

TWO NEW SPECIES OF BOLEODORUS (NEMATODA: TYLENCHIDA) FROM MANIPUR, INDIA

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KEY WORDS

Nematode
Biodiversity
New species
Boleodorus

Received on :
03.01.2012

Accepted on :
22.02.2012

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ABSTRACT

Two new species of *Boleodorus*, *B. longicaudatus* sp. nov. and *B. spinocaudatus* sp. nov. were found associated with mulberry plants in Manipur, India and were described. *Boleodorus longicaudatus* sp. nov. is characterised by body length = 0.43-0.72 mm, a = 25.4-28.3, b = 7.05-9.2, c = 4.88-6.24, c' = 8.66-12.4, V = 64.9%-74.4%, G₁ = 64.9-74.4, oesophagous = 61.2-72.4 μm, spear = 8.5 μm, and tail = 88.5-92.2 μm. *Boleodorus spinocaudatus* sp. nov. is characterised by body length = 0.60-0.69 mm, a = 25.5-36, b = 6.6-10.7, c = 7.5-13, c' = 4.6-6.7, V = 75.9%-82.6 %, G₁ = 48.7 – 81.3, oesophagous = 64.6-96.9 μm, spear = 8.5-10.2 μm, and tail = 49.379.9 μm.

INTRODUCTION

Globally, nematodes are common in almost all soils, with their distribution being determined by temperature, degree of moisture and soil particle size, along with the presence of acceptable food source. Undoubtedly the greatest diversity in nematode specialization can be found in the subtropics and tropics, where climate is less of a determinant of distribution, and where the range and severity of parasitism on all living organisms is greater (Luc *et al.*, 1990). Manipur lies at the heart of Indo-Burma hot-spot of Asia Pacific Region. Due to this, the region is endowed with different climatic conditions and a great diversity in organisms. The present survey was carried out with the view of finding new species of nematodes from this hot spot region in order to fill up the lacunae of knowledge in nematode biodiversity of the region. Among the several genera of nematodes encountered, the genera *Boleodorus* was one of them.

The genus *Boleodorus* Thorne, 1941 is characterized by small to medium sized body which is ventrally arcuate upon fixation with finely striated cuticle. Oral aperture surrounded by six papillae, a second circle of four papillae on the wedges of anterior surface. Cephalic region elevated, weakly to moderately sclerotised. Amphidial apertures oval or crescentic slits, obliquely placed on the head, usually partially covered by a flap. Delicate stylet about 8-10 μm long, conus about half the shaft, about one - third of total stylet length, knobs flanged, rarely rounded, sometimes stylet without knobs. Median bulb with or without thickenings of lumen wall. Basal bulb pyriform. Orifice of dorsal oesophageal gland opening at 1- 4 μm from stylet knob base. Excretory pore behind nerve

ring. Mono-prodelphic reproductive system or amphidelphic. Vulva a transverse generally at 59-75% of body length, spermatheca axial or offset, crustaformeria with few cells. During the study two species of *Boleodorus* were found showing morphometric details different from all the previously known species of *Boleodorus*. Altogether, 30 species of *Boleodorus* have been described world wide, 17 species were described from India among which four species were described from Manipur (Geraert, 2008). Upon detailed study, the two species were found to be new to science. Their descriptions and illustrations are provided.

MATERIALS AND METHODS

For extraction of nematodes, soil samples were processed through Cobb's sieving and decanting method followed by Baermann's funnel technique (Thorne, 1961). After 24h, the nematode suspension was collected in a tube and was allowed to stand for at least 1-2h to allow nematodes to settle at the bottom of the tube. The nematodes were then transferred in a small amount of water in cavity blocks, the excess water was removed and the nematodes were killed and fixed in hot F.A. (4:1). The nematode specimens were dehydrated by Seinhorst's (1959) rapid glycerin method and mounted permanently on slides using dehydrated glycerine as mountant. Then they were observed under a compound microscope, measured using ocular micrometer and diagrams were drawn with a camera lucida attached to a compound microscope. Then, ratios were calculated according to De Man's (1884) formula and De Grisse's (1964) symbols.

