

NEW DATA OF TWO RARE SPECIES OF THE FAMILY AULOLAIMOIDIDAE (DORYLAIMIDA) FROM IRAN

M. Pedram¹, E. Pourjam^{1,*} and R. Peña-Santiago²

¹Department of Plant Pathology, College of Agriculture, Tarbiat Modares University, Tebran, Iran

²Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Campus 'Las Lagunillas' s/n, Edificio B3, 23071 Jaén, Spain

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Summary. *Adenolaimus tropicus* and *Oostenbrinkia parva*, two known members of the rare family Aulolaimoididae, collected in natural areas in northern Iran, are described in detail and illustrated, including SEM pictures. Iranian populations are very similar morphometrically to type ones of both species, only known to occur in Costa Rica and India, respectively, but the ranges of measurements and ratios are significantly widened. New relevant morphological features for their characterization are herein presented, such as the presence of a perioral, disc-like structure at the lip region and the first description of the male in both species. SEM pictures are presented for the first time for the genera *Adenolaimus* and *Oostenbrinkia*.

Key words: *Adenolaimus*, description, Iran, morphology, *Oostenbrinkia*, SEM, taxonomy.

Concerning the members of the family Aulolaimoididae Jairajpuri, 1964, Andrassy (2009, p. 524) stated "... Although they are distributed almost all over the world, they belong to the rarest nematodes". This taxon, which contains four genera and fifteen valid species, is mainly characterized by the peculiar morphology of its pharynx, consisting of three sections. Goseco *et al.* (1975) provided a revision of the group and, more recently, Mushtaq *et al.* (2006) presented a monograph paper devoted to it, with the description of five new species.

During a general nematode survey conducted in 2009-2011 to study the dorylaimid fauna of Iran [for a summary of previous contributions on the matter see Pedram *et al.* (2011a, b)], two populations of two known aulolaimoidid species were collected in the northern region of the country. A detailed examination of this material revealed new relevant data for a better characterization of the two nematode populations, which are presented hereafter.

MATERIALS AND METHODS

Soil and rotten organic samples were collected from several locations in northern Iran. Nematodes were extracted by the Cobb sieving and decanting method by suspending 200 g soil or 100 g rotten organic material in water. A 20 mesh sieve was used to retain debris and root particles. Then, 60 and 200 mesh sieves were used to collect the nematodes. To obtain a cleaner suspension of nematodes, the tray method (Whitehead and Hemming, 1965) was used to extract nematodes from the rotten organic materials. Nematodes were handpicked

under a Nikon SMZ1000 stereomicroscope. The collected specimens were killed in hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse (1969), and mounted on permanent slides. Observations were made under a Nikon E600 light microscope. Some of the best preserved specimens were photographed with a Nikon Eclipse 80i microscope and a Nikon DS digital camera. The photographs were edited using Adobe PhotoDeluxe1.0 software. Drawings were made using a drawing tube attached to a microscope and the scanned files of drawings were managed by CorelDRAW® software version 12 and redrawn. A few specimens preserved in glycerine were recycled for SEM observation, following the protocol of Abolafia and Peña-Santiago (2005). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point dried, coated with gold, and observed with a JEOL JSM-5800 microscope.

DESCRIPTIONS

ADENOLAIMUS TROPICUS

Mushtaq, Baniyamuddin *et Ahmad*, 2006

(Figs 1F-I, 2 and 3A-D)

Material examined. Fifteen females and fifteen males from Sisangan, Iran; in good condition.

Measurements. See Table 1.

Adults. Slender to very slender nematodes of small size, 0.63-0.86 mm long. Body cylindrical, tapering towards both extremities, but more so towards the anterior end because the tail is rounded. Habitus almost straight or slightly curved ventrad upon fixation. Cuticle thin and with equal thickness (0.5-1.0 µm) throughout

* Corresponding author: pourjame@modares.ac.ir

Table I. Morphometric data of *Adenolaimus tropicus* Mushtaq, Baniyamuddin *et* Ahmad, 2006 and *Oostenbrinkia parva* Mushtaq, Baniyamuddin *et* Ahmad, 2006. Measurements in μm (except L, in mm), and in the form: mean \pm standard deviation (range).

