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Description of new stunt nematode species, *Merlinius nagerensis* n. sp. and new records of plant parasitic nematodes from Gilgit-Baltistan, Pakistan

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Abstract

One new species *Merlinius nagerensis* n. sp. and five new records were found during the surveillance of different crop fields of Gilgit-Baltistan, Pakistan. The new species is characterized by punctuation on the entire body and with rounded and smooth tail terminus. This species comes close to *M. montanus* and *M. khuzdarensis*, but differs from *M. montanus* in having pointed tail terminus and from *M. khuzdarensis* by longer stylet. The taxonomic studies on *Boleodorus volutus, Merlinius alboranensis, Paratylenchus (P.) nanus, Pratylenchus alleni, Telomalenchus williamsi* are presented in this paper. The indigenous fauna had similar morphological and morphometrical characters to the corresponding type population.

Keywords: Merlinius nagerensis n. sp., plant parasitic nematodes, taxonomy, Gilgit-Baltistan.

Surveys were conducted during 2014-2015, to study the diverse fauna of nematode associated with different crops of Gilgit-Baltistan. Collected soil and root samples were processed for extracting nematodes by Cobb's wet sieving technique (Cobb,1918) followed by a modified Baermann funnel method (Baermann, 1917). Encountered nematodes were heat killed, fixed in double-strength TAF (Tri-ethanol-amine Formaldehyde) containing 8% formalin and 2% tri-ethanol-amine in distilled water and mounted in dehydrated glycerin (Siddiqi,1986). Illustrations were made by using a drawing tube attached to the compound microscope Nikon Eclipse E400 and photographed with a Nikon DS, Film camera, attached to the same microscope. All identified species belong to suborder Tylenchina. Morphologically, the specimens correspond rather well with original However. description. it was deemed worthwhile to prepare complete illustration from this new material. The objectives of this study were : i) to describe a new species of Merlinius found in soil from the root zone of potato plants (Solanum tuberosum L.) from District Nager, Hoper Valley, Pakistan; and ii) to identify, illustrate, and image other plant-parasitic nematodes found during the survey with some additional morphometric and morphological details.

Merlinius nagerensis n. sp. (Fig. 1a & b; Table 1)

Description

Female: Body slightly arcuate after killing by gentle heat, narrowing towards extremities, Cephalic region offset 3-4 μ m high and 6-8 μ m wide, consists of 4-6 annules, labial frame work moderately sclerotized, lateral fields have six incisures occupying ½ of body width. Punctuations present between lateral lines, punctuated field become obscure at extremities. Stylet slender, moderated, developed with attenuated level 12-14 μ m long. Stylet knobs large 2-3 μ m in diameter, directed slightly posteriorly. Dorsal oesophageal gland orifice 2-3 μ m behind stylet base. Median bulb oval with prominent valvular apparatus, situated at 44.96-46.5% of oesophagus. Isthmus slender, encircled by nerve ring at about 50% of its length. Basal bulb pyriform 21-24 μ m high and 1014 μ m wide. Excretory pore anterior of basal bulb and 88-106 μ m from the anterior end. Hemizonid prominent located 2-3 annules anterior to excretory pore. Vulva transverse, vulval lips not elevated, epiptygma present. Spermatheca oval, filled with sperms. Ovaries paired well developed oocytes arranged in multiple rows. Length from vulva to anus 228-258 μ m. Phasmid prominent located at 10-12 μ m from anus. Rectum short 10-12 μ m in length. Tail rounded, 36-45 μ m long comprised of 35-44 annules, termins smooth. Bursa finely crenate.

Male: Morphologically male is similar to female. Tail portion slightly curved and tapering at the end with pointed tail terminus. Testis stretched out, multinucleate at the apical zone. Spicules curved ventrally at the anterior end, 20 μ m long while gubernaculum 7 μ m in length. Bursa finely crenated.

