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DESCRIPTION OF NEW SPECIES OF *METARHABDITIS LONGICAUDATA* (NEMATODA: RHABDITIDAE) WITH THREE NEW RECORDS FROM SINDH, PAKISTAN

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ABSTRACT

Many rhabditid nematodes like *Metarhabditis* belonging to the bacteriophage group are considered to be necromenic associates of insects and used to be facultative entomopathogenic nematodes or as biocontrol agents. These nematodes can be safely used against insect pests of different crops and vegetables. In the present study, nematode specimens of new and known species were collected from different areas of Sindh and isolated from soil samples by baiting technique using last instar *Galleria mellonella*. One new nematode species *Metarhabditis longicaudata* n. sp. and three new records of *M. adenobia, M. amasactae,* and *M. rainai* (Nematoda: Rhabditidae) were found from Sindh. *M. longicaudata* n. sp. differed from the species of the genus *Metarhabditis* by having long un-covered tail spike. It was also characterized by a large sized male 1286 (1154-1325) µm; longer male tail 69.5 (62-76) µm; longer spicule 43 (40-46) µm; leptoderan bursa; longer gubernaculum 27 (20-34) µm. The female was also of large size of 1507 (1366-1684) µm with larger tail 102 (94-112) µm and longer pharynx 231 (206-265) µm. The genus *Metarhabditis* is reported for the first time from Sindh, Pakistan.

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INTRODUCTION

Soil is an excellent habitat for nematodes and 100 cc of soil may contain several thousands of nematodes. Because of their importance to agriculture, scientists and farmers are well known about plant-parasitic nematodes than about the other kinds of nematodes which are commonly present in soil. Many types of soil nematodes do not parasitize plants, but are beneficial and can be used as biocontrol agents. These nematodes are often referred to as free-living nematodes and are useful for nitrogen fixation in soil. Mass culturing and application of these nematodes will be helpful for growth of plants by inhibiting the growth of insects. Genus *Metarhabditis* (rhabditids) was described in 2004 with type species *M. andrassyana* (Tahseen et al., 2004). In 2011, Sudhaus revised the family rhabditidae in which many species were transferred from and into different genera. Some species from *Rhabditis* (*R. rainai* Carta & Osbrink, 2005; *R. blumi* Sudhaus (1974); *R. adenobia* Poinar, 1971; *R. costai* Martins Júnior (1985); *R. freitasi* Martins Júnior (1985) and other from *Oscheius* (*O. amsactae* Ali, Pervez, Andrabi, Sharma & Verma, 2011) were transferred to *Metarhabditis*. Species of the genus *Oscheius* was synonymized with *M. amsactae* (*O. ciceri*, *O. hussaini*

Shaheen et al. (2011); *O. gingeri* Pervez et al. (2013). In the present study, the extensive survey of different agricultural areas of Karachi, Sindh was conducted during the year 2018 to describe and illustrate *Metarhabditis longicaudata* n. sp. with three new records viz. *M. adenobia, M. rainai* and *M. amasactae* (Nematoda: Rhabditidae). These species are reported for the first time from different fruits of Sindh, Pakistan.

MATERIALS AND METHODS

New species and new records of *Metarhabditis* were found from 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 27-30ºC. 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 ^{o}C) for morphological observations and variation of structures. Specimens were concentrated in a few milliliters of water and fixed in an aqueous solution of 11% formalin + 6% glycerin. The equal volume of boiled fixative to the amount of water was added in a watch glass 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 ^{o}C in 2 ml of 2.5% glycerin. For permanent mount, 10 nematodes were individually picked and transferred to 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, date of making slide and host. Nematode descriptions were accompanied by measurements using a Nikon E-400 in the form of 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

