



# Description of *Paratylenchus manilkarii* n. sp., *P. sindhicus* n. sp., and Observation of *Pratylenchus kralli* Ryss, 1982 from Chikoo (*Manilkara zapota* L. van Royen) Orchards of Balochistan and Sindh, Pakistan

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## ABSTRACT

During the surveys of chikoo (*Manilkara zapota* L. van Royen) plantation at Hub, Balochistan and Gadab, Sindh a number of plant and soil nematodes were encountered. Soil sample analysis revealed two and a known plant parasitic nematode species viz., *Paratylenchus manilkarii* n. sp., *P. sindhicus* n. sp., and *Pratylenchus kralli* Ryss, 1982 as a new record species. *P. manilkarii* n. sp., is characterized by the lateral field with four incisures; stylet 28-29  $\mu$ m long; vulval lips protruding with vulval flap; lip region rounded or truncated without submedian lobes, tail ventrally curved with pointed or acute terminus. *P. sindhicus* n. sp., is characterized by stylet 20-21  $\mu$ m long; vulval lips not protruding with advulval flap; female head with small submedian lobes; tail ventrally curved with finely to broadly rounded terminus. Detailed taxonomical studies of new species have been incorporated herein with measurements, descriptions, illustrations and microphotographs along with brief description, measurements and microphotographs of the new record species.

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## Authors' Contribution

EI and NK identified the specimens and prepared manuscript. NM did survey, processed samples and assisted in drawing. SF supervised the research and reviewed the manuscript.

## Key words

Description, new species, *Paratylenchus*, chikoo, Pakistan

## INTRODUCTION

Chikoo is called as Sapota in English. It is also called as nose berry, mud apples, Sapodilla and Sapodilla plum. It is a brown fruit with a rough skin and sweet brown pulp inside. Each fruit may have 4 or 5 black seeds. The seeds easily sprout into plants and grow into trees in suitable warm climates. The tree can only survive in warm, typically tropical environments, dies easily when temperature drops below freezing. From germination, the sapodilla tree will usually take from five to eight years to bear fruit. The sapodilla trees yield fruit twice a year, though flowering may continue year round.

*Manilkara zapota*, commonly known as the sapodilla (a long-lived, evergreen tree) is native to southern Mexico, Central America and the Caribbean. It was introduced to the Philippines during Spanish colonization. It is grown in large quantities in Pakistan, India, Thailand, Malaysia, Cambodia, Indonesia, Vietnam, Bangladesh and Mexico. In Pakistan sapodilla is cultivated in Karachi, Hyderabad and Gadab, Sind and in Hub, Lasbella district, Balochistan. It is

cultivated on about 564 hectares with production at 2018 metric tons. The plant prefers warm and moist weather and can grow both in dry and humid areas. The coastal climate is best suited for its cultivation (Anon, 2016). Investigations of plant parasitic and soil nematodes in Pakistan have been carried out by several scientists and researchers on various agricultural crops including cereals, fruit and vegetables (Maqbool and Shahina, 2001; Zarina and Shahina, 2013).

In Pakistan the first record of sapodilla nematodes was made by (Saeed and Ashrafi, 1973, 1974) Saeed completed his thesis entitled "Studies on some stylet bearing nematodes associated with sapodilla (*Achras zapota* L.), with special reference to *Hemicriconemoides mangiferae* (Siddiqi, 1961)", the first thesis on sapodilla in Pakistan. Among several species found associated with sapodilla (*Achras zapota* L. now *Manilkara zapota* L. van Royen), five species were reported as new records for Pakistan viz., *Helicotylenchus indicus*, *H. multicinctus*, *Hoplolaimus columbus*, *Psilenchus hilarus*, *Rotylenchulus reniformis* and *Tylenchorhynchus martini* (Saeed, 1974). Later on Saeed and his co-workers published some papers on nematodes of sapodilla during 1973-1987. As very little work has been done on this plant, the present study was conducted with the aim to further investigate the association of nematodes of sapodilla.

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## MATERIALS AND METHODS

During the nematological studies a total of 40 soil and root samples of sapodilla (*Manilkara zapota* L. van Royen) were taken; 25 samples from 8 locations of Hub, Lasbela, Balochistan and 15 samples collected from three locations of Gadap town, Sindh during October, 2018. The samples were placed in polythene bags and sealed tightly with a rubber band with relevant information including date of collection, locality, and crop detail was mentioned on each bag.

