



Description of *Neorhabditis andrassyii* n. sp., and Re-Description of *Poikilolaimus oxycercus* de Man, 1895 from Sindh

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ABSTRACT

During surveys of chikoo (*Manilkara zapota* L. van Royen) plantation at Saedabad, Karachi, Sindh, Pakistan, several soil sample analysis revealed one new and one known free-living soil nematode species, *Neorhabditis andrassyii* n. sp., and *Poikilolaimus oxycercus* which represents a new record of this species from Pakistan. *Neorhabditis andrassyii* n. sp. is characterized by having male (1109-1222) μm long body, spicules 32-44 μm long, gubernaculum slightly curved, boat shaped with flat distal end eight pairs of bursal papillae were present. Tail (46-68) μm long conical with fine tip, leptoderan bursa. Female 1223-1742 μm long, anterior to middle vulva 48.7 (37-55) % tail filiform 156-206 μm long, 7-18.7 times as anal body width long. Detailed taxonomical studies of new species are given with measurements, descriptions, illustrations and photomicrophotographs along with brief description, measurements and microphotographs of *Poikilolaimus oxycercus*.

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Authors' Contribution

TAK conceived and designed the study and drafted the manuscript. NM collected, analyzed and processed samples. SF guide for the article.

Key words

Neorhabditis, *Manilkara zapota*, Description, Soil nematodes, Taxonomy.

INTRODUCTION

Genus *Neorhabditis*, Schuurmans Stekhoven (1954) has single type species *N. flagillicauda* Schuurmans Stekhoven (1951) reported from the roots of African oil palm *Elaeis guineensis* which was transferred from the genus *Pararhabditis* by Sudhaus (2011). *Neorhabditis* has didelphic, amphidelphic reproductive system. Vulva middle in position, vagina with a thin wall covering 20-25% of corresponding body diameter. Gonad didelphic reflexed with tips sometimes reaching level of vulva, anterior un-reflexed part of ovary 400-500 μm long, whereas reflexed part measuring 320-375 μm . Un-reflexed part of the posterior gonad measuring 350-400 μm while reflexed part of posterior gonad 300-350 μm long. Oviduct forming spermatheca, containing large sperm cells of about 2 μm . Ovoviviparous species, uterus with 8-10 eggs at the same time. Rectum 2.5 \pm 0.3 (2.0-3.0) anal body diam., long. Tail elongate, slender, tapering gradually, 3-5 μm anal body diam., long. Phasmids prominent, position variable, located at one-third of tail region posterior to anus. Tail elongate with pointed terminus.

Poikilolaimus Sudhaus (1980) was described in 1930 by Fuchs as a subgenus of the genus *Rhabditis* whereas the genus *Cuticularia* was reported by Van der Lindeas (1938). *Cuticularia mathesoni* was synonymised with *Rhabditis oxycerca* by Osche (1954). Andr ssy (1983)

replaced the genus *Cuticularia* with *C. oxycerca* as its type species, and in 1998 he reported another species *C. firmata* from Antarctica. Khan *et al.* (1999) described *C. brevicorpus* and *C. jenniferae* from India, after that *C. annulata* reported from Siam Bay by Eroshenko (2002).

MATERIALS AND METHODS

During the nematological studies a total of 25 soil samples were taken from the soil around the roots of agrarian site of fruit cultivation of Saedabad, Sindh, Pakistan. Soil was filled in polythene bags and labeled according to the locality, plant name, type of soil and date of collection. Samples reached to lab carefully and were kept at 4°C till further process. Soil nematodes were encountered from soil samples through "Galleria trap" method (Bedding and Akhurst, 1975). Three wax moth larvae were put down into plastic boxes were already occupied with soil of the samples, each box covered with a cap and kept at 25 \pm 2°C temperature. After 2-3 days, dead larvae were rinsed with distilled water and placed on White trap (White, 1927) for isolation of infective juveniles. After the emergence fourth to sixth day the collected juveniles were harvested, and used for identification purpose. These juveniles deposited in a chamber at 15-20°C in a 100-ml beaker having distilled water with a drop of Triton X-100.

For morphological studies male and female were found through white trap and dissections, respectively. First and second generations of soil nematode were obtained after 2-4 and 5-7 days, respectively. Nematodes were killed with boiled water (80°C), and fixed in TAF

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according to Courtney *et al.* (1955). Fixed nematodes washed and transferred in glycerin by following the Seinhorst (1959) method. Permanent slides were made by using glass fiber to avoid flattening of specimens.

