



Research Article

Records of Two Genera and Three Species of Nematodes Belonging to the Order Rhabditida Chitwood, 1933 from Pakistan

Salma Javed, Samreen Khan*, Nasira Kazi and Tabassum Ara Khanum

National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan.

Abstract | Three known species belonging to order Rhabditida were recovered from sewage and stagnant water with sediments from different location of Karachi, Sindh, Pakistan. In this study, two genera, *Rhabditoides* and *Butlerius* and three species identified as *Rhabditoides stigmatus*, *Butlerius micans* and *Demaniella basili* were recorded for the first time in Pakistan. The percentage occurrence of these genera was found as 50% each. These species were identified on the basis of morphological, as well as morphometric characters. In addition, brief description, measurement, line drawings, as well as photomicrographs for Pakistani population of these species are reported.

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***Correspondence** | Samreen Khan, National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan; Email: samreenkhan3336@gmail.com

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Keywords | *Rhabditoides stigmatus*, *Butlerius micans*, *Demaniella basili*, Sewage, Morphometrics

Introduction

Rhabditid nematodes occupy a wide range of habitats. They are found mostly in terrestrial habitats viz., in soil, decaying organic materials, compost, humus and dung etc. The large numbers of Rhabditids are bacterium feeders, but there are also carnivorous species among them (Andrassy, 2005). However, some members are terrestrial and have been noted from fresh water and semiaquatic environments as well. They are also capable to survive and replicate themselves in these habitats and perform significant function particularly, in the nutrient cycling and in biological monitoring for the better environmental health of rivers and lakes (Abolafia, 2006).

De Ley and Blaxter (2002, 2003) laid new outlines of a higher classification. They divided the suborder Rhabditina Chitwood, 1933 into three infraorders Bunonematomorpha, Diplogastromorpha and

Rhabditomorpha.

Rhabditina as a suborder of the order Rhabditida is divided into two super families (i) Rhabditoidea Orley, 1880; (ii) Bunonematoidea Micoletzky, 1922. The sub order Rhabditina comprises 50 valid genera and a total of 329 species (Andrassy, 2005). From Pakistan, until now, a total of 34 genera and 59 species from order Rhabditida have been reported by the numbers of scientists from various locations in Pakistan, out of which 21 species were new to science (Shahina *et al.*, 2019; Zarina and Shahina, 2012; Maqbool and Shahina, 2001).

During the present study three known species including *Rhabditoides stigmatus* (Steiner, 1930), Andrassy, 1984, *Butlerius micans* Pillai and Taylor, 1968a and *Demaniella basili* Pillai and Taylor, 1968b of the order Rhabditida have so far been reported for the first time in Pakistan, in which two genera

Table 1: Location wise occurrence % of each Genus.

Sites	Latitude	Longitude	NS	NPS	Genus	SCG	Occurrence	RF
Drigh Road Nadi	24°53'14.6"N	67°08'09.6"E	02	02	<i>Rhabditoides</i>	01	50%	33.3
					<i>Demaniella</i>	01	50%	33.3
Department of Pharmacy, UoK	24°56'24.3"N	67°07'01.9"E	02	01	<i>Butlerius</i>	01	50%	33.3

NS: Number of Samples; NPS: Number of Positive Samples; SCG: Samples Containing Genus; RF: Relative Frequency.

Butlerius Goodey, 1929 and *Rhabditoides* Rahm, 1928 are reported for the first time. The present taxonomic study provide description, distribution, with illustration for all species.

Materials and Methods

Area of exploration

A total of twenty sewage and stagnant water with sediments were collected from ten different location of Karachi, Sindh, Pakistan viz., Department of Pharmacy, University of Karachi, Drigh Road Nadi, Malir Nadi, Gulistan-e-Jouhar, Shah Faisal Colony, Korangi, Machar Colony, Nazimabad Colony, Safoora Goth and Goli Mar.

Extraction of nematodes

Nematodes were extracted from sewage and stagnant water with sediments by ensuring Cobb's sieving and decanting method (Cobb, 1918) and by modified Baermann funnel technique (Baermann, 1917). The obtained nematodes were killed instantaneously by pouring hot water and preserved immediately in hot 4% formalin, thereafter dehydrated to glycerin by a slow evaporation method and finely mounted in anhydrous glycerin on microscopic glass slides. Measurements were undertaken with the aid of an ocular micrometer. Line drawings were done via drawing tube or camera lucida attached to the Nikon Eclipse E-400 compound microscope. Photomicrographs were captured using digital camera attached to the same microscope.

