

Description of *Discolaimus miniodontii* n.sp. and *Laevides hunderansis* n. sp. with notes on *Discolaimoides spatilabium* (Nematoda: Dorylaimida) from District Ghizer, Gilgit-Baltistan, Pakistan

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Abstract

Soil samples collected from District Ghizer, Gilgit, Baltistan, Pakistan, yielded two new and a known nematode species of the order Dorylaimida viz., *Discolaimus miniodontii* n. sp. and *Laevides manilkarii* n. sp.; and a new report of a known species *Discolaimoides spatilabium* from District Ghizer, Gilgit-Baltistan, Pakistan. *Discolaimus miniodontii* n. sp., is characterized by having smallest odontostyle (9-10µm); relatively smaller and slender body (L=1.0-1.14mm; a= 38.5-45.8); sclerotized odontostyle, its aperture occupying 50-55% of its length; oesophagus with conspicuous muscular sheath in the expended part of oesophagus; basal part of oesophagus expended gradually 51-54% of the total neck length. *Laevides hunderansis* n. sp., collected from district Ghizer village, Hunder of Gilgit-Baltistan is characterized by the smallest body (0.7-0.8mm), small moral tooth (7-8 µm), short spicule, gubernaculum and tail length (24, 5, 14µm, respectively) and by the presence of one supplement within the spicular range.

Keywords: New species, *Discolaimus miniodontii* n. sp., *Laevides hunderansis* n. sp., Dorylaimida, Taxonomy, Gilgit-Baltistan, Pakistan.

The order Dorylaimida is one of the most species-rich groups of free-living Nematoda (Andrassy, 2010). The genus *Discolaimus* is now known to be terrestrial and a predator of nematodes, with a world-wide distribution (Andrassy, 2009).

During a survey of nematodes, soil samples were collected from potato (*Solanum tuberosum* L.) and maize (*Zea mays* L.). After analysis, two un-described species and a known species of the order Dorylaimida were recovered. The new species belonging one each to the genera *Discolaimus* Cobb, 1913 and *Laevides* Heyns, 1968 and a known species *Discolaimoides*

spatilabium Khan & Laha, 1982 are reported as a new record herein.

In Pakistan *Discolaimus* species have been reported by several researchers from different agro-climatic zones from various hosts and localities (Zarina & Shahina, 2012; Maqbool & Shahina, 2001).

Nasira *et al.*, (2008) described a new species *Discolaimus pakistanense* along with a compendium of the genus *Discolaimus* Cobb, 1913.

In the present research, the genera *Laevides* Heyns, 1968 and *Discolaimoides* Heyns, 1963 are reported for the first time from Pakistan.

Materials and Methods

During a survey for diversity of nematodes in district Ghizer, Gilgit-Baltistan, Pakistan, samples were collected from soils around the roots of potato (*Solanum tuberosum* L.) and maize (*Zea mays* L.). Nematodes were extracted by Cobb's sieving and decanting technique (Cobb, 1918) followed by modified Baermann funnel method (Baermann, 1917).

The nematodes recovered were heat killed and fixed in TAF. The nematodes were processed to pure glycerin by Seinhorst's method (Seinhorst, 1959) and mounted on permanent glass slides. The best preserved specimens were observed, photographed, measured and drawn by using ocular micrometer and drawing tube attachment, respectively. For still photo micrography DS L2 camera was used.

Discolaimus miniodontii n. sp. (Figs. 1a & 1b; Table 1)

Description

Female: Body slightly curved ventrally upon fixation. Cuticle with fine transverse striations, 1.5-2.0 μm thick at mid body and 3 μm on tail. Lateral hypodermal chord 10-12 μm wide or about one half of the body diameter at mid body. Lip region well set off by a distinct constriction, 0.5-0.52 times as wide as body diameter at pharyngeal base. Amphid fovea stirrup shaped its aperture diameter about 0.38-0.42 times width at lip region. Odontostyle strongly sclerotized, its aperture 4-5 μm long, 44-50 percent of its length. Odontophore rod like, 1.6-1.8 times the odontostyle length. Guiding ring single, 4-5 μm from anterior end. Nerve ring situated at 28-30% of neck length. Anterior part of pharynx muscular, gradually expanding to form a strongly muscular cylindroid bulb. Basal part is 50-52% of total neck length with a conspicuous sheath of tissue. Dorsal oesophageal gland orifice 10-12 μm and dorsal gland nucleus, 24-26 μm behind anterior end of expanded part of pharynx.

Anterior subventral gland nucleus and orifices are located in the middle, 44-50 μm from the beginning of basal bulb. Orifice of posterior subventral gland located posterior third of basal bulb, 24-30 μm anterior to base of pharynx. Cardia conoid rounded surrounded by large cells. Genital system amphidelphic, ovary reflexed, vulva a transverse slit. Anterior and posterior gonad equally developed 90-104 μm long. Vagina extending inwards about one half of corresponding body width, without sclerotization. Rectum 0.6-0.7 times the anal body diameter. Tail short, dorsally convex, conoid with 3-4 pairs of caudal pores.

Male: Not found.

Type specimens: Holotype and paratype females are deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan.

Type habitat and locality: Specimens were collected from soil around the roots of potato (*Solanum tuberosum* L.) at District Ghizer, village Yasin, Gilgit-Baltistan, Pakistan.

