Aphelenchoides manipurensis sp. nov. (Nematoda: Tylenchina) from Manipur, India

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Abstract *Aphelenchoides manipurensis* sp. nov. was collected from two different localities of Manipur. Though the specimens collected from two different areas show some variations in their morphology and morphometric data, it was taken as the same species due to similarities in many characters. *A. manipurensis* sp. nov. had females with $L = 294.1\text{--}461.91\text{ }\mu\text{m}$, $a = 24.28\text{--}38.14$, $b = 6.3\text{--}8.65$, $b' = 3.61\text{--}4.68$, $c = 12.47\text{--}16.46$, $c' = 3\text{--}4.25$, stylet = $10.38\text{--}13.84\text{ }\mu\text{m}$, $V = 68.88\text{--}71.69$, post uterine sac = $25.98\text{--}53.63\text{ }\mu\text{m}$ long and two lateral lines. Males were common with spicule of $10.38\text{--}17.3\text{ }\mu\text{m}$. Mucro were present in both sexes. Species presented with illustrations and morphometrics.

Keywords *Aphelenchoides manipurensis* sp. nov. · Variations · Manipur and India

Introduction

Species of *Aphelenchoides* Fisher, 1894 are known to occur throughout the world which includes more than 135 species. There are more than 45 species in India alone. A survey for aphelenchid nematodes in Manipur revealed several different species of the genus *Aphelenchoides* Fisher, 1894. This included one new species of the genus collected from rotten tree trunk from different localities.

Materials and methods

The nematodes were extracted from cut wood fragments of rotten tree trunk by Baermann funnel technique. Collected nematodes were fixed in TAF (triethanolamine, formaldehyde and water) and processed by glycerol–ethanol method of Seinhorst (1959). Specimens were mounted in dehydrated glycerine. After slide preparation, measurements were taken using an ocular micrometer and diagrams drawn under a camera lucida.

Results

Aphelenchoides manipurensis sp. nov.

Female

Body cylindrical, ventrally arcuate upon fixation, $294.1\text{--}461.91$ (362.14 ± 54.93) mm long with a maximum body width of $10.38\text{--}13.84$ (11.25 ± 0.86) μm , sometimes anterior body is slender while posterior body is robust with a gradually pointed tail terminus bearing small mucro. Head set off from the body and flat, with a maximum lip width of $5.19\text{ }\mu\text{m}$ and height of $3.46\text{ }\mu\text{m}$, slightly convex rather than straight. Cuticle marked by fine annules about $0.85\text{ }\mu\text{m}$ wide. Lateral field with two incisures about 1/7 as wide as body, stylet short $10.38\text{--}13.84$ (11.53 ± 1.99) μm long with small rounded basal knobs. Median bulb spherical, valves are centrally situated $8.65\text{--}10.38$ (10.09 ± 0.50) μm high and $6.92\text{ }\mu\text{m}$ across. Basal bulb bifurcated into two, widely placed near to body wall, $38.06\text{--}51.9$ (41.52 ± 3.46) μm long. The structure of basal bulb is characteristic of the species. Excretory pore situated

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dimly at posterior base of hind tip of end bulb, 51.9–76.12 (64.01 ± 12.11) μm long from anterior end of body.

Nerve ring behind median bulb, 43.25–60.55 (51.9 ± 8.65) μm from anterior end of body. Ovary single, outstretched, oocytes arranged in single rows, 93.42–121.1 (107.83 ± 0.50) μm long, reaching behind the end bulb. Spermatheca elongated oval filled with sperms, 8.65 μm long. Post vulval uterine sac in some species serve as reservoir of sperms that gradually ends in a rounded tip on the ventral side while in some other species, post vulval uterine sac gradually arcuated ending into a pointed tip at the dorsal side, 25.98–53.63 (36.61 ± 13.98) μm long that is about five times vulval body diameter. Vulva protuberant, flap absent. Vagina transverse, right angle to the body. Tail curved gradually on ventral side ending into a rounded tip, 20.76–31.14 (27.37 ± 2.01) μm long with a small mucro at tip. Hemizonid, deirids and phasmids indistinct.

Male

Body ventrally curved upon fixation. Cuticle, head, excretory pore, median bulb, stylet and basal bulb same as female. Tail conoid, rounded with mucronated tip. Testis 138.4–190.3 (168.67 ± 22.47) μm long. Spermatocytes mostly in single row bent at towards the tip. Spicules are simply arcuate, 10.38–17.3 (15.28 ± 3.49) μm long, rostrum rounded and apex prominent. Three pairs of sub ventral papillae present towards tip of spicule. Bursa and gubernaculum absent.

