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FIVE NEW RECORDS OF FREE-LIVING SOIL NEMATODES (NEMATODA: RHABDITIDA) FROM DIFFERENT FRUITS OF SINDH, PAKISTAN

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ABSTRACT

Nematodes, a diverse group of round worms, exist cosmopolitan in almost all biomes. Saprophytic free-living soil nematodes found as the part of agricultural fauna indicate an important role in the ecosystem. In the present study, nematode specimens of five known species were collected from different fruits (banana, citrus and guava) of Sindh. The nematodes were isolated through soil trap method. A recent survey for plant and soil nematodes of different fruits of Sindh yielded five known species viz. *Eucephalobus iaculo-caudatus* (Bostrom and Holovachov, 2011), *Pelodera punctata* (Cobb, 1914), (Dougherty, 1955), *Panagrolaimus hygrophilus* (Bassen, 1940), *Panagrolaimus rigidus* (Schneider, 1866), (Thorne, 1937), and *Mononchoides change* (Goodrich et al., 1968). Morphometric data, illustrations and brief descriptions of each species are given with references to their host and localities. All species are found for the first time from Sindh, Pakistan.

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INTRODUCTION

Sindh has a subtropical climate and have great agrarian lands for growing new crops. The commercial fruits are mango, guava, citrus, chickoo, and lychee, with a weak agricultural extension service. Effective agronomic production depends on proper crop protection from pests and diseases. Vital role of nematodes in agricultural production is of significant importance. These pests cause huge losses to the farm revenue by reduced yields and quality of production. Nematodes as phyto-parasites play relatively destructive role and cause an average damage of up to 20% or even complete crop loss if associated with other pathogens. Saprophytic nematodes play a significant role in the agricultural fauna of the ecosystem. More than 30% of isolated nematodes present in the field are saprophytic and among them, 80% are bacterial feeder (Gebremikael et

al., 2016; Hu et al., 2018; Youssef and Eissa, 2014). Saprophytic nematodes play a vital role in essentials oil processes such as more nitrogen is available in the ammonium form when bacterivorous and fungivorous nematodes are present (Trofymow and Coleman, 1982). These nematodes are used as biological indicators for soil health because of their different functions in the soil food web (Gebremikael et al., 2016; Hu et al., 2018). A very little work has been done so far. Saprophytic nematodes were found from different agro climatic regions of Sindh viz. *Acrobeles* sp., *Acrobeloides*, *Moshajia qasimii*, *Oscheius niazii*, *O. shamimi* and *O. citri*. In Pakistan earlier researchers have reported the genera *Acrobeles* (Cobb, 1924) and *Acrobeloides* (Thorne, 1937) many times. Zarina and Shahina (2007) detected a new species *Moshajia qasimii* from soil around the roots of banana in the campus of the University of Karachi and at

Mirpurkhas, Pakistan. Khanum and Shahina (2010) described 2 new and 1 new records of the genus *Oscheius* viz. *O. niazii*, *O. shamimi* from around the palm roots (*Cocos nucifera* L.), Karachi. Khanum et al. (2016) described six new species of *Oscheius* from *O. citri* (Citrus spp.) found in the soil of citrus field of Gadap, Sindh. Khanum et al. (2017) re-described two known species of entomopathogenic nematodes. Khanum et al. (2017) reported *Steinernema affine* from cherry (*Prunus aviumhunza*), and *S. cholashanense* was captured from apple (*Malus pumila*) and walnut (*Juglan snigra*) of Gilgit-Baltistan. The present study was focused on nematodes that occur in association with different fruit growing areas of Sindh. The nematodes encountered during the survey were identified up to species level on morphological and morphometric basis.

MATERIALS AND METHODS

New records of Rhabditids were found in collected soil samples through soil trap method (Bedding and Akhurst, 1975). Four larvae of greater wax moth (*Galleria mellonella*) were kept in 300 ml containers with wet soil samples and each container was placed at 27-30°C. The containers were checked daily and dead larvae were selected out and cleaned in distilled water, placed in Petri dish layered with wet filter paper for nematode population production. Different life stages of nematodes viz. juveniles, females and males were killed in a watch glass by hot water (60°C) for morphological observations and variations of structures. Supernatant water was sucked out and in concentrated water added boiled fixative solution (11% formalin + 6% glycerin) and covered to prevent evaporation. The specimens were straightened and well-fixed after 24 hours. For slow dehydration, specimens were kept for 5-6 days in an incubator at 55 °C in 2 ml of 2.5% glycerin. For permanent mount, 10 nematodes were individually picked and placed on a glass slide with pure drop of glycerin and sealed with paraffin wax by gently heating the slide. The slides were labeled with the details of genus, species, and stages, dates of making slide and host. Nematode descriptions were accompanied by measurements using a Nikon E-400 and applied de Man's formula (De Man, 1894). Light photomicrographs were taken with Nikon DS-Fi1. Illustrations of distinguishing features of the nematode specimens were drawn by using drawing tube connected to the compound microscope.

