

HELICOTYLENCHUS SPECIES ASSOCIATED WITH CAJANUS CAJAN (L.) MILLSP. WITH A DESCRIPTION OF A NEW SPECIES FROM MANIPUR

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ABSTRACT

A survey of soil nematodes associated with *Cajanus cajan* (L.) Millsp. from different localities of Manipur revealed the presence of abundant of *Helicotylenchus* species. The present study reveals a new species of *Helicotylenchus*. *Helicotylenchus constrictus* sp. nov. was characterised by having 618.24µm long body, a = 24, b = 5.18, b' = 4.26, c = 34.9, v = 62.76 and Stylet = 20.93.

INTRODUCTION

In India, various pulse crops are being cultivated by farmers every year for livelihood of the community. These crops also play a main role in the agricultural economy as well as in day to day life of millions of people in our country as a source of dietary protein. Among the pulse crops, pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the most important legume which is widely employed in the farming system of the Indian sub continent. This crop is also regarded as one of the most favourite and nutritive food among the pulses. Again such highly economically important plant is not free from threats of various plant pests, either arthropods or nematodes. Few nematode species are almost associated with this plant namely of the genera - *Meloidogyne* sp., *Rotylenchulus* sp., *Heterodera* sp. and very prominently *Helicotylenchus* sp. *Helicotylenchus* Steiner, 1945 commonly called 'spiral nematode' are among the most frequently found plant parasitic nematodes worldwide. Species of *Helicotylenchus* are globally distributed, spanning many climates, and are associated with the root system of diverse crops of agricultural importance (Subotin et al., 2011).

The genus *Helicotylenchus* was first established by Steiner, 1945 with the type species *H. dihystra* (Cobb, 1893) Sher, 1961. The genus *Helicotylenchus* was characterised by lip region - continuous, dorsal oesophageal gland orifice $\frac{1}{4}$ - $\frac{1}{2}$ stylet length behind stylet base. Oesophageal gland overlapping intestine ventrally. Subsequently many species

were added to this genus from different countries of the world. More than 200 species have been described from all over the world, of which about one third of the total is from India alone.

In Manipur, considerable work has been done on the taxonomy of soil and plant nematode of different crop plants. Different workers (Gambhir, 1989; Mohilal, 1996; Romabati, 1998; Solitary, 1998; Victoria, 2008) have reported the presence of many species under this genus from different crop plants of Manipur.

However no detail work has been carried out regarding the nematodes of pigeonpea plant in this state. Thus the present work was undertaken with a view to supplement the knowledge of plant parasitic nematodes of pigeonpea carried out elsewhere in the country and abroad and also to find out any new species. In the present survey of plant parasitic nematodes associated with the pigeonpea occurring in Manipur, many species of *Helicotylenchus* belonging to the family Hoplolaimidae were recorded. The detailed studies of different populations of *Helicotylenchus* revealed a new species and two known species.

MATERIALS AND METHODS

Soil samples were collected from around the rhizosphere of *Cajanus cajan* (L.) Millsp. growing in different localities of Manipur. The nematodes were extracted by using Cobb's sieving and decanting method followed by Baermann funnel technique (Thorne, 1961). The nematode suspension was

collected in a test tube after 24h and were allowed to settle at the bottom. The nematodes were then transfer to a cavity block and were killed by warm F.A. The nematode specimen was then processed to glycerine following the method of Seinhorst (1959). The processed specimen was mounted in dehydrated glycerine using adequate glass wool and subjected to detailed morphological and morphometrical studies. Specimens were measured using ocular micrometer and camera Lucida drawing was done attached to a compound microscope. Body dimensions were calculated using de Man's formula.

RESULTS AND DISCUSSION

Helicotylenchus constrictus sp.nov. (Fig. 1 and 2)

Measurements: Table 1.

Description:

Female: Body open spiral on relaxation by warm F.A. Cuticle bearing fine transverse striation about 1.61µm at midbody. Lateral field with four incisures occupying about 1/3rd of the body width. Lip region rounded with inconspicuous annules. Cephalid not seen. Stylet knob rounded in shape. Orifice of dorsal oesophageal gland almost ½ of spear length from stylet base. Oesophagus typical with procorpus slender measuring 48.3µm, isthmus narrow µm and basal lobe overlapping intestine 19.32µm in length. Median bulb oval 14.49 x 9.66µm in diameter. Excretory pore at the level of base of basal gland. Nerve ring located at the anterior part of the isthmus. Vulva a depressed, transverse slit located at 388.01µm from the anterior end of the body. Epigtygma not seen. Vagina almost ½ body width long. Ovaries paired, out-stretched and oocytes arranged in a single row. Phasmid anterior to anal opening 7 annules upward from anus, 27.37µm from the tip of tail. Tail smooth, broad and conoid ventrally, marked by a constriction at the opposite end of the anus, equal to 1.3 anal body width long having 8 annules and tip pointed without conspicuous annules.

