

# Morphological and molecular characterisation of *Panagrolaimus* Fuchs, 1930 (Nematoda, Rhabditida, Panagrolaimidae) species from Iran

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**Summary.** Seven species of the family Panagrolaimidae are described from natural areas in Kerman province, Iran: *Panagrolaimus concolor*; *P. facetus*; *P. cf. labiatus*; *P. cf. papillosus*; *P. cf. subelongatus*; *P. superbus* and *P. trilabiatus* were recovered. Descriptions, measurements, illustrations and light microscopic photographs are provided for all species. Molecular analysis of three out of seven species based on sequences of the 18S rDNA places *P. facetus* close to *P. davidi* (HQ270131) and an unidentified *Panagrolaimus* (EU040129), only in the Maximum Likelihood (ML) tree. In the Bayesian Inference (BI) tree it groups with an unidentified *Panagrolaimus* (KC522709). *Panagrolaimus trilabiatus* groups with *P. subelongatus* (AY284681) and an unidentified *Panagrolaimus* (FJ590963) in BI tree. *Panagrolaimus cf. papillosus* groups with *P. davidi* (HQ270131) and is placed close to *P. facetus* (KF011488) in the BI tree. In the ML tree it groups with two unidentified *Panagrolaimus* (KC522708; FJ590961). Molecular data of 18S rDNA of *P. facetus*, *P. cf. papillosus* and *P. trilabiatus* are reported for the first time. In addition, Maximum Likelihood and Bayesian analyses revealed the paraphyly of the genus *Panagrolaimus*.

**Key words:** Iran, morphology, Panagrolaimidae, *Panagrolaimus*, 18S rDNA, phylogeny, taxonomy.

The genus *Panagrolaimus* Fuchs, 1930 are bacterial feeding nematodes that occupy different niches, including the cold soil from Antarctica, temperate and semi-arid soils, and mosses (Shannon *et al.*, 2005). These worms are used in developmental studies because they have three reproductive modes (*i.e.*, gonochoristic, hermaphroditic and parthenogenetic) (Lewis *et al.*, 2009). The main study on this group of nematodes has been done by Abolafia & Peña-Santiago (2006) who described several Iberian species and provided a key to species identification. *Panagrolaimus* comprises 44 nominal species (Abolafia & Peña-Santiago, 2006). So far, two species of this genus, namely *P. dendroctoni* (Fuchs, 1932) Rühm, 1956 and *P. rigidus* (Schneider, 1866) Thorne, 1937 were reported from Iran (Shokoohi *et al.*, 2007).

Identification of the species of this genus is frequently very difficult (e.g. molecular analyses executed by Lewis *et al.*, 2009) due to the high intraspecific variation of their size and morphology, and because sometimes the original descriptions of species are very poor or inexact. The present paper

is one of series on nematodes of the order Rhabditida from Iran (province of Kerman) and deals with seven known species belonging to the genus *Panagrolaimus* collected in natural areas. These species are reported for the first time from Iran.

## MATERIALS AND METHODS

**Studies on morphology.** Nematodes were extracted from soil samples by Baermann's (1917) funnel technique. Specimens were fixed with hot 4% formaldehyde solution and processed to anhydrous glycerin by the method of De Grisse (1969). Measurements were taken directly using an ocular micrometer and/or a curvimeter upon drawing the corresponding organ or structure. Drawings were made using a drawing tube attached to the microscope Olympus CH2. LM pictures were made with a Nikon Eclipse 80i microscope equipped with a Nikon Digital Sight DS-5M camera.

Females of *P. trilabiatus* were cultured on water-agar 2% in Petri plates to which ten gravid females were added to each plate. The terminology used to

describe the morphology of the stoma and spicules follows the proposals by De Ley *et al.* (1995) and Abolafia & Peña-Santiago (2006), respectively.

**Phylogenetic analysis.** The sequences of species belonging to the family Panagrolaimidae used for phylogenetic analysis were obtained from the GenBank (73 sequences). DNA extraction was done using an AccuPrep Genomic DNA Extraction Kit (Bioneer Corporation, Korea) (<http://www.bioneer.com>) according to the manufacturer's instructions. Specimens were picked into 1.5 ml tube containing 5 µl double distilled water. The tube was frozen in liquid nitrogen and the nematodes were crushed with vortex; 200 µl Tissue Lysis buffer (TL) and 20 µl proteinase K (20 mg ml<sup>-1</sup>) was added. The homogenate was incubated at 60°C for 2 h. The supernatant was extracted and stored at -20°C. The forward primer SSU\_F\_04 (5'- GCT TGT CTC AAA GAT TAA GCC-3') and the reverse primer SSU\_R\_26 (5'- CAT TCT TGG CAA ATG CTT TCG-3') (Blaxter *et al.*, 1998) were used in the PCR reactions for amplification of the partial 18S region (~900bp). PCR was conducted with 10 µl of the extracted DNA, 4 µl of PCR Master Mix (Kawsar Biotech company, Iran), 1 µl of each primers (10 pmol µl<sup>-1</sup>) and ddH<sub>2</sub>O to a final volume of 25 µl. The amplification was carried out using an Eppendorf master cycler gradient (Eppendorf, Hamburg, Germany), with 3 min at 94°C, 37 cycles of 45 s at 94°C, 45 s at 56°C and 1 min at 72°C, and finally one cycle of 6 min at 72°C followed by a holding temperature of 4°C. After DNA amplification, 5 µl of product was loaded on a 1% agarose gel (40 mM Tris, 40 mM boric acid, and 1 mM EDTA) for DNA checking. The bands were stained with 50 mM ethidium bromide and visualised and photographed on 1% agarose gel under a UV transilluminator. Product was stored at -20°C prior to sequencing. PCR product was purified for sequencing and sequenced using the primers that were used in the amplification step. Sequencing reactions were performed by Bioneer company (South Korea) (<http://eng.bioneer.com>). Sequencing was performed in both directions. The DNA sequence was edited using Chromas version 1.45 (McCarthy, 1997). Primers for the sequencing reaction were those used in the amplification step. The sequence was confirmed in both directions and repeated. Sequences for the ingroups and outgroups were obtained from GenBanks. The ribosomal SSU sequences were analysed and aligned using the program BioEdit (Hall, 1999). The length of alignment is 1857 bp.

Maximum Likelihood (ML) and Bayesian Inference were used to reconstruct the phylogeny. The Maximum Likelihood tree was constructed using the program Mega 5 (Tamura *et al.*, 2011).

The analyses were run for 10000 bootstrap replicates. The Bayesian tree was generated using the Bayesian inference method as implemented in the program MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The analysis under GTR model was initiated with a random starting tree and run with the Markov chain Monte Carlo (MCMC) for 10<sup>6</sup> generations. The distance matrix option of Mega 5 (Tamura *et al.*, 2011) was used to calculate genetic distances according to the Maximum Composite Likelihood model of sequence evolution and bootstrap analysis was implemented with 10000 replicates. The tree was visualised with TreeView program. For phylogenetic relationships analysis of 18S rDNA, *Caenorhabditis japonica* Kiontke, Hironaka & Sudhaus, 2002 (AY602182) was chosen as outgroup. This selection was based on Lewis *et al.* (2009) and van Megen *et al.* (2009) studies. The original partial 18S sequences of *Panagrolaimus* species are deposited in the GenBank under the accession numbers: KF011487, KF011488, KF011489 for *P. trilabiatus* (881 bp), *P. facetus* (915 bp) and *P. cf. papillosus* (906 bp), respectively.

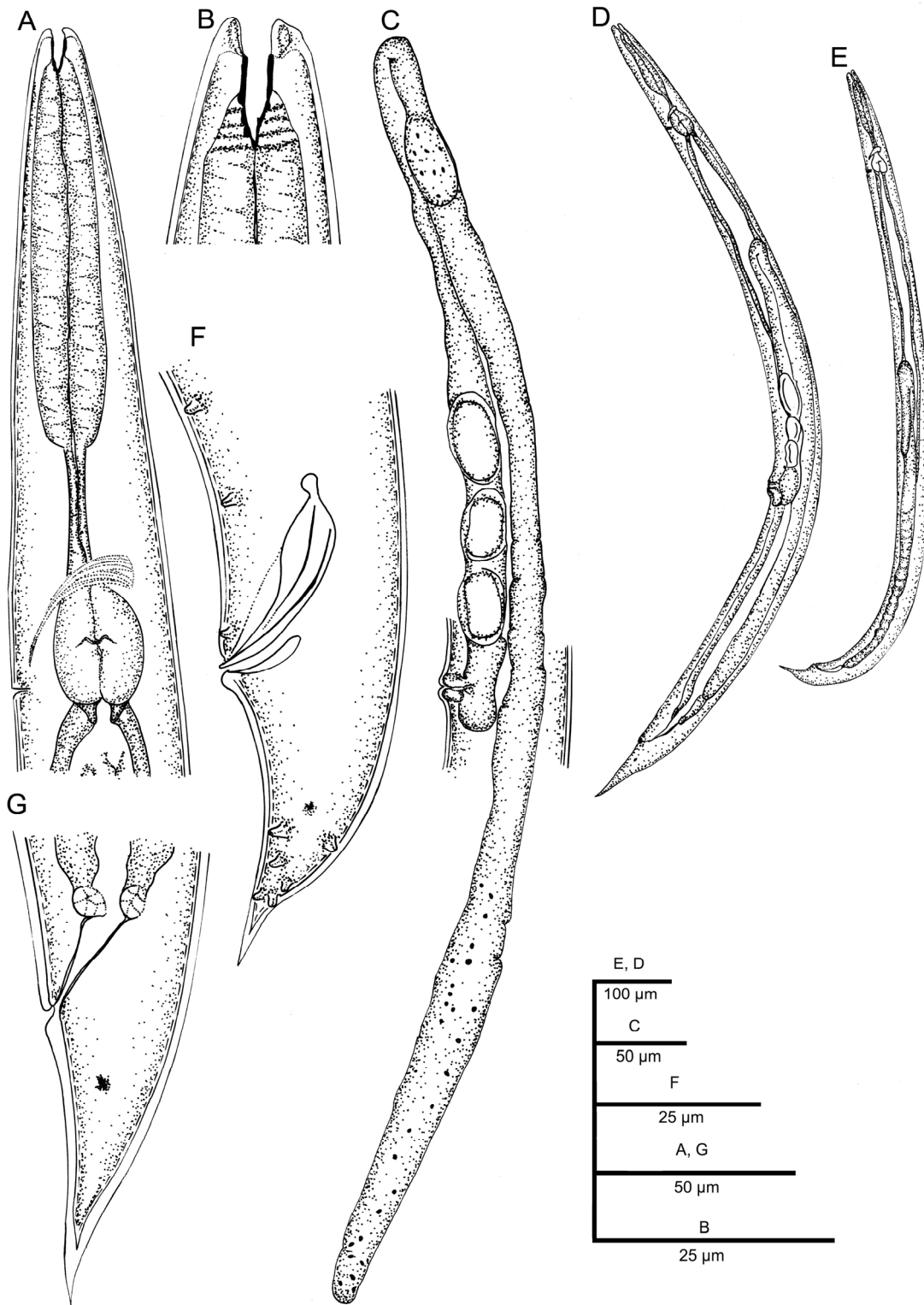
## DESCRIPTIONS

### *Panagrolaimus concolor* Massey, 1964 (Figs 1 & 2A-D)

**Measurements.** See Table 1.

Population collected in Kerman, province of Kerman (3♀♀, 1♂).