Type host and locality

Rotten wood of unidentified trees collected from Manipur University campus, Canchipur, Manipur, India with a latitude of $24^{\circ}45'8''\text{N}$ and longitude $93^{\circ}55'40''\text{E}$.

Fig. 1 *Aphelenchoides manipurensis* sp. nov., *Camera lucida* drawings. **a, c–f**—Female; **a**—Female entire body; **c**—Female anterior body; **d** and **I**—Reproductive system (variations); **e**—Body incisures;

f—Anal region; **b, g–h**—Male; **b**—Male entire body; **g**—Male anterior body region and **h**—Male posterior body

Other host and locality

Cone of *Pinus kesiya* Royle from Nongpok Sekmai Pine Forest, Thoubal District, Manipur, India with a latitude of 24°80'59928"N and longitude of 93°94'79816"E.

Type specimen

Collected on 10th June, 2013 from Manipur University campus and 12th June, 2013 from Nongpok Sekmai. Holotype female on slide BFYS–A₂♀-1/*Aphelenchoides manipurensis* sp. nov., paratype females on slides BFYS–A₂♀-2-12/*Aphelenchoides manipurensis* sp. nov.; BFYS–A₁♀-2-12/*Aphelenchoides manipurensis* sp. nov. and paratype males on slides BFYS–A₂♂-1-6/*Aphelenchoides manipurensis* sp. nov. and BFYS–A₁♂-1-6/*Aphelenchoides manipurensis* sp. nov. The slides are deposited in nematode collection centre of Parasitology Section, Centre of Advanced Study in Life Sciences, Manipur University, Canchipur, Manipur, India (Fig. 1).

Diagnosis and relationships

Aphelenchoides manipurensis sp. nov. is characterised by having short but robust body, ventrally curved upon fixation. Oesophageal gland with basal bulbs bifurcated widely situated very near to the body wall, muscular median bulb, ovary very short with functional uterine sac containing sperms, elongated rose–thorn shaped spicule and a ventrally curved tail to rounded tip with a small mucro (Table 1).

Aphelenchoides manipurensis sp. nov. comes close to *Aphelenchoides trivialis* Franklin & Siddiqi 1963; *Aphelenchoides sacchari* Hooper, 1958; *Aphelenchoides obtusus* Thorne & Malek 1968 and *Aphelenchoides clarus* Thorne & Malek, 1968. A comparative morphometric data among other closely related species of *Aphelenchoides* are given in Table 2.

Aphelenchoides manipurensis sp. nov. comes close to *Aphelenchoides trivialis* Franklin & Siddiqi, 1963 in having similar range of stylet length with ventrally concave

Table 1 Morphometric data of *Aphelenchoides manipurensis* sp. nov.

Characters	Holotype female	Paratype females	Paratype males
N	1	24	13
Stylet	10.38	10.38–13.84 (11.53 ± 1.99)	10.38–13.84 (11.53 ± 1.99)
Length	294.10	294.1–461.91 (362.14 ± 54.93)	264.69–320.05 (289.85 ± 22.63)
a	24.28	24.28–38.14 (32.02 ± 1.92)	25.5–33.39 (28.62 ± 0.72)
b	8.15	6.3–8.65 (7.56 ± 0.39)	5.59–41.52 (28.59 ± 18.93)
b'	3.61	3.61–8.15 (4.21 ± 0.45)	3.47–4.02 (3.93 ± 0.13)
c	12.47	12.47–16.46 (14.38 ± 0.92)	12.0–13.91 (13.33 ± 0.58)
c'	3	3–4.25 (3.41 ± 0.19)	2.4–6 (2.7 ± 0.3)
G ₁	31.76	24.34–33.01 (31.79 ± 6.02)	–
V	69.41	68.88–71.69 (69.81 ± 0.35)	–
T	–	–	50.89–61.09 (54.62 ± 4.31)
PVS/V–A%	77.5	25.43–77.5 (43.92 ± 25.73)	–
Max. body width	12.11	10.38–13.84 (11.25 ± 0.86)	8.65–12.11 (10.66 ± 1.0)
Lip height	3.46	3.46	3.46
Lip diam.	5.19	3.46–5.19 (4.03 ± 1.0)	3.46–5.19 (4.03 ± 1.0)
Median bulb length	8.65	8.65–10.38 (10.09 ± 0.50)	8.65–10.38 (9.51 ± 0.86)
Median bulb diam.	6.92	6.92	5.19–6.92 (6.05 ± 0.86)
Ovary	93.42	93.42–121.1 (107.83 ± 0.50)	–
Testis	–	–	138.4–190.3 (168.67 ± 22.47)
V–A/Tail	–	–	–
PUS	53.63	25.98–53.63 (36.61 ± 13.98)	–
PUS/VBD	5.16	2.14–5.16 (3.26 ± 1.58)	–
Spicule	–	–	10.38–17.3 (15.28 ± 3.49)
Tail	20.76	20.76–31.14 (27.37 ± 2.01)	20.76–25.95 (24.22 ± 3)
Rectum	6.92	6.92–8.65 (7.49 ± 1)	6.92–8.65 (7.78 ± 0.86)
ABD	6.92	6.92–8.65 (8.07 ± 1)	6.92–8.65 (7.78 ± 0.86)

