



Research Article

Description of *Aphelenchoides acacia* n. sp. and *Aphelenchoides naurangiensis* n. sp. (Nematoda: Aphelenchoididae) with Observation on *Aphelenchoides saprophilus* Franklin, 1957 from District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan

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Abstract | Two new species belonging to genus *Aphelenchoides* viz *Aphelenchoides acacia* n. sp. retrieved from soil sample of kikar (*Acacia nilotica* L.), ber (*Ziziphus mauritiana* L.) and paper flower (*Bougainvillea spectabilis* L.) and *Aphelenchoides naurangiensis* n. sp. recovered from soil samples of wheat (*Triticum aestivum* L.), thoroughly described and illustrated from District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The new species namely *Aphelenchoides acacia* n. sp. belongs to the Group 2 of *Aphelenchoides* Species *Sensu* Shahina is characterized by small body length 358–496 µm; cephalic region non offset from the body. Cuticle weakly annulated, approximately 1µm apart in mid body region with transverse striae. Lateral field with four incisures; stylet 11–13 µm long having small basal thickening at the base; tail ventrally curved J-shaped; terminus with very tiny smooth mucro (1µm). *Aphelenchoides naurangiensis* n. sp. belongs to Group 1 of *Aphelenchoides* species and is characterized by small body length 406–492 µm; cephalic region clearly offset from rest of the body; cuticles finely annulated approximately 1µm apart. The lateral field with four incisures; stylet short 09–10 µm long having clear basal knobs at its base; tail subcylindrical, conoid with a rounded terminus. In addition, morphometrics and morphological details are given for *Aphelenchoides saprophilus* which is the first record for Pakistani nematode fauna.

Received | October 30, 2020; **Accepted** | December 08, 2020; **Published** | December 21, 2020

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Citation | Samreen, K., Tabassum, A.K., Nasira, K., Salma, J. and Shahina, F., 2020. Description of *Aphelenchoides acacia* n. sp. and *Aphelenchoides naurangiensis* n. sp. (Nematoda: Aphelenchoididae) with observation on *Aphelenchoides saprophilus* Franklin, 1957 from District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Nematology*, 38(2): 161–170.

DOI | <http://dx.doi.org/10.17582/journal.pjn/2020/38.2.161.170>

Keywords | *Aphelenchoides acacia* n. sp., *Aphelenchoides naurangiensis* n. sp., *Aphelenchoides saprophilus*, New record, Morphology, District Lakki Marwat

Introduction

The genus *Aphelenchoides* Fischer, 1894 belongs to the family of Aphelenchoididae (Skarbilovich, 1947) Paramonov, 1953 is the most diverse family in the order Aphelenchida. This Order having abstemiously limitless and stylets containing nematodes that consisting on four biologically groups including predators, fungal feeders, obligate insect parasites, while some of them are plant pathogens

in the genera *Bursaphelenchus* Fuchs, 1937 and *Aphelenchoides* Fischer, 1894 (Nickle, 1970; Hunt, 1993). The family is characterized by short stylet mostly with small to large basal swellings and narrow lumen, vermiform females with functional anus and rectum but without elongate tails, males with or without a rudimentary bursa (Chanu et al., 2012). The aforesaid genus could be allowing primitive kind of nematode evolution, due to their uniqueness to feed on both plants as well as fungi with maximum

accessible sources of host as compared to another plant pathogenic nematodes (Shurtleff and Averre, 2000). Although, numbers of species belonging to *Aphelenchoides* are fungivores (Kanzaki and Giblin-Davis, 2012).

Numbers of characteristics of the genus *Aphelenchoides* are drawing attention for biologists, plant pathologists and zoologists (Aliramaji *et al.*, 2018) and their association with plants has increased manifold in last few years. Sanchez-Monge *et al.* (2015) looked into plant parasitic forms and introduced 13 plant parasitic nematodes, in which three of them being considered most important in agricultural systems namely *A. besseyi* Christie, 1942, *A. fragariae* (Ritzema Bos, 1890) Christie, 1932 and *A. ritzemabosi* (Schwartz, 1911); Steiner and Buhrer, 1932.

One new species of the genus *Aphelenchoides* viz., *Aphelenchoides turnipi* was described from Pakistan by Israr *et al.*, 2017. Later on, Shahina *et al.*, 2019 compiled nematode fauna of Pakistan including nineteen species of the genus *Aphelenchoides*. In order to study on further presence of species and diversity of this genus in Pakistan, several investigations and scrutiny of numbers of soil samples collected from various locations of District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan was carried out during 2019. After initial analysis, one new recorded species namely *Aphelenchoides macrospica* Golhasan *et al.*, 2017 was reported from paper flower (*Bougainvillea spectabilis* L.) from village Sarai Naurang of the District Lakki Marwat, Khyber Pakhtunkhwa (Salma *et al.*, 2020). Furthermore, enhanced the scope of research work, we explored out two populations of the genus *Aphelenchoides* as new species. These species have been thoroughly examined and it is assured that the new species differentiated morphologically and morphometrically from other *Aphelenchoides* species. Accordingly, the new species is herein described as *Aphelenchoides acacia* n. sp. and *Aphelenchoides naurangiensis* n. sp. along with a new record *Aphelenchoides saprophilus* is briefly redescribed and illustrated.