RESULTS AND DISCUSSION

Eucephalobus iaculo-caudatus Bostrom and

Holovachov, 2011.

Description (Figure 1 and Table 1)

Adult: Small sized nematodes. Body 400-515 µm long, gradually narrow, slightly curved, ventrad after fixation. Cuticle finely annulated, annules 1-1.2 µm wide at mid body. Lateral field with three longitudinal incisures, lip region continuous carrying 6+4 papillae, two amphids, labial probolae present whereas cephalic probolae absent. Stoma 1.2-2 times the lip width long, with the presence of cheilostome, gymnostom and stegostom. Pharynx cylindrical, isthmus narrow, basal bulb oval, with valves. Nerve ring at level of metacarpus-isthmus junction. Excretory pore at level of metacarpus-isthmus junction. Deirids at level of isthmus or at the junction of isthmus and basal bulb.

Female: Ovary monodelphic, prodelphic, vulva a transverse slit, vulval lip not protruding. Ovary reflexed, oviduct short, separated from ovary by a muscular sphincter. Spermatheca offset, project anteriorly between oviduct and uterus, filled with sperm. Post uterine sac about 0.5-0.7 times longer than surrounded body diameter. Tail conoid, terminus having 2-3 µm long mucro.

Male: Reproductive system monarchic, testis reflexed anteriorly. Spicules paired, symmetrical, manubrium oval with narrow shaft. Gubernaculum disk like cornuacrum absent. Tail conoid 30-42 µm long with terminal mucro. Genital papillae are seven pairs and a single, arranged as two pairs are subventral precloacal, one pair subventral ad-cloacal, a single mid-ventral whereas two pairs at mid tail and two pairs situated at tail terminus.

Remarks: Specimens of *Eucephalobus iaculo-caudatus* (Bostrom and Holovachov, 2011) collected from soil around the roots of banana (*Musa acuminata* L.) of Malir Cantt, Karachi Sindh, Pakistan. These specimens are very similar to those of the original description given by Boström and Holovachov (2011). This is the first record of the species from Pakistan.

Pelodera punctata (Cobb, 1914; Dougherty, 1955)

Description (Figure 2 and Table 1)

Adult: Body 945-1125 µm long, slightly curved after fixation. Cuticle finely annulated with punctuations. Lateral field not observed under light microscope. Lip region slightly wider than adjoining body. Labial region with six separate lips, amphid circular. Stoma rhabditoid 20-25 µm long, cheilostome not cuticularised; gymnostome with cuticularied walls, glottoid apparatus

with small denticles. Pharyngeal corpus 80-140 µm long, isthmus 30-55 µm long, with ovoid basal bulb 25-40 µm long. Cardia overlapped by intestine. Nerve ring

surrounded by anterior part of isthmus whereas excretory pore located anterior to the middle of isthmus.

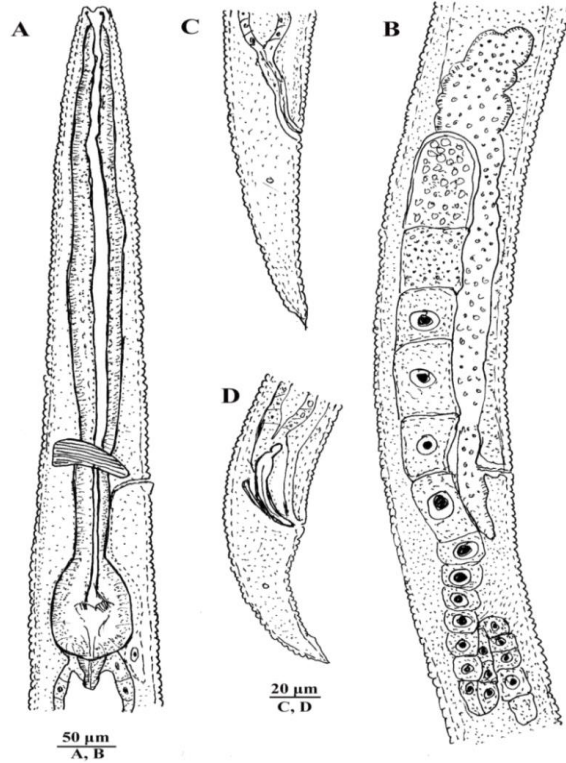


Figure 1 (A-D). *Eucephalobus iaculo-caudatus* Bostrom and Holovachov, 2011 (A-D): Female (A-C): A. Pharyngeal region; B. Tail region; C. Vulval region; Male: D, Ventral view of tail region.