All measurements in µm in the form: mean ± SD (range)

Table 2 Morphometric characteristics differentiating related *Aphelenchoides* species from *Aphelenchoides manipurensis* sp.nov.

Species	Length	a-value	Tail	Stylet	Spicule	Lateral lines	Tail & mucro
<i>A. bicaudatus</i> (Imamura 1931) Filipjev & Stekhoven 1941	380–470	31.3–31.7	50	10–14	14	2	Cylindrical-bifurcated
<i>A. blastophthorus</i> Franklin 1952	680–900	32–47	42–48	14–19	24–31	4	Conoid–central
<i>A. clarus</i> Thorne & Malek 1968	300	21		11		2	Conoid-apical
<i>A. dalianensis</i> Wei et al. 2009	436.8–658	26.3–33.8	29.3–39.2	10–12.7	10–12.9	4	Conoid-ventral
<i>A. ensete</i> Swart et al. 2000	558–754	36.9–61.8	34–43.5	12–14	17–20	4	Conoid–ventral
<i>A. hainanensis</i> (Rahm 1938) Goodey 1951	900–1300	42.4–46.1	–	10.5–11.3	17.5–19		
<i>A. helophilus</i> (De Man 1880) Goodey 1963	800–1300	43–78		12	26	2	Conoid–central
<i>A. macronuclaetus</i> Baranovskay 1963	630–740	34–36.3	40	11–12			
<i>A. paranechaleos</i> Hooper & Ibrahim 1994	630–870	37–46	35–40	9.5–10.5	15–18	4–6	Conoid-pointed
<i>A. parasaprophilus</i> Sanwal 1965	630–750	34–37	45.7	11.7	21	4	Conoid-pointed
<i>A. sacchari</i> Hooper 1958	800	26	–	12	–	3	Conoid-ventral
<i>A. subternuis</i> (Cobb 1926) Stenner & Buhrere 1932	870–1500	–	–	11–13	18–22	3	Conoid-ventral
<i>A. vauhani</i> Maslen 1979	365–435	24–31	23.5–38.5	9.5–11	–	–	–
<i>A. vigor</i> Thorne & Malek 1968	900	33	–	12–15	18	2	Conoid-ventral
<i>A. meghalayensis</i> Bina & Mohilal 2016	493–681	30.7–40.7	28.24–44.96	8.65–10.3	19.03	6	Concave–ventral
<i>A. nachaleos</i> Hooper & Ibrahim 1994	605–931	33–45	–	10.5–11.5	13–17	4–6	Convex-conoid terminal
<i>A. neoechinocaudatus</i> Chanu et al. 2014	530–600	25.5–28.6	45.9–54.4	11.9	–	4	Pointed–central
<i>A. longistylus</i> Chanu & Mohial 1994	590–660	34.4–35.8	41.52–48.44	24.22	24.22	4	Cylindroid–ventral
<i>A. neominoris</i> Chanu & Mohial 1994	351–439	36.29–40.6	12.11–25.95	8.65	–	4	Conoid-central
<i>A. aeralis</i> Chanu et al. 2014	460–510	32.1–33.8	42.5–48.2	15.3	23.8–25.9	2	Conoid–central
<i>Aphelenchoides manipurensis</i> sp. nov.	294.1–461.9	24.28–38.14	20.76–31.14	10.38–13.84	10.38–17.3	2	Conoid–ventral

