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Cover: Orange Oakleaf *Kallima inachus* with colour pencils and watercolor wash by Elakshi Mahika Molur adapted from a workshop by Lenin Raj.



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COMMUNICATION

## A compendium of *Aphelenchoides* (Fischer, 1894) (Nematoda: Tylenchina: Aphelenchoidea) nematodes with the description of a new species from Manipur, India

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**Abstract:** The present compendium is based on the findings of a research work on the survey of nematodes belonging to the family Aphelenchoidea in the northeastern states of India and the literature available on this particular species, mainly from Manipur. During the study, a total of 12 *Aphelenchoides* spp. were found, among which six species were reported for the first time from Manipur. A new species, *Aphelenchoides oryzae* is also described in the present article. The present study will help in making us understand the biodiversity status of *Aphelenchoides* nematodes in the region. Diagnosis of the species and illustrations along with dichotomous keys are provided in the manuscript.

**Keywords:** Aphelenchid, Coconut tree, fungivore, food-web, soil fertility, soil dwelling, *Pinus* sp., *Morus* sp., Orange plant and species richness.

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**Author contributions:** LBC conducted the survey, collected samples, processed the samples, extracted nematodes, identified the nematodes, did statistical calculations and wrote the manuscript. NM helped in designing the survey and collection, in the identification of the nematodes, and in final proofing of the manuscript.

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## INTRODUCTION

Forest conservation improves ecosystem functions and will help to protect natural biodiversity. Plant communities are the critical indicators for forest restoration. Below-ground diversity relates very closely with above-ground biodiversity. Plant parasitic nematodes are found in every soil of varied ecosystems. Nematodes are often sensitive to habitat disturbance, showing the characteristic sequence of recolonization after disturbance. Furthermore, they are represented in a wide array of trophic groups as herbivores (Tylenchid), bacterivores & fungivores (Aphelenchid), omnivores (Dorylaimid), and predators (Mononchid) reflecting resource availability and changes of environmental conditions in the soil providing information on succession and changes in decomposition pathways in the soil food – web, nutrient status & soil fertility, acidity, and the effects of soil contaminants (Yeates & Bongers 1999).

Bacterial as well as fungal feeding nematodes like aphelenchids have a high carbon: nitrogen (C:N) ratio ( $\pm 5.9$ ) than their substrate ( $\pm 4.1$ ), consuming bacteria, they take in more N than necessary for their body structure. The excess nitrogen is excreted as ammonia. The bacterial and fungal feeding nematodes' community in the top 15 cm of the field soil mineralizes N at rates increasing to  $1.01 \mu\text{g} - \text{N g}^{-1} \text{d}^{-1}$  in the rhizosphere (Ferris et al. 1995; 1996; 1997).

Aphelenchid nematodes have diverse habitats. Several aphelenchids are associated with insects, some spending a part of their life - cycle in insects besides being phytophagous, while others are mycetophagous. Some forms of aphelenchids are true plant parasites and are, therefore, economically significant. Of the available aphelenchid nematodes, three species are major pests of agricultural and horticultural crops, i.e., white-tip nematode *Aphelenchoides besseyi*; Red-ring nematode of coconut *Rhadinaphelenchus cocophilus*, and pine-wilt nematode *Bursaphelenchus xylophilus* respectively in the world. So far, 138 species of *Aphelenchoides* (Fischer, 1894) have been identified, of which India contributed more than 12 species.

Recognizing the importance of bacterial and fungal decomposition in forest ecosystems, survey for Aphelenchid nematodes is very important in every region of the Earth. Recently, a survey was conducted on the *Aphelenchoides* nematodes in the different ecosystems of the north-eastern region of India. During the work, 12 *Aphelenchoides* were encountered from Manipur, among which six species were recorded for the first time from this particular region. The species

encountered were *Aphelenchoides aligarhiensis* (Siddiqi et al., 1967); *A. baquei* (Maslen, 1978); *A. confusus* (Thorne & Malek, 1968); *A. minor* (Seth & Sharma, 1986); *A. swarupi* (Seth & Sharma, 1986); *A. vigor* (Thorne & Malek, 1968); *A. dhanachnadi* (Chanu et al., 2012) and *A. neoechinocaudatus* (Chanu et al., 2012); *A. aeralis* (Chanu et al., 2015); *A. longistylus* (Chanu & Mohilal, 2014); *A. neominoris* (Chanu & Mohilal, 2014), and *A. manipurensis* (Chanu & Mohilal, 2018). These species are presented along with their dichotomous keys.

## METHODS

### Study site

Manipur has a geographical area of 22,327 km<sup>2</sup>, situated at an altitude of 790 m with a longitude of 24.733333333333334 and a latitude of 93.96666666666667 (Figure 1). Soil samples were collected from different localities of Manipur.

### Methodology

For collection of soil samples around the rhizospheric region of a particular host, 500 g of soil around the plant from 8 different sides were taken. The soil was mixed together thoroughly. From the thoroughly mixed soil, again 500 g were taken, serving as the sample soil for a particular host plant or tree. The samples were processed for extraction of nematodes through the Cobb's (1918) sieving and decanting method and Baermann's funnel technique. Collected nematodes through the process were fixed with warm formalin alcohol (F.A) (4:1) for 24 hours and afterward, dehydrated under the Seinhorst (1952) dehydration techniques. Dehydrated nematodes were mounted on clean non-greased slides with dehydrated glycerin as mountants. The specimens were studied, measurements taken and diagrams were drawn using a drawing tube attached to a microscope.

## RESULTS

### SYSTEMATICS

#### *Aphelenchoides aeralis* Chanu et al., 2015

(Figure 2, Table 1 & 3)

#### Diagnosis

Female: Body contour S-shaped with fine cuticular striations having a lateral field with two incisures. The cephalic region set off with weak sclerotization, spear  $15.3 \mu\text{m}$  long with small basal thickenings. The median



Figure 1. Expanded map of Manipur.

oesophageal bulb is oblong with centered valvular apparatus. Oesophagus overlap intestine. Nerve ring behind the esophagus-intestinal junction. Excretory pore above nerve ring. Vagina with sphincter and raised vulval lips. Gonads monoprodelfic and oocytes are arranged in single rows. Spermatheca is filled with sperms and with uterine sac. Tail 42.5–48.2  $\mu\text{m}$  long, conical, and with small single mucro.

Male: Body more curved at tail region. Testis long and outstretched. Spicule is typical of the genus. Dorsal limb without knob. The capitulum and rostrum very well developed. Post anal genital papillae, one pair situated above the tail terminus. Tail terminating into a long spine-like mucro.

Remark: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

***Aphelenchoides aligarheinsis* Siddiqi et al., 1967**  
(Table 1)

**Diagnosis**

Female: Body contour slightly curved. Cuticle fine, striations about 1.7  $\mu\text{m}$  at mid-body. Lip region set-off round. Lateral fields with four incisures. Stylet slender 10.2  $\mu\text{m}$  long with weakly developed basal knobs. Oesophagus typical of the genus. Excretory pore at level of the nerve ring. Ovary outstretched and oocytes arranged in single row. Spermatheca oblong with

**Table 1. Morphometric data of female species of *Aphelenchooides* spp. from Manipur.**

Character	<i>A. aeralis</i> Chanu et al., 2013	<i>A. aligar-hiensis</i> Siddiqi et al., 1967	<i>A. ba-guie</i> Maslen, 1973	<i>A. confusus</i> Thorne & Malek, 1968	<i>A. dhanach-adi</i> Chanu et al., 2012	<i>A. longistyl-us</i> Chanu & Mohilal, 2014
L	0.46–0.51	0.42–0.61	0.58–0.74	0.48–0.79	0.37–0.50	0.59–0.66
a	32.1–33.8	25.1–30.2	35.2–38.2	28.6–46.6	25.2–36.1	34.4–35.85
b	6.15–7.2	6.1–8.1	5.2–7.2	2.5–4.4	6.6–7.7	8.53–9.81
b'	3.66–4.1	-	3.6–4.2	-	3.3–7.4	6.03–6.49
c	9.8–10.1	11.4–16.1	14.2–16.2	23.8–38.8	5.3–7.4	13.67–14.58
c'	5–6.4	2.5–3	3.8–4.4	2–2.4	6.6–9.2	5.6–6.14
V	66.7–68.2	64.2–71.8	62.8–68	76.8–78.6	57.9–62.9	67.78–69.76
G <sub>1</sub>	26.9–28.2	30.6–34.8	42.4–48.6	29–42.6	13.6–15.3	24.2
G <sub>2</sub>	-	-	14–16.2	4.3–18.2	-	39.39–43.08
Spear	15.3	10.2	10.2–12.8	10.2–17	-	-
Oesophago-us	125.8	73.1–81.6	146.4–152	134.7–241.4	95.2–129.0	98.61–102.07
Nerve ring	68			90.1–119	56.1–73.1	-
Excretory pore	62.9	52.7	82.4–94.2	86.7–115.6	61.2–85.0	-
Tail	42.5	35.7–39.1	38.4–46.2	8.5–10.2	62.9–85.0	41.52–48.44
ABD	8.5	11.9–15.3			8.5–10.2	6.92–8.65
PUS	-	35.7–37.1	92.4–96.4	20.4	-	-

discoidal spermatozoa. The Uterine sac is five times body width in length. Tail 35.7–39.1 µm long, elongate-conoid, ventrally arcuate, rounded with spine like mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al., (2015).