Materials and Methods

Sampling, extraction, mounting and drawing

The current research study is based on the nematodes belonging to the order of Aphelenchida. Numbers of soil samples were collected for the first time from four

different locations of District Lakki Marwat, Khyber Pakhtunkhwa Pakistan in 2019. Upon completion of pre-requisites procedure as per practice in vogue at laboratory, the collected soil samples were extracted by the prescribed of Cobb's sieving and decanting method (Cobb, 1918). It was further purified by Baermann's funnel technique (Baermann, 1917). The retrieved nematodes were killed instantaneously by pouring a hot water (80-90°C) in a glass cavity block and immediately preserve in TAF (Tri-ethanol Amine Formalin) solution for 24 hours (Courtney *et al.*, 1955). Thereafter, fixed nematodes were sanitized thrice with distilled water. Specimens were kept for 5-6 days in an incubator at 55°C in 2 ml of 1.25 % glycerin for slow dehydration (Seinhorst, 1959). Permanent mounting was done by transferring of nematodes to a clean microscopic glass slide having pure drop of glycerin and sealed with paraffin wax by gently heating the slide. Later on, measurements were undertaken by de Man's, 1884 formula with the assistance of an ocular micrometer and the illustrations were worked out with the help of a drawing tube. Photomicrographs were captured via Nikon DS-fi-1 camera already fixed with Nikon Eclipse-E400 compound microscope.

Systematics

Order Aphelenchida Siddiqi, 1980

Superfamily Aphelenchoidea (Fuchs, 1937), Thorne, 1949

Family: Aphelenchoididae (Skarbilovich, 1947), Paramonov, 1953

Subfamily Aphelenchoidinae Skarbilovich, 1947

Genus *Aphelenchoides* Fischer, 1894

Aphelenchoides acacia n. sp.

(Figure 1a and 1b; Table 1)

Description

Female: Body small, slender, cylindrical, dorsally convex and ventrally concave, highly arcuate at posteriorly upon fixation. Surface of cuticle finely annulated and about 1µm. Lateral field marked with four incisures (i.e. three ridges) occupying about 60% of corresponding body width. Cephalic region low, non-offset. Stylet delicate with small basal swelling having conus with approximately 40-60% of its total length. Procorpus cylindrical. Median bulb oblong, with well sclerotized conspicuous valve situated middle to slightly posteriorly. Excretory pore located just behind the nerve ring; the position is almost

equal to metacarpus length posterior to the base of median bulb. Isthmus short. Nerve ring posteriorly located at about half metacarpus length. Hemizonid indiscernible and seen in few specimens located 8-10 μm (mean=9 \pm 1 μm) from excretory pore. Pharyngeal glands lobe slender *ca* two to five body diam. long, overlapping intestine dorsally. Intestine simple. Gonad short, monodelphic prodelphic, oocytes arranged in single row of cell. Oviduct connecting ovary and spermatheca. Spermatheca well developed filled with small and rounded sperms. Uterus oblong; vagina thick walled, directed anterior. Vulva without any vulval flap apparatus in lateral view, but with slightly protuberant anterior and posterior lips. Rectum and anus functional and visible. Rectum is about as long as anal body width. The anus has slightly elevated anterior lip than posterior one. Post uterine sac (PUS) short usually with no sperms, extending for about 12-26% of vulva-anus distance. Mostly the tail is ventrally curved, approximately 4-6 times anal body diameter long, J-shaped terminus with very small smooth mucro (1 μm).

