INT. J. BIOL. BIOTECH., 11 (4): 497-504, 2014.

FRESH WATER NEMATODES FROM SINDH, PAKISTAN

S. Shamim, K. Nasira and F. Shahina[†]

National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan
[†]Corresponding author emails: shahinafayyaz@gmail.com; nnrcku@gmail.com

ABSTRACT

During the survey of fresh water nematodes in Sindh Pakistan, four known nematodes species viz., *Basirotyleptus (Basirotyleptus) basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992; *Propanagrolaimus hygrophilus* (Bassen, 1940) Andrássy, 2005; *Prismatolaimus intermedius* (Bütschli, 1873) de Man, 1880 and *Achromadora ruricola* (de Man, 1880) Micoletzky, 1925, belonging to four families have been encountered. These species are reported for the first time from Pakistan. *Basirotyleptus* Jairajpuri, 1964 and *Propanagrolaimus* Andrássy, 2005 are new recorded genera from Pakistan.

Key-words: Fresh water nematodes, new record, *Basirotyleptus basiri*; *Propanagrolaimus hygrophilus*; *Prismatolaimus intermedius*; *Achromadora ruricola*, Sindh, Pakistan

INTRODUCTION

Nematodes are one of the most abundant taxa in fresh water benthic communities (Traunspurger, 2000). Nematodes can be used as bio-indicators of physicochemical characteristics of waters and can become useful pollution indicators. Fresh water nematode densities can reach up to $3.5x10^6$ individuals per m^2 (Traunspurger, 2002). They play a major role in ecosystem processes. They usually inhabit the superficial stratum of the sediment, especially those rich in organic material. They are closely connected to benthos sediment of any water environment (river, lakes, springs, dams, ponds etc.) as well as algae or root masses of certain aquatic plants. Among these fresh water inhabitants appear some amphibious species which can inhabit both continental water environments and land ones, and other species typical of fresh water environments. Fresh water nematodes usually inhabit the superficial stratum of the sediment, especially in those rich in organic material. Nematode community structure in river is related to the contamination sources and pollution level (Wu *et al*., 2010).

Several sediments samples along with water were collected from Jamshoro, Karachi canal, Indus River Almanzar Hotel, Hyderabad (Detha), Kot Ghulam Mohammad, (Mir Ki Landhi Stream), Mirpurkhas supply pond and from Kalri water lake, Karachi, Sindh, Pakistan during 2010-11. These areas have tropical climate with temperature ranging from minimum 4 ºC in cold season and 40 °C maximum in hot season. The fresh water nematodes have received little attention in Pakistan. Earlier from Pakistan the genus *Achromadora* Cobb, 1913 was reported from cotton growing areas of Punjab, Pakistan (Anwar and Khan, 1973; Tanveer and Haq, 1975) while the genus *Prismatolaimus* de Man, 1880 reported from Karachi, Sindh (Shahina, 2005).

During the present study, four new record species of nematodes have been identified as *Basirotyleptus (B) basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992; *Propanagrolaimus hygrophilus* (Bassen, 1940) Andrássy, 2005; *Prismatolaimus intermedius* (Bütschli, 1873) de Man, 1880 and *Achromadora ruricola* (de Man, 1880) Micoletzky, 1925. The genera *Basirotyleptus* Jairajpuri, 1964 and *Propanagrolaimus* Andrássy, 2005 were reported for the first time from Pakistan.

MATERIALS AND METHODS

Extraction of sample material was done by Cobb's decanting and sieving techniques (Cobb, 1918) and modified Baermann (1917) funnel technique. They were fixed in 4% formaldehyde solution and processed to anhyderous glycerine according to Hooper *et al*. (2005). Permanent mounts were made in anhydrous glycerol covered with a 19 mm cover slip sealed by paraffin wax. Measurements and drawings were made with an ocular micrometer and a drawing tube attached to a compound microscope. All measurements were made at 100x of compound microscope along the median line. Nematodes were identified according to Eyualem-Abebe *et al*. (2006).

RESULTS / DESCRIPTION

Basirotyleptus (*Basirotyleptus*) *basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992 (Fig. 1 A-E)

Measurements Table 1.

