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DESCRIPTION OF *APHELENCHOIDES MARWATAENSIS* N. SP. (NEMATODA: APHELENCHOIDIDAE) WITH OBSERVATION ON *EKTAPHELENCHOIDES POINARI* ALIRAMAJI *ET AL.*, 2014 AND *PARAPHELENCHUS MYCELIOPHTHORUS* GOODEY, 1958 FROM LAKKI MARWAT DISTRICT, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

The new prescribed species belongs to the genus *Aphelenchoides* namely *Aphelenchoides marwataensis* n. sp. collected from sample of soil around the roots of ber (*Ziziphus mauritiana* L.) is described as well as illustrated from Lakki Marwat district, Khyber Pakhtunkhwa, Pakistan. The new described species belonging to the Group 2 of *Aphelenchoides* species Shahina, 1996 is morphologically characterized by the body length of 408-462 μm in female and 384-460 μm in male; cephalic region rounded and offset. Cuticle finely annulated, thin about less than 1 μm . Lateral field marked by four incisures; stylet 10-12 μm in female, 10-11 μm in male having small basal knobs at the base; female tail terminus bifid with two prong and often with a dorsal process decreased to give angular region adjacent to the ventral mucro. Spicule relatively short, arcuate, apex and rostrum rounded. Male tail conical, sharp mucro and bearing three pairs of sub-ventral caudal papillae. Furthermore, the relevant morphological and morphometric details are provided for *Ektaphelenchoides poinari* and *Paraphelenchus myceliophthorus*. The genus *Ektaphelenchoides* representing new country record for Pakistani nematode fauna.

Keywords: *Aphelenchoides marwataensis* n. sp., *Ektaphelenchoides poinari*, *Paraphelenchus myceliophthorus*, new record genus, new record species, morphology, Lakki Marwat district.

INTRODUCTION

The members belong to super family Aphelenchoidea (Fuchs, 1937) Thorne, 1949 are considered typologically similar, primarily characterized by a strongly developed metacarpus while some time smaller metacarpus does rarely happen (Heydari and Pedram, 2020; Pedram *et al.*, 2018). In this superfamily an exclusive range of feeding habits which includes fungal feeding, predatory, phytoparasitism as well as insect parasitism noticed (Kanzaki and Giblin-Davis, 2012; Hunt, 1993). Aphelenchs are in general highly active nematodes. The richest and widely spread aphelenchs belongs to the genera *Aphelenchus* and

Aphelenchoides (Hunt, 1993). The *Aphelenchoides* is one of the largest genus under the order Aphelenchida and has broad hosts as well as distribution (Hunt, 1993). This genus attained much value due to parasitic potential and quarantine regulation (Jianfeng *et al.*, 2020). Because of the role of mycetophagous lifestyle the genus consists of number of species having more or less importance as an ectoparasites and or endoparasites of plants (Chanu *et al.*, 2013).

In Pakistan, species of *Aphelenchoides* was earlier reported by Akhtar in 1962 from soil samples collected around the roots of sugarcane in the vicinity of Lahore. The incidence of *Aphelenchoides* species in Pakistan are well recognized and include nineteen species (Shahina *et al.*, 2019). In recent studies, four more species including two new and two recorded species were reported in the genus *Aphelenchoides* (Salma *et al.*, 2020; Samreen *et al.*, 2020b). In order to further probe and enhance the scope of research work about Aphelenchid nematode fauna,

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several investigations and analysis of soil samples were conducted from four various location/villages of Lakki Marwat district, Khyber Pakhtunkhwa, Pakistan (Samreen *et al.*, 2020a). During the present study, one new species namely *Aphelenchoides marwataensis* n. sp. has been described along with redescription of two other species *Ektaphelenchoides poinari* Aliramaji *et al.*, 2014 and *Paraphelenchus myceliophthorus* Goodey, 1958. The genus *Ektaphelenchoides* is being reported first time from Pakistan.

MATERIALS AND METHODS

Area explored: A total of 73 samples of soil were taken in year 2019 from various villages including Aba Khel, Aghzar Khel, Sarai Naurang and Tajori of Lakki Marwat district, Khyber Pakhtunkhwa, Pakistan.

Processing of samples: The nematodes from soil were extracted by Cobb’s sieving and decanting method

(Cobb, 1918) and Baermann’s funnel technique (Baermann, 1917). Subsequently, they were killed instantly by pouring hot water (80-90°C) in glass cavity block and preserved in Tri-ethanol Amine Formalin (TAF) solution for 24 hours (Courtney *et al.*, 1955). Later on, samples were washed three times with distilled water, placed for 5-6 days at 55°C in an incubator with 2 ml of 1.25 % glycerin for slow dehydration process (Seinhorst, 1959). Permanent mounting was done in pure drop of glycerin and sealed by paraffin wax.