**Female.** Body length 0.82-1.15 mm, cylindrical, slightly curved ventrad after fixation. Cuticle smooth; having 3.8 µm thickness, annuli 1 µm wide. Lateral field with two incisures occupying 12% of midbody diameter, fading out near phasmids. Lip region continuous with neck, having six small lips amalgamated in pairs, and a circular amphid opening. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, having cuticularised rhabdia differentiated in a shorter anterior part (corresponding with the anterior arcade epidermis), and a longer posterior part (posterior arcade epidermis). Stegostom having four distinct parts, bearing a minute tooth. Pharyngeal corpus cylindrical, 2.1-2.4 times isthmus length, with procorpus longer than metacarpus. Isthmus slenderer than corpus and distinctly separated from metacarpus. Basal bulb spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 85-90% of



**Fig. 1.** *Panagrolaimus concolor* Massey, 1964. A: Neck. B: Anterior end. C: Female reproductive system. D: Entire female. E: Entire male. F: Male posterior end. G: Female posterior end.

neck length. Excretory pore opening at bulb level, at 98% of neck length. Deirid not visible. Reproductive system monodelphic-prodelphic, located on right side of intestine. Vulva protruding, located posterior to middle part of body. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter and well developed. Uterus tubular, about four times the corresponding body diameter long. Post-uterine sac 0.4-0.5 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Rectum 1.0-1.1 times the anal body diameter. Tail conical-elongate, with pointed tip. Phasmid at 53-57% of tail length.

**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad. Two pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail comprising one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded; calamus short, straight; lamina dorsally curved, with pointed terminus, having two longitudinal lines. Gubernaculum well developed, 15  $\mu\text{m}$  long or less than half of the spicule length.

**Locality and habitat.** Material has been found in Mahan (province of Kerman), in association with *Haloxylon persicum* L.

**Remarks.** The material examined is similar to the original population of *P. concolor* described by Massey (1964) in having slightly rounded lips, minute denticle on stegostom, conoid female tail, lateral field with two incisures, corpus/isthmus ratio (2.1-2.4 *vs* 2.3), shape of spicules and gubernaculum. However, they differ in nerve ring position (near bulb level *vs* at middle length of isthmus), excretory pore position (near base of bulb level *vs* slightly more anterior), female tail slightly longer (61-76 *vs* 48  $\mu\text{m}$ ), and precloacal genital papillae (two pairs *vs* only one pair was observed in original description). Other similar species are *P. artyukhovskii* Blinova & Mishina, 1975, *P. detritophagus* Fuchs, 1930 and *P. rigidus* (Schneider, 1866) Thorne, 1937. Our population differs from *P. artyukhoskii* in having two lateral field (*vs* three), shorter corpus/isthmus ratio (2.1-2.4 *vs* 2.9) and spicules shape (lamina not pointed at ventral side *vs* lamina pointed at ventral side in original description) and female tail (61-76 *vs* 51  $\mu\text{m}$  in original description). It differs from *P. detritophagus* in having slightly rounded lips (*vs* conical lips), two lateral fields (*vs* three lateral field) and shape of spicules (lamina not pointed at ventral side *vs* lamina pointed at ventral side in original

description). Also it differs from *P. rigidus* in fused lips (*vs* lips duplex), two lateral field (*vs* three lateral field).

This species is reported for the first time from Iran.

***Panagrolaimus facetus* Massey (1971)  
Andrássy, 1984  
(Figs 2E-H & 3)**

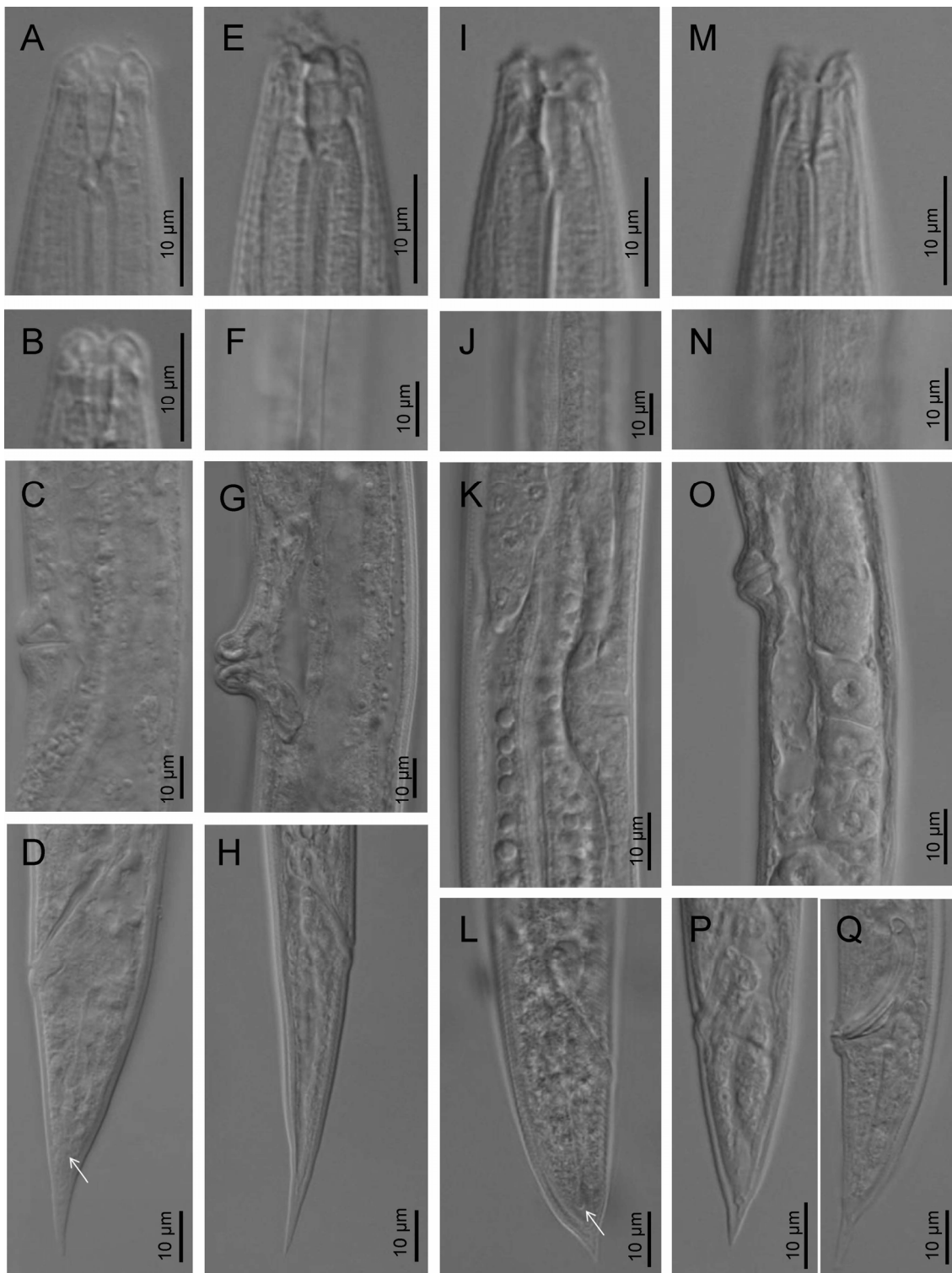
**Measurements.** See Table 1.

Population collected in Kerman, province of Kerman (3♀♀, 1♂).

**Female.** Body length 0.73-0.78 mm, cylindrical, curved ventrad after fixation. Cuticle smooth; having 3  $\mu\text{m}$  thickness, annuli 1  $\mu\text{m}$  wide. Lateral field with two incisures occupying 13-17% of midbody diameter. Lip region continuous with neck, having six lips amalgamated in pair and a circular amphid opening. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, having cuticularised rhabdia. Stegostom having four distinct parts. Pharyngeal corpus cylindrical, 2.2-3.2 times isthmus length, with procorpus longer than metacarpus. Isthmus slenderer than corpus and separated with metacarpus distinctly. Basal bulb spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 66-78% of neck length. Excretory pore opening at isthmus level, at 76-84% of neck length. Deirid at 95% of neck length, at bulb level. Reproductive system monodelphic-prodelphic, located on right side of intestine. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter. Uterus tubular, about five times the corresponding body diameter long. Postuterine sac 0.6-0.8 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Vulva protruding, located posterior to middle part of body. Rectum 0.8-1.3 times the anal body diameter. Tail conical-elongate, with pointed end and mucro. Phasmid at 30-40% of tail length.

**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad, bearing a thin mucro. Two pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail comprising one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded, straight; calamus as wide as manubrium; lamina ventrally curved, with





**Fig. 2.** *Panagrolaimus concolor* Massey, 1964 (LM). A, B: Anterior end. C: Vagina region. D: Female posterior end (arrow points at phasmid). *Panagrolaimus facetus* (Massey, 1971) Andr ssy, 1984 (LM). E: Anterior end. F: Lateral field. G: Vagina region. H: Female posterior end. *Panagrolaimus* cf. *labiatus* (Kreis, 1929) Andr ssy, 1960 (juvenile, LM). I: Anterior end. J: Lateral field. K: Vagina region. L: Posterior end. *Panagrolaimus* cf. *papillosus* Loof, 1971 (LM). M: Anterior end. N: Lateral field. O: Vagina region. P: Female posterior end. Q: Male posterior end.

pointed terminus and three longitudinal lines. Gubernaculum well developed, 14  $\mu\text{m}$  long or about 50% of the spicule length.

**Locality and habitat.** Material has been found in Kerman (province of Kerman), in soil.

**Remarks.** Our population is similar to *P. facetus* in having high lips, two lateral fields, location of nerve ring and excretory pore (both at isthmus level), conical elongated female tail. However, the material examined compared with the original description of this species provided by Massey (1971), differs in having slightly rounded lips (*vs* slightly acute lips), slightly shorter body (0.73-0.78 mm in females and 0.55 mm in males *vs* 0.83-0.86 mm in females and 0.78-0.81 mm in males in the original description), and slightly longer spicules (29 *vs* 23  $\mu\text{m}$  in the original description). On the other hand, Andr ssy (1984) reported this species with longer body (*vs* 0.80-0.90 mm in females and 0.80 mm in males), and shorter c value ( $c=13-14$  *vs* 16-21).

This species also slightly resembles *P. magnivulvatus* Bostr m, 1995. However, it differs in lateral field (two incisures *vs* three incisures) and longer female tail ( $c' = 4$  *vs*  $c' = 2-3$ ) (see Abolafia & Pe na-Santiago, 2006). Massey (1971) mentioned *P. margaretae* (Massey, 1964) Andr ssy, 1984 as similar species with *P. facetus*. However, their lips are very different (more or less acute in *P. facetus* *vs* acute and prominent in *P. margaretae*), and lateral field (two *vs* three incisures).

This species is reported for the first time from Iran.

### ***Panagrolaimus cf. labiatus* (Kreis, 1929)**

**Andr ssy, 1960**

**(Figs 2I-L & 4)**

**Measurements.** See Table 1.

Population collected in Kerman, province of Kerman (3♀♀, 2♂♂).

**Female.** Body length 0.92-0.97 mm, cylindrical, curved ventrad after fixation. Cuticle smooth; having 1  $\mu\text{m}$  thickness, annuli 1  $\mu\text{m}$  wide. Lateral field with two incisures occupying 8-10% of midbody diameter, fading out near tail terminus. Lip region continuous with neck, having six small lips amalgamated in pairs, and a circular amphid opening. Labial papillae well observed. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, having cuticularised rhabdia. Stegostom having four distinct parts. Pharyngeal corpus cylindrical, 2-3 times isthmus length, with procorpus longer than

metacarpus. Isthmus slender than corpus and distinctly separated from metacarpus. Basal bulb ovoid to spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 65-80% of neck length. Excretory pore opening at bulb level, at 67-97% of neck length. Deirid at 88-94% of neck length, at bulb level. Reproductive system monodelphic-prodelphic, located on right side of intestine. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter. Uterus tubular, about five times the corresponding body diameter long, consisting of a long proximal tubular part and a short distal part with thinner walls. Post-uterine sac 0.8-0.9 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Vulva protruding, located posterior to middle part of body. Rectum 1.3-1.5 times the anal body diameter. Tail conical-elongate, with pointed end. Phasmid at 17-31% of tail length.

