# DESCRIPTION OF *MELOINEMA ODESANENS* SP. N. (NEMATODA: HETERODERIDAE) FROM KOREA

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**Summary.** High infection rates of *Liriodendron tulipifera* L. feeder roots and soil by a new species of *Meloinema* were found during a nematode survey in the Odae Mountain area of the Gwangweon Province of the Korean Peninsula. Morphometric observations made by light and scanning electron microscopy showed that the specimens differed clearly from the known *Meloinema* species. Therefore a new species is described and illustrated herein, and is named as *M. odesanens* n. sp. It is characterized by: long pharyngeal glands largely overlapping the intestine in all developmental stages and an excretory pore position consistently mid-way between the basal stylet knobs and median bulb. As *Meloinema odesanens* n. sp., posses the generic characteristics referred to above, marked sexual dimorphism, sexual dimorphism in the position of the excretory pore, the presence of head plates, the structure of the lateral field, the posterior position (about 90%) of the vulva in adult females, and the presence of two functional gonads, becomes the forth species of the genus *Meloinema* Choi *et* Geraert, 1974, Choi Y.E., 1981. A number of characters readily differentiate the new species from *M. kerongense*, *M. silvicolum*, *M. maritimum* and *M. chitwoodi*. Roots infected by *M. odesanens* n. sp. show small swellings (2.5 times the diameter of uninfected roots) at its feeding sites.

The genus Meloinema in the family Heteroderidae was first proposed by Choi and Geraert (1973) when they described the type species of the genus, Meloinema kerongense. Until the present study, in addition to the type species only three other species of Meloinema have been assigned to the genus. They are M. maritimum Eroshenko, 1990, M. silvicolum Kleynhans, 1998 and M. chitwoodi (Golden et Jensen, 1974) Stone, 1978 (Luc et al., 1988). During a recent survey for plant nematodes in the Korean peninsula, a large population of *Meloine*ma was recovered from the rhizosphere and roots of Liriodendron tulipifera L. in the Odae Mountain area of Gwangweon Province. Studies of juveniles, immature females and adults convinced us that this population is morphologically and morphometrically different from the known species assigned to the genus Meloinema and it is described herein as *Meloinema odesanens* sp. n.

### MATERIALS AND METHODS

The source of material for morphological studies was a large population collected from the type locality, in the rhizosphere and roots of tulip trees. Male and juvenile nematodes were extracted by the modified Baermann's funnel method while females were collected by dissecting the roots under a stereomicroscope.

Extracted nematodes were fixed with hot F:G 4-1, transferred to glycerin according to the Seinhorst (1959) rapid method, and permanently mounted using the paraffin ring method for examination by light microscopy. The neck and posterior body portion of adult females were excised and cleared in lactic acid before mounting, to provide a better illustration of vulva, anus, location of the excretory pore and other morphological features with high taxonomic value.

For scanning microscopy studies, freshly isolated nematodes were pre-fixed with 4% glutaraldehyde-2% formaldehyde (pH 7.2, 20 hours) and post-fixed in 2% osmium tetroxide solution (pH 7.2, for 4 hours). They were then dehydrated in an ethanol series, critical point dried in liquid  $CO_2$ , mounted on studs, sputter coated with gold palladium, and examined in a Leo 1450Vp SEM using an accelerating voltage of 10 kV (Eisenback, 1986).

## DESCRIPTION

#### MELOINEMA ODESANENS sp. n. (Table I; Figs. 1-5)

Holotype (male in glycerin). Length = 1615  $\mu$ m; body width = 35.3  $\mu$ m; a = 44.5; b = 9.9; c = 62.9; stylet = 29  $\mu$ m; spicules = 45.5  $\mu$ m; gubernaculum = 11  $\mu$ m; excretory pore = 135  $\mu$ m; DGO = 3.5  $\mu$ m; median bulb = 12 × 20  $\mu$ m; tail length = 26  $\mu$ m; hyaline tail part = 13  $\mu$ m.