Measurement, illustration and Photomicrograph: Accordingly, measurements were taken by de Man’s, 1884 formula, however, illustrations were prepared by drawing tube or camera lucida. Photomicrographs were captured via Nikon DS fi 1 camera attached with Nikon Eclipse E-400 compound microscope.

Table 1. Morphometric data of *Aphelenchoides marwataensis* n. sp. Measurements are in µm (range) Mean±SD.

Morphological Characters	Holotype ♀	Paratype (07♀♀)	Paratype (04 ♂♂)
L(mm)	408	440.6 ± 23.45 (408-462)	415.4 ± 32.37 (384-460)
a	28.3	28.9 ± 0.53 (28.3-29.6)	31.4 ± 1.40 (29.5-32.8)
b'	4.0	4.23 ± 0.26 (4-4.6)	4.4 ± 0.21 (4.2-4.7)
c	13	13.9 ± 0.94 (13-15.2)	12.6 ± 1.10 (11.2-13.9)
c'	3.9	3.83 ± 0.32 (3.4-4.2)	3.36 ± 0.28 (3-3.7)
V or T%	69.4	69.46 ± 0.57 (68.8-70.2)	47.8 ± 2.90 (43.7-50)
Lip width	4	3.9 ± 0.09 (3.8-4)	3.9 ± 0.09 (3.8-4)
Lip height	2	1.83 ± 0.23 (1.5-2)	1.83 ± 0.23 (1.5-2)
G1 %	49	53.1 ± 3.03 (49-56.2)	-
Stylet	12	10.8 ± 0.86 (10-12)	10.6 ± 0.47 (10-11)
Conus length	4	(4 ± 0)	(4 ± 0)
Pharyngeal length	101	103 ± 3.5 (100-108)	92.8 ± 3.71 (88-97)
Median bulb	52	52.6 ± 0.94 (52-54)	53 ± 0.81 (52-54)
Median bulb length	10	10.3 ± 0.47 (10-11)	10.3 ± 0.47 (10-11)
Median bulb width	8	8.1 ± 0.23 (8-8.5)	8.1 ± 0.23 (8-8.5)
Median bulb ratio (L/W)	1.2	(1.2 ± 0)	(1.2 ± 0)
Width at median bulb	12.5	13 ± 0.70(12-14)	12.8 ± 0.65 (12-13.6)
Nerve ring	56	55.3 ± 0.94 (54-56)	56 ± 1.6 (54-58)
Excretory pore	54	52.6 ± 0.94 (52-54)	52.5 ± 0.75 (52-53.6)
Max. body width	14.4	15.7 ± 0.99 (14.4-16.8)	13.2 ± 0.86 (12-14)
Width at vulva	12	12.8 ± 0.8 (12.5-14)	-
Post uterine sac (PUS)	34	36 ± 1.41 (34-38)	-
Ovary or Testis length	200	235 ± 25.49 (200-260)	183.3 ± 12.47 (170-200)
Tail	31.2	31.6 ± 1.8 (29.6-34)	32.9 ± 3.03 (28.8-36)
ABW/CBW	8	8.3 ± 1.24 (7-10)	9.7 ± 0.18 (9.6-10)
Vulva-anus distance	93.6	95.8 ± 10.73 (84-110)	-
Mucro	-	-	3.06 ± 0.09 (3-3.2)
Spicule	-	-	14.6 ± 0.94 (14-16)

RESULTS AND DISCUSSION

***Aphelenchoides marwataensis* n. sp.: Description**

Female: Body usually small, cylinder-shaped, slightly ventrally concave and dorsally convex tapering at both

ends. Cuticle finely annulated slightly less than 1µm wide. Lateral field having four incisures. Cephalic region rounded, offset, about 3.8-4 µm in width and 1.5-2 µm high. Stylet 10-12 µm long and delicate with slight basal knobs. Conus about 33-40% of total stylet length. Procorpus slender. Metacarpus rounded, having well sclerotized conspicuous valve located centrally, about 10-11 µm in length and 8-8.5 µm wide.

Excretory pore situated just opposite to the base of metacarpus; the distance from anterior region is about 5 times to metacarpus length. Isthmus short. Nerve ring located at about 5-6 times of metacarpus length posterior to it. Hemizonid not visible. Pharyngeal gland slender, about 6-7 times body diameter long, overlying intestine dorsally. Intestine simple.