Diagnosis and relationship: *Discolaimus miniodontii* n. sp., differs from all the other species of the genus *Discolaimus* by having smallest odontostyle (9-10 μm). The new species is characterized by relatively smaller and slender body. Odontostyle strongly sclerotized, its aperture occupying 50-55% of its length. Oesophagus with conspicuous muscular sheath in the expanded part of oesophagus. Basal part of oesophagus expanded gradually 51-54% of the total neck length. According to the detailed key given by Wu *et al.*, 2006 the new species comes close to *D. laski* Khan & Laha, 1982 in most measurements but can be differ from it by smaller odontostyle and lip width (9-10 vs. 15-16; 13-14 vs. 20 μm), more slender body ($a=$ 38.5-45.8 vs. 25.3-36.8) and more anteriorly located nerve ring (67-75 vs. 90 μm).

It also comes close to *D. labiatus* Pena Santiago *et al.*, 2002 and *D. microdorus* Siddiqi, 2005 in length of body and in “a” and “b” values. It differs from *D. labiatus* by having small odontostyle (9-10 vs. 10-12.5 μ m), more posteriorly located vulva (47-52.4

vs. 39-43%) and lower c` value (1.2-1.3 vs. 1.5-1.7). It differs from *D. microdorus* in b and c` values (b=4.3-4.6 vs. 3.2-3.9, c`=1.2-1.3 vs. 1-1.2) in smaller odontostyle (9-10 vs. 11-13 μ m) and more behind vulva (V= 47-52.4 vs. 43-46%).

Table 1. Morphometric data of *Discolaimus miniodontii* n. sp. All measurements are in μ m except L and in the form: Mean \pm S.D (range).

Characters	Female	
	Holotype	Paratypes (n=10)
L	1.13	1.07 \pm 0.04 (1.0-1.14)
a	42	40.9 \pm 2.0 (38.5-45.8)
b	4.4	4.44 \pm 0.12 (4.3-4.6)
c	47.2	46.9 \pm 1.96 (44-50.3)
c`	1.33	1.3 \pm 0.02 (1.27-1.35)
V%	52	50.7 \pm 1.51 (47-52.4)
G ₁	8.1	8.78 \pm 0.61 (7.8-9.9)
G ₂	7.9	8.76 \pm 0.80 (7.8-10.3)
Lip width	14	13.5 \pm 0.49 (13-14)
Lip height	4	4.8 \pm 0.4 (4-5)
Amphid aperture	6	5.9 \pm 0.3 (5-6)
Odontostyle length	9	9.3 \pm 0.45 (9-10)
Odontophore length	18	16.3 \pm 1.0 (15-18)
Guiding ring from anterior end	4	4.75 \pm 0.40 (4-5)
Nerve ring from anterior end	74	74.6 \pm 2.76 (67-77)
Pharyngeal length	254	238.3 \pm 4.1 (232-254)
Expanded part of pharynx	128	123.5 \pm 2.72 (120-128)
Cardia length	9	9.5 \pm 0.67 (8-10)
Body diameter at neck base	26	25.8 \pm 0.6 (25-27)
Body diameter at mid body	27	26.1 \pm 0.7 (25-27)
Body diameter at anus	18	17.7 \pm 0.45 (17-18)
Vaginal depth	12	11.3 \pm 0.64 (10-12)
Vulva from anterior end	590	548.9 \pm 22.1 (531-590)
Prerectum length	24	27.2 \pm 2.71 (24-30)
Rectum length	11.5	12.05 \pm 0.56 (11.5-13)
Tail length	23	22.8 \pm 0.97 (21-25)