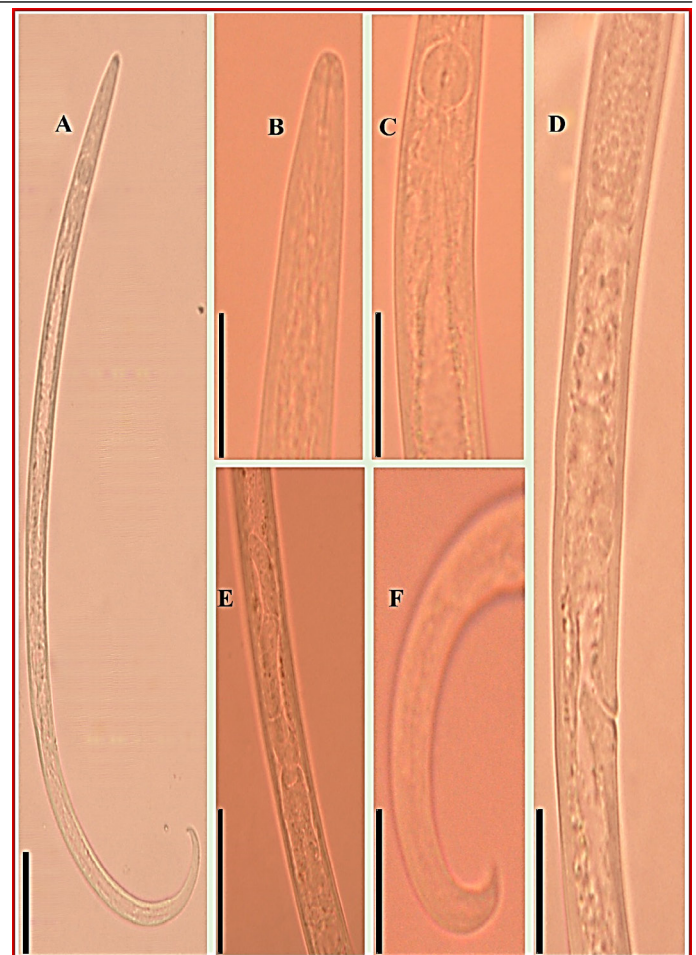


Figure 1b: (A-F). *Aphelenchoides acacia* n. sp. Female: A, whole body; B, Anterior region; C, Stylet Posterior pharynx region showing excretory pore; D, Vulva position; E, Anterior ovary; F, Tail; (Scale: A=20 μm ; B-F=100 μm).

Male: Not ascertained.

Differential diagnosis and relationship

Aphelenchoides acacia n. sp. is differentiated from other species of *Aphelenchoides*, in having curved J-shaped tail with very tiny smooth mucro (1 μm). The new species has been characterized by small body length 358-496 μm ; non offset cephalic region; cuticle annulation approximately 1 μm ; lateral field with four incisures; stylet 11-13 μm long having small basal swelling at the base; tail ventrally curved J-shaped terminus with very tiny smooth mucro.

According to grouping of *Aphelenchoides* species (Shahina, 1996) the new species categorized in Group 2 having "one or some time two mucronate structure on tail terminus." On the basis of body, stylet and tail length and in lateral field, the new species is close to two species of Group 2, including *Aphelenchoides richardsoni* Grewal et al., 1992 and

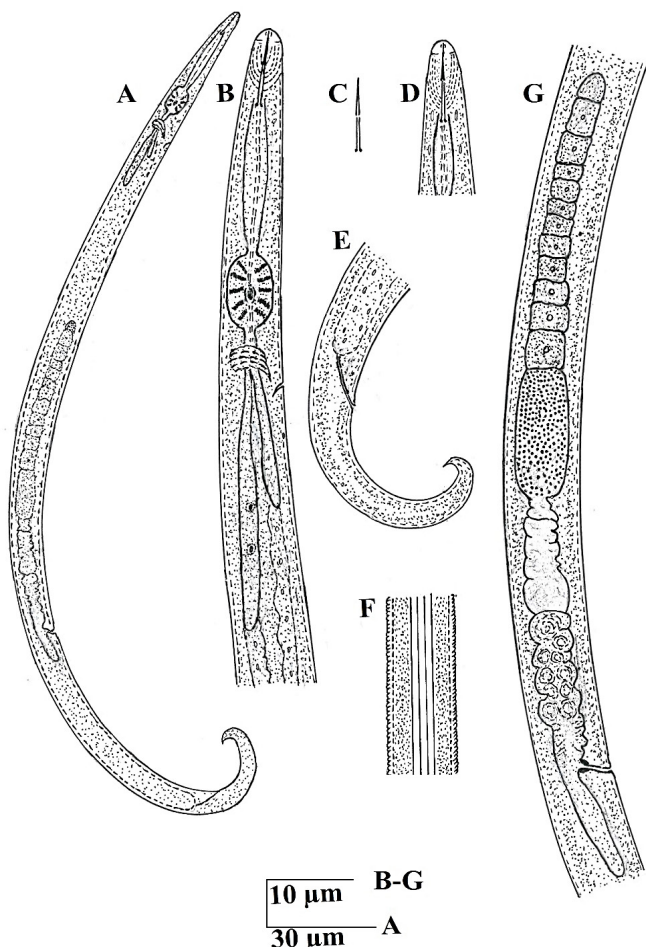


Figure 1a: (A-G). *Aphelenchoides acacia* n. sp. Female: A, whole body; B, Anterior region; C, Stylet; D, Cephalic region; E, Tail; F, Lateral lines; G, Female gonad.