Character	<i>A. tropicus</i> (From Sisangan forests)		<i>O. parva</i> (From Kelardasht forests)	
	15 females	15 males	12 females	7 males
L	0.72 \pm 0.07 (0.63-0.86)	0.70 \pm 0.03 (0.65-0.75)	0.46 \pm 0.05 (0.39-0.53)	0.50 \pm 0.02 (0.47-0.53)
a	39.5 \pm 3.0 (36-47)	44.0 \pm 3.3 (39-51)	27.5 \pm 2.0 (25-30)	31.0 \pm 2.0 (29-34)
b	5.4 \pm 0.5 (5.0-6.5)	5.3 \pm 0.3 (5-6)	4.2 \pm 0.5 (3.5-5.0)	4.5 \pm 0.2 (4.0-4.5)
c	57.0 \pm 7.5 (43.0-70.5)	54 \pm 4 (46.5-60.0)	44.5 \pm 6.0 (35-52)	38.0 \pm 1.0 (36-40)
c'	1.0 \pm 0.1 (0.9-1.2)	1.0 \pm 0.1 (0.9-1.0)	0.9 \pm 0.1 (0.8-1.0)	1.0 \pm 0.1 (0.9-1.1)
V	43.3 \pm 2.0 (36-47)	-	58.5 \pm 1.5 (55-61)	-
Lip region diameter	7.5 \pm 0.5 (7-9)	7.5 \pm 0.5 (7-8)	7.0 \pm 0.1 (6.5-7.0)	7.0 \pm 0.5 (6.0-7.5)
Odontostyle length	4.7 \pm 0.5 (4.5-5.0)	4.3 \pm 0.3 (4.0-4.5)	4.5 \pm 0.5 (4.0-5.0)	4.0 \pm 0.5 (3.0-4.5)
Odontophore length	12.0 \pm 0.8 (11-14)	12.0 \pm 1.0 (10-13)	13.0 \pm 1.0 (12-15)	13.0 \pm 1.0 (12-15)
Guide ring from anterior end	4.0 \pm 0.3 (4.0-4.5)	3.7 \pm 0.3 (3-4)	4.3 \pm 0.3 (4.0-4.5)	4.3 \pm 0.3 (4.0-4.5)
Neck length	134 \pm 7 (119-147)	133 \pm 7 (124-145)	116.0 \pm 5.5 (109-122)	115 \pm 5 (110-122)
Pharyngeal expansion length	34.0 \pm 3.0 (29-38)	32.0 \pm 3.3 (27-37)	29.5 \pm 1.0 (27-30)	27.5 \pm 2.5 (25-31)
Body diameter at neck base	17 \pm 1(14.5-20.0)	17.0 \pm 1.0 (16.0-18.5)	16.0 \pm 1.5 (14-18)	15.5 \pm 1.0 (14-17)
Body diameter at mid-body	18.5 \pm 1.5 (15-21)	16.0 \pm 1.5 (14-18)	17.0 \pm 1.5 (14-20)	16.0 \pm 1.0 (15-17)
Body diameter at anus/cloaca	13.0 \pm 1.5 (10-15)	14.0 \pm 0.5 (13-15)	13.0 \pm 0.5 (10-14)	13.0 \pm 0.5 (12-14)
Pre-rectum length	77 \pm 6.5 (72-89)	55.5 \pm 5.5 (50-64)	71.0 \pm 13.5 (60-90)	65 \pm 5 (60-70)
Rectum/cloaca length	13.0 \pm 1.2 (11-14)	28.5 \pm 3.5 (22-33)	11.5 \pm 1.5 (10-13)	18.5 \pm 2.5 (16-20)
Tail length	12.5 \pm 1.5 (10-15)	13.0 \pm 0.5 (12-14)	10.5 \pm 1.5 (9-13)	13.0 \pm 1.0 (12-14)
Spicules length	-	31.0 \pm 1.5 (28.0-32.5)	-	12.5 \pm 0.5 (11-13)
Ventro-median supplements	-	2	-	0

the body; its precise nature difficult to distinguish, but apparently dorylaimoid; outer layer (under SEM) with very fine transverse striations, often smooth. Lateral chord 5.0-5.5 μm wide or occupying one-fifth to one-third (26-32%) of mid-body diameter; body pores inconspicuous. Lip region continuous, rather rounded, about twice as wide as high and two-fifths to one-half (43-51%) of body diameter at neck base; lips amalgamated, very weakly angular under LM but SEM observations (Fig. 3A, B, arrowhead) show that they protrude in six small bulges and that their inner, perioral area is elevated and differentiated in a hexagonal, disc-like structure nearly 3 μm wide. Amphid fovea obscure in the specimens examined. Cheilostom short, thick-walled; in some specimens, especially in fresh individuals, it is possible to observe a few rib-like structures surrounding it. Odontostyle small but with distinct lumen and aperture, 4-6 times as long as wide, shorter (about three-fifths) than lip region diameter or 0.62% of total body length; aperture one-fourth to one-fifth its length. Guide ring weakly conspicuous, presumably simple, at 4.0-4.5 μm or 0.6 times the lip region diameter from anterior end. Odontophore distinctly longer (2.2-3.0 times) than odontostyle, with very well developed basal knobs or flanges. Pharynx typical of the genus, *i. e.* consisting of a rather muscular anterior region 62-70 μm long or 49-52% of total neck length; an intermediate, isthmus-like portion 11-14 μm long or 8-10% of total

neck length, which is surrounded by glandular tissue; and basal (posterior), bulb-like, cylindrical portion 31-34 μm long, 2.5-3.5 times as long as wide, 1.5-2.2 times as long as body diameter, and occupying 23-26% of total neck length; pharyngeal gland nuclei difficult to observe. Cardia short and rounded, 3.5-5.0 \times 7.0-8.0 μm , surrounded by intestinal tissue.