#### ***Aphelenchooides baguei* Maslen, 1978**

(Table 1)

##### **Diagnosis**

Female: Body contour slightly ventrally curved. Fine cuticularisation with four incisures throughout body length. Cephalic region set off flattened. Spear 10.2–12.8 µm long with a small basal knob. Oesophagus typical of aphelenchoid. Mono-prodelphic reproductive system and ovary outstretched. Vulva is a transverse slit with slightly protruding lips. Oocytes in single row with a uterine sac. Tail 38.4–46.2 µm long, about 4–5 times anal body width long, terminus with a small ventral mucro which is multi-papillate almost to its tips.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

#### ***Aphelenchooides confusus* Thorne & Malek, 1968**

(Table 1)

##### **Diagnosis**

Female: Body contour gradually tapering near extremities. Cuticle with fine striations and lateral fields marked by four fine lines. Cephalic region set-off. Spear 10.2–17 µm long. Oesophagus typical aphelenchoid with massive valvular apparatus. Nerve ring is behind the oesophageal bulb. Excretory pore at level of the nerve ring. Hemizonid posterior to excretory pore.

Vulva with protuberant labia and vagina directed forward. Uterus spheroid-shaped, filled with sperm. Ovary outstretched and post uterine sac collapsed. Tail 20.4–25.3 µm long without a mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

#### ***Aphelenchooides dhanachandi* Chanu et al., 2012**

(Figure 3, Table 1)

##### **Diagnosis**

Female: Body contour slender ventrally curved. Lateral field marked by three incisures. The body cuticle is fine. The cephalic framework is high. Spear 13.6–15.3 µm long, slender with indistinct basal knobs. Oesophagus typical with tamarind seed-shaped median bulb. Nerve ring behind the median bulb, 59.5–69.0

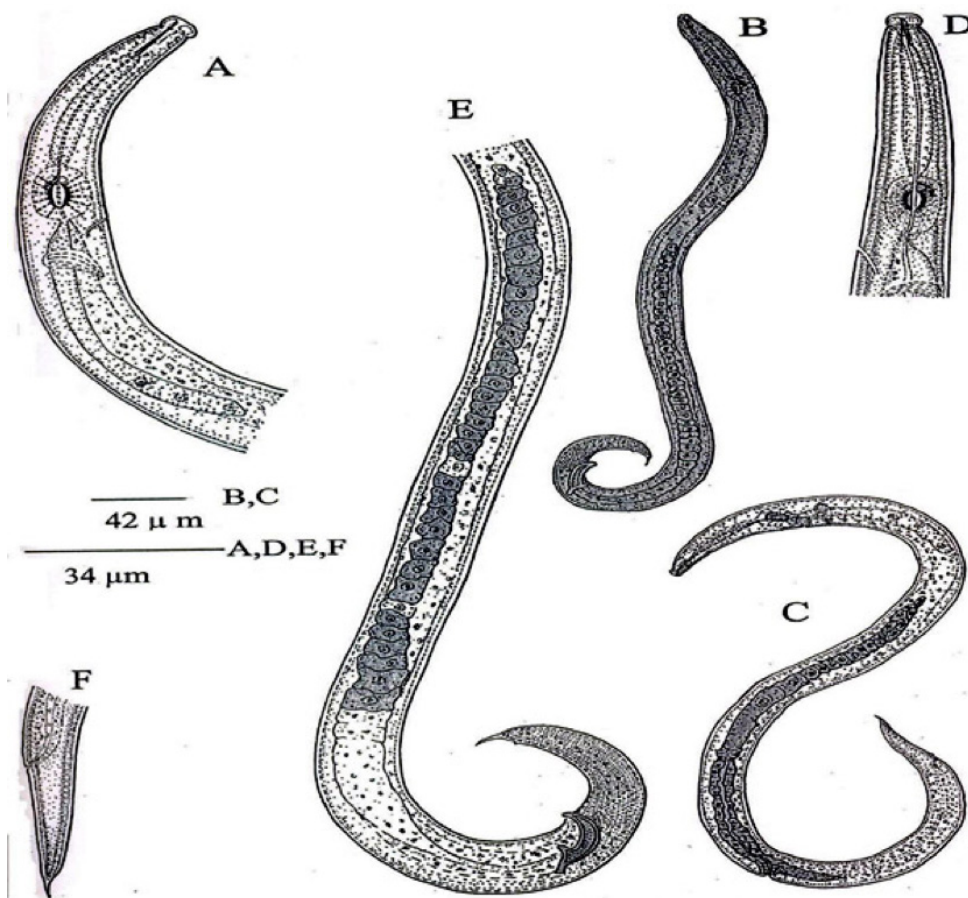


Figure 2. *Aphelenchooides aeralis* Chanu et al., 2013: A—Anterior part of Female body | B—Entire body of Male | C—Entire body of Female | D—Anterior body part of male | E—Posterior end of body (enlarged) | F—Tail region of Female.

$\mu\text{m}$  long. Excretory pore at the level of the nerve ring. Oesophageal gland lying dorsally along the intestine.

Monoprodelfic reproductive system and oocytes arranged in a single row with uterine sac. Vulva protrudes in some species. Tail 62.9–98.0  $\mu\text{m}$  long, highly curved ventrally tapering into a pointed terminus.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2012).

#### *Aphelenchooides longistylus* Chanu & Mohilal, 2014

(Figure 4, Table 1 & 3)

##### Diagnosis

Female: Body contour slightly curved with fine annulation. Lateral fields with four longitudinal lines merge into two lines at around the tail region. The cephalic region indistinctly set off with six equal lips. Spear 24.22  $\mu\text{m}$  long with indistinct basal knobs. Procorpus straight, median bulb spherical to pyriform in shape. Excretory pore at the base of the median

bulb. The vulva is a transverse slit. The monoprodelfic reproductive system and oocytes are arranged in a single row. Uterine sac well developed. The tail gradually tapers into a cylindrical tube, terminating in a ventral prong tip.

Male: Slightly smaller than female. Tail slender with single terminal mucro. Spicules absent about 24.22  $\mu\text{m}$  long. Testis single, 335.62–342.45  $\mu\text{m}$  long.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2014).