Data analysis

The aim of area explored in Karachi, Sindh, was to ascertain the biodiversity of Rhabditid nematodes and their occurrence percentage as follows:

$$\text{Occurrence (\%)} = \frac{\text{Total number of positive samples}}{\text{Total numbers of soil sample}} \times 100$$

$$\text{Relative Frequency (\%)} = \frac{\text{Frequency of each Genus}}{\text{Sum of frequencies of all genus}} \times 100$$

Results and Discussion

Occurrence percentage (%)

Two sewage sample out of twenty were found positive (10%) and analysis of overall samples revealed three types of genera viz., *Rhabditoides*, *Butlerius* and *Demaniella* (50%) each. In term of overall occurrence percentage of these genera, relative frequency and Global Positioning System (GPS) of each site was illustrated in Table 1.

Rhabditoides stigmatus (Steiner, 1930) Andrassy, 1984

(Figures 1 and 2; Table 2)

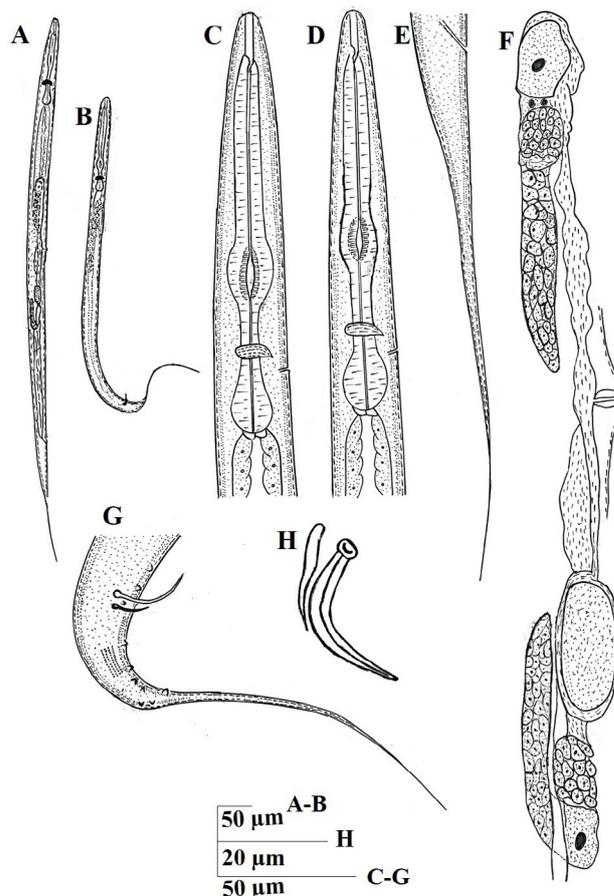


Figure 1: (A-H). *Rhabditoides stigmatus* (Steiner, 1930) Andrassy, 1984. A. Female whole body; B. Male whole body; C. Female pharynx; D. Male pharynx; E. Female tail; F. Female gonad; G. Male tail; H. Spicule and Gubernaculum.