Description

Female: Body slightly ventrally curved upon fixation. Body tapering anterior to base of oesophagus. Cuticle smooth, inner finely striated, loose in many regions, radial element absent. Cuticle 1.6-2 µm thick at mid body and 3-4 µm on tail. Lateral hypodermal chords about ½ as wide as body. Lateral body pores indistinct. Lip region distinctly set off from body, three times as wide as high. Amphids stirrup shaped almost occupying the lip width 9-10 µm long. Six small liplets around the cuticularized oral opening. Stoma long narrow conical. Odontostyle solid needle like with narrow lumen and obscure aperture, 8-10 µm or about 1.3-1.4 lip width long. Guiding ring 10 µm from anterior end. Odontophore rod like 11-12 µm about as long as odontostyle. Oesophagus a slender tube with a pyriform basal bulb measuring 18 x10 µm in dimension, occupying about 16-17% of oesophageal length. Nerve ring surrounding anterior slender part of oesophagus, slightly posterior to the middle of oesophagus at 59-64 µm from anterior end. Cardia small round to hemispheroid 3-4 µm long.

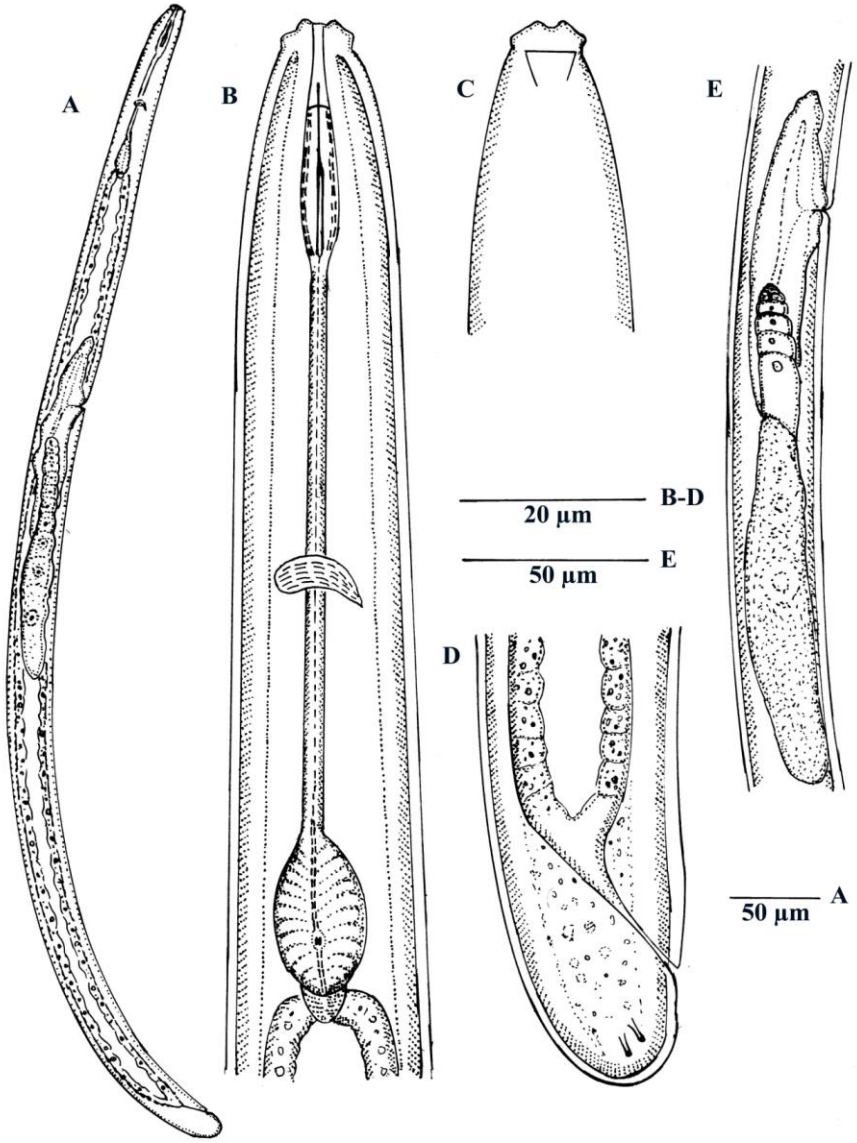

Fig. 1 (A-E). *Basirotyleptus* (*B*) *basiri* Jairajpuri, 1964. Female: A. Whole body; B. Oesophageal region; C. Anterior region showing amphid; D. Tail region; E. Reproductive system.