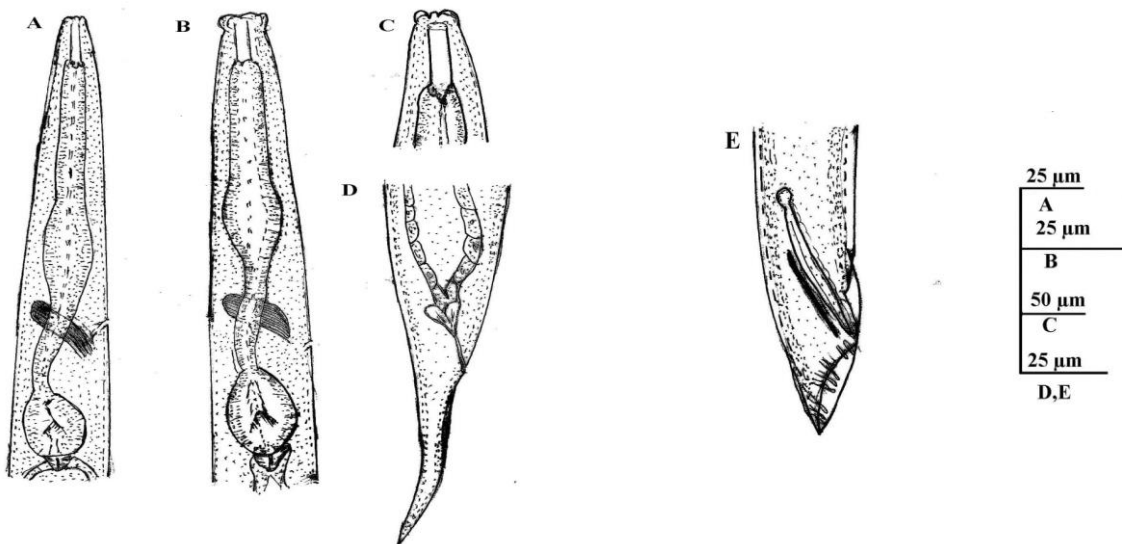


Figure 2 (A-F). *Pelodera punctata* (Cobb, 1914; Dougherty, 1955) (A,B): Male: A. Pharyngeal region; E. Ventral view of tail region showing papillae. Female (B-D): B. Pharyngeal region; C. Anterior region; D. Tail region.

Table 1. Measurements (μm) of *Eucephalobus iaculo caudatus* Bostrom and Holovachov, (2011) and *P. punctata* (Cobb, 1914; Dougherty, 1955). Female (n= 10), Male (n = 05).

Characters	<i>P. punctata</i>		<i>E. iaculo caudatus</i>	
	Female Mean \pm SD (Range)	Male Mean \pm SD (Range)	Female Mean \pm SD (Range)	Male Mean \pm SD (Range)
Total body length	1000 \pm 75 (945-1125)	789 \pm 82.6 (700-890)	375 \pm 10 (350-412)	410 \pm 25 (400-515)
Max. body width	51 \pm 3.6 (55-65)	32 \pm 2.8 (30-39)	15.5 \pm 1.0 (14-17)	11 \pm 0.3 (10-11.5)
Lip width	7.9 \pm 0.5 (7-10)	7.2 \pm 0.9 (6.5-8)	5.6 \pm 0.3 (5.7-6.1)	5.8 \pm 0.1 (5-6)
Stoma length	23.1 \pm 0.9 (20-25)	20.6 \pm 0.8 (20-25)	11 \pm 0.3 (10-11)	110 \pm 5 (105-125)
Pharynx	200 \pm 4.7 (188-225)	174.5 \pm 6.4 (168-190)	112 \pm 2.5 (100-120)	88 \pm 3.5 (80-90)
Nerve ring	68 \pm 2.6 (64-70)	65.8 \pm 18 (65-69)	72 \pm 3.5 (65-75)	78 \pm 3.2 (70-85)
Excretory pore	74.5 \pm 2.5 (70-82)	73 \pm 1.4 (70-78)	80 \pm 3.0 (73-80)	11.5 \pm 0.7 (10.5-12.5)
Tail	68 \pm 2.6 (58-85)	24.5 \pm 1 (23-30)	30 \pm 1.5 (28-31)	35 \pm 3.2 (30-42)
Abd	17 \pm 1 (13-25)	21 \pm 1.1 (17-28)	13.5 \pm 0.9 (12-14)	20 \pm 1.0 (17-21)
Spicules	--	50 \pm 2.5 (40-55)	--	19 \pm 0.7 (16-20)
Gubernaculum	---	32 \pm 1.4 (27-35)	--	13.8 \pm 0.9 (12.8-13.9)
a	23.5 \pm 2.1 (17-25)	23 \pm 2.5 (19-26)	23.5 \pm 1.1 (22.0-26.0)	22 \pm 0.6 (20-24)
b	5.0 \pm 0.2 (5.2 - 6.5)	4.3 \pm 0.5 (4-8.5)	3.1 \pm 0.2 (3.0-3.5)	3.4 \pm 0.1 (3.0-3.6)
c	15.4 \pm 1.4 (13.5-15.8)	30 \pm 3.2 (26-37)	12.8 \pm 0.6 (12.5-13.9)	12.0 \pm 0.3 (11.5-12.5)
c'	4.1 \pm 0.6 (3.4-5.2)	0.8 \pm 0.1 (0.78-1.16)	2.1 \pm 0.1 (2.0-2.5)	2.8 \pm 0.1 (2.5-3.2)
V%	55.3 \pm 2.5 (51-60)	---	63 \pm 1.5 (60-70)	--