RESULTS AND DISCUSSION

Boleodorus longicaudatus sp. nov.

Descriptions

Female: Body cylindrical, tapering towards both ends, slightly ventrally arcuate when relaxed by warm F. A. (4:1). Cuticle finely and transversely striated, each stria being $1.7\mu\text{m}$ wide at mid body. Lateral fields with four incisures, with non crenated outer lines, occupying one third of body width at mid body. Lip region continuous, conical, anterior end with a slight depression, smooth, $5.1\mu\text{m}$ wide and $1.7\mu\text{m}$ across. Amphidial apertures prominent, oblique slits, post-labial in position. Cephalic framework weak. Stylet slender, weak; conus needle like, $8.5\mu\text{m}$ long; knobs flange like, $1.7\mu\text{m}$ width and $0.8\mu\text{m}$ high. Orifice of dorsal oesophageal gland openings at $3.4\mu\text{m}$ behind the spear base. Procorpus $35.7\mu\text{m}$ long, slender, enlarging slightly to metacorpus, metacorpus $1.2\text{--}15.3\mu\text{m}$ long. Phasmids indistinct. Metacorpus not distinctly separate from procorpus, non valvate, non-muscular, $8.5\mu\text{m}$ high and $4.2\mu\text{m}$ wide. Isthmus, somewhat narrower than the procorpus, tubular, $20.4\mu\text{m}$ long. Basal bulbs elongate pyriform, $17\mu\text{m}$ long and $6.8\mu\text{m}$ wide. Cardia hemispherical, $4\mu\text{m}$ long. Nerve ring $54.4\mu\text{m}$ from anterior end encircling the isthmus just at the base of neck of median bulb. Excretory pore at the base of the end of the bulb, $71.4\mu\text{m}$ from the anterior end of the body (Table 1, Fig. 1).

Gonad mono-prodelphic, oocytes arranged in a double row up to the tip and in a single row slightly above the spermatheca. Vulva a transverse slit. Vagina at right angles to body wall and muscular. Uterus with proximal, distal muscular parts curved. Spermatheca large, cylindrical filled with sperms, $47.6\mu\text{m}$ long and $15.3\mu\text{m}$ wide. Post-uterine sac $6.8\mu\text{m}$ or 2.25 times vulval

Table 1: Morphometric data of the females of *Boleodorus longicaudatus* sp. nov. All measurements in μm except body length in mm

Character	Holotype	Paratype
N	1	6
Body length	0.43	0.43 - 0.72 (0.54 ± 0.11)
a	25.4	25.4 - 28.3 (26.8 ± 1)
b	7.05	7.05 - 9.2 (7.9 ± 0.8)
c	4.88	4.88 - 6.24 (5.4 ± 0.5)
c'	8.66	8.66 - 12.4 (10.4 ± 1.3)
V	64.9	64.9 - 74.4 (69 ± 3.7)
G ₁	46.8	64.9 - 74.4 (69 ± 3.7)
Uterine sac	6.8	8.66 - 2.4 (10.4 ± 1.3)
Procorpus	35.7	34.5 - 35.7 (35.1 ± 0.6)
Mesocorpus	8.5	8.5
End bulb	17	17
Oesophagous	61.2	61.2-72.4 (66.1 ± 3.7)
Lip width	5.1	5.1
Lip height	1.7	1.7
Spear	8.5	8.5
Knob width	1.7	1.7
Knob height	0.8	0.8
DGO	3.4	3.4
Exc. Pore	71.4	71.4 - 74.3 (72.8 ± 1.05)
Nerve ring	54.4	51.3 - 54.4 (52.8 ± 1.1)
Rectum	15.3	14.2 - 15.3 (14.7 ± 0.5)
V-A dist.	62.9	62.9 - 74.6 (68 ± 5)
Tail	88.4	88.5 - 92.2 (90.2 ± 1.4)
ABD	10.2	7.4 - 10.2 (8.4 ± 0.9)

