Aphelenchoides meghalayensis sp. nov. (Nematoda: Aphelenchoididae) from Meghalaya, India

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Abstract During a survey for the aphelenchid nematodes of North Eastern States of India, a new species of *Aphelenchoides* nematode was found from Cherrapunji, Meghalaya. The morphological and morphometric characteristics of the species were studied using light and SEM microscopy. *Aphelenchoides meghalayensis* sp. nov. is characterized by having 493.03–681.62 (604.0 ± 80.52) µm long body, strong stylet of 8.65–10.38 (9.8 ± 0.81) µm long with small basal swellings, six lateral lines, elongated uterine sac and tail which is slightly arcuate, gradually become cylindroids and bluntly pointed, 28.24–44.96 (37.09 ± 6.86) µm long with a strong and elongated curved mucro situated ventrally.

Keywords Aphelenchoididae · *Aphelenchoides* · Cherrapunji · Meghalaya · India

Introduction

A survey for the species of Aphelenchoididae nematode in the North Eastern states of India was carried out during 2014. In one of such collections, the presence of an *Aphelenchoides* Fischer 1894 nematode species from the

unidentified grasses of Thangkharang Park, Cherrapunji, Meghalaya was encountered. Detailed morphological and morphometric data studies indicated the nematode as new to science and it is named as *Aphelenchoides meghalayensis* sp. nov. The species is described and illustrated here supported with photographs.

Materials and methods

The samples were collected and processed through Cobb's (1919) decanting and sieving method followed by modified Baermann's 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 glycerin. The specimens that were mounted in permanent slides were used for morphological and morphometric observations under an ocular micrometer fixed on Nikon, Trinocular Research Microscope, model (ECLIPSE E200) and diagrams were drawn under a drawing tube attached to the same microscope. Light photographs were taken using digital camera.

For Scanning electron microscopy images, adult females and males were collected; heat killed, placed in 3 % glutaraldehyde for 4 h and washed in 0.1 M Sodium Cacodylate Buffer for three changes at 4 °C. Then the specimens are post fixed in 1 % Osmium Tetroxide in 0.1 M Sodium Cacodylate Buffer and again washed in 0.1 M Sodium Cacodylate Buffer for three changes at 4 °C. Specimens were dehydrated up to 100 % acetone and immersed in Tetra Methyl Silane for 5–10 for two changes at 4 °C. Then they were brought to room temperature (25–26 °C) to dry. Coating was carried out using Gold up to about 35 nm thick and observed with a JEOL JSM 6360 at 20 kv.

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Results and discussion

Aphelenchoides meghalayensis sp. nov. (Figs. 1, 2 and 3).

Dimensions

Table 1.

Female

Body slender, cylindrical, slightly arcuate ventrally upon fixation, 493.03–681.62 (604.0 ± 80.52) μm long. Cuticle roughly annulated under SEM microscope, 0.7–0.9 μm wide near mid-body. Lateral fields one and half times maximum body widths, with six lateral lines throughout the body length under light and SEM microscopy. Cephalic

region high, well set off from the body with 6–7 annuli, 5.19 μm wide, about twice as wide as high, outer margins convex in lateral view. Well defined labial disc demarcated by a groove seen in *en face* view with SEM. Oral aperture surrounded by six small inner labial papillae. Amphidial apertures not visible. Six outer elongated labial papillae located external to oral disc. Labial cuticularisation rough. Stylet strong, 8.65–10.38 (9.8 ± 0.81) μm long, with small basal swellings, guiding ring encircle the conical shaft.

Oesophagus typically aphelenchoid, the glands in a lobe overlapping the intestine dorsally or dorso—laterally. Procorpus long and broad throughout the body length, about 46.71–51.9 (48.44 ± 2.44) μm long from the anterior body. Medianoesophageal bulb rounded to slightly oval with the refractive thickenings usually placed centrally, 12.11–13.84 (12.68 ± 0.81) μm high and

Fig. 1 *A. meghalayensis* sp. nov., camera lucida drawings. **a** Female entire body, **b** female anterior body, **c** female reproductive system, **d** female tail region, **e** lateral lines, **f** male entire body, **g** male anterior body and **h** male tail region

Fig. 2 *A. meghalayensis* sp. nov., light microscopy photographs. **a** Female anterior body, **b** female vulval region, **c** female tail region, **d** female gonadal tip, **e** spermatheca, **f** male tail region and **g** male tail tip

6.92–10.38 (8.65 ± 1.41) μm across. Nerve ring behind the median bulb at around 64.01–69.2 (66.87 ± 2.15) μm from the anterior end of the body. Excretory pore behind nerve ring at about 64.01–69.2 (66.87 ± 2.15) μm from the anterior end of the body.