Diagnosis and relationship: Merlinius nagerensis n. sp. can be separated from all the known species of the genus by the presence of punctuations throughout the body with rounded and smooth tail terminus, but this species comes close to M. montanus Maqbool & Shahina (1987) and M. khuzdarensis Handoo et al., (2007) by the presence of punctuation in body. This species differs from M. montanus due to pointed and small tail and body length (tail length=36-45 vs $42-53\mu$ m, L = 0.45-0.56 mm vs 0.52-0.65 mm); less body width (21.1-24.4 vs 23.2-27.04) and "c" ratio (10.7-12.1 vs11.81-15.66) while as M. khuzdarensis has larger body length (0.73-0.87 vs 0.52-0.65); longer stylet (20-22 vs 12-14) and "a" ratio (32.0-37.0 vs 23.2-27.04).

Type habitat and locality: Samples were collected from the root zone of potato plants (*Solanum tuberosum* L.) from District Nager, Hoper Valley.

Type material: Holotype (female): *Merlinius nagerensis* n. sp. Slide No. 1 deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan. Paratype females (n=10), Male (n=1): on Slides No.2-10, are

also deposited in the above said nematode collection.

Etymology: The species name refers to the type locality of Nager.

Boleodorus volutus Lima & Siddiqi, 1963 (Fig. 2a & b; Table 2)

Description

Female: Body C shape or spiral in structure, tapers at both ends. Body cuticle marked with fine annules, about 1µm apart near vulva. Head raised, offset, smooth, anteriorly flattened 5µm wide and 2.5 µm high. Stylet 9-10 µm, conus 5µm whereas shaft 4-5 µm in length. Stylet knobs flanged like 2 um wide and in height about half to its width. Dorsal gland orifice located behind the base of stylet knobs at 1-2 µm. Lateral fields contain four incisures approximately 1/3-1/4 of the mid body width. Median bulb non valvular, elongated, fusiform swelling, 16 µm in length and 4 µm in width. Excretory pore located at upper part of the oesophagus at 73-76 µm from the anterior end. Nerve ring 18-25 µm anterior to excretory pore. Basal bulb elongated, pyriform 100-101 µm in length. Cardia oval shaped, 5 µm wide and 4 µm in length. Vulval slit transverse occupying half of the body width. Post uterine sac 10 µm long. Spermatheca oval shaped filled with sperms. Ovary short, multinucleate at the tip. Vulva to anus distance 80-104 µm. Tail twisted from anus to tail tip, hook shaped, unstriated and rounded at the tip.

Male: Male less coiled as compared to female. Spicules cephalated, ventrally arcuate 18 μ m long, gubernaculum short, trough shaped, about 5 μ m. Bursa adanal, starts adjacent to the head of spicules and up to 2 anal body width long. Tail terminus pointed.

Remarks: Samples were collected from the root zone of potato plants (*Solanum tuberosum* L.) from District Nager, Hoper Valley. Morphology and measurements of this population correspond well to the original description except in spicule length (18 vs 14-15 μ m).



Fig. 1a (A-H). *Merlinius nagerensis* n. sp., A-B. Whole body of female and male; C. Oesophageal region of female; D. Vulva and reproductive part; E. Male tail; F-H. Variations of female tail shape.



Fig. 1b (**A-H**). *Merlinius nagerensis* n. sp. A. Whole male body; B. Whole female body; C. Oesophageal region of female; D. Vulval region; E, F. Lateral lines with punctations; G. Female tail end; H. Male tail showing spicules and bursa (scale: A, B=50 μm; C-H=10 μm).

Characters	Holotyne female	Paratypes			
Characters	Holotype Temale	Female (n=10)	Male (n=1)		
L (mm)	0.60	0.56±0.04 (0.520-0.656)	0.530		
a	26.08	25.02±1.60 (23.2-27.04)	27.41		
b	5.03	4.93±0.29 (4.46-5.55)	5.30		
с	14.63	13.91±1.07 (11.81-15.66)	13.16		
c'	2.27	2.46±0.32 (1.16-3)	4.16		
V%	56.5	56.56±2.63 (54-63.84)	-		
Stylet	12	12.7±0.64 (12-14)	12		
Excretory pore	90	87±4.73 (79-96)	92		
Oesophagus	118	114±6.02 (106-127)	121		
Tail length	41	39.6±2.61 (36-45)	50		
Tail annules	40	37±2.5 (35-40)	-		
Head annules	5	5±0.44 (4-5)	5		
Maximum body width	23	22.6±2.38 (20-29)	6		
Anal body width	18	16.1±1.86 (13-18)	12		
Gubernaculum	-	-	7		
Spicules	-	-	20		