Female. Genital system monodelphic-opisthodelphic. Anterior branch reduced to a pre-vulval uterine sac 25-38 μm long, 1.2-2.0 times the body diameter long or 2.9-5.5% of total neck length. Posterior branch well developed, 134-167 μm or 19-28% of total body length. Ovaries comparatively large, reflexed, often reaching and surpassing the sphincter level, 71-107 μm long; oocytes first in two or more rows, then in a single row. Oviduct 76-120 μm or 4.2-6.5 times the body diameter long, consisting of a slender section and a moderately developed *pars dilatata*, which often contains sperm cells. Oviduct-uterus junction not very conspicuous. Uterus a simple tube-like structure, 52-75 μm or 3-4 times the body diameter long. Uterine egg 83-87 \times 10-14 μm . Sperm cells spindle-shaped, 7-11 \times 1.5-2.0 μm . Vagina 7-8 μm long, as wide as long and extending inwards about one-half (39-58%) of body diameter; *pars refringens* lacking and *pars proximalis* and *pars distalis* difficult to distinguish. Vulva an oval transverse slit, with a depression or small cavity of body surface behind

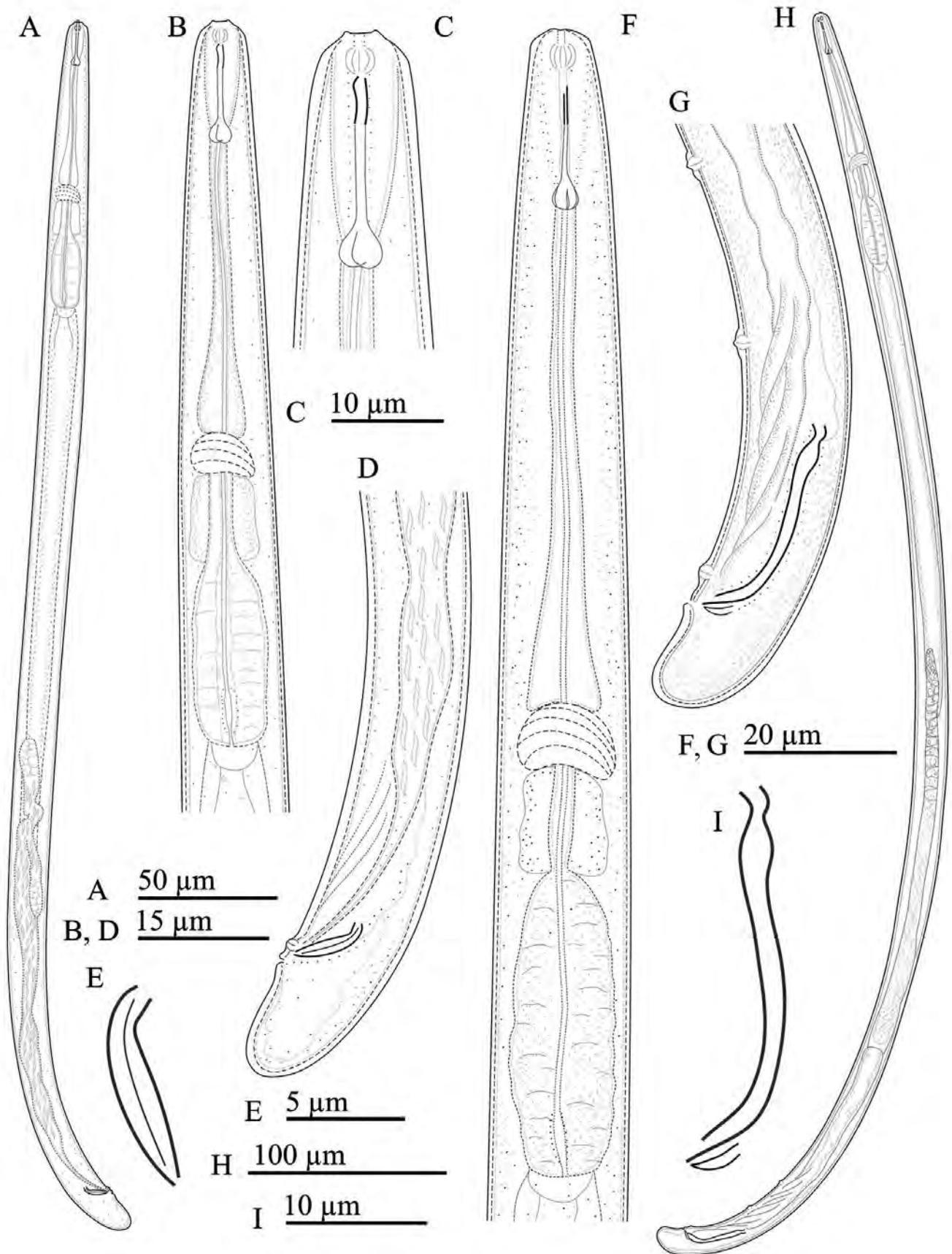


Fig. 1. A-E: *Oostenbrinkia parva* Mushtaq, Baniyamuddin *et* Ahmad, 2006 (male). A: Entire. B: Neck region. C: Anterior region. D: Posterior region. E: Spicules. F-I: *Adenolaimus tropicus* Mushtaq, Baniyamuddin *et* Ahmad, 2006 (male). F: Neck region. G: Posterior region. H: Entire. I: Spicules.