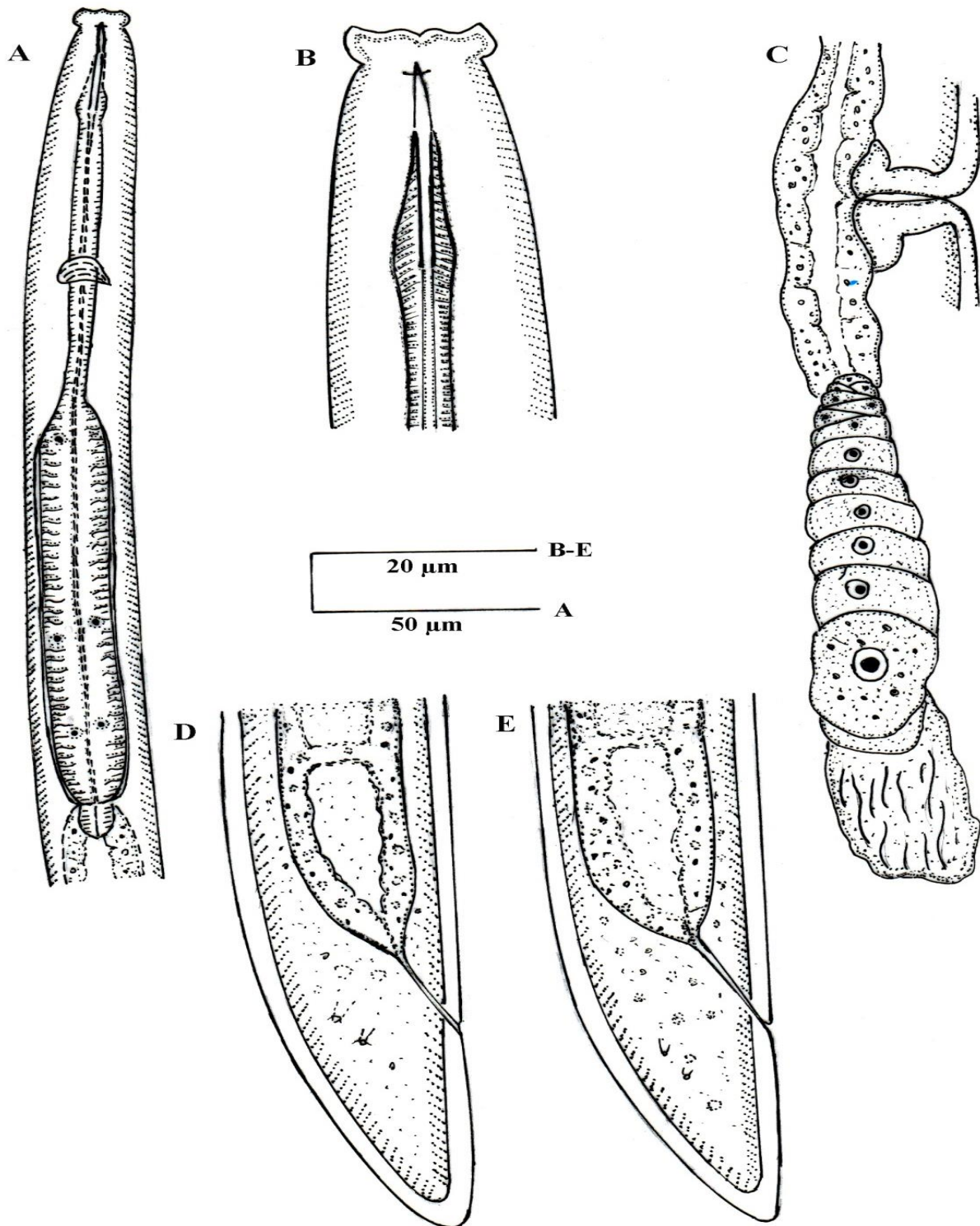


Fig. 1a. (A-E). *Discolaimus miniodontii* n. sp. Female: A. oesophageal region; B. Anterior region; C. Female gonad; D, E. Tail region.