Table 1. Morphometrics of *Basirotyleptus* (*B*) *basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992; *Propanagrolaimus hygrophilus* (Bassen, 1940) Andrássy 2005; *Prismatolaimus intermedius* (Bütschli, 1873) de Man, 1880 and *Achromadora ruricola* (de Man, 1880) Micoletzky, 1925. All measurements in µm.

Characters	***Basirotyleptus* (*B*) *basiri***	***Propanagrolaimus hygrophilus***		***P. intermedius***	***Achromadora ruricola***
	Females n=8	Female n=4	Male n=2	Females n =5	Females n=6
L (Total body length)	690±44.9 (630-780)	908±102.5 (800-1030)	840-970	556±22.9 (530-591)	489.8±37.6 (450-551)
a (Total body length / maximum body width)	25.12±3.30 (19.9-28.3)	38.6±1.52 (36.5-40)	40-42	35.08±1.17 (33.5-37.4)	20.3±1.05 (19-22)
b (Total body length / oesophageal length)	5.88±0.51 (5.0-6.8)	6.15±0.6 (5.5-6.8)	5.3-5.5	4.4±0.4 (3.9-4.9)	5.96±0.28 (5.9-6.5)
c (Total body length / tail length)	59.9±3.99 (56.5-69)	8.1±0.62 (7.5-8.8)	9.8-12	3.86±0.17 (3.7 – 4.1)	7.25±0.18 (7.0-7.5)
c´ (Tail length / anal body width)	0.67±0.06 (0.59-0.75)	7.38±1.13 (6.3-8.4)	3.9-5.00	12.4±46.6 (11.7-13.3)	5.52±0.54 (5.1-6.3)
V% (Vulva from anterior end / Total body length x 100)	36.1±1.08 (33.9-37.7)	54.9±1.19 (54-56.5)	-	58.24±2.26 (55.9-61.9)	47.3±1.36 (46- 49)
Lip width	8.75±0.89 (8.0-10)	8.75±0.96 (8.0-10)	8-11	8.4±0.54 (7-9)	10±0.89 (9-11)
Stoma length	9.5±0.53 (9.0-10)	9.5±1.29 (8-11)	10-12	11±1.0 (10-12)	10±1.4 (8.0-12)
Pharyngeal corpus length	-	93±1.83 (91-95)	90-91	-	-
Isthmus length	-	37.5±6.45 (30-45)	30-42	-	-
Bulb length	19.0±3.27 (18-20)	20±0.82 (19-21)	20-22	-	12.33±1.03 (11-14)
Pharyngeal length	121.25±11.94 (105-130)	149.5±3.42 (146-154)	145-158	119.6±14.54 (108-145)	78.3±1.63 (76-80)
Nerve ring	60.25±2.49 (57-64)	111.2±2.99 (108-115)	100-112	62.4±2.07 (60-65)	42.5±1.9 (40-45)
Body diameter, at base of oeso.	22.5±1.19 (21-24)	20±0.82 (19-21)	20-21	14.8±0.84 (14-16)	17.9±0.91 (17-19)
Body diameter, at mid body	26.88±3.72 (25-36)	20.5±1.29 (19-22)	20-22	16.8±0.84 (16-18)	23±0.89 (22-24)
Body diameter, at anus	17.88±1.64 (16-20)	14±0.82 (13-15)	20-22	11.7±0.45 (11-12)	12.8±0.98 (12-14)
Rectum length	15.75±1.49 (14-18)	14.5±1.29 (13-16)	-	7.8±0.84 (7-9)	10.8±0.98 (10-12)
Tail length	11.75±1.16 (10-13)	98.8±8.54 (90-110)	90-96	143±12.04 (130-160)	63.67±2.07 (60-66)
Vulva to anterior end	258±7.29 (243 -266)	467±.2±22.7 (454-500)	-	320 ±12.81 (305-330)	216.8±10.6 (208-236)
Spicule length	-	-	20-22	-	-
Gubernaculum length	-	-	11-14	-	-

Female reproductive system mono-opisthodelphic. Vulva a transverse slit. Anterior branch of reproductive organs sac like, slightly longer than vulvar body width. Posterior reproductive branch well developed, reflexed at the oviduct. Oocytes arranged in a single row. Prerectum not distinct. Rectum about one anal body width long. Tail dorsally convex, obtusely rounded, slightly less than one anal body width long with 2-3 caudal pores on each side.