*Female.* Measurements and description (n = 4): Body 1003.9 (820-1150)  $\mu$ m long, slender anteriorly and swollen posteriorly; slender part 44.7-47.2% of the body length; swollen part 390-445  $\mu$ m long, 350-549  $\mu$ m wide. Cuticle 6-7  $\mu$ m thick on slender part and 11-17  $\mu$ m on swollen part. Head well offset from body by a deep groove, without annules; cephalic framework prominent.

Stylet 24.5-26  $\mu$ m with well-developed knobs. Dorsal pharyngeal gland opens at 3.5-5.6  $\mu$ m behind the stylet base. Median pharyngeal bulb 35.7-36.4  $\mu$ m long, 24.5-28  $\mu$ m wide, located at 73-74  $\mu$ m (to middle of valve) from head end, with well developed valve. Hemizonid and excretory pore not seen. Vulva a transverse slit 36.4-37.4  $\mu$ m long, situated at 77-105  $\mu$ m from the tail terminus. Anus distinct, 27-41  $\mu$ m from the tail terminus. The phasmids are very large (diameter 5.4  $\mu$ m) and situated at 10  $\mu$ m from tail end. The very faint circular striae occur around the vulva and tail. Tail tip nipple-like projecting 9-15.5  $\mu$ m long.

Measurements of second stage juveniles (n = 15); males (n = 10); and immature females (n = 4) all infiltrated with glycerin are reported in Table I.

Immature female. Body length about 1 mm, vermiform with a cylindrical tail, much shorter than in males. Head 5.6-6.3 µm high and 10.5-12.5 µm wide, rounded, well offset from body by a constriction; stylet well developed with the conical part 61.4-75% of the total stylet length of 26.3 µm (25.9-26.6 µm); stylet knobs large, rounded, anterior surface flattened. Dorsal pharyngeal gland opens at 3.5-6.3 µm from the stylet base. Median pharyngeal bulb elongate with extremely large, crescentic valves 8.4-12.6 µm wide and 16.1-21 µm long. Pharyngeal gland lobe 215-345 µm long. Nerve ring surrounding the isthmus; hemizonid at 120-140 µm from anterior end. Excretory pore located very anteriorly at two to three stylet lengths posterior to the knobs, at 58.1-72.1 µm from the anterior end. Lateral field with four incisures. Vulva very posterior; gonads didelphic, amphidelphic outstretched, not functional. Tail cylindrical, between 1 and 1.5 times anal body width long; tail end broadly rounded; annulations on the tail end less developed, cuticle on tail end very thick. Phasmids at the anterior half of the tail.

Second-stage juveniles. Long, cylindrical body with a tapering tail. Head low, offset by a constriction; without annules and apparently without head plates. Cephalic framework and stylet well developed; anterior conical part of the stylet about 58-66.6% of the total stylet length; stylet knobs large, rounded, with flattened surfaces. Dorsal pharyngeal gland opening at 2.8-4.9 µm from stylet base. Median pharyngeal bulb elongate with long crescentic valves. Isthmus, nerve ring, hemizonid as in male and female; the excretory pore at 85.4-102.2 µm from anterior end. Lateral field with three lines at middle of body and four lines at posterior region (Fig. 5I, J). Tail conical, two to three times the anal body diameter; towards the end the annules become large and are more irregular; the finely to broadly rounded tail end shows a very thick cuticle. Phasmids small, located at anterior half of the tail, 21-38 um from tail terminus.

