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Journal of Threatened Taxa



Open Access

10.11609/jott.2026.18.9.29559-29790

www.threatenedtaxa.org

26 September 2026 (Online & Print)

18(9): 29559-29790

ISSN 0974-7907 (Online)

ISSN 0974-7893 (Print)





ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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Cover: Indian Grey Wolf *Canis lupus pallipes* and Smooth-coated Otter *Lutrogale perspicillata* sharing the territory in Chambal basin. Digital art on Procreate by Aakanksha Komanduri.



Characterization of *Aphelenchoides confusus* Thorne & Malek, 1968 (Nematoda: Rhabditida: Aphelenchoididae) occurring in a marine habitat of eastern India

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Abstract: *Aphelenchoides confusus* Thorne & Malek, 1968, was isolated and described during a survey on the east coast mangroves from Odisha, India. The females characterized by a short body ($596.6 \pm 20.1 \mu\text{m}$), a short stylet ($9.7 \pm 0.3 \mu\text{m}$) with small basal knobs, a vulva at 64.7–69.6% of body length with an unmodified lip, a well-developed post-uterine sac, and a conoid, rounded tail without a mucro. Morphometrics closely agree with the original description and other populations. Principal component analysis explained 91.67% of total variation and clustered the present population with previously reported populations, indicating morphometric congruence. This record extends the known host association of the species to mangrove plants.

Keywords: *Avicennia marina*, mangroves, morphotaxonomy, parasitic, PCA, pneumatophores.

Abbreviations: MBL—Median bulb length | MBW—Median bulb width | PCA—Principal component analysis | PUS—Post-uterine sac | SD—Standard deviation.

The genus *Aphelenchoides* Fischer, 1894 (Aphelenchoididae) includes nematodes exhibiting diverse feeding habits, ranging from mycophagy to phytophagy (Handoo et al. 2020). Members of this genus are distinguished by vermiform females possessing a functional anus and rectum, a relatively short stylet with basal swellings, a well-developed median bulb, a posteriorly positioned vulva, heat relaxed males assume a walking-stick shape with the tail region curled

ventrally, and bursa absent (Chanu et al. 2015). Globally, more than 200 species of *Aphelenchoides* have been described, with 54 species reported from India to date (Chanu & Mohilal 2023). Several species, including *A. besseyi*, *A. fragariae*, and *A. ritzemabosi* are recognised as economically important plant parasites that cause severe damage to agricultural and horticultural crops worldwide (Sanchez-Monge et al. 2015). Detection of foliar and root-associated *Aphelenchoides* species is often challenging, as external disease symptoms may not become apparent for extended periods (Jagdale & Grewal 2006).

Aphelenchoides confusus was first described by Thorne & Malek (1968) and was later recorded from *Morus alba* in Manipur, India, by Chanu et al. (2015). However, there have been no prior records of *Aphelenchoides* species associated with mangrove plants. The present study documents the occurrence of *A. confusus* in pneumatophores of *Avicennia marina*, representing the first evidence of an *Aphelenchoides* species in a mangrove habitat.

MATERIALS AND METHODS

Necrotic and damaged pneumatophores of *Avicennia marina* (Image 1) were collected on 22.vii.2025 from

Editor: Biplob Kumar Modak, Sidho-Kanho-Birsha University, Purulia, India.

Date of publication: 26 September 2026 (online & print)

Citation: Das, S. & S.K. Ghorai (2026). Characterization of *Aphelenchoides confusus* Thorne & Malek, 1968 (Nematoda: Rhabditida: Aphelenchoididae) occurring in a marine habitat of eastern India. *Journal of Threatened Taxa* 18(9): 29736–29742. <https://doi.org/10.11609/jott.10449.18.9.29736-29742>

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Funding: No external funding was received for the conduct of this research.

Competing interests: The authors declare no competing interests.

Acknowledgements: The authors thank the Coastal Laboratory members for technical assistance and Ardhendu Das Mahapatra and Subhankar Das for field and laboratory support.