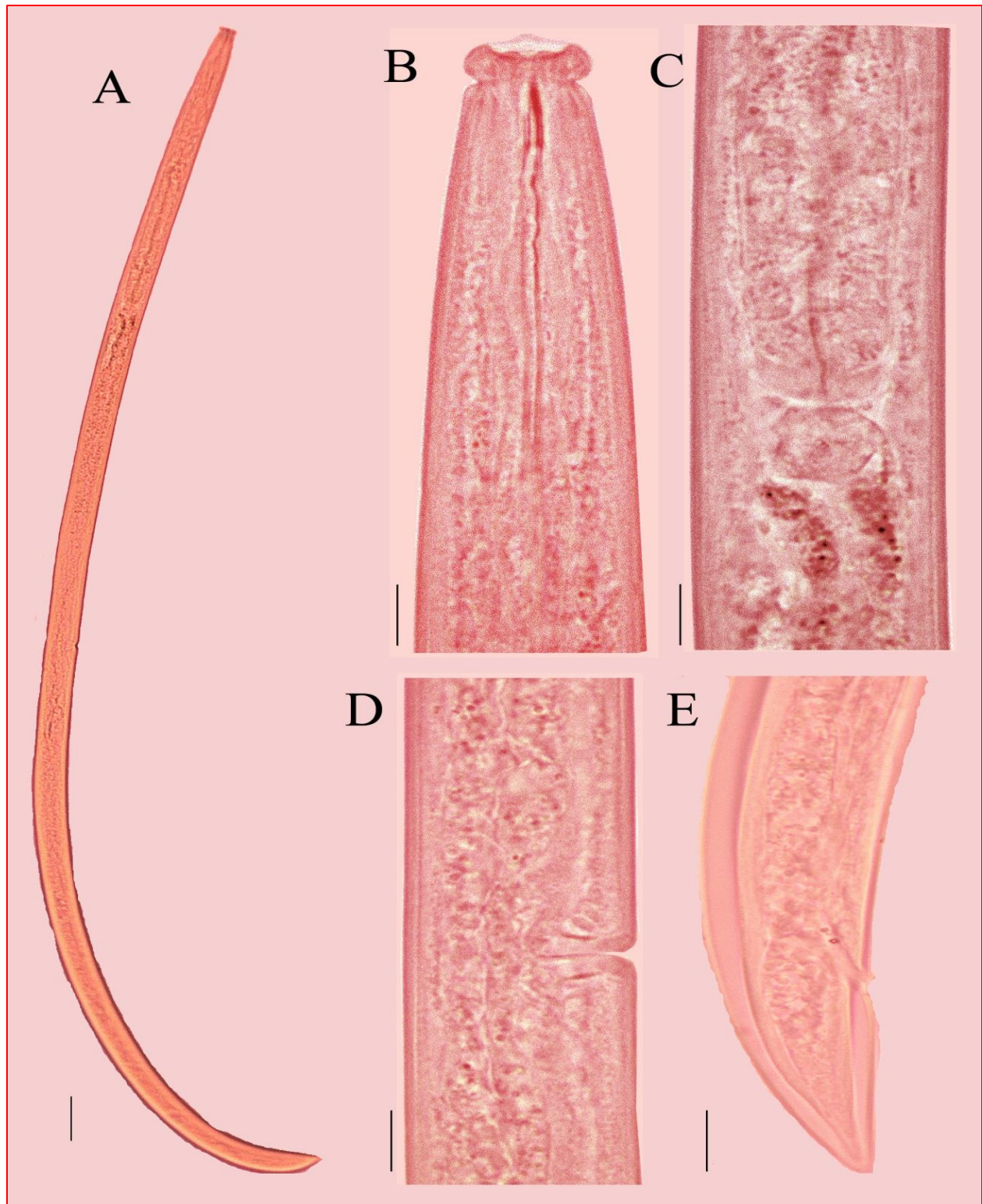


Fig. 1b. (A-E). *Discolaimus miniodontii* n. sp. Female: A. Whole body; B. Anterior region; C. Oesophageal region; D. Vulval region; E. Tail region (Scale: A=10 µm; B-E= 100 µm).

***Laevides hunderansis* n.sp.**
(Figs. 2a & 2b, Table 2)

Description

Female: Body almost straight when relaxed, tapering anterior to base of oesophagus. Cuticle smooth 2.0-2.5µm thick at mid body and 3 – 3.5µm on tail. Lip region 8-9 µm wide, continuous with body contour; lips amalgamated, labial papillae not elevated. Body pores are indistinct; lateral chords about one third of body width at mid body. Amphid aperture slit like, occupying about half of corresponding body width. Mural tooth small 7-8 µm long, dorylaimoid with a short oblique dorsal aperture. Body at posterior end of oesophagus 2.6-2.7 times as wide as head. Oesophagus 190-204µm long. Expanded part of oesophagus occupying 46-49% of oesophageal length. Nerve ring surrounding anterior slender part of oesophagus, 64-68 µm from anterior end. Cardia hemispherical short 5-6 µm long with three large spherical glands enclosed within oesophageal sheath. Oesophageal gland nuclei rather inconspicuous. Female reproductive system amphidelphic well developed. Vulva a transverse slit, its lips not sclerotized, vagina 7-8 µm long about one third vulval body width long or extending over 30-35% of body width. Genital branch 3.2-4.6 times the body width long or occupying 10-11% of body length. Ovaries reflexed, oocytes arranged in a single row. A distinct sphincter present at oviduct-uterus junction. Distance between posterior end of oesophagus and vulva 1-2 times as long as oesophagus. Vulva-anus distance equal to twice the tail lengths. Prerectum 2.3-3.1 times longer than anal body diameter. Rectum about half anal body width long. Tail short, hemispherical or slightly clavate, with broadly rounded terminus 14 µm long or 1.7-1.8% of entire length of body.

Male: Similar to female in general morphology. Two testes, spicules 24 µm long along the curved axis. Gubernaculum 5 µm long. Supplements an adanal pair and three regularly spaced ventromedians. Prerectum 3.3 anal body width long. Tail 14 µm long, broadly rounded with 2-3 pairs of caudal pores.

Type habitat and locality: Specimens were collected from soil around the roots of potato (*Solanum tuberosum* L.) at, District Ghizer, village Hunder, Gilgit-Baltistan, Pakistan.

Type specimens: Holotype and paratype females and male deposited in the Nematode Collection of National Nematological Research Centre, University of Karachi, Karachi, Pakistan.

Diagnosis and Relationship: *Laevides hunderansis* n. sp., differs from all other species of the genus *Laevides* among the nygolaimid nematodes in having smallest body length (0.7-0.8mm). *Laevides hunderansis* n. sp., from village Hunder, District Ghizer, Gilgit-Baltistan, Pakistan is characterized by the very small body (0.7-0.8 mm), continuous lip region, small mural tooth. Short spicules, gubernaculum and tail and by the presence of three regularly spaced ventromedian supplements. According to the key to species given by Ahmed & Jairajpuri (1982) this new species comes close to *L. laevis* (Thorne, 1939) Thorne, 1974 but differs from it in having smaller mural tooth; shorter oesophagus, tail length, rectum, shorter spicules and gubernaculum and in lower number of ventromedian supplementary papillae in males (Tooth=7-8 vs 8-12 µm; oesophagus = 190-204 vs 390-520 µm, tail length=14 vs 17-29 µm; ABD=16-17 vs 23-28 µm; spicules=24 vs 35-42 µm; gubernaculum=5 vs 6-8 µm; ventromedian supplements=4 vs 5). The genus *Laevides* Heyns, 1968 is a new record genus and species from Pakistan.