Table 1. Morphometrics of *Merlinius nagerensis* n. sp. Measurements (except where indicated) are in µm and in the form mean±SD (range).



Fig. 2a (A-D). *Boleodorus volutus*. A. Oesophageal region of female; B. Tail end of female; C. Tail end of male; D. Whole body of female.



Fig. 2b (**A-D**). *Boleodorus volutus*. A. Oesophageal region; B. Vulval region; C. Lateral lines; D. Male tail with spicule and bursa (scale: A-D=10 μm).

Characters	Measure	ements	Original measurements (Lima & Siddiqi,1963)		
	Female n=6	Male n=1	Female	Male	
L (mm)	0.430±0.02	0.500	0.39-0.51	-	
	(0.40-0.45)				
a	30.71±1.86	50.3	18-37	-	
	(28.85-32.57)				
b	4.27±0.23	4.90	-	-	
	(4.04-4.51)				
С	9.43±0.21	13.5	8.4	-	
	(8.76-10.1)				
c'	3.21±0.21	4.75	3.4-5.4	-	
	(3-3.43)				
V%	71.77±0.5	-	67-72	-	
	(71.27-72.27)				
Stylet	9.5±0.5	7	8-10	-	
	(9-10)				
Excretory pore	74.5±1.5	83	69-85	-	
	(73-76)				
Tail length	46±6	38	-	-	
	(40-50)				
Anal body width	9±1	8	-	-	
	(8-10)				
Maximum body width	14 ± 0	10	-	-	
	(14)				
Oesophagus	100.5±0.4	-	-	-	
	(100-101)				
Gubernaculum	-	5	-	5	
Spicules	-	18	-	14-15	

Table 2. Morphometric data of *Boleodorus volutus*. Measurements (except where indicated) are in µm and in the form mean±SD (range).

Merlinius alboranensis (Tobar-Jimenez, 1970) Tarjan, 1973 (Fig. 3a & b; Table 3)

Description

Female: Body open C-shaped. Head off set contains 4-6 annules, 3-4 µm high and 7-8 µm in width. Stylet 12-18 µm long, strongly developed. Stylet knobs prominent, rounded in shape. Stylet knobs 1-2 x 2-3 µm in size. Dorsal gland orifice at the distance of 3-4 um from the stylet base. Excretory pore 78-96 µm from the anterior end whereas nerve ring at 76-84 µm. Median bulb oval shape 12-14 x 8-10 µm in size. Basal bulb elongate, 24-26 µm long. Cardia prominent, rounded located between the base of basal bulb and intestine 2-3µm in length. Vulval slit transverse, ovary outstretched, oocytes arranged in a single row. Annules prominent at vulval and tail region about 0.7-1 µm in length. Body tapers from vulva to tail tip, the distance from vulva to anus 106-127 µm. Anus prominent, distance between anus to tail tip 36-44 µm and contain 32-40 annules. Rectum 10-12 µm long. Phasmids 18-24 um from anus. Tail cylindrical to sub cylindrical in shape with smooth terminus.

Male: Morphologically males are similar to female but the difference is due to male reproductive parts (spicules and gubernaculum). Spicules curved at the anterior, $18-20 \mu m$ in length whereas gubernaculum crescent like 6 μm long. Tail 42-48 μm long, terminus narrow and pointed. Phasmids 18-28 μm apart from anus, bursa crenated enveloping tail.