***Metarhabditis longicaudata* n. sp.:** (Figure, 1 and 2), Measurements: See table 1.

Female: Body straight to slightly arcuate ventrally, when heat-relaxed. Cuticle smooth finely transversely annulated, 0.5-1 µm thick with fine annulations, longitudinal striation in conspicuous. Lateral field with 4 ridges (5 incisures), occupying about 7-9 µm of the corresponding diameter at mid body. Head continuous to slightly offset from adjoining body. Lips round forming 3 parts, arranged around oral aperture in the form of doublets. Amphids inconspicuous under light microscope. Stoma tubular, moderately cuticularized, 1.5-2 times the lip region diameter in length. Pharyngeal sleeves surrounding 40-50% of stoma length. Cheilostome cuticularized, gymnostome smaller than stegostome (pharyngeal collar). Glottoid apparatus isomorphic and isotopic with plate bearing 4-6 setose denticles. Telotegostom isoglottoid. Pharynx 206-265 µm long, pharyngeal corpus cylindrical 30-40% of pharyngeal length. Median bulb weak or absent, slightly narrower isthamus and a round valvate basal bulb. Nerve ring situated at 65-74% of pharyngeal length. Excretory pore slightly anterior to basal bulb 60-70% of pharyngeal length. Deirids and hemizonid inconspicuous, cardia small, 5-10 µm long.

Gonads didelphic amphidelphic, ovaries moderately developed, dorsally reflexed often extending as far as vulva, ovoid spermatheca contains round sperm, demarcated from uterus by distinct sphincters. Ovaries dorsally reflexed, sometimes reaching level of vulva. Eggs present in different embryonic stages with in uterine. Vulva in the form of transverse slit with protruding lips, weak epiptygma but distinct cuticular flap. Rectum about 2-3 times as long as anal body width. Tail elongate 2.1-3.7 times longer than the body width at anus, gradually tapering to a fine terminus. Phasmid tubular located about one anal body diameter posterior to anus.

Male: Body slender, arcuate curved posteriorly 1286 (1154- 1325) µm long (Table 1), testis single reflexed ventrally on the left side of intestine. Vas-deference a broad tube filled with sperms without demarcation of seminal vesical. Ejaculatory gland absent. Tail conical, with sharp posterior end, 62-76 µm long. Bursa leptoderm, anteriorly open not, enclosing long tail spike. Bursa bearing eight pairs of genital papillae of different length arranged as 1+1/1+3+2 (three of them pre-cloacal and six post-cloacal). P_1 is well anterior to the cloaca whereas pair 4-6 (P_{4-6}) and 7-9 (P_{7-9}) posterior to cloaca. Tubular phasmid present. Copulatory muscle bands mostly faint and weakly developed. Spicule paired, long, separate, symmetrical, slightly curved ventrally having round or beak like capitula. Gubernaculum slightly curved boat shape with flat distal end.