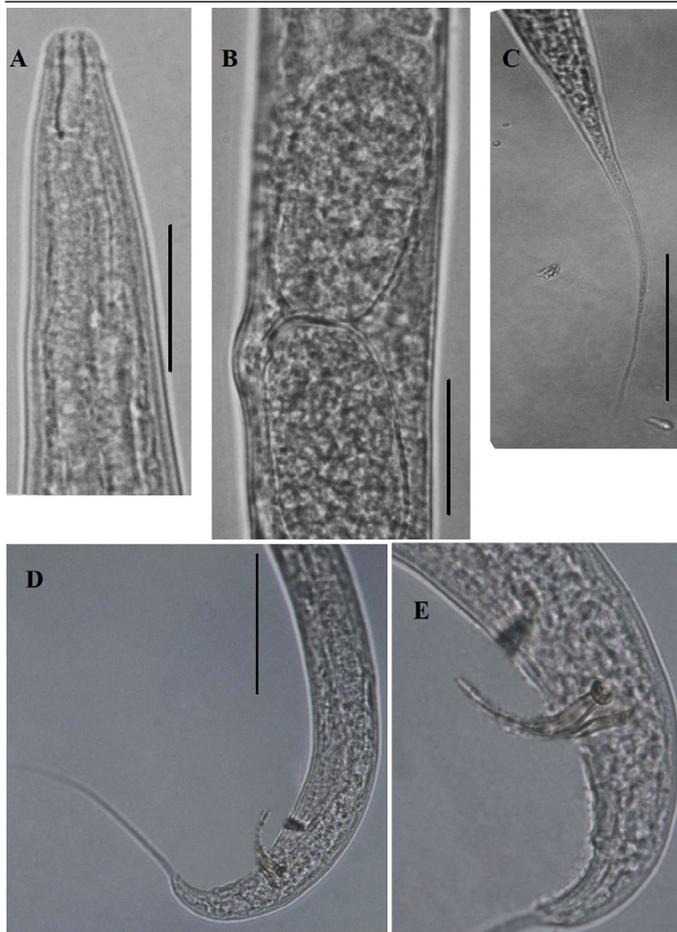


Figure 2: (A-E). *Rhabditoides stigmatus* (Steiner, 1930), Andrassy, 1984. A. Female anterior region; B. Female vulval region; C. Female tail; D. Male posterior region showing spicules and gubernaculum. (Scale: A, B, D=100 μ m; C=40 μ m).

Description

Female: Body usually straight, narrowing at the both ends. Cuticle with fine longitudinal and transverse striations. Lip region 6-7 μ m wide, fused, non-offset. Small labial papillae protruding from labial contour. Amphid situated at junction of cheilostom and gymnostom, oval, 4 μ m from anterior end of body. Stoma tubular, six time longer than wide, curved ventro-dorsally in the posterior region. Stegostom anisomorphic, a large ventrally directed flap-like tooth present on the dorsal wall, surface weakly cuticularized. Oesophagus typically diplogasteroid. Procorpus muscular. Metacarpus well developed having large valve plates, 20-22 μ m long and 15-17 μ m in width, lumen of procorpus sclerotized, corpus twice the combined length of isthmus and terminal bulb. Terminal bulb conspicuous, ellipsoidal, slightly smaller than median bulb, 15-20 μ m long and 14-15 μ m wide. Corpus 63-70% of pharyngeal length. Nerve ring near middle of isthmus, located at 81.4-82.6% of pharynx length. Excretory pore and hemizonid

difficult to observed. Oesophago-intestinal valve conoid 4-6 μ m long.

Table 2: Morphometric data of *Rhabditoides stigmatus* (Steiner, 1930); Andrassy, 1894. All measurements are in μ m except L (mm) in the form of Mean \pm SD (range).

Characters	Female (n=7)	Male (n=1)
L	820 \pm 0.07 (750-980)	750
a	26 \pm 2.04 (22.6-29)	27.9
b	5.8 \pm 0.29 (5.4-6.3)	5.2
c	4.5 \pm 0.32 (4.0-5.0)	4.7
c'	11.2 \pm 0.78 (10.2-12.3)	7.2
V %	44.5 \pm 0.77 (43.2-45.2)	-
Stoma width	2.9 \pm 0.06 (2.8-3.0)	3
Stoma length	18.5 \pm 0.44 (17-20)	16
Median bulb length	19.2 \pm 2.29 (17-22)	20
Median bulb width	15.3 \pm 1.43 (14-18)	17
Nerve ring	120.1 \pm 4.42 (110-124)	110
Excretory pore	126.7 \pm 3.36 (120-130)	131
Isthmus	29.0 \pm 0.86 (28-30)	30
Pharynx	141.7 \pm 4.87 (135-150)	142
Maximum body width	32 \pm 2.20 (29-36)	27
Tail	189 \pm 12.46 (170-200)	160
Anal body width	16 \pm 1.19 (14-18)	22
Spicule	-	30
Gubernaculum	-	19

Reproductive system amphidelphic, didelphic, anterior branch present on the right side while posterior branch present on the left side of intestine. Ovaries reflexed to vicinity of vulva sometimes anterior and posterior ovaries overlapped around the vulva. Oocytes organized in two to three rows. Spermatheca filled with sperms. Vulva a transverse slit, always protruding to some extent. Vagina almost half vulval body diameter. Anterior branch of reproductive system usually slightly longer than posterior branch. Eggs oval shaped, 20-25 μ m long and 37-40 μ m wide. Rectum straight, equal to the corresponding body width. Rectal glands present. Phasmid situated about 1.3-1.6 anal body diameter from anus. Tail filiform and 0.5-0.8 times vulva-anus distance long.