#### *Aphelenchooides manipurensis* Chanu & Mohilal, 2018

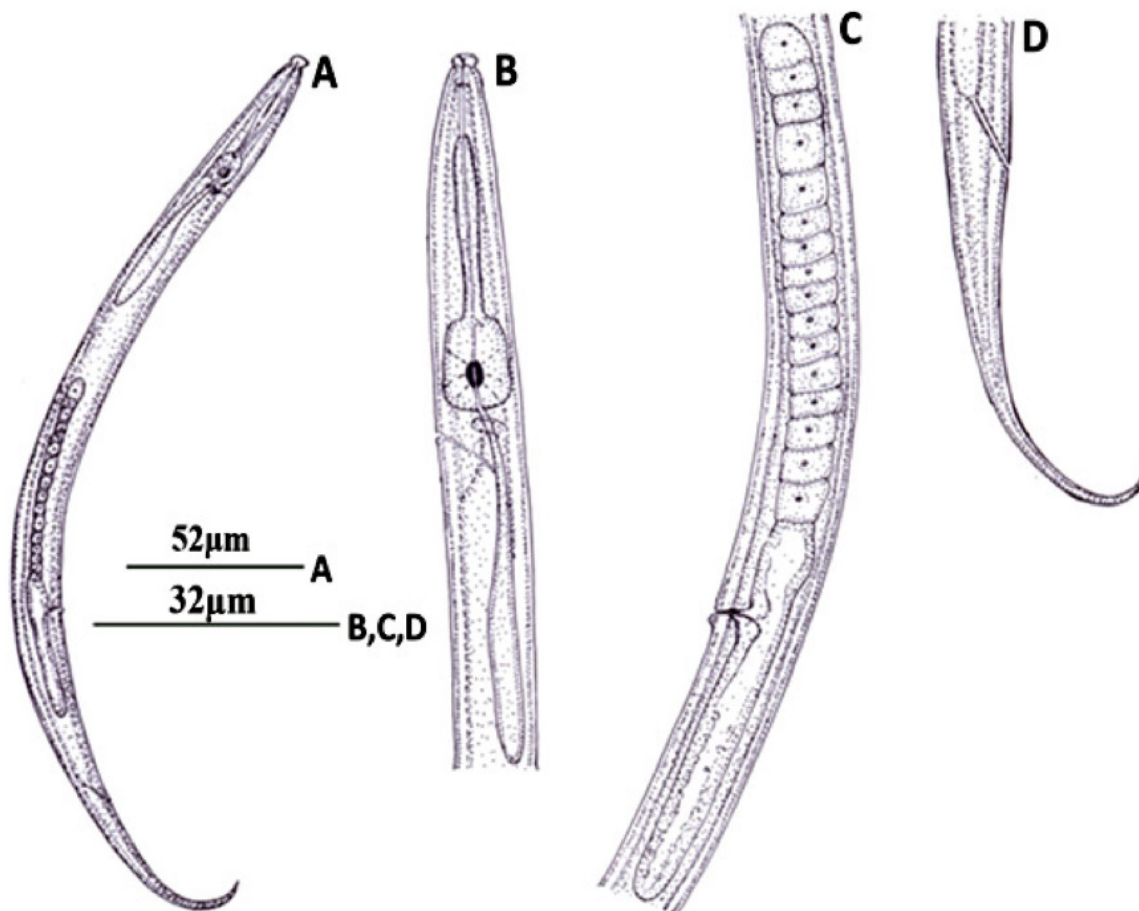
(Figure 5 & 6, Table 2 & 3)

##### Diagnosis

Female: Body contour cylindrical, ventrally arcuate, the cephalic framework set off and flat. Cuticle marked by fine annulus. Lateral fields with two incisures. Spear short, 10.33–13.84  $\mu\text{m}$  long with small rounded basal knobs. The median bulb is spherical and basal bulb bifurcated. Excretory pore at 51.9–76.12  $\mu\text{m}$  from anterior end of body. Nerve ring behind median bulb,

**Table 2.** Morphometric data of female species of *Aphelenchooides* spp. from Manipur.

Characters	<i>A. manipurensis</i> Chanu & Mohilal, 2018	<i>A. minor</i> Seth & Sharma, 1986	<i>A. neoechino-</i> <i>caudatus</i> Chanu et al., 2012	<i>A. neominoris</i> Chanu & Mohilal, 2014	<i>A. swarupi</i> Seth & Sharma, 1986	<i>A. vigor</i> Thorne & Malek, 1968
Length	0.294–0.461	0.28–0.36	0.53–0.60	0.35–0/43	0.52–0.68	0.44–0.49
a	24.28–38.14	18.2–26.2	25.5–28.6	36.29–40.6	34.4–42.4	24.8–29.3
b	6.3–8.65	4.8–7.2	8.3–9.0	4.23–6.51	5.8–8.2	2.7–4.8
b'	3.61–8.15	2.4–4.4	4.5–5.0	6.03–6.49	-	5.6–8.6
c	12.47–16.46	12.2–16.2	11.0–11.7	13.53–36.29	12.2–16.2	12.4–30.3
c'	3–4.25	2.2–2.6	5.3–5.4	1.75–3.75	2.2–2.6	1.5–3.5
V	68.88–71.69	66.4–72.2	64.4–64.9	69.95–71.65	66.4–72.2	69.2–88.6
Stylet	10.38–13.84	48.2–56.2	11.9	8.65	8.2–10.2	11.9–13.6
G <sub>1</sub>	24.34–33.01	4.2–5.2	-	41.87–50.0	32.4–36.4	33.5–45.9
G <sub>2</sub>	-	4.8–8.2	-	-	-	10.5–13.3
Oesophagus	-	-	119.0122.4	67.47–83.04	-	102–164.9
Nerve ring	-	-	66.3–68.0	-	-	74.8–88.4
Exc. Pore	-	47.6–54.6	62.9–64.6	-	-	61.2–78.2
Tail	20.76–31.14	22.4–30.8	45.9–54.4	12.11–25.95	32.3–37.4	15.3–35.7
ABD	6.92–8.65	-	8.5–10.2	6.92	8.5–12.4	10.2



**Figure 3.** *Aphelenchooides dhanachandi* Chanu et al., 2012: Female A—Entire body | B—Anterior region | C—Reproductive system | D—Tail region.



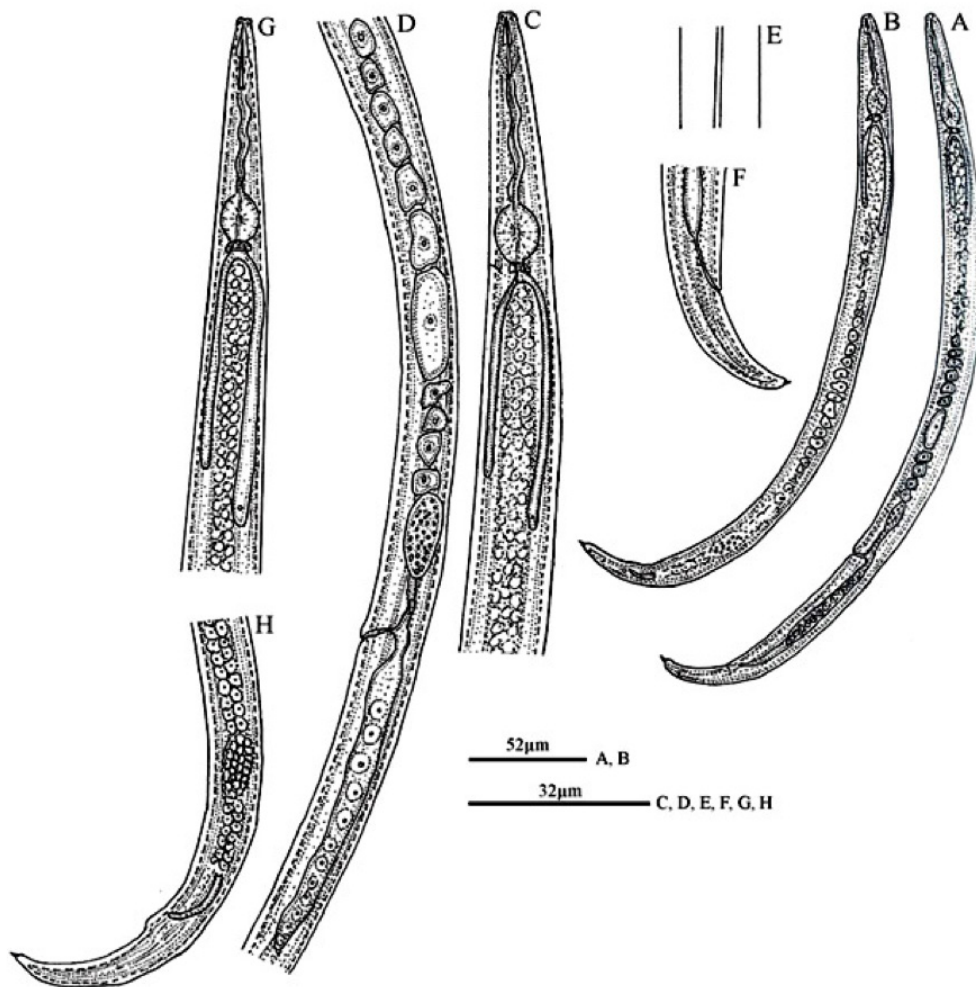
ovary single, outstretched, and oocytes arranged in single row reaching the basal bulb. Spermatheca elongated oval with sperms. Uterine sac filled with sperms and ventral rounded tip. Vulva protuberant and vagina at right angle to the body. Tail curved ventrally with rounded tip with a small mucro at tip.