*Males.* Long, vermiform with a short hemispherical tail; posterior body portion sometimes twisted (Fig. 1 E-H). Head 6.3-7 µm high and 13.3-14 µm wide, rounded,

well offset from body by a deep and wide groove, without distinct annulation; labial disc not raised above the medial lips. Medial lips as wide as the head region, of round hexagonal shape, wider than the labial disc. Labial lips are large and separated from the hexagonal medial lips. Amphidial apertures slit-like, oblique, close to oral opening (Fig. 3A-C). Cephalic frame-work very well developed with a heavy ring at the base. Stylet 29 (27.3-30.8) µm long and stylet knobs strongly developed; anterior conical part 57.1-65% of the stylet length; Stylet knobs rounded, anteriorly flattened. Dorsal pharyngeal gland opens at 2.8-4.2 µm from the stylet base. Median oesophageal pharyngeal bulb elongate at 84-110 µm from anterior end, with well developed crescentic valves 8.4-16.1 µm wide and 14.7-16.2 µm long. Pharynx 175-379 µm long, with the 2-3 gland nuclei more or less regularly spaced. Nerve ring surrounding the isthmus; hemizonid two annules long, located at one or two body widths posterior to the excretory pore. Excretory pore at about one body width posterior to median bulb. Excretory canal extended very long. Lateral field with three lines at anterior region, four lines at posterior region (Fig. 3F, G), then an additional irregular fifth line at middle of body (Fig. 2D). The lateral field is very pronounced at posterior region, continues on the tail but is slightly twisted; lateral lines may end at anterior of the anus. Spicule narrow, elongated, slightly curved with finely rounded tips; gubernaculum short, straight (Fig. 1E, F). Tail hemispherical, slightly shorter than anal body diameter, cuticle very thick at the tip; annulations on tail tip less developed, irregular. Phasmids at about anal level with usually a very fine pore (Fig. 1G, H). No bursa but the ventral side is flattened.

*Eggs (n* = 23). Deposited in a gelatinous matrix forming a large egg mass containing 115 (33-264) eggs. L = 132 (128-136)  $\mu$ m; maximum width = 51 (49-54)  $\mu$ m; and L/W (maximum egg length/maximum egg width) = 2.6 (2.5-2.7). Egg shell hyaline and usculptured when observed under light microscope. Juvenile stage in fully embryonated eggs folded three to four times.

*Type host and locality.* Roots and soil around the deciduous tree *Liriodendron tulipifera* L. in the Odae Mountain area of Gwangweon Province, South Korea. Roots infected by *M. odesanens* showed small swellings (2.5 times the diameter of the roots) at the feeding sites.

*Etymology.* The species epithet refers to the type locality, the Odae mountain area of the Korea peninsula.

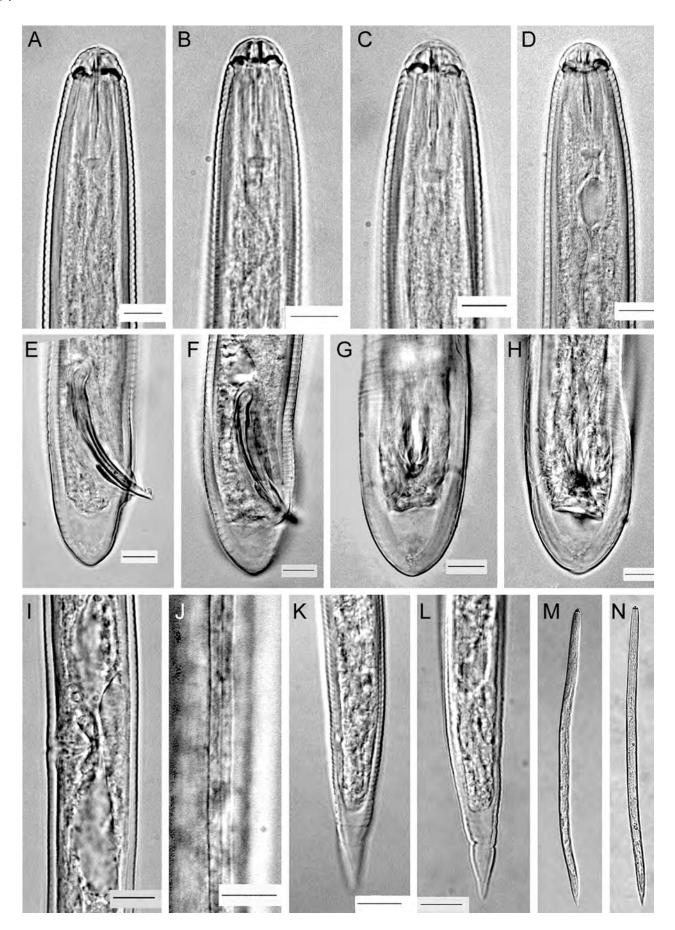
*Type designations.* Holotype male on slide 490 at the nematode collection of Gyeongbuk Agricultural Technology Administration, Daegu, Korea.

