

## Description of *Aphelenchoides turnipi* n. sp. and redescription of *A. siddiqii* with notes on *A. bicaudatus* (Nematoda: Aphelenchoididae) from Pakistan

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### Abstract

A new species of the genus *Aphelenchoides* is described from soil around the roots of turnip (*Brassica rapa* L.) plants collected from Mianwali, Punjab, Pakistan. *Aphelenchoides turnipi* n. sp. belongs to the Group 2 of *Aphelenchoides* species *sensu* Shahina with one or sometimes two mucronate structures in female tail terminus and is characterized by small body size (0.29-0.38 mm); two lateral incisures in the lateral field; small stylet with minute basal swellings (stylet: 7-9 µm); vulva at 67-69 percent of body, tail short with pointed mucro (tail = 25-30 µm); and excretory pore situated just behind the median bulb, anterior to nerve ring. Female have a short post vulval uterine sac extending 25-34% of vulva-anus distance. Also included is the first record of *A. siddiqii* Fortuner, 1970 from around the roots of carrot (*Daucus carota* L.), from Hasan Abdal, Punjab, Pakistan. Morphometric data of a known species *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941 is also given.

**Keywords:** Taxonomy, *Aphelenchoides turnipi* n. sp., *A. siddiqii*; *A. bicaudatus*, *Brassica rapa*, *Daucus carota*, *Raphanus sativus*.

Under modern classification based on molecular technologies De Ley & Blexter (2002; 2004) placed all aphelenchs including *Aphelenchoides* Fuchs, 1937 under the infra order Tylenchomorpha De Ley & Blexter, 2002, suborder Tylenchina Thorne, 1949, order Rhabditida Chitwood, 1933 of the subclass Chromadoria Pearse, 1942 and class Chromadorea Inglis, 1983. The genus *Aphelenchoides* was proposed by Fischer, 1894 with *A. kuehnii* as the type species. It belongs to the super family Aphelenchoidea Fuchs, 1937 of the family Aphelenchoididae Skarbilovich, 1947 and subfamily Aphelenchoidinae Skarbilovich, 1947 (Hunt, 2008); in the checklist he included 153 valid species of the genus *Aphelenchoides*. The diagnostic compendium of the genus *Aphelenchoides* consisted of 144 valid species (Shahina, 1996). Hunt (1993) reported 138

nominal species in his book “*Aphelenchida, Longidoridae and Trichodoridae: Their Systematics and Bionomics*”.

Fifteen species of the genus *Aphelenchoides* have so far been reported from Pakistan (Zarina & Shahina, 2012). *Aphelenchoides* species were first recorded in Pakistan by Akhter (1962) from soils around the roots of sugarcane, Lahore. These are *Aphelenchoides bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941, *A. blastophthorus* Franklin, 1952, *A. caprifici* (Gasparrini, 1864) Filipjev, 1934, *A. parietinus* (Bastian, 1865) Steiner, 1932 and *A. winchesi* Goodey, 1927. Among them two species viz., *A. caprifici* and *A. winchesi* have now been transferred to the genera *Schistonchus* Cobb, 1927 as *S. caprifici* (Gasparrini, 1864) Cobb, 1927 and *Seinura* Fuchs, 1931 as *S. winchesi* (Goodey,

1927) Goodey, 1960, respectively. Since then these nematode species have been reported by many researchers from different hosts and localities of Pakistan (Maqbool & Shahina, 2001).

Other species of the genus *Aphelenchoides* viz., *A. aligarhensis* Siddiqi *et al.*, 1967 and *A. involutus* Minegawa, 1992 were detected from banana soils of Sindh (Shahina, 1996). *A. besseyi* Christie, 1942 was first recorded from rice (Maqbool, 1984) and maize (Maqbool, 1986). Khan & Bilqees (1985) reported two species viz., *A. dactylocercus* Hooper, 1958 and *A. goodeyi* Siddiqi & Franklin, 1967 from date palm plantations in Balochistan. *A. helicossoma* Maslen, 1979 was detected from betel vine (Amer-Zareen *et al.*, 1999). *A. ritzemabosis* (Schwartz, 1911) Steiner & Buhner, 1932 was reported from pyrethrum (Anwar, 1989; Maqbool, 1992) while Saeed *et al.*, (1986) reported *A. sacharii* Hooper, 1958 from tobacco nurseries of Pakistan. Two species viz., *A. asterocaudatus* Das, 1960 and *A. rutgersi* Hooper & Myers, 1971 were reported by Erum & Shahina (2010) collected from wheat (*Triticum aestivum* L.) and ashok (*Polyalthia longifolia* L.) trees of Tandojam and Karachi, respectively.