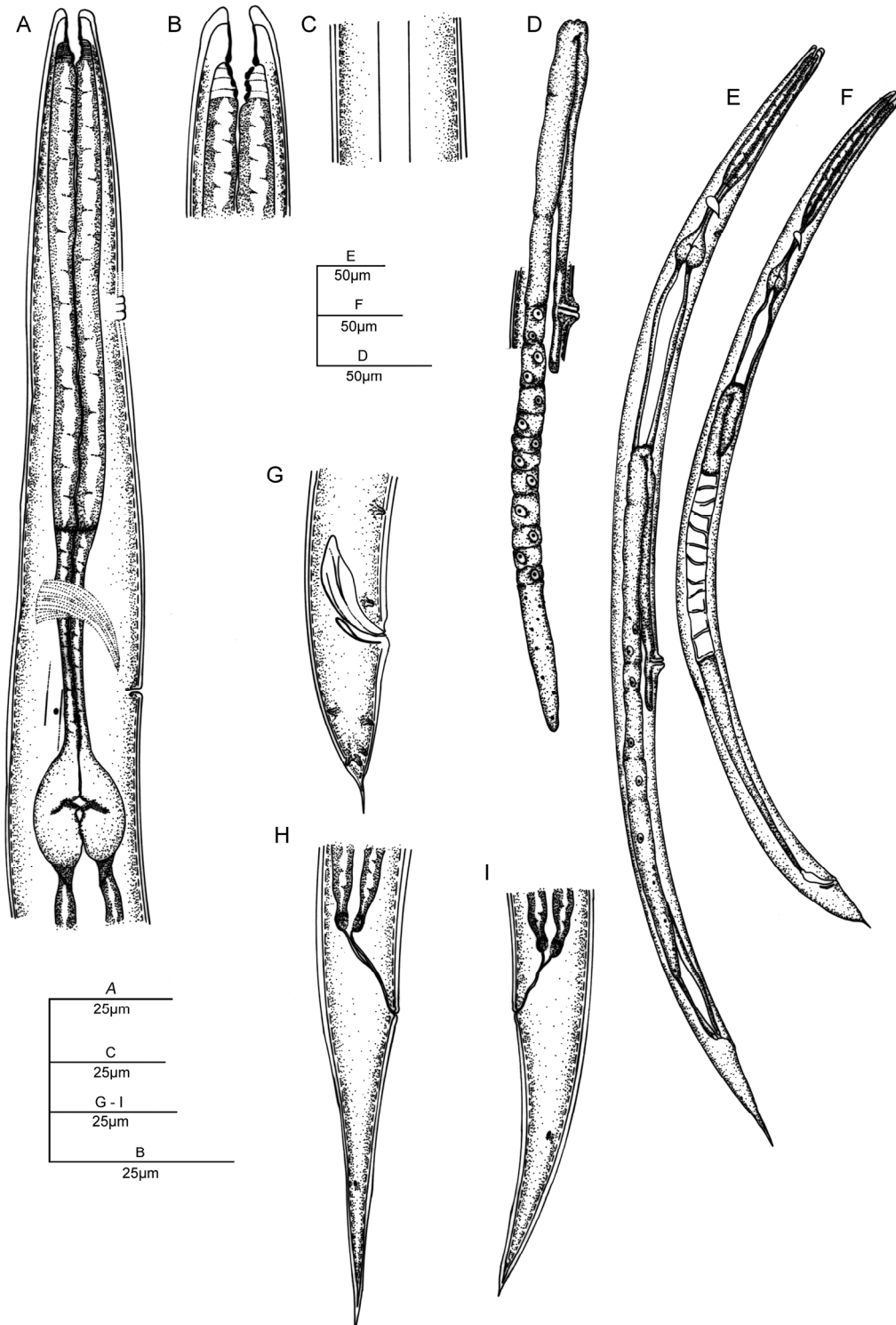
**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad. Two pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail comprising one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded, straight; calamus slenderer than manubrium; lamina ventrally curved, with rounded terminus and three longitudinal lines. Gubernaculum well developed, 14-16  $\mu\text{m}$  long or about 50% of the spicule length.

**Locality and habitat.** Material has been found in Kerman (province of Kerman), in association with *Haloxylon persicum* L.

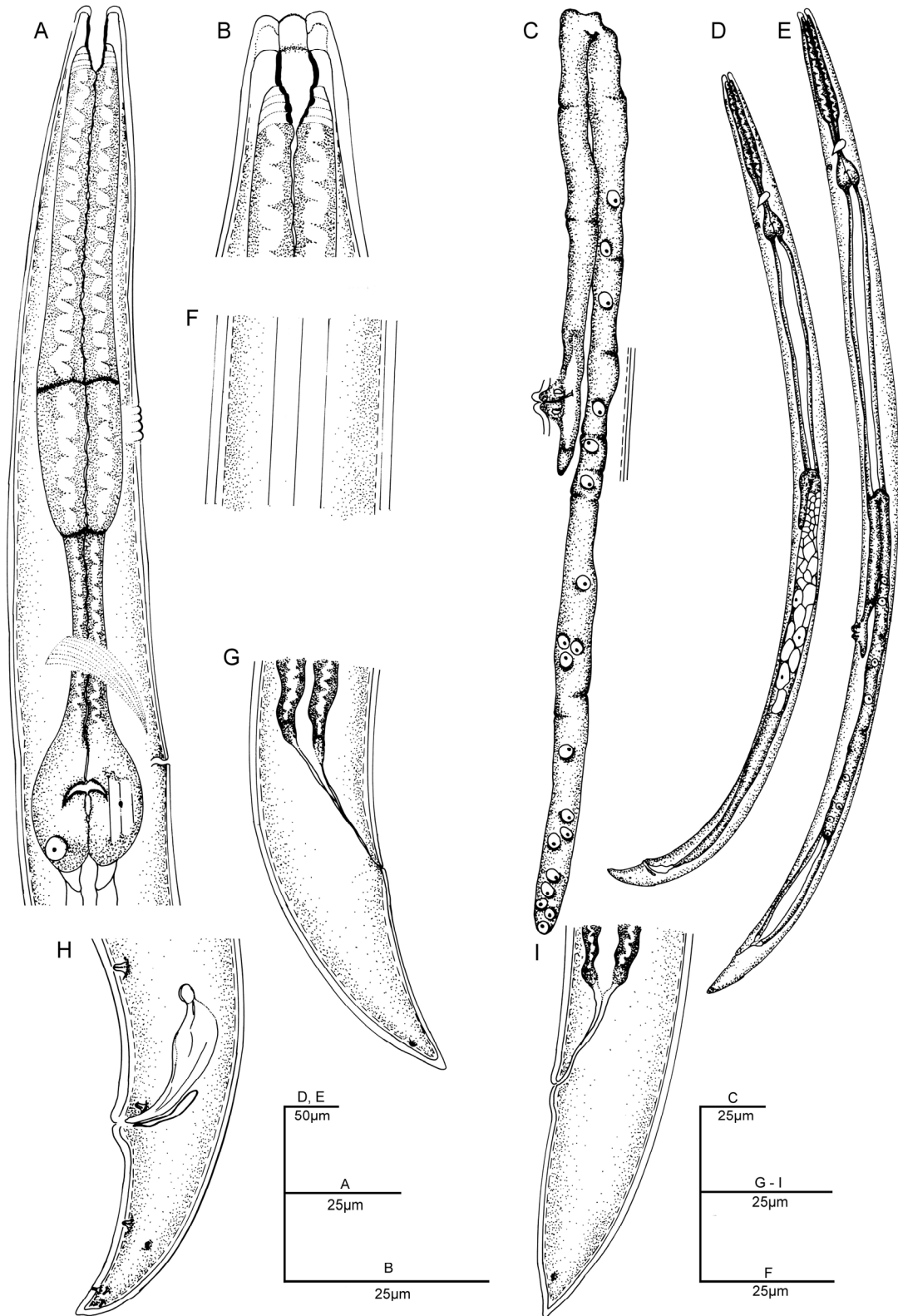
**Remarks.** Our specimens are similar to *P. labiatus* (Kreis, 1929) Andr ssy, 1960 in rounded lip and conical female tail with convex in dorsal and ventral side. This species resembles in the measurements with *P. labiatus*. However, it differs from that species by having longer females (925-972 *vs* 480-680  $\mu\text{m}$  in the original description), and female tail tip (not differentiated at tip *vs* with a marked narrowing at its posterior half in the original description). From its junior synonym *P. burdwanensis* Chaturvedi and Khera, 1979 it differs by longer body (*vs* 0.74-0.78 mm in females and 0.56-0.62 mm in males), longer spicules (31-34 *vs* 18-20  $\mu\text{m}$ ), longer gubernaculum (14-15 *vs* 8-10  $\mu\text{m}$ ) and fewer precloacal genital papillae (one *vs* two precloacal genital papillae). Previous descriptions of *P. labiatus* are incomplete, and therefore it is impossible to be absolutely sure about the identity

**Table 1.** Measurements of *Panagrolaimus concolor* Massey, 1964, *P. facetus* (Massey, 1971) Andrassy, 1984 and *P. cf. labiatus* (Kreis, 1929) Andrassy, 1960 from Iran. All measurements in  $\mu\text{m}$ , and in the form; mean  $\pm$  standard error (range).

