Two new species of soil nematodes from Manipur, India

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Abstract Survey for soil nematodes associated with mulberry plants in valley districts of Manipur revealed the presence of two new species of soil nematodes of the genus *Tylenchus* sp. and *Telotylenchus* sp. The two new species are described and illustrated here.

Keywords Manipur · Nematode · Biodiversity · New species

Introduction

Manipur lies at the heart of North-Eastern biodiversity hot spot area. The region encompasses a great floral diversity and thus it can also be thought to have a great diversity of rare fauna which are supported by the floral diversity. A survey for soil nematodes associated with mulberry plants in valley districts of Manipur was conducted in view to find out the most economically important soil nematodes affecting the wellness of the plants. The study encountered two new species of soil nematodes *Tylenchus conicaudatus* sp. nov. and *Telotylenchus manipurensis* sp. nov. from the rhizospheric regions of the plants which is an added information in the rich diversity of the region.

The genus *Tylenchus* was re-established by Filipjev (1936) which was originally proposed by Bastian (1865). The type species under the genus was characterised by small body that curved ventrally upon fixation. Lateral fields each with four incisures, often crenated on borders, phasmid dorso-sublateral and post-median behind vulva.

Cephalic region continuous with body, annulated and framework with light or no sclerotisation. Stylet moderately developed 8–12 µm long with conus half or less of stylet length. Distinct and rounded basal bulb, vulva a transverse-slit, usually at 60–79 % of body length, prodelphic reproductive system and tail ventrally arcuate, often hooked, regularly tapering to a pointed or minutely rounded terminus.

Siddiqi (1960) proposed *Telotylenchinae* with *Telotylenchus* as its type species. The genus was also characterised by small to medium sized body, lateral fields with four incisures, plain or areolated. Lip region hemispherical to broadly rounded, stylet moderately developed, conus solid appearing anteriorly, straight, knobs prominent; dorsal oesophageal gland elongate, extending over intestine mostly laterally, vulva flush with body contour or rarely in a small body cavity; ovaries paired, outstretched and female tail elongate conical to subcylindrical, 2–4 anal body widths long.

Study of the prevailing literatures showed that *Tylenchus conicaudatus* sp. nov. and *Telotylenchus manipurensis* sp. nov. encountered during the survey of soil nematodes differed from all existing species of *Tylenchus* spp. and *Telotylenchus* spp. The two species are illustrated here with their morphometric details and line diagrams.

Materials and methods

Collection of soil samples

Soil samples were collected from around the rhizosphere of mulberry plants, *Morus alba* L. (Local name: Kabrangchaak) from different localities of Manipur, India. Samples were taken from a depth of 10–30 cm after discarding the top layer.

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At least ten sub-samples were collected from the locality of the same host plant from each area forming a bulk soil sample. The collected sub-samples were mixed thoroughly and from this bulk sample, about 500 g of soil was taken as the representative soil sample of that particular locality.

Processing of soil samples

The collected soil samples were processed for the extraction of nematodes by modified Baermann's funnel technique (Thorne 1961). For this, soil sample was put in a plastic bucket after a thorough breakage of the big chunks. Tap water was added to half the bucket and the suspension was gently stirred by hand breaking the bigger soil clods. More water was added allowing all the nematodes to float free of the soil particles in the suspension. The suspension was allowed to stand for 15–20 s to allow heavier soil particles to settle to the bottom. The suspension was then passed through a basal sieve size of 350 numbers. The collected residue containing the nematodes was washed thoroughly under tap water and transferred to a beaker. A double layer of tissue paper was placed on a strainer onto which the residue collected from the sieve 350—meshes was poured. After that, the strainer was put into the Baermann's funnel, already filled with clean filtered water in such a way that the strainer touches the bottom water. It was left undisturbed for about 24 h. The nematode suspension was collected after 24 h from the collecting tube by opening the clip.

Killing and fixing

The nematodes were killed and fixed in hot F.A. (4:1).