Male: Body ventrally curved. Tail conoid with mucronated lip. Testis 138.4–190.3  $\mu\text{m}$ . spermatocytes in single row, spicules simply arcuate, rostrum rounded, and prominent apex. Three pairs of sub ventral papillae present towards tip of spicule. Bursa and gubernaculum absent.

Remarks: The morphometric details of the present species conform well with those described by Chanu & Mohilal (2018).

**Table 3. Morphometric data of male species of *Aphelenchooides* spp. from Manipur.**

Characters	<i>A. aeralis</i> Chanu et al., 2013	<i>A. longistylus</i> Chanu & Mohilal, 2014	<i>A. manipurensis</i> Chanu & Mohilal, 2018
L	0.46–0.51	0.562–0.62	264.69–320.05
a	29–29.41	38.2–42.0	25.5–33.39
b	5.9–6.92	7.24–8.33	25.59–41.52
b'	3.4	3.25–5.60	3.47–4.02
c	8.7	13.54–17.45	12.0–13.91
c'	4.2	4–6.2	2.4–6
T	46.71–47.8	92.61–102.84	50.89–61.09
Testis	46.71–47.8	335.62–342.45	138.4–190.3
Spicule	23.8–25.95	24.22	10.38–17.3
Tail	51.0–55.36	41.52–46.23	20.76–25.95
ABD	11.9–13.84	10.32	6.92–8.65
Stylet	15	24.22	10.38–13.84



**Figure 4. *Aphelenchooides longistylus* Chanu & Mohilal, 2014: Female. A—Entire body | C—Anterior body | D—Reproductive system | E—Lateral lines | F—Tail region. Male. | B—Entire body | G—Anterior body | H—Tail region.**

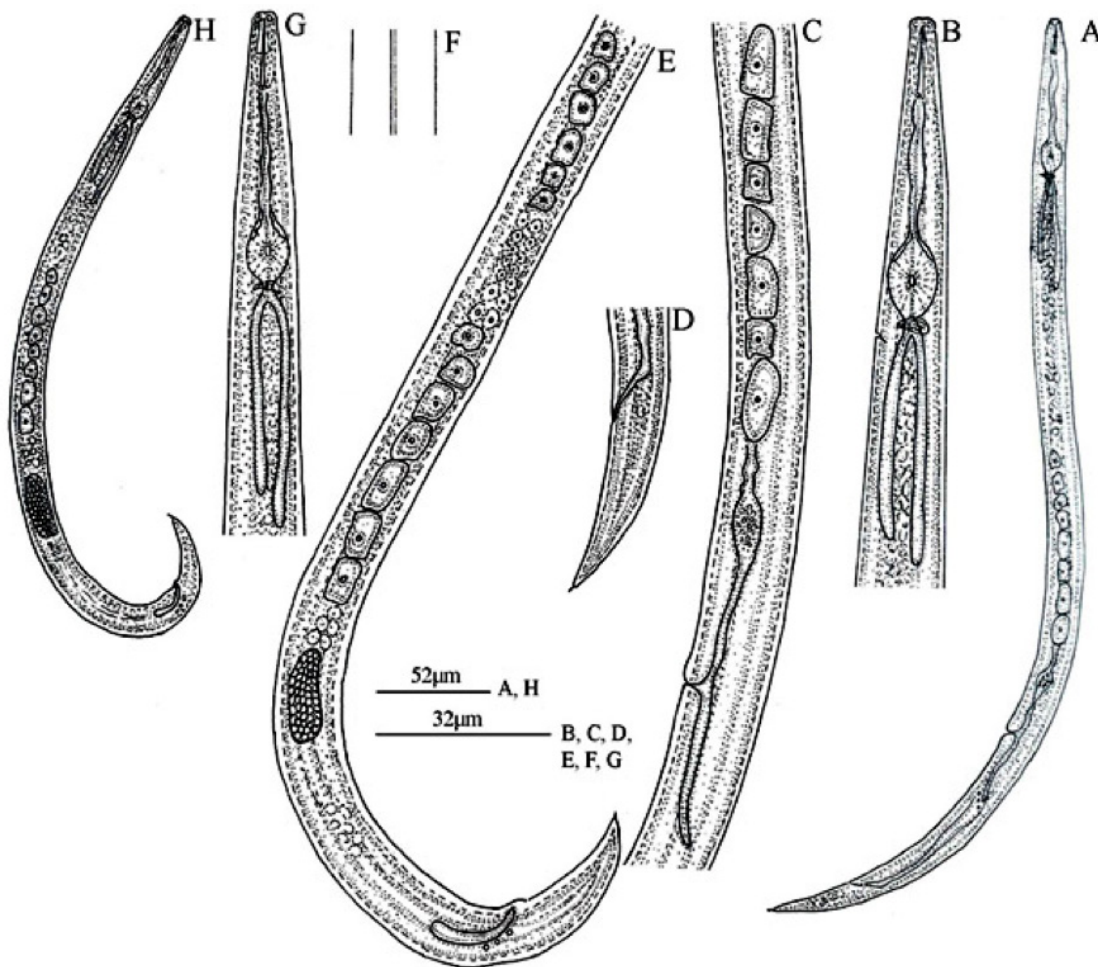


Figure 5. *Aphelenchoides manipurensis* Bina & Mohilal, 2018 from Manipur University Campus: Female. A—Entire body | C—Anterior body | D & I—Reproductive system (variation) | E—Body incisure | F—Anal region. Male. B—Male | Entire body | G—Anterior region | H—Posterior body.

Table 4. Species of *Aphelenchoides* spp. with their hosts and localities.

Species	Host	Locality
<i>A. aeralis</i> Chanu et al., 2015	Pine tree, <i>Pinus roxburghii</i> Sarg	Nongpok Sekmai, Thounal District
<i>A. aligarhiensis</i> Siddiqi et al., 1967	Pine tree, <i>Pinus kesiya</i> Royle	Khuga Dam, Churhandpur District
<i>A. baguei</i> Maslen, 1973	<i>Morus alba</i> Lin, <i>Pinus kesiya</i> Royle	Kakwa Naorem Leikai, Imphal West District; Keibul Lamjao, Bishnupur District
<i>A. confusus</i> Thorne & Malek, 1968	<i>Morus alba</i> Linn	Matai garden, Imphal East District
<i>A. minor</i> Seth & Sharma, 1986	<i>Morus alba</i> Linn	Kalika Village, Iribung, Kyamgei, Imphal East District; C. I. College, Bishnupur district; Govt. Silkfarm Wangbal, Thoubal district; Regional Tasar Research Station, Chingmeirong, Imphal West district.
<i>A. swarupi</i> Seth & Sharma, 1986	<i>Morus alba</i> Linn, <i>Morus indica</i> Linn, <i>Pinus roxburghii</i> Sarg	Regional Tasar Research Station, Chingmeirong, Imphal West District; Bishnupur ward no. 4, Bishnupur district; Nongpok Sekmai, Thoubal District
<i>A. vigor</i> Thorne & Malek, 1968	<i>Morus indica</i> Linn	Bishnupur ward no. 4, Bishnupur District
<i>A. dhanachandi</i> Chanu et al., 2012	Mulberry plant, <i>Morus alba</i> L.	Yurembam Rose Garden, Imphal West District
<i>A. neoehinocaudatus</i> Chanu et al., 2012	Mulberry plant, <i>Morus alba</i> L.	Yurembam Rose Garden, Imphal West District
<i>A. neominoris</i> Chanu & Mohilal, 2014	Orange plants	Siblong, Chandel district
<i>A. longistylus</i> Chanu & Mohilal, 2014	Coconut tree ( <i>Cocos nucifera</i> )	Ningsing Khul, Jiri, Imphal West District
<i>A. manipurensis</i> Chanu & Mohilal, 2018	Rooten wood lock	Manipur University campus, Canchipur.