Female reproductive system monoprodelphic, vulva a transverse slit with weakly protuberant lips. Ovary with oocytes arranged in irregular double row with nuclei. Spermatheca elongated spherical filled with sperms. Post uterine sac prominent with a length of 34.6–51.9 (43.25 ± 7.06) μm or 27.17–35.42 (31.12 ± 3.16) % of distance from vulva to anus which is 22.76–32.18 (28.24 ± 3.78) μm in length. Rectum about 20.76 μm in length.

Tails are slightly arcuate, gradually cylindroid, bluntly pointed, 28.24–44.96 (37.09 ± 6.86) μm long with a strong and elongated curved ventral mucro.

Male

Body showed open J-shaped upon fixation. General morphology and *en face* view as in female, except for sexual dimorphism. Lateral region with six incisures forming three ridges. Spicule large, 19.05 μm long, characteristic rose- thorn shaped. Rostrum and apex well developed, rostrum high. Bursa absent. One caudal papillae present.

Type habitat and locality

Collected in May, 2014 from rhizospheric regions of unidentified grasses from Thangkharang Park, Cherrapunji, Mawmai-Nongthymmai, Meghalaya with a latitude of 25°15'N, longitude of 91.44°E and altitude of 1300 m.

Fig. 3 *A. meghalayensis* sp. nov., Scanning electron microscopy images. **a** *En-face* view of mouth, **b** closer *en-face* view of mouth, **c** vulval opening, **d** lateral lines, **e** tail tip showing mucro and **f** tail region of female

Type materials

Holotype female on the slide FTY5♀- 1/*A. meghalayensis* sp. nov., paratype females on the slides FTY5♀- 2- 12/*A. meghalayensis* sp. nov. and paratype males on the slides FTY5♂- 1- 7/*A. meghalayensis* sp. nov. and deposited in the nematode collection of Parasitology Section, Centre of Advanced Study in Life Sciences, Manipur University, Canchipur—795003, Manipur, India.

Diagnosis and relationship

A. meghalayensis sp. nov. is characterized by having 493.03–681.62 (604.0 ± 80.52) mm long body which is to be considered as short body length, strong stylet of 8.65–10.38 (9.8 ± 0.81) µm long with small basal

swellings, six lateral lines, elongated uterine sac and tail slightly arcuate, gradually cylindroid, bluntly pointed, 28.24–44.96 (37.09 ± 6.86) µm long with a strong and elongated curved mucro situated ventrally.

A. meghalayensis sp. nov. can be easily distinguished from similar species by the following characters—short body with long stylet and indistinct basal swellings, gradually—cylindroid tail with a blunt pointed terminus bearing a single curved mucro situated ventrally and presence of six lateral lines in the lateral field throughout the body.

When Sanwal (1961) published the first identification key to the species of *Aphelenchoides*, *A. subtenuis* and *A. martini* Rühm, 1955 were the only species known with a single mucro situated ventrally and terminally respectively. However, the latter species was transferred to a separate genus *Ruehmaphelenchus* by Goodey (1963). Since 1961,

