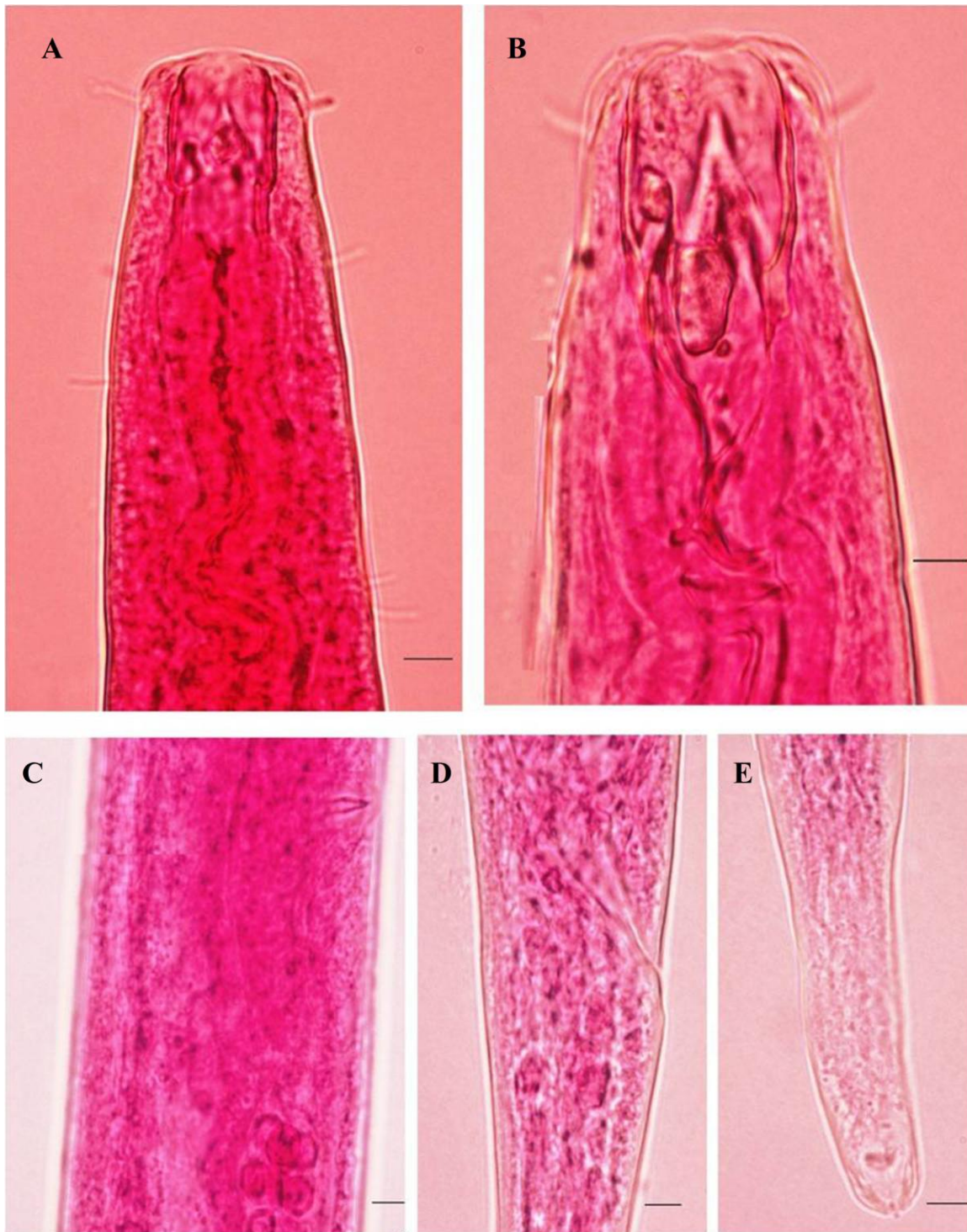


**Fig. 12 (A-H).** *Metoncholaimus medispiculatum* n. sp. A. Anterior region of male; B. Anterior region of female; C. Uvette and terminal pore; D. Pharyngo-intestinal junction of male; E. Male tail; F. Female tail; G. Vulval region; H. Demanian system.





**Fig. 13 (A-E).** *Metoncholaimus medispiculatum* n. sp. Male: A. Pharyngeal region; B. Anterior region; C-E. Tail region showing spicules and papillae (Scale: A-D=100  $\mu$ m; E= 20  $\mu$ m).



**Fig. 14 (A-E).** *Metoncholaimus medispiculatum* n. sp. Female: A. Pharyngeal region; B. Anterior region; C. Demanian system; D. Anal region; E: Tail posterior region (Scale: A-E=100  $\mu$ m).

strongly sclerotized hook-like formation situated at distal end. Tail elongate conoid, ventrally curved, 7.0 times as long as anal body diameter. Caudal glands and spinneret well developed. Tail terminus simple, without setae.