Species	<i>P. concolor</i>		<i>P. facetus</i>		<i>P. cf. labiatus</i>	
Province, Locality	Kerman, Mahan		Kerman, Kerman		Kerman, Kerman	
Habitat	<i>Haloxylon persicum</i>		Soil		<i>Haloxylon persicum</i>	
n	3♀♀	1♂	3♀♀	1♂	3♀♀	2♂♂
Body length	963.6 $\pm$ 170.3 (827-1154)	936	766.7 $\pm$ 26.2 (736-782)	554	943.4 $\pm$ 25.0 (925-972)	811, 783
a	18.9 $\pm$ 2.6 (16.7-21.8)	20.9	26.5 $\pm$ 3.3 (23.0-29.6)	25.6	24.3 $\pm$ 2.1 (22.1-26.3)	28.7, 24.6
b	6.2 $\pm$ 1.1 (5.4-7.5)	6.6	4.5 $\pm$ 0.5 (4.2-5.1)	3.9	5.3 $\pm$ 0.0 (5.2-5.3)	4.0, 5.0
c	14.4 $\pm$ 0.8 (13.5-15.1)	19.9	13.0 $\pm$ 0.5 (12.6-13.6)	15.1	23.5 $\pm$ 1.4 (22.6-25.1)	18.9, 22.08
c'	2.4 $\pm$ 0.4 (2.1-2.9)	1.8	3.0 $\pm$ 0.7 (2.2-3.6)	1.9	1.7 $\pm$ 0.0 (1.7-1.8)	1.8, 1.6
V	55.1 $\pm$ 4.9 (50-60)	–	58.1 $\pm$ 1.2 (57-59)	–	60.7 $\pm$ 2.4 (58.6-63.3)	–
Lip region width	10.5 $\pm$ 1.1 (9-12)	9	8.8 $\pm$ 0.5 (8-9)	7	10.3 $\pm$ 0.5 (10-11)	9, 10
Stoma	11.3 $\pm$ 0.8 (10-12)	12	12.6 $\pm$ 0.5 (12-13)	9	14.1 $\pm$ 1.1 (13-16)	12, 13
Pharyngeal corpus	81.6 $\pm$ 3.2 (79-85)	78	102.2 $\pm$ 7.3 (96-110)	78	97.3 $\pm$ 2.7 (94-99)	97, 87
Isthmus	36.1 $\pm$ 1.8 (35-38)	28	39.9 $\pm$ 9.2 (29-45)	39	34.5 $\pm$ 4.9 (31-40)	26, 30
Bulb	26.5 $\pm$ 0.03 (26-26)	26	24.5 $\pm$ 0.9 (24-25)	20	29.8 $\pm$ 2.7 (28-33)	26, 23
Pharynx length	144.2 $\pm$ 2.7 (141-147)	132	166.7 $\pm$ 16.2 (149-181)	137	163.1 $\pm$ 5.9 (158-169)	158, 146
Neck	156.2 $\pm$ 4.8 (153-162)	141	171.0 $\pm$ 23.8 (143-186)	143	177 $\pm$ 6 (171-183)	202, 155
Nerve ring-ant. end	134.5 $\pm$ 4.9 (131-138)	116	121.7 $\pm$ 25.5 (95-146)	103	134.9 $\pm$ 13.0 (120-144)	127, 125
Excretory pore-ant. end	155.5 $\pm$ 6.3 (151-160)	141	135.8 $\pm$ 24.1 (109-157)	112	152.6 $\pm$ 25.1 (124-167)	114, 97
Deirid-ant. end	–	–	185.8 $\pm$ 0.0 (185.8)	127	169 $\pm$ 1.6 (159-162)	144, 143
Cuticle thickness	3.8 $\pm$ 0.00 (3.8)	–	2	1.5	1.4 $\pm$ 0.6 (1-2)	1.5
Body width: neck base	33.0 $\pm$ 1.3 (32-34)	35	27.0 $\pm$ 2.7 (25-30)	22	13.0 $\pm$ 1.4 (12-15)	10, 12
Body width: midbody	50.8 $\pm$ 1.8 (49-53)	45	29.2 $\pm$ 4.1 (26-34)	22	39.1 $\pm$ 3.3 (36-42)	28, 32
Body width: anus	27.4 $\pm$ 1.7 (26-29)	26	20.4 $\pm$ 5.3 (16-26)	20	23.0 $\pm$ 1.4 (22-25)	23, 21
Lateral field	–	–	4.7 $\pm$ 0.0 (4.7)	1.9	3.6 (n=1)	4
Vagina	40.6 $\pm$ 2.1 (38-42)	–	13.8 $\pm$ 0.5 (13-14)	–	13 $\pm$ 2.9 (11-16)	–
Ovary	570.2 $\pm$ 54.7 (511-618)	–	288.6 $\pm$ 34.1 (254-322)	–	316.4 $\pm$ 59.3 (259-377)	–
Postuterine sac	23.3 $\pm$ 2.6 (21-26)	–	22.6 $\pm$ 0.9 (22-24)	–	33.9 $\pm$ 2.3 (32-36)	–
Rectum	30.0 $\pm$ 2.09 (28-32)	–	21.4 $\pm$ 2.7 (20-24)	–	32.1 $\pm$ 0.5 (31.8-32.7)	–
Tail	66.5 $\pm$ 8.6 (61-76)	47	58.8 $\pm$ 1.4 (57-60)	37	40.3 $\pm$ 2.8 (37-42)	43, 35
Vulva- anterior end	525.1 $\pm$ 46.6 (496-579)	–	445.5 $\pm$ 24.1 (418-464)	–	572.3 $\pm$ 21.8 (547-585)	–
Spicules	–	37	–	29	–	31, 34
Gubernaculum	–	15	–	14	–	14, 15



**Fig. 3.** *Panagrolaimus facetus* (Massey, 1971) Andrassy, 1984. A: Neck. B: Anterior end. C: Lateral field. D: Female reproductive system. E: Entire Female. F: Entire male. G: Male posterior end. H, I: Female posterior end.



**Fig. 4.** *Panagrolaimus* cf. *labiatus* (Kreis, 1929) Andr assy, 1960. A: Neck. B: Anterior end. C: Female reproductive system. D: Entire male. E: Entire female. F: Lateral field. G, I: Female posterior end. H: Male posterior end.

of our population. In view of close similarity of our specimens with *P. labiatus*, we consider it closely related to it and identify it as *P. cf. labiatus*.

Furthermore, this material is also similar to *P. goodeyi* Rühm, 1956. However they differ in phasmid position (near tail terminus in our population vs near mid tail in *P. goodeyi* in the original description).

***Panagrolaimus cf. papillosus* Loof, 1971  
(Figs 2M-Q & 5)**

**Measurements.** See Table 2.

Population collected in Kerman, province of Kerman (3♀♀, 1♂♂).

**Female.** Body length 0.75-0.80 mm, cylindrical, curved ventrad after fixation. Cuticle annulated, having 1 µm thickness, annuli 1 µm wide. Lateral field with three incisures occupying 17-30% of midbody diameter. Lip region continuous with neck, having six rounded lips amalgamated in pairs, and a circular amphid opening. Labial papillae well observed. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, having cuticularised rhabdia. Stegostom having four distinct parts. Pharyngeal corpus cylindrical, 2.5-3.0 times isthmus length, with procorpus longer than metacarpus. Isthmus slenderer than corpus distinctly separated from metacarpus. Basal bulb ovoid to spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 70-76% of neck length. Excretory pore opening at isthmus level, near to bulb, at 81-84% of neck length. Deirid at 88-97% of neck length, at bulb level. Reproductive system monodelphic-prodelphic, located on right side of intestine. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter and well developed. Uterus tubular, about five times the corresponding body diameter long, consisting of a long proximal tubular part and a short distal part with thinner walls. Post-uterine sac 0.7-0.8 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Vulva protruding, located posterior to middle part of body. Rectum 1.1-1.4 times the anal body diameter. Tail conical. Phasmids at 65-80% of tail length.

**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad, having different colour and distinguished tip. Two pairs of precloacal genital papillae. Five

pairs of caudal genital papillae are present along the tail comprising one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded; calamus as wide as manubrium, straight; lamina ventrally curved, with dorsally hump proximally, pointed terminus, and having two longitudinal lines. Gubernaculum well developed, 14 µm long or less than half of the spicule length.

**Locality and habitat.** Material has been found in Andoohjerd (province of Kerman), in association with *Vitis vinifera* L.

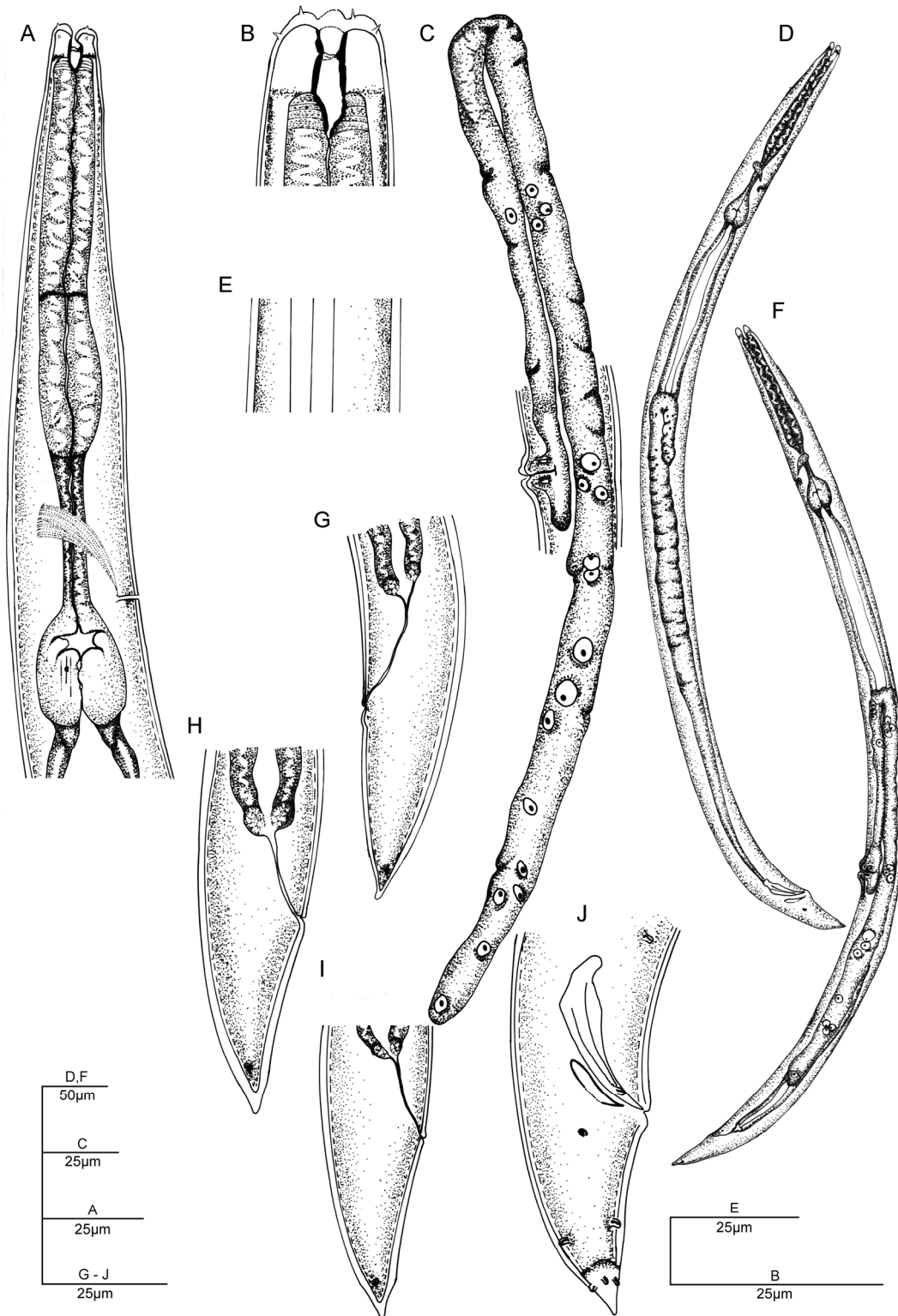
**Remarks.** This material is similar to *P. papillosus* described by Loof (1971) from Spitzbergen, in having prominent lips with conspicuous papillae, three lateral field, protruded vulva, symmetrical uterus, large posterior anal lips; however, it differs from that by having longer female body (754-800 vs 510-690 µm in the original description), although this difference may be due to geographical variations. According to Loof (1971), this material also is similar to *P. subelongatus*; however they differ in conspicuous labial papillae and location of phasmid (near tail terminus in *P. papillosus* vs located in posterior third of tail in *P. subelongatus*).

