

***Aphelenchoides subbotini* sp. n. (Nematoda: Aphelenchoididae) from roots of mangrove plant *Excoecaria agallocha* in India**

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Summary. *Aphelenchoides subbotini* sp. n. was isolated and described during a survey on the east coast mangroves from West Bengal, India. The species was isolated from the necrotic and damaged roots of the blind-your-eye mangrove plant *Excoecaria agallocha*. The new species can be differentiated from the other known *Aphelenchoides* species by having a relatively shorter body length ($538.9 \pm 47.6 \mu\text{m}$), weak stylet with distinct basal swelling, reproductive tract stretched approximately 0.45 times of the body length including postvulval uterine sac, vulval position at around 65-70% of the body length with unmodified vulval lips, and ventrally arcuate conical tail bearing a tetramucronate (rarely trimucronate) spine with blunt processes. Molecular characterisation was carried out based on small subunit 18S rDNA, ITS rDNA and D2-D3 expansion segment of 28S rDNA genes. The phylogenetic analyses based on the ribosomal 18S, ITS and D2-D3 genes confirmed clear separation of *A. subbotini* sp. n. as a new species. This is the first report of any *Aphelenchoides* species from a blind-your-eye mangrove plant from this locality.

Key words: mangrove, molecular phylogeny, morphology, new species.

The genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchoididae) contains around 200 nominal species (Handoo *et al.*, 2020). Some of these nematodes cause considerable losses to agricultural and horticultural products, whereas the majority are found in association with fungi, decaying organic matter and plant tissues, and also insects (Kanzaki, 2006; Kanzaki & Giblin-Davis, 2012; Sanchez-Monge *et al.*, 2015; Kanzaki *et al.*, 2021). The genus *Aphelenchoides* is a conserved one as its species lack a large variety of diagnostic morphological and anatomical features for authentic taxonomic identification. This bottleneck has complicated the aphelenchoid taxonomy, and as a result a large number of species have been poorly or inadequately described (Mobasserri *et al.*, 2018). DNA-based diagnostics and phylogenetic analyses with small subunit 18S ribosomal DNA (rDNA), ribosomal internal transcribed spacer 1 and 2 regions (rDNA ITS), D2-D3 expansion segment of large subunit 28S

rDNA, and mitochondrial *COI* genes provide valuable information for reliable species identification and evolutionary inferences, when used complementarily with morphological data (Mobasserri *et al.*, 2018; Gu *et al.*, 2025a, b).

During a survey at the coastal mangrove belt of West Bengal, India, a population of *Aphelenchoides* was found associated with the roots of blind-your-eye mangrove *Excoecaria agallocha* Linn. Nearly, 54 species of *Aphelenchoides* have been reported from India (Chanu & Mohilal, 2023) but previous reports on the diversity of plant-parasitic nematodes from the mangrove plants are scarce. In the present study, detailed morphological and morphometric analyses revealed a clear distinction of the nematode population from other known *Aphelenchoides* species. Additionally, the molecular characterisation based on 18S SSU rDNA, ITS region of rDNA, and D2-D3 of 28S rDNA was carried out for robust and authentic identification of the species.



Fig. 1. Blind-your-eye mangrove plant with roots at collection site.

MATERIALS AND METHODS

Nematode population. The roots of *Excoecaria agallocha* (Fig. 1) were primarily collected from Shankarpur beach of West Bengal, India (21.6366° N, 87.5624° E). A few of the roots showed necrotic and damaged lesions, but other roots showed no distinguishable signs of infection. The roots were cut into small pieces, thoroughly washed with running tap water, and finely chopped using a sterilised scalpel. The nematode specimens were extracted from the chopped roots using a modified tray technique (Whitehead & Hemming, 1965), with incubation duration of 3–4 days.

Morphological studies. The nematode specimens extracted from the roots were killed using hot water bath at 60°C, and fixed in TAF fixative (triethanolamine: 2 ml, formalin: 7 ml, distilled water: 91 ml). Post-fixation, the specimens were processed by glycerin-ethanol method (Seinhorst, 1959), and permanently mounted in anhydrous glycerin on clean glass slides for morphological and morphometric studies. Photomicrographs were acquired using a Zeiss Axiolab 5 compound microscope (Carl Zeiss, Germany) equipped with Axiocam 208 colour camera. The morphometric

measurements were obtained *via* ocular micrometer and ZEN 3.0 blue edition software (Carl Zeiss Microscopy GmbH). The linear measurements were obtained as described previously (Miraeiz *et al.*, 2017; Mobasseri *et al.*, 2018; Handoo *et al.*, 2020), and de Man's ratios were calculated. Line drawings were made manually using photographs taken with an Axiocam colour camera attached to a compound microscope as templates.

Molecular characterisation. For molecular characterisation, a single nematode specimen (with three replicates) was processed with worm lysis buffer following standard methods (Waghmare *et al.*, 2022). Briefly, a single nematode specimen was transferred to a 0.5 ml microcentrifuge tube containing 25 µl of sterilised nuclease free water, together with an equal volume of worm lysis buffer (0.2 M NaCl, 0.2 M Tris-HCl at pH 8.0, 1% (v/v) β-mercaptoethanol, and 2 µl proteinase K from 10 mg ml⁻¹ stock solution), incubated in 62°C for 2 h with intermittent flicking, followed by incubation at 95°C for 15 min. Two µl of the lysate was used as DNA template in PCR reaction. The 18S, ITS and D2-D3 molecular markers of rDNA were PCR amplified using the primers listed in Floyd *et al.* (2002), Subbotin *et al.* (2000) and Nunn (1992). The thermal

cycling condition consisted of denaturation at 94°C for 5 min; followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 54°C (for 18S), 51°C (for ITS) and 52°C (for D2-D3) for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 10 min. The amplified PCR products were cleaned using GeneJET PCR purification kit (ThermoFisher Scientific) following the manufacturer's protocol, electrophoresed on 1% agarose gel for confirmation, and sequenced by Sanger dideoxy method at Barcode Biosciences, Bangalore, Karnataka, India. The raw sequences were quality checked against chromatograms, aligned, and consensus sequences were generated for each gene (for all three replicates) using BioEdit software (Ibis Biosciences). The newly obtained sequences were BLAST searched against NCBI database to check their authenticity. For phylogenetic analyses, corresponding rDNA 18S, ITS and D2-D3 sequences of *Aphelenchoides* species (Miraeiz *et al.*, 2017; Mobasser *et al.*, 2018; Gu *et al.*, 2025a, b) were retrieved from NCBI database, aligned using ClustalW, and evolutionary history was deduced using the maximum likelihood (ML) method following described methods (Phani *et al.*, 2019). *Panagrellus* sp. and *C. elegans* (for ITS tree only) were used as outgroups (Mobasser *et al.*, 2018).