**Female:** General appearance similar to that of male. Lips well developed 24-25  $\mu\text{m}$  wide and 5-6  $\mu\text{m}$  high. Stoma funnel shaped. Amphidial fovea occupying 31-36% of corresponding body diameter, and situated at a distance of 12-14  $\mu\text{m}$  or 0.5-0.58 labial region diameters from anterior body end. Pharynx muscular. Rectum about 0.8-1.1 times as long as anal body diameter. Reproductive system monodelphic, prodelphic situated to left side of intestine. Vulva at 67-68 of body length. Tail elongate conical, ventrally curved. Caudal gland and spinneret well developed.

**Type specimens:** Holotype (male) slide No. NNRC 25/1 and paratype slide No. NNRC 25/2-4 (five males and six females) are deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan. One paratype male and one paratype female have been deposited in the nematode collection at Nematode Taxonomy Laboratory, 24 Brantwood Road, Luton, England, UK.

**Type habitat and locality:** Samples containing *Theristus* n. sp. were recovered from sandy beach of Mubarak Village and Kaemari, Sindh.

**Diagnosis and relationship:** *Theristus (Penzancia) karachiense* n. sp. belongs to “*Theristus flevensis*” group (Wieser & Hopper, 1967). This group is of old genus *Theristus* may be characterized by the structure of the spicular apparatus; the spicules are arched with twisted lateral edges, and the gubernaculum has a distal triangular plate on both sides. *T. (P) karachiense* n. sp. is characterized by its long and robust body (L = 2.4 -2.6 mm, a = 50 - 52.8), long and slender tail (c = 7.2-7.8; c' = 7-8). The new species is close to *Theristus (Penzancia) levensis* Schuurmans-Stekhoven, 1935 and *T. longisetifer*

Kitu & Arguthkar, 1998 in structure of spicular apparatus. From both the species it differs in the longer and thicker body (L = 0.93-1.4 mm; a = 42-49.7 in *T. (P) flevensis* L = 1.2-1.66 mm; a = 38.4-50 in *T. longisetifer*. L = 2.0-2.5 mm; a = 50-53 in *T. (Penzancia) n. sp.*). In longer spicule and tail length (spicules = 37-43  $\mu\text{m}$ ; tail = 130-160  $\mu\text{m}$  in *T. (Penzancia) flevensis*; spicules = 41.6-45.6  $\mu\text{m}$ ; tail = 160-192  $\mu\text{m}$  in *T. longisetifer*; spicules = 100-105 m; tail = 308- $\mu\text{m}$ ).

**Molecular identification of *Theristus (Penzancia) karachiense* n. sp.:** Sequence of the *Theristus (Penzancia) karachiense* n. sp., 18S ribosomal RNA gene has been submitted to Genbank under accession No. KY979968. The sequence length of new species is 792 bp with nucleotide composition A = 0.249, C = 0.229, G = 0.284 and T = 0.237. KY979968 showed up to 89 % similarity with closely related species.

#### *Theristus cylindricus* n. sp.

#### Morphological characters

(Fig. 18-20; Table 6)

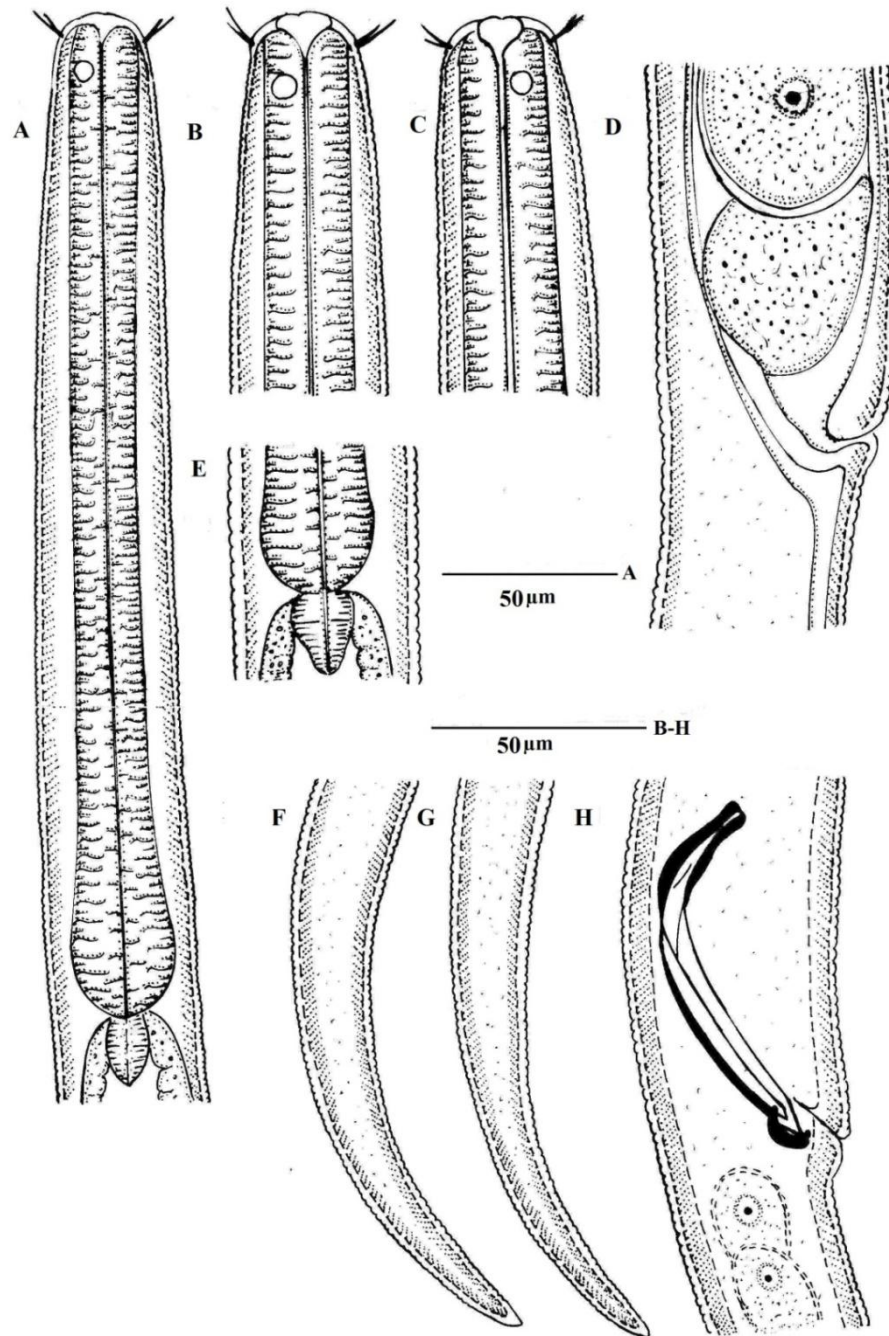
**Male:** Body relatively short and slender, tapering at both ends cuticle annulated, thin. Somatic and cervical setae absent. Labial region low. Labial sensilla not seen. Cephalic setae 5-6  $\mu\text{m}$  long 41-42 % of labial region width, stoma short funnel shaped. Amphidial fovea circle in shape 4-5  $\mu\text{m}$  in diameter, occupying 28-33 % of corresponding body diameter. Anterior margin of amphidial fovea situated at a distance of 6-10  $\mu\text{m}$  or 1.4-1.8 labial region diameters from anterior body end.