Identification of nematodes was made through measurements given by de Man (1884) formula with an ocular micrometer under a compound microscope (Nikon E400 light microscope) and identification was based on the systematics given by Sudhaus (2011). Illustrations were drawn by drawing tube attached to the Nikon Eclipse E400 microscope. Photomicrographs were also captured compound microscope using Nomarski's interference contrast system (Nikon DS-Fi1).

***Neorhabditis andrassyi* n. sp.**

(Fig. 1, Supplementary Fig. S1, Table 1)

Description

Female

Medium size nematode 1223-1742 μm long, body cylindrical, straight after fixation. Cuticle smooth finely annulated. Lips closed and offset by a constriction around the labial region. Six separate well developed lips present each with one-minute terminal papilla. Lateral field with 6 incisures or 5 ridges at mid body. Stoma tubular, 22-26 μm long 7.3 -9 times as long as wide with prominent cheilostome. Glottoid apparatus, pharyngeal collar absent. Pharyngeal corpus cylindrical and narrow, 120-144 μm long, procorpus cylindrical; metacorpus not differentiated from corpus and isthmus, median bulb absent. Nerve ring 142-179 μm away from labial region, usually surrounding mid-part of isthmus, whereas excretory pore at 178-206 μm from anterior region, or at the posterior end of basal bulb ovoid to pyriform. Hemizonid not clearly observed. Basal bulb weak, cardia hemisphericle surrounded by intestinal tissue. Cardia present protruding into intestine. Rectum 2.5-3 times long as anal body width. Reproductive system didelphic, amphidelphic, ovaries reflexed, oviduct short, vagina thin walled surrounded by glands. Eggs 15-20 x 25-30 μm , vulva approximately median. The tail thin and filiform 7-18.7 times as anal body width length.

Male

Body straight, slightly curved posteriorly. Morphological characters similar to females. Testis single ventrally reflexed, vas deference well developed. Tail (46-68) μm long with fine tip, bursa leptoderan. Bursal cuticle transversely striated. Spicule paired, 32-44 μm long, fused at proximal end, slightly curved ventrally having round or beak like capitula. Gubernaculum slightly curved boat shape with flat distal end. Eight pairs of bursal papillae arranged as 1+1+1+2+1+2+1. First pair well anterior to

the cloaca pair 2 and 3 immediately anterior to the cloaca, whereas pair 4-6 and 7-9 posterior to cloaca. Tubular phasmid present.

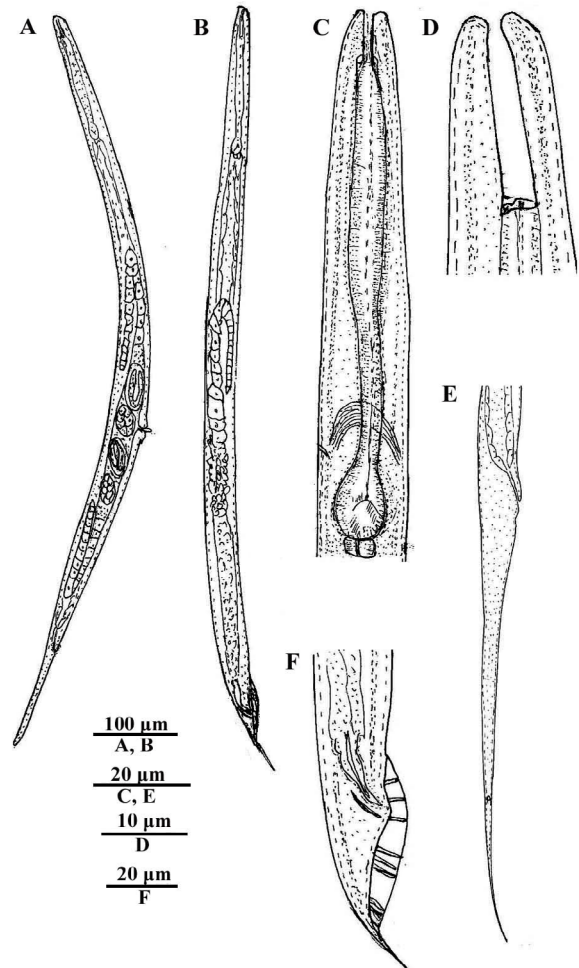


Fig. 1. Light microphotographs of *Neorhabditis andrassyi* n. sp. Female (A, C-E): A, whole body; C, pharyngeal region; D, anterior region; E, tail region and male; B, whole body; F, ventral view of tail region showing papillae.