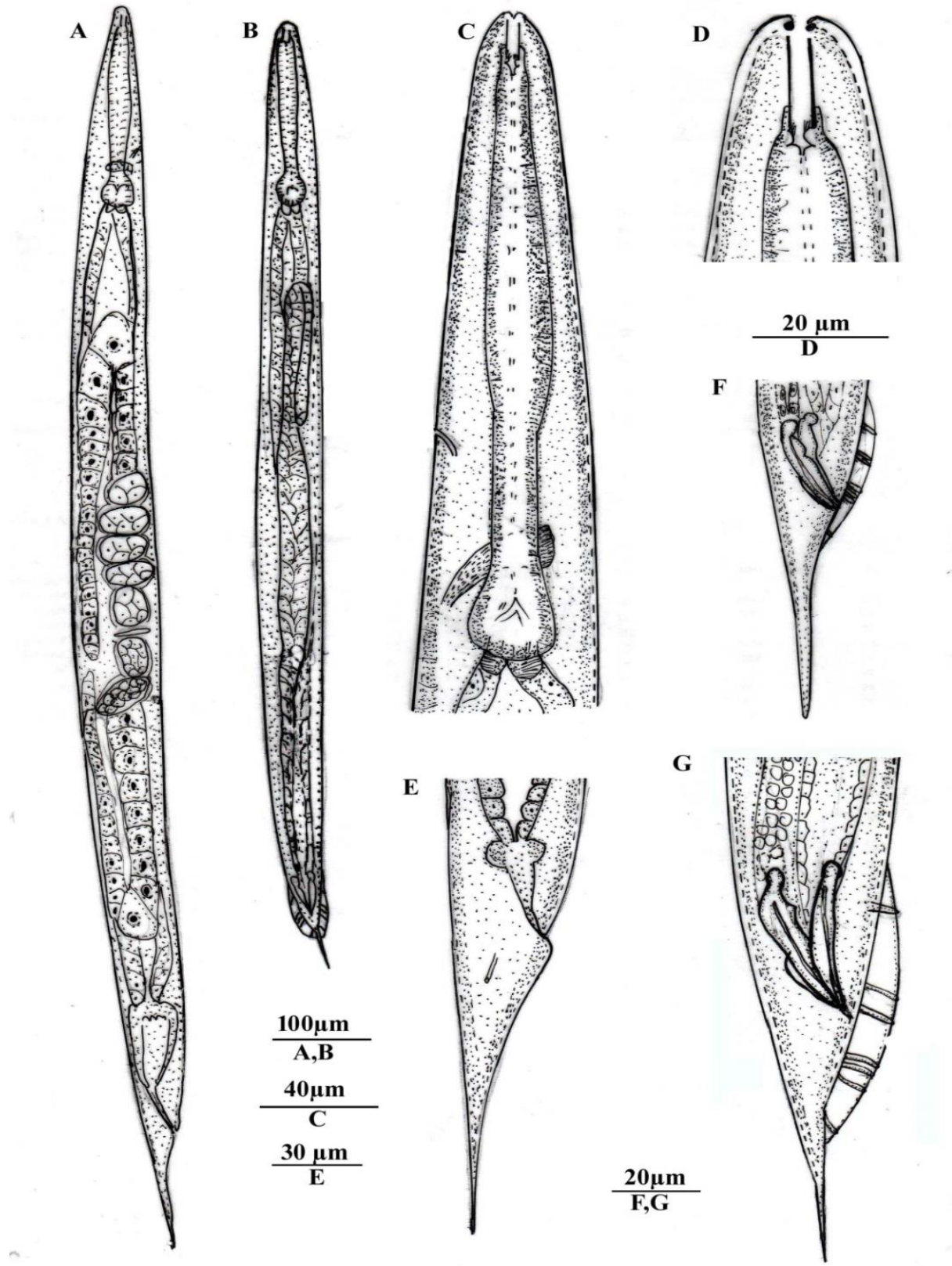

Figure 1 (A-F). *Metarhabditis longicaudata* n. sp. Female (A,C-E): B, whole body; C. Pharyngeal region; D. Anterior region; E, Tail region; Male: (B,F,G); B. whole body; F,G. Ventral view of tail region showing papillae.