Pharynx slender, muscular surrounding buccal cavity swollen proximally. Cardia short separate from oesophagus. Ventral gland cell not observed Testis single, outstretched, comparatively long and situated to right side of intestine. Spicules strongly sclerotized, proximally cephalated, 38-40  $\mu\text{m}$  long, with twisted lateral edges.

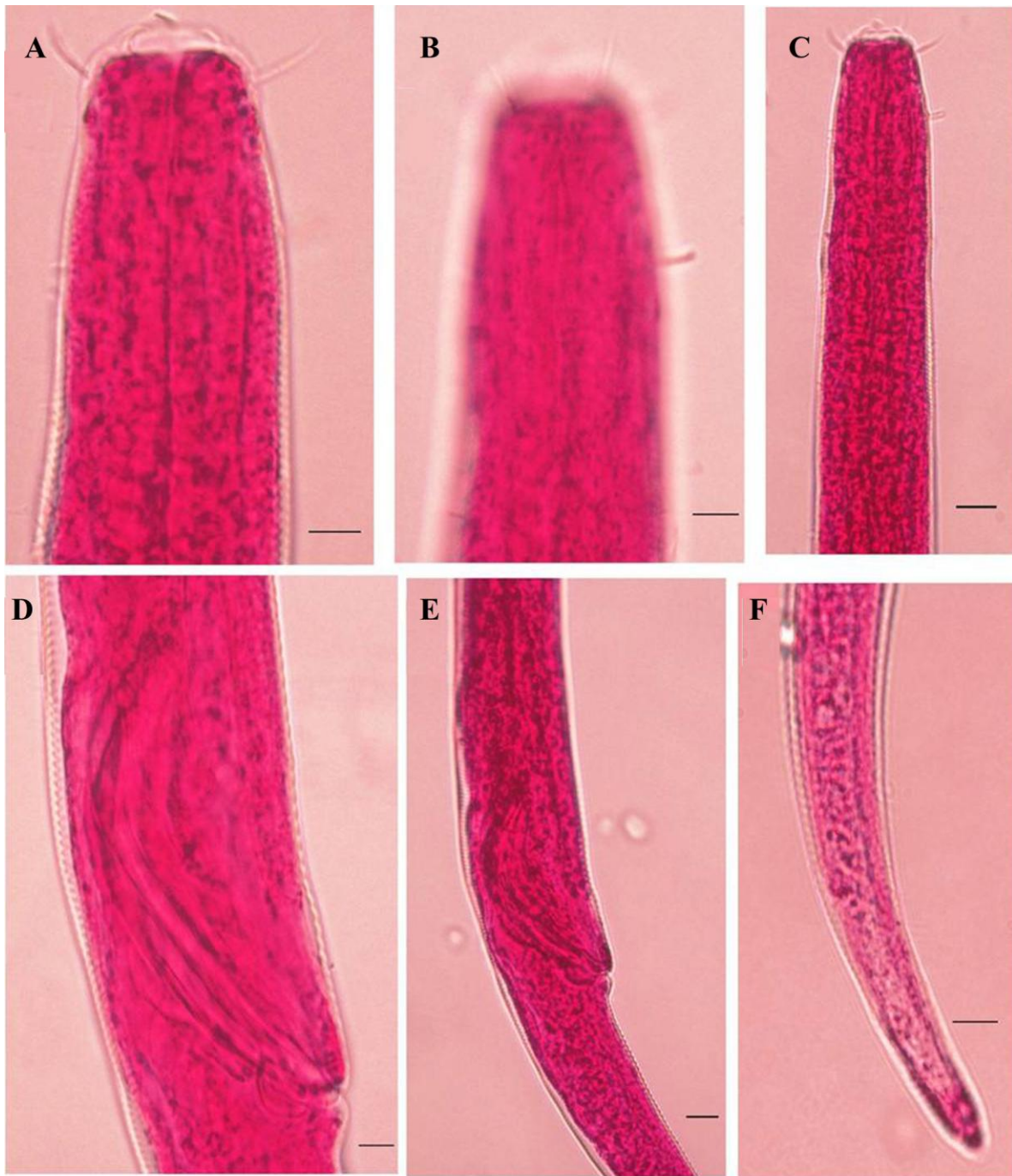
**Table 5. Measurements of *Theristus (Penzancia) karachiense* n. sp. in  $\mu\text{m}$  except L.**