***Panagrolaimus cf. subelongatus* (Cobb, 1914)  
Thorne, 1937  
(Figs 7A-E & 6)**

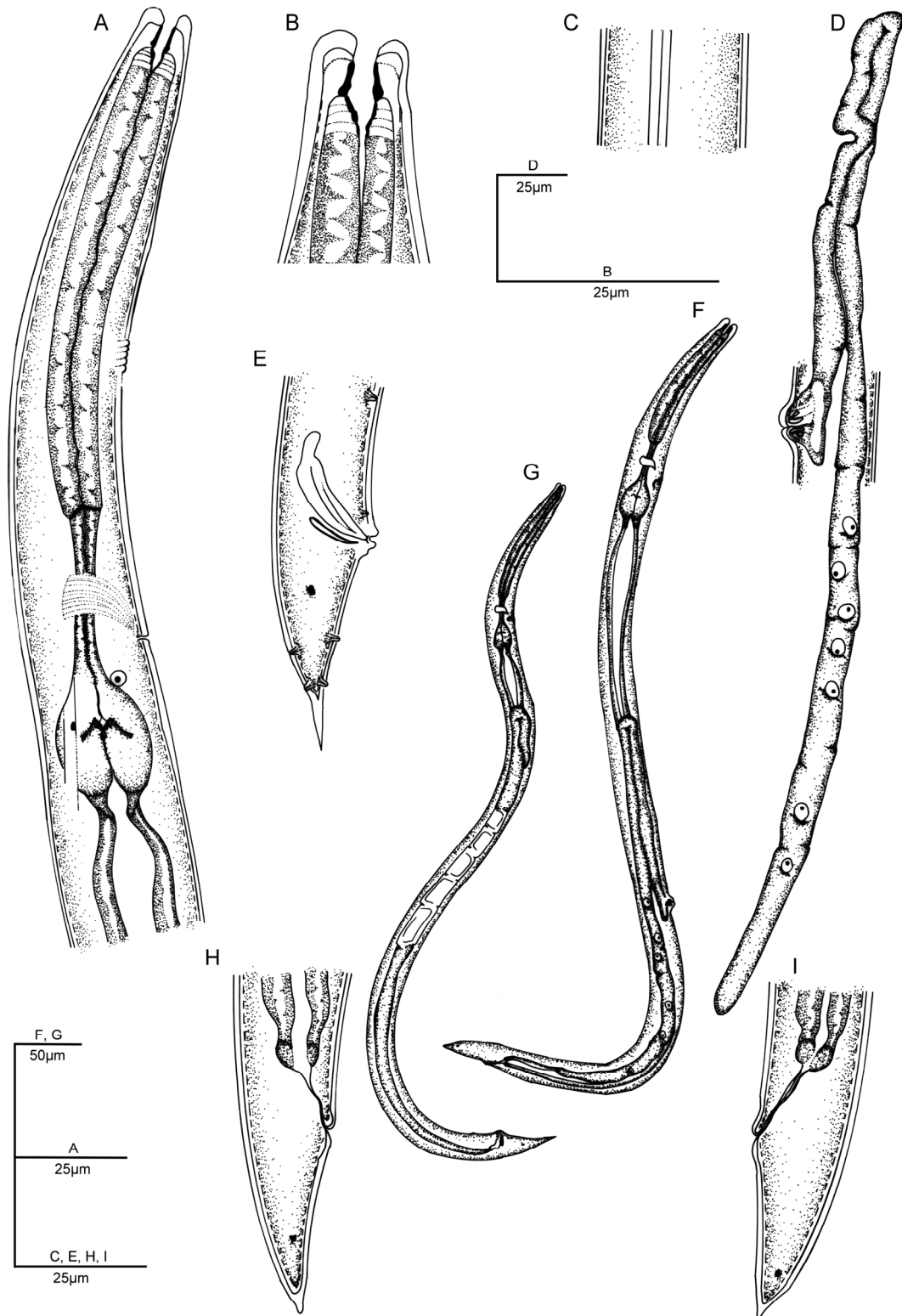
**Measurements.** See Table 2.

Population collected in Shahdad, province of Kerman (3♀♀, 2♂♂).

**Female.** Body length 709-764 µm, cylindrical, curved ventrad after fixation. Cuticle smooth; having 2.8 µm thickness, annuli 1.9 µm wide. Lateral field with three incisures occupying 20-24% of midbody diameter, fading out near tail terminus. Lip region continuous with neck, having three lips and a circular amphid opening. Labial papillae well developed. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, having cuticularized rhabdia. Stegostom having four distinct parts. Pharyngeal corpus cylindrical, 2.8-3 times isthmus length, with procorpus longer than metacarpus. Isthmus slenderer than corpus distinctly separated from metacarpus. Basal bulb ovoid to spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 65-80% of neck length. Excretory pore opening at bulb level, at 80-90% of neck length. Deirid at 90% of neck length, at bulb level. Reproductive system



**Fig. 5.** *Panagrolaimus* cf. *papillosus* Loof, 1971. A: Neck. B: Anterior end. C: Female reproductive system. D: Entire male. E: Lateral field. F: Entire female. G-I: Female posterior end. J: Male posterior end.



**Fig. 6.** *Panagrolaimus* cf. *subelongatus* (Cobb, 1914), Thorne, 1937. A: Neck. B: Anterior end. C: Lateral field. D: Female reproductive system. E: Male posterior end. F: Entire female. G: Entire male. H, I: Female posterior end.



monodelphic-prodelphic, located in right side of intestine. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter. Uterus tubular, about five times the corresponding body diameter long, consisting of a long proximal tubular part and a short distal part with thinner walls. Post-uterine sac 0.4-0.6 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Vulva protruding, located posterior to middle part of body. Rectum 0.5-0.7 times the anal body diameter. Tail conical, with pointed end. Phasmid at 21-23% of tail length.

**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad, with a mucro less than half of tail length. Two pairs of precloacal genital papillae and five pairs of caudal genital papillae are present along the tail comprising one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded, straight; calamus slender than manubrium; lamina ventrally curved, with somewhat rounded terminus and two longitudinal lines. Gubernaculum well developed, 11-13  $\mu\text{m}$  long or about 30-40% of the spicule length.

**Locality and habitat.** Material has been found in Shahdad (province of Kerman), in association with *Citrus sinensis* (L.).

**Remarks.** Our specimens are similar to *P. subelongatus*, especially the material described by Steiner (1924) and that described as *P. obesus* by Thorne (1937), its junior synonym, in rounded lips, gymnostom shape, female tail, phasmid location (at posterior third of tail), two precloacal papillae, arrangement of poscloacal papillae (four near tail terminus), spicules and gubernaculum shape. However, it differs from that by having longer female body length (709-764 vs 650  $\mu\text{m}$ ), and male tail with longer mucro (vs very short). Cobb (1914) and Andr ssy (2005) described populations of this species with shorter female body (vs 600  $\mu\text{m}$  in Cobb (1914) and 600-680  $\mu\text{m}$  in Andr ssy (2005)), and pharynx (155-158 vs 138  $\mu\text{m}$  in Cobb (1914) and 130-140  $\mu\text{m}$  in Andr ssy (2005)).

This material also resembles to *P. labiatus* in having rounded lips and phasmid location (posterior third part of tail in both species); however, it differs in longer ovary (vs shorter ovary), and female tail (tapering gradually to the end vs marked narrowing near its terminus, see Abolafia & Pe na-Santiago, 2006).

### *Panagrolaimus superbis* Fuchs, 1930 (Figs 7F-J & 8)

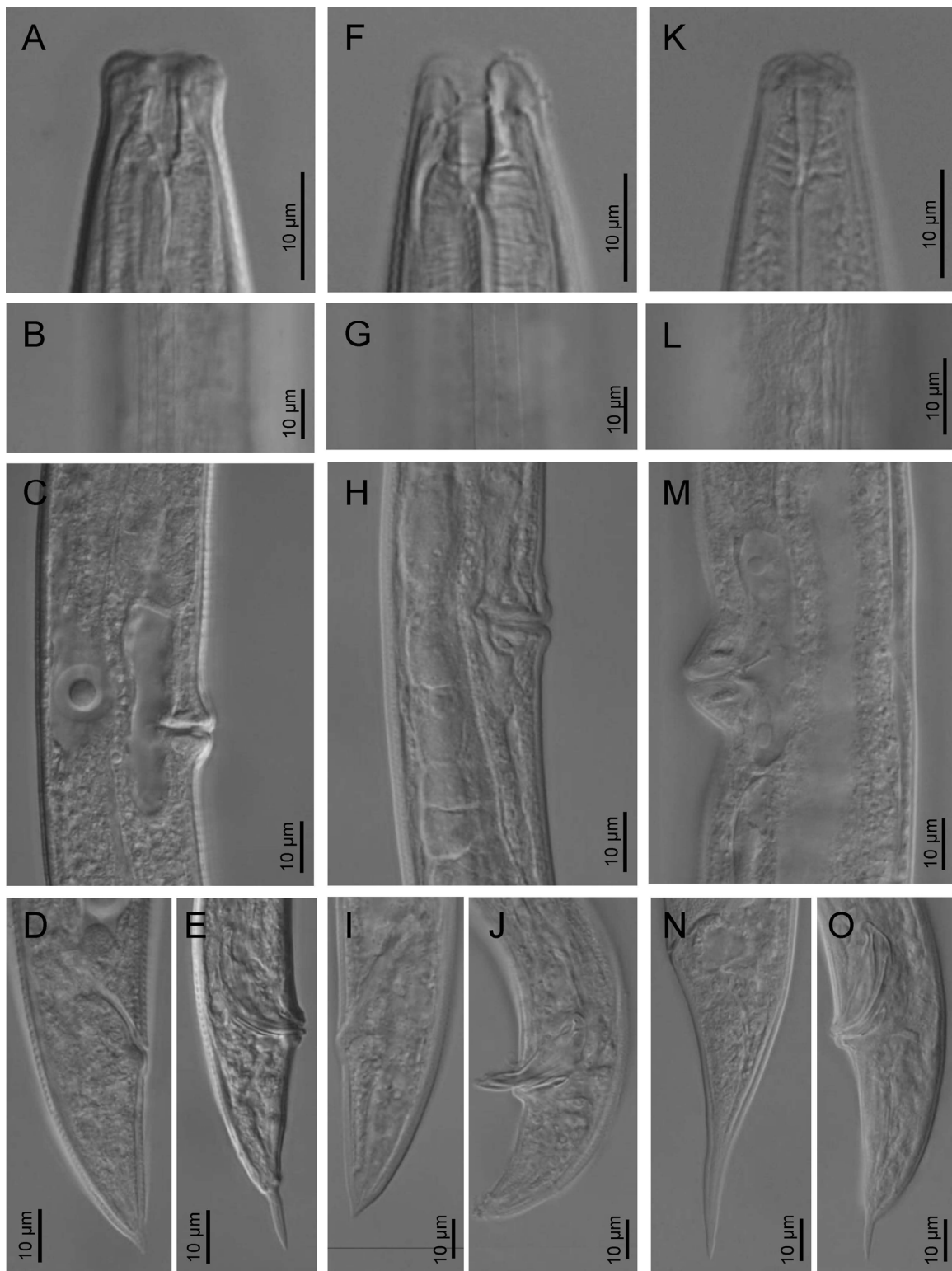
**Measurements.** See Table 2.

Population collected in Kerman and Gharyatolarab, province of Kerman (3♀♀, 2♂♂ and 3♀♀, 2♂♂, respectively).