Male: Body extremely bent in the posterior end in fixed specimens. Anterior part morphologically similar to the female. Genital system monorchic with testis overlapping the intestine ventrally, anterior end reflexed dorsally. Spicules well developed, arcuate, with proximal knobs. Gubernaculum slender and proximal

end anteriorly directed; central part is fusiform while distally it is needle like. 10 pairs of genital papillae arranged as two pairs precloacal, one pair adanal and seven post cloacal. Tail consist of two parts, one short conoid and posteriorly long filiform.

Remarks: Specimens were collected from sewage water at Drigh road nadi, Karachi, Sindh. The morphology of the specimens well corresponds with the description given by (Steiner, 1930) Andrassy, 1894 and Dassonvilla and Heyns, 1984.

***Butlerius micans* Pillai and Taylor, 1968**
(Figures 3 and 4; Table 3)

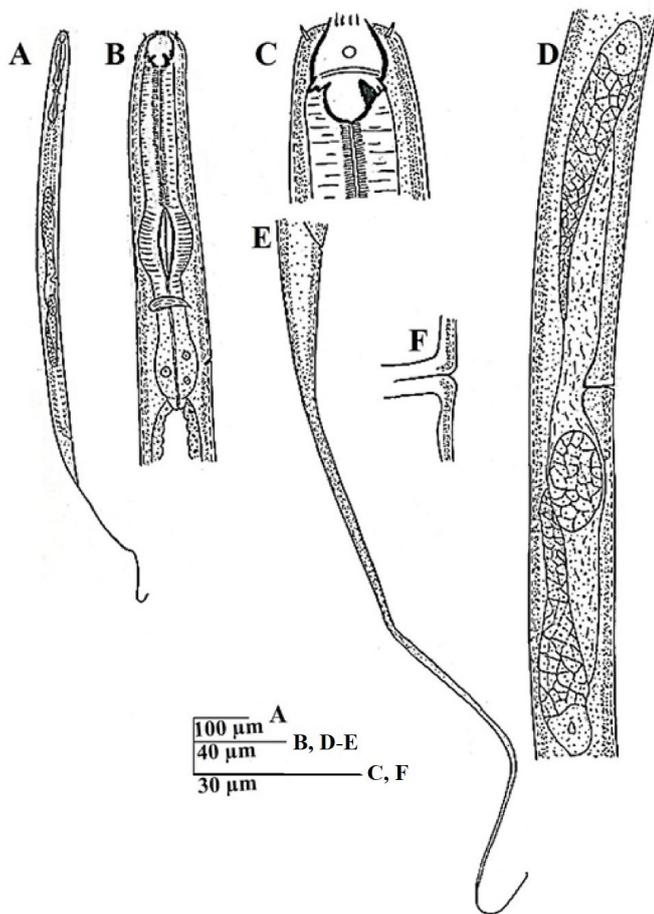


Figure 3: (A-F). *Butlerius micans* Pillai and Taylor, 1968. Female. A. Whole body; B. Pharyngeal region; C. Cephalic region; D. Gonad; E. Tail region; F. Vulval region.

Description

Female: Body usually straight when heat killed, narrowing towards both ends, more towards posteriorly having long filamentous tail. Cuticle with transverse striations, longitudinal lines and faint dot like punctation. Longitudinal striations more discrete

on post anal part. Labial region wide, continuous and flattened. Lips amalgamated, each bearing slightly an elevated outer labial sensilla, 2.5-3 μm long. Rest of sensilla papilliform. Amphidial aperture oval to round shape, located at cheilostomal base, about 2 μm wide. Stoma large, spacious, in two parts, anterior wider and posterior narrower part. Stoma about 1.1-1.2 times longer than wide, about 14-18 μm deep in females. Cheilostom with eight narrow rib like plates, extending beyond labial contour, inner wall of cheilostom cuticularised. Stegostome anisotropic, surrounded by pharyngeal tissue. Matestegostome with a large dorsal tooth, 4-4.5 μm long and 2.5-3 μm wide and a smaller tooth on each subventral wall. Pharynx diplogasteroid. Pharyngeal procorpus tubular 1.7-1.9 times metacarpus length; metacarpus swollen, ovoid having valve plates, distinctly separated from tubular isthmus and small glandular basal bulb without a valve plate or grinder. Cardia conoid, surrounded by intestinal tissue, 5-6 μm long. Nerve ring surrounds the pharyngeal region at 72-91% of pharynx length measured from anterior end. Deirid present at level of basal bulb. Excretory pore behind to the nerve ring in isthmus region or about 81-87.4% of pharynx length from anterior end. Intestine granular with wide lumen.