Characters	Holotype male	Paratypes	
		Males (n=2)	Female (n= 6)
L (mm)	2.43	2.43, 2.47	2.34 $\pm$ 0.20 (2.0-2.5)
a	52.8	52, 53	45.86 $\pm$ 3.36 (42.4-50)
b	8.4	8.4, 8.0	9.24 $\pm$ 0.89 (8-10.4)
c	7.8	7.8, 7.2	8.64 $\pm$ 0.24 (8.3-8.9)
c'	7.0	7.0, 6.9	8.2 $\pm$ 0.42 (7.8 - 9.0)
V	-	-	67.9 $\pm$ 1.05 (67.2-70.0)
Lip width	24	24, 26	24.8 $\pm$ 0.89 (24-26)
Cephalic setae length	12	10, 12	9.83 $\pm$ 1.21 (9-12)
Amphid width	6	5, 6	5.5 $\pm$ 0.44 (5-6)
Amphid from anterior end	12	12,14	12 $\pm$ 0.81 (11-13)
Amphid cbd	21	21, 23	27.5 $\pm$ 0.5 (27-28)
Body width	46	46, 48	58 $\pm$ 4.1 (50-62)
Pharynx length	288	288, 290	270 $\pm$ 20.66 (240-288)
Posterior end of pharynx to vulva	-	-	1474.1 $\pm$ 6.28 (1462-1480)
Posterior end of pharynx to cloaca	1831	1830-1831	-
Vulva- anus	-	-	245 $\pm$ 40.45 (184-296)
Body diameter at base of pharynx	42	42, 44	44.4 $\pm$ 1.01 (43-46)
Tail length	310	310, 313	280 $\pm$ 5.57 (270 - 286)
Cloaca / anal body width	44	44, 46	33.8 $\pm$ 2.0 (30 - 36)
Spicule length	100	100, 102	-
Gubernaculum length	12	12, 11.5	-