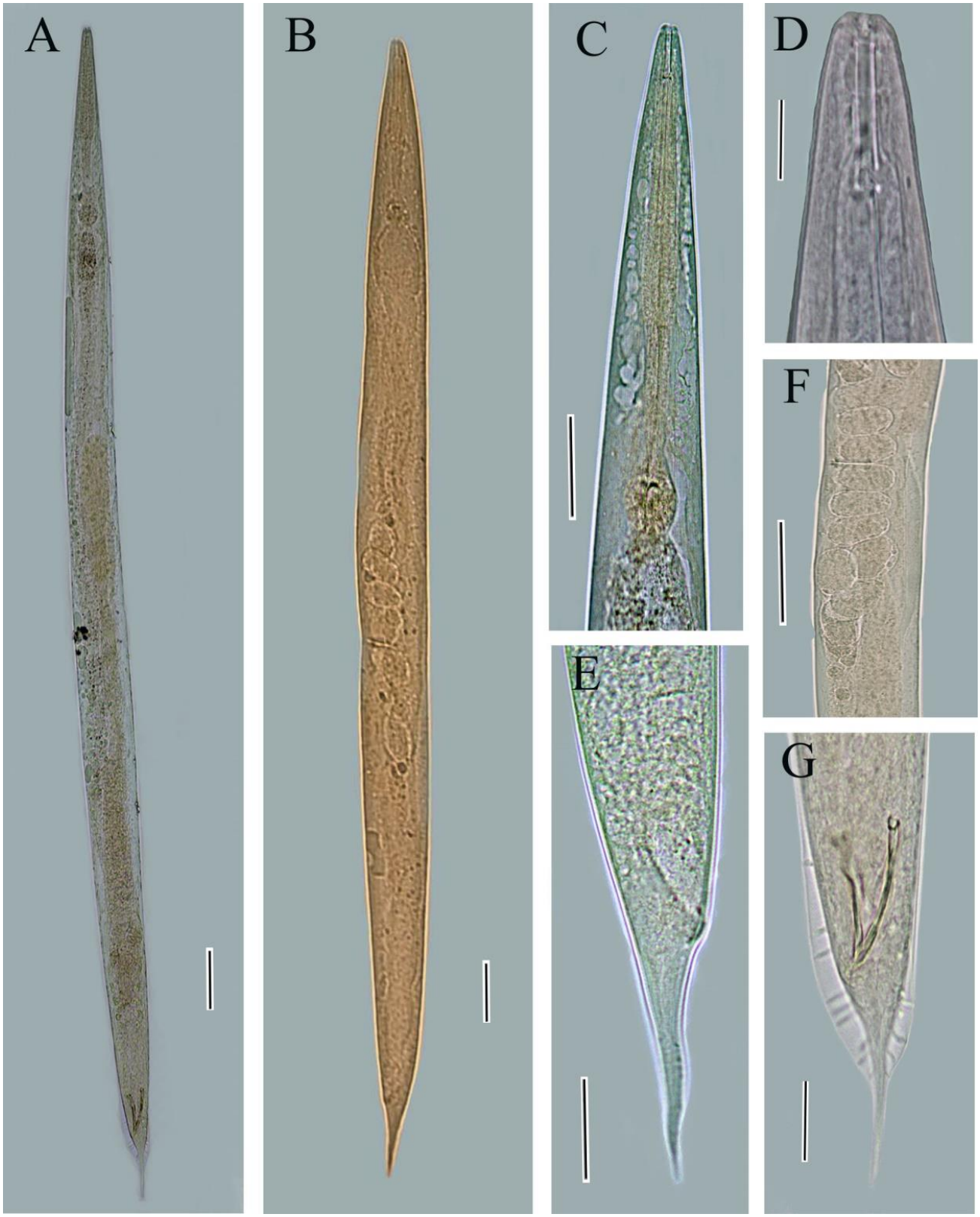

Figure 2 (A-G). Light microphotographs of *Metarhabditis longicaudata* n. sp. Female (B-F): B, whole body; C. Pharyngeal region; D. Anterior region; E, Tail region; F. Vulval region; Male: A, G; A. whole body; G. Ventral view of tail region showing papillae (scale: A,B, =100µm; C-H=20µm).

Table 1. Measurements of *Metarhabditis longicaudata* n. sp. (all measurements except a-c' and V in µm).

Characters	Holotype male	Paratype male	Paratype female
Total Length	1325	1286 ± 137 (1154-1325)	1507 ± 96.6 (1366-1684)
Stoma length	24	23 ± 2.0 (20-28)	24.5 ±1.7 (22-28)
Stoma width	04	3 ± 0.5 (3-4)	3.8 ± 0.6 (3-5)
Lip width	12	12 ± 0.9 (11-14)	11.4 ± 3.3 (11-15)
Oesophagus	241	212 ± 18.9(184-256)	231 ± 16.7 (206-265)
Excretory pore	160	162 ± 14 (137-179)	187.7 ± 26 (148-250)
Nerve ring	152	158 ± 12.6 (143-185)	170 ± 12.3 (154-190)
Anal body width	34	26 ± 4.3 (21- 4)	28 ± 3.1 (25-33)
Tail length	72	69.5 ± 3.9 (62-76)	102 ± 7.8 (94-112)
Vulva	-	43 ± 2.0 (40-46)	785 ± 58 (725-899)
Max. B.W	76	74.4 ± 4.1 (64-69)	93 ± 7.0 (78-108)
a	17.4	16.3 ± 0.8 (14.4-19.4)	16.2 ± 1.1 (14-18)
b	5.4	5.8 ± 0.5 (5.3-6.6)	6.5 ± 0.7 (5.5-7.8)
c	18.4	17.5 ± 1.2 (14.9-19)	14.8 ± 1.6 (12-16.4)
c'	2.1	2.7 ± 0.41 (2.1-3.7)	3.4 ± 0.4(2.5-4.4)
V%	-	-	52 ± 2.0 (48.5-55.7)
Spicule	46	43 ± 2.0 (40 -46)	-
Gubernaculum	25	26.7 ± 3.3 (20-34)	-

Juveniles: Body short, slender tapering gently toward both ends. Cuticle with longitudinal striations; head rounded lip region smooth, mouth closed. Stoma and pharynx morphology similar to adult. Cardia protruding into intestine. Excretory pore located at the middle of basal bulb. Nerve ring surrounding the isthmus. Tail elongate conical, tapering to a pointed terminus.