Male: Not found

Type host and locality: Collected around the roots of *Cajanus*

cajan (L.) Millsp. from Canchipur, Manipur, India (24°82'NL and 93°90'EL).

Type specimen: Holotype female on the slide no.CT₆N₁₅/1 and paratype (CT₆N₁₅/1-5) deposited in the Laboratory of Parasitology, Department of Life Sciences, Manipur University, Canchipur.

Etymology: The species name '*constrictus*' is given after the body constriction at the opposite end of the anus of the present species.

Diagnosis and relationship: *Helicotylenchus constrictus* sp. nov. comes close to *Helicotylenchus caipora* Monteiro and

Table 1: Morphometric data of female species of *Helicotylenchus constrictus* sp. nov. All measurements in µm except L in mm

Characters	<i>Helicotylenchus constrictus</i> sp. nov	
	Holotype	Paratype
n	1	5
L	0.61	0.61 – 0.62 (0.61±0.00)
a	24	23.75 – 24.25(23.4±0.63)
b	5.18	5.17 – 5.20(5.26±0.11)
b'	4.26	4.26 – 4.43 (4.31±0.11)
c	34.9	34.9 – 36.2 (35.6±0.31)
c'	1.37	1.37 – 1.49(1.38±0.12)
v	62.76	62.63 – 62.88(62.6±0.24)
G ₁	23.69	23.15 – 23.71(23.5±0.23)
G ₂	26.04	25.26 – 26.28 (25.5±0.38)
Lip height	4.83	3.22 – 4.83 (4.1±0.79)
Lip width	6.44	6.44 – 7.24 (6.7±0.39)
Stylet length	20.93	20.93 – 22.54 (21.8±0.79)
DGO	11.27	8.05 – 12.88 (11.27±1.01)
Oesophagus	112.7	112.7 - 120.82 (117.6±2.71)
Body width at mid body	25.76	25.76 – 27.37 (25.7±0.71)
Median bulb length	12.88	12.88 – 14.49 (13.8±0.79)
Median bulb width	9.66	9.66 – 11.27(10.3±0.78)
Nerve ring	93.38	91.7 – 94.9 (93.3±1.02)
Excretory pore	106.26	104.69–107.32 (109.4±3.52)
Tail	17.71	16.9 – 17.71 (17.3±0.41)
ABD	12.88	11.27 – 12.88(17.3±0.41)
Phasmid	7 annules	6 – 8 annules
	anterior	anterior
Tail annules	8	7 – 8