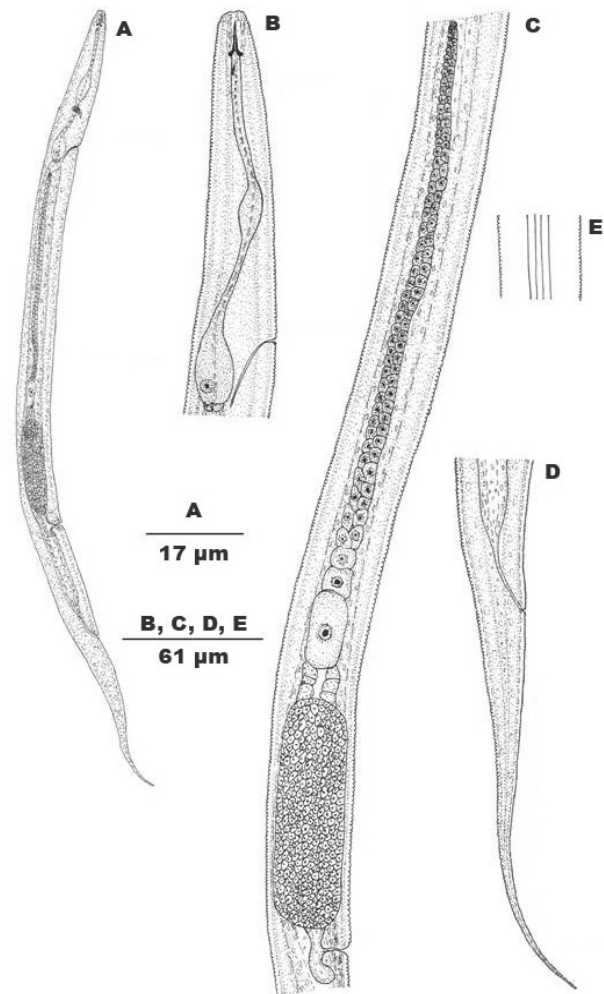


Figure 1: *Boleodorus longicaudatus* sp. nov.

Camera lucida drawings (Female) (A) entire body; (B) anterior region; (C) reproductive system; (D) tail region and (E) lateral lines.

body width long. Distance between vulva and anus $62.9\mu\text{m}$ or 1.4 times tail length. Body gradually narrowing downwards ending into an elongate and pointed tail terminus. Tail $88.4\mu\text{m}$ long or 8.6 times anal body width.

Male: Not found.

Type host and locality: *Morus alba* L. from Jiri, Imphal East District, Manipur, India, latitude $24^{\circ}51'21.76''\text{N}$ to $24^{\circ}33'13.08''\text{N}$ and longitude of $93^{\circ}4'22.973''\text{E}$.

Type specimen: Collected in October, 2009. Holotype female on the slide PBB-1-*Boleodorus longicaudatus* sp. nov./1. Paratype on the slides PBB-1-*Boleodorus longicaudatus* sp. nov./2-5 and deposited in the nematode collection of the Parasitology Section, Department of Life Sciences, Manipur University, Canchipur, Imphal, India.

Diagnosis and relationship: *Boleodorus longicaudatus* sp. nov. differs from all other species of *Boleodorus* in having a long slender tail gradually tapering into a pointed terminus.

Boleodorus longicaudatus sp. nov. comes close to *B. filiformis* Husain and Khan, 1977 and *B. impar* Khan & Basir, 1964.

Table 2: Morphometric data of the females of *Boleodoros spinocaudatus* sp. nov. All measurements in μm except body length in mm