Type habitat and locality: Samples containing *M. longicaudata* n. sp. collected from Mango tree (*Mangifera indica* L.) of Malir Cantt, Karachi, Sindh, Pakistan.

Type specimen: Holotype male, 10 female and male paratypes deposited in the National Nematode Collection of NNRC, University of Karachi, Karachi 75270 Pakistan.

Etymology: The name of the species refers to its long tail character i.e *longicaudata*.

Diagnosis and Relationship: *M. longicaudata* n. sp. can be different from all species of the genus *Metarhabditis* by having long un-covered tail spike. It is also characterized by a large size male 1154-1325 µm; longer male tail 62-76 µm; longer spicule 40-46 µm; leptoderan bursa; longer gubernaculum 20-34 µm; in female large size of whole body 1366-1684 µm; and larger tail 94-112 µm; longer pharynx 206-265 µm (Table 1). According to key described by Tahseen et al. (2004), *M. longicaudata* n. sp. comes close to *M. amasactae* by having uncovered long tail spike but this species distinguish by having the characters in male larger body size (1154-1325 µm vs 515-868 µm); long spicule (40-48 µm vs 25-40 µm); and gubernaculum (20-34 µm vs 10.3-20.0 µm). In female larger body (1366-1684 µm vs 631-1022 µm); larger pharynx (206-265 µm vs 150-218) and longer tail (94-112 vs 69-100) µm; larger distance from head to excretory pore (148-250 vs 104-160 µm); slightly longer stoma (22-28 µm vs 15.8-25 µm). Other species of the genus *Metarhabditis* by having short un-covered tail spike, i.e., *M. blumi* differs from *M. longicaudata* n. sp. by having gubernaculum large in size (20-34 µm vs 16-22 µm); shorter a ratio (14.4-19.4 vs 17.3-22.3); higher c ratio (14.9-19.0 vs 17-27). This new species also distinguish from female by having shorter tail (94-112 µm vs 156-221 µm); shorter a ratio (14-18 vs 18-23); higher c ratio (12.0-16.4 vs 7.0-9.7). *M. andrassyana* differs from *M. longicaudata* n. sp. by having male with smaller body length (841-1073 vs 1154-1325 µm), gubernaculum small in size (16-20 vs 20-34 µm). In female *M. andrassyana* differs from *M. longicaudata* n. sp. by having (964-1515 vs 1366-1684 µm), long tail (150-195 vs 94-112 µm). Other species of the genus *Metarhabditis* differ from the new species viz., *M. adenobia*, *M. freitasi*, and *M. costai* by having spicules stout, ≥ 50 µm long.

Metarhabditis adenobia (Poinar, 1971) Sudhaus, 2011 (Figure 3).

Measurements:

Male: L = 1284 ± 92.8 (1181- 1475) µm; a = 17.5 ± 1.39 (16-20); b = 5.4 ± 0.49 (5-6); c = 29 ± 4.0 (23 - 35); c' = 1.2 ± 0.04 (1-2); spicule = 43.2 ± 4.6 (38-50) µm ; Gubernaculum = 25 ± 3.2 (18-28) µm; stoma length = 23.7 ± 2.86 (20-30) µm; Tail length = 44.4 ± 4.3(40-51) µm; Anal body width = 29 ± 1.27 (28-32) µm; Maximum body width = 73 ± 6.4(62-80) µm; Pharynx length = 241.5 ± 19.5 (216-280).