Four paratype females on slide 490-1-5, 10 immature females and 10 males on slides 490-2-10, and fifteen second-stage juveniles on slide 490-3-5, all in the same collection as the holotype. Additional specimens (one female, three young females, five males and six juveniles) have been deposited at the U.S. Department of Agriculture Nematode Collection, Beltsville, MD, USA. The following type numbers of the USDA collection have

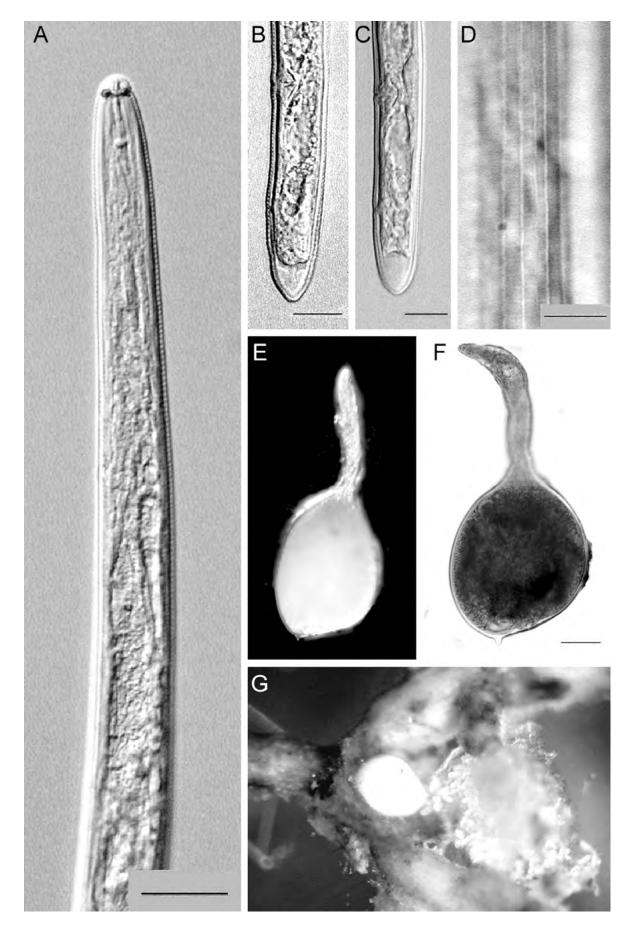
Character	Juveniles ± SD (range)	$\frac{Males \pm SD}{(range)}$	Immature females ± SD (range)
I	507.0 ± 21.4	1604.8 ± 193.6	961.6 ± 123.7
L	(469.0-542.5)	(1298.0-1932.0)	(794.5-1060.5)
a	$28.1 \pm 1.0$	$45.8 \pm 3.4$	$40.0 \pm 2.1$
	(26.2-29.8)	(39.4-50.1)	(37.8-42.8)
Ь	$4.8 \pm 0.6$	9.7 ± 2.3	$7.8 \pm 0.6$
	(3.6-5.7)	(6.6-13.2)	(7.1-8.4)
b'	$2.8 \pm 0.5$	$6.5 \pm 1.3$	$3.7 \pm 1.4$
	(1.9-3.6)	(4.8-8.7)	(2.2-4.9)
c	$13.8 \pm 1.6$	$61.9 \pm 8.0$	$28.3 \pm 6.2$
	(11.7-18.0)	(49.7-76.2)	(23.5-36.5)
c'	$3.0 \pm 0.6$	$0.9 \pm 0.1$	$1.3 \pm 0.5$
	(2.1-5.1)	(0.6-1.0)	(0.7-1.8)
MB%	$38.1 \pm 6.6$	$38.1 \pm 5.6$	$37.9 \pm 12.0$
	(25.9-47.6)	(28.9-48.6)	(23.3-52.6)
V			$90.5 \pm 1.3$
	-	-	(89.5-92.3)
Stylet	$22.3 \pm 0.6$	$29.0 \pm 1.2$	$26.3 \pm 0.4$
	(21.7-23.1)	(27.3-30.8)	(25.9-26.6)
DGO	$3.6 \pm 0.6$	$3.8 \pm 0.5$	$4.2 \pm 0.6$
	(2.8-4.9)	(2.8-4.2)	(3.5-4.9)
Con.	$14.4 \pm 1.8$	$17.6 \pm 0.5$	$16.5 \pm 0.4$