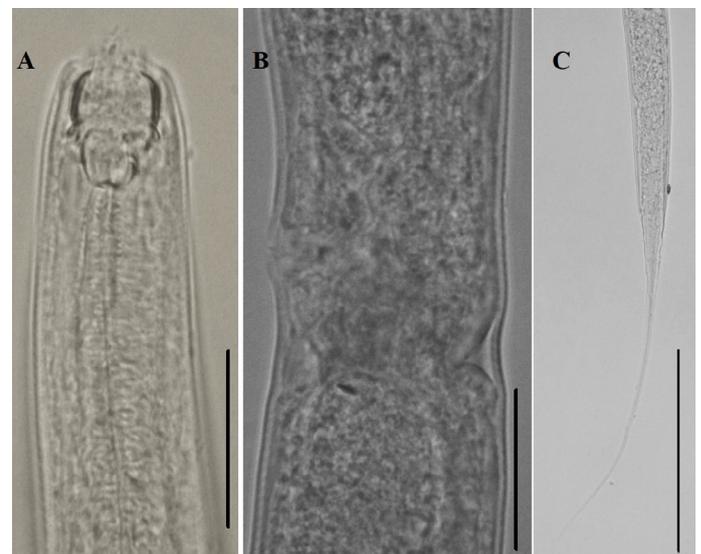


Figure 4: (A-C). *Butlerius micans* Pillai and Taylor, 1968. Female A. Anterior region; B. Vulval region; C. Tail region (Scale: A, B=100μm; C=40μm).

Genital system amphidelphic, didelphic with reflexed ovaries, posterior branch typically smaller than the anterior one. Oocytes organized in 2 or more rows. Uterus having 1-3 eggs of 46-47 x 24-25 μm dimension. Vagina 10-12 μm long, occupying about

1/3rd of the corresponding body diameter, with a pair of circularized pieces and small ovoid glands. Vulva a small transverse slit. Vulva to anus distance is about 0.6 times to the tail length. Phasmid less than one anal body diameter and located posteriorly from anus or situated at 2.7-3.1% of tail length. Rectum 0.7-0.8 times long to that of anal body diameter. Tail initially conical followed by filiform, 1.5-1.6 times long to vulva-anus distance.

Male: Not found.

Table 3: Morphometric data of *Butlerius micans* Pillai and Taylor, 1968. All measurements are in μm except L (mm) in the form of Mean \pm SD (range).

Characters	Female (n=10)
L	987 \pm 99.7 (861-1109)
a	25.8 \pm 2.81 (20.7-30)
b	6.9 \pm 0.55 (6.5-8.2)
c	3.1 \pm 0.19 (2.9-3.6)
c'	19.8 \pm 1.78 (17-23)
V%	43.4 \pm 2.40 (40-47.5)
Lip width	18 \pm 1.61 (16-20)
Stoma length	16.4 \pm 1.49 (14-18)
Stoma width	12.9 \pm 0.91 (12-14)
Corpus length	96.2 \pm 13.94 (80-111)
Isthmus length	26.1 \pm 1.74 (24-28)
Pharynx length	142.2 \pm 13.42 (126-167)
Nerve ring	117.7 \pm 2.0 (115-120)
Excretory pore	140 \pm 3.47 (130-146)
Cardia	5.3 \pm 0.43 (5-6)
Basal bulb length	26.6 \pm 1.67 (24.5-30)
Basal bulb width	18.9 \pm 0.91 (18-20)
Maximum body width	38.3 \pm 3.52 (30-42)
Tail	319.7 \pm 26.08 (290-360)
Anal body width	16.8 \pm 2.52 (14-20)
Rectum length	13 \pm 0.85 (12-14)

Remarks: The specimens were collected from stagnant water near Department of Pharmacy, University of Karachi, Sindh. The morphometric data are closely resembling the original description given by Pillai and Taylor, 1968a.