Female: L = 1760 ± 77.9 (1676-1926) µm; a = 17.3 ± 1.2(15-19); b = 7.1 ± 0.31(7-8); c = 13 ± 1.4 (11-15) c'= 4.4 ± 0.68 (3-5); stoma length = 22 ± 1.2(20-23) µm; Tail length = 136 ± 12.3(121-155) µm; Anal body width = 31.4 ± 2.7 (25-35); Maximum body width = 102 ± 8.7 (90-120) µm; V% = 52 ± 0.94(51-54) µm; Pharynx length = 241.7 ± 12.3 (224-266).

Remarks: The specimens of *M. adenobia* (Poinar, 1971) Sudhaus (2011) were collected from decomposed fruit of guava (*Psidium guajava* L.) from vegetative areas of Sindh. The morphological characters showed similarity with previously described *M. adenobia* and is being reported for the first time from Pakistan.

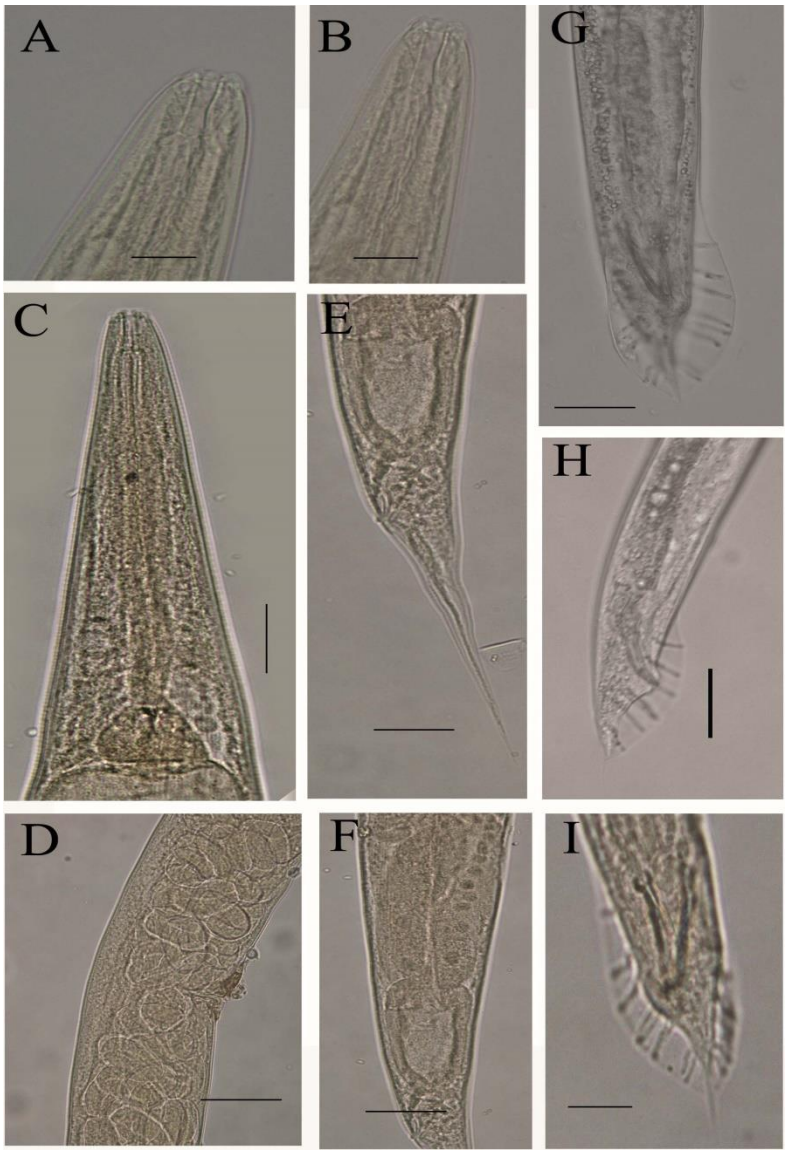

Figure 3 (A-G). Light microphotographs of *Metarhabditis adenobia* (Poinar, 1974), Sudhaus, 2011. Female (A-F): A,B. Anterior region; C. Pharyngeal region; D. Vulval region; E,F. Tail region; Male: G-I Ventral view of tail region showing papillae (scale: A,B,I =10µm; C-H=20µm).