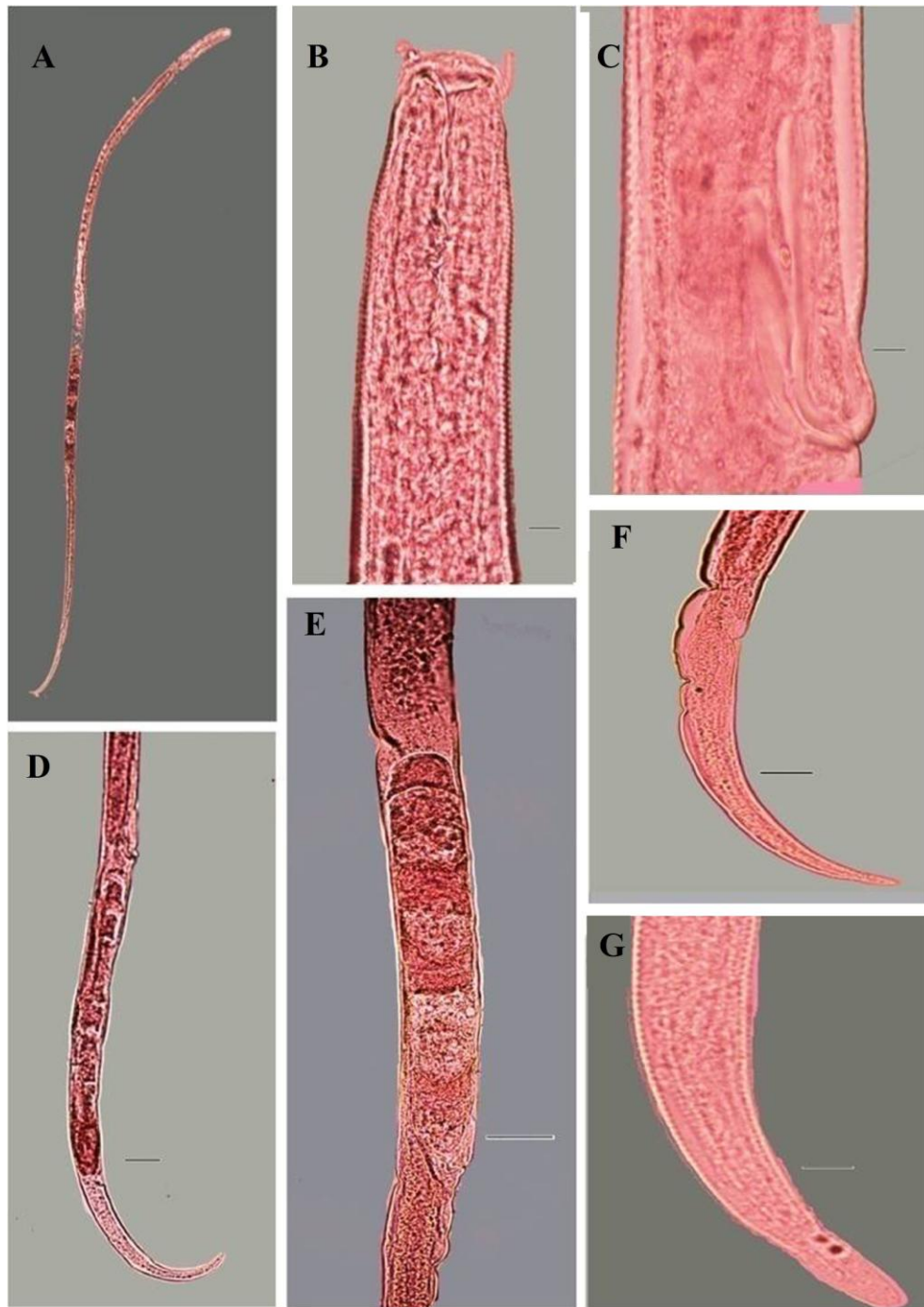




**Fig. 15 (A-H).** *Theristus (Penzancia) karachiense* n. sp. A. Pharyngeal region of male; B. Anterior region of male; C. Anterior region of female; D. Vulval region; E. Pharyngo-intestinal junction of male; F. Male tail; G. Female tail; H. Spicules.



**Fig. 16 (A-F).** *Theristus (Penzancia) karachiense* n. sp. Male: A. Anterior region; B. Anterior region showing amphid; C. Pharyngeal region; D-E. Spicule; F. Tail region (Scale: A, B, D, F= 100  $\mu$ m; C, E= 20  $\mu$ m).