Male: Not found.
Remarks: *Basirotyleptus* (*B*) *basiri* (Jairajpuri, 1964) Jairajpuri & Ahmad, 1992 collected from sediments of Kalri lake, Karachi. Specimens of *Basirotyleptus* (*B*) *basiri* well agree with the original description, however it is slightly longer (0.63-0.78 vs 0.50-0.58 mm) in size and in lower c´ value 0.59-0.75 vs one anal body width long.

Propanagrolaimus hygrophilus (Bassen 1940) Andrássy, 2005
(Fig. 2 A-G)

Measurements Table 1.

Description

Female: Body straight or slightly curved, tapering at both ends.Cuticle finely annulated.Lips amalgamated, continuous with neck, 7.5-11µm wide. Stoma panagrolaimoid 7-12 µm long, with cheilo-gymno and stegostom, gymnostom some what longer than cheilostom. Metastom with a distinct tooth. Pharyngeal corpus cylindrical about twice or more isthmus length, occupying 61-64% of neck length. Junction of pharyngeal corpus isthmus is distinctly marked. Isthmus slender 30-45µm long, narrow than pharyngeal corpus and is surrounded by nerve ring which is at 65-68% of neck length. Excretory pore opposite isthmus at 74-79% of neck length. Deirid at level of isthmus at 72-85% of neck length. Cardia, conoid surrounded by intestinal tissue. Reproductive system monodelphic-prodelphic. Postvulval uterine sac absent. Vulva not protruding located posterior to mid body. Oviduct well developed, uterus tubular, consisting of long proximal tubular part and short distal part with thinner walls. Vagina extending inwards to one third of body width. Vulva-anus distance equal to 1.7-2.5 tail lengths. Tail elongate conoid with acute tip. Phasmid at 53-58% of tail length.

Male: General morphology similar to female. Reproductive system monorchic. Testis reflexed. Spicules 20-22 µm long, gubernaculum 11-13 cm long. Three preanal and three postanal pairs of papillae. Tail elongate conical with sharp tip.

Remarks: The description and morphometric data were based on the specimens collected from Kot Ghulam Mohammad (Mir Ki Landhi) area of Sindh, Pakistan. Measurements and morphological characters are quite similar to *Propanagrolaimus hygrophilus* (Bassen, 1940) Andrássy, 2005 given by Andrássy (2005) and Abolafia & Pena Santiago (2005). This species is reported for the first time from Pakistan.

Prismatolaimus intermedius (Bütschli, 1873) de Man, 1880
(Fig. 3 A-E)

Measurements Table 1.

Description

Female: Body slender 16-18 µm wide, almost straight or slightly curved ventrally, tapering gradually into filiform tail. Cuticle thin finely annulated but distinctly marked. Somatic setae short 1-2 µm long. Labial region not separated from adjacent body, 7-9 µm wide at level of setae. Cephalic setae consist of six longer and four shorter submedial setae, arranged in one circle. Amphidial apertures slit like located at 25-32 µm from head end of body. Stoma moderately spacious with sclerotized wall armed with a small dorsal tooth and two small minute subventral teeth at the base. Stoma 10-12 µm long and 3-5 µm wide. Oesophagus more or less cylindrical 108-145 µm long occupying 20-24% body length. Cardia spherical in shape with large adjacent gland cells. Nerve ring at about 60-65 µm from anterior end. Reproductive system monodelphic-prodelphic without post vulval uterine sac. Vulva transverse, 55.9-61.9 % of body length. Vulva to anus distance is 70-75 µm. Vulval lips not raised, and are in a depression of body. Vagina directed inward and forward. Tail 130-160 µm long, filiform and has distinct terminal mucron. Rectum 7-9 µm long.

Male: Not found.

Remarks: The specimens of *Prismatolaimus intermedius* (Bütschli, 1873) de Man, 1880 were collected from tube well and stagnant pond water from Mirpurkhas. The measurements and general morphological characters well fit with the measurements of Andrássy (2005). This species is reported for the first time from fresh water in Sindh, Pakistan.