Remarks: Specimens found around the roots of potato from District Hunza villages; Khudabad, Sost, Gojal and District Nager, village Jafarababd in District Nager, Gilgit-Baltistan. The population of *M. alboranensis* has morphological and morphometrical characters corresponding to those of the type population described by (Tobar-Jimenez, 1970) Tarjan, 1973. In the present population the only difference is in vulva percentage (53.09-57.44 vs 57.7-60.5).

Paratylenchus (P.) nanus Cobb, 1923 (Fig. 4a & b; Table 4)

Description

Female: Body curved, ventrally bent at posterior end. Lateral fields consist of four incisures. Body annulation prominent 1 µm apart. Head continuous, truncated, annulated (3-4 annules) about 6-8 µm wide. Stylet 25-28 µm long with well developed sloping knobs 2x4 µm in height and width. Dorsal gland orifice 6-8 µm behind the base of the stylet. Median bulb occupy about 1/2 of the oesophageal length. Excretory pore located 64-78 µm from the lip, opposite to the anterior end of basal bulb. Basal bulb small pyriform 14-18 µm long whereas width is half to its length. Cardia attached at the base of basal bulb and overlap anterior end of intestine. Annules prominent at vulval region 1 µm wide. Vulva transverse slit covered with massive lateral membrane i.e. 8-10 um in length. Vagina directed forward covers more half of body width, than join to quadricolumella i.e. 4-5 cells long. Spermatheca filled with sperms. Ovary outstretched, oocytes in single column except at apical zone. Vulva-anus distance equal to tail length. Post uterine sac absent. Deirid not seen. Rectum short 7-8 µm in length. Tail digitate with rounded terminus, 13-20 µm long, consists of 12-20 annules.

Male: Not found.

Remarks: Samples have been collected from the root zone of pomegranate (*Punica* granatum L.) from District Nager, Village Thole Nager No 2. Gilgit-Baltistan. Measurements and description correspond with those of Cobb, 1923. The only difference was found in stylet length (25-28 vs 28-32 μ m).



Fig. 3a (**A-F**). *Merlinius alboranensis*. A. Oesophageal region; B, C. Whole female and male body; D. Vulva and reproductive system; E. Female tail; F. Male tail.



Fig. 3b (A-F). Merlinius alboranensis. A. Male whole body; B. Female whole body; C. Vulval region; D. Oesophageal region; E. Spicules and tail end; F. Female tail. (scale: A, B=50 μm; C-F=10 μm).

	Measurements			
Characters -	Female n=9	Male n=5		
L (mm)	0.63±0.06	0.59±0.04		
	(0.57-0.76)	(0.53-0.66)		
a	31.07±2.58	30.28±2.43		
	(26.22-34.2)	(26.9-33.3)		
b	5.11±0.30	4.86±0.34		
	(4.84-5.61)	(4.53-5.20)		
с	14.30±0.85	12.88±0.03		
	(13.17-15.83)	(12.45-13.32)		
c'	3.04±0.56	4.11±0.28		
	(2.26-4.33)	(3.66-4.54)		
V%	55.82±1.40	-		
	(53.09-57.44)			
Stylet	12.22±0.41	11.2 ± 0.74		
	(12-18)	(10-12)		
Excretory pore	98.33±9.09	93±3.68		
	(80-110)	(88-96)		
Oesophagus	125.1±7.59	122±8.07		
	(107-136)	(108-132)		
Tail length	46.5±6.56	46.4±2.33		
	(34-58)	(42-48)		
Tail annules	37.55±4.69	-		
	(32-48)			
Head annules	4.6 ± 0.48	4.6 ± 0.48		
	(4-5)	(4-5)		
Maximum body width	21.22±0.91	18.8 ± 0.97		
	(20-22)	(18-20)		
Anal body width	14.77 ± 0.9	11±0.63		
	(13-16)	(10-12)		
Gubernaculum	-	7.1 ± 0.48		
		(6.5-8)		
Spicules	-	17.6±0.8		
		(16-18)		

Table 3.	Morphometrics	data	of	Merlinius	alboranensis	(Tobar	-Jimenez,	1970)	Tarjan,	1973.
	Measurements	(exce	pt v	where indic	cated) are in µ	um and i	n the forn	n mean	±SD (ran	.ge).