Image 1. The pneumatophore of *Avicennia marina*.

from Talsari Beach, Odisha, India (21.6019° N, 87.4546° E). The roots were cut into small pieces, thoroughly washed with running tap water, and ground with a domestic mixer grinder. Nematodes were extracted by modified Baermann funnel technique (Whitehead & Hemming 1965). Finally, we hand-picked individual specimens under a stereomicroscope (MS Z-TR, Magnus) using a fine hairbrush. Specimens were heat-killed in hot triethanolamine-formalin fixative (TAF), and processed in Seinhorst's solutions for morphological analysis (Ravichandra 2014). After fixation, specimens were permanently mounted in anhydrous glycerin on clean glass slides for morphological and morphometric analyses. All morphological and morphometric measurements, along with the photomicrographs, were obtained using a Dewinter Ultima phase-contrast microscope equipped with a camera integrated with DIGI-4K software.

RESULTS

Aphelenchoides confusus Thorne & Malek, 1968 (Images 2 & 3, Tables 1 & 3)

Measurements: All the morphometric measurements

and de Man's ratio values are listed in Table 1.

Description: Females: Body short in length (571.4–616.4 μm), ventrally curved after fixation. Cuticle finely annulated. Lateral field with four incisures occupying approximately one-eighth of body width. Lip region slightly offset, 1.9–2.4 μm high and 4.1–5.4 μm wide. Stylet short (9.2–10.2 μm) with small basal knobs. Median bulb well-developed, oval to spheroid shaped (10.3–18.2 μm long and 7.3–15.8 μm wide), occupying about three-fourth of body width, with a centrally located refringent valve plate. Nerve ring located anterior to the median bulb. Excretory pore situated 62.4–68.1 μm from the anterior and situated posterior to the median bulb. Esophageal gland lobe aphelenchoid in form, extending dorsally over the intestine and provided with a short, arm-like ventral projection near the pharyngo-intestinal junction, extending 35–48 μm posterior to the base of the median bulb. Reproductive system monodelphic, anteriorly outstretched, occupying 35.9–39.8 % of body length, occasionally reaching median bulb. Vagina slightly anteriorly directed, uterus well developed, vulva a transverse slit located at 64.7–69.6 % of body length, with slightly elevated labial part. Post-uterine sac well