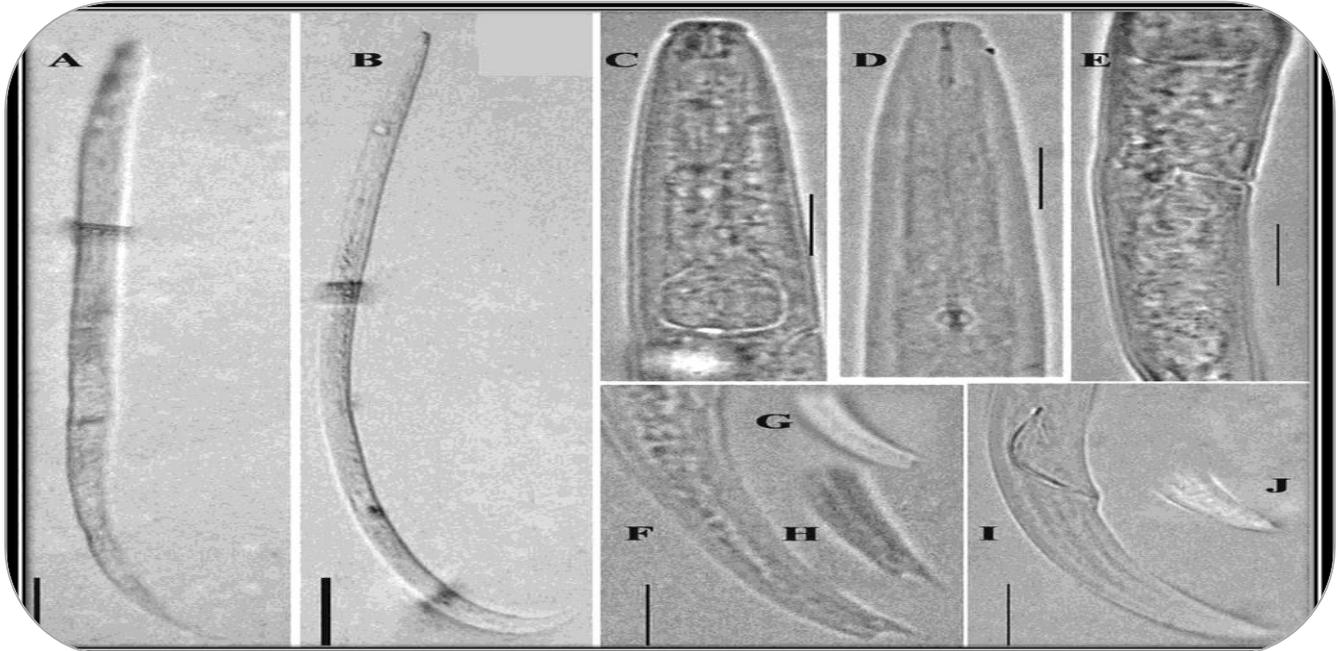


Figure 1. *Aphelenchoides marwataensis*. A. Female. Whole body; B. Male whole body; C. Female pharynx; D. Male Pharynx; E. Female vulval position and PUS; F-G. Female tail variations; I. Male tail; J. Male tail tip. (Scale: A, B=20µm; C-J=100µm).