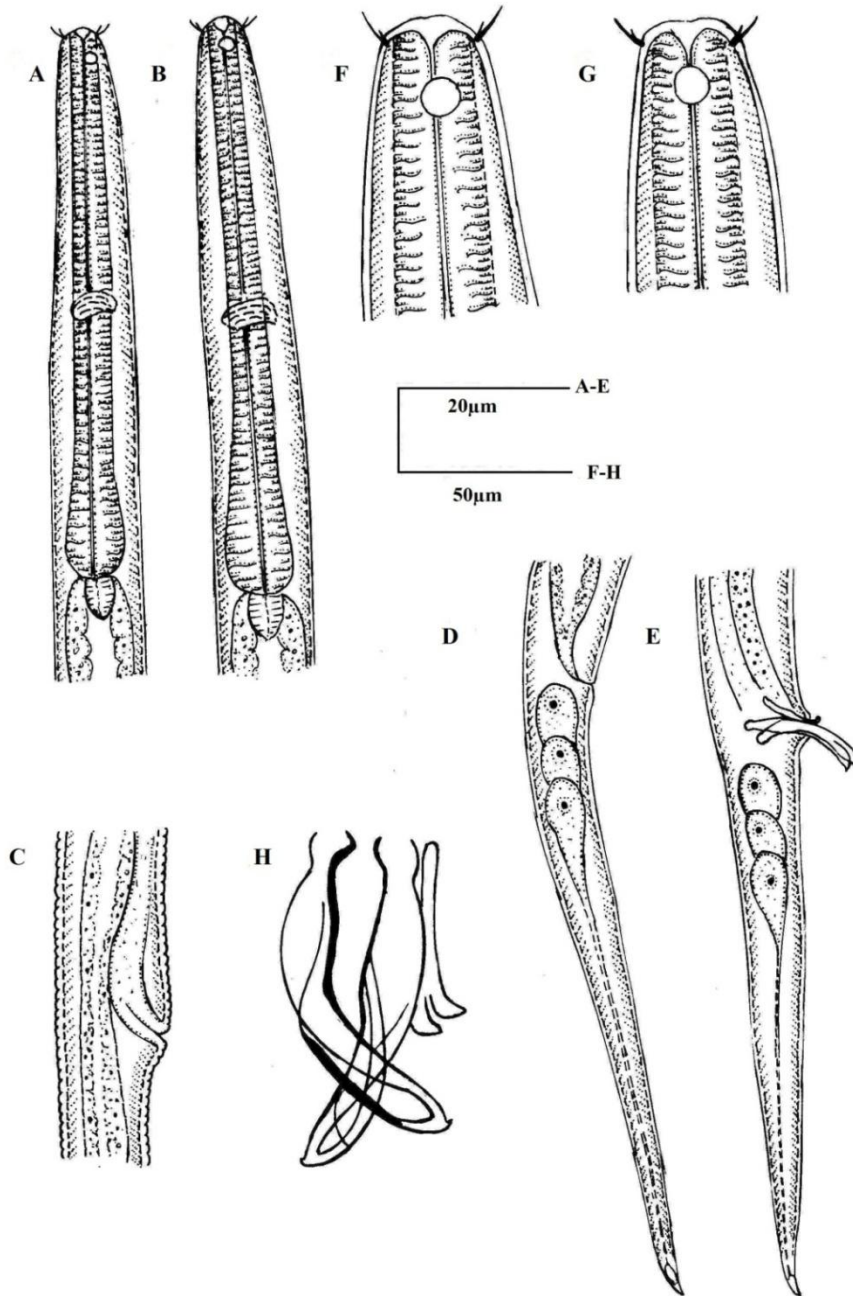


**Fig. 17 (A-G).** *Theristus (Penzancia) karachiense* n. sp. Female: A: Whole body; B. Anterior region; C. Vulval region; D-E. Female gonad; F-G. Tail region (Scale: A= 4  $\mu$ m; B, C, G= 100  $\mu$ m; D= 10  $\mu$ m; E, F= 20  $\mu$ m).

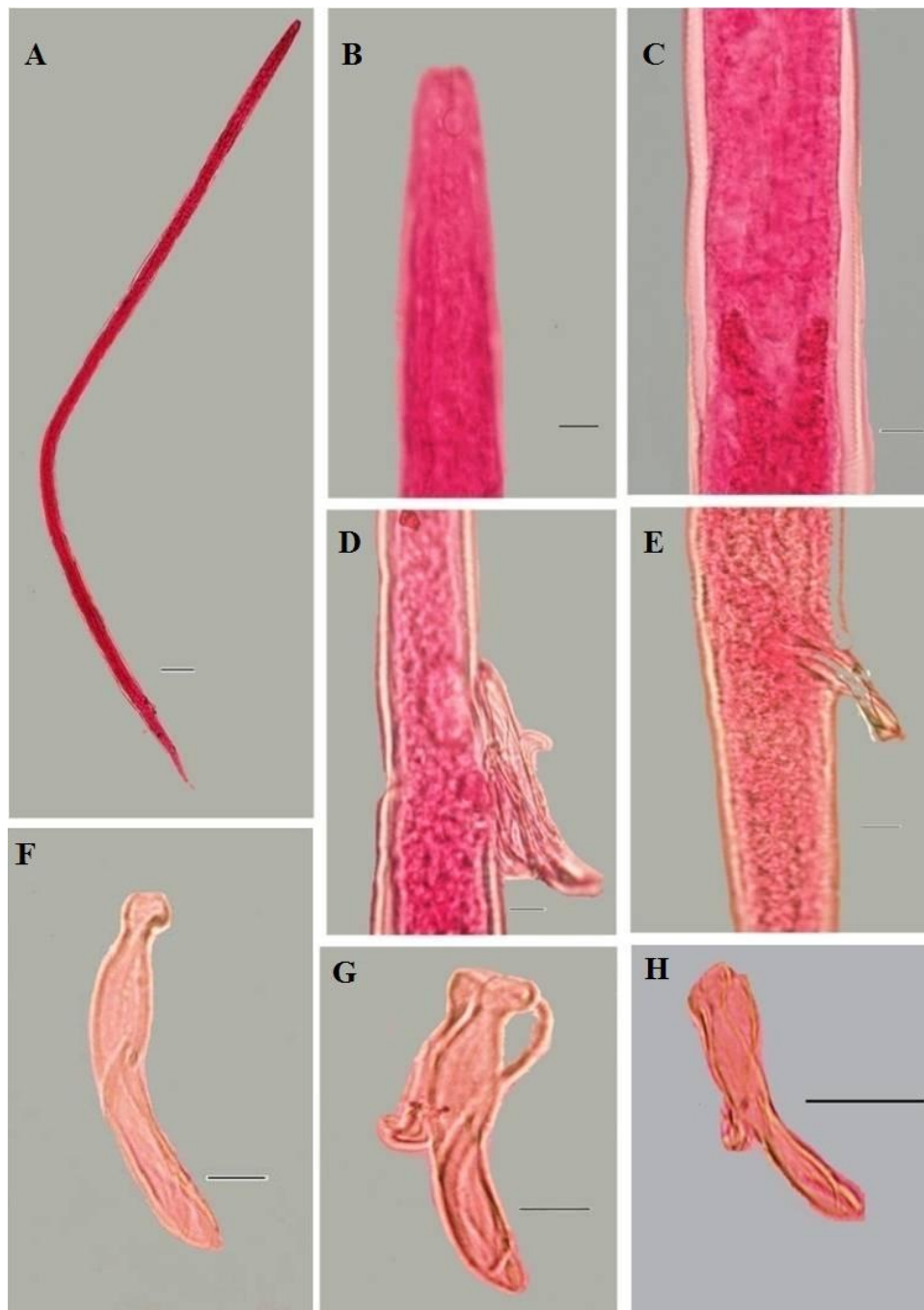
**Table 6. Measurement of *Theristus cylindricus* n. sp. in  $\mu\text{m}$  except L.**