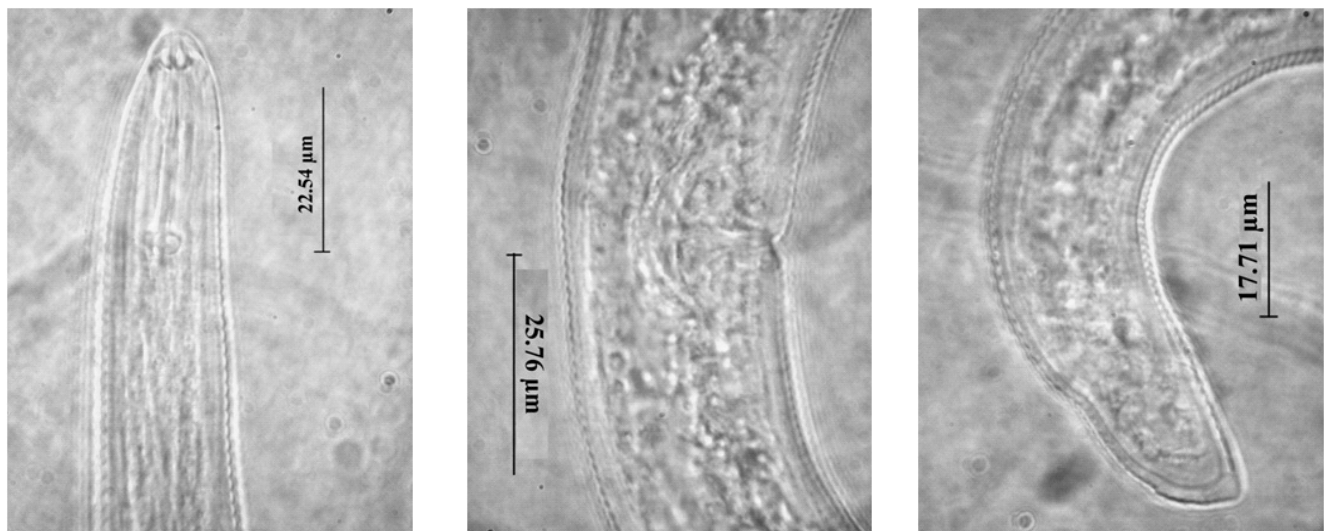


Figure 1: Photomicrograph of *Helicotylenchus constrictus* sp. nov. A. anterior end, B. vulval region and C. tail region

Mendonca, 1972; *Helicotylenchus truncatus* Roma'n, 1965. *Helicotylenchus constrictus* sp. nov. comes close to *Helicotylenchus caipora* Monteiro and Mendonca, 1972 in having similar range of body length, body with oesophageal length and anal body diameter and stylet length. But the present species differ from the species in having rounded cephalic region with inconspicuous annules, indistinct cephalid, indistinct hemizonid, position of excretory pore and tail smooth, conoid broadly at ventral side marked with 8 annules (Cephalid region semispherical with four or five indistinct rings, presence of cephalid, well developed hemizonid, excretory pore somewhat anterior to oesophago-intestinal valve, and tail conically convex on dorsal side without outgrowth, terminus semispherical and smooth (non-annulated) in *Helicotylenchus caipora* Monteiro & Mendonca, 1972).

Helicotylenchus constrictus sp.nov. comes close to *Helicotylenchus truncatus* Roma'n, 1965 in having similar value of a, b and similar length of stylet length, larger value of b, c, c', v, m, shape of lip region, hemizonid indistinct, absence of epiptigma, position of phasmid and shape of tail (L = 0.43 – 0.50, b = 3.7 – 5.2, c = 34– 50, c' = 0.9– 1.0, v = 60 – 65, m = 49 – 50, anteriorly blunt lip region, presence of hemizonid, presence of lateral membrane in the vulva, phasmid at the level of anus and blunt tail with 12 to 13 cuticular rings, cuticular annulations extending upto the end of tail in *Helicotylenchus truncatus* Roma'n, 1965).

***Helicotylenchus digonicus* Perry, 1959**

Measurements: Table 2.

Description:

Female: Body in open spiral shape when relaxed. Lip region not demarcated from the general shape of the body with inconspicuous annules. Stylet knob anteriorly flat. Width of cuticular ring 1.61µm in middle of the body. Duct of dorsal oesophageal gland opens into oesophageal lumen at 8.05 – 11.27µm behind base of stylet. Oesophageal bulb oval in

shape. Nerve ring situated at the anterior part to middle of the isthmus. Excretory pore situated at 96.6 – 99.82 µm from the anterior end of the body. Oesophageal gland overlaps commencement of the intestine from ventral. Hemizonid not distinct. Vulva a transverse slit. Ovaries straight and oocyst

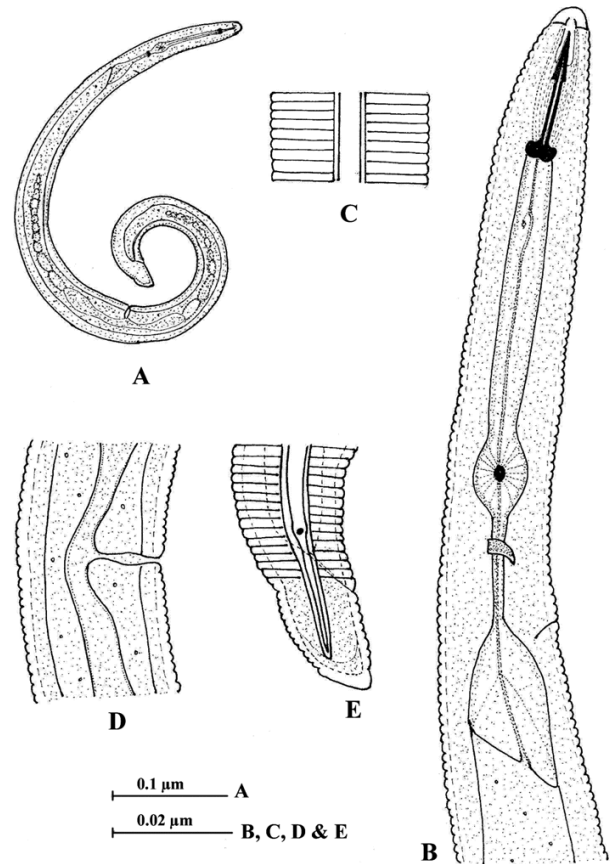


Figure 2: *Helicotylenchus constrictus* sp. nov. A. entire female, B. anterior end, C. lateral field, D. vulval region and E. tail region