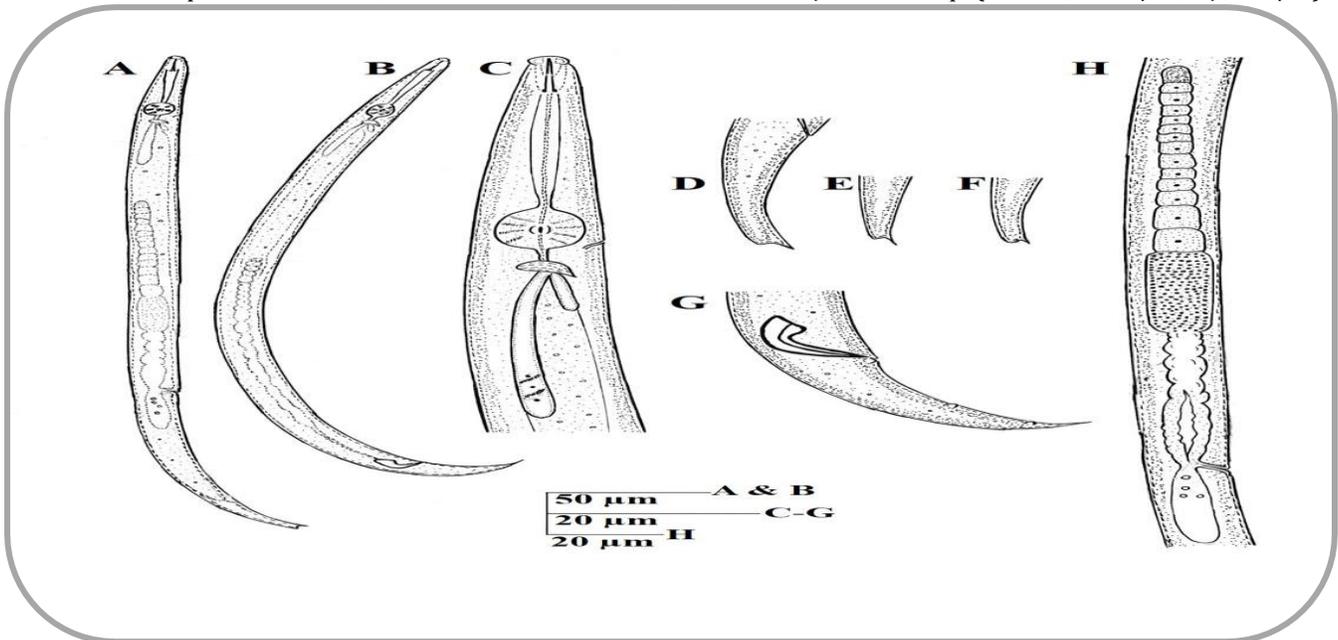


Figure 2. *Aphelenchoides marwataensis* n. sp. A. Female whole body; B. Male whole body; C. Female pharyngeal region; D. Tail; E, F. Female tail variation; G. Male tail; H. Female gonad showing vulval position and PUS.

Reproductive system mono-prodelphic, oocytes organized in one row. Spermatheca well developed, oblong, filled with small rounded sperms. Uterus long. Vagina thick walled, directed anteriorly. Vulva slightly protuberant lips. Rectum and anus functional. Post uterine sac usually with sperms, about 35-42% to vulva-anus distance. The tail terminus variable, bifid with two prongs, with the dorsal process decreased to give angular region adjacent to the ventral mucro. Tail is approximately 3-4 times anal body diameter long.

Male: Body slender, J-shaped when heat killed. Anterior part and cuticle similar to female. Reproductive system monorchic, testis outstretched with spermatocytes designed in one row. Spicule short, arcuate, apex and rostrum rounded, end of dorsal limb is simple. Gubernaculum absent. Tail conical, with sharp mucro. Three pairs of sub ventral caudal papillae present; first pair positioned just posterior to cloacal aperture, second pair is slightly posterior to the mid of tail region and third one just anterior to tail end.

Differential diagnosis and relationship: According to key given by Shahina, 1996, the new described species belongs to genus *Aphelenchoides* of Group 2 as "Tail mostly with one or sometimes two mucronate structure on the tail end". Upon basis of bifid nature of tail terminus, the new species comes close to four species of Group 2 of genus *Aphelenchoides* viz., *A. bicaudatus* (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941; *A. cibolensis* Riffle, 1970; *A. sacchari* Hooper, 1958 and *A. varicaudatus* Ibrahim and Hooper, 1994. From *A. bicaudatus* it differs in having slender female body vs robust, greater a value in male ($a=29.5-32.8$ vs 22.6), in lateral field (4 vs 2); shorter tail length of female ($29.6-34$ vs $50 \mu\text{m}$); in position of excretory pore (anterior to nerve ring vs usually opposite anterior margin or behind nerve ring); larger value of c and smaller value of c' ($c=13-15.2$ vs $9.4-12.6$; $c'=3.4-4.2$ vs $4.5-5$). In addition, the shape of the tail terminus is quite different from that of *A. bicaudatus*. From *A. cibolensis* it differentiates in slightly larger value of c' ($3.4-4.2$ vs 3.2); longer tail length ($29.6-34$ vs $26.6 \mu\text{m}$), lateral lines (4 vs 3) and presence of male vs absent. From *A. sacchari* it differentiates in having smaller body length ($0.40-0.46$ vs $0.51-0.75$ mm), and in number of lateral lines (4 vs 3). From *Aphelenchoides varicaudatus* it varies in body length ($0.40-0.46$ vs $0.58-0.71$ mm); smaller value of b ratio ($4.0-4.6$ vs $8.6-10.9$); shorter tail length ($29.6-34$ vs $42.2 \mu\text{m}$); shorter stylet length ($10-12$ vs $12.7-14.6 \mu\text{m}$)

and presence of male vs absent.

Type habitat and locality: Specimens were retrieved from ber (*Ziziphus mauritiana* L.) from Sarai Naurang village of Lakki Marwat district, Khyber Pakhtunkhwa, Pakistan (GPS of sampling site= $32^{\circ}49'43''\text{N}$ and $70^{\circ}46'33''\text{E}$).