Female: Gonads didelphic, amphidelphic, ovaries reflexed, vulva transverse, vagina short, uteri with thin walls with oval spermatheca filled with sperms. Rectum longer than anal body diameter. Distance between vulva and anus 8-12 times as long as tail. Conical tail, with acute end, 58-85 μm long.

Male: Smaller in size but similar to females in general body morphology. Testis reflexed. Spicules 40-55 μm , long separate. Gubernaculum simple, 27-35 μm long.

Nine pairs of papillae present in group 2+4+3. Tail conical curved ventrally, anteriorly open peloderan bursa present.

Remarks: Specimens of *P. punctata* (Cobb, 1914; Dougherty, 1955) collected from soil around the roots of citrus fields of Nawabshah is very similar to those of the original description given by De Man (1894). This is the first record of this species from Pakistan.

Panagrolaimus hygrophilus Bassen, 1940

Description (Figure 3 and Table 2)

Female: Medium size body straight to slightly curved ventrally upon heat relaxation, tapering at both extremities. Lateral field with three crenate lines. Head continuous with body contour, lips fused, amphids small, located posterior to cheilostom. Stoma narrow, tubular about 1–1.5 lip diameter long. Cheilostom not cuticularized, telostegostom continuous with pharyngeal lumen. Pharynx 193–200 μm long, corpus cylindrical, isthmus narrow than corpus separated from metacarpus. Nerve ring located at about 60–74% of

pharyngeal length, surrounding the isthmus. Excretory pore at 60–80% of pharynx length situated at isthmus level. Basal bulb ovoid, cardia small, flat, 4–6 μm long with narrow lumen. Rectum with markedly cuticularized lumen, 1.3–1.8 anal body diameter long. Gonads monodelphic, prodelphic. Ovary reflexed, reflexed portion straight, reaches up to vulva. Vulval lips slightly protruded, having transverse slit, thin walled vagina present. Post-uterine sac 20–35 μm long. Vulva–anus distance 180–251 μm . Phasmids located at middle of tail. Tail elongate, straight to a narrow, subacute terminus.