Soil and root samples were processed by the methods of Cobb (1918) and Baermann (1917) funnel method. After qualitative and quantitative analysis of nematodes process of killing, fixing, and slow dehydration were done for permanent slide mounts (Hooper *et al.*, 2005). Identification of nematodes was made through measurements given by De Man (1884) formula with an ocular micrometer under a compound microscope and identification was based on the systematics given by Siddiqi (2000). Illustrations were prepared with the help of a drawing tube attached to the compound microscope and photographed of nematodes were made with a Nikon DS, Film camera, attached to the same Nikon Eclipse E400 microscope.

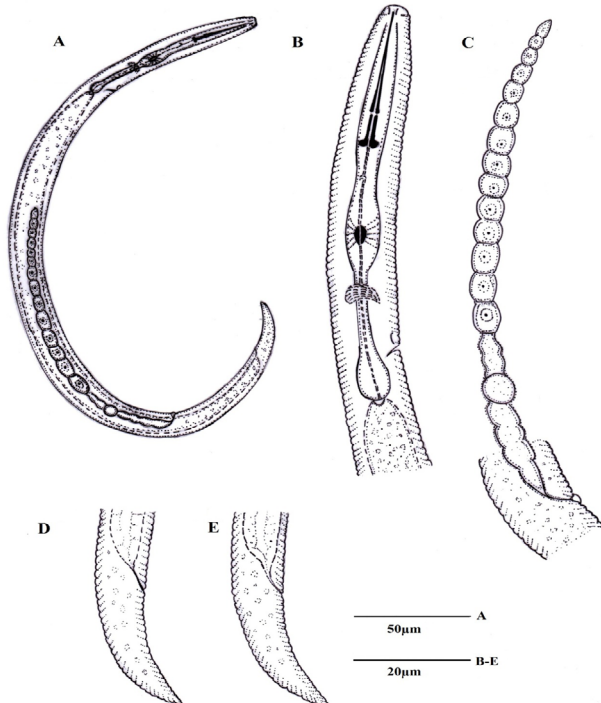


Fig. 1. *Paratylenchus manilkarii* n.sp. A, whole female body; B, oesophageal region; C, vulval and reproductive structure; D-E, tail region.

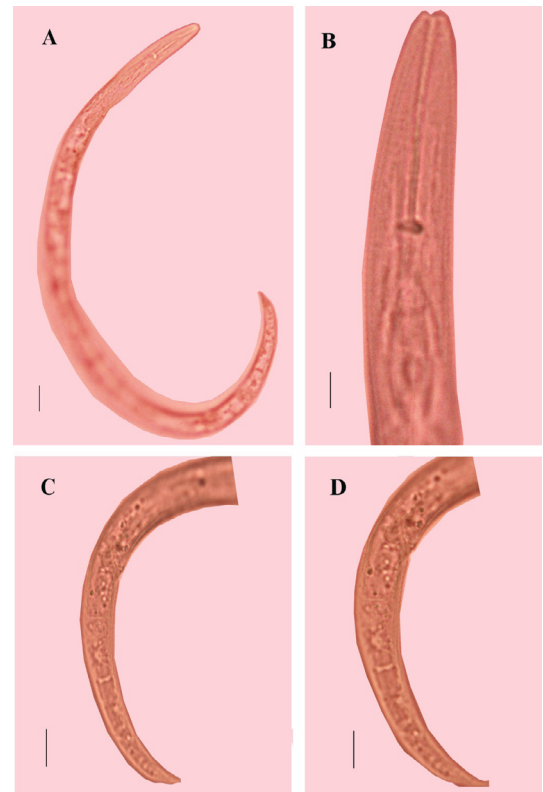


Fig. 2. *Paratylenchus manilkarii* n.sp. A, whole female body; B, oesophageal region; C, vulval part; D, vulva with tail region (scale A= 10 µm; B-D=100 µm).