Type material: The holotype female and paratype female and male has been submitted in Nematode Collection of National Nematological Research Centre, University of Karachi, Pakistan.

Etymology: The new identified species was designated its name according to the type area "Lakki Marwat".

***Ektaphelenchoides poinari*: Aliramaji et al., 2014:**

Description: Female: Body usually cylindrical, narrowing gradually at both ends, ventrally arcuate when heat killed. Cuticle finely annulated. Lateral field obscure. Head off set from rest of the body, width 2 times the height. Stylet without basal knobs. Procorpus cylindrical, connected to a muscular and rectangular median bulb with the granular part. Pharyngeal gland well developed, overlapping intestine dorsally, 2.6-2.7 times the distance from anterior region to the base of metacarpus. Nerve ring situated posterior to the base of metacarpus, about 4.3 times to metacarpus length. Excretory pore situated at the level of metacarpus base to slightly posterior. Hemizonid 5.4-5.5 times metacarpus length from anterior region. Reproductive system mono-prodelphic, occupying 41.8-47.8% of the body length, located at left of intestine. Oocytes mostly in double row. Oviduct small, spermatheca oval to irregular, filled with rounded sperm. Crustiformerian unclear, details hardly visible. Uterus with thick-walled. Vagina not sclerotized, slightly inclined anteriorly. Post-uterine sac short. Intestine ends in blind sac. Anus and rectum indistinct. Posterior region conical, ending to a rounded to pointed tip.

Male: Not ascertained.

Remarks: Earlier, *E. poinari* was originally described by Aliramaji et al., 2014 from the bark of a dead *Pinus sylvestris* L. as well as from soil around its trunk from Tehran, Iran. Presently, this species was recovered from soil around the rhizosphere of banana (*Musa paradisiaca* L.) from village Sarai Naurang of Lakki Marwat district, KPK, Pakistan. The dimensions closely fit within the original description except in pharynx length ($173-205$ vs $65-83 \mu\text{m}$). The genus *Ektaphelenchoides* are reported as new record from Pakistan.

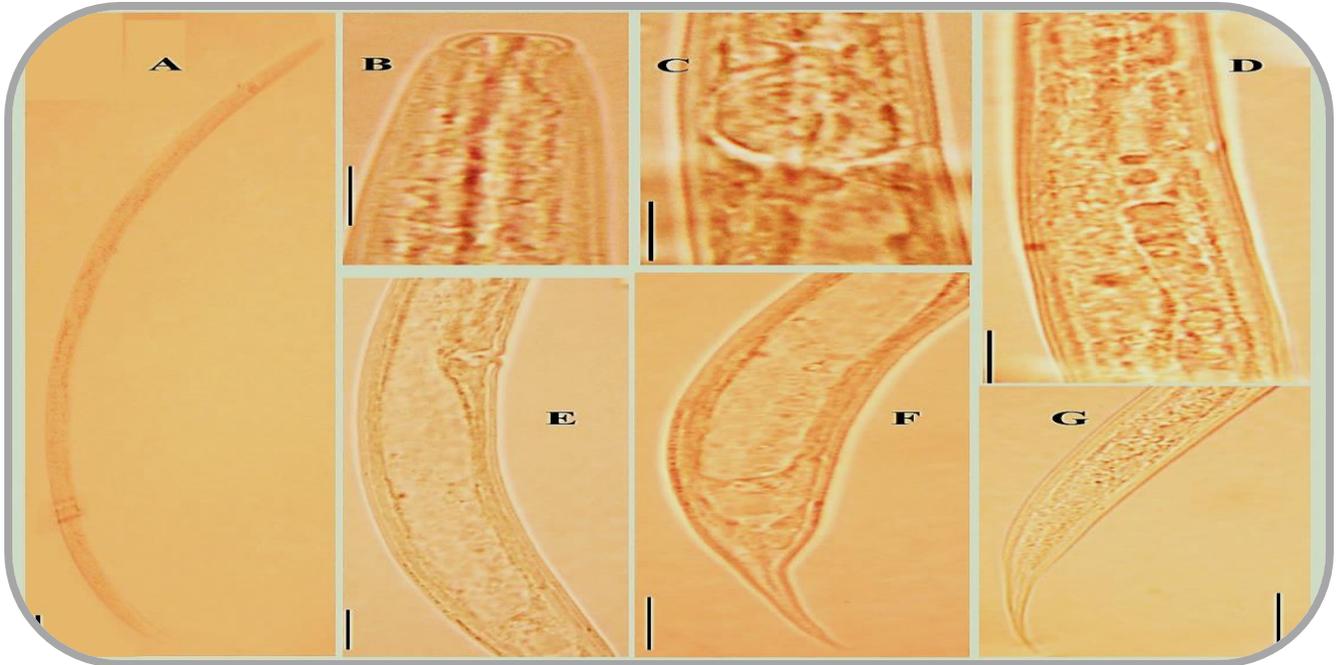


Figure 3. *Ektaphelenchoides poinari* Aliramaji *et al.*, 2014 Female: A. Whole body; B. Cephalic region; C. Oesophageal region showing excretory pore; D. Anterior region showing hemizonid; E. Posterior part showing vulva, PUS and blind end of intestine; F-G. Tails (Scale: A=20µm; B-G=100µm).