DESCRIPTION

Aphelenchoides subbotini sp. n. (Figs 2 & 3)

Measurements. See Table 1.

Female. Body cylindrical, gently narrowing towards both ends; mostly curved ventrally to open C-shape when heat relaxed, but few specimens curved on dorsal side. Body cuticle striations fine and smooth. Lateral field with two lateral lines, weakly recognisable at mid-body region. Lip region rounded, not annulated, separated from body contour by slight depression, slightly truncated in oral area. Stylet short and weak, conus length being around 45-50% of the stylet length, shaft with visible lumen throughout the length, having conspicuous swellings at base. Procorpus long and slender, median bulb broadly ovoid with length approximately 1.4-1.5 times of the width or almost rounded, having valve centrally located and well sclerotised. Pharyngeal glands overlapping dorsally typical to aphelenchoid type, not visibly separated, having a very small arm-like ventral stretch near the pharyngo-intestinal junction visible under light microscope, junction located around 20-22 µm posterior to base of median bulb. Intestine simple. Location of secretory-excretory pore variable, located around 5-10 µm

posterior to base of median bulb, generally above the level of pharyngo-intestinal junction. Nerve ring located at 12-18 µm posterior to base of median bulb, generally below the level of secretory-excretory pore, difficult to observe under bright field microscope. Reproductive tract monodelphic prodelphic; consisting of outstretched ovary, oviduct, spermatheca, crustaformeria, uterus, vagina + vulva, and postvulval uterine sac (PUS); oviduct tubular, oocytes arranged in single row but in two/irregular rows at terminal end; spermatheca not prominent, crustaformeria formed with rounded cells and well developed; uterus cylindroid broadly opening at vagina; vagina simple without sclerotisation. PUS elongated cylindrical, difficult to observe, around 2.5 times shorter in length than the anterior gonad tract, *ca* 4.1-5.2 times the maximum body width, 44-55% in lengthwise to vulva-anus distance, without any spermatozoa within it. Vulva located at around 65.3-70.0% of the body length, with slit like opening and simple lips without any modification, or having very small protuberant lips (observed in one specimen). Anus distinct, may be with slight posterior swelling at the opening. Tail conical, ventrally arcuate, having visible refractive granules inside almost up to tail tip, tapering to a rounded terminus bearing a stellate or tetramucronate (rarely trimucronate) spine with blunt processes.

Male. Not found.

Type host and locality. Collected from the roots of blind-your-eye mangrove, *Excoecaria agallocha*, grown at Shankarpur beach of West Bengal, India (21.6366° N, 87.5624° E) in 2024.

Type material. Holotype female and three female paratypes were deposited at the Nematelminthes Section of National Zoological Collections (NZC), Zoological Survey of India, Kolkata, West Bengal, India (NZC Registration Nos. N12542-N12544). The Life Science Identifier (LSID) for ZooBank submission is C913DE03-8C3D-458E-96E0-8199 E76BAD16.

Etymology. The species epithet has been given in honour of Dr Sergei A. Subbotin for his outstanding contribution in nematode taxonomy and systematics.

Diagnosis and relationships. *Aphelenchoides subbotini* sp. n. can be characterised by having a body length of 538.9 ± 47.6 µm, body posture curved on both ventral and dorsal side(s) when heat relaxed, weak stylet of 9.8 ± 0.3 µm length with conspicuous basal swelling, two lateral lines, outstretched reproductive tract with well-developed PUS, posteriorly located vulva at around 65-70% of the body length, unmodified vulval lips almost at the same level to body contour or having very small protuberance, and tail elongated and conical with