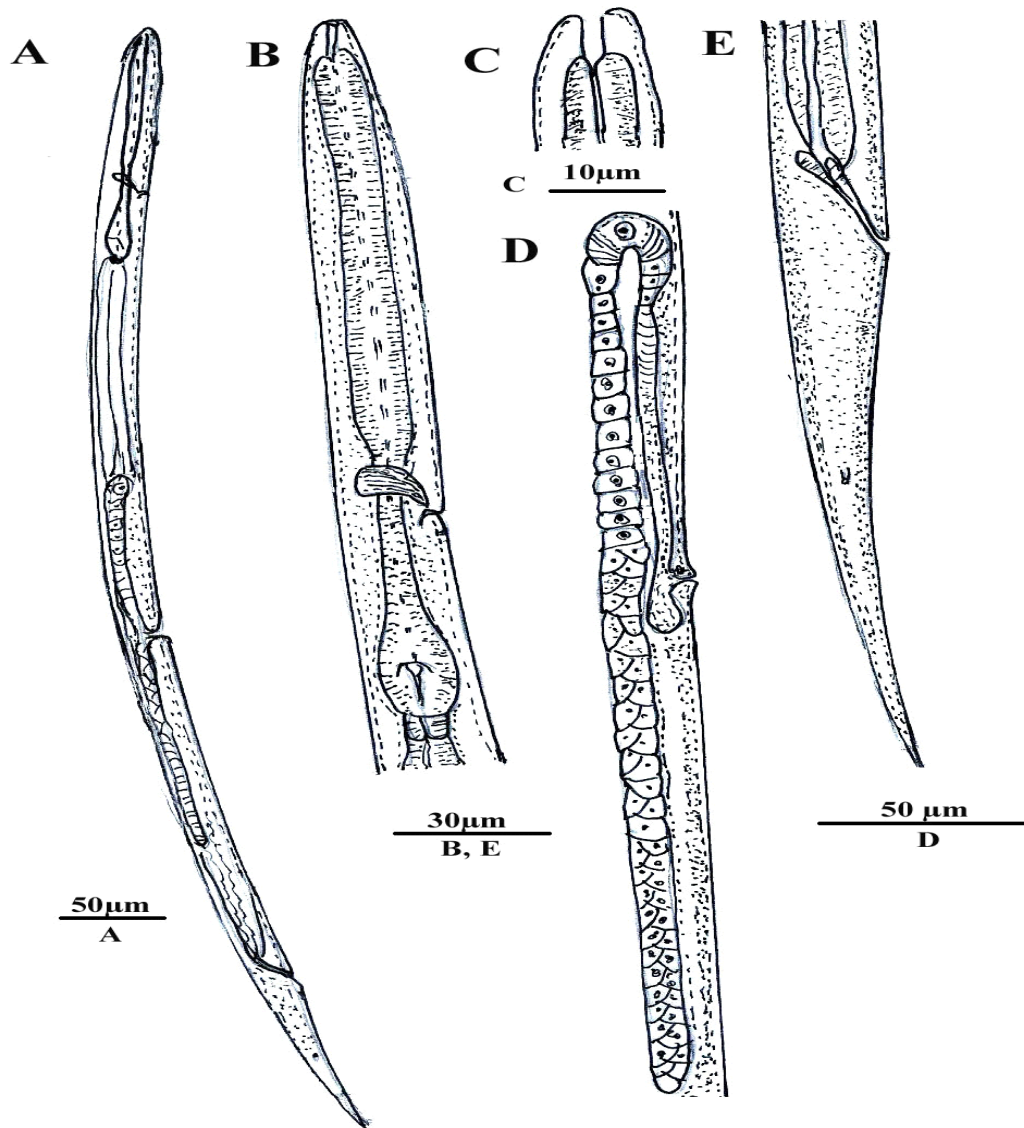


Figure 3. *Panagrolaimus hygrophilus* Bassen, 1940. Female: A-E; A. Whole body; B. Pharyngeal region; C. anterior region; D. vulval region; E. tail region.

Table 2. Measurements (μm) of *Panagrolaimus hygrophilus* Bassen, 1940 and *P. rigidis* (Schneider, 1866; Thorne, 1937).