### *Paratylenchus manilkarii* n.sp. (Figs. 1, 2; Table I)

#### Description

##### Female

Body slender, vermiform, ventrally arcuate when heat relaxed. Cuticular annulation distinct 1-1.5 µm wide at mid body. Lateral field marked by four incisures occupying about 1/3 of the body width. Female head rounded or truncate, sub median lobes indistinct, not offset from the body contour. Cephalic sclerotization weak. Stylet robust, averaging 28-29 µm long, conus 75% of the total stylet length; knobs small 1.5-2.0 x 3.0-4 µm rounded, slightly posteriorly directed. Dorsal oesophagel gland orifice about 6 µm behind the stylet base. Median pharyngeal bulb elongate, bearing distinct large valve. Isthmus slender encircled by nerve ring. Basal bulb pyriform 5-6 x 8-9 µm. Cardia small conoid, inconspicuous. Excretory pore anterior to the level of basal bulb or 67 µm from anterior end. Hemizonid just anterior to excretory pore. Gonad short, prodelphic, spermatheca short rounded empty. Vulva a transverse slit occupying about half of the body

width. Vulval lips slightly protruding with advulval flaps. Post uterine sac absent. Oocytes arranged in a single row. Anus indistinct. Tail slender, conoid, ventrally curved, annulated, gradually tapers to sub acute terminus.

**Table I. Morphometric data for *Paratylenchus manilkarii* n. sp. (All measurements are in  $\mu\text{m}$  except L).**

Morphological characters	Holotype female	Paratype females (n=4)
L (mm)	0.36	0.37 $\pm$ 0.01(0.36-0.40)
a	22.5	21.8 $\pm$ 0.49 (21-22.5)
b	4.5	4.42 $\pm$ 0.08 (4.3-4.5)
c	15	14.75 $\pm$ 0.43 (14-15)
c'	3.0	2.95 $\pm$ 0.08 (2-3)
V	78.6	78.32 $\pm$ 0.50 (77.7-79)
Lip region height	1.0	1.05 $\pm$ 0.08 ( 1.0-1.2)
Lip region width	4.0	3.87 $\pm$ 0.21 ( 3.5-4)
Stylet	28	28.3 $\pm$ 0.41 (28-29)
Median bulb length	14	14.5 $\pm$ 0.5 (14-15)
Median bulb width	5	5.5 $\pm$ 0.5 ( 5-6)
Median bulb valve length	5	4.87 $\pm$ 0.21 (4.5-5)
Median bulb valve width	3	2.95 $\pm$ 0.08 (2-3)
Anterior end to excretory pore	67	68.8 $\pm$ 1.43 (67-70.5)
Pharynx length	80	79.25 $\pm$ 0.82 (78-80)
Maximum body width	16	15.6 $\pm$ 0.41 (15-16)
Vulval body diameter	12	12 $\pm$ 0.35 (11.5-12.5)
Vulva to tail terminus	72.4	72.8 $\pm$ 0.75 (72-74)
Anal body width	8	8.2 $\pm$ 0.25 (8-8.5)
Tail length	24	23.6 $\pm$ 0.41(23-24)

#### Male

Not found.

#### Type host and locality

The type material was collected from rhizospheric soil samples of sapodilla (*Manilkara zapota*) from Hub, Lasbela, Balochistan, Pakistan.

#### Type specimens

Holotype and paratype females are deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi Pakistan.

#### Diagnosis and relationship

*Paratylenchus manilkarii* n. sp., is characterized by the lateral field with four incisures; stylet 28-29  $\mu\text{m}$  long;

vulval lips protruding with vulval flap; lip region rounded or truncated without submedian lobes, continuous; excretory pore located at the anterior end of basal bulbs; gonad prodelphic with small spermatheca; tail ventrally curved with pointed or acute terminus.

According to the grouping scheme of *Paratylenchus* given by Ghaderi *et al.* (2014), this new species belongs to group 3 (stylet < 40  $\mu\text{m}$ ; 4 lateral lines; advulval flaps present) and is most similar to *P. concavus* (Eroshenko, 1978). The new species differs from *P. concavus* in longer body (L= 360-365 vs 280-300)  $\mu\text{m}$ ; greater a, b and c values (a=21-22.5 vs 17-20; b= 4.5-4.7 vs 3.9-4.1; c=15-16 vs 9-10), slightly longer stylet (28-29 vs 25-28)  $\mu\text{m}$ . Cuticular annulation distinct vs fine cuticular annulations, ovary small vs long.