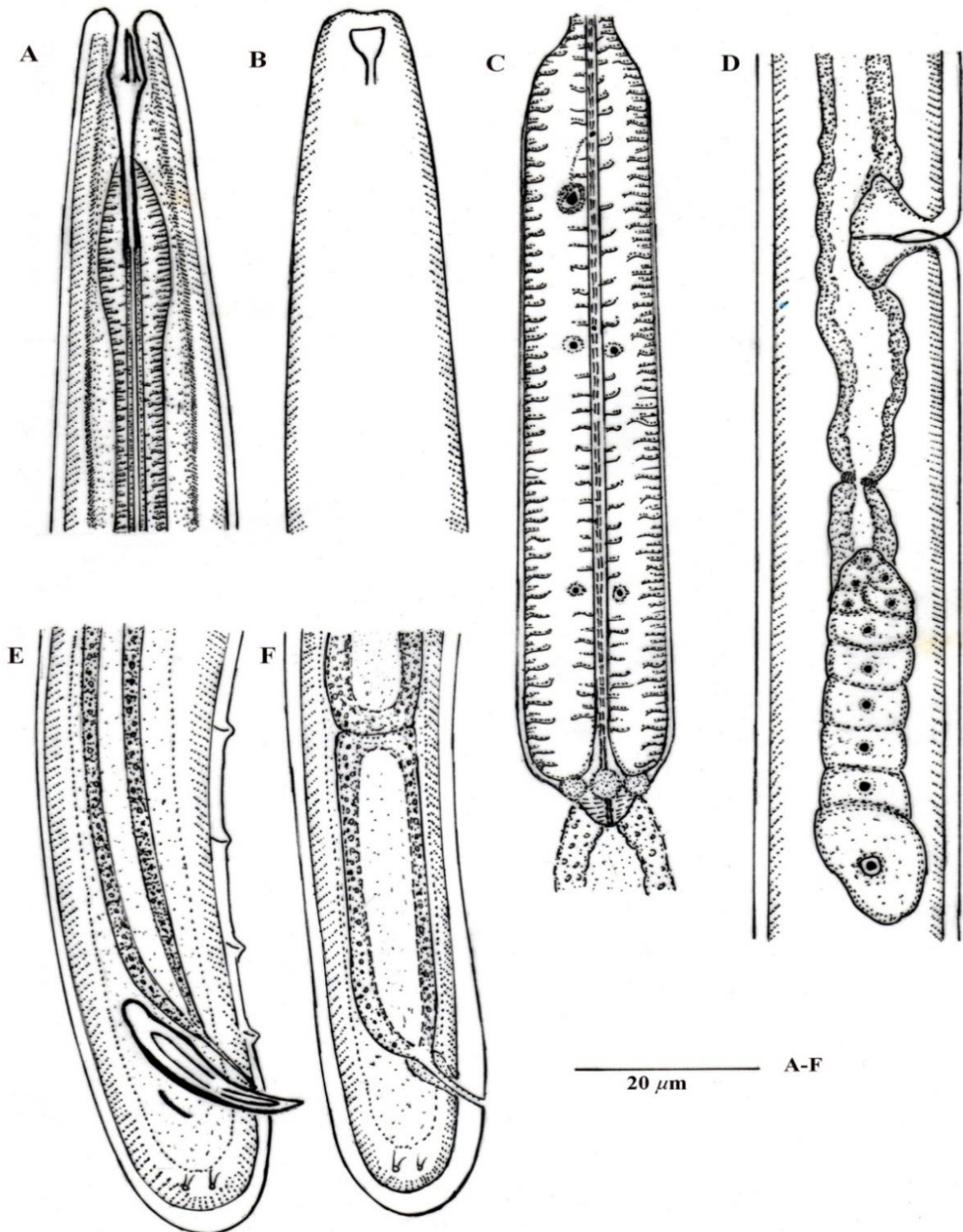


Fig. 2a (A-F). *Laevides hunderansis* n. sp: Female. A. Anterior region; B. Anterior region showing amphid; C. Expanded part of oesophagus; D. Female gonad; E. Male tail; F. Female tail.