***Demaniella basili* Pillai and Taylor, 1968**
(Figures 5 and 6; Table 4)

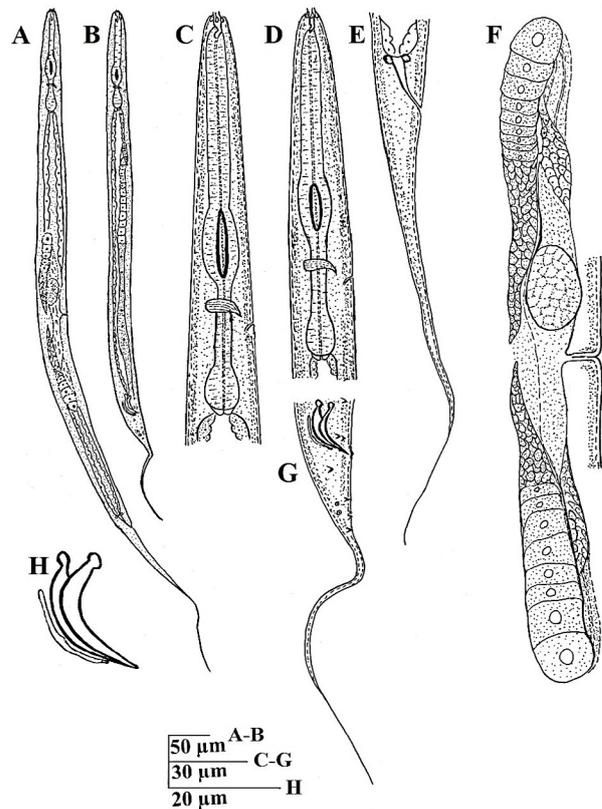


Figure 5: (A-H). *Demaniella basili* Pillai and Taylor, 1968. A. Female whole body; B. Male whole body; C. Female pharyngeal region; D. Male pharyngeal region; E. Female tail; F. Female gonad; G. Male tail; H. Spicules and Gubernaculum.

Description

Female: Body upto 1mm, straight on heat relaxation, narrowing at both ends, more towards posteriorly by a long filiform tail in both sexes. Cuticle fine, longitudinally and transversely striated approximately 2 μm apart. Crossing of transverse and longitudinal striations display appearance of punctation. Annules more prominent at caudal region. Amphidial aperture round to oval at the level of metarhabdions. Posterior to stegostome. Lip region non-offset, 6-7 μm in width, having six lips, tips are drawn out to fine projections, inarching oral inaugural. A small papillae present in the middle of each lip. Stoma three times longer than wide. Mesorhabdions more sclerotized than prorhabdions. Posterior part concave having sclerotized arch. Procorpus muscular, gradually expanding posteriorly to an elongate, highly muscular metacarpus with valve plates, longer than wide. Isthmus long and expending to an ovoid basal bulb. Pharyngeal corpus 66-81% of entire pharyngeal length. Hemizonid located near to nerve ring. Nerve ring surrounding at anterior part of isthmus, located at 80.2-96.2% of total neck length. Excretory

pore situated in mid region of isthmus behind the nerve ring, 83.7-96.2% of total neck length. Cardia hemispherical. Intestinal lumen wide, containing six to eight rows of cells.