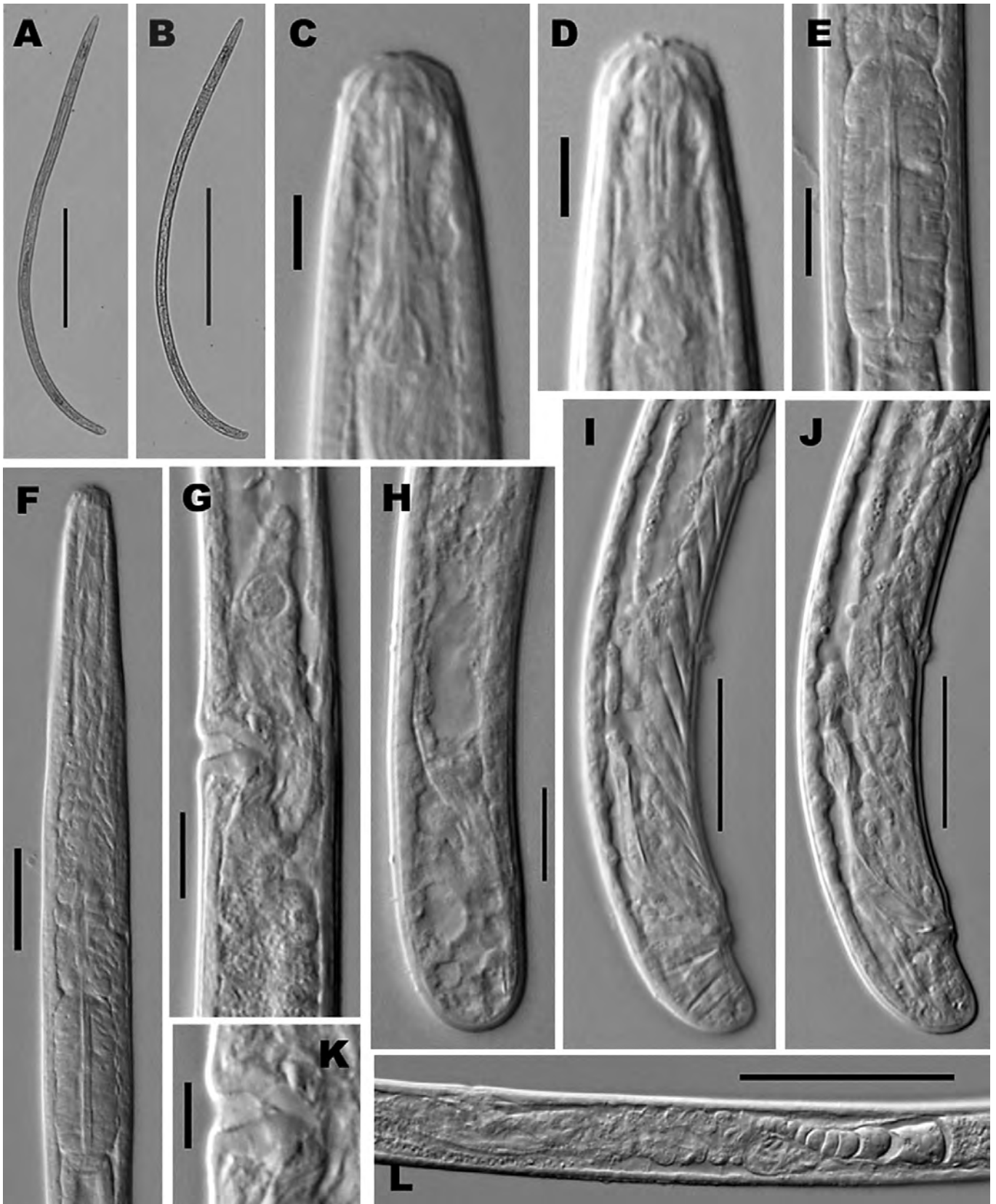


Fig. 2. *Adenolaimus tropicus* Mushtaq, Baniyamuddin *et* Ahmad, 2006 (LM). A: Female, entire. B: Male, entire. C, D: Anterior region in median view. E: Pharyngeal bulb and cardia. F: Neck region. G: Female, genital system in part. H: Female, posterior body region. I, J: Male, posterior body region. K: Vagina. L: Female, genital system. (Scale bars: A, B: 200 μ m; C, D, K = 5 μ m; E, G, H = 10 μ m; I, J = 20 μ m; L = 50 μ m).

