Aphelenchus assamensis sp. nov. (Nematoda: Aphelenchida: Aphelenchoidea: Aphelenchidae) from Assam, North East India

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Abstract During a survey for the aphelenchid nematodes of North Eastern States of India, a new species of *Aphelenchus* nematode was found from Kahilipara, Assam associating with the decaying plant materials of *Areca catechu*. The morphological and morphometric characteristics of the species were studied using light and Scanning Electron microscopy. *Aphelenchus assamensis* sp. nov. is characterized by having 700.65–776.77 (733.30 ± 28.85) μm long body, stylet 12.11 μm long without basal swellings, two lateral lines, elongated narrow uterine sac and a cylindrical 20.76–36.33 (27.03 ± 5.03) μm long tail or about 1.36–2.37 (1.94 ± 0.43) times anal body widths long.

Keywords *Aphelenchus assamensis* sp. nov. · *Areca catechu* · Assam · North East India

Introduction

Aphelenchus Bastian 1865 contains about 30 nominal species of which very few are adequately characterized to allow reliable identification. Anderson and Hooper (1980), having studied various populations, erected the subgenus *Anaphelenchus* on the basis of differences in the shape or proportions of the vagina. *Aphelenchus* is characterized by having a of unequal proportions (=anisomorphic) whereas

Aphelenchus (*Anaphelenchus*) has a vagina with equal development (=isomorphic). However, this proposal has not met with general acceptance and Ebsary (1991) synonymised the subgenus. The nematode group is widespread in temperate and tropical soils. Males are only present in some populations usually those from warmer soils. The nematodes are mycetopgous, but are often found in damaged or decaying plant materials where they presumably feed on fungal hyphae.

During a survey for the aphelenchid nematodes in the North Eastern states of India, an *Aphelenchus* nematode was found associated with the decaying plants materials of Tamul plant, *Areca catechu* from Odalpara region of Kahilipara, Kamrup District, Assam. Studies on the available literatures on *Aphelenchus* Bastian 1865 revealed the present species as new in comparison with those existing species. The morphometric data and morphology of the nematode were studied using light microscopy and Scanning Electron Microscopy.

Materials and methods

The samples were collected and processed through Cobb's (1918) 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 and sealing done with Dextrin plasticized xylene. 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

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Fig. 1 Line drawings of *A. assamensis* sp. nov.

same microscope. Light photographs were taken using digital camera.

For Scanning electron microscopy observation, 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 3 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.

Fig. 2 Light photomicrographs of *A. assamensis* sp. nov.

Results and discussions

Aphelenchus assamensis sp. nov.

Figures 1 and 2.

Dimensions

Table 1.

Descriptions

Female

Medium sized body, 700.65–776.77 (733.30 ± 28.85) μm long. Linear to ventrally arcuate upon fixation. Cephalic region low, flattened and slightly offset, 6.92–8.64 (8.21 ± 0.749) μm wide and 3.46–5.12 (4.75 ± 0.74) μm high. SEM studies shows 5 annuli in the cephalic region. Body cuticle coarsely annulated, 1.7 μm wide at mid body width. Lateral fields each with a single ridge (2 incisures) running throughout the length of the body. Stylet slender, 12.11 μm long without basal swellings. Procorpus cylindrical, 57.09–65.64 (64.20 ± 10.06) μm long from the anterior end of the body, somewhat narrowing just before joining the large muscular, ovoid median bulb which is 12.11–15.57 (13.40 ± 1.67) μm wide and 19.03–20.76 (19.67 ± 0.83) μm long with centrally placed, crescentic

valve plates. Oesophageal glands on a dorsal lobe, oesophago-intestinal junction at 98.61–152.24 (115.42 ± 15.79) μm from the anterior cephalic region. Nerve ring behind the median bulb at 108.99–124.56 (116.31 ± 5.08) μm from the anterior end of the cephalic region. Excretory pore above the nerve ring, at 86.5–100.34 (93.11 ± 4.63) μm from the cephalic end. Vulva posterior at about 70–75 % of the body length. Vulval lips slightly protuberant and body often narrowing sharply just behind the vulva. Vagina sloping anteriorly. Genital tract monoprodelphic, outstretched, spermatheca 68.4–78.2 (74.38 ± 4.28) μm long, not fully segmented, filled with sperms, oocytes arranged in single row, tip of the oocytes sometimes reaching the base of the end bulb. Post uterine sac narrow elongated 51.9–83.04 (67.80 ± 11.54) μm long (Fig. 3).

Tail long, cylindrical, sometimes slightly ventrally concave, ending in a broadly rounded terminus, 20.76–36.33 (27.03 ± 5.03) μm long or about 1.36–2.37 (1.94 ± 0.43) times anal body widths long.

Male

Spicules paired, slender, ventrally arcuate 25.95–29.14 (27.47 ± 1.3) μm long. Gubernaculum linear, 8.65 μm long. Bursa not clearly seen.

Tailed elongated a caudal papilli with a large mucro like structure, 34.6–37.73 (36.19 ± 1.27) μm long.

Table 1 Morphometric data of *A. assamensis* sp. nov.