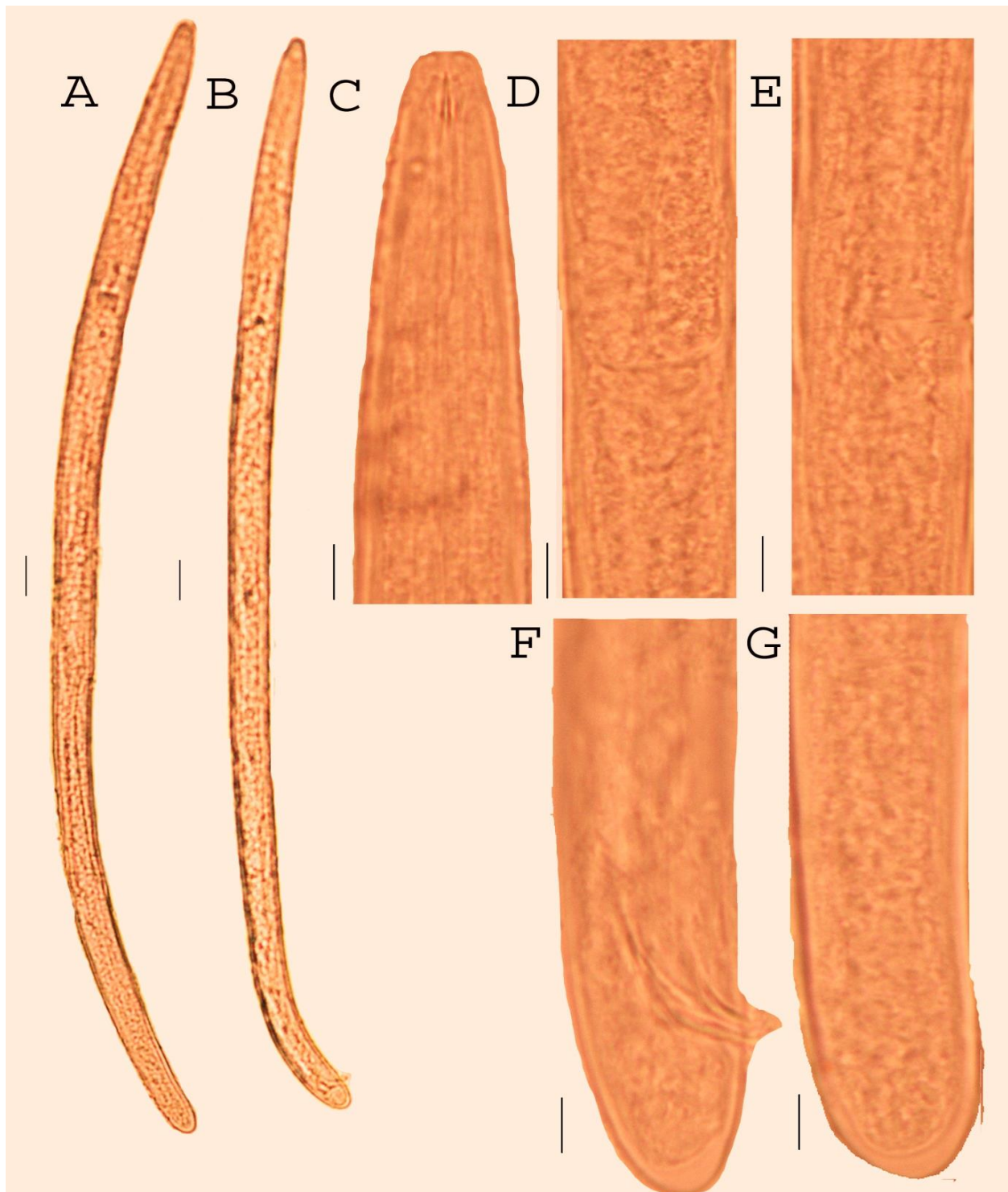


Fig. 2b (A-G). *Laevides hunderansis* n. sp.: A. Whole body of female; B. Whole body of male; C. Anterior region of female; D. Oesophageal region; E. Vulval region; F. Male tail; G. Female tail (Scale: A,B =10 μ m; C-G = 100 μ m).

Table 2. Morphometric data for *Laevides hunderansis* n. sp. All measurements are in μm except L. and in the form: Mean \pm S.D (range).

Characters	Holotype female	Paratypes	
		Female (n=5)	Male (n=1)
L	0.77	0.78 ± 0.01 (0.76-0.81)	0.81
a	35.1	33.9 ± 2.37 (31.3-38)	33.8
b	4.0	3.9 ± 0.09 (3.8-4.0)	4.4
c	55.2	55.8 ± 1.36 (54-58)	58
c'	0.8	0.82 ± 0.04 (0.8-0.9)	0.77
V	46.1	46.7 ± 1.06 (45-48)	-
G ₁	10.1	10.4 ± 0.28 (10.1-10.8)	-
G ₂	10.8	10.87 ± 0.42 (10.5-11.5)	-
Tooth	8	7.8 ± 0.4 (7-8)	8
Oesophagus	190	196.4 ± 5.8 (190-204)	184
Prerectum	40	40.8 ± 1.6 (38-42)	60
Rectum	8	8.9 ± 0.91 (8-10)	10
Tail	14	14 ± 0 (14-14)	14
ABW	17	16.3 ± 0.44 (16-17)	18
Spicule	-	-	24
Gubernaculum	-	-	5
Lateral guiding pieces	-	-	8
Ventromedian supplements	-	-	3
Body width	22	22.5 ± 2.0 (20-26)	24

***Discolaimoides spatilabium* Khan & Laha, 1982**
(Figs. 3a & 3b; Table 3)

Description

Female: Body slightly curved ventrad upon fixation. Cuticle 2-2.5 μm thick at mid body and 2-3 μm on tail, tapering at both the ends. Lateral hypodermal chord assuming maximum width at the mid body. Lip region expanded, measuring 10-11x3 μm in diameter. Guiding ring single, anterior, located 5-6 μm from head end. Odontostyle 12-13 μm long, its aperture occupying 1/3 of stylet length. Odontophore simple and equal to odontostyle length. Amphid stirrup shaped occupying about 40-50% of corresponding body diameter. Anterior oesophagus tubular, basal oesophageal bulb occupying 50% of the oesophageal length. Nerve ring located at 90-108 μm from anterior end.

Cardia conoid, 4-5 μm long. Vulva transverse, vagina extending to about half of the corresponding body width deep. Gonad paired, symmetrical, well developed, ovaries reflexed at oviduct. Rectum 16-18 μm in length 0.8-0.9 of anal body width in length. Prerectum 3 times anal body width in length. Tail dorsoventrally curved, conoid terminus. Caudal pores not observed.