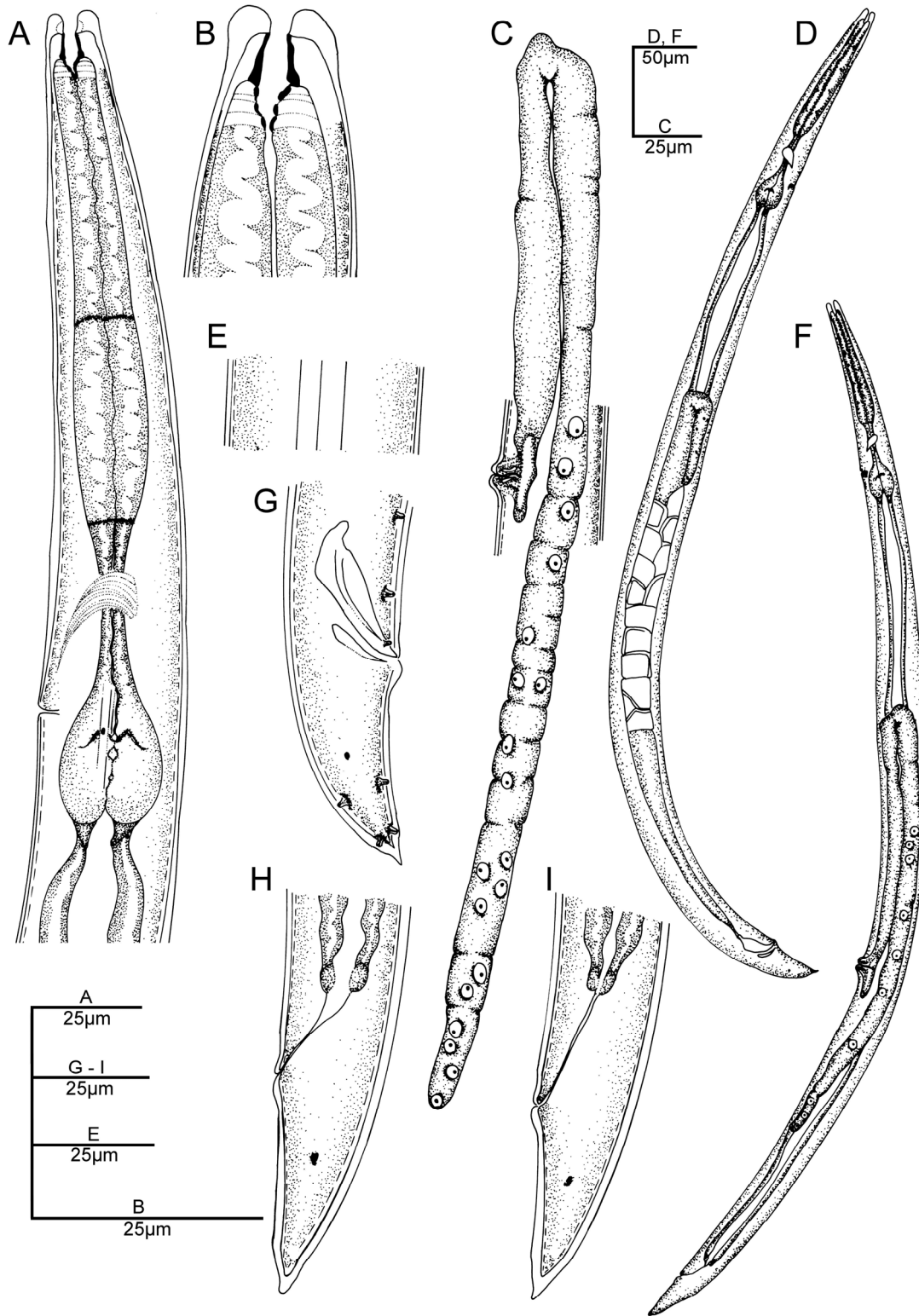
**Female.** Body length 0.86-0.89 mm, cylindrical, curved ventrad after fixation. Cuticle smooth; having 1-3  $\mu\text{m}$  thickness, annuli 1  $\mu\text{m}$  wide. Lateral field with three incisures occupying 19-22% of midbody diameter. Lip region continuous with neck, having six lips amalgamated in pairs, and a circular amphid opening. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, with cuticularised rhabdia. Stegostom having four distinct parts. Pharyngeal corpus cylindrical, 2.3-3.0 times isthmus length, with procorpus longer than metacarpus. Isthmus slenderer than corpus and distinctly separated from metacarpus. Basal bulb ovoid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 71-75% of neck length. Excretory pore opening at bulb level, at 86-87% of neck length. Deirid at 87-90% of neck length, at bulb level. Reproductive system monodelphic-prodelphic, located on right side of intestine. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter. Uterus tubular, about four times the corresponding body diameter long. Post-uterine sac 0.3-0.4 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Vulva protruding, located posterior to middle part of body. Rectum 1.2-1.3 times the anal body diameter. Tail conical, with pointed or rounded terminus. Phasmid at 50% of tail length.

**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad. Three pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail comprising one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded, straight; calamus as wide as manubrium; lamina ventrally curved, with dorsal hump proximally, pointed terminus, and two longitudinal lines. Gubernaculum well developed, 15  $\mu\text{m}$  long or about less than half of the spicule length.

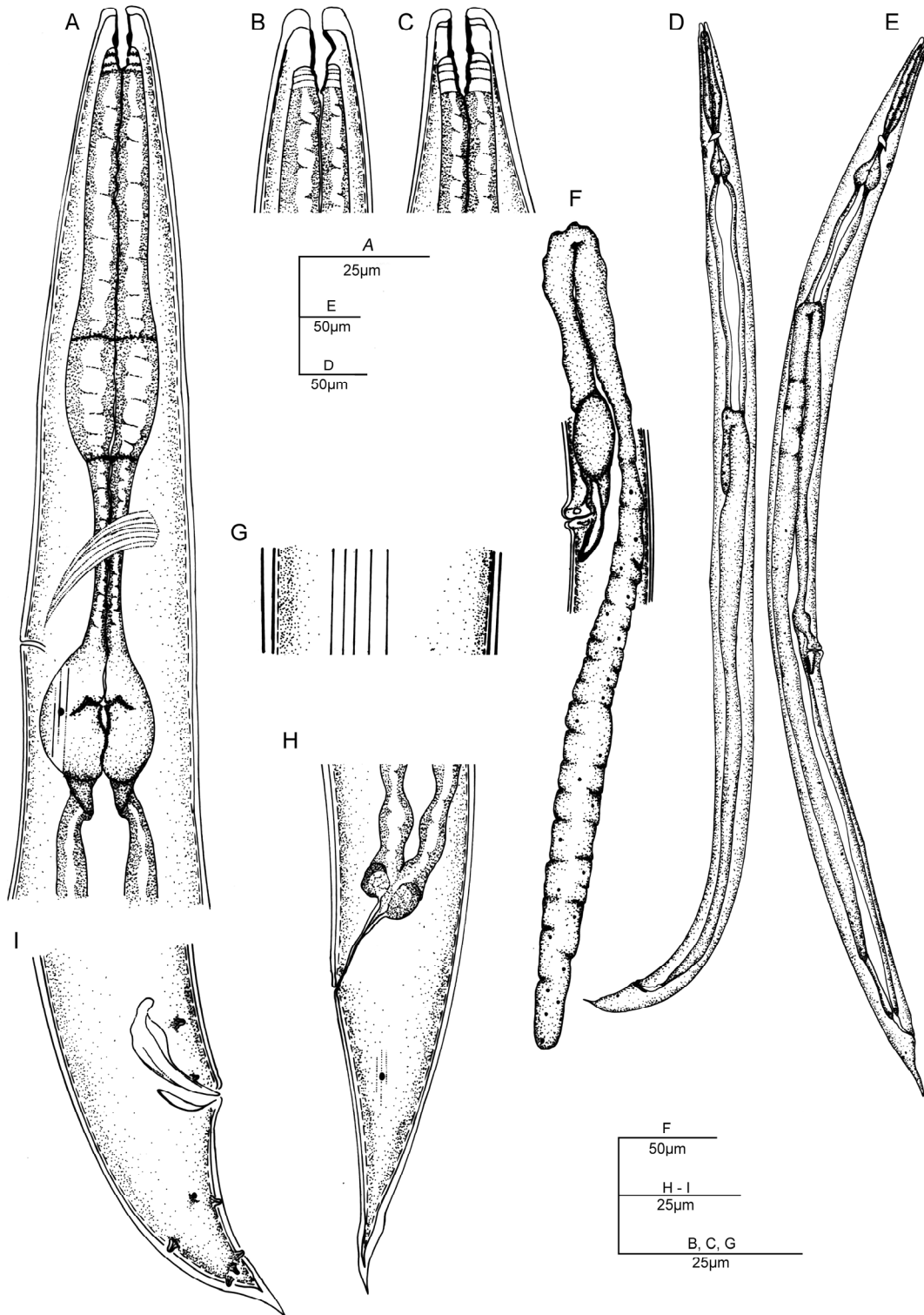
**Locality and habitat.** Material has been found in Kerman and Gharyatolarab (province of Kerman), in association with *Haloxylon persicum* L.



**Fig. 7.** *Panagrolaimus* cf. *subelongatus* (Cobb, 1914), Thorne, 1937 (LM). A: Anterior end. B: Lateral field. C: Vagina region. D: Female posterior end. E: Male posterior end. *Panagrolaimus superbus* Fuchs, 1930 (LM). F: Anterior end. G: Lateral field. H: Vagina region. I: Female posterior end. J: Male posterior end. *Panagrolaimus trilabiatus* Zell, 1987 (LM). K: Anterior end. L: Lateral field. M: Vagina region. N: Female posterior end. O: Male posterior end.



**Fig. 8.** *Panagrolaimus superbus* Fuchs, 1930. A: Neck. B: Anterior end. C: Female reproductive system. D: Entire male. E: Lateral field. F: Entire Female. G: Male posterior end. H, I: Female posterior end.



**Fig. 9.** *Panagrolaimus trilabiatus* Zell, 1987. A: Neck. B, C: Anterior end. D: Entire male. E: Entire female. F: Female reproductive system. G: Lateral field. H: Female posterior end. I: Male posterior end.

**Remarks.** Our population is similar to material of *P. superbis* previously studied (Fuchs, 1930; Boström, 1989; Abolafia & Peña-Santiago, 2006) in having six free and separated lips, three lateral field, corpus/ isthmus ratio (2.3-3.0 vs 2.0-3.1 in Abolafia & Peña-Santiago, 2006), spicules shape and length (35-37  $\mu\text{m}$ ), gubernaculum shape and length (13-15  $\mu\text{m}$ ), and phasmid position (mid tail) and similar tail in both sexes. However, it differs with the material examined by Fuchs (1930) in having shorter body (0.73-0.89 mm in females and 0.62-0.88 mm in males vs 1.20 mm in female and 1.06 mm in male). In comparison with the material examined by Boström (1989) it differs by having shorter body (vs 0.86-1.32 mm in females and 0.74-1.05 mm in males). In addition, Abolafia & Peña-Santiago (2006) reported this species with longer body (vs 0.75-0.95 mm in females and 0.74-0.86 mm in males). Abolafia and Peña-Santiago (2006) studied this species with more posterior phasmids position (50% vs 62-69% of tail length). Compared with specimens studied by Thorne (1937) and Rühm (1956) as *P. subelongatus*, it differs slightly in body length range (vs 0.7-1.0 mm in females and 0.7-0.90 mm in males reported by Thorne, 1937; and 0.98-1.22 in females and 0.96-1.18 mm in males reported by Rühm, 1956).

This species is reported for the first time from Iran.

***Panagrolaimus trilabiatus* Zell, 1987  
(Figs 7K-O & 9)**

**Measurements.** See Table 2.

Population collected in Tehran, province of Tehran (5♀♀, 3♂♂).