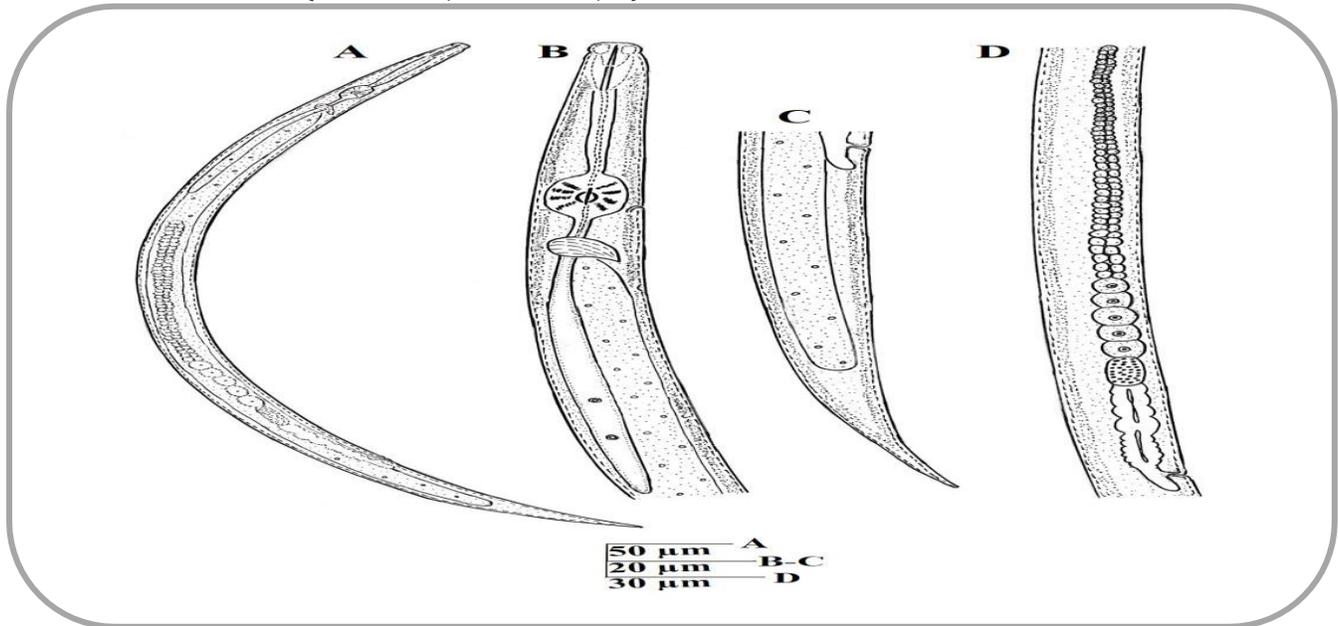


Figure 4. *Ektaphelenchoides poinari* Aliramaji *et al.*, 2014. Female. A. Whole body; B. Pharyngeal region; C. Posterior region showing vulva, PUS, blind end of intestine and tail; D. Gonad.

***Paraphelenchus myceliophthorus*: Goodey, 1958:**

Description: Female: Body long, slender, slightly ventrally curved upon heat killing. Cuticle finely annulated, about less than 1µm. Lateral field with six incisures. Cephalic region rounded, non-offset and about 4 µm in width and 4 µm high. Stylet 14 µm long and delicate without basal knobs. Conus is about as long as

shaft. Procorpus cylindrical. Metacarpus rounded with well sclerotized conspicuous valve located centrally, about 21 µm in length and 16 µm wide. Excretory pore and nerve ring located almost parallel to each other posterior to the median bulb, the position of both approximately 1/3 of the metacarpus length. Isthmus long. Hemizonid not seen. Pharyngeal glands forming a

spatulate bulb and abuts the intestine, about 1/3 of body diameter long. Cardia about 3 µm. Intestine simple. Reproductive system mono-prodelphic, oocytes organized in one row of cells. Oviduct leading to ovary and spermatheca. Spermatheca oblong and well developed, packed with small rounded sperms. Uterus oblong, vagina thick walled and directed arterially.