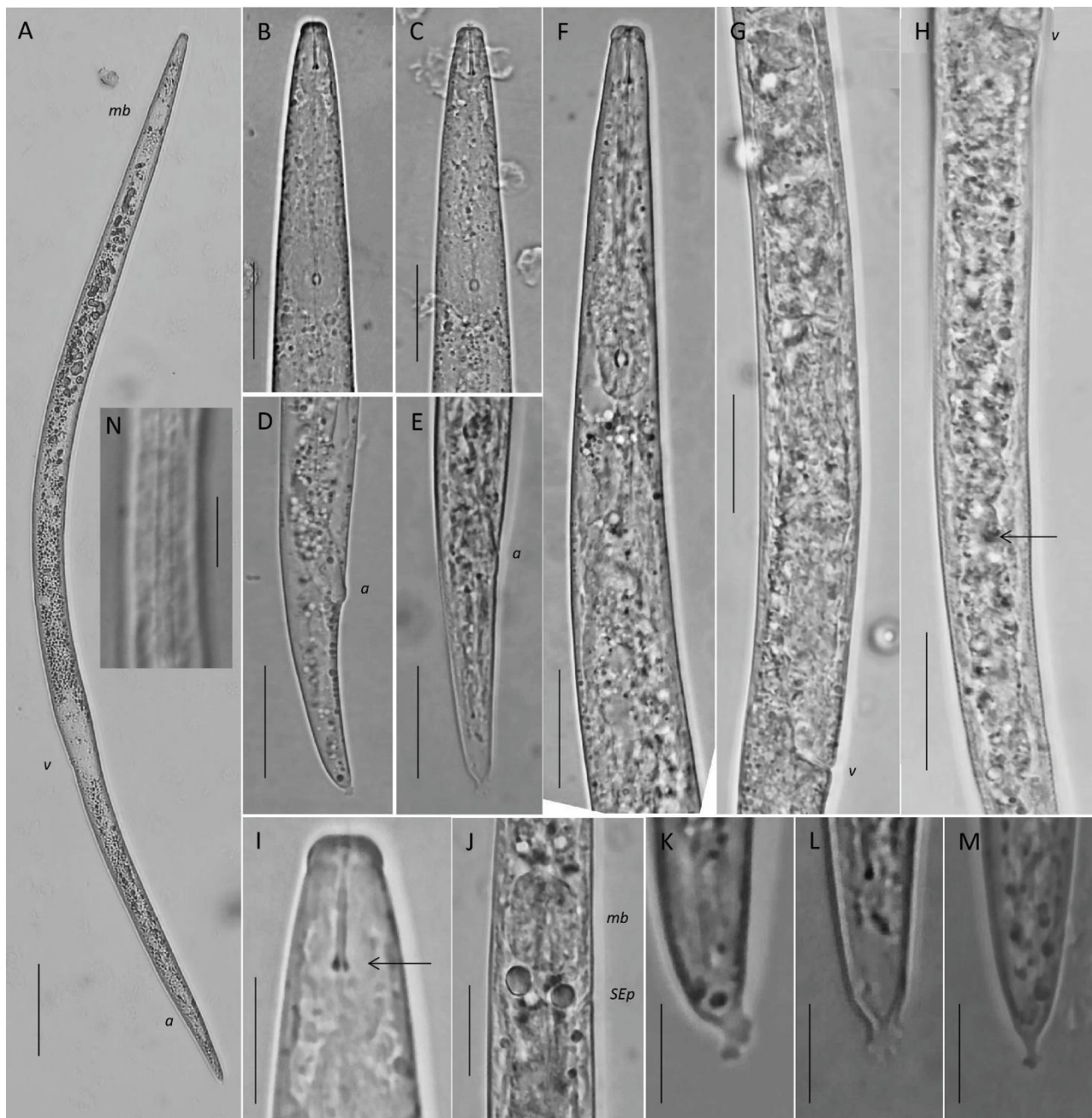


Fig. 2. Light photomicrographs of *Aphelenchoides subbotini* sp. n. Female. A: Entire body; B, C: Anterior region; D, E: Posterior region; F: Anterior body region with pharyngeal overlapping; G: Vulva with part of anterior branch of gonad showing crustaformeria; H: Vulva with postvulval uterine sac (arrow pointing terminal end); I: Magnified anterior region showing stylet with basal swelling (arrow); J: Body region showing median bulb and secretory-excretory pore; K-M: Magnified posterior region showing tail mucro; N: Lateral field with two lateral lines. *mb*: median bulb, *SEp*: secretory-excretory pore, *v*: vulva, *a*: anus. Scale bars: A = 50 µm; B-H = 20 µm; I-N = 10 µm.

a tetramucronate (rarely trimucronate), blunt-ended spine. According to the *Aphelenchoides* groups made by Shahina (1996) based on the shape of mucro on nematode tail, *A. subbotini* sp. n. belongs to 'Group 3' for having tetramucronate spine or star-shaped mucro on tail. However, comprehensive comparison

of gross body morphology and morphometrics (not restricted to tail and mucro shape only) with other *Aphelenchoides* spp. revealed close resemblance of the new species with *A. aligarhiensis* Siddiqi, Husain & Khan, 1967; *A. andrassyi* Husain & Khan, 1967; *A. appendurus* Singh, 1967; *A. asterocaudatus* Das,