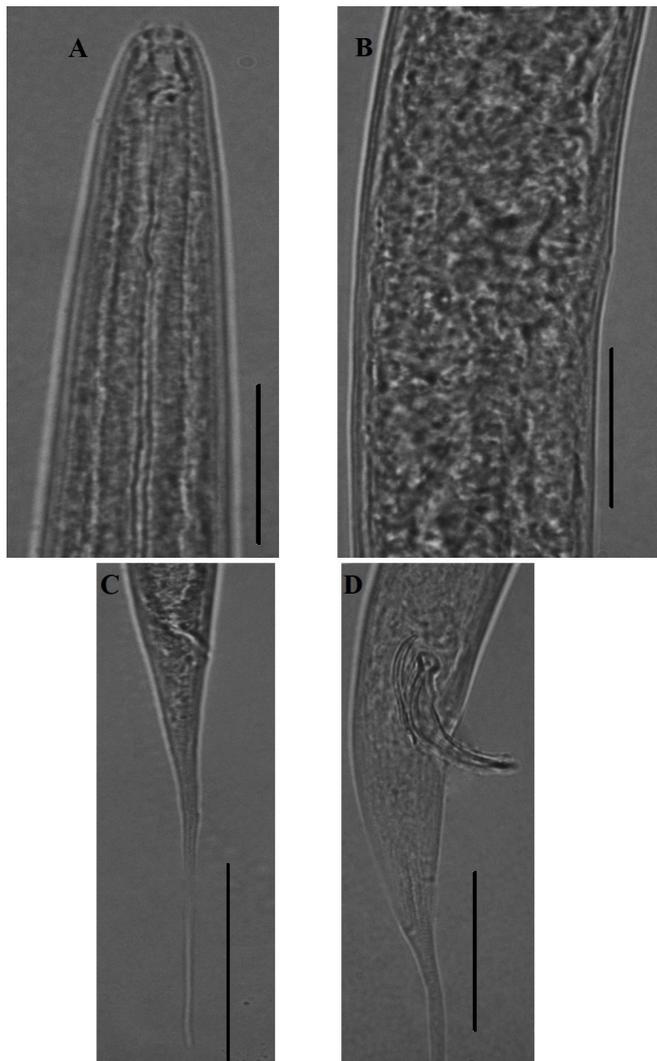


Figure 6: (A-D). *Demaniella basili* Pillai and Taylor, 1968. Female: Anterior region; B. Vulval region; C. Tail region. Male: D. Spicules and gubernaculum. (Scale: A, B, D=100µm; C=40µm).

Reproductive system didelphic-amphidelphic, with reflexed ovaries, reaching up to the vulval region. Oocytes designed more than in one row in maturation region. Oviduct is long, slender, enlarging at proximal area from where it connects the distal part of uterus. Vagina muscular, short. Vulva a transverse slit. One egg exists in a uterus. Rectum is approximately 1.3-1.4 times long to anal body diameter. Tail is long, filamentous and 11-15 times longer than anal body width.

Male: The general morphology of male is similar to that of the female apart from reproductive system.

Testis monorchic, occupying 43-49% of total body length, distal part reflexed. Germ cells organized in a several rows. Spicules ventrally arcuate, paired, and proximally knobbed. 10 pairs of caudal papillae; three pairs preanal and seven posts anal.

Table 4: Morphometric data of *Demaniella basili* Pillai and Taylor, 1968. All measurements are in µm except L (mm) in the form of Mean±SD (range).

Characters	Female (n=10)	Male (n=10)
L	782±58.17 (701-835)	703±60.20 (614-780)
a	25.93±0.92 (24-26.2)	32.5±0.28 (30-34.7)
b	5.06±0.26 (4.7-5.3)	4.5±1.19 (4.3-4.8)
c	4.3±0.36 (3.9-4.8)	4.8±0.60 (3.9-5.6)
c'	13.3±1.73(11.1-15.3)	7.4±0.87 (6.1-8.5)
V or T%	47.3±1.31(45.6-48.8)	46.9±2.18 (43-49.1)
Stoma width	3.3±0.47 (3-4)	3.3±0.47 (3-4)
Stoma length	10.8±1.31 (9-12)	9.8±0.73 (9-11)
Excretory pore	137±3.26 (130-144)	119.3±1.19 (115-125)
Nerve ring	134±8.6 (130-138)	129±4.30 (124-135)
Head-corpus	107±8.83 (95-116)	105±03 (100-108)
Head-basal bulb	149±16.39 (135-172)	152.2±6.49 (142-160)
Max. body width	33.3±0.94 (32-34)	23.3±2.16 (20-26)
Tail	218 ±19.8 (190-234)	145.5±13.73(122-156)
Anal body width	16.3±0.94 (15-17)	19.5±0.86 (18-20)
Rectum length	22.1±1.64 (20-24)	20.8±0.73 (20-22)
Spicule	-	30.1±1.06 (28.4-31)
Gubernaculum	-	20.5±0.86 (20-22)

Remarks: The specimens were collected from sewage water at Drigh road nadi, Karachi, Sindh. The morphometric data are closely fit with the original description given by Pillai and Taylor, 1968b.

Conclusions and Recommendations

It is concluded that three known genera and species of free living nematodes were retrieved from samples of sewage water which have significant biological importance. It is therefore recommended that this research being guidelines for researchers to further probe and identified undiscovered species from Pakistan.

Novelty Statement

In this study, species namely *Demaniella basili* was found as new record, while *Butlerius micans* and *Rhabditoides stigmatus* were studied as a new

country record of genera and species.

Author's Contribution

Salma Javed supervised entire research and critically examined the article. Samreen Khan carried out surveys, processed samples followed by measurements, made line drawing, took photomicrographs and designed the manuscript. Nasira Kazi identified the species and reviewed the manuscript. Tabassum Ara Khanum helped in photography.

Conflict of interest

The authors have declared no conflict of interest.

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