Table 2: Morphometric data of female species of *Helicotylenchus digonicus* and *Helicotylenchus dihystra*. All measurements in µm except L in mm

Characters	<i>Helicotylenchus digonicus</i> Perry, 1959	<i>Helicotylenchus dihystra</i> (Cobb,1893) Sher,1961
n	5	5
L	0.57 – 0.59 (0.58±0.004)	587.65 – 611.8 (0.59±0.01)
a	25.92 – 30.0 (27.2±1.94)	23 – 25 (24.2 ± 1.16)
b	5.7 – 5.76 (5.7±0.06)	3.9 – 4.13 (4.05 ± 0.09)
b'	4.5 – 4.6(4.5±0.06)	4.4 – 4.8 (4.6 ± 0.14)
c	40.33 – 45.6 (42.9±2.11)	36 – 45.3 (40.3±4.19)
c'	0.8 – 1.0 (0.9±0.08)	0.8 – 1.25 (1.01±0.15)
V	66.39 – 66.57 (65.6±1.04)	62.1 – 70.7 (64.2±3.22)
Lip height	3.22 – 4.83(3.96±0.72)	3.22 – 4.83 (4.18±0.78)
Stylet length	20.93 – 22.54 (21.3±0.62)	20.93 – 24.15 (22.2±1.46)
DGO	8.05 – 11.27 (11.33±2.32)	8.05 – 11.27 (9.54±1.44)
Oesophagus	101.43 – 104.65 (102.54±1.20)	117.53 – 123.97(120.42±5.25)
Body width at mid body	19.32 – 24.15(21.4±1.90)	23.46 – 26.6 (24.74±1.03)
Median bulb length	11.27 – 12.88 (12.1±0.72)	11.27 – 14.49 (13.20±1.20)
Median bulb width	9.65 – 9.67(9.66±0.006)	6.44 – 9.66(8.05±1.01)
Nerve ring	85.33 – 91.77 (88.04±1.93)	96.6 – 114.31 (102.71±6.78)
Tail	12.88 – 14.49 (13.69±0.71)	12.88 – 16.1(14.49±1.44)
ABD	14.49 – 16.1(15.06±0.71)	12.88 – 14.49 (14.16±1.20)
Phasmid	5 – 7 annules anterior	2 – 5 annules anterior
Tail annules	6 – 8	9 – 10

arrange in a single row. Phasmid five to seven annules anterior to anus. Tail dorsally more arced than ventrally with 6 – 8 cuticular ring on ventral side. Tail terminus has a short ventral outgrowth. In some specimen tail terminus is rounded with two large cuticular rings.

Male: Not found.

Host and locality: Collected around the roots of *Cajanus cajan* (L.) Millsp. from Canchipur, Kanglatongbi and Kakching, Manipur.

Remarks: The dimension and characters of the mentioned population confirm with those of the description of *Helicotylenchus digonicus* Perry, 1959 as given by Perry, 1959 except for slight variation in stylet, tail length and more posterior position of vulva.

***Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961**

Dimensions: Table 2

Descriptions:

Female: Body spiral in shape when relaxed. Lip region hemispherical, with 4 annules. Stylet well developed. Stylet knob rounded with anteriorly flattened surface, 4.83 x 3.22 μ m. Median oesophageal bulb oval in shape. Excretory pore near oesophago-intestinal junction. Ovaries paired, outstretched and oocyte arranged in a single row. Tail bluntly conoid with slight ventral outgrowth, bearing 9 – 10 cuticular annules. Phasmid 2 – 5 annules anterior to anus.

Male: Not found.

Habitat and locality: Soil around the roots of *Cajanus cajan* (L.) Millsp. from Canchipur, Kanglatongbi, Sagoltongba and Tera, Manipur.

Remarks: The present specimen conform well to the morphological characters and measurements of *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961 except for slight variation in stylet length.

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