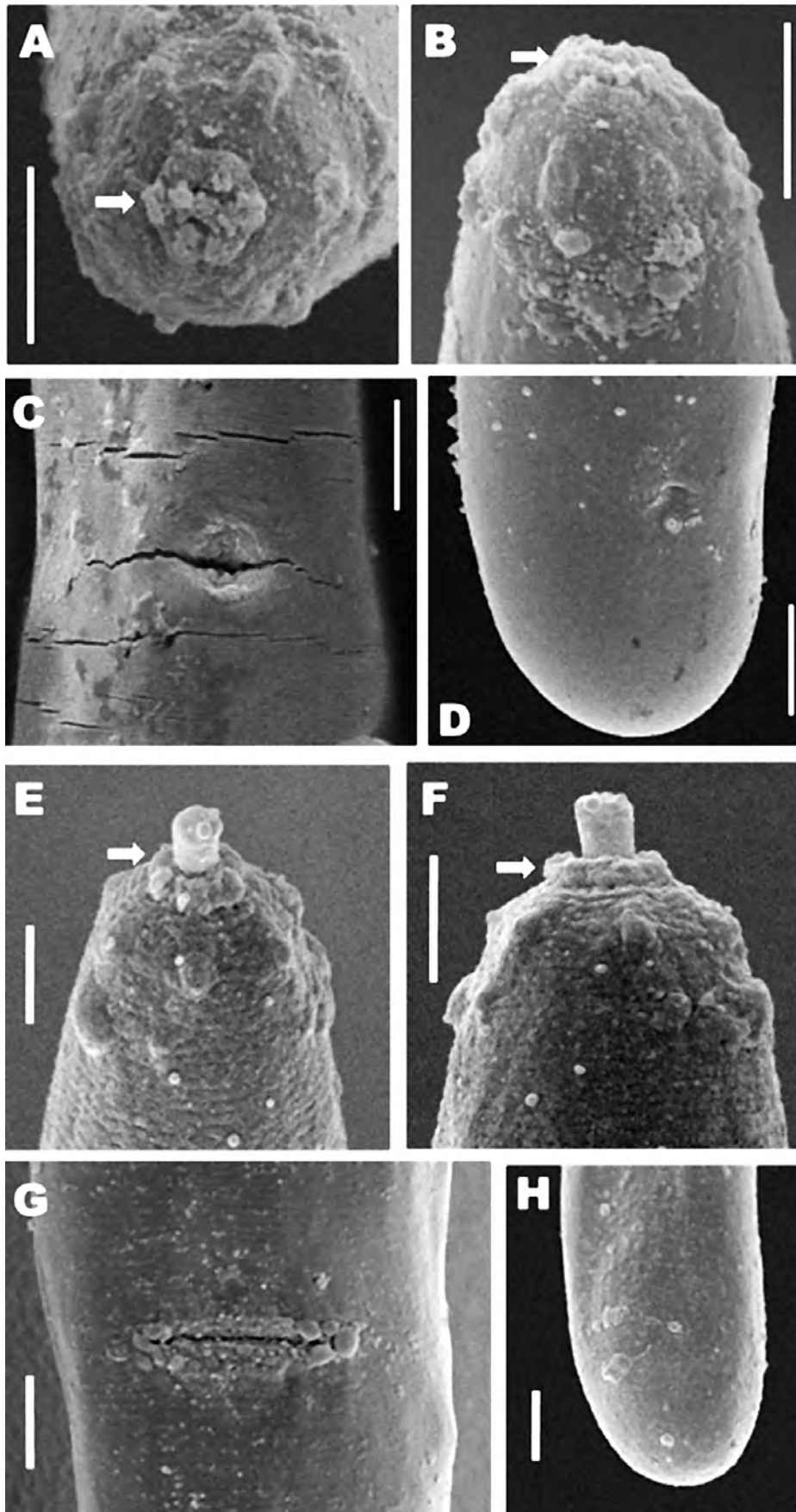


Fig. 3. A-D: *Adenolaimus tropicus* Mushtaq, Baniyamuddin *et* Ahmad, 2006 (female, SEM). A: Lip region in frontal view; arrowhead pointing at perioral disc. B: Lip region in ventral view; arrowhead pointing at perioral disc. C: Vagina in ventral view. D: Caudal region in ventral view. E-H: *Oostenbrinkia parva* Mushtaq, Baniyamuddin *et* Ahmad, 2006 (female, SEM). E, F: Lip region in ventral view, arrowhead pointing at perioral disc. G: Vagina in ventral view. H: Caudal region. (Scale bar: 5 μ m; E, F = 3 μ m).

it. Pre-rectum 4.3-7.2 times as long as anal body diameter, usually consisting of a shorter posterior portion with thin walls and wide lumen and a longer anterior portion with thicker walls and narrower lumen. Rectum almost equal (1.0-1.3 times) to anal body diameter. Anus (under SEM) a very small, transverse slit, about 1 μm broad. Tail short and rounded, nearly hemispherical.

Male. Genital system diorchic, with opposed testes. In addition to the pre-cloacal pair, situated at 3-5 μm from the cloacal aperture, there are two widely spaced ventro-median supplements, the posteriormost of which is situated at 27-35 μm from the pre-cloacal pair, beyond the range of the spicules. Spicules slightly curved ventrad, unusually long and slender, more than ten times as long as wide and 2.0-2.5 times longer than body diameter. Lateral guiding pieces (or gubernaculum, see remarks) 3.0-4.5 μm long. Tail short and rounded, ventrally straighter, dorsally more convex.

Diagnosis. The Iranian population herein studied is characterized by its body 0.63-0.86 mm long, lip region continuous, 7.0-9.0 μm wide and bearing a perioral disc, odontostyle 4.0-5.0 μm long with aperture occupying one-fifth of its length, odontophore 10-14 μm long and with distinct basal flanges, neck 119-147 μm long, basal pharyngeal bulb cylindrical and 27-38 μm long or occupying 24-26% of total neck length, female genital system mono-opisthodelphic, pre-vulval uterine sac 25-38 μm long or 1.2-2.0 times the body diameter, vulva (V = 36-47) preceded by a depression of the body surface, tail short and rounded in both sexes (10-15 μm , $c = 43-70$, $c' = 0.9-1.2$), spicules 28-32 μm long, and two well spaced ventromedian supplements.