Metarhabditis amasactae

(Ali, Pervez, Andrabi, Sharma, Verma 2011), Sudhaus, 2011 (Figure 4).

Description

Measurements:

Male: L = 1347 ± 95.8 (1234-1498) µm; a = 16.3 ±1.8 (14-20); b = 6.4 ± 0.69 (6-8); c = 18.8 ± 1.39 (16-20); c' = 2.6 ± 0.5(2-3); spicule = 50.2 ± 5.02(42-60) µm ; Gubernaculum = 19 ± 2.5 (16-22) µm; stoma length = 21.8 ± 1.3(20-24) µm; Tail length = 71.6 ± 3.9(66-78) µm; Anal body width = 29 ± 1.6 (27-32); Maximum body width = 83.3 ± 8.2(70-98) µm; Pharynx length = 215.5 ± 20.6 (204-236).

Female: L = 1603 ± 52.9 (1546-1694) µm; a = 16.5 ± 1.05(15-18); b = 7 ± 0.6 (6-8); c = 14.9 ± 2.07 (13-19); c'= 3.4 ± 0.5(3-4); stoma length = 22.9 ±1.28(22-26) µm; Tail length = 109 ± 13.8 (84-132) µm; Anal body width = 31.8 ± 1.9 (30-36); Maximum body width = 97.4 ± 5.6(88-105) µm; V% = 51.7 ± 0.6(50-54) µm; Pharynx length = 231 ± 10.8 (218-247).

Remarks: Specimens of new species were collected from soil around the roots of Chicko (*Manilkara zapota*, L.) fields of Malir Cantt, Karachi, Sindh, Pakistan. Measurements and morphological characters are similar with reported species *M. amasactae* given by Ali et al. (2011).

Figure 4 (A-G). Light microphotographs of *Metarhabditis amasactae* (Ali *et al*., 2011), Sudhaus, 2011 Female (A-D): A. Pharyngeal region; B. Anterior region; C. Vulval region; D. Tail region; Male: (E-G): E. Lateral view of tail papillae; F,G: Ventral view of tail region showing papillae (scale: A, F, D, G =20µm; B,C, E=20µm).

Metarhabditis rainai: (Carta and Osbrink, 2005), Sudhaus (2011)(Figure 5).

Measurements:

Male: L = 1281.8 ± 96.5 (1100- 1392) µm; a = 19.3 ± 2.57 (15-24); b = 5.5 ± 0.6 (4-6); c = 29.8 ± 4.3 (23-37); c'= 1.4 ± 0.4(1-2); spicule = 44.8 ± 8.4 (32-60) µm ; Gubernaculum = 17.8 ± 2.9 (13-23) µm; stoma length = 25.9 ± 1.37(24-28) µm; Tail length = 44.4 ± 6.9(32-56) µm; Anal body width = 30.2 ± 2.56 (26-34); Maximum body width = 67 ± 8.8(54-88) µm; Pharynx length = 233.8 ± 12.2 (211-256).

Female: Length = 1863 ± 94.08 (1769-2078) µm; a = 18.5 ± 1.3(117-20); b = 7.1 ± 0.3(7-8); c = 15.8 ± 2.09 (12-18); c'= 6.3 ± 1.5(4-8); stoma length = 27.8 ±1.2(26-30) µm; Tail length = 117.7 ± 19.6 (100-148) µm; Anal body width = 20 ± 7.0 (12-30); Maximum body width =102 ± 7(92-111) µm; V% = 51 ± 3.1(49-58) µm; Pharynx length = 261.2 ± 8.1 (250-279) (Figure 5).

Remarks: The present specimens were collected from decomposed fruit of guava from vegetative areas of Sindh. Measurements and morphological characters are similar with previously described *M. rainai* given by Carta and Osbrink (2005).