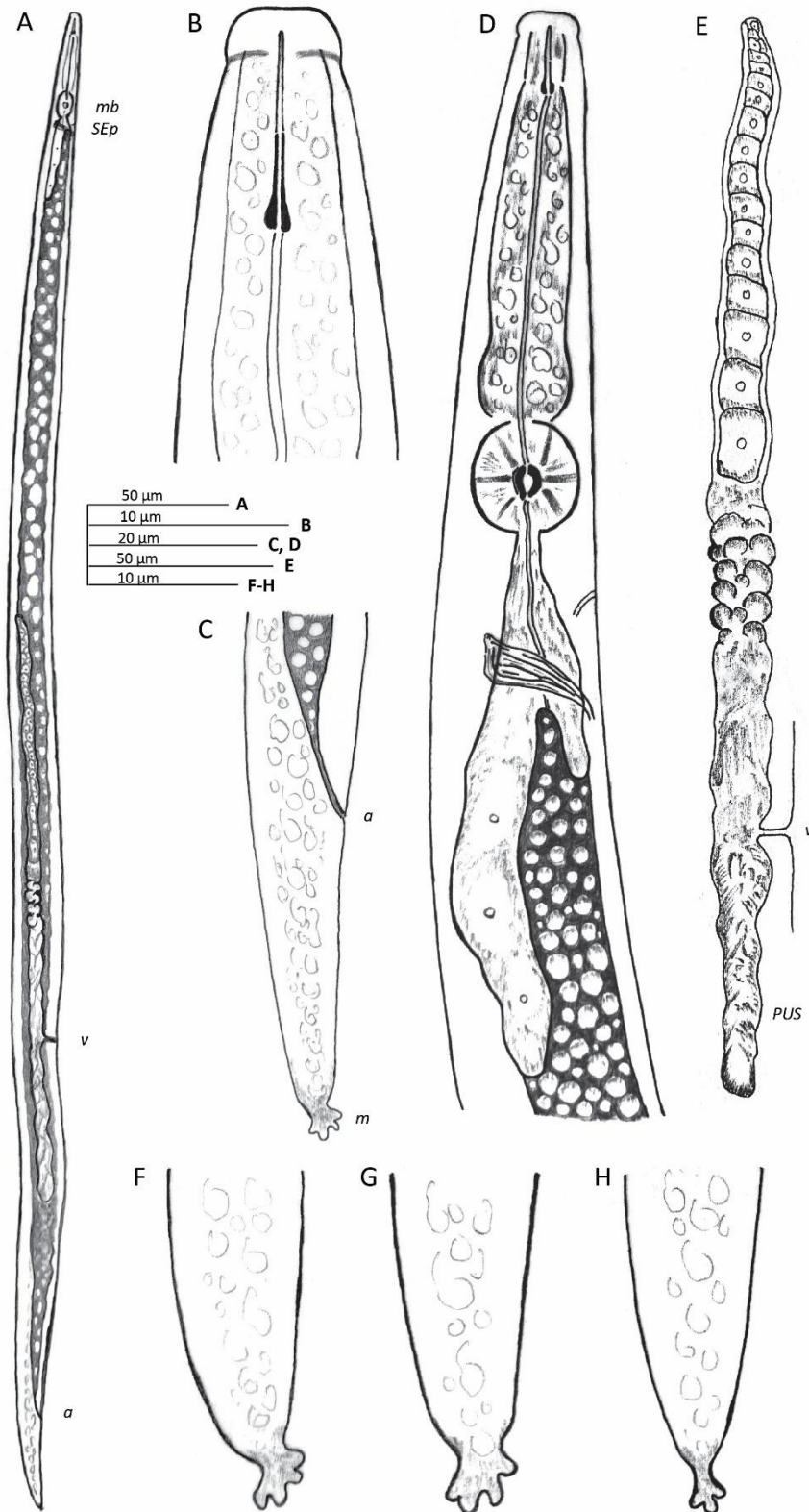


Fig. 3. Line drawings of *Aphelenchoides subbotini* sp. n. Female. A: Entire body; B: Anterior region; C: Posterior region; D: Anterior region with pharyngeal overlapping; E: Entire gonad with vulva; F-G: Variations in tail mucro. *mb*: median bulb, *SEp*: secretory-excretory pore, *v*: vulva, *a*: anus, *m*: mucro, *PUS*: postvulval uterine sac.

Table 1. Morphometrics of *Aphelenchoides subbotini* sp. n.
All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Character	Female	
	Holotype	Paratypes
n	—	20
Body length	558.6	538.9 $\pm$ 47.6 (473.4-601.3)
a	41.6	37.3 $\pm$ 3.2 (32.3-41.6)
b	6.3	5.9 $\pm$ 0.3 (5.3-6.3)
b'	4.9	4.4 $\pm$ 0.3 (3.9-5.1)
c	15.3	15 $\pm$ 0.4 (14.1-15.7)
c'	4.6	4.4 $\pm$ 0.5 (4.0-5.1)
Maximum body width	13.4	14.4 $\pm$ 1.3 (13.1-17.2)
Lip height	2.6	2.3 $\pm$ 0.4 (1.9-3.1)
Lip width	5.3	5.1 $\pm$ 0.5 (4.5-6.1)
Stylet length	9.6	9.8 $\pm$ 0.3 (9.3-10.4)
Conus length	4.1	4.2 $\pm$ 0.2 (4.0-4.4)
Median bulb length (MBL)	11.4	11.8 $\pm$ 0.5 (11.0-12.8)
Median bulb width (MBW)	8.2	7.7 $\pm$ 0.6 (7.1-8.3)
MBL/MBW	1.4	1.5 $\pm$ 0.1 (1.4-1.7)
Distance from anterior end to median bulb	48.2	53.9 $\pm$ 4.6 (48.2-60.5)
Distance from anterior end to excretory pore	55.1	60.2 $\pm$ 5.9 (53.8-69)
Distance from anterior end to nerve ring	58.7	63.4 $\pm$ 5.4 (58.7-65.3)
V%	68	66.7 $\pm$ 1.6 (65.3-70.0)
Distance from vulva to anus	142.2	143.8 $\pm$ 17.4 (109.3-167.2)
Distance from anterior end of gonad to vulva	196.5	175.1 $\pm$ 25.5 (148.7-215.8)
Length of postvulval uterine sac (PUS)	70.9	68.8 $\pm$ 3.8 (61.3-74.2)
PUS/Maximum body width	5.2	4.7 $\pm$ 0.3 (4.1-5.2)
Anal body width	7.9	8.0 $\pm$ 0.5 (7.7-9.1)
Tail length	36.6	35.6 $\pm$ 3.2 (31.3-42.5)

1960; *A. asteromucronatus* Eroshenko, 1967; *A. besseyi* Christie, 1942; *A. fujianensis* Zhuo *et al.*, 2010; *A. goodeyi* Siddiqi & Franklin, 1967; *A. nonveilleri* Andr  ssy, 1959; *A. pseudobesseyi* Subbotin *et al.*, 2021; *A. pseudogoodeyi* Oliveira *et al.*, 2019; *A. ritzemabosi* (Schwartz, 1911) Steiner & B  hrer, 1932; and *A. unisexus* Jain & Singh, 1984.

In this context, *A. subbotini* sp. n. differs from *A. aligarhiensis* with respect to b value (5.3-6.3 vs 7-9), shape of procorpus (attenuated vs cylindrical), shape of median bulb (broadly rounded vs oval), length of PUS (4.1-5.2 times of maximum body width vs 5.5 times and more of maximum body), anal opening (generally unmodified vs raised anterior lip), and number of lateral lines (2 vs 4); from *A. andrassyi* with respect to body length (473.4-601.3 μm vs 390-440 μm), a value (32.3-41.6 vs 23-28), b value (5.3-6.3 vs 3.2-3.9), c value (14.1-15.7 vs 6-12), position of secretory-excretory pore (generally above the nerve ring vs below the nerve ring), tail length (nearly