Dehydration process

The nematodes were collected in a cavity block containing G.A. (Glycerine alcohol). The cavity block was kept in desiccators containing Calcium chloride for a minimum of 15 days (Seinhorst 1959).

Mounting and sealing

The dehydrated nematodes were mounted in dehydrated glycerin and sealed with DPX (Seinhorst 1959).

Measurements and drawings

The measurements of the nematodes were done using the De Man's formula and De Grisse's symbol. The diagrams were drawn with the help of camera lucida attached to an Olympus compound microscope, Model No. 76627.

Results and discussion

Tylenchus conicaudatus sp. nov. (Fig. 1)

Dimensions

Refer to Table 1.

Description *Female* Female body slightly curved ventrally upon fixation (Fig. 1) and robust being 0.45–0.62 (0.51 ± 0.08) mm long and greatest body width about 13.6–15.3 (13.8 ± 0.6) μm , tapering more posteriorly than anteriorly (Table 1). Cuticle finely annulated, each annule about 1.7 μm wide at middle body and narrower near tail. Four lateral lines distinct and outer line non-crenated. Head slightly narrower than body, flattened, offset, with four annules, about 5.1 μm wide and 2.5 μm high. Amphidial aperture longitudinal slits, originating on the top of lateral

Fig. 1 *Tylenchus conicaudatus* sp. nov. **a** Entire body, **b** Anterior region, **c** Reproductive system, **d** Tail region and **e** Lateral lines

Table 1 Morphometric data of the females of *Tylenchus conicaudatus* sp. nov.

Characters	Holotype	Paratypes
n	1	7
Length	0.53	0.45–0.62 (0.51 ± 0.08)
a	39.3	32.4–46 (37.8.3 ± 4.4)
b	4.5	3.6–5.4 (4.5 ± 0.5)
c	9	7.1–18.4 (9.8 ± 3.8)
c'	5.8	5.3–8.2 (6.6 ± 0.9)
V	75.5	67.4–75.5 (71 ± 2.5)
G ₁	27.6	67.4–75.5 (72 ± 2.5)
Uterine sac	25.5	11.9–25.5 (19.5 ± 4.4)
Spear	8.5	8.5
DGO	5.1	5.1–5.18.5 (8.5 ± 0)
Oesophagous	119	102–127.5 (113.6 ± 8.4)
Knob width	2.5	2.5
Knob height	1.7	1.7
Nerve ring	69.7	61.2–69.7 (66 ± 2.8)
Excretory pore	96.9	66.3–96.9 (63.4 ± 28.1)
Max. body width	13.6	13.6–15.3 (13.8 ± 0.6)
Tail	59.5	34–69.7 (56.3 ± 10.9)
ABD	10.2	34–69.7 (56.3 ± 10.9)
VAD/TL	1.2	1.2–3.8 (1.6 ± 0.9)

All measurements in µm except length in mm

lips. Spear moderately developed about 8.5 µm long with rounded spear knobs measuring about 1.7 µm across and 1.7 µm high. Procorpus wider at anterior part and 35.7–51.0 (41.9 ± 5.5) µm long. Median bulb rounded with indistinct central crescentic valve plates with 11.9–17.0 (15.0 ± 1.8) µm high. Isthmus long, slender wide, 25.5–42.5 (37.1 ± 6.2) µm. Basal bulb elongated, tamarind seed-shaped, 11.9–27.2 (19.5 ± 4.9) µm high. Nerve ring about 61.2–69.7 µm from anterior end of the body. Excretory pore 66.3–96.9 (63.4 ± 28.1) µm long from labial disc of body and about 1.6–2.6 total isthmus length behind base of median bulb, hemizonid anterior to excretory pore, about two annules long. Intestine with anterior wall thicker than rest of body. Rectum a tube bent posterior, one to two anal body widths.

Vulva transverse slit, vagina at right angles to body axis. Ovary single, anteriorly outstretched, oocytes arranged in single row at the anterior part of spermatheca and behind are arranged in double row, spermatheca with sperms. Ovary filled with cells. Uterus thick-walled, elongated, with a posterior branch measuring about one-quarter to two and half of vulval body width long. Tail short, gradually tapering to a rounded tip, 34.0–69.7 (56.3 ± 10.9) µm long, about seven to eight quarter times anal body diameter.