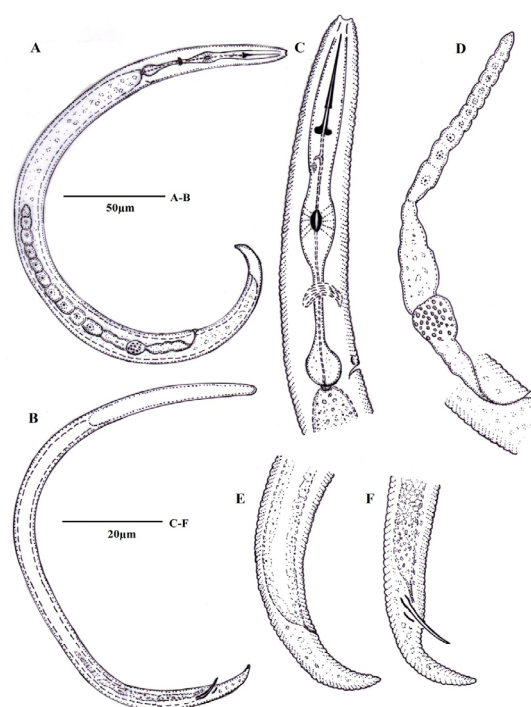


Fig. 3. *Paratylenchus sindhicus* n.sp. A, whole female body; B, whole male body; C, oesophageal region; D, vulval and reproductive structure; E, female tail region; F, male tail region.

#### *Paratylenchus sindhicus* n.sp.

(Figs. 3, 4; Table II)

#### Description

##### Female

Body ventrally curved to open C- shaped when heat relaxed. Cuticle finely annulated, annules 0.5-1  $\mu\text{m}$  wide at mid body. Lip region narrow, truncated with

small submedian lobes; not offset from body. Cephalic sclerotization weak. Stylet well developed, almost straight with rounded to laterally directed knobs; the conus about three fourth of the total stylet length. Orifice of dorsal gland opening at 4-5  $\mu\text{m}$  posterior to stylet knobs. Pharynx criconematoid with well developed corpus with valvular apparatus large and muscular. Relatively short, narrow isthmus surrounded by nerve ring. Basal bulb pyriform, set off from intestine. Cardia small 2-3  $\mu\text{m}$  in length Excretory pore at mid or slightly posterior to mid of pharyngeal basal bulb. Gonad prodelphic, short and outstretched, in one female it is reflexed; spermatheca oval containing spheroid sperms. Vulva a broad transverse slit with distinct but relatively small ad vulval flaps. Post uterine sac absent. Post-vulval region tapering gradually. Anus distinct or vestigial. Tail ventrally curved tapering with conical pointed to slightly rounded tail tip.

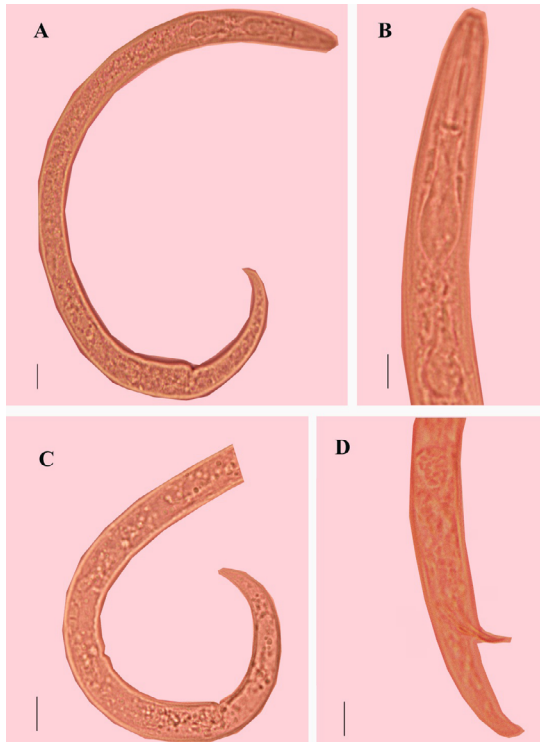


Fig. 4. *Paratylenchus sindhicus* n.sp. A, whole female body; B, oesophageal region; C, vulva with tail region; D, male tail region. (Scale A= 10  $\mu\text{m}$ ; B-D=100  $\mu\text{m}$ ).