Aphelenchoides tuzeti B'Chir, 1979. However, it differs from these two species in some morphometric and allometric characters. From *A. richardsoni*, the new species differs in lower c ratio (9.6–12.3 vs 14–19), greater c' ratio (4.3–5.7 vs 2.5–4), anteriorly located vulva (60.3–67.5 vs 66–72.5%), head structure (non-offset vs offset), shorter PUS (15–22 vs 13–38 μ m) and female tail shape (J-shaped with smooth mucro vs almost straight or slightly curved with ventral mucro). From *A. tuzeti*, it differs in greater c' ratio (4.3–5.7 vs 2.7–3.3), cephalic region (non-offset vs offset), and in shape of female tail (J-shaped with tiny smooth mucro vs almost straight, slight ventrally curved with or without mucro). The new species is also comes close in stylet length and lateral lines with other three species of Group 2 i.e. *Aphelenchoides cyrtus* Paesler, 1959, *Aphelenchoides haguei* Maslen, 1979 and *Aphelenchoides absari* Hussain and Khan, 1967. From *A. cyrtus* the new species is differ in smaller body length and smaller c value ($L=0.35-0.49$ vs $0.50-0.57$, $c=9.6-12.3$ vs 16.8); greater a and c' ratio ($a=27.5-42.5$ vs $24-28$, $c'=4.3-5.7$ vs 3.3); female tail shape (J-shaped with smooth mucro vs shallow constriction narrowed sharply with a very fine ventral mucro) and in anterior located vulva (60.3–67.5 vs 70–73). From *A. haguei*, the new species differ in smaller body length ($0.35-0.49$ vs $0.56-0.76$); slightly greater a and c' ratio ($a=27.5-42.5$ vs $28-38$, $c'=4.3-5.7$ vs $3-5$) and V% (60.3–67.5 vs 66–70); vagina angled obliquely forward vs at 90° to the body axis and female tail terminus (J-shaped with smooth mucro vs short ventral mucron minutely multi-papillate almost to the tip). From *A. absari* the new species differed in smaller c ratio (9.6–12.3 vs 16–20); greater c' ratio (4.3–5.7 vs 3); tail length (35.2–45 vs 25) and in V% (60.3–67.5 vs 69–77). Furthermore, some relationships were also detected with *Aphelenchoides vaughani* Maslen, 1979 of Group 4 *sensu* Shahina, 1996. The new species is come close to *A. vaughani* in body length and in lateral lines but differed in smaller c value (9.6–12.3 vs 12.1–16.9); greater c' value (4.3–5.7 vs 2–2.5); V% (60.3–67.5 vs 70.5–73.1) and in smaller G1% (20.9–28.4 vs 32–44).

Type habitat and locality: Specimens were retrieved from kikar (*Acacia nilotica* L.), from village Tajori, District Lakki Marwat, Khyber Pakhtunkhwa. Further, specimens were also collected from ber (*Ziziphus mauritiana* L.) and paper flower (*Bougainvillea spectabilis* L.) from village Sarai Naurang of District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The geographical location of the sampling sites were Tajori

(32°37'51"N and 70°34'59"E) and Sarai Naurang (32°49'43"N and 70°46'33"E).

Table 1: Morphometric data of *Aphelenchoides acacia* n. sp. All measurements are in μ m excluding L(mm) in the form of Mean \pm S.D (range).

Characters	Female	
	Holotype	Paratype (n=10)
L	472	414.6 \pm 42.31 (358-496)
a	31.4	34.36 \pm 4.20 (27.5-42.5)
b'	4.6	4.28 \pm 0.36 (3.5-4.7)
c	10.4	10.78 \pm 0.78 (9.6-12.3)
c'	5.6	4.94 \pm 0.52 (4.3-5.7)
V%	65	63.81 \pm 1.99 (60.3-67.5)
Lip width	3	4.1 \pm 0.83 (3-5)
Lip height	2.5	3.05 \pm 0.35 (2.5-3.5)
G1 %	27.5	26.18 \pm 2.04 (20.9-28.4)
Stylet	13	12 \pm 0.77 (11-13)
Conus length	06	6.33 \pm 0.45 (6-7)
Procorpus	40	40.22 \pm 3.84 (32-46)
Pharyngeal length	102	95.7 \pm 4.14 (90-103)
Distance to the base of median bulb	42	49.72 \pm 4.05 (42-57)
Median bulb length	11	11.5 \pm 0.5 (11-12)
Median bulb width	08	8.55 \pm 0.5 (8-9)
Median bulb ratio (L/W)	1.3	1.3 \pm 0 (1.3)
Width at Median bulb	12	12.2 \pm 0.6 (11-13)
Nerve ring	60	57.8 \pm 3.68 (55-67)
Excretory pore	63	59.48 \pm 3.89 (55-69)
Max. body width	15	12.2 \pm 1.69 (10-15)
Width at vulva	12	12.66 \pm 0.81 (12-14)
Post uterine sac (PUS)	15	18 \pm 2.56 (15-22)
Overall ovary length	130	109 \pm 15.66 (77-130)
Tail	45	38.32 \pm 3.17 (35.2-45)
ABD	08	7.8 \pm 0.87 (7-10)
Rectum length	10	9.4 \pm 0.91 (7-10)
Vulva-anus distance	124	109.84 \pm 16.75 (80-139)
Mucro	1	1 \pm 0

Types Specimens: Holotype and paratype females are submitted to Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan.

Etymology: The new species designated its named related to the type host (*Acacia nilotica* L.).