**Female.** Body length 0.85-1.12 mm, cylindrical, slightly curved ventrad after fixation. Cuticle smooth; having 4-5  $\mu\text{m}$  thickness, annuli 1  $\mu\text{m}$  wide. Lateral field with five incisures occupying 10% of midbody diameter, fading out near the phasmid. Lip region continuous with neck, having three lips and a circular amphid opening. Labial papillae distinct. Stoma panagrolaimoid, with distinct cheilo-, gymno- and stegostom. Cheilostom without refringent rhabdia. Gymnostom longer than cheilostom, with well cuticularised rhabdia. Stegostom having four distinct parts. Pharyngeal corpus cylindrical, 1.8-3.2 times isthmus length, with procorpus longer than metacarpus. Isthmus slenderer than corpus and distinctly separated from metacarpus. Basal bulb ovoid to spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 65-75% of neck length. Excretory pore opening at isthmus level, near the bulb, at 75-85% of neck

length. Deirid at 90-95% of neck length, at bulb level. Reproductive system monodelphic-prodelphic, located on right side of intestine. Ovary without flexure posterior to vulva level. Oviduct short, less than a half of the corresponding body diameter. Uterus tubular, about five times the corresponding body diameter long. Post-uterine sac 0.2-0.4 times the corresponding body diameter long. Vagina extending inward less than a half of the body width. Vulva protruding, located posterior to middle part of body. Rectum 0.8-0.9 times the anal body diameter. Tail conical, with lateral field ending at phasmid level. Phasmid at 40-50% of tail length.

**Male.** Body curved ventrally after fixation. Reproductive system monorchic, testis reflexed dorsad anteriorly. Tail conical, distally curved ventrad. Two pairs of precloacal genital papillae. Five pairs of caudal genital papillae are present along the tail comprising: one ventral pair, one lateral pair, one dorsal pair, and two pairs near tail terminus. Spicules curved ventrally: manubrium rounded; calamus as wide as manubrium, straight; lamina ventrally curved, very wide proximally and pointed terminus, having two longitudinal lines. Gubernaculum well developed, 15  $\mu\text{m}$  long or less than half of the spicule length.

**Locality and habitat.** Material has been found in Kerman (province of Kerman), in association with seed coat of wheat (*Triticum aestivum* L.).

**Remarks.** This species description agrees with the material of *P. trilabiatus* described by Zell (1987) in rounded lips, lateral field with five incisures, corpus/isthmus ratio (1.8-3.2 vs 3.0), vulval position (56-62 vs 54-64) (see Zell, 1987; Abolafia & Peña-Santiago, 2006). However, it differs from that by having slightly longer body (0.85-1.12 mm in females and 0.76-0.79 mm in males vs 0.55-0.90 mm in females and 0.49-0.78 mm in males), tail slightly less slender ( $c^2=2.1-2.7$  in females and 1.7-2.1 in males vs  $c^2=2.4-3.1$  in females and 2.0-2.7 in males in type specimens), and spicules longer (36 vs 23-24  $\mu\text{m}$ ). This species with its robust body and five lateral field incisures could be recognized from other species of the *Panagrolaimus*.

This species is reported for the first time from Iran.

## DISCUSSION

**On the phylogeny of the family Panagrolaimidae.** In this study, we tried to analyse the 18S rDNA to understand the evolutionary relationships of *Panagrolaimus* species. Relating to the family Panagrolaimidae, the consensus tree inferred from 18S rDNA (Figs 10 & 11) appears to

**Table 2.** Measurements of *Panagrolaimus* cf. *papillosus* Loof, 1971, *P. cf. subelongatus* (Cobb, 1914) Thorne, 1937, *P. superbus* Fuchs, 1930 and *P. trilabiatus* Zell, 1987 from Iran. All measurements in  $\mu\text{m}$ , and in the form; mean  $\pm$  standard error (range).

Species Locality Province Habitat	<i>P. cf. papillosus</i> Anduhjerd Kerman		<i>P. cf. subelongatus</i> Shahdad Kerman		<i>P. superbus</i> Kerman Kerman   Gharyatolara Kerman				<i>P. trilabiatus</i> Kerman Kerman	
	<i>Vitis vinifera</i>		Soil		<i>Haloxylon persicum</i>				Seed coat of wheat ( <i>Triticum aestivum</i> )	
n	3♀♀	1♂	3♀♀	2♂♂	3♀♀	2♂♂	3♀♀	2♂♂	5♀♀	3♂♂
Body length	772.7 $\pm$ 24.1 (754-800)	791	733.3 $\pm$ 27.7 (709-764)	627, 682	875 $\pm$ 14.1 (864-891)	882, 828	800 $\pm$ 80.8 (736-891)	628, 709	1001.7 $\pm$ 96.2 (854-1118)	778.8 $\pm$ 13.8 (764-791)
a	23.4 $\pm$ 0.7 (22.9-24.2)	22.7	23.5 $\pm$ 3.8 (19.8-27.5)	26.7, 31.4	19.8 $\pm$ 1.2 (18.5-21.0)	24.0, 21.9	20.3 $\pm$ 3.01 (17.7-23.6)	22.3, 22.1	19.9 $\pm$ 1.1 (18.8-21.6)	20.3 $\pm$ 1.1 (19.3-21.5)
b	4.6 $\pm$ 0.3 (4.3-4.9)	4.4	4.3 $\pm$ 0.2 (4.1-4.6)	4.3, 4.6	5.0 $\pm$ 0.1 (4.8-5.2)	4.9, 5.1	5.4 $\pm$ 0.5 (5.0-6.0)	5.1, 5.1	6.6 $\pm$ 0.3 (6.1-7.2)	5.4 $\pm$ 0.2 (5.2-5.7)
c	19.8 $\pm$ 0.6 (19.0-20.2)	18.6	20.5 $\pm$ 1.09 (19.3-21.4)	14.8, 15.4	20.4 $\pm$ 1.4 (18.8-21.8)	19.5, 17.5	23.3 $\pm$ 2.9 (20.0-25.2)	19.6, 19.3	15.3 $\pm$ 1.4 (13.5-16.8)	15.8 $\pm$ 2.4 (13.1-17.6)
c'	1.8 $\pm$ 0.1 (1.7-2.0)	1.6	1.8 $\pm$ 0.2 (1.6-2.1)	2.1, 1.9	1.8 $\pm$ 0.1 (1.7-2.0)	1.7, 1.7	1.7 $\pm$ 0.2 (1.5-2.0)	1.5, 1.4	2.3 $\pm$ 0.2 (2.1-2.7)	1.9 $\pm$ 0.2 (1.7-2.1)
V	63.8 $\pm$ 2.4 (62-67)	-	62.2 $\pm$ 3.6 (58-66)	-	64.0 $\pm$ 6.9 (58-72)	-	58.6 $\pm$ 4.01 (54-62)	-	59.0 $\pm$ 2.4 (56-62)	-
Lip region width	11.0 $\pm$ 0.5 (10.4-11.3)	10	11.3 $\pm$ 0.9 (10-12)	7, 7	11.0 $\pm$ 0.5 (10-11)	11	10.4 $\pm$ 0.9 (9-11)	9, 10	8.9 $\pm$ 0.8 (8-10)	7.9 $\pm$ 0.5 (7-8)
Stoma	12.3 $\pm$ 0.9 (11-13)	13	11.9 $\pm$ 1.08 (11-13)	10, 12	14.8 $\pm$ 1.0 (14-16)	14, 13	12.9 $\pm$ 0.5 (12-13)	11, 10	10.9 $\pm$ 0.8 (10-12)	9.4 $\pm$ 0.0 (9.4)
Pharyngeal corpus	97.2 $\pm$ 1.4 (96-99)	40	97.2 $\pm$ 1.4 (96-99)	73, 73	98.1 $\pm$ 2.4 (95-100)	103, 87	78.6 $\pm$ 2.6 (75-80)	71, 76	85.1 $\pm$ 10.2 (68-95)	81.4 $\pm$ 1.1 (80-82)
Isthmus	35.3 $\pm$ 2.3 (33-38)	13	33.6 $\pm$ 1.1 (33-35)	39, 39	35.8 $\pm$ 5.7 (32-42)	39, 35	30.3 $\pm$ 2.8 (27-33)	24, 25	34.0 $\pm$ 4.6 (26-39)	31.1 $\pm$ 3.4 (28-35)
Bulb	29.4 $\pm$ 1.1 (28-31)	11	26.1 $\pm$ 2.3 (23-28)	18, 24	29.2 $\pm$ 4.1 (24-32)	32, 31	27.0 $\pm$ 1.4 (25-28)	21, 26	26.2 $\pm$ 1.6 (24-28)	22.6 $\pm$ 0.9 (22-24)
Pharynx length	161.9 $\pm$ 1.6 (160-163)	160	156.8 $\pm$ 1.3 (155-158)	129, 135	163.2 $\pm$ 3.7 (159-167)	173, 153	136.8 $\pm$ 1.7 (136-139)	121, 128	145.8 $\pm$ 12.5 (129-162)	134.2 $\pm$ 6.4 (127-140)
Neck	166.7 $\pm$ 7.5 (162-175)	181	170.0 $\pm$ 3.3 (167-174)	147, 148	174.6 $\pm$ 4.8 (172-180)	181, 162	147.8 $\pm$ 1.4 (146-149)	122, 139	152.5 $\pm$ 14.8 (132-169)	143.5 $\pm$ 4.7 (139-148)
Nerve ring- ant. end	124.2 $\pm$ 0.5 (124-124)	118	130.7 $\pm$ 2.09 (128-132)	112, 116	128.0 $\pm$ 3.8 (124-130)	136, 124	107.5 $\pm$ 4.0 (105-110)	89, 93	109.6 $\pm$ 8.8 (99-119)	103.5 $\pm$ 8.5 (97-113)
Excretory pore-ant. end	138.1 $\pm$ 5.1 (133-143)	129	141.1 $\pm$ 3.4 (139-143)	123, 126	152.2 $\pm$ 3.9 (149-157)	157, 141	139.6 $\pm$ 0.0 (139.6) n=1	110	121.9 $\pm$ 6.3 (112-128)	119.3 $\pm$ 4.6 (116-123)
Deirid-ant. End	154.7 $\pm$ 9.1 (144-161)	158	161.3 $\pm$ 0.0 (161)	119	155.7 $\pm$ 2.8 (153-158)	145	132.1 $\pm$ 0.0 (132.1) n=1	110	139.6 $\pm$ 20 (125-154)	142.5 $\pm$ 0.0 (142.5)
Cuticle thickness	2	2	2.8 $\pm$ 0.0 (2.8)	2.8	1.5	1.6	1.4	1	3	3
Body width: neck base	33.3 $\pm$ 1.4 (32-35)	-	27.4 $\pm$ 2.8 (24- 30)	23, 20	35.8 $\pm$ 1.8 (34-38)	32, 32	30.8 $\pm$ 0.5 (30-31)	26	38.7 $\pm$ 1.4 (37-41)	31.8 $\pm$ 3.1 (28-34)
Body width: midbody	33.0 $\pm$ 1.8 (31-35)	35	31.8 $\pm$ 4.8 (27- 36)	23, 22	44.3 $\pm$ 3.4 (41-48)	37, 38	39.6 $\pm$ 1.8 (38-41)	28, 32	50.2 $\pm$ 3.4 (45-55)	38.4 $\pm$ 1.4 (37-40)
Body width: anus	21.4 $\pm$ 1.4 (20-23)	26	19.8 $\pm$ 1.8 (18- 22)	20, 24	23.6 $\pm$ 0.0 (23.6)	27, 28	20.1 $\pm$ 0.5 (20-21)	22, 25	28.7 $\pm$ 3.1 (23-32)	26.1 $\pm$ 1.1 (25-27)
Lateral field	7.5 $\pm$ 2.6 (6-9)	7	7.1 $\pm$ 0.6 (6.6-7.5)	3.8	9.4 $\pm$ 0.0 (9.4)	-	4.7 $\pm$ 0.0 (4.7)	6.6	3.8 $\pm$ 0.0 (3.8)	4.2 $\pm$ 0.6 (3.8-4.7)
Vagina	13.8 $\pm$ 0.5 (13-14)	-	11.3 $\pm$ 0.9 (10-12)	-	15.7 $\pm$ 3.8 (12-20)	-	10.7 $\pm$ 0.5 (10-11)	-	13.6 $\pm$ 2.2 (10-16)	-
Ovary	354.0 $\pm$ 9.1 (345-364)	-	334.9 $\pm$ 52.92 (275-376)	-	369.4 $\pm$ 56.5 (329-409)	-	304.3 $\pm$ 67.1 (228-355)	-	457.1 $\pm$ 26.7 (428-491)	-

**Table 2.** Measurements of *Panagrolaimus* cf. *papillosus* Loof, 1971, *P.* cf. *subelongatus* (Cobb, 1914) Thorne, 1937, *P. superbis* Fuchs, 1930 and *P. trilabiatus* Zell, 1987 from Iran. All measurements in  $\mu\text{m}$ , and in the form; mean  $\pm$  standard error (range) (**continued**).