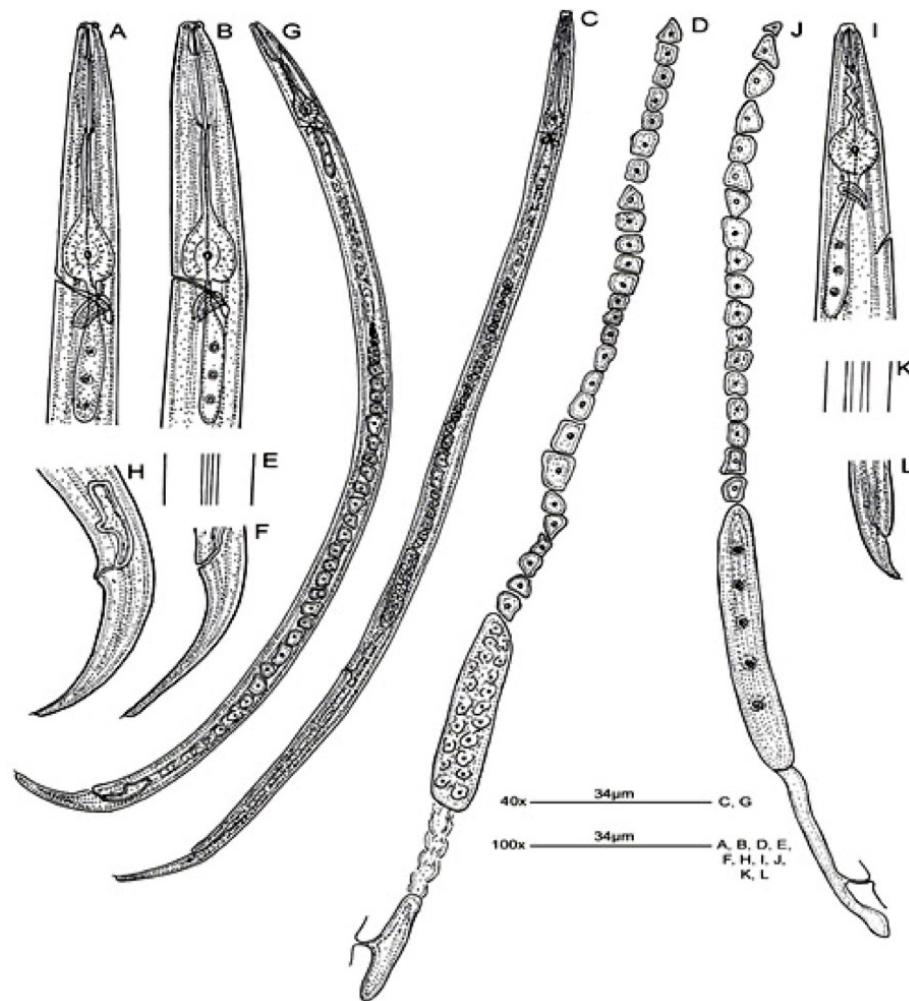


Figure 6. *Aphelenchooides manipurensis* Bina & Mohilal, 2018 from Nongpok Sekmai Pine Reserve Forest. Female: A—Entire body | B—Anterior body | C—Reproductive system | D—Anal region | E—Body incisures. Male: E—Reproductive system | G—Anterior region | H—Entire body.

#### *Aphelenchooides minor* Seth & Sharma, 1986

(Table 2)

##### Diagnosis

Female: Body contour straight to slightly curved ventrally. Lateral fields with three incisures. Cephalic framework set off without annulation. Spear 4.8–8.2  $\mu\text{m}$  long with indistinct basal thickenings. Oesophagus is aphelenchoid type with squarish muscular median bulb with a flat base and crescentic wave. Excretory pore at level of nerve ring. Vulva, a transverse slit with prominent lips. Oocytes are arranged in single row with a uterus. Tail 22.4–30.8  $\mu\text{m}$  long, rounded with ventral mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2018).

#### *Aphelenchooides neoechinocaudatus* Chanu et al., 2012

(Figure 7, Table 2)

##### Diagnosis

Female: Body contour is slender with three lateral incisures. The cephalic region is slightly set off. Stylet slender 11.9  $\mu\text{m}$  long without basal swellings. Oesophagus typical, median bulb elongated pear-shaped. Nerve ring 66.3–68.0  $\mu\text{m}$  long. Excretory pore at 62.9–64.4  $\mu\text{m}$ , at the level of the nerve ring. The oesophageal gland was dorsal to the intestine. Reproductive system monoprodelfic and oocytes arranged in a single row, and uterine sac well developed. Tail 45.9–54.4  $\mu\text{m}$  long, short, and pointed with a ventral mucro.

Male: not found

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2012).

***Aphelenchooides neominoris* Chanu & Mohilal, 2014**

(Figure 8, Table 2)

**Diagnosis**

Female: Body contour straight, tapering towards both extremities with four incisures in lateral field. Cephalic framework smooth and set-off. Spear 8.65  $\mu\text{m}$  long with distinct stylet knobs. Procorpus zig-zag, coiled, strongly rounded corpus with sclerotized plates and elongated gland lobe, dorsal to the intestine. The excretory pore is close to the nerve ring. Vulval lips protrude with an inclined vagina. Monoprodelfic reproductive system,

oocytes are arranged in a single row reaching up to the oesophageal bulb. Spermatheca large elongated and uterine sac empty. The anterior lip of the anus protrudes, the tail bluntly rounded, 12.11–25.95  $\mu\text{m}$  in length, with a small hair-like mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu & Mohilal (2014).

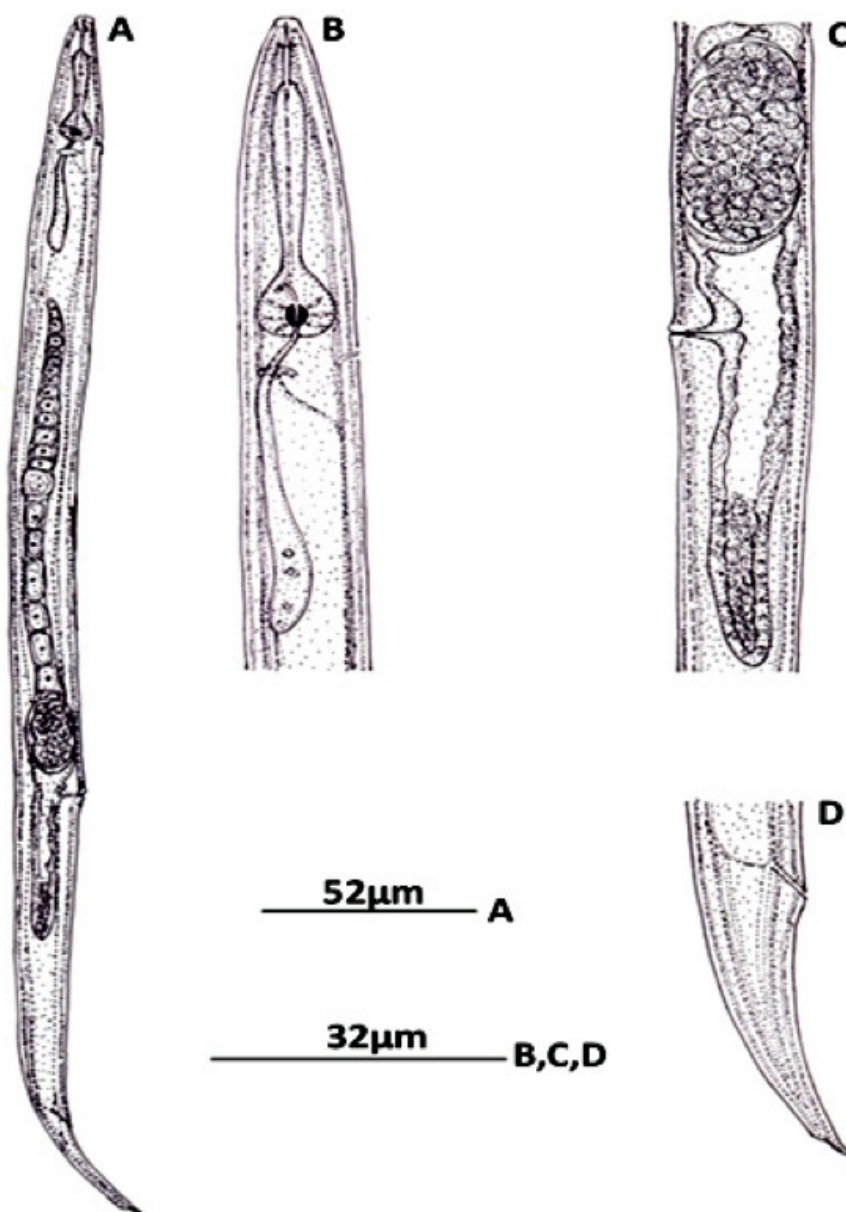


Figure 7. *Aphelenchooides neoechinocaudatus* Chanu et al., 2012. Female: A—Entire body | B—Anterior region | C—Reproductive system | D—Tail region. Photomicrographs: E—Anterior region | F—End bulb | G—Reproductive system | H—Tail region.