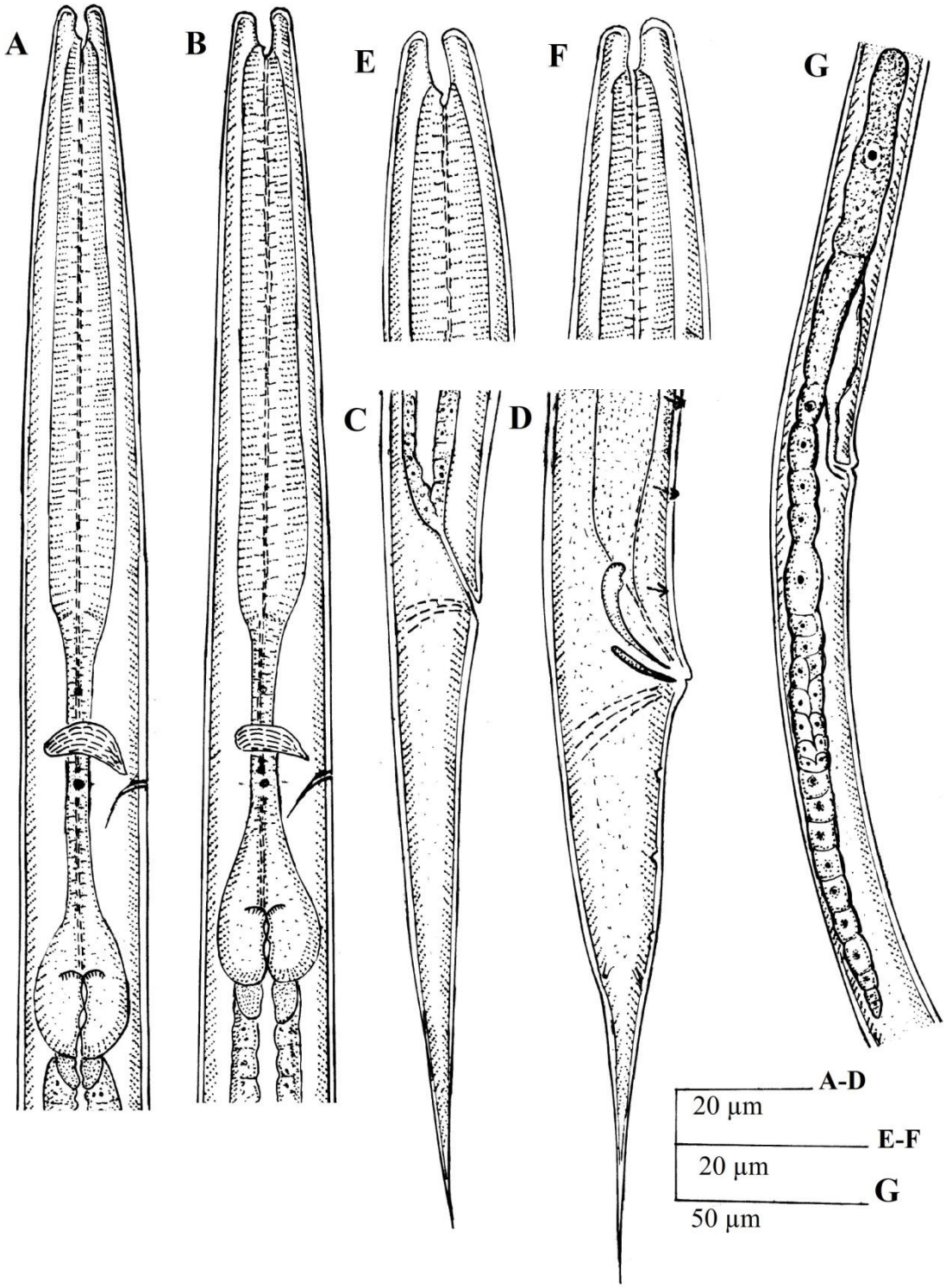

Fig. 2 (A-G). *Propanagrolaimus hygrophilus* (Bassen, 1940) Andrássy, 2005. Male: A. Oesophageal region; F. Anterior region; D. Tail region; Female: B. Oesophageal region; C. Tail region; E. Anterior region; G. Reproductive system.