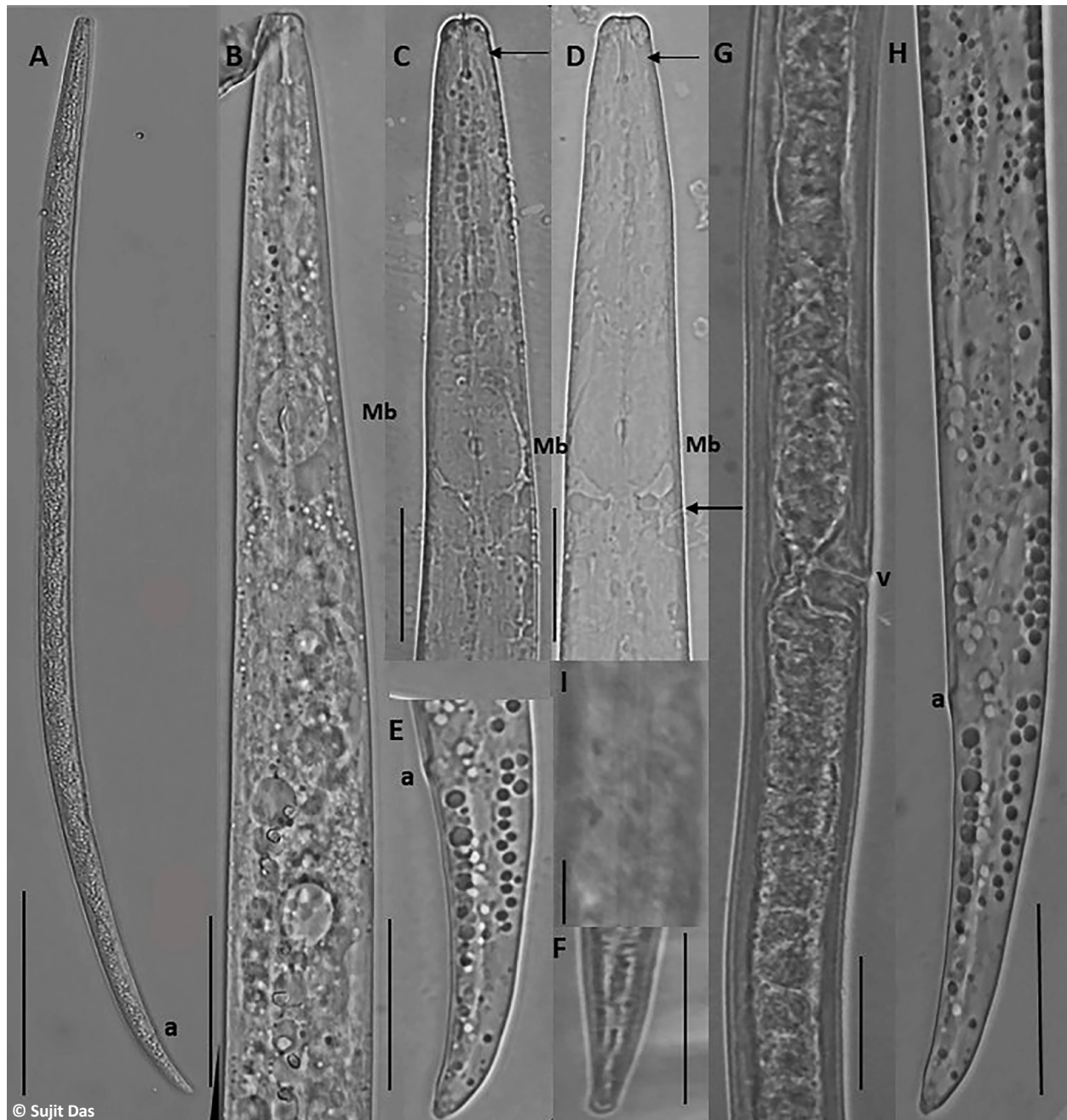


Image 2. Light photomicrographs of *Aphelenchoides confusus*: A—Entire female body | B—Anterior region with intestinal junction | C, D—Anterior region with secretory excretory pore (arrow) | E, F—Tail region | G—Female gonad and vulva with pus | H—Posterior region | I—Lateral line. (mb—median bulb | v—vulva | a—anus). Scale bars: A—100 μ m | B–I_20 μ m.