#### Male

Body slender, similar to female in general appearance but with smaller body size. Cephalic frame work less developed, narrower than female, continuous from body. Pharynx rudimentary, procampus, metacampus and basal bulb inconspicuous, Gonad about 65-70

$\mu\text{m}$  long; testis outstretched with small spermatozoa. Cloacal opening not elevated. Gubernaculum about 3  $\mu\text{m}$  long. Bursa absent. Tail terminus similar to that of female, tapering gradually to a bluntly pointed to rounded tip.

**Table II. Morphometric data for *Paratylenchus sindhicus* n. sp. (All measurements are in  $\mu\text{m}$  except L).**

Morphological characters	Holotype female	Paratypes	
		Female (n=54)	Male (n=2)
L (mm)	0.30	0.29-0.35 (0.32 $\pm$ 0.02)	0.27,0.32
a	25	23-28 (25 $\pm$ 1.51)	27,32
b	4.2	4.2-4.8 (4.4 $\pm$ 0.18)	-
c	21.4	18-21 (20.4 $\pm$ 0.87)	16.8,16.8
c'	2.3	2.0-2.5 (2.2 $\pm$ 0.16)	2,3
V/T	82	79.8-82.4 (81.0 $\pm$ 0.84)	33,20
Lip region height	2	1-2 (1.52 $\pm$ 0.45)	3,3
Lip region width	2	2-3 (2.5 $\pm$ 0.42)	2, 2
Stylet	21	20-21 (20.5 $\pm$ 0.49)	20,20
Median bulb length	13	13-16 (14.6 $\pm$ 1.08)	-
Median bulb width	5	5-7 (5.9 $\pm$ 0.70)	-
Valvular apparatus length	3	3-4 (3.5 $\pm$ 0.45)	-
Valvular apparatus width	2	2-3 (2.6 $\pm$ 0.41)	-
Excretory pore from anterior end	63	61-71 (63.6 $\pm$ 2.5)	60,56
Pharynx length	70	70-76 (71.4 $\pm$ 2.57)	-
Maximum body width	12	12-15 (12.6 $\pm$ 0.98)	10,10
Vulval body width	10	10-13 (11.6 $\pm$ 1.04)	-
Vulva to tail terminus	50	50-70 (57.8 $\pm$ 6.88)	-
Anal/cloacal body width	6	6-8 (6.8 $\pm$ 0.72)	6,8
Tail length	14	14-17 (15.5 $\pm$ 0.93)	18,16
Spicule	-	-	16,15
Gubernaculum	-	-	3,3
St / L%	7	5.8-6.7 (6.3 $\pm$ 0.41)	7.4,6.2

#### Type host and locality

The type material was collected from rhizospheric soil samples of sapodilla (*Manilkara zapota*) from Gadap



town, Sindh, Pakistan

#### *Type specimens*

Holotype female, 54 paratype females and 2 paratype males were deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan.

#### *Diagnosis and relationship*

*Paratylenchus sindhicus* n. sp., is characterized by the combination of characters: stylet 20-21 µm long; vulval lips not protruding with advulval flap; female head with small submedian lobes; excretory pore located at the middle of basal bulb; gonad prodelphic with round to oval spermatheca filled with sperms; tail ventrally curved with finely to broadly rounded terminus.

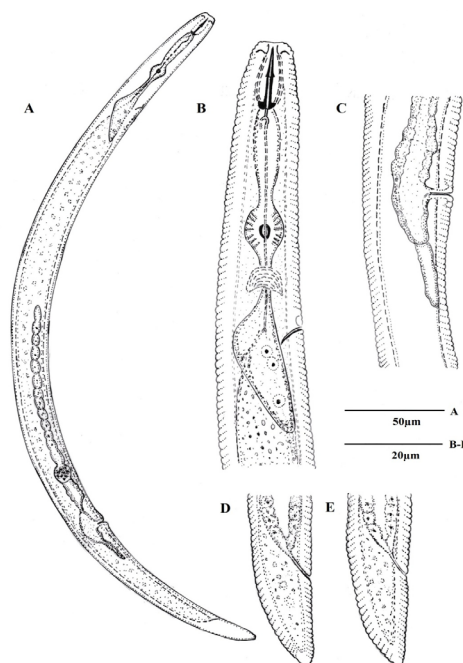
According to the grouping scheme of *Paratylenchus* species given by Ghaderi *et al.* (2014), this new species belongs to group 5 (stylet ≤ 40 µm; lateral lines indistinct; advulval flaps present). There is only one species in this group, namely *Paratylenchus leioderms* (Raski, 1975). The new species differs from it by smaller body length (L= 0.03-0.35 vs 0.34-0.42 mm), in higher st/L% ratio (5.8-6.7 vs 4.5-5); excretory pore located anteriorly (61-71 vs 70-84 µm); cuticular annulation (cuticle with distinct annules vs smooth or indistinct), head with small submedian lobes vs bluntly rounded head almost hemispherical.