During a nematological survey of vegetable fields in different localities of Punjab, Pakistan, a new species *Aphelenchoides turnipi* n. sp. and a known species *A. siddiqii* Fortuner, 1970 were found, which are here in described and redescribed, respectively along with additional morphometric details given of a reported species of *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941.

### Materials and Methods

Nematodes were extracted from soil by Cobb's wet sieving technique (Cobb, 1918) followed by a modified Baermann funnel method (Baermann, 1917). Extracted nematodes were killed by gradual heat, fixed in TAF and mounted in dehydrated glycerine (Siddiqi, 1986). Illustrations were made by using a drawing tube attached to the compound microscope Nikon Eclipse E400.

### *Aphelenchoides turnipi* n.sp (Fig. 1, Table 1)

#### Description

**Female:** Body slender, cylindrical ventrally arcuate when heat relaxed. Cuticle thin about 1 µm apart in mid body region with transfer striae. Surface of cuticle finely annulated, annuli indistinct. Lateral field with two incisures occupying 1/4-1/5 of the body width. Cephalic region rounded slightly offset, about twice as wide as high. Labial frame weakly sclerotized. Stylet delicate 7-9 µm with small basal swellings. Procorpus cylindrical. Median bulb round to oval 9-10 x 6-7 µm in size, with conspicuous valve situated centrally slightly posteriorly.

Excretory pore located just behind the median bulb anterior to the nerve ring. Nerve ring situated posterior to metacarpus, 50-55 µm from anterior end. Hemizonid invisible. Pharyngo-intestinal junction immediately posterior to nerve ring. Oesophageal gland lobe slender overlapping intestine dorsally. Oesophagus extending 90-104 µm from anterior region of the body. Female reproductive system monodelphic, prodelphic, outstretched, occupying 25-34 % of body length. Oocytes arranged in a single row of cell. Spermatheca round to oval filled with round sperms. Vagina directed anteriorly, walls not sclerotized, vulval flap absent. Post-vulval uterine sac short about 1-2 vulval widths long or 14-27% of vulva-anus distance, usually not containing any sperm. Rectum and anus visible. Rectum 10-12 µm long. Tail short conoid, slight ventral curvature, 6-7 anal body diameters long. Tail terminus bearing single small mucron, 2-3 µm long.

**Male:** Not found.

**Type habitat and locality:** Specimens were collected from soil around the roots of turnip (*Brassica rapa* L.) from Mianwali, Punjab, Pakistan.

**Type specimens:** Holotype (female) slide no.

NNRC 125/724 and paratype slides no. NNRC 125/725-728 (nine females and four juveniles) deposited in the National Nematode Collection of NNRC, University of Karachi, Karachi, Pakistan. Slide no. NNRC 125/729 (one female) deposited in the British Nematode Collection at The Food and Environmental Research Agency, Sand Hutton York, England.

**Diagnosis and Relationship:** *Aphelenchoides turnipi* n. sp. is characterized by small body length (L = 0.29-0.38 mm). The cuticle is finely annulated and bears two incisures in the lateral field. The stylet is 7-9  $\mu$ m long and has small basal swellings. The excretory pore is located posterior to the median bulb, anterior to the nerve ring or 44-51  $\mu$ m from the anterior end. Vulva at 67-69 percent of body. The spermatheca axial and oblong, contains round sperms in multiple rows. Female have a short vulval uterine sac (10-20  $\mu$ m), tail short with pointed mucro (Tail = 25-30  $\mu$ m).

According to the category of *Aphelenchoides* species (Shahina, 1996), the new species belongs to Group 2 which has been defined as having the female tail terminus with “one or sometimes two mucronate structures”. Based on two lateral lines, body length and vulva percentage, it is close to three species of Group 2, including *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941; *A. clarus* Thorne & Malik, 1968 and *A. tagetae* Steiner, 1941.