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

Characters	Holotype female	Paratype females	Paratype males
n	1	12	7
Length	637.37	493.03–681.62 (604.0 ± 80.52)	595.12–615.88 (576.09 ± 39.79)
a	30.7	30.70–40.71 (34.74 ± 4.30)	17.3–21.37 (19.34 ± 1.43)
b'	5.94	4.91–5.94 (5.86 ± 0.07)	5.54–7.32 (6.37 ± 0.73)
b ₁	10.52	8.38–10.64 (9.85 ± 1.04)	8.82–9.34 (9.12 ± 0.22)
c	3.66	2.72–4.33 (3.57 ± 0.66)	13.76–15.21 (14.59 ± 0.54)
c'	3.66	2.72–4.33 (3.57 ± 0.66)	4.166–7.53 (5.64 ± 1.40)
V	69.054	69.05–94.27 (85.57 ± 11.68)	–
T	–	–	92.73–102.21 (97.56 ± 3.43)
G ₁	33.65	2.72–4.33 (3.57 ± 0.66)	–
Post uterine sac	43.25	34.6–51.9 (43.25 ± 7.06)	–
PVS/V-A %	27.173	27.173–35.42 (31.12 ± 3.165)	–
Oesophagous	107.26	100.34–117.64 (108.41 ± 7.10)	107.26–122.93 (115.52 ± 6.74)
Stylet	10.38	8.65–10.38 (9.80 ± 0.81)	8.65
Lip width	5.19	5.19	5.19
Lip height	3.46	3.46	3.46
Median bulb length	13.84	12.11–13.84 (12.68 ± 0.81)	6.92
Median bulb diam	8.65	6.92–10.38 (8.65 ± 1.41)	13.84
Ovary	214.52	184.72–234.02 (211.08 ± 0.81)	–
PUS/VBD	2.27	1.81–2.27 (2.02 ± 0.18)	–
Nerve ring	69.2	64.01–69.2 (66.87 ± 2.15)	68.45–74.39 (71.41 ± 2.25)
Excretory pore	74.39	69.2–74.39 (71.98 ± 2.13)	58.45–65.74 (61.58 ± 2.83)
Spermatheca	–	–	–
Rectum	20.76	20.76	15.57–20.76 (18.04 ± 1.89)
Spicule	–	–	19.03
Tail	38.06	28.24–44.96 (37.09 ± 6.86)	31.14–43.25 (35.63 ± 4.47)
ABD	10.38	10.38	8.65–12.11 (11.10 ± 1.74)

All measurements in µm

more species possessing a single mucro on the tail tip have been described, but the shape of the mucro, its position and the tail shape of the present species is highly different from them.

On the other hand, nematodes in *Aphelenchoides* were separated into four groups based on the shape of their tails (Shahina 1996). Nematodes of group 1 have simple tails without any outgrowth or mucronated structure. Group 2 nematode has tail with one or sometimes two mucronate structures on tail terminus. Group 3 has tail with spine or star-shaped terminus while group 4 has tail outgrowth other than spine or starred. Based on this the present species belonged to group 2.

Among the members of this group, *A. meghalayensis* sp. nov. is very close to *Aphelenchoides blastophthorus* Franklin 1952; *A. helophilus* (De Man 1880) Goodey 1933; *Aphelenchoides scalacaudatus* Sudokova, 1958; *Aphelenchoides suipingensis* Feng and Li 1986 and *Aphelenchoides submersus* Truskova 1973 in the tail shape.

The present species differs from *A. blastophthorus* Franklin 1952 in having smaller body length, smaller value of 'a', 'c', larger value of 'c' smaller stylet length, shorter tail, spicule, more lateral lines and cylindroid blunt tail (L = 680–900, a = 32–47, c = 16–21, c' = 3.3, Tail = 42–48, Stylet = 14–19, spicule = 24–31, lateral lines = 4 and conoid-central tail in *A. blastophthorus*).

From *Aphelenchoides helophilus* (De Man 1880) Goodey, 1933; it differs in smaller body length, smaller values of 'a', 'c', 'c'', stylet length, longer spicule and cylindroids blunt tail (L = 800–1300, a = 43–78, c = 14–20, c' = 5.5, stylet = 12, spicule = 26 and conoid central tail in *A. helophilus*).

Again, it differs from *A. scalacaudatus* Sudokova 1958; in having shorter body length, 'c', larger c', shorter tail length, stylet, spicule and cylindroids blunt tail (L = 700–900, a = 32–38.9, c' = 2.5, Tail = 51, stylet = 15, spicule = 22 and conoid central tail in *A. scalacaudatus*).