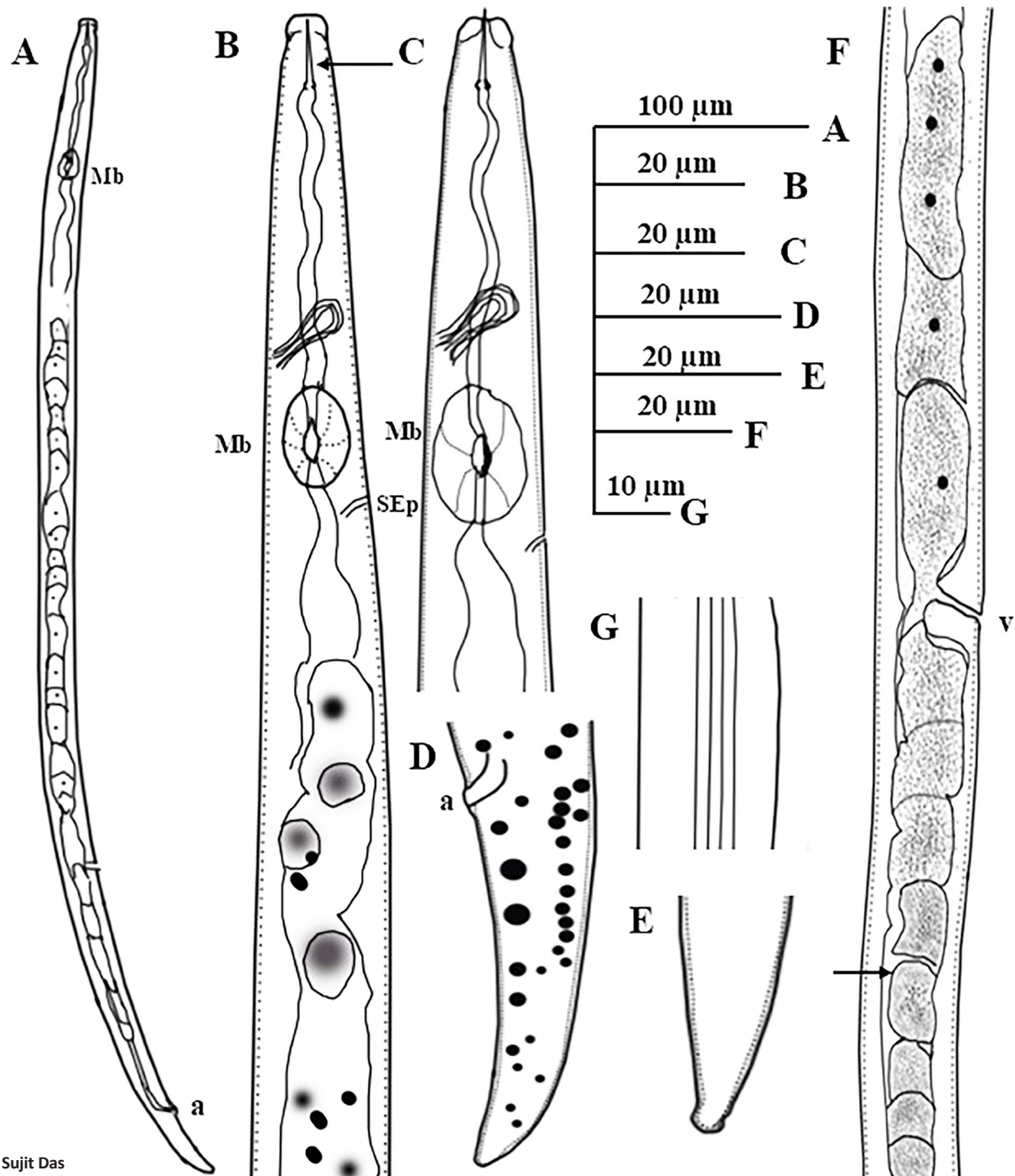
developed, extending posterior towards anus. Tail conoid, rounded, 39.3–43.7 μ m long, without mucro.

Male: Not observed.

Host and locality: Collected on 22.vii.2025 from pneumatophores of the white mangrove *Avicennia marina* at Talsari Beach, Odisha, India (21.6019° N, 87.4546° E).

Relationship

Based on the present morphological and morphometric observations, the studied population was identified as *Aphelenchoides confusus* Thorne & Malek, 1968. Comparative analysis showed close agreement with the original description in the principal diagnostic characters, including body length, a ratio, stylet length, and conoid tail shape. Minor differences were observed



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Image 3. Line drawings of *Aphelenchoides confusus*: A—Entire female body | B, C—Anterior region with secretory excretory pore | D, E—Tail with rounded tail terminus | F—Female gonad with vulva | G—Lateral line. (Mb—median bulb | Sep—secretory-excretory pore | v—vulva | a—anus).

in the de Man indices, with higher *b* (12.4–14.9 vs. 7.8–9.4) and *c'* (4.8–5.5 vs. 2.5–3.2) values, and a lower *c* value (9.4–9.9 vs. 17.5–19.0). These differences most likely reflect geographical or intraspecific variation (Table 2). *Aphelenchoides confusus* is visually similar to *A. obtusus* Thorne & Malek, 1968, and *A. avenae*

Kumari, 2012. However, *A. confusus* differs from *A. obtusus* with respect to larger body size (571.4–616.4 μm in *A. confusus* vs. 401–550 μm in *A. obtusus*), nerve ring location (Anterior to median bulb in *A. confusus* vs. posterior to median bulb in *A. obtusus*), phasmid presence at the base of the tail, higher *a* value (32.8–

Table 1. Morphometric parameters of *Aphelenchoides confusus*. All values are in micrometers (μm), and in the form of mean $\pm$ SD (range).