Locality and habitat. Sisangan forests, Mazandaran province, northern Iran, where it was collected in rotten organic materials and superficial soils by A. Houshmand in May 2010.

Remarks. The Iranian material is very similar to the type population for this species (four females, holotype and three paratypes) from Costa Rica, with minor ($c' = 0.9-1.2$ vs $c' = 0.8-0.9$ in the type population) as well as more relevant differences: lip region nearly continuous with the adjacent body (vs "offset from body by slight depression"), presence of a low (but well perceptible under SEM) perioral disc (vs not mentioned), longer female pre-rectum (72-89 vs 18-25 μm), and males as frequent as females (vs males absent). However, such differences might not be real or significant. By courtesy of Prof. W. Ahmad (Aligarh Muslim University, India), a series of pictures of type specimens were available to the authors, which do not differ from those shown in Fig. 2 and suggest that a low (difficult to appreciate under LM) perioral disc is present. As mentioned above, the female pre-rectum consists of two portions, the posteriormost of which is shorter and with wider lumen (Fig. 2H), and is very similar to that originally illustrated.

Concerning the presence of males in Iranian population, it should be mentioned that females from Costa Rica were described bearing sperm cells in their anterior uterine sac (confirmed by W. Ahmad, *in lit.*), evidence that males also exist in this population. The differences in lip region demarcation and tail length are provisionally regarded as geographical variations.

OOSTENBRINKIA PARVA

Mushtaq, Baniyammuddin *et* Ahmad, 2006

(Figs 1A-E, 3E-H & 4)

Material examined. Twelve females and seven males from Kelardasht, Iran; in good condition.

Measurements. See Table I.