*Aphelenchoides turnipi* n. sp. differs from *A. bicaudatus* in having shorter stylet (7-9 vs 10-12  $\mu$ m), lower b value (5.2-6.2 vs 6.8-8.4), in tail shape (single mucro on tail vs bifurcate tail tip), in small tail length (25-30 vs 50  $\mu$ m) and the absence of males vs present.

It differs from *A. clarus* in body width (a = 29-36 vs 21), in c and c' values (c=10-12.3 vs 19; c'= 3.9-4.8 vs 2.8) and in smaller stylet length (7-9 vs 11  $\mu$ m). From *A. tagetae*, the new species differs by having shorter stylet (7-9 vs 10  $\mu$ m), higher a

and c' ratio (a = 29-36 vs 28; c' = 3.9-4.8 vs 2.6), lower b and c ratios (b = 5.2-6.2 vs 9.3; c = 10.9-12.3 vs 16.6) and in vulva percentage (67-69 vs 71).

**Etymology:** The species is named “turnipi” after its type host.

### *Aphelenchoides siddiqii* Fortuner, 1970

(Fig. 2, Table 2)

#### Description

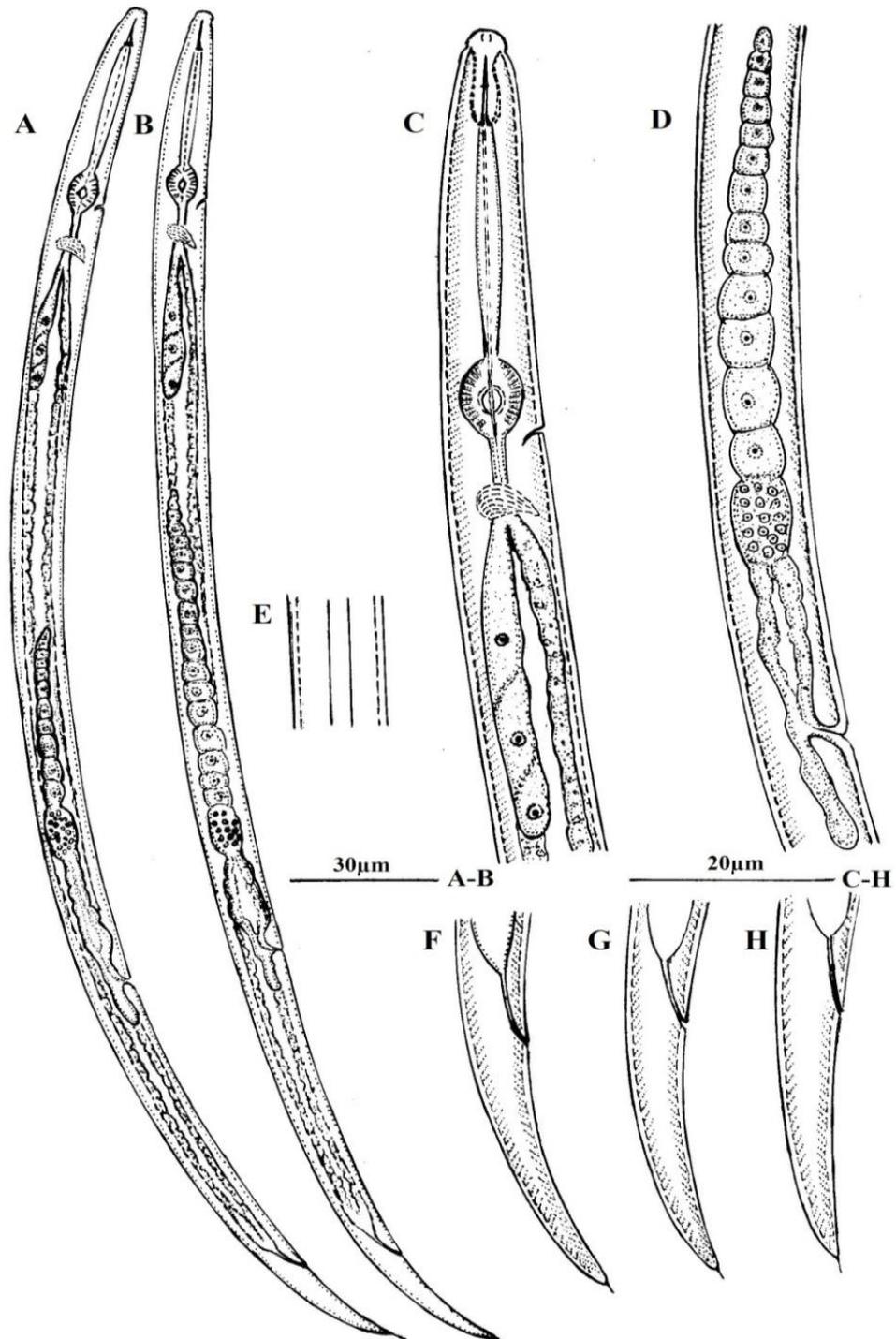
**Female:** Body cylindrical, ventrally arcuate when relaxed by gentle heat, tapering at both ends. Cuticle annulated, annules about 1 $\mu$ m apart at mid body region, the striae being interrupted at lateral fields which occupy about 1/4-1/5 of body width and marked by four incisures. Labial framework weakly developed. Lip region off set, without annulation, 3-4  $\mu$ m high and 4-5  $\mu$ m wide at its base. Stylet with distinct knobs. Median bulb oval to spheroid 11-12  $\times$  9-10  $\mu$ m, with well developed valvular apparatus in centre.