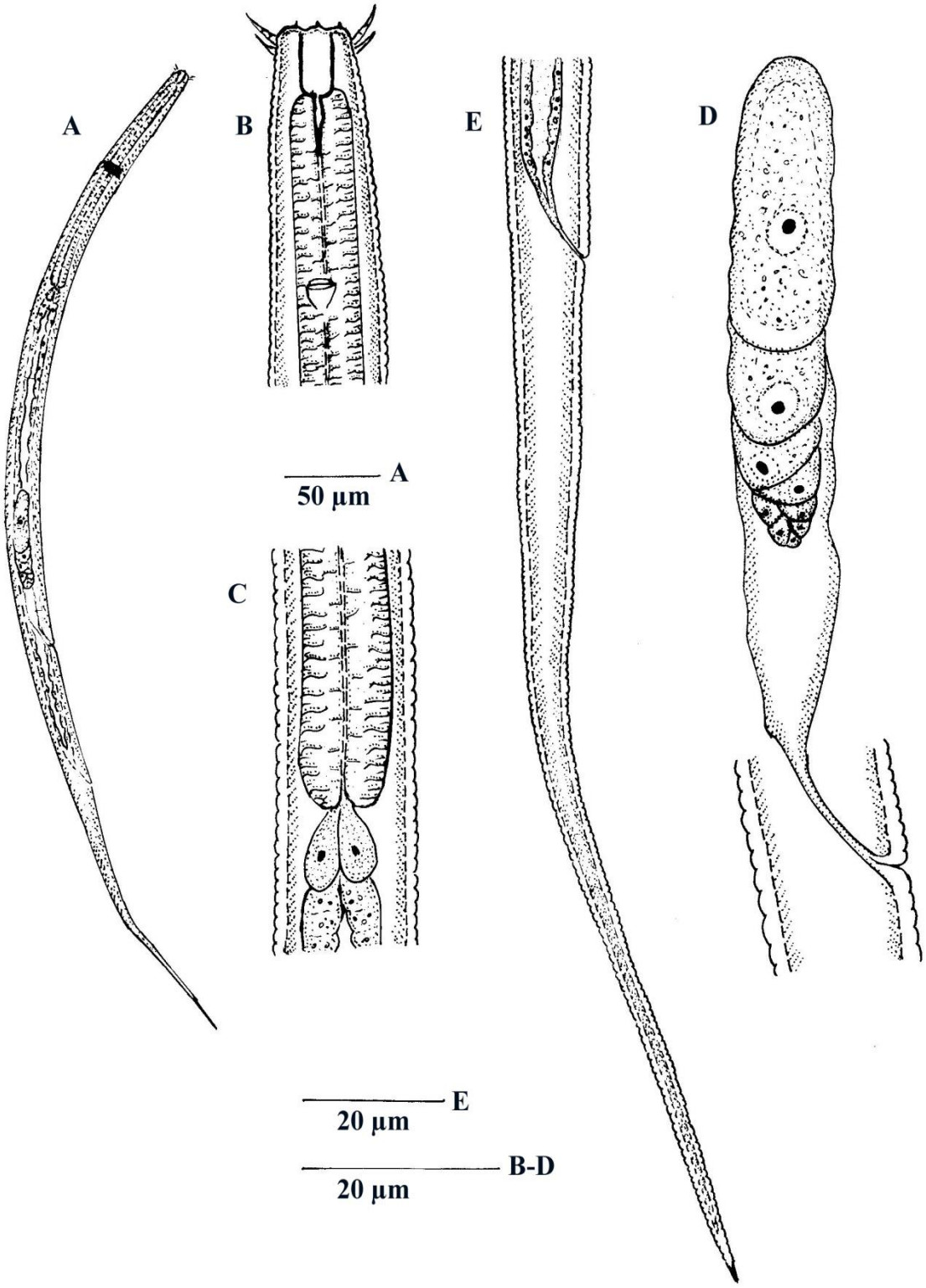

Fig. 3. (A-E). *Prismatolaimus intermedius* (Bütschli, 1873) de Man, 1880. Female: A. Whole body; B. Anterior region; C. Basal region of oesophagus; D. Reproductive system; E. Tail region.

***Achromadora ruricola* (de Man, 1880) Micoletzky, 1925**
(Fig. 4 A-D)

Measurements Table 1.