4.5 times the anal body width vs 7 times the anal body width), and length of PUS (longer vs shorter); from *A. appendurus* with respect to body length (473.4-601.3 μm vs 720-880 μm), b value (5.3-6.3 vs 9-10.5), stylet length (9.3-10.4 μm vs 16.5-17.5 μm), stretch of anterior gonad (much below the posterior margin of pharyngeal gland lobe vs extending up to posterior margin of pharyngeal gland lobe), and length of PUS (4.1-5.2 times of maximum body width vs 1.5-1.75 times of maximum body); from *A. asterocaudatus* with respect to body length (473.4-601.3 μm vs 630-700 μm), b value (5.3-6.3 vs 7.5-7.7), c value (14.1-15.7 vs 16.5-17.95), length of PUS (61.3-74.2 vs 29.6-40), and shape of vulval lips (unmodified vs protuberant); from *A. asteromucronatus* with respect to number of lateral lines (2 vs 4), c' value (4.0-5.1 vs 3), and stylet length (9.3-10.4 μm vs 9 μm); from *A. besseyi* with respect to body length (473.4-601.3 μm vs 660-750 μm), stylet length (9.3-10.4 μm vs 10-12.5 μm), b value (5.3-6.3

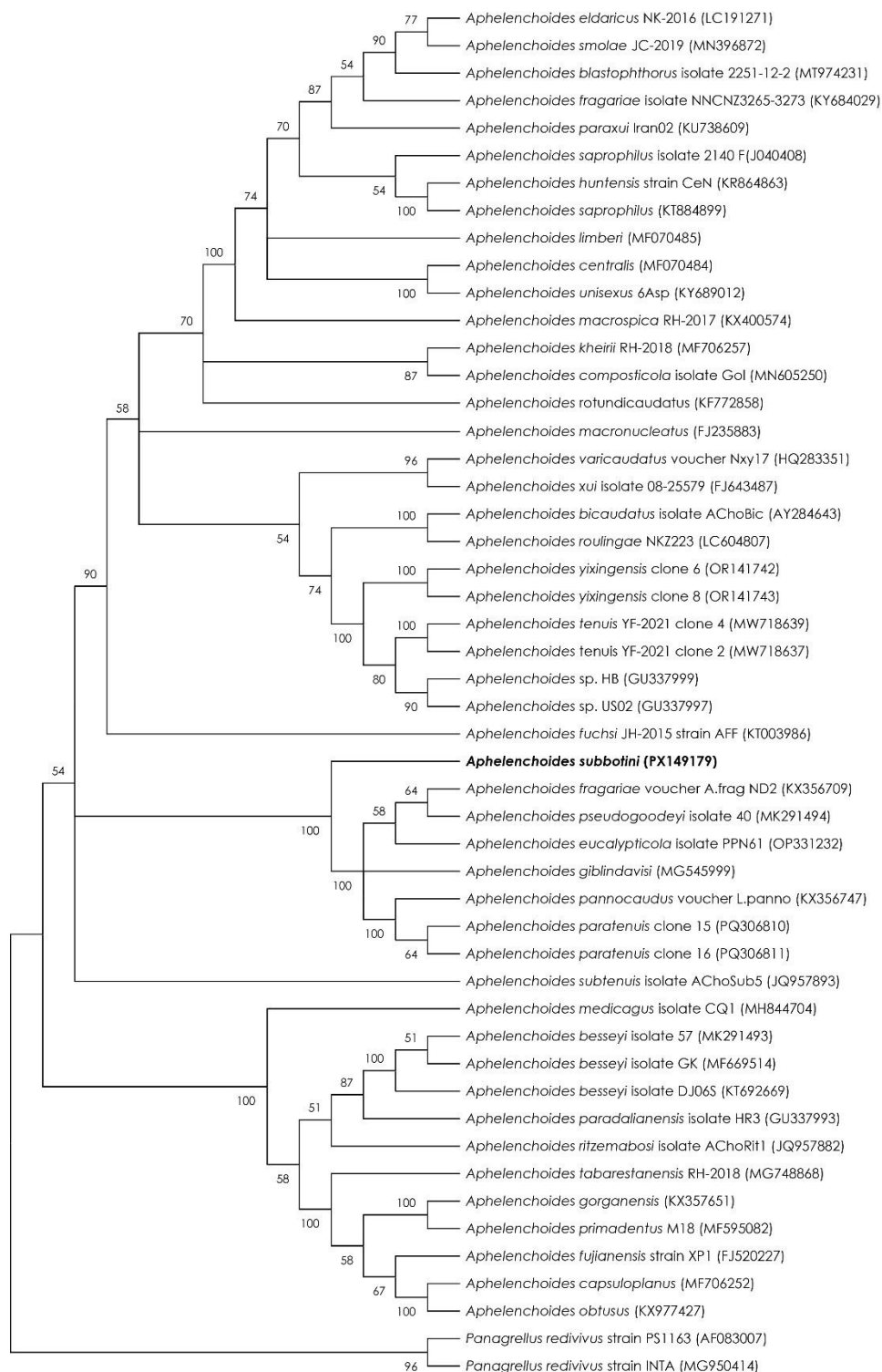


Fig. 4. Evolutionary relationships of *Aphelenchoides subbotini* sp. n. with other *Aphelenchoides* using 18S SSU rDNA sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura 3-parameter model. The bootstrap consensus tree inferred from 1000 replicates. Branches with less than 50% bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using maximum composite likelihood approach and then selecting the topology with superior log likelihood value (-22740.03). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.8292)).