Oesophageal glands extending back over intestine dorsally 120-128  $\mu$ m in length, about 4 body diameter at region of median bulb. Excretory pore 69-70  $\mu$ m from head end, opposite the anterior margin of the nerve ring. Hemizonid 10  $\mu$ m from excretory pore. Nerve ring located at 68  $\mu$ m from anterior end.

Reproductive system single outstretched, extending nearly to base of oesophageal gland. Ovary with 2 to 4 rows of oocytes. Vulva slightly protruding to body contour, slit like, two fifths of body width long. Post-vulval uterine sac about one body width in length. Tail subcylindrical with a broadly rounded tip, 3-4 anal body width long, with star shaped mucro. Phasmid not observed.

**Male:** Not found.

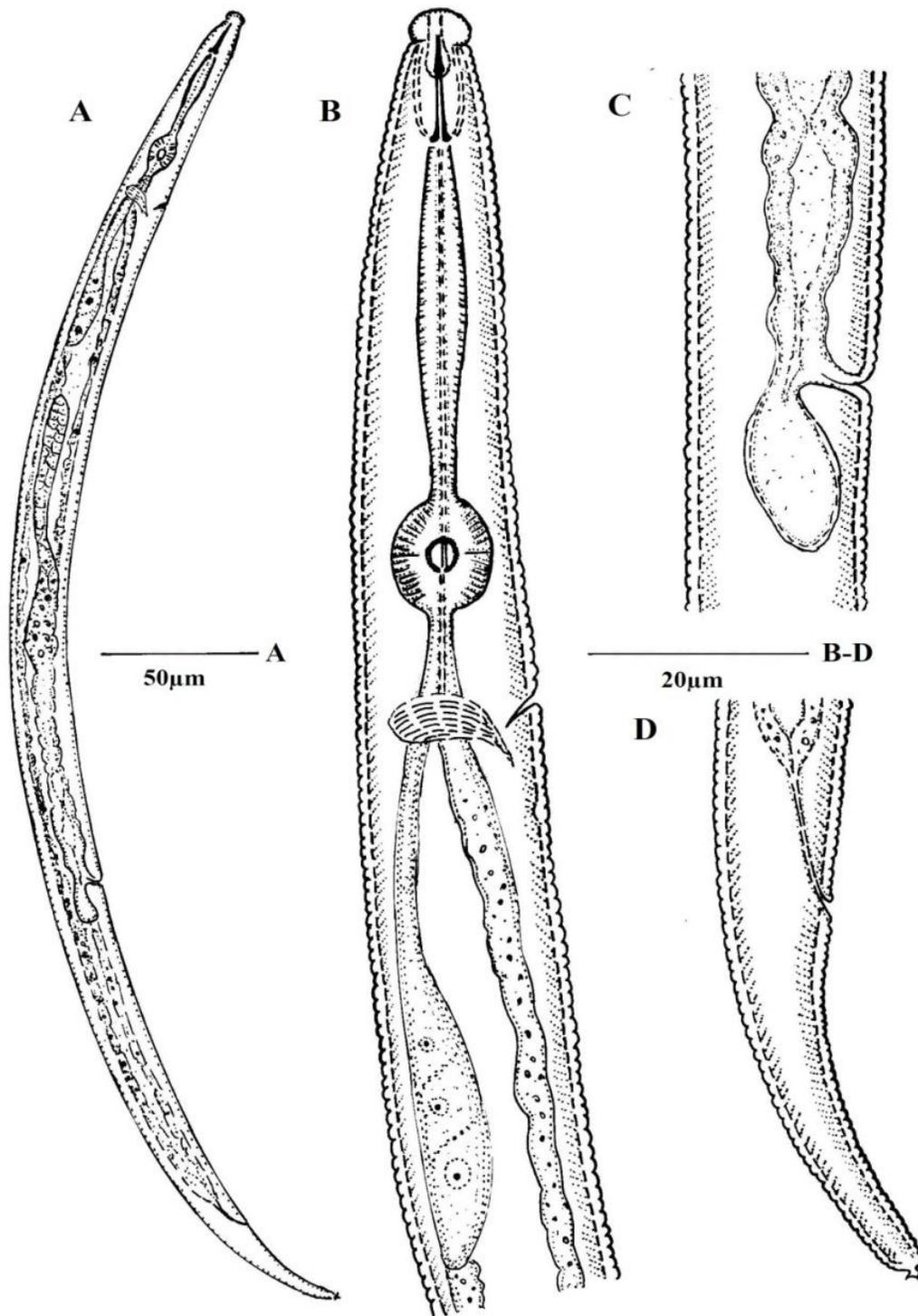
**Remarks:** Specimens of *Aphelenchoides siddiqii* Fortuner, 1970 were collected from soil around the roots of carrot (*Daucus carota* L.) from Hasan Abdal, Punjab, Punjab, Pakistan. The measurements closely fit to the original description of the species.