Male: Not found.

Remarks: Specimens were collected from maize (*Zea mays* L.) during the survey of district Ghizer, village Thawoos Gilgit-Baltistan, Pakistan. The dimensions and characters of the above mentioned populations agree well with those of *Discolaimoides spatilabium* Khan & Laha, 1982. This is the first record genus and species from Pakistan.

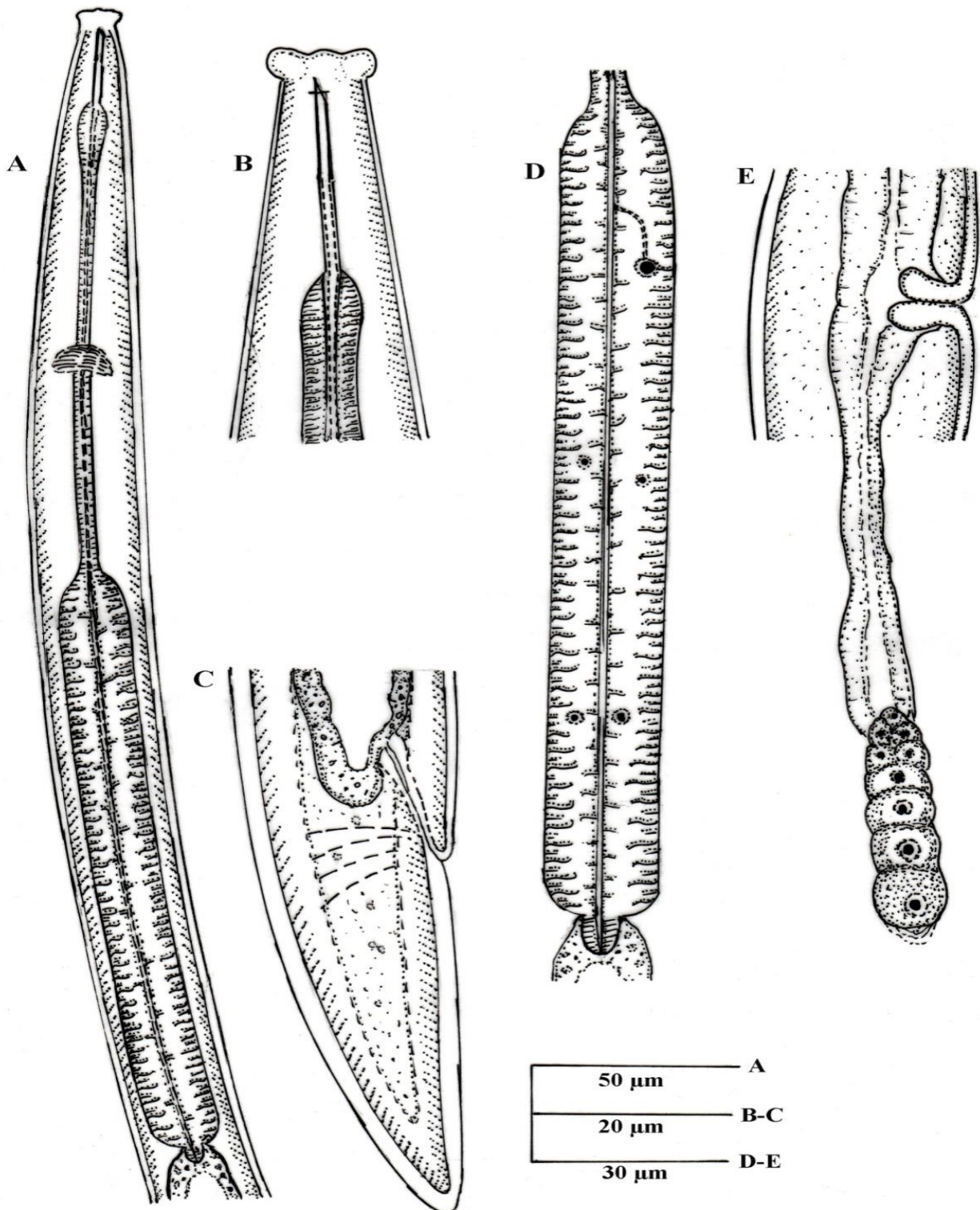


Fig. 3a (A-E). *Discolaimoides spatilabium* Khan & Laha, 1982. Female. A. Oesophageal region; B. Anterior region; C. Tail region; D. Expanded part of oesophagus; E. Female gonad.