Vulva with prominent anterior and posterior lips. Rectum about two anal body diameter long. Post uterine sac long, with no sperms, covering about 32-33% of vulva-anus distance. Tail conical, about 3 times anal body diameter long, ending to a bluntly rounded terminus.

Male: Not ascertained.

Table 2. Morphometric data of *Ektaphelenchoides poinari* Aliramaji *et al.*, 2014 and *Paraphelenchus myceliophthorus* Goodey, 1958. All measurements are in µm (range) Mean±SD.

Characters	<i>Ektaphelenchoides poinari</i> Aliramaji <i>et al.</i> , 2014	<i>Paraphelenchus myceliophthorus</i> Goodey, 1958.
	Female (n=10)	Female (n=02)
L (mm)	583.75±60.12 (526-702)	704, 709
a	29.36±1.52 (27.1-31.6)	27, 30.8
b	7.51±0.62 (6.6-8.7)	4.8, 5.1
b'	3.07±0.27 (2.8-3.7)	-
c	-	22.7, 21.4
c'	-	2.8, 3.3
V%	77.27±1.43 (74.6-79.5)	75.9, 77
Lip width	7.87±0.59 (7-9)	8, 8.5
Lip height	3.75±0.43 (3-4)	4, 4.2
Stylet	17.37±2.95 (14-21)	14, 14
Pharyngeal length	187.5±10.53 (173-205)	144, 138
Median bulb	71±4.03 (63-77)	90, 96
Median bulb length	18.5±0.86 (17-20)	21, 20
Median bulb width	10.92±0.73 (10-12)	15.5, 15.2
Median bulb ratio (L/W)	1.6±0.13 (1.4-1.8)	1.3, 1.3
Nerve ring	80.12±4.25 (74-86)	96, 95
Excretory pore	71.6±6.5 (62-82)	96, 90
Max. body width	19.87±1.96 (18-24)	26, 23
Post uterine sac (PUS)	7±1 (6-8)	45, 44
ABD	-	9, 10
Tail	-	31, 33

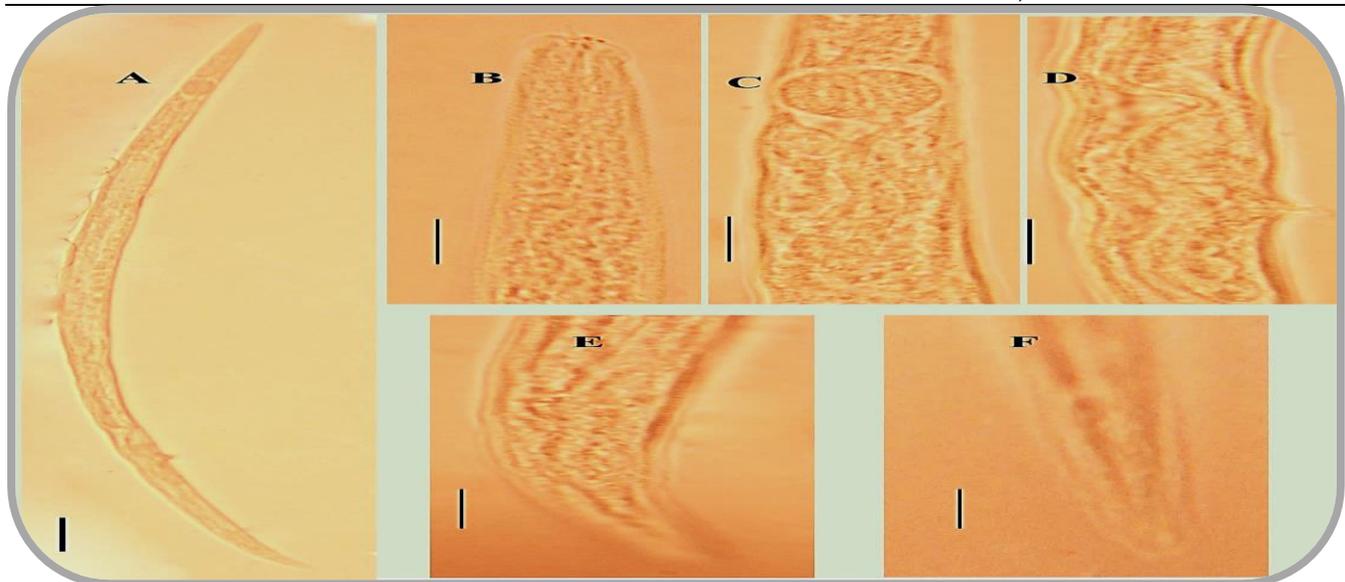


Figure 5. *Paraphelenchus myceliophthorus* Goodey, 1958. Female: A. Whole body; B. Cephalic region; C. Esophageal region showing excretory pore; D. Vulval region and PUS; E. Anus; F. Tail (Scale: A=20µm; B-F=100µm).