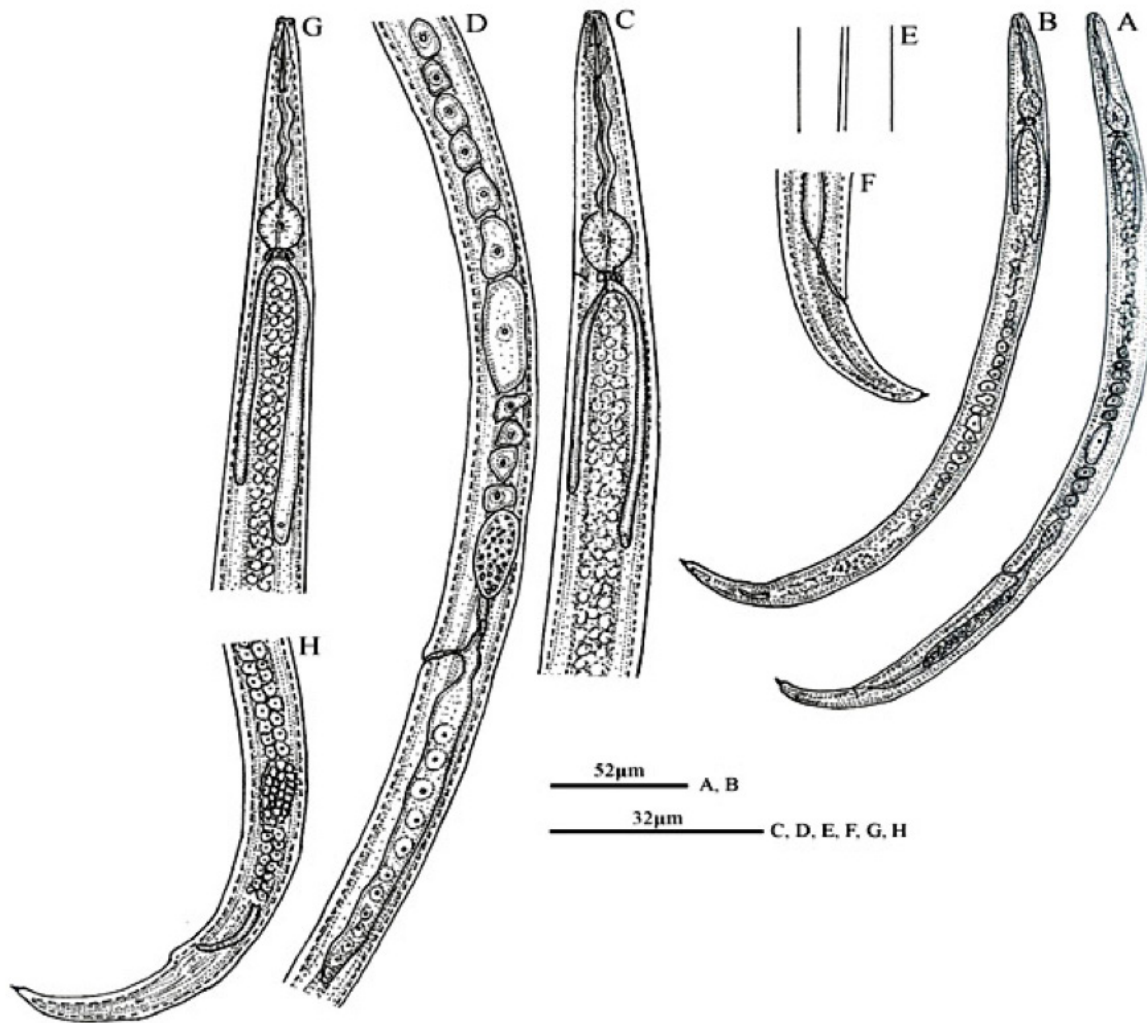


Figure 8. *Aphelenchooides neominoris* Chanu & Mohilal, 2014; A—Female whole body | B—Male whole body | C—Anterior portion of female | D—Body portion showing reproductive organs of female | E—Lateral lines | F—Female tail region | G—Anterior body region of male | H—Posterior body region of male showing testes.

***Aphelenchooides swarupi* Seth & Sharma, 1986**

(Table 2)

**Diagnosis**

Female: Body contour cylindrical to slightly curved, with three incisures in the lateral lines. Cephalic region set off without annules. Spear 8.2–10.2 µm long with basal thickenings, procorpus muscular. Nerve ring at 72.8 µm from anterior body. Excretory pore ventral at level of nerve ring. Tail 32.8–37.4 µm long, bluntly rounded with a ventral mucro. Vulva a transverse slit with vulval lips. Post vulval uterine sac well developed.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

***Aphelenchooides vigor* Thorne & Malek, 1968**

(Table 2)

**Diagnosis**

Female: Body cylindrical with coarse annulations. Lateral filed with two incisures. The cephalic framework is set off by constriction. Spear 11.9–13.6 µm long with distinct knobs. Nerve ring at 74.8–88.4 µm long and excretory pore at 78.2 µm from the anterior body region. Vulva sclerotized, overlapping with jointed flap. Ovary outstretched, tubular uterine sac filled with sperms. Tail 15.3–35.7 µm long, arcuate, blunt tip without mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

The hosts and localities of all the species are

mentioned in Table 4.

***Aphelenchooides oryzae* sp. nov.**

(Figure 9, Table 5)

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**Material examined**

**Holotype:** Collected on August, 2015 from paddy fields (*Oryza sativa* L. growing field) by L. Bina chanu, from Thoubal Khekman, Thoubal District, Manipur with a longitude of 24.5036 and latitude of 93.9116. The specimen is deposited on nematode collection of Parasitology Section, Centre for Advanced Study in Life Sciences, Manipur University, Canchipur, Manipur under the Voucher no. ZoDMU\_MN02 with holotype female on slide FTY<sub>4</sub> ♀1.

**Paratype:** Females on slides FTY<sub>4</sub> ♀2-12 and males on the slides FTY<sub>4</sub> ♂1-7, same data as holotype.

**DESCRIPTIONS**

**Holotype female:** Body straight, cylindrical, and robust upon fixation, 685.08 µm long. Lip region offset with rounded sides & flattened anteriorly, 5.19 µm wide and 1.7 µm high, and smooth in appearance. Body elongate with fine transverse annulations, 0.8 µm at mid body region. Lateral lines extend almost to tail tip with two ridges having four evenly spaced lines in the middle of the body.