Characters	Holotype male	Paratypes	
		Males (n=10)	Female (n=4)
L (mm)	1.44	1.38 $\pm$ 0.09 (1.29-1.6)	1.53 $\pm$ 0.08 (1.44-1.65)
a	48.1	44.7 $\pm$ 3.11 (40.3-49.0)	43.9 $\pm$ 5.1 (36-50)
b	8.5	8.1 $\pm$ 0.32 (7.9-8.9)	8.53 $\pm$ 0.45 (7.9-9.1)
c	8.2	8.21 $\pm$ 0.61 (7.7-9.9)	8.0 $\pm$ 0.45 (7.9-8.4)
c'	6.2	5.9 $\pm$ 0.50 (5.0-6.9)	6.05 $\pm$ 2.95 (7.0-1.0)
V	-	-	65.2 $\pm$ 7.7 (55-73.6)
Lip width	12	12.33 $\pm$ 0.81 (11-13)	13 $\pm$ 0.70 (12-14)
Cephalic setae length	5	5.7 $\pm$ 0.45 (5-6)	5.5 $\pm$ 0.5 (5-6)
Amphid width	4	4.1 $\pm$ 0.2 (4-5)	4.25 $\pm$ 0.43 (4-5)
Amphid from anterior end	8	8.2 $\pm$ 1.07 (6-10)	7.75 $\pm$ 0.43 (7-8)
Amphid cbd	14	14.6 $\pm$ 0.48 (14-15)	14.5 $\pm$ 0.5 (14-15)
Poster end of pharynx to vulva	-	-	816 $\pm$ 65.0 (730-880)
Poster end of pharynx to cloaca	1000	1098.6 $\pm$ 121.8 (992-1320)	-
Vulva to anus	-	-	253 $\pm$ 6.40 (244-262)
Body diameter at base of pharynx	28	27.4 $\pm$ 2.90 (25-30)	28.75 $\pm$ 0.82 (28-30)
Spicule length	40	39.8 $\pm$ 1.16 (38-40)	-
Gubernaculum length	21	22.1 $\pm$ 1.37 (20-24)	-
Tail length	174	168.5 $\pm$ 8.58 (150-180)	192.2 $\pm$ 7.81 (180-200)
Anal body width	28	28.5 $\pm$ 1.77 (25-32)	23.2 $\pm$ 2.04 (20-25)
Body width	30	31.1 $\pm$ 1.70 (29-34)	32.7 $\pm$ 1.63 (30-34)
Pharynx	168	1.69 $\pm$ 9.7 (156-190)	176.2 $\pm$ 9.95 (160-187)