Type host and locality Collected from soil around the rhizospheric regions of *Morus alba* L. (Local name:

Kabrangchaak) from Govt. Silk Farm, Wangbal, Thoubal District, Manipur, India with a latitude of 24.62306 and longitude of 94.00896.

Material examined Type specimen collected on April 2008. Holotype: female on the slide BWT₃/2-*Tylenchus conicaudatus* sp. nov.; paratypes: females mounted on the slides BWT₃/1–6 *Tylenchus conicaudatus* sp. nov. and deposited with nematode collection of Parasitology section, Life Sciences Department, Manipur University, Canchipur, Manipur, India.

Etymology The species was named *Tylenchus conicaudatus* sp. nov. considering the conical shaped tail of the species.

Diagnosis and relationships *Tylenchus conicaudatus* sp. nov. differs from all other species of *Tylenchus* in having short and gradually tapering tail to a rounded tip, elongated basal bulb and uterine sac.

Tylenchus conicaudatus sp. nov. comes close to *Tylenchus neoandrassyi* Geraert and Raski 1987 and *Tylenchus madarpurensis* Sultan et al. 1991.

The species is similar to *Tylenchus neoandrassyi* Geraert and Raski 1987 in having very posterior vulva, but the present species differs in having smaller body and stylet length, smaller value of c', larger value of V and shorter tail (L = 0.55–0.58 mm, c' = 10, V = 53–59 %, stylet = 10 and elongated tail in *T. neoandrassyi* Geraert and Raski 1987). *Tylenchus conicaudatus* sp. nov. is similar to *T. madarpurensis* Sultan et al. 1991 in having finely annulated cuticles and similar range of body and stylet length. But the present species differ from the species in having larger value of a, c, V, VAD/TL; smaller value of b and tail structure (L = 0.41–0.43 mm, a = 31–33, c = 4.0–4.2, V = 52–64, stylet = 8.5–9, VAD/TL = 0.5 and elongated tail in *T. madarpurensis* Sultan et al. 1991).

Telotylenchus manipurensis sp. nov. (Fig. 2)

Dimensions

Refer to Table 2.

Description *Female* Body straight or slightly arcuate upon fixation, tapering gradually towards both extremities, 0.37–0.46 (0.41 ± 0.04) mm in length (Fig. 2; Table 2). Cuticle marked with transverse striae, about 2.2 µm apart. Lateral fields marked with four equidistant longitudinal lines occupying about one-third of body width at a point mid way in body length. The outer lateral lines are non-crenated. Labial region slightly set off, flat apex, marked with 7–8 fine annules. Cephalic framework moderately sclerotised with its outer margins, extending 3–4 body annules posteriorly. Spear 6.8–10.2 µm long with rounded spear knobs. Orifice of dorsal oesophageal gland 5.1 µm long from base of spear. Oesophagous typical of the genus

with procarpus 30.6–35.7 μm long, median oesophageal bulb spheroid with 8.5–13.6 μm high and 4.2–6.8 μm across, with a prominent valvular apparatus, isthmus 11.9–13.6 μm long and basal lobe elongated, 39.1–51 μm long extending laterally over the intestine and absence of post intestinal sac. Hemizonid 2–3 annules wide, 51–54.4 μm long from anterior end. Nerve ring near middle of isthmus, 51–56.1 μm from anterior end, located at 10–12 annules anterior to oesophago-intestinal junction.

Vulva a transverse slit, vagina extending at right angles to body axis and half way into body. Spermatheca rounded, axial filled with sperms. Ovaries asymmetrical,

outstretched with single row of oocytes in each. Anterior gonad is longer than posterior. Spermatheca spherical, packed with sperms. Rectum less than half of total body width long. Tail elongated, gradually tapering into a pointed tip. Phasmids pore-like, located at anterior part of body and 45.9–59.5 μm from tail tip.

Male Not found.