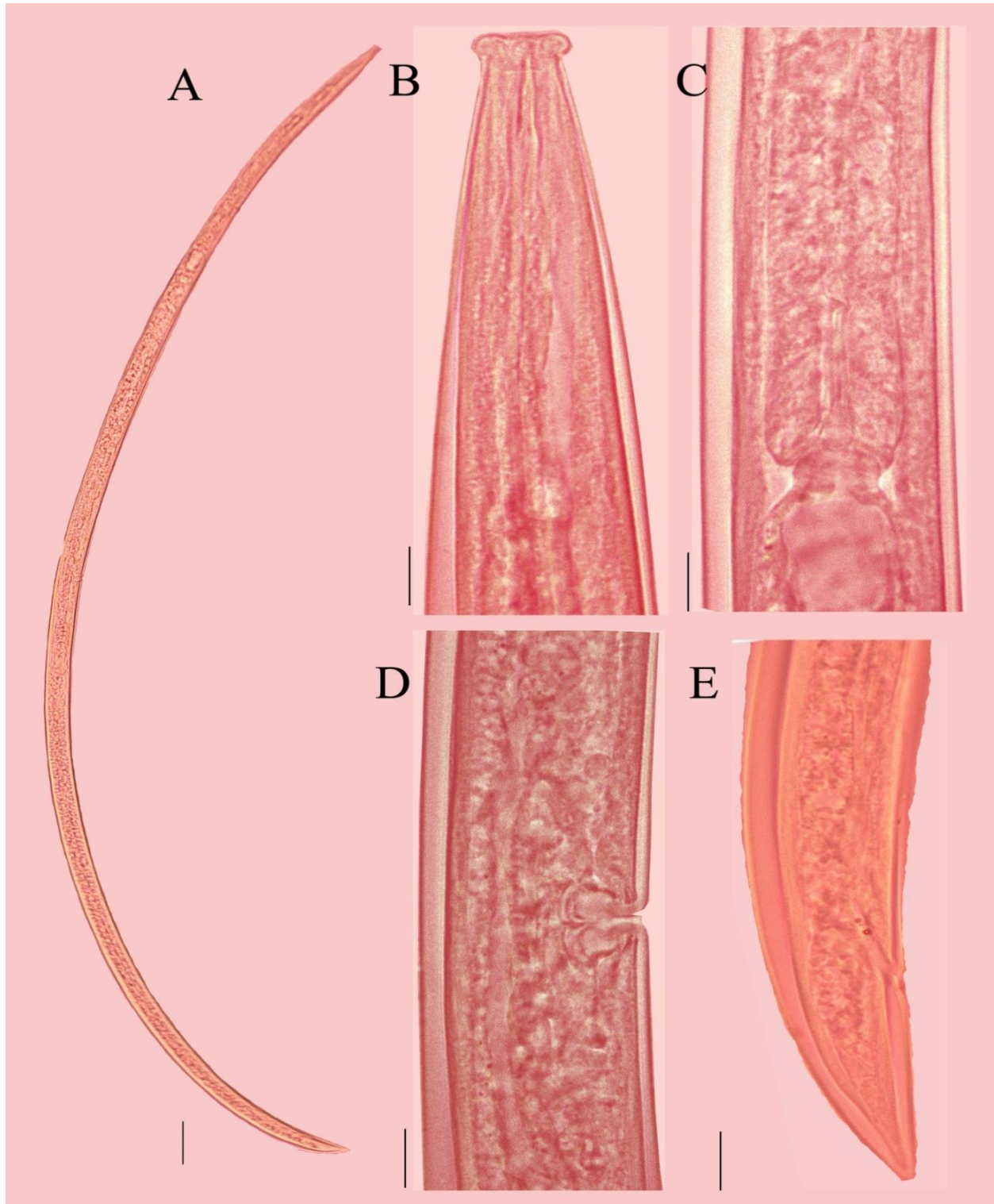


Fig. 3b (A-E). *Discolaimoides spatilabium* Khan & Laha, 1982. Female: A. Whole body; B. Anterior region; C. Oesophgeal region; D. Vulval region; E. Tail region (Scale: A= 10 μ m; B-E= 100 μ m).

Table 3. Morphometric data of *Discolaimoides spatilabium* Khan & Laha, 1982. All measurement are in μm except L. and in the form: Mean \pm SD (range).

Characters	Female (n=6)
L	1.54 \pm 0.08 (1.47-1.7)
a	51.3 \pm 4.3 (44.4-56)
b	4.62 \pm 0.32 (4.2-5.0)
c	34.7 \pm 1.40 (32.6-36.3)
c`	2.3 \pm 0.20 (2.1-2.7)
V%	45.45 \pm 1.91 (41.8-47.6)
G ₁	109.6 \pm 14.4 (90-128)
G ₂	120 \pm 14.1 (100-130)
Lip height	3.4 \pm 0.43 (3-4)
Lip width	10.7 \pm 0.71 (10-11.2)
Odontostyle length	12.7 \pm 0.36 (12-13)
Odontophore length	13.8 \pm 1.34 (12-16)
Total stylet	26.8 \pm 1.14 (24-28)
Guiding ring from anterior end	5.1 \pm 0.68 (5-6)
Nerve ring from anterior end	101.6 \pm 6.1 (90-108)
Pharynx length	333.7 \pm 18.9 (296-353)
Expanded part of pharynx	169.1 \pm 14.4 (148-182)
Cardia length	5.0 \pm 0.65 (4-7)
Body diameter at neck base	28.8 \pm 0.68 (28-30)
Body diameter at mid body	29.8 \pm 1.89 (27.5-33.6)
Body diameter at anus	19.45 \pm 0.58 (18.5-20)
Vulva from anterior end	676.0 \pm 31.6 (645-725)
Prerectum length	66.6 \pm 5.6 (60-74)
Tail length	44.3 \pm 2.78 (40.8-50)
Rectum	17.9 \pm 1.5 (16-20)

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