Character	Holotype	Paratype
N	1	7
Length	0.64	0.60 - 0.69 (0.63 \pm 34.4)
a	29	25.5 - 36 (31.5 \pm 4.4)
b	6.6	6.6 - 10.7 (8.3 \pm 1.4)
c	13	7.5 - 13 (10.1 \pm 2)
c'	4.8	4.6 - 6.7 (5.2 \pm 0.8)
V	82.4	75.9 - 82.6 (79 \pm 2.5)
G ₁	59.6	48.7 - 81.3 (61.9 \pm 11.9)
Uterine sac	6.8	6.8
Procorpus	56.1	30.6 - 56.1 (40.8 \pm 9.3)
Mesocorpus	13.6	13.6 - 17 (15.3 \pm 1.7)
End bulb	18.7	11.9 - 18.7 (14.8 \pm 2.5)
Oesophagous	91.8	64.6 - 96.9 (78.4 \pm 11.6)
Lip width	6.8	5.1 - 6.8 (5.5 \pm 0.7)
Lip height	3.4	3.4
Spear	10.2	8.5 - 10.2 (9.3 \pm 0.8)
Knob width	3.4	3.4
Knob height	1.7	1.7
DGO	5.1	5.1
Excretory pore	88.4	61.8 - 88.4 (74.9 \pm 12.6)
Nerve ring	68	52.7 - 68 (61.1 \pm 6.2)
Rectum	17	17
V - A dist.	62.9	68 - 73.1 (68.2 \pm 3.2)
Tail	49.3	49.3 - 79.9 (64.7 \pm 10.9)
ABD	10.2	10.2 - 13.6 (12.3 \pm 1.4)

From *B. filiformis*, it differs in having larger body length, greater b value, smaller c value, greater c' value and elongated spermatheca with double-rowed oocytes and tail (L = 0.46 - 0.55, b = 5 - 5.6, c = 7 - 9, c' = 6 - 7, rounded spermatheca with single outstretched oocytes in *B. filiformis*).

Boleodoros longicaudatus sp. nov. differs from *B. impar* in having longer body length, larger b, c, c', V value, shorter stylet, elongated spermatheca with double-rowed oocytes and tail (L = 0.50-0.60, b = 4.8-6.2, c = 5-7, c' = 9, V = 63-66, stylet = 13- 4, rounded spermatheca with single oocytes and filiform tail in *B. impar*).

Boleodoros spinocaudatus sp. nov.

Descriptions

Female: Body cylindrical, tapering towards both extremities upon fixation. Cuticle thin and fine transversely striated with $1\mu\text{m}$ width. Cephalic framework continuous with body with indistinct striations. Lip region $3.4\mu\text{m}$ high and $5.1-6.8\mu\text{m}$ wide, continuous with the body. Lateral fields with four incisures equally spaced occupying one third of body width at mid body. Amphids posterior to lip region, aperture slit like $3.4-4.2\mu\text{m}$ long with flanged knobs, $3.4\mu\text{m}$ across and $1.7\mu\text{m}$ high. Orifice of dorsal oesophageal gland opening at 5.1m behind the base of stylet knobs. Procorpus $30.6 - 56.1\mu\text{m}$ long gradually narrows towards the mesocorpus. Mesocorpus $5.1-6.8 (5.5 \pm 0.7)\mu\text{m}$ across and $13.6-17 \mu\text{m}$ high, elongated without valve plates. Isthmus very short not separated from the basal bulb. Basal bulb pyriform, $5.1-10.2 (6.3 \pm 2.2)\mu\text{m}$ wide and $11.9-18.7\mu\text{m}$ high. Cardia hemispherical $2-3 (2.5 \pm 0.5)\mu\text{m}$ long and $3.8\mu\text{m}$ wide. Nerve rings $52.7-68\mu\text{m}$ from the base of the anterior end of the body. Excretory pore $61.9-$