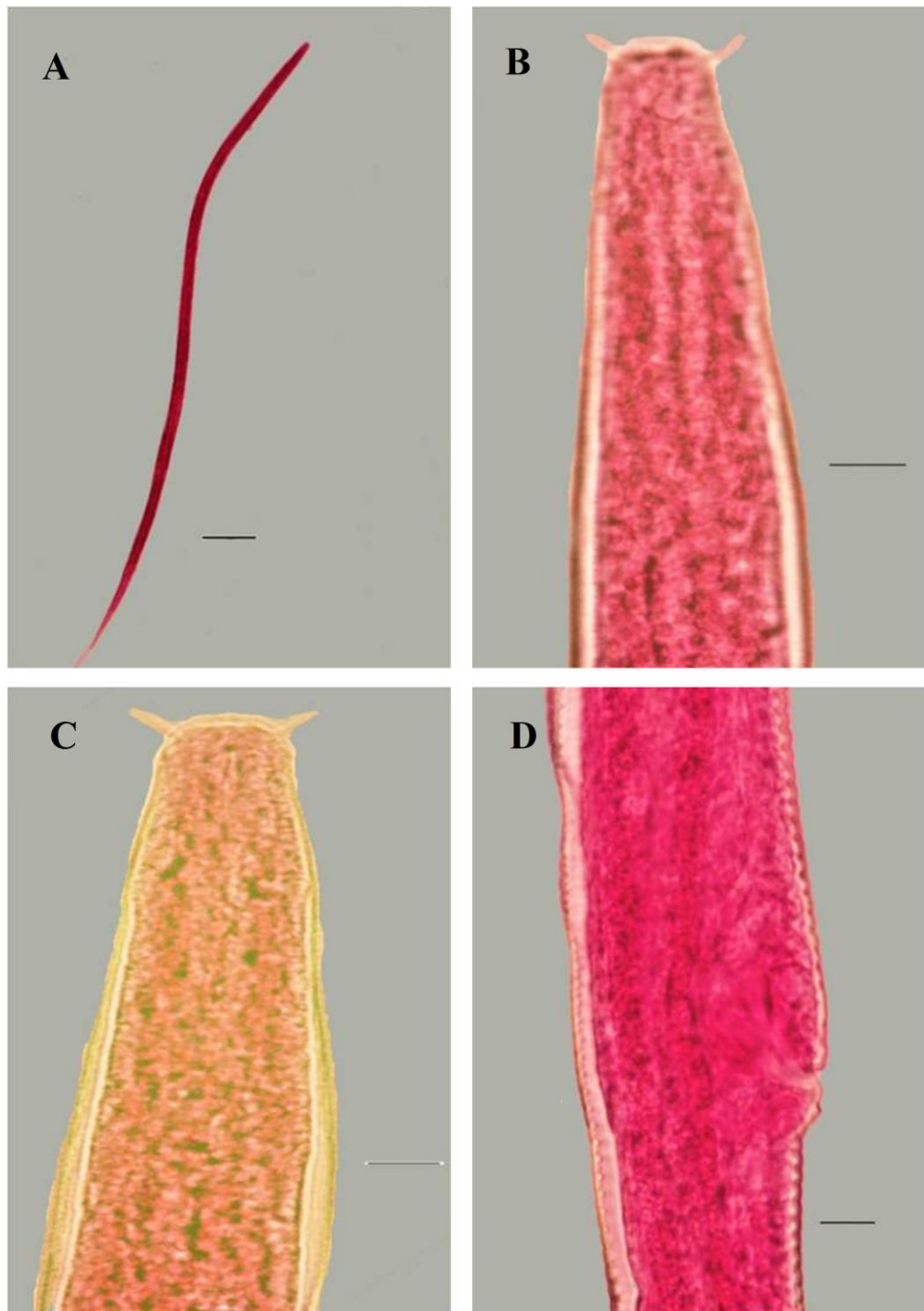




**Fig. 18 (A-H).** *Theristus cylindricus* n. sp. A. Pharyngeal region of male; B. Pharyngeal region of female; C. Vulval region. D. Female tail; E. Male tail; F. Anterior region of male; G. Anterior region of female; H. Spicules.



**Fig. 19 (A-H).** *Theristus cylindricus* n. sp. Male: A. Whole body; B. Anterior region showing amphid; C. Pharyngo-intestinal junction; D-E. Tail region showing spicules; F-H. Spicules (Scale: A= 4  $\mu$ m; B-H= 100  $\mu$ m).



**Fig. 20 (A-D).** *Theristus cylindricus* n. sp. Female: A. Whole body; B. Anterior region showing amphid; C. Anterior region; D. Vulval region (Scale: A= 4 μm; B-D= 100 μm).

Gubernaculum about 20-24  $\mu\text{m}$  long, characteristic in shape. Tail almost straight slightly ventrally curved. Caudal setae absent. Caudal glands and spinneret well developed subterminal setae absent.

**Female:** General appearance, structure of cuticle and anterior portion of body similar to male. Lips well developed. Stoma funnel shaped. Amphidial fovea occupying 26-28% of corresponding body diameter. Anterior margin of amphidial fovea situated at a distance of 6 - 8  $\mu\text{m}$  or 0.5-0.6 labial region diameters from anterior end. Pharynx muscular, slender, slightly widening at the posterior end. Reproductive system monodelphic, prodelphic and left of intestine. Vagina short curved to anterior end of the body contour. Vulval glands and posterior sac absent. Tail elongate conoid, curved ventrally. Caudal glands and spinneret well developed. Caudal setae absent.

**Type specimens:** Holotype (male) slide No. NNRC 26/1 and paratype slide No. NNRC 26/2-4 (nine males and three females) are deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi Pakistan. One paratype male and one paratype female have been deposited in the nematode collection at Nematode Taxonomy, Laboratory, 24 Brantwood Road, Luton, England, UK.

**Type habitat and locality:** Samples containing *Theristus cylindricus* n. sp. were recovered from the Balochistan coastal area of Gadani, Sonmiani and Mubarak Village and Kaemari of Sindh coast.

**Diagnosis and relationship:** *Theristus cylindricus* n. sp. belongs to “*Theristus flevensis*” group (Wieser & Hopper, 1967). This group of the genus *Theristus* characterized by the structure of the spicular apparatus, the spicules are arched with twisted lateral edges, and the gubernaculum has a distal triangular plate on distal end. *T. cylindricus* n. sp. is characterized by its medium size and thin body

( $L = 1.3-1.6$  mm,  $a = 40-49$ ), long and slender tail ( $c = 7.7-9.9$ ,  $c' = 5.0-6.9$ ). The new species is close to *T. flevensis* Schuurmans-Skethoven, 1935 and *T. (P) karachiense* n. sp. in structure of spicular apparatus. From *T. flevensis* it differs in maximum body width (29-34 vs 42-58 $\mu\text{m}$  ( $a = 40-49$  vs 20-31) and in shorter length of cephalic setae (six longer setae 5-6 vs 9-11  $\mu\text{m}$ ). The new species differs from *T. (P) karachiense* n. sp. in shorter body, spicules and tail length ( $L = 1.3-1.6$  vs 2.43, 2.47 mm; spicules = 38-40 vs 100, 102  $\mu\text{m}$ , tail length = 150-180 vs 310, 313  $\mu\text{m}$ ).

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