Table 2 Key morphometric differences of *A. meghalayensis* sp. nov. from other related species of *Aphelenchoides*

<i>Aphelenchoides</i> spp.	Group	Length	a-value	Tail	Stylet	Spicule
<i>A. baguei</i> Maslen (1979)	3	560–765	28–38	42–54	11.5–13	16–25
<i>A. bicaudatus</i> (Imamura 1931) Filipjev and Schuurmans Stekhoven (1941)	2	380–470	31.3–31.7	50	10–12	14
<i>A. blastophthorus</i> Franklin, (1952)	2	680–900	32–47	42–48	14–19	24–31
<i>A. clarus</i> Thorne and Malek (1968)	2	300	21	–	–	–
<i>A. composticola</i> Franklin (1957)	4	440–610	30–42	35.7	11	21
<i>A. dalianensis</i> Wei et al. (2009)	2	436.8–658	26.3–33.8	25.1–31.1	10.0–12.7	10.0–12.9
<i>A. ensete</i> Swart, Bogale and Tiedt (2000)	2	558–754	36.9–61.8	34–43.5	12–14	17–20
<i>A. goodeyi</i> Siddiqi and Franklin (1967)	3	460–610	29–39	–	11.5–12.5	–
<i>A. hainanensis</i> (Rahm 1938) T. Goodey (1951)	2	900–1300	42.4–46.1	–	10.5–11.3	17.5–19.0
<i>A. helicosoma</i> Maslen (1979)	1	525–915	25–32	26.5–49	13–15.5	21.5–27
<i>A. helophilus</i> (De Man 1880) Goodey (1963)	2	800–1300	43–78	–	12	26
<i>A. kungradensis</i> Karimova (1957)	4	650	37.6	36.7	10.7	–
<i>A. lilium</i> Yokoo (1964)	4	640–750	25.8–34.5	–	12.5	17.5
<i>A. macronucleatus</i> Baranovskaya (1963)	2	630–740	34–36.3	40	11–12	–
<i>A. microstylus</i> Kaisa (2000)	1	370–485	25.4–48.5	–	6–7.5	–
<i>A. nechaleos</i> Hooper and Ibrahim (1944)	4	600–930	33–45	42.5–50	10.5–11.5	–
<i>A. paranechaleos</i> Hooper and Ibrahim (1944)		630–860	37–46	35–40	9.5–10.5	15–18
<i>A. parasaprophilus</i> Sanwal (1965)	2	630–750	34–37	45.7	11.7	21
<i>A. sacchari</i> Hooper (1958)	2	800	26	–	–	–
<i>A. scalacaudatus</i> Sudokova (1958)	4	700–900	32.0–38.9	51	15	22
<i>A. saipingensis</i> Feng and Li (1986)	4	640–980	27.3–38.2	60–62	11.8–13	23–26
<i>A. subternuis</i> (Cobb 1926) Steiner and Buhrere (1931)	2	8.70–1.50	–	–	–	–
<i>A. submersus</i> Truskova (1973)	4	490–720	30.8–48.5	–	13	–
<i>A. tsalolikhini</i> Ryss (1993)	4	540–640	38–42	43	11–12	18–19
<i>A. vughani</i> Maslen (1979)	2	365–435	24–31	23.5–38.5	9.5–11	–
<i>A. vigor</i> Thorne and Malek (1968)	2	900	33	–	–	–

All measurements in μm

From *Aphelenchoides suipingensis* Feng and Li 1986, the present species differs in having shorter body length, smaller ‘c’, shorter tail length, stylet, spicule and 6 lateral lines ($L = 640\text{--}980$, $c = 14.6\text{--}21$, Tail = $60\text{--}62$, stylet = $11.8\text{--}13$, spicule = $23\text{--}26$ and 4 lateral lines in *A. suipingensis*).

And from *Aphelenchoides submersus* Truskova 1973; it differs in having smaller value of ‘c’, stylet, six lateral lines and cylindroids blunt tail ($c = 15.7\text{--}17.5$, stylet = 13, 4 lateral lines and conoid central tail in *A. submersus*).

In addition, *A. meghalayensis* sp. nov. differs from them in the shape of tail, stylet and spicule lengths, in the shape of mucro and number of lateral lines.

The morphometric differences of the present species with other closely related species are provided in Table 2.

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