Description

Female: Body slightly curved ventrally, cylindrical, tapering at both ends. Cuticle finely annulated, annules 1 µm wide, with distinct fine punctations. Somatic setae scattered. Lip region not offset, 9-11 µm wide. Labial papillae and ten cephalic setae 2 µm long, at the base of labial region. Amphid spiral or unispiral, at the level of stoma, 3 µm in diameter and its corresponding body diameter (cbd) is 10-12 µm. Stoma 8-12 µm long, funnel shaped with dorsal tooth medially situated and 1 or 2 smaller subventral teeth situated posteriorly. Oesophagus 76-80 µm long, with moderately basal bulb. Oesophagus intestinal valve is spheroid shaped. Nerve ring at 40-45 µm from anterior end. Vulva more equitorally placed at 46.2-49% of body length. Vulva to anus distance is 180-184 µm and 2.8 times long as tail. Vulval lips slightly protruding from body. Ovary paired, didelphic, reflexed. Rectum length is 10-12 µm equal to anal body width. Tail 60-66 µm long conical and has a small terminal process.

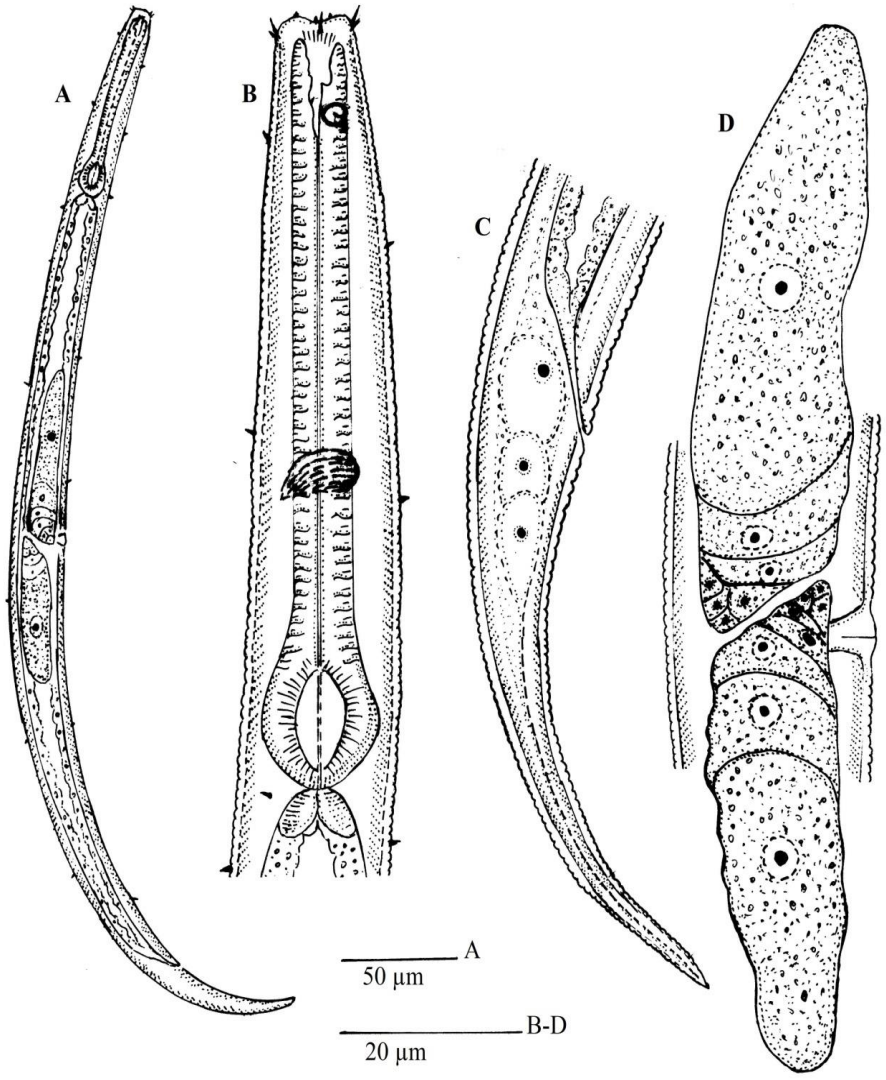

Fig. 4 (A-D). *Achromadora ruricola* (de Man, 1880) Micoletzki, 1925. Female: A. Whole body; B. Oesophageal region; C. Tail region; D. Reproductive system.

Male: Not found.

Remarks: The description and morphometric data of species was based on the specimens collected from Karachi Canal Sindh, Pakistan. Morphometrics of specimen well agreed with the description as described by Andrássy (2005). This is the first reported species from Sindh, Pakistan.