Type host and locality

Soil around the roots of chickoo (*Manilkara zapota*) from Saedabad, Karachi, Sindh Pakistan.

Type specimen

Holotype male and 15 paratypes of each male and female specimens deposited in the Nematode collection of the NNRC, University of Karachi, Karachi, Pakistan.

Etymology

This species is dedicated to late Dr. Istivan Andrassy of Budapest, Hungry.

Table I.- Measurements (in μm) of *Neorhabditis andrassyii* n. sp. The values are Mean \pm SD (with ranges).

Characters	Holotype male	Male	Female
Total length	1012	1154.6 \pm 35.51 (1009-1222)	1480.88 \pm 153.79 (1223-1742)
Stoma length	25	24.6 \pm 2.057 (22-28)	23.88 \pm 1.45 (22-26)
Stoma width	3	3.75 \pm 0.43 (3-4)	4.38 \pm 0.484 (4-5)
Lip width	10	10.8750 \pm 0.92 (9-12)	11.75 \pm 0.968 (11-14)
Max.B.W	40	42.6 \pm 3.11 (38-48)	49.50 \pm 3.27 (46-56)
Pharynx	240	233.2 \pm 7.57 (226-250)	247.13 \pm 9.01 (238-265)
Excretory pore	178	180.8 \pm 10.78 (162-198)	191 \pm 10.29 (178-206)
Nerve ring	146	159.3 \pm 7.12 (146-171)	164.13 \pm 13.95 (142-179)
Vulva	--	--	724.38 \pm 127.38 (545-962)
Tail length	50	58.5 \pm 6.38 (46-68)	187.50 \pm 19.15 (156-206)
Anal body width	22	24.25 \pm 1.63 (22-27)	16.13 \pm 5.66 (11-24)
a	25.3	27.12 \pm 1.96 (25-31)	29.88 \pm 3.88 (25-36)
b	4.21	4.5 \pm 0.1 (4-5)	6.00 \pm 0.5 (5-7)
c	20.2	20 \pm 2.29 (17-25)	7.88 \pm 1.05 (7-10)
c'	2.2	2.37 \pm 0.484 2-3	12.63 \pm 3.9 (7-18)
V%	--	--	48.75 \pm 4.96 (37-55)
Spicule	40	36.75 \pm 3.99 32-44	--
Gubernaculum	17	14.2 \pm 2.38 (12-19)	--

V%, distance of vulva from lips \times 100/body length; a, body length/greatest width; b, body length/oesophagus length; c, body length/tail length; c', Tail length/body width at anus or cloaca.

Diagnosis and relationship

Neorhabditis andrassyii n. sp. is characterized by presence of male (1109-1222) μm long, spicules 32-44 μm long, Gubernaculum slightly curved boat shape with flat distal end. Eight pairs of bursal papillae. Tail (46-68) μm

long conical with fine tip, leptoderan bursa. Female 1223-1742 μm long, anterior to middle vulva 48.7 (37-55) % tail filiform 156-206 μm long, 7-18.7 times as anal body width long. *Neorhabditis andrassyii* n. sp. is differ from it type species *N. flagillicauda* Schuurmans Stekhoven, (1951), by having male; also distinguish from female by having longer body (1223-1742) vs 736 μm ; Stoma 7.2 times vs 6.5 times as long as wide; vulva%= 48.7 (37-55) vs 37 %; shorter tail 7-18.7 times vs 21.4 times anal body width long.