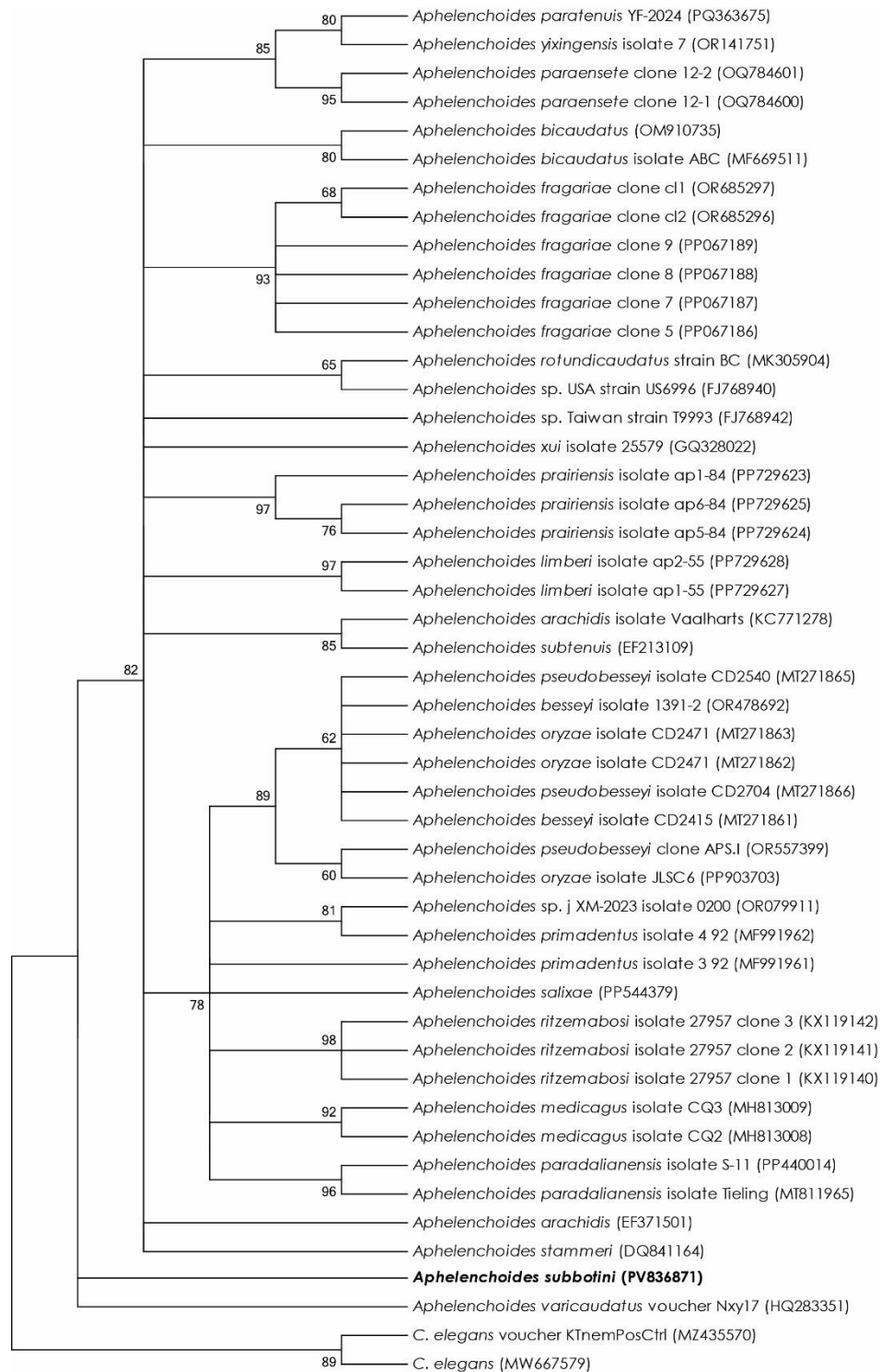


Fig. 5. Evolutionary relationships of *Aphelenchoides subbotini* sp. n. with other *Aphelenchoides* using ITS rDNA sequences. The evolutionary history was inferred by using the maximum likelihood method based on the General Time Reversible model. The bootstrap consensus tree inferred from 1000 replicates. Branches with less than 60% bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using maximum composite likelihood approach and then selecting the topology with superior log likelihood value (-924.9426). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 2.3101)).