	(12.6-20.3)	(16.8-18.2)	(16.1-16.8)
Shaft	$8.4 \pm 0.5$	$11.2 \pm 1.2$	$9.8 \pm 0.6$
	(7.7-9.1)	(9.5-12.6)	(9.1-10.5)
Con %	$62.6 \pm 2.0$	$61.3 \pm 2.7$	$62.9 \pm 1.5$
	(58.0-66.6)	(57.1-65.0)	(61.4-64.8)
Pharynx	$185.8 \pm 38.2$	$269.9 \pm 64.5$	$263.0 \pm 59.0$
	(143.5-282.8)	(175.7-379.4)	(214.2-347.2)
BW	$18.1 \pm 0.9$	$35.0 \pm 2.8$	$23.6 \pm 1.8$
	(16.8-19.6)	(30.8-39.9)	(21.0-25.2)
Ex. pore	$93.2 \pm 5.0$	$139.0 \pm 11.2$	$62.0 \pm 4.8$
	(85.4-102.2)	(120.4-150.5)	(58.1-68.6)
Hemizonid	99.4 ± 9.3	$168.3 \pm 11.6$	$128.1 \pm 8.1$
	(90.3-125.0)	(155.4-189.0)	(118.3-137.9)
Hyaline	$19.3 \pm 4.5$	$11.5 \pm 4.7$	$17.2 \pm 2.0$
	(9.1-27.3)	(6.3-20.3)	(14.7-19.6)
Tail	$37.1 \pm 4.1$	$25.9 \pm 2.7$	$34.5 \pm 4.7$
	(30.1-44.1)	(22.4-30.1)	(28.7-39.9)
ABW	$12.5 \pm 1.6$	$29.8 \pm 3.7$	$23.1 \pm 1.2$
	(8.4-14.7)	(21.7-33.6)	(22.4-24.5)
Anus-Phasmid distance	$7.7 \pm 2.8$	_	9.3 ± 4.2
	(2.1-12.6)	-	(4.9-13.3)
Tail-Phasmid distance	$27.1 \pm 4.5$	_	$28.0\pm2.5$
	(21.0-37.8)	-	(25.9-30.8)
MBW	$9.5 \pm 1.7$	$11.8 \pm 2.3$	$11.6 \pm 2.2$
	(7.7-13.3)	(8.4-16.1)	(8.4-13.3)
MBL	$16.2 \pm 2.0$	$20.2 \pm 3.9$	$21.5\pm4.1$
	(14.0-20.3)	(14.7-26.2)	(16.1-25.9)
MB	$68.7 \pm 5.6$	95.9 ± 8.9	
	(50.8-74.0)	(84.0-109.9)	-
Spicule		$46.2 \pm 3.8$	
	-	(39.9-51.1)	-
		$10.9 \pm 1.6$	
Gubernaculum	-	(8.4-12.6)	-

Table I. Measurements of juveniles, males and immature females of Meloinema odesanens sp. n. (in µm).