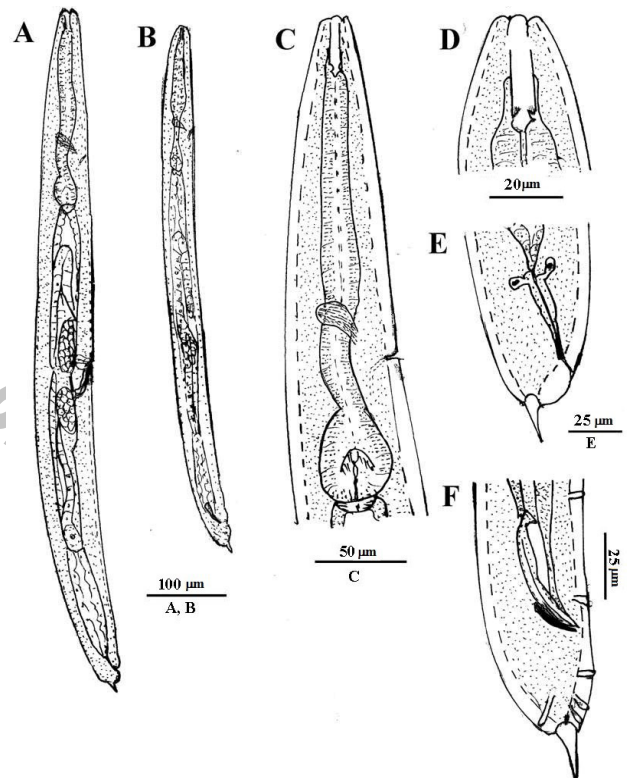


Fig. 2. *Poikilolaimus oxycercus* de Man, 1895 (A-F). Female (A,C-D): A, whole body; C, pharyngeal region; D, anterior region; E, tail region. Male: B, whole body; F, ventral view of tail region showing papillae.

Poikilolaimus oxycercus, de Man, 1895
(Fig. 2, Supplementary Fig. S2, Table II)

Description

Female

Body straight when killed by gentle heat. Body medium sized 746-840 μm long, cuticle thin loose sac like, finely annulated. Lateral field simple inconspicuous. Head continuous 12-16 m wide. Stoma rhabditoid, 20-23 μm long tubular 10-13 % of pharynx. Cheilostome not cuticularised pro-mesostome tubular, metastome anisoglottoid with

denticles. Thin oesophageal collar present, reaching up-to middle of stomal tube. Pharynx 188-200 μm long 20-25% of body length. Isthmus tubular 40-50 μm long. Basal bulb pear shaped slightly longer than its width. Excretory pore 72-82 μm from anterior end located at level of isthmus.

Gonads didelphic, amphidelphic, ovaries reflexed, vulva transverse, vagina short, uteri with thick muscular walls with oval spermatheca filled with sperms. Rectum longer than anal body diameter and characteristically curved in anterior half. Distance between vulva and anus 7-8 times as long as tail. Tail conoid, obtusely rounded, copula shaped, 15-25 μm long, 3-5 % of total body length.

Table II.- Measurements (in μm) of *Poikilolaimus oxycercus* de Man, 1895. The values are Mean \pm SD (with ranges).

Measurements	Female (n=10)	Male (n=5)
Total body length	637 \pm 39.1 (646-740)	608 \pm 82.6 (560-670)
Max. body width	51 \pm 3.6 (55-65)	42.5 \pm 2.8 (40-60)
Head width	13 \pm 0.5(12-16)	19.3 \pm 0.9 (19-21)
Stoma length	22.1 \pm 0.9(20-23)	20.6 \pm 0.8 (20-22)
Corpus	117.5 \pm 8 (103-125)	108 \pm 19.6 (90-125)
Isthmus	45 \pm 4.3 (40-50)	41 \pm 10.2 (30-50)
Bulbus	27.5 \pm 3.3 (25-33)	26.5 \pm 1.7 (25-28)
Pharynx	192.8 \pm 4.7 (188-200)	174.5 \pm 6.4 (168-190)
Nerve ring	68 \pm 2.6 (64-70)	65.8 \pm 18 (65-69)
Excretory pore	74.5 \pm 2.5 (70-82)	73 \pm 1.4 (70-78)
Vulva	446.2 \pm 27 (388-470)	---
Vulva-anus distance	285 \pm 7 (280-295)	---
Tail	20 \pm 2.6 (15-25)	24.5 \pm 1 (23-25)
Abd	30.8 \pm 1 (30-32)	27 \pm 1.1 (23-32)
Spicules	--	33.7 \pm 2.5 (30-35)
Gubernaculum	---	12 \pm 1.4 (10-13)
a	12.5 \pm 1.1 (11-13.5)	12.8 \pm 2.5 (10-14)
b	3.0 \pm 0.2 (3.2-3.9)	3.3 \pm 0.5 (2.8-3.9)
c	30 \pm 1.49 (28-32)	22 \pm 3.2 (19-26)
c'	1 \pm 0.06 (1-1.1)	0.8 \pm 0.14 (0.78-1.16)
V%	57.3 \pm 3.1 (51-60)	---

For abbreviations, see Table I.