Stylet is slender, 17.3 µm long, the conus slightly shorter than the shaft with indistinct swellings. Median oesophageal bulb rounded to slightly oval with the refractive thickenings usually placed centrally, 13.84µm

**Table 5. Morphometric data of species of *Aphelenchooides oryzae* sp. nov. All measurements in µm except L in mm.**

Characters	Holotype female	Paratype females	Paratype males
n	1	18	7
Length	685.08	569.17–750.82 (675.045±58.74)	536.3–615.88 (576.09±39.79)
a	44	36.55–44 (42.39±2.93)	34.44–39.55 (36.99±2.65)
b	7.33	6.18–7.33 (6.72±0.51)	5.74–5.83 (5.78±0.04)
b'			-
b <sub>1</sub>	9.65	9.65–11.12(10.26±0.55)	7.75–9.12 (8.43±0.68)
c	36	16.45–36 (25.70± 8.59)	15.5–19.77 (17.63±2.13)
c'	1.83	1.83–4 (3.144±0.94)	2.57–4 (3.28±0.71)
T	-	-	81.86–93.54 (94.24±0.69)
V	69.44	68.20–70.7 (69.65±0.89)	-
G <sub>1</sub>	49.24	29.95–49.24 (43.55±7.66)	-
Post. Uterine sac	46.71	46.71–86.5 (62.97± 15.61)	-
PVS/ V-A%	24.54	24.54–51.02 (34.65±14.15)	-
Oesophagous	93.42	91.69–114.18 (101.2±8.91)	91.69–114.18 (101.2±8.91)
Stylet	17.3	17.3	17.3
Lip width	5.19	5.19	5.19
Lip height	1.73	1.73	1.73
Median bulb length	13.84	10.38–13.84 (12.68±1.28)	10.38–13.84 (12.68±1.28)
Median bulb diam.	8.65	6.92–8.65 (7.49± 0.81)	-
Spicule	-	-	15.57–17.3(16.43±0.86)
Ovary	337.35	224.9–337.35 (293.05±54.2)	-
PUS/ VBD	3	3–6.25 (4.27±1.2)	-
Nerve ring	74.39	69.2–76.12 (72.94±2.72)	69.2–76.12 (72.94±2.72)
Excretory pore	72.66	72.66–77.85 (744.04±2.01)	72.66–77.85 (744.04±2.01)
Spermatheca	29.41	29.41	-
Rectum	8.65	5.19–8.65 (7.26± 1.69)	-
Tail	19.03	19.03–41.52 (29.41± 8.59)	31.14–34.6 (32.87±1.73)
ABD	10.38	8.65–10.38 (9.51± 0.94)	8.65–12.11 (10.38± 1.73)

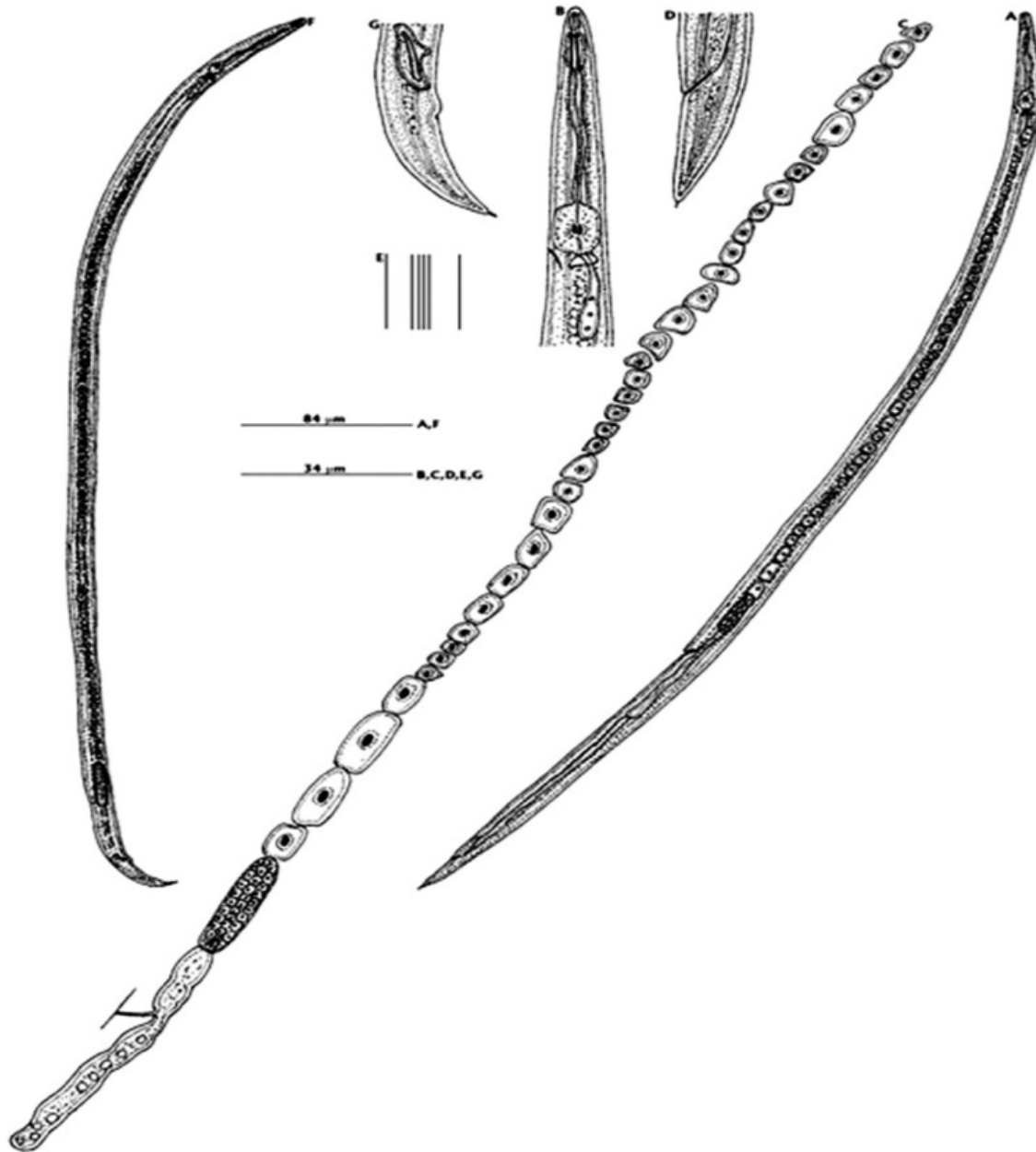


Figure 9. *Aphelenchooides oryzae* sp. nov.: Female, A—Entire body length | B—Anterior region | C—Female reproductive system | D—Tail region | E—Lateral lines | Male, F—Entire body length | G—Tail region.

high and  $8.65\mu\text{m}$  across in length. Excretory pore and nerve ring, one to two body widths posterior to median bulb, excretory pore at  $72.66\mu\text{m}$  from the anterior end and nerve ring at  $74.39\mu\text{m}$  from the anterior end of the body. Oesophageal glands overlap the intestine.

Vulva is at about two thirds of the body length from the anterior end. Reproductive system with a single anterior ovary, oocytes arranged in single rows, with spermatheca,  $29.41\mu\text{m}$  long filled with sperms and a prominent post-vulval uterine sac which usually extends just half the distance from the vulva to the anus. Post

uterine sac is about  $46.71\mu\text{m}$  in length.

Tail convex-conoid,  $19.03\mu\text{m}$  long and straight, usually  $10.35\mu\text{m}$  anal body widths long with a rounded tail tip bearing a small terminal mucro.

Paratype males: Lip region, stylet and oesophagus similar to female. Tail ends curls ventrally through  $45\text{--}90^\circ$  when killed by heat and usually with simple terminal mucro. Spicules well developed;  $15.57\text{--}17.3$  ( $16.43 \pm 0.86$ )  $\mu\text{m}$  long, the dorsal limb smoothly curved in its proximal half but flattened to concave tip; the ventral limb appears much weaker than the dorsal limb. The