Aphelenchoides naurangiensis n. sp.
(Figure 2a, 2b and Table 2)

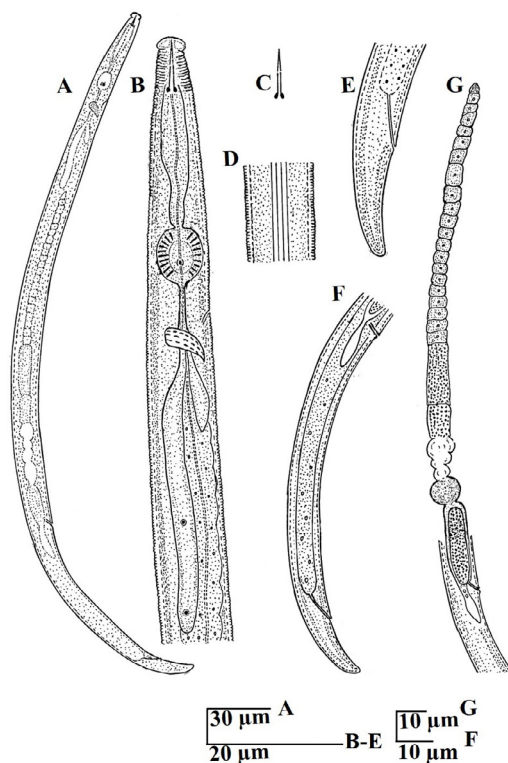


Figure 2a: (A-G). *Aphelenchoides naurangiensis* n. sp. Female: A, whole body; B, Anterior region; C, Stylet; D, Lateral lines; E, Tail; F, Posterior region showing post uterine sac; G, Gonad.

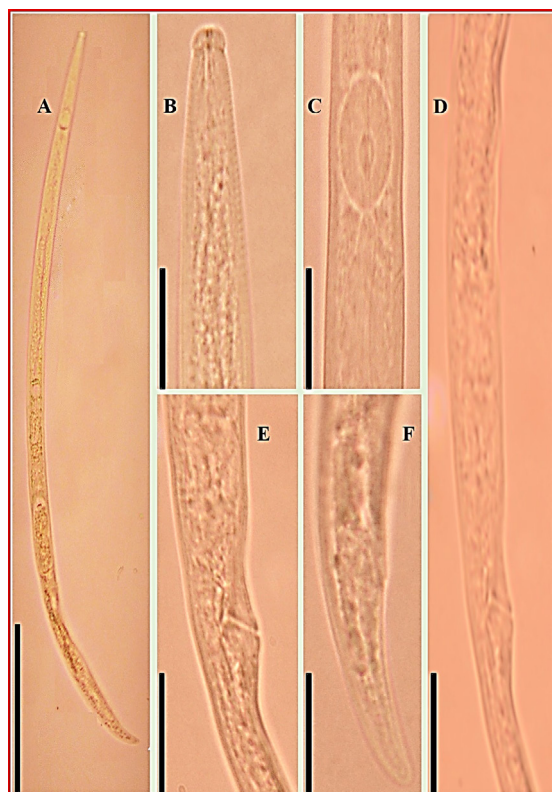


Figure 2b: (A-F). *Aphelenchoides naurangiensis* n. sp. Female: A, whole body; B, Cephalic region; C, Oesophageal region showing excretory pore; D, Female gonad showing ovary; E, Vulval position and PUS; F, Tail (Scale: A=20µm; B-F=100µm).

Table 2: Morphometric data of *Aphelenchoides naurangiensis* n. sp. All measurements are in µm excluding L(mm) in the form of Mean ± S.D (range).

Characters	Female	
	Holotype	Paratype (n=11)
L	490	463.54±25.44 (406-492)
a	32.6	29.92±2.68(25.3-34)
b'	4.9	4.57±0.37(4.0-5.0)
c	31.3	20.70±1.04(18.4-22.1)
c'	2.5	2.32±0.14(2-2.5)
V%	69.5	70.45±1.64(66.4-73)
Lip width	5	5.72±0.86 (5-7)
Lip height	2	2.27±0.44 (2-3)
G1 %	61.6	51.92±6.57 (40.1-61.6)
Stylet	10	9.7±0.44(9-10)
Conus length	4	4.90±0.66 (4-6)
Procorpus	40	42.09±2.96 (36-48)
Pharyngeal length	100	100.54±8.44 (90-124)
Distance to the base of median bulb	52	54.9±2.39 (50-59)
Median bulb length	11	10.81±0.71 (10-12)
Median bulb width	8	9.0±0.60 (8-10)
Median bulb ratio(L/W)	1.3	1.17±0.07 (1.1-1.3)
Width at median bulb	14	14±1.04 (12-16)
Nerve ring	62	62.36±3.02 (56-66)
Excretory pore	60	56.90±3.23 (52-62)
Max. body with	15	15.54±1.07 (14-18)
Width at vulva	14	14±1.04 (12-16)
Post uterine sac (PUS)	12	14.36±2.77 (12-20)
Overall ovary length	302	241±33.89 (184-302)
ABD	9	9.2±0.86 (8-10)
Rectum length	10	10.72±1.28 (10-14)
Vulva-anus distance	126	11.3±6.31 (105-126)
Tail	23	22.36±1.06(20-24)