Male

Smaller in size but similar to females in general body morphology. Testis reflexed, posterior to the basal

oesophageal bulb. Spicules 30-35 μm long separate. Gubernaculum simple, 10-13 μm long. 5 pairs of papillae present in group 1+1+3. Phasmid small, tail similar to that of female, conoid rounded as long as anal body width.

Remarks

Specimens of *Poikilolaimus oxycercus* de Man (1895) collected from soil around the roots of citrus fields of Nawabshah is very similar to those of the original description given by de Man (1895). This is the first record of this species from Pakistan.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <http://dx.doi.org/10.17582/journal.pjz/2017.x.x.xxx.xxxxxxxx>

Statement of conflict of interest

The authors have declared no conflict of interests.

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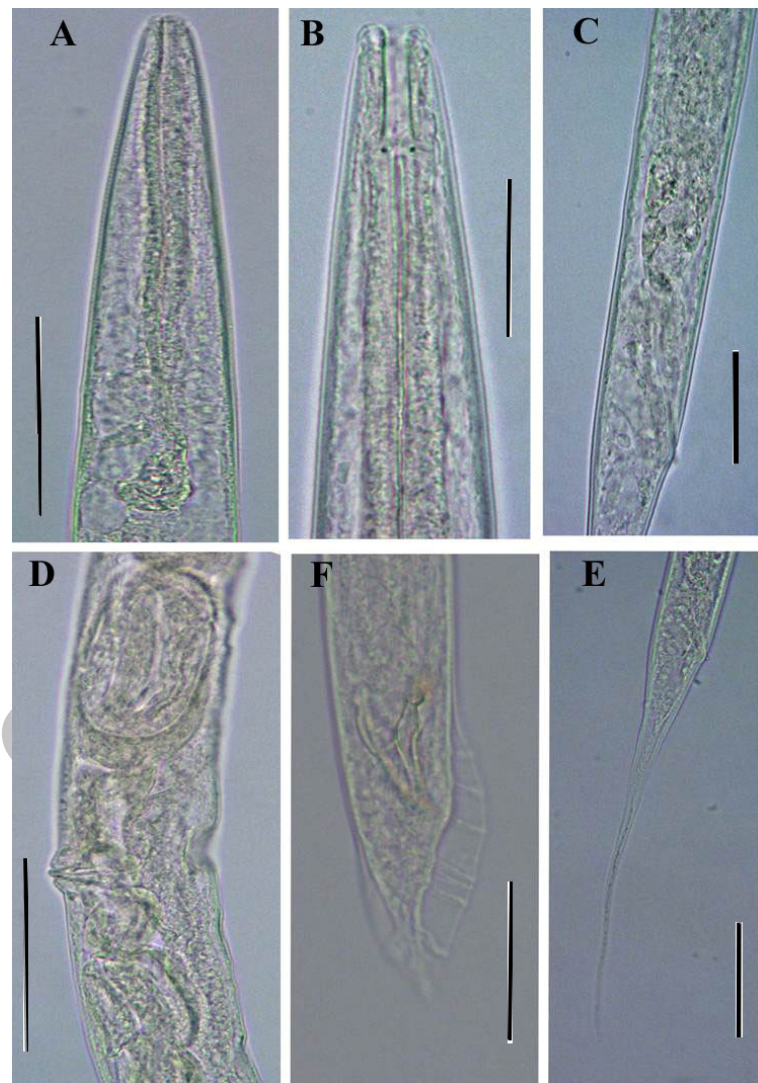


Supplementary Material

Description of *Neorhabditis andrassyi* n. sp., and Re-Description of *Poikilolaimus oxycercus* de Man, 1895 from Sindh

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Supplementary Fig. S1. Light microphotographs of *Neorhabditis andrassyi* n. sp. Female (A-E): A, pharyngeal region; B, anterior region; C, tail region; D, vulval region; E, filiform tail; F, tail region showing papillae (scale: A, E = 20 μ m; B, G = 10 μ m; C, F = 50 μ m).

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Supplementary Fig. S2. Light photomicrograph of *Poikilolaimus oxycercus* de Man, 1895 (A-F): Female (A, C-D): A, whole body; C, pharyngeal region; D, tail region; E, vulval region; Male: B, whole body; F, ventral view of tail region showing papillae (scale: A, B =10 μ m; C-F=50 μ m).