**Table 6. Characters differentiating *Aphelenchoides oryzae* sp. nov. from other related *Aphelenchoides* species.**

<i>Aphelenchoides</i> spp.	Character differentiation
<i>A. blastophthorus</i> Franklin, 1952	Shorter stylet (15–16 μm) with prominent knobs
<i>A. brassicae</i> Edward & Misra, 1969	Excretory pore opposite median bulb base, female tail, shorter (c' = 3) with longer mucro, spicules more smoothly curved.
<i>A. baguei</i> Maslen, 1979	The female tail is ventrally concave with a longer mucro, and spicules more smoothly curved with a more prominent apex.
<i>A. hamatus</i> Thorne & Malek, 1968 [After Vovlas, 1982]	Shorter stylet (12–13 μm), female tail ventrally curved with a ventral mucro, spicule larger
<i>A. helophilus</i> (de Man, 1880) Goodey, 1933	Female body length over 1mm, stylet 14–16 μm with prominent knobs, spicules smoothly curved
<i>A. lanceolatus</i> Tandon & Singh, 1974	Lip region continuous, stylet shorter (12.5–13 μm), female body thinner (a=33), shorter post-vulval sac, spicules smoothly curved
<i>A. lichenicola</i> Siddiqi & Hawksworth, 1982	Female body was shorter (L=610 μm), female tail longer (c' = 3.5), spicules were characteristically swollen near the distal end of the dorsal limb
<i>A. liliium</i> Yokoo, 1964	Excretory pore a body width posterior to nerve ring, shorter stylet (12.5 μm), shorter post-vulval sac, female tail ventrally curved, spicules smoothly curved
<i>A. saprophilus</i> Franklin, 1957	Shorter female body (L= 546 μm), ventrally curved female tail and larger spicules
<i>A. sexlineatus</i> Eroskenko, 1967	Shorter stylet (9 μm), shorter female body (L = 605–645 μm), longer post vulval sac, female tail with a longer mucro.
<i>A. submerses</i> Truskova, 1973	Lip region narrower than the adjacent body; female tail more curved ventrally; excretory pore anterior to median bulb
<i>A. suipingensis</i> Feng & Li, 1986	Female with the thinner body (a=32); excretory pore opposite median bulb base; female tail ventrally curved with hair-like mucro
<i>A. Tumulicaudatus</i> Truskova, 1973	Lip region not offset, post vulval sac shorter; female tail with characteristic terminal swelling.
<i>A. nechaleos</i> Hooper & Ibrahim, 1994	Female body longer (L=10.5–11.5 mm); shorter stylet (10.5–11.5 μm) and shorter tail (3.9–4.6 μm)
<i>A. paranechaleos</i> Hooper & Ibrahim, 1994	Longer body (631–860 mm); shorter stylet (9.5–10.5 μm); thinner body (a=37–46) and shorter tail (c' = 2.6–3.6)

rostrum and apex are moderately developed; a tangent drawn from the apex to the spicule tips is separated from the tangent from the apex through rostrum.

**Etymology:** The species name is derived from the host plant.

**Diagnosis and Relationships**

*Aphelenchoides oryzae* sp. nov. is characterized by narrow cylindrical body, adults being 569.17–750.82 (675.04±53.74) μm long with a stylet about 17.3 μm long with indistinct basal swellings, a prominent median bulb and short end bulb, 4 lateral lines throughout the body, and tail straight, convex-conoid with a simple terminal mucro.

Males are common and functional with prominal spicules with dorsal limb flattened to indent in its distal half and the tip curled ventrally. Lateral fields of adults usually with four lines.

In view of its association with paddy plants, *Aphelenchoides oryzae* sp. nov. might be confused with *Aphelenchoides besseyi* Christie, 1942 the rice nematode. However, *Aphelenchoides oryzae* sp. nov. is separated from *A. besseyi* Christie, 1942 in having a single, simple, tail mucro instead of three–four processes as in *A. besseyi*. The present species also has longer oesophagus and stylet than *A. besseyi* Christie,

1942 whereas oesophagus ranges from 64–68 μm and stylet 10.0–12.5 μm in *A. besseyi* Christie, 1942.

*Aphelenchoides oryzae* sp. nov. differed from other species of *Aphelenchoides* in having a female body length of 569.17–750.82 (675.04 ± 58.74) μm, with a slender stylet length of 17.3 μm and a convex conoid tail with a simple terminal mucro with four lateral lines along the whole body length.

The differences of characters between closely related species of *Aphelenchoides* is provided in Table 6. Based on these morphometric differences the present species is reported as new to science.

The Shannon - Wiener species diversity index and Evenness for all the mentioned species are given in the table 7.

**CONCLUSIONS**

The richness of the species in the region may be due to warm climatic conditions, suitable habitats, and hosts as well as due to the absence of drastic changes in the climatic conditions during the past few years. But the present work could not cover all the varied ecosystems of Manipur. Since, nematodes are soil dwelling living around the roots of plants as well as plant parasitic



**Table 7. Shannon-Wiener species diversity index and evenness of the species of *Aphelenchooides*. Shannon-Wiener index is denoted by *H* and evenness by *E*. Total number of species is 255 and the Shannon-Wiener species index is 3.783.**

Species	No. of individuals (n)	Proportion, $pi=n/N$	$\ln(pi)$	$pi \times \ln(pi)$	$H = -\sum[(pi) \times \ln(pi)]$	$\ln k$	$E = H / \ln(k)$
<i>A. aeralis</i> Chanu et al., 2015	20	20/40=0.5	-0.6931	-0.346	0.346	3.688	0.093
<i>A. aligarheinsis</i> Siddiqi et al., 1967	5	5/35=0.1428	-1.9463	-0.277	0.277	3.555	0.077
<i>A. baguei</i> Maslen, 1973	18	18/90=0.2	-1.609	-0.321	0.321	4.499	0.071
<i>A. confusus</i> Thorne & Malek, 1968	10	10/32=0.3125	-1.1631	-0.321	0.321	3.465	0.092
<i>A. dhanachandi</i> Chanu et al., 2012	12	12/53=0.2264	-1.485	-0.336	0.336	2.484	0.135
<i>A. longistylus</i> Chanu & Mohilal, 2014	17	17/46=0.3695	-0.995	-0.367	0.367	3.850	0.095
<i>A. manipurensis</i> Chanu & Mohilal, 2018	38	38/58=0.6551	0.422	-0.277	0.277	4.060	0.068
<i>A. minor</i> Seth & Sharma, 1986	51	51/200=0.255	-1.366	-0.348	0.348	5.298	0.065
<i>A. neoechinocaudatus</i> Chanu et al., 2012	4	4/53=0.0754	-2.5849	-0.194	0.194	3.970	0.048
<i>A. neominoris</i> Chanu & Mohilal, 2014	11	11/47=0.2340	-1.452	-0.339	0.339	3.850	0.088
<i>A. swarupi</i> Seth & Sharma, 1986	36	36/143=0.251	-1.382	-0.346	0.346	4.962	0.069
<i>A. vigor</i> Thorne & Malek, 1968	7	7/38=0.1842	-1.6917	-0.311	0.311	3.687	0.084
<i>A. oryzae</i> sp. nov.	26	26/43=0.4651	-0.7655	-0.356	0.356	5.366	0.074

**Key to the species of *Aphelenchooides* spp. from Manipur**

1. Cephalic region set-off from body ..... 2  
 Cephalic region not set-off from body ..... *A. longistylus* Chanu & Mohilal, 2014
2. Lateral fields with 2 incisures ..... 3  
 Lateral fields with 3–4 incisures ..... 6
3. Basal bulb elongated ..... 4  
 Basal bulb bifurcated ..... *A. manipurensis* Chanu & Mohilal, 2018
4. Vagina with lips ..... *A. aeralis* Chanu et al., 2015  
 Vagina without lips ..... 5
5. Distinct spear knob, spear 11.9–13.6 µm long, arcuated blunt tail ..... *A. vigor* Thorne & Malek, 1968  
 Indistinct spear knob, spear 17.3 µm long, tail convex-conoid with blunt tip ..... *Aphelenchooides oryzae* sp. nov.
6. Lateral fields with 3 incisures ..... 7  
 Lateral fields with 4 incisures ..... 10
7. Tail tip pointed ..... 8  
 Tail tip bluntly rounded ..... 9
8. Spear 4.8–8.2 µm, indistinct knob, tail 22.4–30.8 µm ..... *A. minor* Seth & Sharma, 1986  
 Spear 8.2–10.2 µm, distinct knob, tail 32.8–37.0 µm long ..... *A. swarupi* Seth & Sharma, 1986
9. Tail with a mucro ..... *A. neoechinocaudatus* Chanu et al., 2012  
 Tail without a mucro ..... *A. dhanachandi* Chanu et al., 2012
10. With simple single tail mucro ..... 11  
 With multi-papillated tail mucro ..... *A. baguei* Maslen, 1973
11. Uterine sac collapsed/absent ..... *A. confusus* Thorne & Malek, 1968  
 Uterine sac well-developed ..... 12
12. Body contour curved, weak knob, tail elongated conoid with spine like mucro .....  
 ..... *A. aligarhiensis* Siddiqi et al., 1967  
 Body contour straight, tapering at extremities, knob distinct, tail blunt conoid with hair like mucro .....  
 ..... *A. neominoris* Chanu & Mohilal, 2014

forms, there is potential for availability of the organism in various other plant and tree varieties in different ecosystems of Manipur. There are various forest ecosystems found in Manipur with varied tree species along the Himalayan range. Further survey and in-depth taxonomic works incorporating molecular taxonomic techniques can reveal the rich diversity of the nematode group in Manipur.

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