Characters	Holotype female	Paratype females	Paratype males
n	1	12	7
Length	762.93	700.65–776.77 (733.3 ± 28.85)	717.95–730.06 (724.84 ± 5.08)
a	29.4	21.38–32.07 (28.11 ± 4.16)	32.69–37.72 (35.58 ± 2.12)
b	6.68	4.73–7.10 (6.44 ± 0.69)	5.86–6.28 (6.03 ± 0.18)
b'	5.37	3.53–5.47 (4.55 ± 0.64)	4.00–4.32 (4.17 ± 0.13)
c	36.75	19.53–37.41 (28.2 ± 6.01)	19.34–20.75 (20.04 ± 0.57)
c'	1.5	1.36–2.37 (1.94 ± 0.43)	2.5–3.49 (3.03 ± 0.40)
V	75.96	74.61–77.2 (76.33 ± 0.82)	–
G ₁	48.75	47.88–57.32 (52.01 ± 3.33)	–
G ₂	7.58	7.19–10.97 (9.24 ± 1.53)	–
Lip width	8.65	6.92–8.65 (8.21 ± 0.74)	5.19
Lip height	5.19	3.46–5.19 (4.75 ± 0.74)	3.46
Oesophagous	141.86	141.86–204.14 (163.68 ± 19.82)	166.08–182.11 (173.61 ± 6.58)
Stylet	12.11	12.11	12.11
Nerve ring	108.99	108.99–124.56 (116.31 ± 5.08)	–
Excretory pore	86.5	86.5–100.34 (93.11 ± 4.63)	–
Hemizonid	86.5	86.5–91.69 (89.28 ± 1.79)	–
Max. body width	25.95	22.49–36.33 (27.03 ± 5.03)	19.03–22.22 (20.44 ± 1.32)
Tail	20.76	20.76–36.33 (27.03 ± 5.03)	34.6–37.73 (36.19 ± 1.27)
ABD	13.84	10.38–19.03 (14.27 ± 2.96)	10.38–13.84 (12.11 ± 1.41)

Fig. 3 SEM photomicrographs of *A. assamensis* sp. nov.

Type habitat and locality

Collected in October, 2013 from rotten stem of Tamul paan, *Areca catechu* from Kahilipara, with latitude of 26.1428694 and longitude of 91.768487, Kamrup District, Assam, North East India.

Type materials

Holotype female on the slide FTY5♀- 1/*Aphelenchus assamensis* sp. nov., paratype females on the slides FTY5♀- 2- 12/*Aphelenchus assamensis* sp. nov. and paratype males on the slides FTY5♂- 1- 7/*Aphelenchus assamensis* sp. nov.

and deposited in the nematode collection of Parasitology Section, Centre for Advanced Study in Life Sciences, Manipur University, Canchipur-795003, Manipur, India.

Differential diagnosis

Aphelenchus assamensis sp. nov. differs from all other species of *Aphelenchus* Bastian (1865) in having narrow and least number of lateral lines i.e. two, while in all the other species, lateral fields are broad with numerous incisions, usually in excess of ten exceptionally fewer (*Aphelenchus sparsus* Thorne and Malek 1968 only has four to six, for example).

Table 2 Differences of *A. assamensis* sp. nov. from *A. avenae* described by different authors

Characters	<i>Aphelenchus assamensis</i> sp. nov.	<i>Aphelenchus avenae</i> (Bastian 1865)			
		After Christie (1942)	After Allen (1952)	After Goodey and Hooper (1965)	After Fortuner (1970)
Length	0.70–0.77	0.66–0.75	0.62–0.88	0.55–0.86	0.57–0.84
Wide	22.49–36.33	17.22	–	–	–
Stylet	12.11	–	10	15	10–12.5
Oesophagous	141.86–204.14	64–68	–	–	–
Tail	20.76–36.33	36–42	–	–	–
a	21.38–32.07	32–42	38–58	25–39	39–53
b	4.73–7.10	10.2–11.4	9–12	4.9–6.9	9.2–13.1
b'	3.95–54.7	–	–	7.2–9.2	4.06–5.77
c	19.53–37.41	17–22	15–20	27–35	13.8–20.4
V%	74.61–77.20	68–70	66–72	74–78	68.7–73.6

Aphelenchus assamensis sp. nov. is similar to *Aphelenchus isomerus* Anderson and Hooper (1980) in having similar range of b', c and c'. But, the present species differs from *Aphelenchus isomerus* Anderson and Hooper (1980) in having smaller value of a, longer oesophagous, longer tail, larger body widths and smaller number of lateral lines [$a = 30.0\text{--}54$ (37.8 ± 4.5), oesophagous = $84\text{--}130$ (111 ± 11.9) μm , tail = $15.0\text{--}31.5$ (22.5 ± 2.6) μm , body widths = $14\text{--}24$ (18) μm and 12–16 incisures in *Aphelenchus isomerus*].

The present species is similar to *A. sparsus* Thorne and Malek (1968) in having low and moderate sclerotised cephalic region and rounded tail terminus. But, *A. assamensis* sp. nov. differs from *A. sparsus* Thorne and Malek (1968) in having shorter body length, larger value of a, b, smaller value of c, shorter stylet, different shape of anal body region and smaller number of lateral fields ($L = 0.9$ mm, $a = 25$, $b = 3.7$, $c = 34$, stylet = 12.11 μm , anus continuous with the body contour, tail bluntly rounded with radial striae and lateral fields marked by four to six incisures near middle of body, decreasing to two near head and four at terminus in *A. sparsus* Thorne and Malek 1968). From *Aphelenchus mirzai* Das (1960); it differs in having only one caudal papilli (three pairs of caudal papillae in *A. mirzai*).

Aphelenchus assamensis sp. nov. is similar to *Aphelenchus avenae* Bastian (1865) in having similar body length range, body contour and cephalic region structure. But, it differs from *Aphelenchus avenae* Bastian (1865) in being narrow lateral fields with two incisures in mid-body region (lateral fields are wide in mid body region with 12 (10–14) incisures, posteriorly the field often widens in front of vulva and narrows just posterior to it, the incisures decreasing in about eight at about anal level although the field often expands again in the tail region in *A. avenae*). The morphometric differences of the present

specimen from *Aphelenchus avenae* Bastian (1865) described by different authors are given in the table below (Table 2).

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