Morphometric characters	Females (n = 14)
	Mean $\pm$ SD (Range)
L	596.6 $\pm$ 20.1 (571.4–616.4)
a	33.6 $\pm$ 1.1 (32.8–35.9)
b	13.6 $\pm$ 1 (12.4–14.9)
c	9.8 $\pm$ 0.3 (9.4–9.9)
c'	5.2 $\pm$ 0.3 (4.8–5.5)
Lip height	2.25 $\pm$ 0.2 (1.9–2.4)
Lip width	4.7 $\pm$ 0.5 (4.1–5.4)
Body diameter	17.1 $\pm$ 1.1 (15.9–18.7)
V%	67.5 $\pm$ 1.6 (64.7–69.6)
Stylet	9.7 $\pm$ 0.3 (9.2–10.2)
MBL	15.3 $\pm$ 2.2 (10.3–18.2)
MBW	13 $\pm$ 2.4 (7.3–15.8)
MBL/MBW	1.1 $\pm$ 0.1 (1.1–1.4)
Anterior to median bulb	61.3 $\pm$ 2.4 (57.6–65.2)
Anterior to excretory pore distance	64.6 $\pm$ 2.2 (62.4–68.1)
Distance of nerve ring from anterior	40.7 $\pm$ 4.1 (38.2–46.9)
Vulva to anus distance	194.2 $\pm$ 5.3 (185.2–201.4)
Length of female gonad	208.6 $\pm$ 26 (186.2–245.7)
Post uterine sac length (PUS)	98.4 $\pm$ 11.6 (81.3–110.5)
PUS/maximum body width	5.5 $\pm$ 0.2 (5.1–5.9)
Tail length	42.2 $\pm$ 2 (39.3–43.7)

35.9 μm in *A. confusus* vs. 25–31 μm in *A. obtusus*), higher b value (12.4–14.9 μm in *A. confusus* vs. 7.3–9.6 μm in *A. obtusus*), smaller stylet (9.2–10.2 vs. 11–12); from *A. avenae* with respect to shorter body length (571.4–616.4 μm in *A. confusus* vs. 710–910 μm in *A. avenae*), number of the lateral line (Four in *A. confusus* vs. 10–14 in *A. avenae*), higher b value (12.4–14.9 μm in *A. confusus* vs. 6.2–7.5 μm in *A. avenae*), lower c value (9.4–9.9 μm in *A. confusus* vs. 26.5–45.4 μm in *A. avenae*), and position of the vulva (Table 3).

Statistical analysis

The first two principal components explained 91.67% of the total variation in female morphometrics, PC1 explained 68.11% of the variance, while PC2 explained 23.56%. PC1 represents the principal axis explaining the largest proportion of total variance and is predominantly influenced by b ($r = -0.426$), tail length ($r = -0.420$), c' ($r = -0.412$), c ($r = 0.408$), and V% ($r = 0.408$). These loadings indicate that variation in tail and body proportions accounts for most of the observed population differentiation, with moderate contributions from body

Table 2. Morphometric differentiation of *Aphelenchoides confusus* (India) with previously reported species. All values are in μm and in the form of mean $\pm$ SD (range).

	Present study	Thrace	Chanu et al. 2015	Cheri et al. 2016
n	14	4	10	7
L	596.6 $\pm$ 20.1 (571.4–616.4)	610 $\pm$ 26 (590–650)	620 $\pm$ 99.3 (480–790)	640 $\pm$ 76 (580–770)
a	33.6 $\pm$ 1.1 (32.8–35.9)	32.4 $\pm$ 1.8 (30–34.6)	36.5 $\pm$ 5.8 (28.6–46.6)	30.1 $\pm$ 1.2 (29–32)
b	13.6 $\pm$ 1 (12.4–14.9)	4.8 $\pm$ 0.2 (3.91–5)	3.5 $\pm$ 0.6 (2.5–4.4)	8.4 $\pm$ 0.5 (7.8–9.4)
c	9.8 $\pm$ 0.3 (9.4–9.9)	24.1 $\pm$ 2.2 (21.2–26.4)	30.4 $\pm$ 4.8 (23.8–38.8)	18.2 $\pm$ 0.5 (17.5–19)
c'	5.2 $\pm$ 0.3 (4.8–5.5)	2.0 $\pm$ 0.1 (1.9–2.3)	2.2 $\pm$ 0.2 (2–2.4)	2.9 $\pm$ 0.2 (2.5–3.2)
Stylet	9.7 $\pm$ 0.3 (9.2–10.2)	18.2 $\pm$ 1.8 (16.2–20.4)	15 $\pm$ 2.2 (10.2–17)	12.4 $\pm$ 0.7 (11–13)
Tail	42.2 $\pm$ 2 (39.2–43.7)	24.8 $\pm$ 0.1 (24.7–24.9)	9.3 $\pm$ 0.8 (20.4–25.5)	31.7 $\pm$ 2.1 (29–35)
V%	67.5 $\pm$ 1.6 (64.7–69.6)	75.3 $\pm$ 0.47 (75–76)	77.7 $\pm$ 0.7 (74–78)	71.4 $\pm$ 1.6 (68.4–73.6)