Characters	<i>Panagrolaimus hygrophilus</i>		<i>Panagrolaimus rigidis</i>	
	Male Mean \pm SD (Range)	Female Mean \pm SD (Range)	Female Mean \pm SD (Range)	Male Mean \pm SD (Range)
Total Length	1149 \pm 27.7 (1110-1174)	1200 \pm 14.39 (1183-1213)	1145 \pm 58.4 (1073-1267)	963
Stoma length	7 \pm 0.25 (6-8)	7.5 \pm 0.3 (7-8)	10.5 \pm 3.8 (10-12)	12
Stoma width	2.5 \pm 0.1 (2-3.5)	3 \pm 0.1 (3-3.5)	3.5 \pm 0.6 (03-04)	03
Lip width	7.5 \pm 0.3 (7-8)	7.6 \pm 0.2 (7-8)	9 \pm 1.2 (8-10)	08
Pharynx	183 \pm 14 (166-199)	196 \pm 3.0 (193-200)	182 \pm 25 (178-188)	170
Excretory pore	149 \pm 10 (135-160)	145 \pm 4.3 (140-150)	147 \pm 14 (143-153)	143
Nerve ring	133 \pm 8.5 (125-145)	136 \pm 3.1 (133-140)	139 \pm 12 (133-148)	130
Anal body width	21 \pm 0.4 (20-22)	18.5 \pm 0.4 (18-19)	24 \pm 3.5 (21-28)	21
Tail length	106 \pm 2.5 (104-110)	118 \pm 2.5 (115-131)	87 \pm 4.5 (82-90)	70
Vulva	--	665 \pm 3.1 (663-670)	590 \pm 34 (580-671)	--
Max.B.W	23 \pm 0.2 (22-24)	28 \pm 0.6 (26-31)	37 \pm 6.2 (34-47)	34
a	6.1 \pm 0.1 (5.8-6.6)	42 \pm 3.1 (38-46.6)	27 \pm 3.8 (25.3-29)	28.3
b	10.2 \pm 0.1 10-10.6	6.1 \pm 0.1 (6.0-6.2)	5.9 \pm 2.4 (5.7-6.4)	5.6
c	10.2 \pm 0.1 (10-10.7)	9.5 \pm 0.2 (9.2-10.2)	12 \pm 2.8 (11.8-14)	13.7
c'	4.9 \pm 0.1 (4.7-5.2)	6.5 \pm 0.2 (6-7)	3.6 \pm 1.5 (3.2-4.2)	3.3
V%	--	55 \pm 0.3 (55-56)	55 \pm 7.2 (54.0-56.5)	--
Spicule	25 \pm 1.5 (25-26)	--	--	27
Gubernaculum	12.5 \pm 0.3 (12-13)	--	--	13

Male: Body J-shaped with heat relaxation. Testis single, reflexed ventrally. Tail conical and distally curved. Spicules curved, head rounded, calamus short, lamina curved dorsally bearing proximal hump. Gubernaculum well developed less than half of the spicule long.

Remarks: Specimens of *Panagrolaimus hygrophilus*

Bassen, 1940 collected from soil around the roots of banana (*Musa acuminata* L.) of Malir Cantt, Karachi, Sindh, Pakistan. Specimens are very similar to those of the original description given by Bassen (1940). This is the first record of this species from Pakistan.

Panagrolaimus rigidis (Schneider, 1866; Thorne, 1937)

Description (Figure 4 and Table 2)

Female: Body (1073-1267) μm long, slightly curved ventrally when killed by gentle heat. Cuticle smooth, about 1.5 -2 μm wide at corresponding body diameter. Lateral fields with three incisures at mid-body. Lip region consists of three doublets, stoma narrow tubular; cheilostom not cuticularized; stegostom with weak cuticularization. Pharynx consists of cylindrical corpus, isthmus slender, basal bulb ovoid with well-developed valves. Nerve ring situated at mid of isthmus. Deirids at level of basal bulb, cardiaconoid, surrounded by intestinal tissue. Gonad monodelphic-prodelphic, uterus about 2.0-2.5 times long at corresponding body diameter; vagina less than a half of the body width, vulva a transverse slit with protruding lips. Tail conical with

acute tip.

Male: Body "J"-shaped after heat relaxation. General morphology similar to female. Gonad monorchic, testis reflexed ventrally. Tail conical, ventrally curved with acute tip. Spicules curved ventrally: manubrium rounded, bent ventrally, calamus short, lamina well-developed having ventral wing and rounded terminus. Gubernaculum well developed, less than a half of spicules length.

Remarks: Specimens of *Panagrolaimus rigidis* (Schneider, 1866), Thorne, 1937 captured from soil of guava (*Psidium guajava* L.) of Malir Cantt, Karachi, Sindh, Pakistan. Specimens are quite similar to the original description given by Thorne (1937). This is the first record of this species from Pakistan.