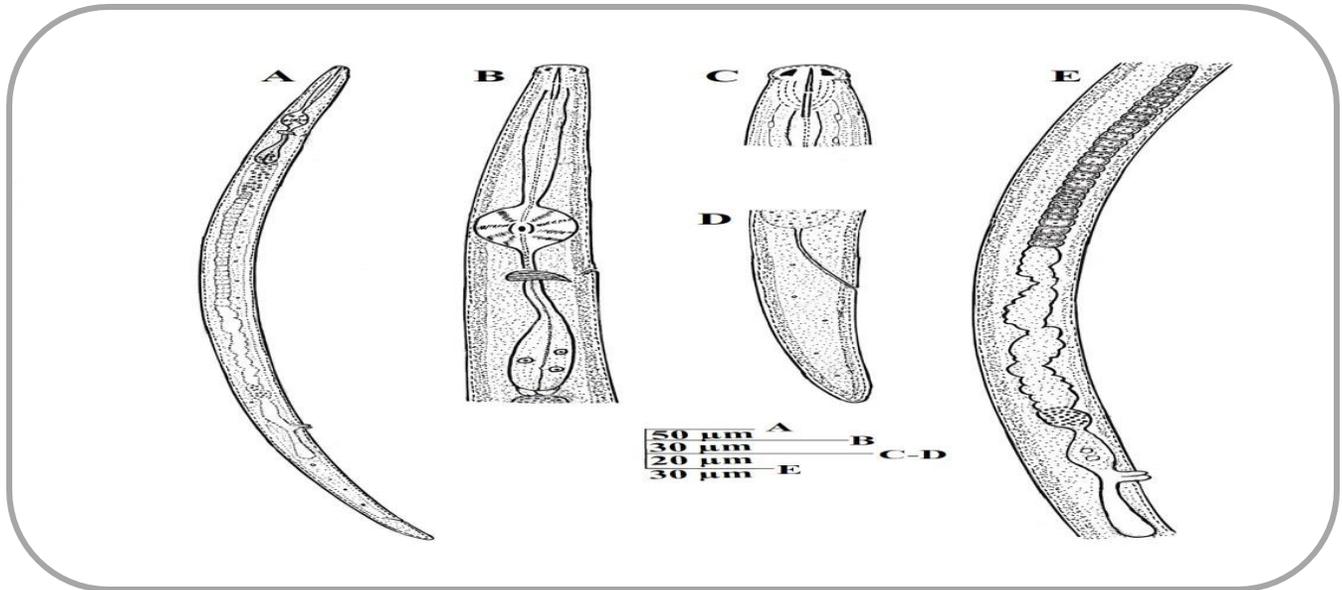


Figure 6. *Paraphelenchus myceliophthorus* Goodey, 1958. Female. A. whole body; B. Pharyngeal region; C. Cephalic region; D. Tail; E. Gonad showing vulval position and PUS.

Remarks: Earlier, Goodey in 1958 originally described *Paraphelenchus myceliophthorus* from mushroom compost. In Pakistan, the genus *Paraphelenchus* (*Paraphelenchus* spp) was reported by Akhtar, 1962 from wheat and sugarcane field, Lahore (Zarina and Shahina, 2012; Maqbool and Shahina, 2001). It is pertinent to highlight that no species has been reported/identified in this genus until now. Hence, *Paraphelenchus myceliophthorus* is the first new record species reported under the genus i.e., *Paraphelenchus* from Pakistan. Currently, it was recovered from sample of soil collected around the rhizosphere of chickpea (*Cicer arietinum* L.) from village Aghzar Khel of Lakki Marwat district, KPK, Pakistan (GPS of sampling site: 32°23'37.0"N and 70°44'16.0"E). The dimensions closely fit within the original description given by Goodey, 1958.

CONCLUSION

It is considered to highlight that this research study was carried out on identification of nematodes up to species level from Lakki Marwat district, Khyber Pakhtunkhwa, Pakistan. The presence of new and known species is an indicator for the existence of other undiscovered species in this region. Much more intention should be needed to get more valuable evidences regarding the incidence of nematodes especially from Order Aphelenchida.

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Contribution of Authors:

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| J. Salma | : Supervised the work. |
| K. Samreen | : Conceived the idea and personally conducted surveys, processed samples, taken measurement, made line drawing, took photographs, identified the species and wrote/designed the manuscript. |
| K. Nasira | : Guided in identification of species and reviewed the manuscript. |