Abbreviations used for characters in Table I: L = total body length; a = L/the greatest body width; b = L/the distance from anterior end to junction of pharynx and intestine; b' = L/the distance from anterior end to posterior end of pharyngeal glands (used when glands overlap intestine); c = L/tail length; c' = tail length/body width at anus or cloaca; MB% = distance of median bulb from anterior end × 100/total pharyngeal length; V = distance of vulva from anterior end × 100/body length; Stylet = stylet length; DGO = distance from dorsal gland outlet to stylet base; Con. = length of stylet cone; Shaft = length of stylet shaft; Con % = cone × 100/stylet; pharynx = pharynx from anterior end; BW = body width; Ex. pore = excretory pore from anterior end; Hemizonid = Hemizonid from anterior end; Hyaline = Hyaline tail length; Tail = tail length; ABW = anal body width; MBW = median bulb width; MBL = median bulb length; MB = distance of median bulb from anterior end; Spicule = Spicule length; Gubernac-thene. ulum = Gubernaculum length.



**Fig. 1.** Photomicrographs of *Meloinema odesanens* sp. n. A, B: immature female head; C, D: male head; E-F: posterior part of male, lateral view; G, H: posterior part of male, ventral view; I: immature female vulva region; J: Lateral field of second-stage juvenile; K, L: Second-stage juvenile tail; M, N: Second-stage juvenile. Scale bar =  $10 \mu m$ .



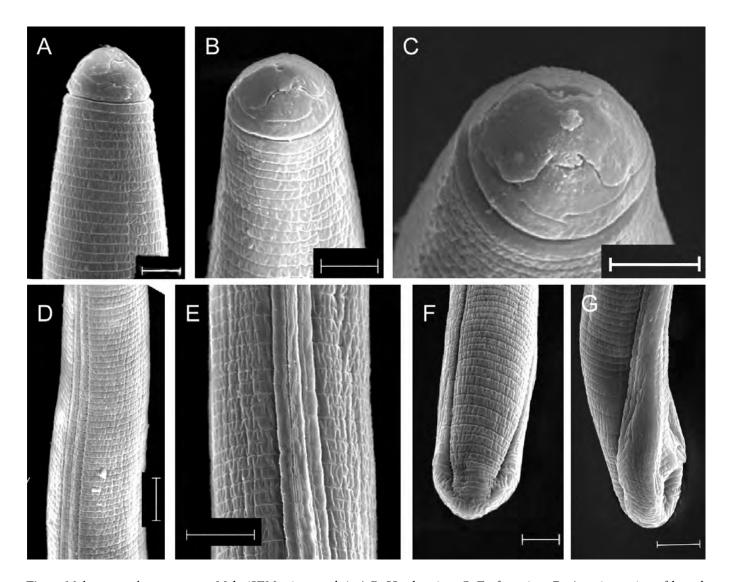
**Fig. 2.** Photomicrographs of *Meloinema odesanens* sp. n. A: Male, anterior part of body showing elongated oesophageal lobe overlapping intestine; B-C: Posterior part of immature female; D: Male lateral field, middle of body; E-F: Entire female; G: Female posterior swollen part protruding from the rootlet and a large egg mass. Scale bar:  $A = 40 \mu m$ ;  $B, C = 20 \mu m$ ;  $D = 10 \mu m$ ;  $F = 100 \mu m$ .

been assigned to these slides: *Meloinema odesanens* – T-5324p and T-5327p.

*Diagnosis and Relationships. Meloinema odesanens* sp. n. can be distinguished from all other *Meloinema* spp. by the following characteristics: (i) the long oesophageal glands largely overlapping the intestine in vermiform immature females, males, and juveniles; (ii) the consistent location of the excretory pore at about mid-way between the basal knobs and metacorpus; and (iii) a number of morphometric parameters that readily differentiate it from the other species.