Type host and locality Collected from soil around the rhizospheric regions of *Morus alba* L. from Jiri, Imphal West District, Manipur, India with a latitude of 24.68813 and longitude of 93.14139.

Materials examined Collected on October, 2009. Holotype: female mounted on the slide BP Te/1, *T. manipurensis* sp. nov.; paratypes: females mounted on slides BP Te/2–6. *T. manipurensis* sp. nov. and deposited at Nematode collection of Parasitology Section, Department of Life Sciences, Manipur University, Canchipur.

Etymology The new species is designated *T. manipurensis* sp. nov. considering the fact that the revelation of the species was first record of the genus from Manipur, India.

Diagnosis and relationships *Telotylenchus manipurensis* sp. nov. differs from all other species of *Telotylenchus* Siddiqi 1960 in having shortest body and stylet lengths but having conically elongated tail which is longest than other existing species of *Telotylenchus*.

Fig. 2 *Telotylenchus manipurensis* sp. nov. **a** Entire body, **b** Anterior region, **c** Reproductive system, **d** Tail region and **e** Lateral lines

Table 2 Morphometric data of the females of *Telotylenchus manipurensis* sp. nov

Characters	Holotype	Paratypes
n	1	5
Length	0.46	0.37–0.46 (0.41 $\pm$ 0.04)
a	30.55	24.3–30.5 (27.1 $\pm$ 2.3)
b	4.29	3.5–4.4 (4 $\pm$ 0.3)
c	5	5–6.4 (5.6 $\pm$ 0.5)
c'	9.16	6.8–9.1 (7.9 $\pm$ 0.8)
V	63.6	63.6–71.6 (66.5 $\pm$ 3.1)
G ₁	20	20–31 (26 $\pm$ 3.9)
G ₂	15.27	10.4–15.5 (12.7 $\pm$ 2.6)
Spear	10.2	6.8–10.2 (8 $\pm$ 1.4)
DGO	5.1	5.1
Oesophagus	10.2	6.8–10.2 (8 $\pm$ 1.4)
Nerve ring	52.7	51–56.1 (53.5 $\pm$ 1.9)
Excretory pore	74.8	59.5–56.1 (53.5 $\pm$ 1.9)
Lip width	5.1	5.1
Lip height	2.2	1.7–2.2 (1.8 $\pm$ 0.2)
V–A distance	71.4	54.4–71.4 (61.6 $\pm$ 6.1)
Max. body width	15.3	15.3
ABD	10.2	8.5–10.2 (9.3 $\pm$ 0.8)
Tail	93.5	64.6–93.5 (74.3 $\pm$ 11.2)
Scutellum	59.5	45.9–59.5 (52.7 $\pm$ 5.5)

All measurements in μm except length in mm

Telotylenchus manipurensis sp. nov. comes close to *Telotylenchus areolatus* Baqri and Jairajpuri 1969 and *Telotylenchus impar* Khan and Darekar 1979.

Telotylenchus manipurensis sp. nov. differs from *T. areolatus* Baqri and Jairajpuri 1969 in having shorter body and stylet length, smaller value of *c*, larger value $\acute{c}$ and *V*, non - crenated outer incisures and conically elongated tail with smooth terminus ($L = 0.71\text{--}0.80$, $c = 15\text{--}19$, $\acute{c} = 2.9\text{--}3.7$, $V = 52\text{--}55$, crenated outer incisures and bluntly pointed tail with smooth terminus in *T. areolatus* Baqri and Jairajpuri 1969). From *T. impar* Khan and Darekar 1979, the present species differs in having shorter body and stylet length; smaller *c* value; greater value of $\acute{c}$ and *V* and conically elongated tail with smooth terminus ($L = 0.90\text{--}1.0$, $c = 15\text{--}18$, $\acute{c} = 4\text{--}5$, $V = 52\text{--}54$, stylet = 15–17, sub hemispherical tail with smooth terminus in *T. impar* Khan and Darekar 1979).

The presence of the species is the first record for its occurrence from Manipur, North East India.

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