Description

Female: Habitus of body usually straight; arcuate when heat relaxed. Body gently narrowing towards both ends. Cuticle finely annulated about 1µm. The lateral field with four incisures (i.e. three ridges) consisting of 23% of corresponding body width. Cephalic region high, offset with a constriction. Stylet short sized with clear basal knobs. Conus length approximately 40-65% of its total length. Procorpus is slightly swollen anteriorly and then narrowing to connect with prominent muscular, almost round shaped median bulb with conspicuous valve situated posteriorly. Isthmus long. Excretory pore located anterior to nerve ring and

located 1.5 times of metacarpus length and measured 8 to 12 μm posterior to the base of median bulb. Nerve ring sighted at about one third to the metacarpus length posterior to it. Hemizonid indiscernible. Pharyngeal glands lobe slender *ca* three to five body diameter long, overlapping intestine dorsally. Intestine simple. Gonad outstretched, monodelphic-prodelphic, oocytes arranged in single row of cell. Anterior end of ovary may reach near the posterior end of oesophageal glands, sometimes its tip is recurved. Spermatheca well filled with small and rounded sperms. Uterus oblong containing many small eggs. Vagina directed anteriorly. Vulva a simple slit in ventral view, without any vulval flap with slightly protuberant anterior and posterior lips. Rectum and anus functional and visible. Anus has slightly elevated lips. Post uterine sac (PUS) short usually with no sperms, extending for about 9-18% of vulva-anus distance. Tail is approximately 2-3 times anal body diameter long. Tail subcylindrical, medium conoid with rounded terminus.

Male: Not ascertained.

Differential diagnosis and relationship:

Aphelenchoides naurangiensis n. sp. is differentiated from all other species of *Aphelenchoides* belonging to Group 1 due to its smallest tail length. The new species characterized by small body length 406 to 492 μm ; cephalic region offset from rest of the body; cuticle annulation is about 1 μm . The lateral field with four incisures (i.e. three ridges). Stylet short 9-10 μm long having clear basal knobs at its base. Tail subcylindrical, conoid with a rounded terminus.

Sequel to grouping of *Aphelenchoides* species (Shahina, 1996) the new species categorized in Group 1 as its tail simple without any outgrowth or mucronate structure. On the basis of body as well as stylet length, four lateral lines and vulva percentage, the new species having close similarity with four species belonging to group 1, i.e. *Aphelenchoides capsuloplanus* (Haque, 1967; Andrassy, 1976), *Aphelenchoides obtusus* Thorne and Malek, 1968; *Aphelenchoides orientalis* Eroshenko, 1968 and *Aphelenchoides rotundicaudatus* Fang et al., 2014. The new species *Aphelenchoides naurangiensis* n. sp. differs from *A. capsuloplanus* by slightly higher a ratio (25.3-34 vs 24-30.2); higher c value (18.4-22.1 vs 16.8-19); slightly lower c' ratio (2-2.5 vs 2.6); smaller tail length (20-24 vs 28.8 μm) and in absence of male vs present. The new species also differs from *A. obtusus* in higher c value (18.4-22.1 vs 15); slightly

lower c' ratio (2-2.5 vs 3); smaller tail length (20-24 vs 30-34 μm); position of metacarpus valve (posterior vs central); Posterior uterine branch about as long as body width vs about twice as long as body width and in absence of male vs present. Further, it also differs from *A. orientalis* in greater a and c value (a=25.3-34 vs 19.7-21.9, c=18.4-22.1 vs 13.5-15.4) and in female tail shape (subcylindrical vs conoid). It also differentiate from *A. rotundicaudatus* having shorter c' value (2-2.5 vs 2.9-4.0); higher body diameter (14-18 vs 10.1-12.7 μm); shorter PUS (12-20 vs 52-89 μm), shorter tail length (20-24 vs 21-30 μm), female tail (subcylindrical and rounded terminus vs small cuticular thickening or bearing a blunt peg); excretory pore position (posterior to the median bulb vs anterior to the median bulb) and in absence of male vs present. The new species is also comes close to the species of the genus *Robustodorus* viz., *R. helicus* (Heyns, 1964) Andrassy, 2007 but differ in greater pharynx length (90-124 vs 49-65); smaller PUS (12-20 vs 28-55) and in lateral lines (4 vs 3)

Type habitat and locality: Specimens of new species were retrieved from wheat (*Triticum aestivum* L.) from Sarai Naurang of District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The geographical location of the sampling site of Sarai Naurang (32°49'43"N and 70°46'33"E).

Types Specimens: Holotype and paratype females are submitted to Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan.

Etymology: The aforesaid new species designated its named related to the locality Sarai Naurang.

Aphelenchoides saprophilus Franklin, 1957 (Figure 3a and 3b; Table 3)

Description

Female: Habitus of body usually straight, slightly arcuate when heat relaxed. Body gently narrowing towards both ends. Cuticle finely annulated about 1 μm . The lateral field with four incisures (i.e. three ridges) consisting of 37% of corresponding body width. Cephalic region rounded, offset. Stylet short sized 10-11 μm long with small basal knobs. Conus length is approximately 36-40% of its total length. Procorpus cylindrical. Well-developed prominent muscular, almost round shaped median bulb with

conspicuous valve situated posteriorly and *ca* 11-14 μ m in length and 9-11 μ m measured in width. Isthmus short. Excretory pore located at the level of nerve ring; the position is half of the metacarpus length. Nerve ring is sighted at approximately half of the metacarpus length posterior to it. Hemizonid indiscernible. Pharyngeal glands lobe slender *ca* four to five body diameter long, overlapping intestine dorsally. Intestine simple. Gonad outstretched, monodelphic-prodelphic, oocytes arranged in single row of cell. Anterior end of ovary may reach some time near the posterior end of oesophageal glands, sometimes its tip is recurved. Spermatheca well developed filled with compact disc like sperms. Vagina directed anteriad. Vulva transverse with slightly raised lips. Rectum and anus functional and visible. Anus has slightly anterior elevated lip. Post uterine sac (PUS) well developed usually with many rounded sperms, extending for about 30-70% of vulva-anus distance. Tail is approximately 3-5 times anal body diameter long. Tail conical, usually with single tiny nodular protuberance.