Fig. 4a (A-F). *Paratylenchus (P.) nanus* A. Whole female body; B. Oesophageal region; C-F. Variations in tail shape.

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Fig. 4b (**A-D**). *Paratylenchus* (*P*.) *nanus*. A: Whole female body; B: Head portion; C: Vulval part; D: Vulva with tail portion (scale A= 50 μm; B-D=10 μm).

Character	Massuramonts	Original measurements		
Character	Wieasurements	(Cobb, 1923)		
-	Female n=7	n=10		
L (mm)	0.32±0.01	0.35-0.42		
	(0.30-0.34)			
a	19.24±1.17	19-22		
	(17.47-20.55)			
b	3.58±0.31	3.6-4.3		
	(2.87-3.90)			
c	18.41±3.77	17		
	(14.26-25.30)			
c'	1.66±0.23	-		
	(1.45-2.16)			
V %	84.67±1.64	83-88		
	(81.33-87.26)			
Stylet	26.42 ± 1.04	27-32		
	(25-28)			
Excretory pore	70.28±3.23	-		
	(64-75)			
Oesophagus	91±6.69	-		
	(86-106)			
Tail length	18.14±3.56			
	(13-20)			
Tail annules	16.57±2.49			
	(12-20)			
Head annules	4 ± 0			
	(4)			
Anal body width	10.87±0.63			
	(10-12)			
Maximum body width	17.1±1.35			
	(15-18)			

Table 4. Morphometrics data of *Paratylenchus (P.) nanus* Cobb, 1923. Measurements (except where indicated) are in µm and in the form mean±SD (range).

Pratylenchus alleni Ferris, 1961 (Fig. 5a &b; Table 5)

Description

Female: Body posture open 'C' shape, tapering from both ends. Whole body finely annulated, lip region comprised of 2 annules or one striation. Head continuous bluntly rounded 3-4 μ m high and 7-8 μ m in width. Stylet 12-16 μ m long, stylet knobs rounded, slightly flattened anteriorly about 3-4 μ m in diameter, length of conus 5-6 μ m whereas shaft is 7-8 μ m. The length of annules near vulval region is 1 μ m.

Median bulb highly muscular, valvular apparatus prominent, 10-14 μ m long and 8-9 μ m in width. DGO clearly seen 4 μ m from the base of stylet knobs. The length of procorpus 50-52 μ m, metacorpus 40-44 μ m, nerve ring located at 56-60 μ m from the anterior end. The length of isthmus 20-26 μ m, position of hemizonid just anterior to the excretory pore. Lateral fields with 4 lines about 1/3 the body width at vulval region, visible behind the phasmids.

Vulva transverse slit, vagina covers 1/3 of the body width. Spermatheca rounded functional as long as uterus, while the length of ovary is 117-120 μ m. Post uterine sac 14-16 μ m long whereas vulva at 46-58 μ m from anus. Tail rounded, consists of 14-18 annules, tail terminus with one or two indentations usually unstraited.

Male: Not found.

Remarks: Specimens of *Pratylenchus alleni* were collected from different hosts and localities i.e., Okara from Juital Village, District Gilgit, The high population was recovered from the roots and soil around the plants of plum, Jafarabad Village and walnuts from Thole Nager of Gilgit-Baltistan. Measurements and morphology of this population fits with the original description given by Ferris, 1961.

Telomalenchus williamsi (Geraert & Raski, 1986) Siddiqi, 2000 (Fig. 6a & b; Table 6)

Description

Female: Body slightly arcuate ventrad. tapering from both ends. Body has prominent annules throughout its length. Head slightly offset, truncated, 3 µm high and 8 µm wide, comprised of 4 annules. Amphidial apertures slit like, slightly curved. Stylet 12 µm with very slender conus and delicate flanged knobs. DGO at the distance of 2 µm from the base of stylet knobs. Median bulb fusiform to slightly spherical, valvular apparatus distinct, at 46 % of total oesophageal length. Isthmus long, slender 28 µm in length while pyriform basal bulb 90 µm in length.