**Fig. 1 (A-H).** *Aphelenchoides turnipi* n. sp. Female: A & B. Whole body; C. Oesophageal region; D. Female gonad; E. Lateral field; F-H. Tail regions.

**Table 1. Morphometrics of *Aphelenchoides turnipi* n. sp. (Measurements in  $\mu\text{m}$ , except L).**

<b>Morphological characters</b>	<b>Holotype female</b>	<b>Paratype females (n=10) Mean <math>\pm</math> SD (range)</b>
L (mm)	0.29	0.32 $\pm$ 0.02 (0.29-0.33)
a	29.5	32.27 $\pm$ 2.83 (29-36.4)
b	6.2	5.69 $\pm$ 0.33 (5.2-6.2)
b'	3.4	3.26 $\pm$ 0.21 (2.9-3.6)
c	11.8	11.64 $\pm$ 0.49 (10.9-12.3)
c'	4.1	4.37 $\pm$ 0.29 (3.9-4.8)
V%	68.4	68.22 $\pm$ 0.61 (67-69)
G <sub>1</sub> %	25.6	27.63 $\pm$ 3.81 (22-33)
Body diameter	10	9.9 $\pm$ 0.73 (9-11)
Lip height	1.8	2.01 $\pm$ 0.19 (1.8-2.5)
Lip width	4	4.3 $\pm$ 0.48 (4-5)
Stylet length	8	8.4 $\pm$ 0.69 (7-9)
Median bulb length	9	9.27 $\pm$ 0.41 (9-10)
Median bulb width	6	6.7 $\pm$ 0.48 (6-7)
Median bulb length/width	1.5	1.30 $\pm$ 0.19 (1.28-1.5)
Distance from anterior end to distal end of median bulb	48	47.8 $\pm$ 2.14 (44-51)
Anterior end to excretory pore	44	48.3 $\pm$ 3.33 (44-51)
Anterior end to nerve ring	52	52.9 $\pm$ 2.0 (60-55)
Anterior end to vulva	220	220.8 $\pm$ 8.19 (202-232)
Ovary length	84	90 $\pm$ 10.13 (80-109)
Distance from vulva to anus	74	73.3 $\pm$ 1.56 (72-77)
Post uterine sac length	14	13.8 $\pm$ 3.58 (10-20)
Post uterine sac length/ vulva anus distance%	18.9	18.74 $\pm$ 4.90 (13.8-27.0)
Oesophageal length	92	96.6 $\pm$ 4.92 (90-104)
Oesophageal intestinal junction	56	56.7 $\pm$ 2.58 (54-62)
Tail length	28	27.9 $\pm$ 1.72 (25-30)
Anal body width	6	6.1 $\pm$ 1.72 (6-7)
Mucro	2	2.13 $\pm$ 0.50 (2-3)



**Fig. 2 (A-D).** *Aphelenchoides siddiqii* Fortuner, 1970. Female: A. Whole body; B. Oesophageal region; C. Vulval region; D. Tail region with star shaped mucro.