Table 3: *Morphometric data of Aphelenchoides saprophilus Franklin, 1957; All measurements are in μ m excluding L(mm) in the form of Mean \pm S.D (range).*

Characters	Female (n=12)	Male (n=10)
L	561.1 \pm 41.4 (502-641)	544.9 \pm 43.88 (477-609)
a	35.66 \pm 2.72 (31.2-40.4)	34.1 \pm 3.22 (29.8-40.6)
b'	4.5 \pm 0.44 (3.6-5.4)	4.33 \pm 0.43 (3.8-5.0)
c	14.42 \pm 0.98 (12.1-15.6)	14.54 \pm 1.37 (12.6-17.4)
c'	4.21 \pm 0.38 (3.5-4.7)	2.98 \pm 0.37 (2.3-3.7)
V or T%	67.23 \pm 1.73 (65-70.5)	52.22 \pm 3.90 (45.4-58.11)
Lip width	5 \pm 0.71 (4-6)	5 \pm 0.71 (4-6)
Lip height	2.16 \pm 0.23 (2-2.5)	2.16 \pm 0.23 (2-2.5)
G1 %	41.66 \pm 4.52 (38.1-48.2)	-
Stylet	10.22 \pm 0.41 (10-11)	10.22 \pm 0.41 (10-11)
Conus length	4 \pm 0 (4)	4 \pm 0 (4)
Procorpus	53 \pm 3.23 (50-60)	52.3 \pm 2.00 (50-55)
Pharyngeal length	123.77 \pm 9.35 (112-143)	125 \pm 7.22 (116-139)
Median bulb	63.33 \pm 4.61 (53-68)	65 \pm 4.24 (55-70)
Median bulb length	12.4 \pm 0.8 (11-14)	12.4 \pm 0.8 (11-14)
Median bulb width	10.02 \pm 0.66 (9-11)	10.02 \pm 0.66 (9-11)
Nerve ring	73.22 \pm 3.85 (65-77)	73.22 \pm 3.85 (65-77)
Excretory pore	68.11 \pm 2.33 (64-72)	70.7 \pm 2.60 (66-76)
Max. body width	15.77 \pm 1.47 (14-18)	16 \pm 1 (15-18)
Post uterine sac (PUS)	68.22 \pm 20.23 (45-110)	-
Overall ovary/ testis length	227.11 \pm 29.28 (180-280)	295.5 \pm 53.10 (220-315)
ABD	9.22 \pm 0.78 (8-10)	12.5 \pm 1.02 (11-15)
Vulva-anus distance	144.22 \pm 16.75 (116-168)	-
Tail	38.66 \pm 3.71 (32-45)	36.8 \pm 4.33 (31-45)
Spicule	-	21.1 \pm 0.83 (20-22)

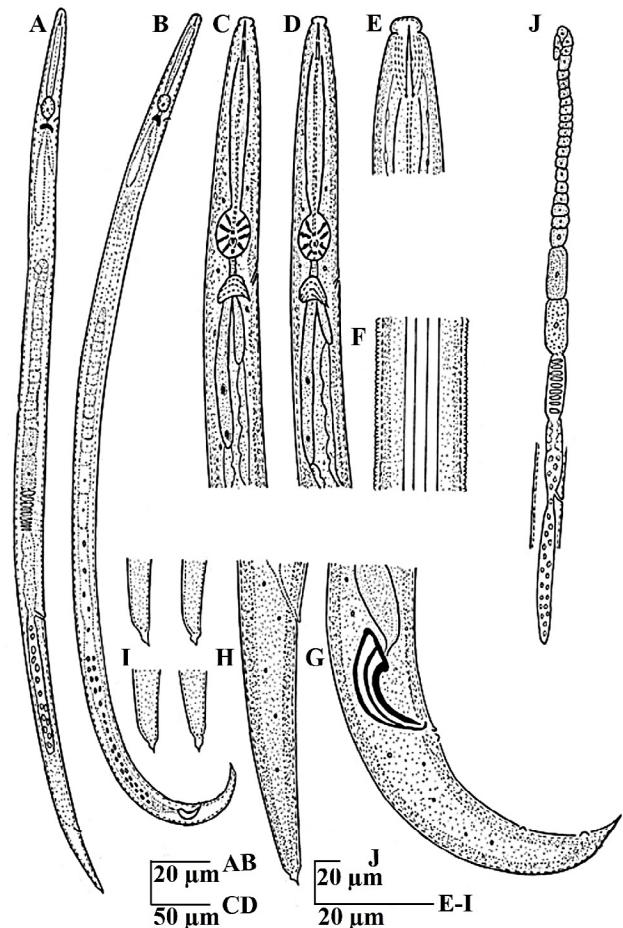


Figure 3a: (A-J). *Aphelenchoides saprophilus*. A, Female whole body; B, Male whole body; C, Female anterior region; D, Male anterior region; E, Cephalic region; F, Lateral lines; G, Male tail; H, Female tail; I, Female tail variations; J, Female gonad.