Adult. Moderately slender to slender nematodes of very small to small size, 0.39-0.53 mm long. Body cylindrical, tapering towards both extremities, but more so towards the anterior end because the tail is rounded. Habitus almost straight or slightly curved ventrad upon fixation, to an open C. Cuticle thin and with equal thickness (1.0-1.5 μm) throughout the body; its precise nature difficult to distinguish, but apparently dorylaimoid; outer layer (under SEM) bearing very weak transverse striation throughout the body. Lateral chord 4.0-5.5 μm wide or occupying one-fifth to one-third (26-35%) of mid-body diameter; body pores inconspicuous. Lip region rounded or slightly angular, two-fifths to one-half (41-53%) of body diameter at neck base; under SEM it appears nearly continuous with the adjacent body, with amalgamated lips, but their perioral region differentiated in a disc-like structure, and labial and cephalic papillae prominent, conferring to lip region an angular contour. Amphid aperture not observed under SEM due to deposition of material on it, but certainly small. Cheilostom wide, bearing relatively thick supporting ribs that appear as refractive rods (Fig. 4C,D). Odontostyle short (3-5 μm) but robust, with distinct lumen and aperture, 4-6 times as long as wide, shorter (about two-thirds) than lip region diameter or 1.0% of total body length; it is somewhat irregular since its dorsal side is slightly sigmoid and the anterior tip appears visibly broader than the other part, aperture about one-fifth its length. Guide ring simple but distinct, at 4.0-5.5 μm or 0.5-0.6 times the lip region diameter from anterior end. Odontophore distinctly longer (about three times) than odontostyle, with very well developed basal knobs or flanges. Pharynx typical of the group, tripartite, *i. e.* consisting of a rather muscular anterior region 51-58 μm long or 45-51% of total neck length; an intermediate, isthmus-like portion 11-12 μm long or 8-10% of total neck length, which is surrounded by glandular tissue; and basal (posterior), bulb-like, cylindrical portion 24-31 μm long, 2.3-2.6 times as long as wide, 1.8 times as long as body diameter, and occupying 22-26% of total neck length; pharyngeal gland nuclei difficult to

observe. Nerve ring located at 45-65 μm or 41-52% of total neck length from anterior end. Cardia short and rounded, surrounded by intestinal tissue.

Female. Genital system didelphic-amphidelphic, with both branches equally developed and very short, the anterior 43-57 μm or 7-11% of total body length and the