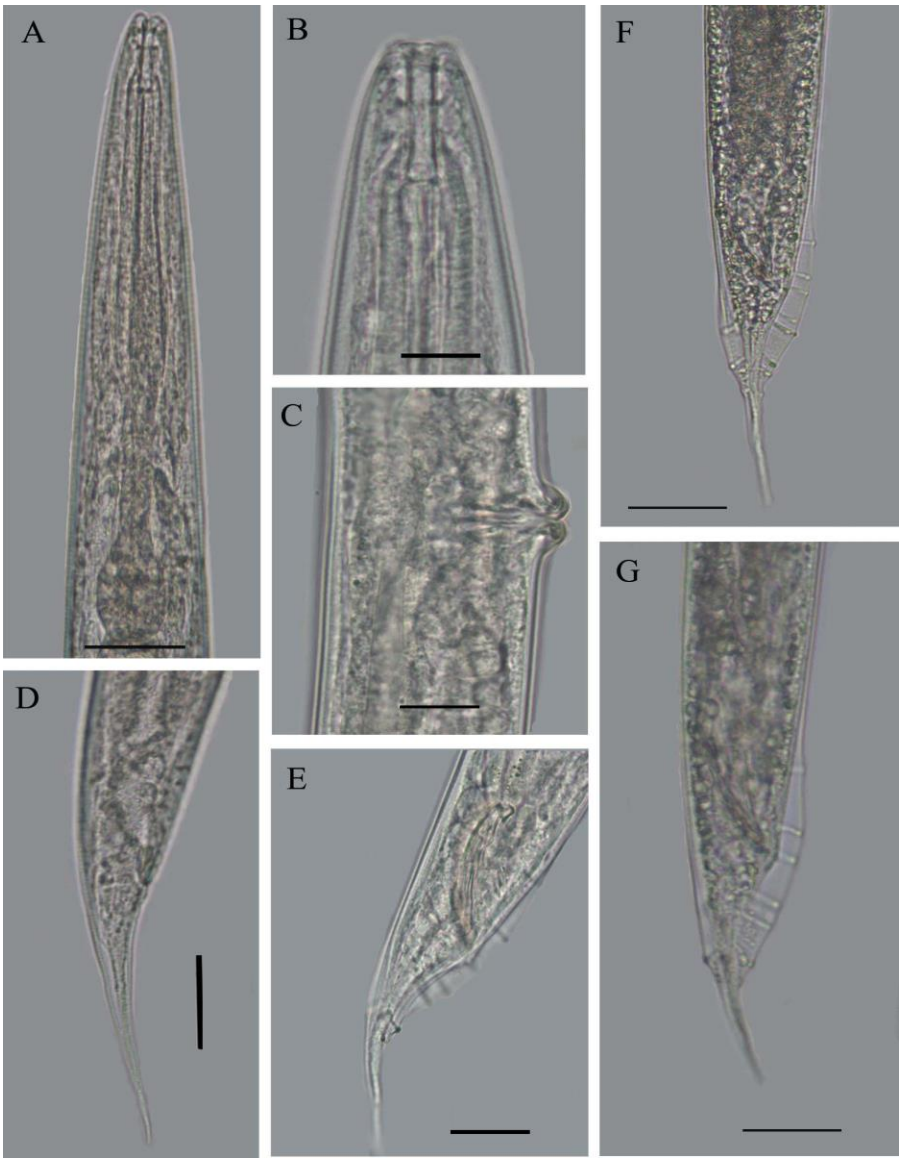

Figure 5 (A-G). Light microphotographs of *Metarhabditis rainai* (Carta and Osbrink, 2005) Sudhaus, 2011. Female (B-E):, B. Anterior region; C. Pharyngeal region; D. Vulval region; E. Tail region; Male: A. whole body; F,G: Ventral view of tail region showing papillae (scale: A, G-I=10µm; B-F=20µm).

CONCLUSION

One new species of soil nematode viz. *Metarhabditis longicaudata* n. sp., along with three new record species viz. *M. adenobia* (Poinar, 1971), Sudhaus (2011), *M. amasactae* Ali et al. (2011), Sudhaus (2011) and *M. rainai* Carta & Osbrink (2005) Sudhaus, 2011 were isolated and identified. Genus *Metarhabditis* was for the first time reported from Pakistan. This study help will in isolating more new species of this genus.

Authors' contribution: TAK designed the study, identified the nematodes and wrote the manuscript; SJ helped in the identification of nematodes and reviewed the manuscript; NM collected the nematode samples.

Conflict of Interest: The authors declare no conflict of interest.

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