REFERENCES

Abolafia, J. and R. Pena Santiago (2005). Nematodes of the order Rhabditida from Andalucia Oriental, Spain. The family Panagrolaimidae, with a compendium of species of *Panagrolaimus* and key to their identification. *Journal of Nematode Morphology and Systematics*, 8: 133-160.

Andrássy, I. (2005). *Free-living nematodes of Hungary* (*Nematoda Errantia*). Vol. 1, Hangarian Natural History Museum & Systematic Zoology Research Group of the Hangarian Academy of Sciences, Budapest, 518 pp.

Anwar, S.A. and I. U. Khan (1973). Some studies on cotton wilt complex. *Journal of Agricultural Research* (*Punjab*), 11: 94-98.

Baermann, G. (1917). Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) larven in Erdprobem. *Geneeskundig Tijdschrift voor Nederlandsch-Indie Batavia*, 57: 131-137.

Bassen, J.L. (1940). *Panagrolaimus hygrophilus* n. sp., a nematode found in decayed tubers of water lily root, *Nelumbium nucifera* Gaerin. *Proceedings of the Helminthological Society of Washington*, 7: 101-103.

Bütschli, O. (1873). Beitrage zur kentnis der freilebenden Nematoden. *Nova Acta Academiae Caesarea Leopoldino Carolinae Germanicae Naturae Curiosorum*, 36: 1-124.

Cobb, N.A. (1918). *Estimating the nema population of soil*. Agricultural Technical Circular 1. Bureau of Plant Industry. United States Department of Agriculture 1, 48 pp.

Cobb, N.A. (1913). New Nematode genera found inhabiting fresh water and non brakish soils *Journal of Washington Academy of Science*, 3: 430-444.

de Man, J.G. (1880). Die einheimischen, frein in der reinen Erde und im Sussen Wasser lebenden Nematoden. Vorlaufiger Bericht und descriptive seystematischer Theil. *Tijdschrift der Nederladsche dierkundige Vareeniging*, 5: 1-104.

Eyualem-Abebe, W. Traunspurger and I. Andrássy (2006). *Fresh water nematodes: Ecology and Taxonomy*. CABI Publication, Wallingford, UK, 752 pp.

Hooper, D.J., J. Hallmann and S.A. Subbotin (2005). Methods of extraction, processing and detection of plant and soil nematodes. In: *Plant parasitic nematodes in subtropical and tropical agriculture*. 2nd Edition (Eds. Luc, M., Sikora, R.A. and Bridge, J.) CABI Publishing, Wallingford, UK, 53-86 pp.

Jairajpuri, M.S. (1964). Studies on Campydoridae and Leptonchidae (Nematoda: Dorylaimoidea) with description of *Basirotyleptus basiri* n. gen. n. sp., from India. *Proceedings of the Helminthological Society of Washington*, 31: 59-64.

Jairajpuri, M. S. and W. Ahmad (1992). *Dorylaimida*: *Free-living, predaceous and plant parasitic nematodes*. Oxford and IBH Publishing Company Private Limited, New Delhi, India, 458 pp.

Micoletzky, H., (1925). Die Freilebende Süßasser- und Moornematoden Danemarks, *Mem. Acad. Royale Sci. et Lett. Danemark*. T. 10: 57–308.

Shahina, F. (2005). New records of plant parasitic and free-living nematodes of Pakistan. 5th. Nat. Nem. Conf. *Pakistan Journal of Nematology*, 23: 3-6.

Tanveer, M. and E.U. Haq (1975). Prevalence of nematodes in cotton fields of Pakistan. *Pakistan Cottons* 20, 45-54.

Traunspurger, W. (2000). The biology and ecology of lotic nematodes. In special issue: The role of meiofauna in lotic ecosystem (edited by Robertson, A.L., Rundle, S.D., Schmid- Aroya, J.M.). *Fresh water Biology*, 44: 29-45.

Traunspurger, W. (2002). Nematoda. In*: Freshwater meiofauna: Biology and ecology* (Rundle, S.D., Robertson, A.L. & Schmid-Araya, J.M. Eds.). Leiden, spec. 63-104 pp.

Wu, H.C., P.C. Chen and T.T. Tsay (2010). Assessment of nematode community structure as a bioindicator in river monitoring. *Environment Pollution*, 158: 1741-1747.

(*Accepted for publication September 2014*)