All measurements in μm except length in mm

tail, tapering to rounded tip bearing a small, simple mucro. But the species differs from *Aphelenchoides trivialis* Franklin & Malek, 1968 in having smaller body length, greater value of a, smaller value of b and c, greater tail,

very short ovary, filled post uterine sac and having two lateral lines ($L = 369\text{--}497$, $a = 19\text{--}26$, $b = 9\text{--}11$, $c = 17\text{--}21$, tail = $2\text{--}2\frac{1}{2}$ ABD, very long ovary reaching to the oesophageal region. Post vulval sac having no contents

and three lateral lines in *Aphelenchoides trivialis* Franklin & Malek, 1968).

Aphelenchoides manipurensis sp. nov. is similar to *Aphelenchoides sacchari* Hooper, 1958 in having set off lip region, posterior uterine branch reaching more than half way to anus, serving as reservoir for many sperms and conoid tail, abruptly rounded bearing a mucro. But *Aphelenchoides manipurensis* sp. nov. differs from *Aphelenchoides sacchari* Hooper, 1958 in having smaller body length, lesser body wide in female, greater oesophageal length in female, greater tail and smaller stylet length, lateral fields with two incisures, lesser vulva–anus distance, indistinct papillae and ovary at middle of body length behind basal bulb ($L = 0.8$ mm, $a = 26$, $b = 6.7$, $c = 15$, stylet = 12 μ m, lateral fields with three incisures, $V-A = 5ABD$ versus $V-A = 2.52-3.33$ (present species) and presence of three pairs of submedian papillae and ovary reaching up to almost bulb in *Aphelenchoides sacchari* Hooper, 1958).

Aphelenchoides manipurensis sp. nov. is similar to *Aphelenchoides obtusus* Thorne & Malek, 1968 in having set off lip region, similar range of stylet with small rounded knobs, post uterine branch serving as spermatheca and rounded tail. But the present species differs from *Aphelenchoides obtusus* Thorne & Malek, 1968 in having lesser body length in both male and female, greater body width, lesser oesophageal length; longer tail in female, rounded tail with a mucro and two lateral lines ($L = 0.46$ mm♀, 0.43 mm♂; $a = 26$, $b = 6.4$, $c = 15$, rounded tail without mucro and four lateral lines in *Aphelenchoides obtusus* Thorne & Malek, 1968).

Aphelenchoides manipurensis sp. nov. is similar to *Aphelenchoides clarus* Thorne & Malek, 1968 in having similar range of body, posterior uterine branch with spermatozoa, similar stylet range, ovary arranged in single row and tail terminus rounded with mucro. But *Aphelenchoides manipurensis* sp. nov. differs from *Aphelenchoides clarus* Thorne & Malek, 1968 in having lesser body wide, greater oesophageal length in females, greater tail length, indistinct phasmids and two lateral lines more anterior vulva ($a = 21$, $b = 6.2$, $c = 19$, phasmids almost terminal located sublaterally and a plain lateral line and $V = 67$ in *Aphelenchoides clarus* Thorne & Malek, 1968).

Aphelenchoides manipurensis sp. nov. comes close to *Aphelenchoides vigor* Thorne & Malek, 1968 in having set off lip region, small stylet knobs, posterior uterine branch filled with sperms and tail with blunt tip. But the present species differs from *Aphelenchoides vigor* Thorne & Malek, 1968 in having shorter body length in both male and female, greater oesophagus, longer tail in both male and female, more anterior vulva, greater length of ovary, shorter stylet, presence of two lateral lines, non-sclerotised vulva without flap, indistinct papillae, longer testis and

shorter spicule ($L = 0.9$ mm♀, 0.72 mm♂; $b' = 9.2$ ♀, 8.2 ♂; $c = 22$ ♀, 16 ♂; $V = 63$, stylet = 12–15 μ m; lateral field a plain band, sclerotised vulva with jointed flaps, male with three pairs of papillae, $T = 54$ and spicule = 18 μ m in *Aphelenchoides vigor* Thorne & Malek, 1968).

A comparative study on the morphometric details of the present species with other related species of *Aphelenchoides* is given in Table 2.

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Authors contributions Loukrakpam Bina Chanu conducted survey, extracted nematodes from the samples, identified and compared the nematodes. Naorem Mohilal identified and compared the nematodes with other existing group and help in compiling the manuscript.

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