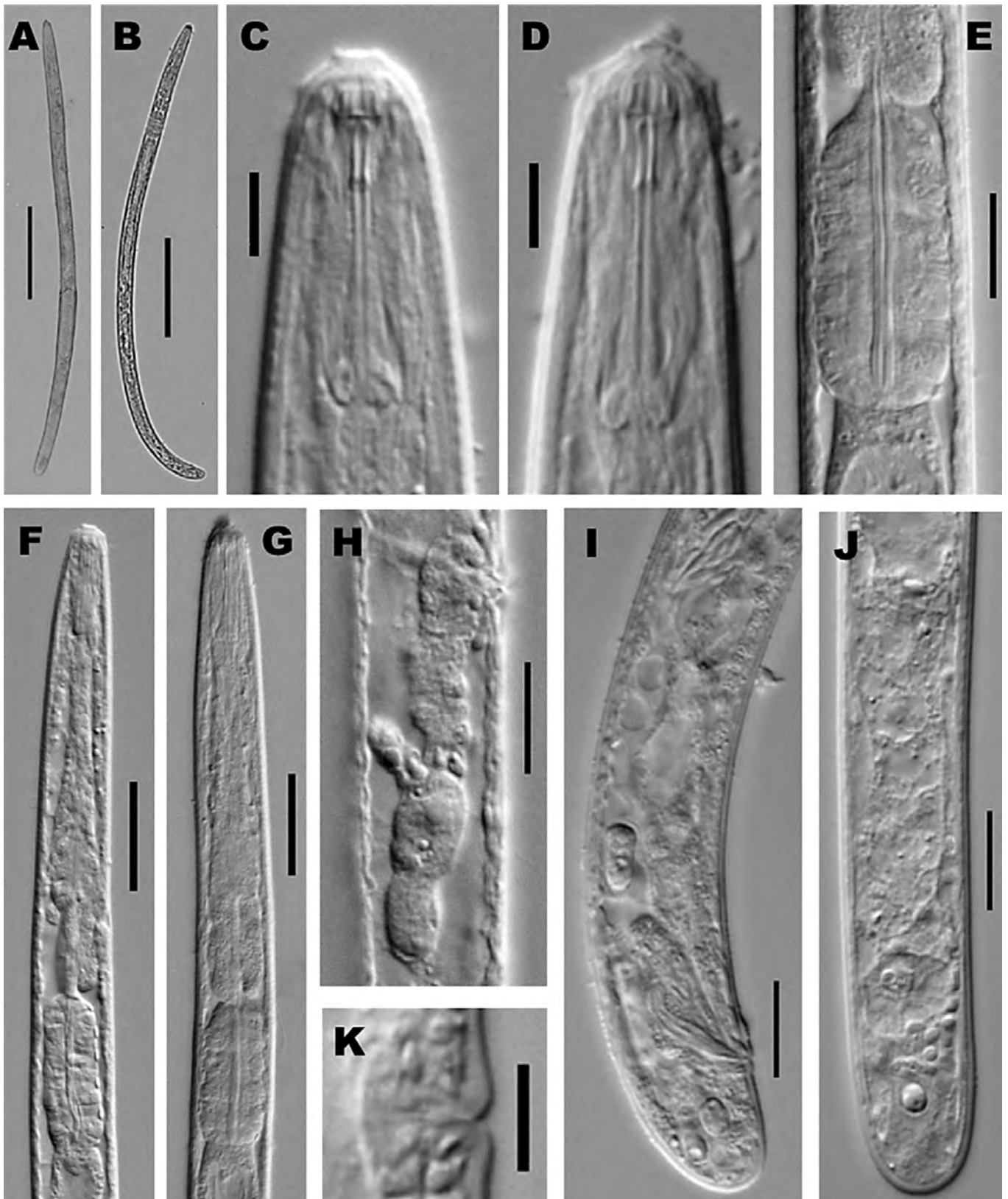


Fig. 4. *Oostenbrinkia parva* Mushtaq, Baniyamuddin *et* Ahmad, 2006 (LM). A: Female, entire. B: Male, entire. C, D: Anterior region in median view. E: Pharyngeal bulb and cardia. F, G: Neck region. H: Female, posterior genital branch. I: Male, posterior body region. J: Female, posterior body region. K: Vagina. (Scale bars: A, B: 100 μm ; C, D, K: 5 μm ; E, H-J: 10 μm , F, G: 20 μm).

posterior 38-57 μm or 7-11% of total body length. Ovaries comparatively well developed, the anterior 22-30 μm and posterior 21-38 μm long, reflexed, almost reaching the vulva level; oocytes first in two or more rows, then in a single row. Genital tract very poorly differentiated since a distinct sphincter is lacking and both oviduct and uterus are apparently about one body diameter long; one uterine egg observed, $54 \times 17 \mu\text{m}$. Vagina also very short and poorly differentiated, extending inwards about 4 μm or one-fifth of body diameter. Vulva post-equatorial, under SEM a large transverse slit, about 10 μm long. Anus not observed under SEM, certainly a very small transverse slit. Pre-rectum 5.5-6.0 times as long as rectum, almost equal to anal body diameter. Tail short and rounded, nearly hemispherical.

Male. Genital system diorchic, with opposed testes. Pre-cloacal pair of supplements situated at 2-3 μm from cloacal aperture, and no ventro-median supplement. Spicules small (almost equal to anal body diameter long), relatively slender (about 6.8 times as long as wide) and bent ventrad at level of the anterior fifth, then almost straight. Lateral guiding pieces not distinctly distinguishable in the specimens examined, certainly small. Tail short and rounded.

Diagnosis. The Iranian material is characterized by its body 0.39-0.53 mm long, lip region continuous and 6.0-7.5 μm wide or about two-thirds of body diameter at neck base and bearing a perioral disc, odontostyle 4-5 μm long with aperture occupying one-fifth of its length, odontophore 12-15 μm long and with distinct basal flanges, neck 109-122 μm long, basal pharyngeal bulb cylindrical and 25-31 μm long or occupying 22-26% of total neck length, female genital system amphidelphic but poorly differentiated, vulva transverse ($V = 55-61$), tail short and rounded in both sexes (9-14 μm , $c = 35-52$, $c' = 0.8-1.0$), spicules 11-13 μm long and no ventro-median supplement.

Locality and habitat. Kelardasht forests, Mazandaran province, northern Iran, where it was collected in rotten organic materials and superficial soils in August 2010.

Remarks. The Iranian population is nearly identical to the type material (two females, holotype and paratype, and two juveniles) from India, but there are some differences: lip region bearing a low perioral disc-like structure (*vs* not mentioned in type material), odontostyle somewhat irregular (*vs* apparently regular), tail slightly shorter (*vs* 14-15 μm) and males are as frequent as females (*vs* male unknown, although only a few specimens were available to study for the type material). The assistance of Prof. W. Ahmad was also essential to clarify the identity of the Iranian material, since his observa-

tions (*in lit.*) after re-examination of type specimens and a series of pictures taken by him have revealed that a perioral disc as well as irregular odontostyle are also present in the Indian females. Other differences are considered of minor relevance taking into consideration the small number of specimens in the type material.

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