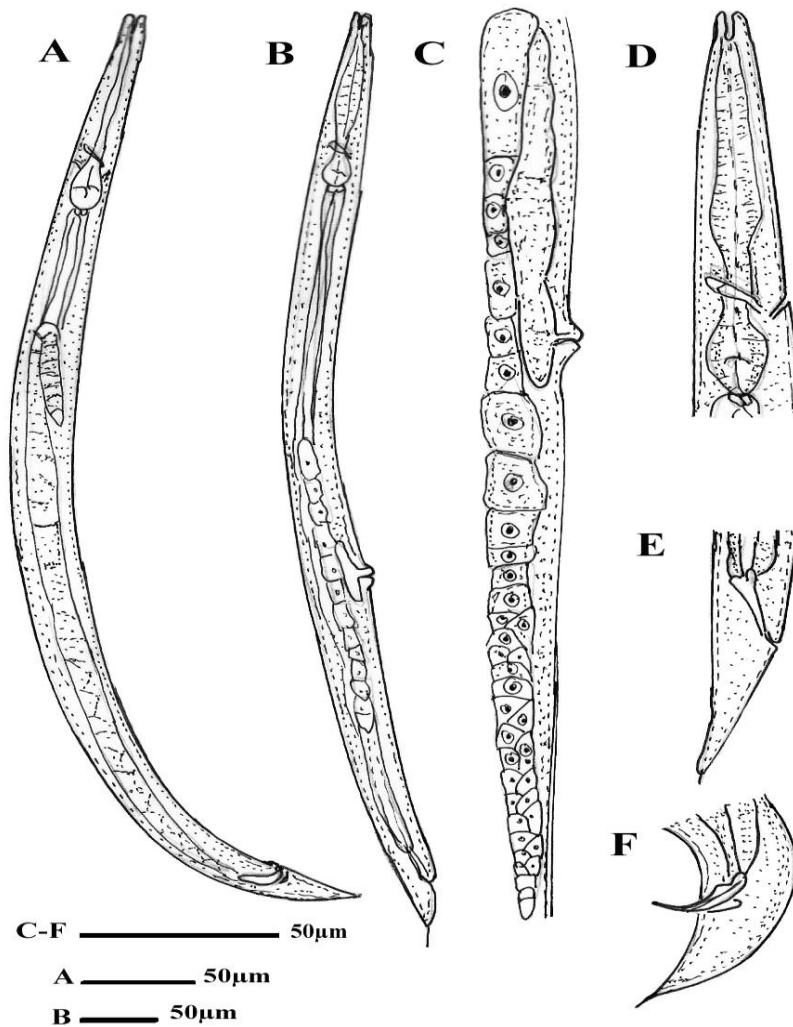


Figure 4. *Panagrolaimus rigidis* (Schneider, 1866; Thorne, 1937). Female: B-E; B. Whole body; C. vulval region; D. Pharyngeal region; E. tail region; Male: A. Whole body; F. tail region.

Mononchoides change (Goodrich et al., 1968)

Description (Figure 5 and Table 3)

Female: Body almost straight after heat relaxation. Fine transverse striations present on cuticle having 14 longitudinal ridges. Head round, continuous with body contour. Lips fused, with a small papilla. Amphids present, located at base of dorsal tooth. Stoma longer than wide, chielostom cuticularised. Cheilostom divided into some rod like plates. Other part of stoma divided into gymnostom and stegostom. Two dorsolateral denticles present on inner wall of gymnostom, large claw like dorsal tooth situated on stegostome. Pharynx having

muscular procorpus slightly expanding posterior with valves and a short, slender, glandular isthmus with nonmuscular elongated oval basal bulb without valves. Nerve ring encircling isthmus in anterior half. Excretory pore 150-219 μm posterior to anterior end. Reproductive system amphidelphic, uterine sac 20 μm long, connecting both uteri. Vulva anterior in position (38-40%), vulval lips weakly cuticularised, not protruding. Vagina muscular, with narrow lumen. Phasmids prominent, located posterior to anus. Tail long, filiform.