In females of *M. odesanens* n. sp., the slender part is much shorter than that of *M. kerongense* [388-444  $\mu$ m (44.7-47.2% of body length) *vs* 1,180-1,550  $\mu$ m (77.1-79.4% of body length)]. In addition, compared to *M. kerongense*, the total body length of *M. odesanens* n. sp. is distinctly shorter (794.5-1,060.5 *vs* 1,400-1,940  $\mu$ m); also, the stylet length is shorter (25.9-26.6  $\mu$ m *vs* 32-35  $\mu$ m), while the vulva position is slightly anteriorly positioned (89.5-92.3% at the new species vs 92-95% in *M. kerongense*). Male morphology of *M. odesanens* sp. n. closely resembles that of *M. kerongense* in its general shape except for the tail shape, which is hemispherical vs more conical in *M. kerongense*. In summary, all measurements of *M. odesanens* sp. n. are much shorter than those of *M. kerongense* except the stylet cone, which is slightly longer (57.1-65% vs 58-60% of stylet length).

Compared to *M. silvicola*, the body length of second stage juveniles of *M. odesanens* sp. n. is much shorter (469-542.5  $\mu$ m vs 907-1,144  $\mu$ m) and the phasmids are located anteriorly from the tail end (21-37.8  $\mu$ m vs 44.1-54.  $\mu$ m). The new species has a more hemispherical tail than *M. silvicola* and its tail tip is not annulated, vs the subcylindrical tail end irregularly annulated and broadly rounded in *M. silvicola*. *Meloinema odesanens* sp. n. is shorter than *M. silvicola* in almost all measurements: body length (1,298-1932  $\mu$ m vs 1,954-2,59  $\mu$ m), stylet length (27.3-30.8  $\mu$ m vs 29.1-35.9  $\mu$ m), tail length (22.4-30.1  $\mu$ m



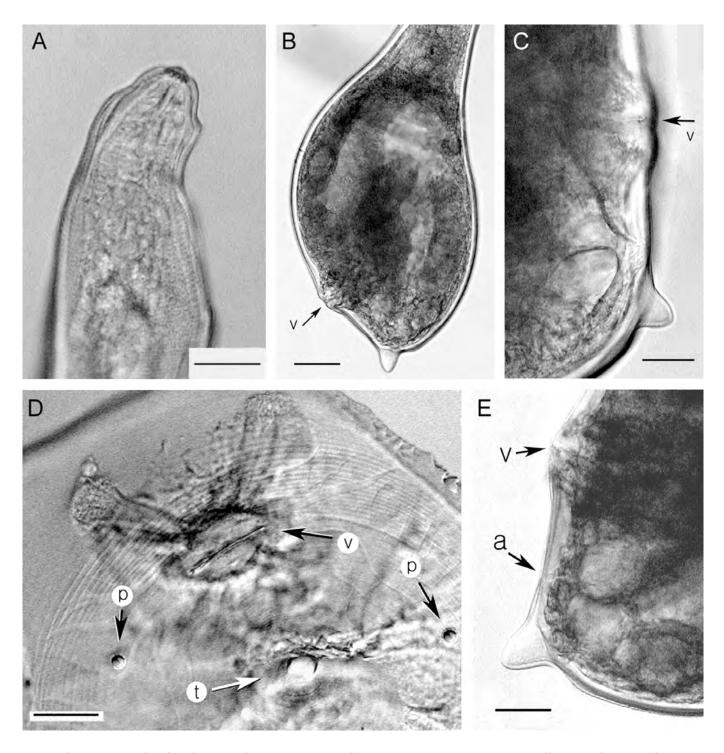
**Fig. 3.** *Meloinema odesanens* sp. n. Male (SEM micrographs): A-B: Head region; C: En-face view; D: Anterior region of lateral field; E: Lateral field, middle of body; F: Posterior part of tail, dorsal view; G: Posterior part of tail, lateral view. Scale bar:  $A-C = 5 \mu m$ ; D-G = 10  $\mu m$ .