length ($r = 0.256$) and a ($r = 0.225$). PC2 explains the second-largest proportion of variance and is mainly driven by a ($r = 0.563$) and stylet length ($r = -0.684$), followed by body length ($r = 0.326$) (Table 5). Principal component analysis (PCA) of morphometric characters from the present and previously reported populations of *Aphelenchoides confusus* revealed evident intraspecific variation (Table 4). The present population formed a distinct cluster, primarily associated with tail length and the b and c' ratios, whereas the population of Chanu et al. (2015) was characterized by greater body length and higher a and c values. The populations reported by Cheri et al. (2016) and Thrace occupied separate positions, with the latter showing a close association with stylet length. These differences likely represent geographical variation in morphometric traits rather than species-level divergence (Image 4).

Overall, PCA revealed clear intraspecific morphometric variation among populations, likely reflecting geographic and environmental influences.

Remarks

The present study contributes to the existing knowledge of *Aphelenchoides* taxonomy by providing updated polyphasic data for *A. confusus* found in a mangrove-associated habitat in eastern India. This record expands current knowledge of *Aphelenchoides* and provides a framework for future studies on its adaptation, host associations, and ecological distribution.

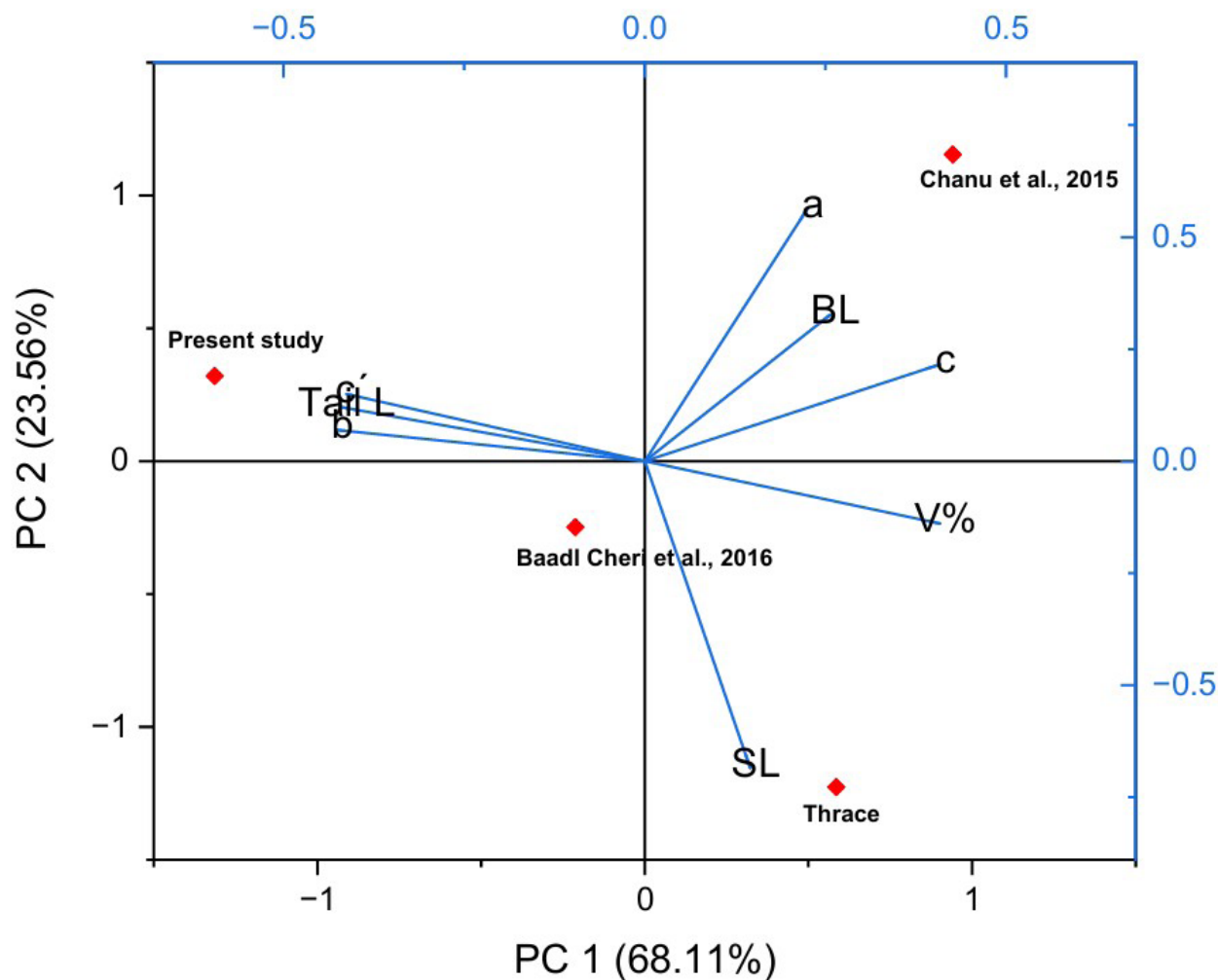


Image 4. PCA biplot of standardized morphometric characters of *Aphelenchoides confusus* populations. PC1 and PC2 explained 68.11% and 23.56% of the total variance, respectively.

Table 3. Comparative morphometric data of *Aphelenchoides confusus* from closely related species.