Excretory pore near the middle of the basal bulb 81 um from the anterior end. Nerve ring in the posterior region of isthmus, the distance from the tip of head to never ring is 60 µm. At oesophageal position annules become wide about 2 µm. Cardia rounded 4 µm wide and 9 µm high. Lateral fields comprised of four incisures started from head region below stylet and ends below phasmids. The distance between each lateral line is 2 µm at mid of the body. Vulva posterior, covered with lateral flaps that occupying 3 body annules. Ovary prodelphic, spermatheca rounded and filled with sperms. Distance from vulva to anus 83 µm about equal to tail length. Tail elongate, filiform 90 µm in length. Annules width become slightly reduced and ends abruptly near to the tail terminus.

Male: Not found.

Remarks: Morphometric data and description above are based on the single specimen isolated from the sample of potato from District Hunza Village, Khudabad Sost, Gilgit-Baltistan. The description of present specimen agrees with the original description of *Telomalenchus williamsi* by Geraert & Raski (1986).



Fig. 5a (A-F). *Pratylenchus alleni*. A. Whole female; B. Oesophageal region; C.Vulval and reproductive structure; D-F. Variations in tail shapes.



Fig. 5b (**A-D**). *Pratylenchus alleni*. A. Whole female body; B. Vulval region; C. Tail region; D. Oesophageal region (scale: A=20 μm; B-D=10 μm).

Characters	Measurements	Original measurements (Ferris, 1961)
	Female n=9	Female n=10
L (mm)	0.38±0.02	4.7-6.1
	(0.33-0.42)	
a	23.72±1.09	19-27
	(21.23-25.25)	
b	4.31±0.29	4.7-6.1
	(4.02-4.85)	
c	21.58±1.84	15-25
	(18.09-24.14)	
c'	1.98±0.33	-
	(1.6-2.62)	
V %	81.67±6.97	78-83
	(79.41-85)	
Stylet	13.88±0.56	13.5-15
	(13-15)	
Excretory pore	72.33±5.73	-
	(60-80)	
Oesophagus	90±6	-
	(84-100)	
Tail annules	16.22±1.93	-
	(12-18)	
Head annules	2 ± 0	2
	(2)	
Anal body width	8.88±0.73	-
	(8-10)	
Tail length	18.11±2.46	-
	(14-22)	
Maximum body width	15.66±1.24	
	(14-18)	

Table 5. Morphometrics data of *Pratylenchus alleni* Ferris, 1961. Measurements (except where indicated) are in µm and in the form mean±SD (range).



Fig. 6a (A-E). *Telomalenchus williamsi*. A. Whole female body; B. Oesophageal region; C. Vulva and reproductive structure; D. Lateral lines at mid of body; E. Tail end.





Fig. 6b (A-D). *Telomalenchus williamsi*. A. Whole female body; B. Head portion; C. Tail portion; D. Vulval region (scale: $A=20 \ \mu m$; B-D =10 μm).

		Original measurements (Geraert & Raski, 1986)		
Characters	Measurements			
		Siddiqi, 2000		
	Female n=1	Female n=10		
I (march)	0.400	452±18.3		
L (mm)	0.498	(425-495)		
a	23.71	24.33±2.02		
		(21-29)		
b	5.53	4.73±0.17		
		(4.5-5)		
с	5.65	5.59±0.25		
		(5.2-6.0)		
c'	8.8	4.73±0.17		
		(4.5-5)		
V %	66.42	79.0±1.87		
		(74-81)		
Stylet	12	11.5 ± 0.15		
,		(11-12)		
Excretory pore	81	76.6±3.21		
		(70-83)		
Oesophagus	90	94.4±2.6		
1 0		(92-98)		
Tail length	88	82.2±2.5		
6		(79-85)		

Table 6. Morphometrics data of *Telomalenchus williamsi* (Geraert & Raski, 1986) Siddiqi, 2000. Measurements (except where indicated) are in µm and in the form mean±SD (range).

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