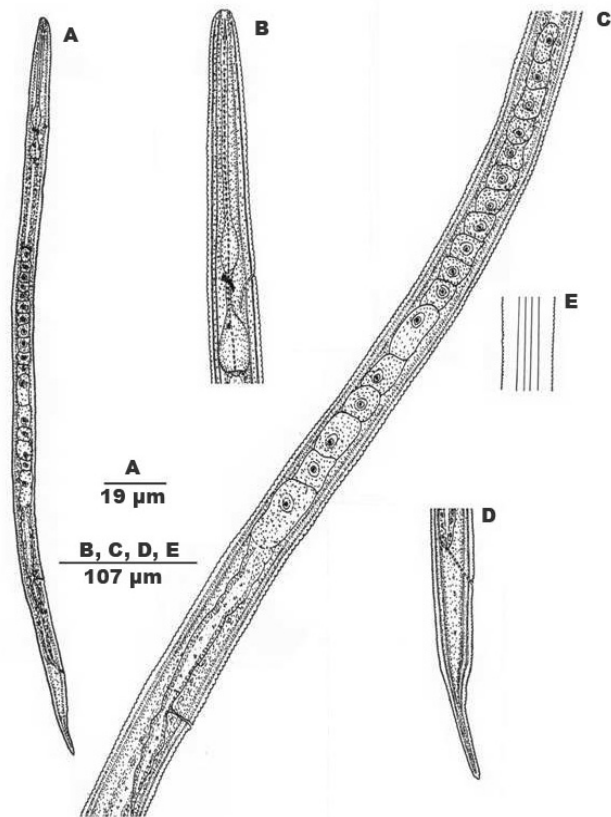


Figure 2: *Boleodoros spinocaudatus* sp. nov.

Camera lucida drawings (Female) (A) entire body; (B) anterior region; (C) reproductive system; (D) tail region and (E) lateral lines.

$88.4\mu\text{m}$ from anterior end of the body and at the base of basal bulb (Table 2; Fig. 2).

Mono-prodelphic reproductive system, vulva a transverse slit. Vagina closed type, at right angles to the body and thick walled, one third vulval body width long. Ovary single, arranged in a single row, spherical filled with sperms. Uterus with proximal and distal muscular part $6.8\mu\text{m}$ long, or about two and a half of total length from anus. Distance between vulva and anus $0.8-1.2 (1.0 \pm 14)$ times tail length. Body gradually tapering towards a narrow tail ending into a round pointed terminus, $49.3-79.9\mu\text{m}$ long or $4.6-6.7$ times anal body diameter long.

Male: Not found.

Type host and locality: *Morus alba* from at Mayang Imphal Konchak Makha Leikai, Imphal West District, Manipur, India, latitude of $24^{\circ}39' 32.40''\text{N}$ and longitude of $93^{\circ}54' 11.13''\text{E}$.

Type specimen: Collected in March, 2009. Holotype female on the slide PBB-2-*Boleodoros spinocaudatus* sp. nov./1. Paratype on slides, PBB-2-*Boleodoros spinocaudatus* sp. nov. /2-5 and deposited in the nematode collection of Parasitology Section, Department of Life Sciences, Manipur University, Canchipur, Imphal.

Diagnosis and relationship: *Boleodoros spinocaudatus* sp. nov. differs from all other species of the genus *Boleodoros* in having indistinctly demarcated mesocorpus from the basal bulb and a round pointed tail.

Boleodorus spinocaudatus sp. nov. comes close to *B. brevistylus* Khera, 1970; *Boleodorus rafiqi* Husain and Khan, 1965. With *B. brevistylus*, *B. spinocaudatus* sp. nov. shares the same stylet length, but differs from it in having smaller values of L, a, b and tail shape and oesophagous (L = 0.70 - 0.81, a = 37 - 40, b = 5.2 - 5.6 and arcuate with bluntly rounded tail tip and demarcated oesophagous in *B. brevistylus*). *Boleodorus spinocaudatus* sp. nov. is similar to *B. rafiqi* Husain and Khan, 1965, in having same ranges of body and stylet length, but differs from it in having greater value of b, V, smaller value of c', tail and oesophagous shapes (b = 4.2 - 5, V = 65 - 68, c' = 6 - 7, arcuate with ventrally tip tail and demarcation of mesocarpus and end bulb in *B. rafiqi*).

ACKNOWLEDGEMENTS

The authors are thankful to the Head of the Department of Life Sciences, Manipur University, Canchipur, Manipur, India, for providing necessary laboratory facilities, and UGC for the grant provided to the first author in the form of fellowship. The soil collector, Mr. L. Ritendro Singh is also acknowledged with thanks.

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