Male: Body slender, cylindrical, J-shaped when heat relaxed. Anterior region and cuticle similar to female. Genital system monorchic, testis outstretched with spermatocytes arranged in a single row. Spicule arcuate, relatively short, apex and rostrum rounded and well developed, the end of the dorsal limb is curved ventrally like a hook. Gubernaculum absent. Tail conical, bearing a short sharp mucro. Three pairs of subventral caudal papillae present: first pair located just posterior to cloacal aperture, second pair in mid of tail region and the third one just anterior to tail end.

Remarks: *Aphelenchoides saprophilus* was originally described from Doking village, Norfolk city of England in a soil sample by Franklin, 1957. Presently, this population was recovered from soil sample of paper flower (*Bougainvillea spectabilis* L.) from villages Sarai Naurang (32°49'43"N and 70°46'33"E) and Aghzar Khel (32°23'37"N and

70°44'16"E) of District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan. The aforementioned species is first time reported from Pakistan. Morphological and morphometric characters are in range with those of original description except in slightly higher 'a' ratio (31.2-40.4 vs 26-33); slightly b ratio (3.6-5.4 vs 4-6.7). *Aphelenchoides saprophilus* also compared with Iranian population described by Adeldoost *et al.*, 2017. The species slightly differ in higher b ratio in male (3.9-5.0 vs 3.7-3.9); slightly greater cloacal body width (11-15 vs 11-12 μ m) and in spicule length (20-22 vs 22-24 μ m).

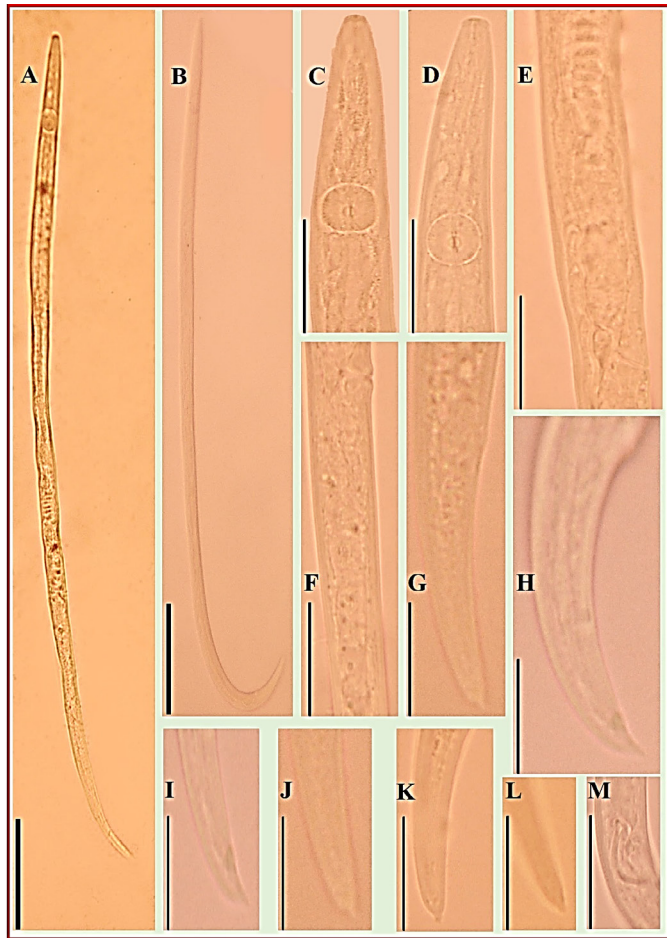


Figure 3b: (A-M). *Aphelenchoides saprophilus*: A, Female whole body; B, Male whole body; C, Female anterior region; D, Male anterior region; E, Female gonad; F, Female post utrine sac; G, Female tail; H, Male tail; I-L, Female tails variation; M, Spicule (Scale: A-B=20 μ m; C-M=100 μ m).

Novelty Statement

The said research article described two new and one known plant parasitic nematodes of agricultural importance for the first time from District Lakki Marwat, Khyber Pakhtunkhwa. This research work is

meaningful because new morphological information obtained from these new species and the same will be assisted/ helpful in future identifications of plant-parasitic nematodes and the said article will be used being a guideline for researcher in the field of plant nematology.

Author's Contribution

Samreen Khan performed surveys, processed samples, line drawing, photography and drafted the manuscript. Tabassum Ara Khanum identified the species *Aphelenchoides acacia* and guided in line drawing and photography. Nasira Kazi guided in identification and manuscript writing. Salma Javed supervised the research and critically reviewed the manuscript. Shahina Fayyaz assigned the research topic Order Aphelenchida and learned the basic keys for identification of the nematodes.

Conflict of interest

The authors have declared no conflict of interest.

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