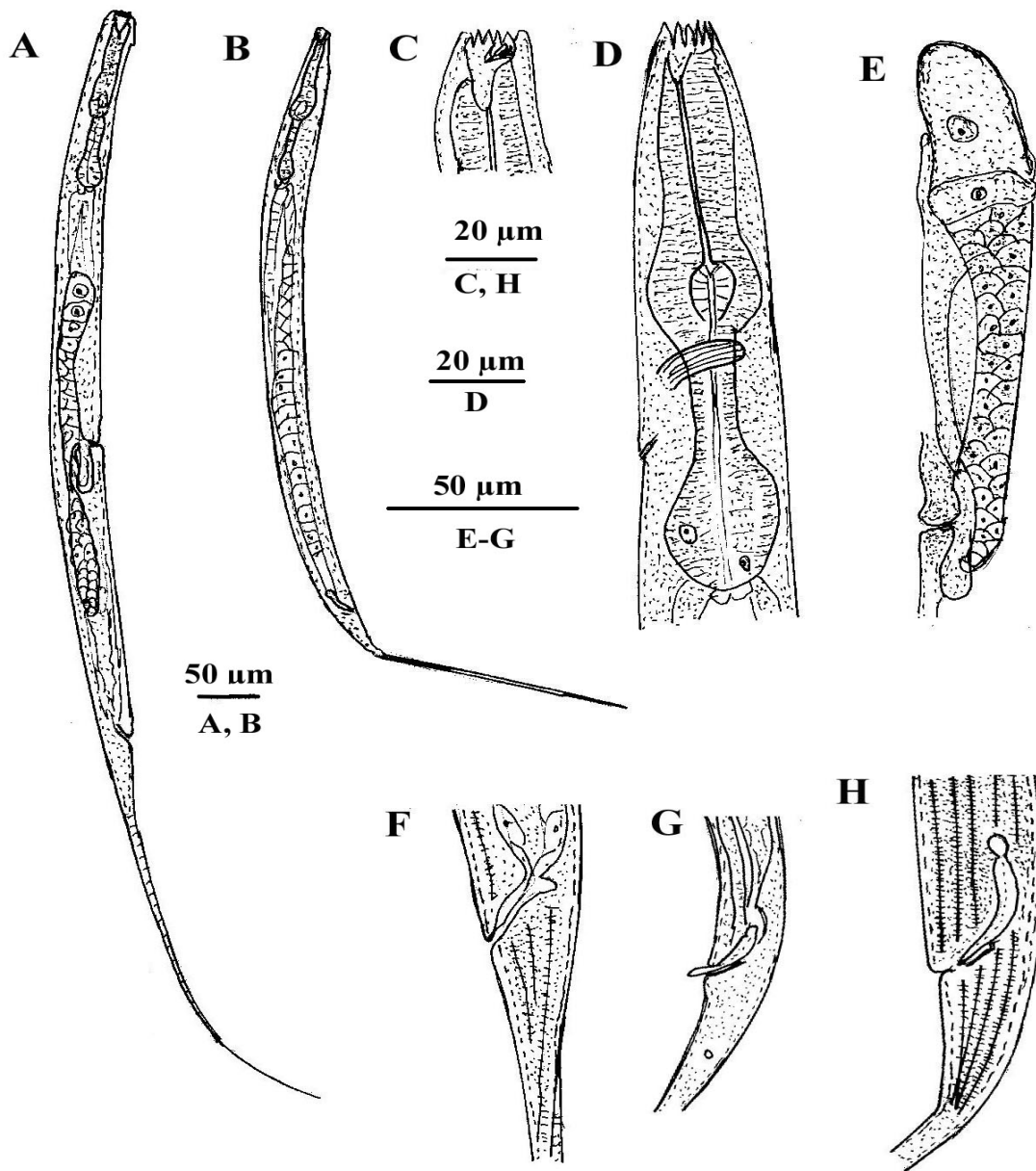


Figure 5. *Monochoides changi* (Goodrich et al., 1968). Female: A-E; A. Whole body; C. anterior region; D. Pharyngeal region; E. vulval region; F. tail region; Male: B. Whole body; G,H. tail region.

Table 3. Measurements (μm) of *Monochoides changi* (Goodrich et al., 1968).

Character	Male	Female Mean \pm SD (Range)
Total Length	942	1267.5 \pm 22.3 (1243-1301)
Stoma length	18	2.14 \pm 1.6 (19-23)
Mesostome width	07	6 \pm 0.7(5-7)
Lip width	15	16 \pm 0.7 (15-17)
Telostome	10	12.5 \pm 0.5 (12-13)
Pharynx	130	177 \pm 7.6 (165-185)
Excretory pore	115	171 \pm 28 (150-219)
Anal body width	26	28.7 \pm 1.7 (27-31)
Tail length	395	441 \pm 70 (360-550)
Vulva	--	500 \pm 10 (483-509)
Max. B. W	30	43.5 \pm 2.0 (41-46)
a	31.2	28.6 \pm 0.9 (28-30)
b	7.2	7 \pm 0.3 (6.8-7.5)
c	8.3	2.8 \pm 0.4(2.7-3.5)
c'	5	15.2 \pm 1.7(13.3-17.7)
V%	--	38.9 \pm 0.7(38-40)
Spicule	40	--
Gubernaculum	20	--

Male: General morphology similar to female but body slightly smaller, typically J-shaped after fixation. Testis single, anteriorly ventrally reflexed. Spicules separate slightly arcuate. Gubernaculum with inconspicuous proximal appendage. Nine pairs of genital papillae present: three pairs precloacal, six pairs postcloacal. Tail mostly shorter than female, filiform.

Remarks: Specimens of *Mononchoides changi* (Goodrich et al., 1968) encountered from soil of guava (*Psidium guajava* L.) of Malir Cantt, Karachi, Sindh, Pakistan. Specimens are quite similar to the original description. This is the first record of this species from Pakistan.

CONTRIBUTION OF THE AUTHORS

All the authors equally participated in designing the study, collecting soil samples, identification of nematodes, performing micrometry, and writing and editing the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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