**Table 2. Morphometrics of *Aphelenchoides siddiqii* Fortuner, 1970 and *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941 (Measurements in  $\mu\text{m}$ , except L).**

Morphological characters	<i>A. siddiqii</i> Fortuner, 1970	<i>A. bicaudatus</i> (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941	
	Female (n=2)	Female (n=2)	Male (n=1)
Length (mm)	0.48 , 0.50	0.36 , 0.36	0.40
a	25.5 , 27	30.1 , 32.7	30.7
b	3.7 , 4.0	8.8 , 7.2	4.3
b'	6.7 , 6.7	5.6 , 5.8	6.2
c	13.1 , 14.5	11.3 , 12	10
c'	3.7 , 4.0	2.9 , 3.7	3.9
V/T %	65 , 66	66.8 , 67.2	52
G <sub>1</sub> %	35.8 , 35.2	25, 26.2	-
Body diameter	19 , 20	12 , 12.5	13
Lip height	3, 4	2 , 2	2
Lip width	4 , 5	4 , 4.3	5
Stylet	11 , 11.5	10 , 11	10
Spicule	-	-	14
Median bulb length	11, 12	10, 10	12
Median bulb width	9 , 10	7 , 8	9
Median bulb length/ width	1.2 , 1.2	1.4 , 1.3	1.3
Distance anterior end to distal end of median bulb	60, 61	51 , 52	54
Anterior end to excretory pore	69 , 70	50 , 51	62
Anterior end to nerve ring	68 , 68	55, 56	60
Anterior end to vulva	316 , 315	242 , 248	-
Ovary length	174 , 176	95 , 84	-
Distance from vulva to anus	330 , 328	85 , 84	-
Post uterine sac length	19 , 20	24 , 22	-
Post uterine sac length/ vulva anus distance%	5.7 , 8.0	22.4 , 24	-
Oesophageal length	120 , 128	90 , 92	92
Oesophageal intestinal junction	72 , 74	62 , 64	64
Tail length	37 , 36	31 , 30	39
Anal body width	10 , 10.4	11 , 8	10

***Aphelenchoides bicaudatus* (Imamura, 1931)  
Filipjev & Schuurmans Stekhoven, 1941  
(Fig. 3, Table 2)**

**Description**

**Female:** Body slightly curved ventrally when killed by gentle heat. Slightly narrowing anteriorly but markedly towards the posterior side. Lip region set off by constriction. Cuticle marked by fine transverse striae about 0.8-1  $\mu\text{m}$  apart at mid body region. Lateral field with two incisures, occupying 16-17 % of body width. Stylet 10-11  $\mu\text{m}$  long with distinct basal knobs. Median bulb pyriform to spheroid and occupying 4/5 of body width, 10  $\mu\text{m}$  long and 7-8  $\mu\text{m}$  wide. Oesophageal gland extends dorsally over intestine 50-55  $\mu\text{m}$  in length. Excretory pore 50-51  $\mu\text{m}$  from head end about opposite the nerve ring or slightly anterior. Nerve ring 56  $\mu\text{m}$  from anterior end. Vulva post-equatorial, vagina directed anteriorly, occupying about two fifths body width long. Ovary single, anterior outstretched, 84-95  $\mu\text{m}$  long, reaching close to oesophageal gland. Oocytes arranged in a single row. Post uterine sac 2-3 anal body width long. Tail 2.9-3.7 anal body width long, regularly tapering to a narrowly bifurcate tip. The ventral prong is long than the dorsal one.