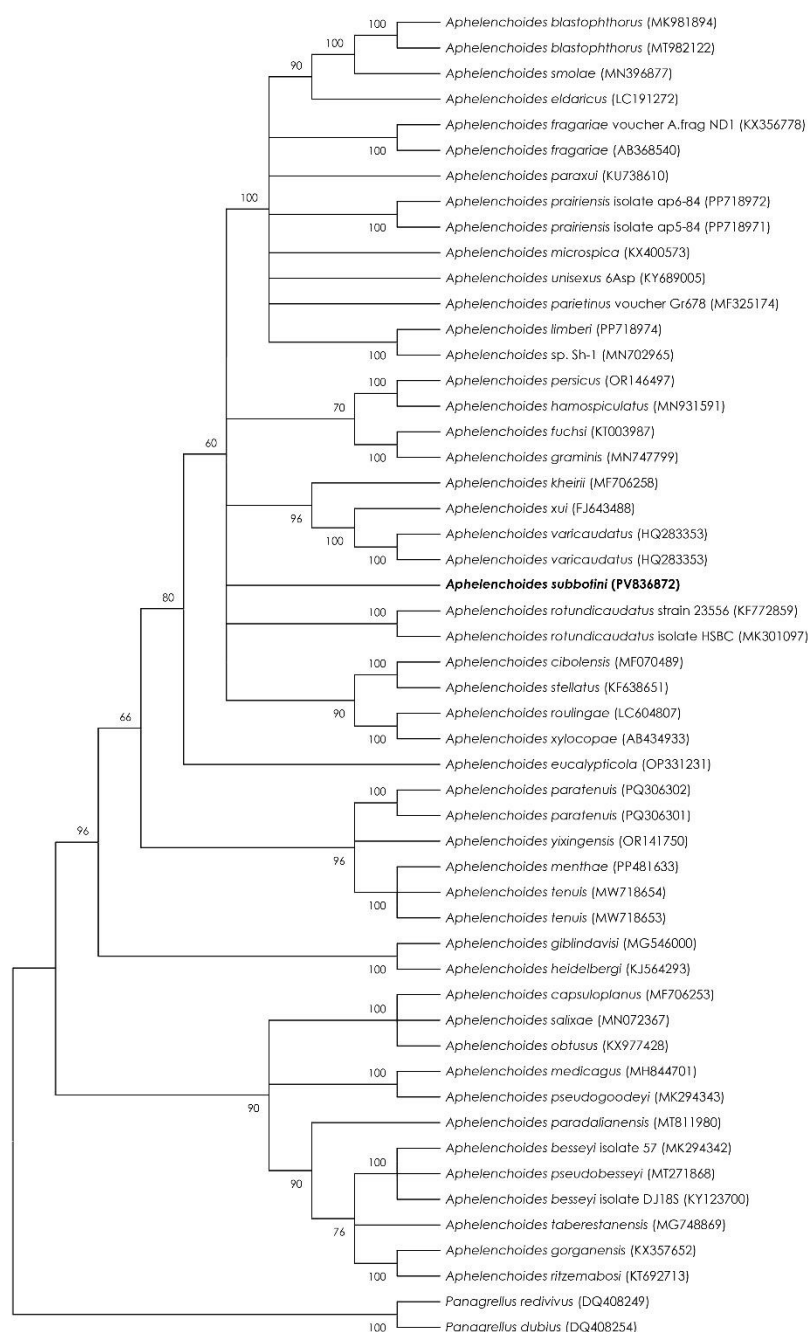


Fig. 6. Evolutionary relationships of *Aphelenchoides subbotini* sp. n. with other *Aphelenchoides* using D2-D3 expansion segment of 28S rDNA sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model. The bootstrap consensus tree inferred from 1000 replicates. Branches with less than 60% bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using maximum composite likelihood approach and then selecting the topology with superior log likelihood value (-19370.49). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4351)).

vs 10.2-11.4), c value (14.1-15.7 vs 17-21), head shape (weakly set-off vs deeply set-off), number of lateral lines (2 vs 4), arrangement of oocytes (single row vs 2-4 rows), length of PUS (1/4th the length of

vulva-anus distance vs less than 1/3rd the length of vulva-anus distance), shape of vulva (unmodified lips vs slightly raised lips); from *A. fujianensis* with respect to body length (473.4-601.3 μ m vs 803-941

µm), b value (5.3-6.3 vs 9.8-13), stylet length (9.3-10.4 µm vs 12.5-14 µm), vulva-anus distance (109.3-167.2 µm vs 205-250 µm), tail length (31.3-42.5 µm vs 46-58 µm), shape of vulval lips (unmodified vs slightly protruding), and number of lateral lines (2 vs 4); from *A. goodeyi* with respect to stylet length (9.3-10.4 µm vs 11.5-12.5 µm), b' value (3.9-5.1 vs 8-10), stretch of anterior gonad (longer vs shorter), length of PUS (4-5 times of body width vs 3 times of body width), arrangement of oocytes (single row vs multiple rows), number of lateral lines (2 vs 4), vulval shape (unmodified vs slightly protruding), position of excretory pore (anterior to nerve ring vs posterior to nerve ring), structure of uterus (broad single-part vs two-part having distinguishable muscular and thick-walled region); from *A. nonveilleri* with respect to body length (473.4-601.3 µm vs 751.4-842.5 µm), b value (5.3-6.3 vs 8.27-9.1), c value (14.1-15.7 vs 18-20), stylet length (9.3-10.4 µm vs 11.8-14.2 µm), position of secretory-excretory pore (generally anterior to nerve ring vs posterior to nerve ring), length of PUS (61.3-74.2 µm vs 84.4-103.0 µm), stretch of anterior gonad (shorter vs extended almost to pharyngeal gland lobe); from *A. pseudobesseyi* with respect to body length (473.4-601.3 µm vs 611-757 µm), b value (5.3-6.3 vs 9.4-11.2), stylet length (9.3-10.4 µm vs 12-12.8 µm), number of lateral lines (2 vs 4); from *A. pseudogoodeyi* with respect to body length (473.4-601.3 µm vs 663.8-776.0 µm), b value (5.3-6.3 vs 9.6-10.8), stylet length (9.3-10.4 µm vs 12-12.7 µm), length of PUS (61.3-74.2 µm vs 32.6-47.5 µm), and number of lateral lines (2 vs 4); from *A. ritzemabosi* with respect to body length (473.4-601.3 µm vs 768-1027 µm), a value (32.3-41.6 vs 43.4-60.5), b value (5.3-6.3 vs 9.4-11.2), c value (14.1-15.7 vs 16.8-21.2), position of secretory-excretory pore (generally anterior to nerve ring vs posterior to nerve ring), and length of PUS (61.3-74.2 µm vs 105-160 µm); and from *A. unisexualis* with respect to body length (473.4-601.3 µm vs 600-710 µm), b value (5.3-6.3 vs 7.5-8.9), position of secretory-excretory pore from anterior body end (53.8-69 µm vs 73-83 µm), and length of PUS (61.3-74.2 µm vs 50-61 µm).

Molecular characterisation and phylogenetic relationships. The amplification and sequencing of 18S, ITS, and D2-D3 genes yielded *ca* 661 bp, 433 bp and 698 bp sequences, respectively. For all the three replicates and each of the genes, the sequences were aligned and showed no significant variations; hence, single replicate sequences (for 18S, ITS and D2-D3 genes) were processed for submission. The sequences were submitted to GenBank under the accession numbers PX149179 (18S SSU rDNA), PV836871 (ITS rDNA), and PV836872 (D2-D3

expansion segment of 28S rDNA). The top three BLAST hit results against NCBI database showed the 18S SSU rDNA sequence of *A. subbotini* sp. n. to have 86.02% identity with *Aphelenchoides* sp. G027 from Belgium (KX356746; query coverage = 100%, e-value = 0.0), 86.06% identity with *Aphelenchoides* sp. G024 from Belgium (KX356744; query coverage = 99%, e-value = 0.0), and 85.56% identity with *Aphelenchoides* sp. G026 from Belgium (KX356745; query coverage = 94%, e-value = 0.0). The top three BLAST hit results for ITS rDNA sequence of *A. subbotini* sp. n. showed 81.19% identity with *A. varicaudatus* voucher Nxy17 from China (HQ283351; query coverage = 93%, e-value = 2e-80), 90.78% identity with *Bursaphelenchus minutus* from Italy (LR746542; query coverage = 33%, e-value = 1e-41), and 90.78% identity with *Aphelenchoides* sp. 1 FDL-2017 from Italy (LR890127; query coverage = 33%, e-value = 1e-41). Alternatively, the top three BLAST hit results for D2-D3 expansion segment sequence showed 84.51% identity with *Aphelenchoides* sp. Asp6266 from Japan (KY769083; query coverage = 100%, e-value = 0.0), 84.11% identity with *Aphelenchoides* sp. 12Asp from China (KX356748; query coverage = 100%, e-value = 0.0), and 83.15% identity with *Aphelenchoides epiphyticus* from Japan (LC876838; query coverage = 100%, e-value = 6e-172).