#### *Paratylenchus kralli* Rass, 1982

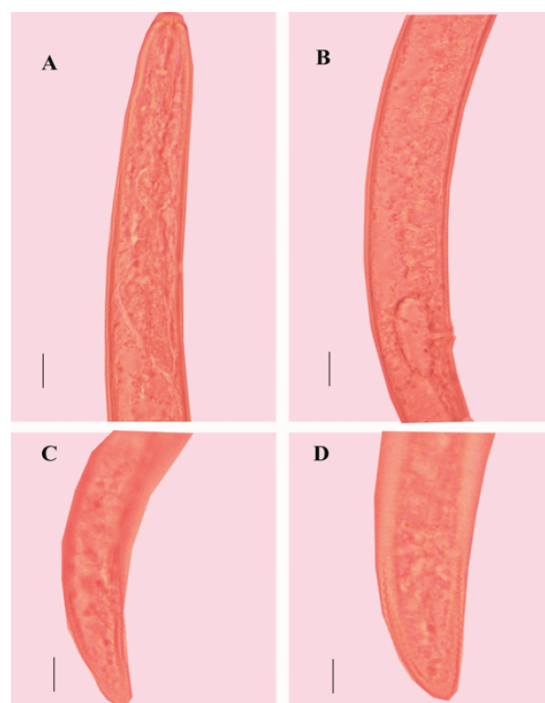
(Figs. 5, 6; Table III)

**Table III. Morphometric data of *Paratylenchus kralli* Ryss, 1983. (All measurements are in µm except L).**

Morphological characters	Female (n=8)	
	Mean ±SD	Range
L (mm)	0.37±0.02	0.34-0.40
a	20.6±1.88	20.2-24.6
b	6.1±0.41	5.5-6.5
b'	4.2±0.25	4.0-4.7
c	18.6±1.0	17.5-20.3
c'	1.9±0.19	1.6-2.1
V%	75.4±0.93	74.5-77
Stylet	13.7±0.37	13-14
Excretory pore	63.2±3.1	59-68
Oesophageal length	87.7±2.94	85-93.6
Tail length	20.2±1.36	18.4-22
Anal body width	10.6±0.69	10-12
Maximum body width	17.3±1.37	16-20
Post uterine sac	23±1.63	20-25



**Fig. 5. *Paratylenchus kralli* (Rass, 1982). A, whole female body; B, oesophageal region; C, vulval region; D-E, different female tail regions.**



**Fig. 6. *Paratylenchus kralli* (Rass, 1982). A, oesophageal region; B, vulval region; C-D, different tail regions. (Scale A-D =100 µm).**

*Brief description**Female*

Body slender and vermiform, slightly curved ventrally when killed. Body annuli 0.8-1.0 µm wide at mid body. The head region continuous with 3 lips. Stylet small robust and strongly sclerotized. Stylet conus 50% of the entire stylet length; stylet shaft tubular, basal knobs forwardly directed and wide.

Pharynx with rounded median bulb and rather small glandular lobe that overlaps the intensive ventrally. Isthmus slender and encircling by nerve ring. Hemizonid located just above the excretory pore at the level of pharyngeal- intestinal junction. Hemizonion not observed. Reproductive tract mono-prodelphic, germinative zone outstretched. Spermatheca rounded, filled with small spermatozoa. Post uterine branch long, more than corresponding body diameter. The vulva position is high (75-78%). The tail shape conoid with smooth terminus, bearing 17-23 annules. Phasmid pore like and located at one third from the tail tip.

*Male*

Not found.

*Remarks*

Specimens were collected from soil around rhizopheric region of sapodilla (*Manilkara zapota*) from Gadab town, Sindh. The present specimens agree well in all dimensions and morphological characters with those described by Ryss (1982).

*Statement of conflict of interest*

The authors declare no conflict of interest.

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