Characters	<i>A. confusus</i> (Present study)	<i>A. confusus</i> (Thorne & Malek, 1968 / Chanu et al., 2015)	<i>A. obtusus</i> Thorne & Malek, 1968	<i>A. avenae</i> Kumari, 2012
Body length (μm)	571.4–616.4	Comparable	401–550	710–910
Body posture	Straight to slightly curved	Similar	Similar	Similar
Lateral field	4 incisures	4 incisures	4 incisures	10–14 incisures
Stylet length	Short	Short	Longer	Longer
Nerve ring position	Anterior to median bulb	Anterior to median bulb	Posterior to median bulb	Anterior
Vulva position (V%)	64.7–69.6	Similar	Different	Different
Phasmids	Present near tail base	Present	Absent or indistinct	Present
Tail shape	Conoid, rounded	Conoid	Conoid	Conoid
Tail length (μm)	39.3–43.7	Similar	Shorter	Variable
a (μm)	32.8–35.9	Similar	25–31	Lower
b (μm)	12.4–14.9	7.8–9.4	7.3–9.6	6.2–7.5
c (μm)	9.4–9.9	17.5–19.0	Higher	26.5–45.4
c' (μm)	4.8–5.5	2.5–3.2	Lower	Lower

Table 4. Factor scores of the variable of the different populations of *Aphelenchoides confusus*.

Observation	PC1	PC2
Present study	-1.314	0.320
Thrace	0.584	-1.226
Chanu et al. 2015	0.941	1.154
Cheri et al. 2016	-0.211	-0.248

Table 5. Factor scores of the different morphometric features for different populations of *Aphelenchoides confusus*.

Characters	PC1	PC2
BL	0.256	0.326
a	0.225	0.563
b	-0.426	0.069
c	0.408	0.215
c'	-0.412	0.149
SL	0.145	-0.684
V%	0.408	-0.138
Tail L	-0.420	0.120

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Journal of Threatened Taxa is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64



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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

September 2026 | Vol. 18 | No. 9 | Pages: 29559–29790

Date of Publication: 26 September 2026 (Online & Print)

DOI: 10.11609/jott.2026.18.9.29559-29790

www.threatenedtaxa.org

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