The evolutionary phylogenetic analyses using the sequences of 18S SSU, ITS, and D2-D3 genes revealed that they were sufficiently divergent from the other molecularly characterised *Aphelenchoides* species. The phylogenetic relationship as inferred from maximum likelihood (ML) method using the 18S SSU rDNA sequence (Fig. 4) showed *A. subbotini* sp. n. to form a sub-clade with *A. fragariae*, *A. pseudogoodeyi*, *A. eucalypticola*, *A. giblindavisi*, *A. pannocaudus* and *A. paratenuis* with strong bootstrap support. Additionally, the phylogenetic inferences deduced by ITS rDNA (Fig. 5) and D2-D3 expansion segment of 28S rDNA (Fig. 6) sequences confirmed the status of *A. subbotini* sp. n. as a separate species, where the species branched out separately in both cases from the other *Aphelenchoides* species.

DISCUSSION

Mangrove flora serves as one of the most diversified eco-habitats to favour abundant number of heterogeneous living resources. In India, mangrove forests cover 4921 sq Km spanning the states of West Bengal, Odisha, Andhra Pradesh, Tamil Nadu, Andaman and Nicobar Islands, Kerala, Goa, Maharashtra and Gujarat (Sreelekshmi *et al.*,

2020). Despite the presence of large microhabitat diversities within mangroves, the study of mangrove-associated nematodes with respect to generic and specific distribution is a rarity. The majority of the studies in this direction are focused towards the mangrove sediments. For example, Gwyther (2003), while studying the nematode assemblages from *Avicennia marina* leaf litter in south-east Australia, found the dominant feeding group to be the non-selective deposit feeders. Pinto *et al.* (2013) found the pneumatophores colonised by epiphytic algae to harbour mainly the non-selective deposit feeders like *Oncholaimus*, *Microlaimus* and *Thalassomonhystera* in Brazil. In another study, Balan and Boyles (2016) found the root-knot nematode incidences to be higher in more disturbed ecosystem than in less disturbed areas within the mangroves. In relation specifically to the aphelenchid nematodes, Timm and Franklin (1969) described *Aphelenchoides gynotylurus* from the bottom mud of saline pool at Cox's Bazar, East Pakistan (now Bangladesh). Otherwise, any previous report on direct association of an aphelenchid species with the mangrove plant roots is absent from this area.

Classical species identification within the genus *Aphelenchoides* is based primarily upon morphometric differences with comparison of intricate morphological details. However, morphological similarity and morphometric plasticity often create ambiguities in using sole source of microscopy-based data for species diagnosis (Kanzaki, 2006). Disputes in *Aphelenchoides* species identification also arise due to inadequate description of several species, inaccessibility of literature, and lack of reliable details. In this regard, the molecular diagnoses based on ribosomal 18S SSU, ITS, and D2-D3 genes often provide reliable data for authentic species identification (Kanzaki & Giblin-Davis, 2012; Rybarczyk-Mydlowska *et al.*, 2012; Miraeiz *et al.*, 2017; Mobasser *et al.*, 2018; Handoo *et al.*, 2020; Gu *et al.*, 2025a, b), but such data are unavailable for the majority of the nominal *Aphelenchoides* species. Here, the combination of morphological and morphometric distinctions along with phylogenetic analyses based on 18S SSU, ITS, and D2-D3 genes confirmed the status of *A. subbotini* as a new species. The new species was found associated with a poisonous mangrove plant growing in a saline environment. Comprehensive studies in future may be directed in this area towards enumeration of the nematode assemblages associated with the root systems of the mangrove plant to understand clearly their survival strategies, adaptations and ecological specialisations.

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S. Das, A. Giri, S. K. Bera, S. K. Ghorai and V. Phani. *Aphelenchoides subbotini* sp. n. (Nematoda: Aphelenchoididae) из корней мангрового растения *Excoecaria agallocha* из Индии.

Резюме. *Aphelenchoides subbotini* sp. n. был выделен и описан в ходе исследования мангровых зарослей на восточном побережье Западной Бенгалии, Индия. Вид был выделен из некротизированных и повреждённых корней мангрового растения *Excoecaria agallocha*, известного как мангровый кустарник «ослепляющий глаз». Новый вид можно отличить от других известных видов рода *Aphelenchoides* по относительно небольшой длине тела ($538,9 \pm 47,6$ мкм), небольшим стилетом с хорошо выраженным базальным утолщением, репродуктивному тракту, протяжённость которого вместе с поствulварным маточным мешком составляет приблизительно 0,45 длины тела, положению вульвы примерно на уровне 65–70% длины тела при неизменённых вульварных губах, а также по вентрально изогнутому коническому хвосту, несущему тетрамуcronатный (редко тримуcronатный) шип с тупыми отростками. Молекулярная характеристика была проведена на основе анализа генов малой субъединицы 18S рДНК, ITS рДНК и D2–D3-расширяющего сегмента 28S рДНК. Филогенетический анализ, основанный на последовательностях генов 18S, ITS и D2-D3 рибосомальной ДНК, подтвердил чёткое обособление *A. subbotini* sp. n. как нового вида. Это первое сообщение о каком-либо виде рода *Aphelenchoides*, обнаруженном на мангровом растении *Excoecaria agallocha*, для данной местности.