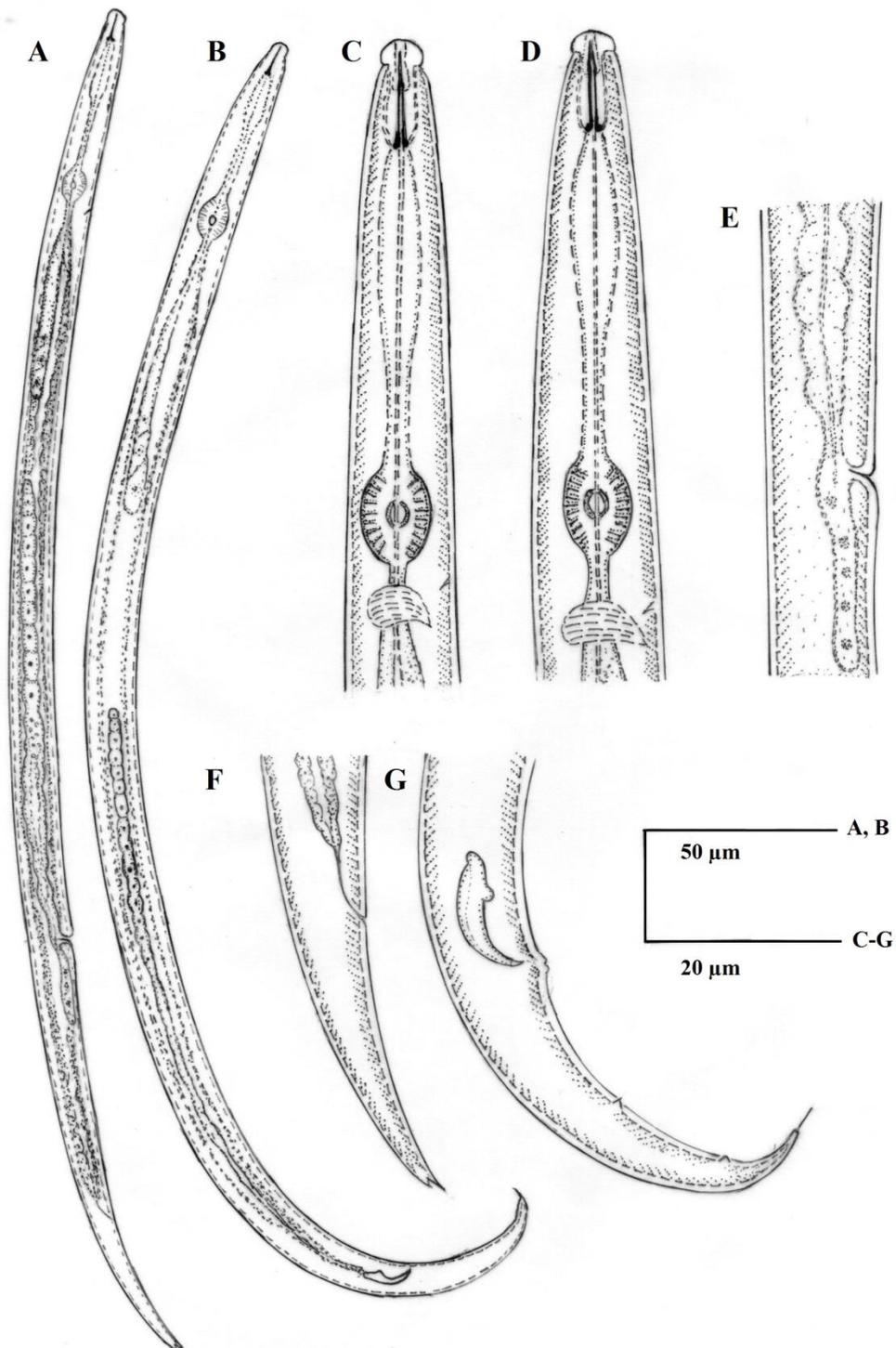
**Male:** General morphology similar to that of female. Testis extending upto the posterior end of oesophageal gland lobe. Spicules equal in size. Tail without bifurcation and gradually narrows to form a centrally located mucro. Two caudal papillae distinct.

**Remarks:** The specimens fit in well with the original description of the species *Aphelenchoides bicaudatus* given by (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941. The specimens were recovered from the rhizosphere of radish (*Raphanus sativus* L.) from Faisalabad, Punjab, Pakistan.

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**Fig. 3 (A-G).** *A. bicaudatus* (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941. A. Whole body of female; B. Whole body of male; C. Anterior region of female; D. Anterior region of male; E. Vulval region; F. Female tail; G. Male tail.

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