*vs* 54.3-80 µm). The conical part of the stylet is much longer (57.1-65 % *vs* 43-48 %) and the excretory pore much more anterior (120.4-150.5 µm *vs* 205-233 µm) compared to *M. silvicola*. The stylet knobs are anteriorly flattened compared to backward-sloped in *M. silvicola*.

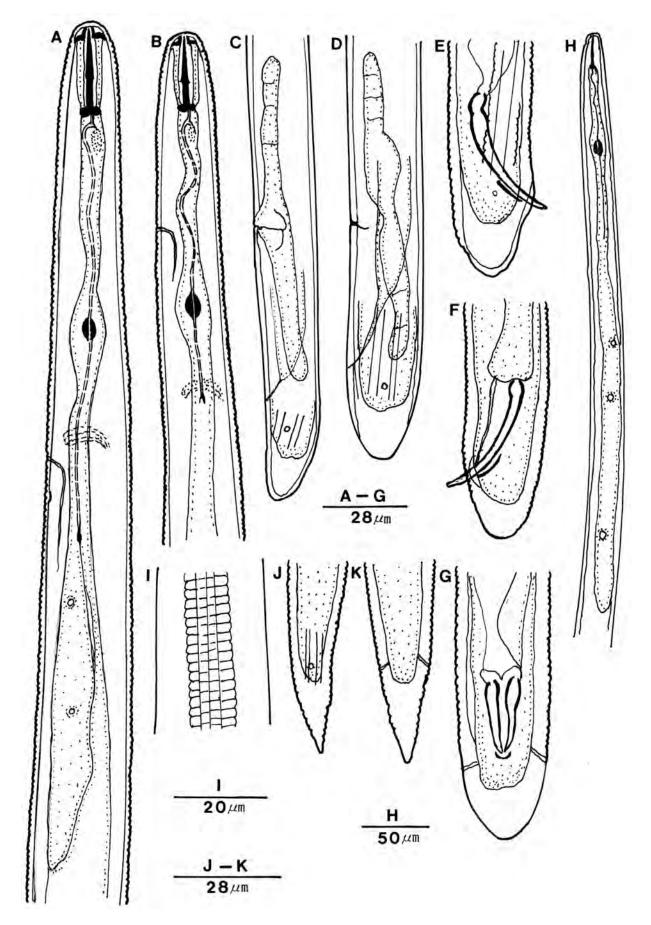
*Meloinema odesanens* n. sp. differs from *M. maritima* in having a longer stylet in juveniles (21.7-23.1 *vs* 18-21 µm) and a posteriorly positioned excretory pore, at me-

dian bulb level.

Finally, the new species differs from *M. chitwoodi* by having shorter stylets in juveniles (21.7-23.1 *vs* 24-25  $\mu$ m) and males (27.3-30.8 *vs* 34-40  $\mu$ m), a posteriorly positioned excretory pore in all developmental stages, and a consistently shorter body length in females (822-1,142 *vs* 1,311-2,601  $\mu$ m) and males (1,298-1,932 *vs* 1,964-2,922  $\mu$ m).



**Fig. 4.** Photomicrographs of *Meloinema odesanens* sp. n. Female: A: Anterior part; B: Posterior swollen part, showing vulva, anus and nipple-like tail tip; C, E: Enlargement of anus vulva region, showing vulva (v) and anus (a); D: Surface view of tail tip (t) and vulval region (v), showing phasmids (p) and faint circle striae around them. Scale bar: A, C-E =  $30 \mu m$ ; B =  $50 \mu m$ .



**Fig. 5.** *Meloinema odesanens* sp. n. A: Male anterior part; B: Immature female anterior region; C, D: Posterior part of immature female; E-G: Male posterior part, E, F: Lateral view, G: Ventral view; H: Second-stage juvenile anterior part; I: Lateral field at middle of juvenile body; J, K: Posterior part of second-stage juvenile, J: Lateral view, K: Ventral view.

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