Species Locality Province Habitat n	<i>P. cf. papillosus</i> Anduhjerd Kerman <i>Vitis vinifera</i>		<i>P. cf. subelongatus</i> Shahdad Kerman Soil		<i>P. superbis</i> Kerman Kerman <i>Haloxylon persicum</i>				<i>P. trilabiatus</i> Kerman Kerman Seed coat of wheat ( <i>Triticum aestivum</i> )	
	3♀♀	1♂	3♀♀	2♂♂	3♀♀	2♂♂	3♀♀	2♂♂	5♀♀	3♂♂
Postuterine sac	24.0 $\pm$ 3.5 (22-28)	–	15.1 $\pm$ 0.9 (14-16)	–	18.9 $\pm$ 0.9 (18-20)	–	11.9 $\pm$ 0.5 (11-12)	–	18.6 $\pm$ 2.4 (15-21)	–
Rectum	26.4 $\pm$ 1.8 (24-28)	–	18.6 $\pm$ 0.5 (18-19)	–	29.2 $\pm$ 1.8 (27-31)	–	25.5 $\pm$ 0.9 (24-26)	–	23.8 $\pm$ 1.5 (22-25)	–
Tail	39.0 $\pm$ 1.1 (38-40)	42	35.8 $\pm$ 1.6 (34-37)	–	43.1 $\pm$ 3.3 (40-46)	45, 47	34.6 $\pm$ 4.8 (36-39)	32, 37	65.7 $\pm$ 3.2 (61-69)	50.0 $\pm$ 7.4 (44-58)
Vulva- anterior end	493.2 $\pm$ 20.1 (471-509)	–	455.3 $\pm$ 10.23 (445-466)	–	559.4 $\pm$ 56.3 (518-623)	–	466.7 $\pm$ 13.8 (454-482)	–	590.7 $\pm$ 64.5 (508-673)	–
Spicules	–	32	–	29, 30	–	35, 37	–	30, 33	–	28.9 $\pm$ 3.3 (25-32)
Gubernaculum	–	14	–	11, 13	–	15, 13	–	11, 14	–	11.3 $\pm$ 0.9 (10-12)

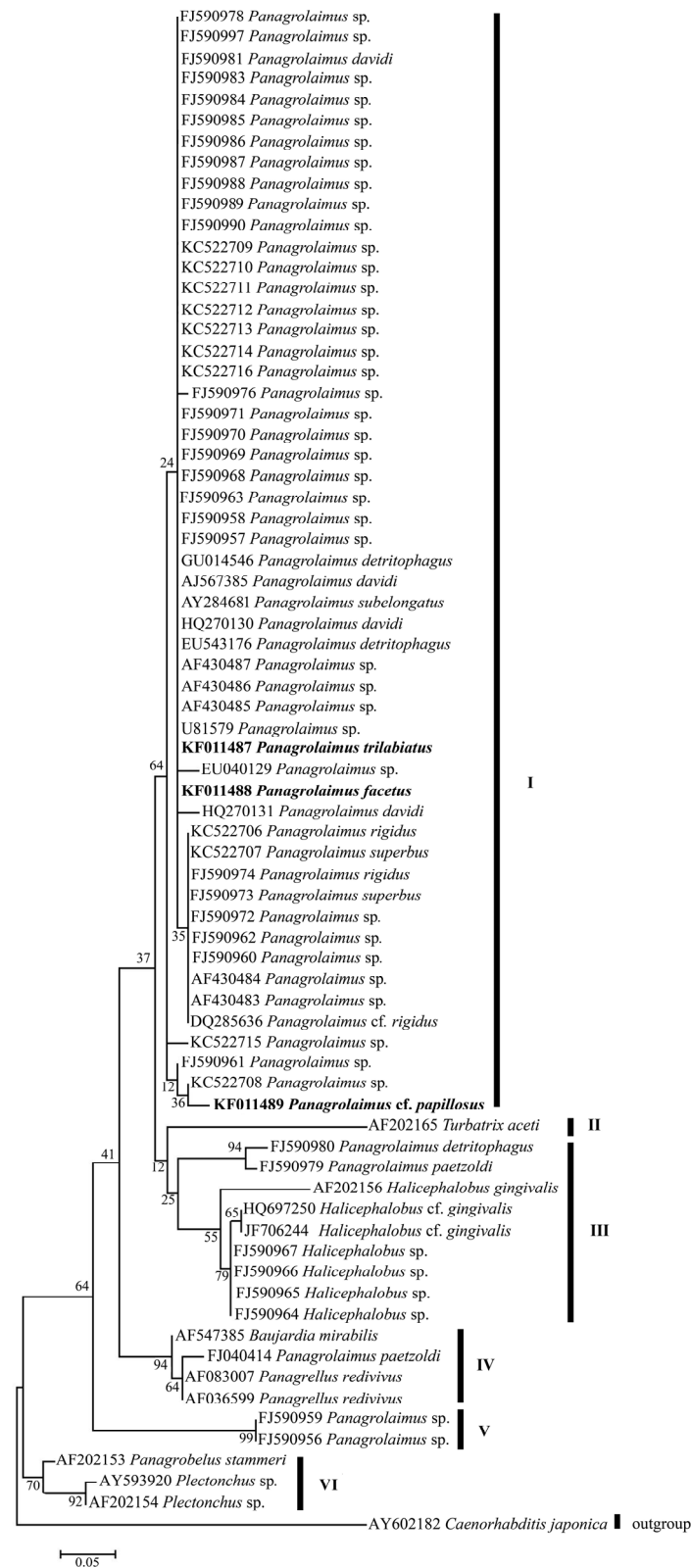
be divided into five and six main clades based on the Bayesian tree and Maximum Likelihood tree respectively. According to the Bayesian tree clades are: I) *Panagrolaimus* spp. which form a robust clade (1.00 posterior probability); II) *Halicephalobus* spp., *P. detritophagus*, *P. paetzoldi* and two unidentified *Panagrolaimus* spp.; III) *Panagrellus redivivus*, *Baujardia mirabilis* and *P. paetzoldi* which is very consistent (1.00 posterior probability); IV) *Turbatrix acetii*; and V) *Plectonchus* spp. and *Panagrobelus stammeri*, set near the base of the tree.

The consensus tree obtained by ML is similar to that elaborated by BI (Bayesian Inference), however two populations of unidentified *Panagrolaimus* (FJ590956; FJ590959) form a separate clade in ML tree.

The genetic distance among the identified species of *Panagrolaimus* ranges from 0.00–0.542 for SSU rDNA gene. Genetic distance using Maximum Composite Likelihood method among *Panagrolaimus* species showed that *P. trilabiatus* (KF011487) and *P. subelongatus* (AY284681) have no genetic distance. The maximum genetic distance was observed between two populations of *P. paetzoldi* (FJ040414; FJ590979).

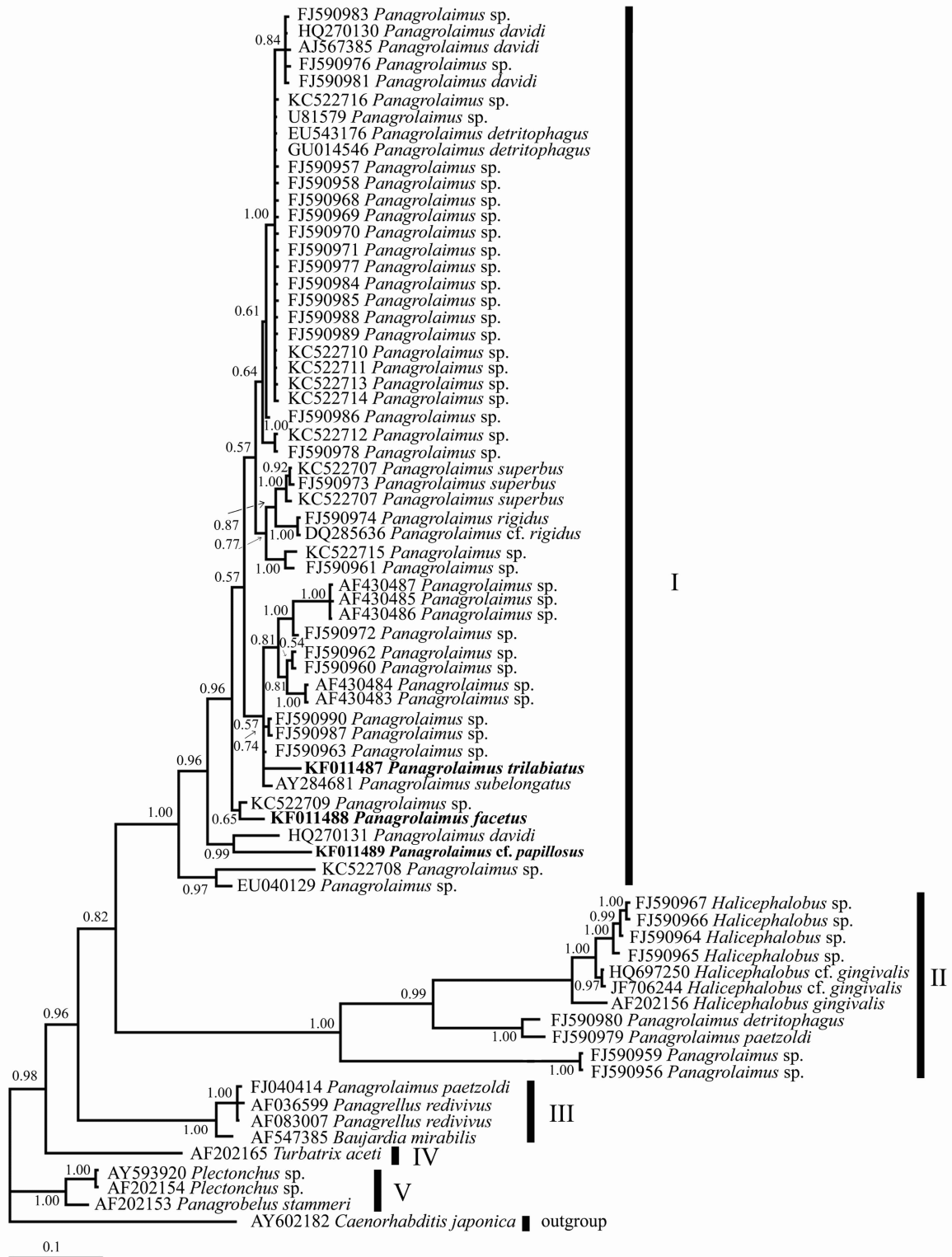
**On *Panagrolaimus* species phylogeny.** The phylogenetic tree inferred from SSU sequences of *Panagrolaimus* species indicated that this genus is a paraphyletic group, agreeing with Shannon *et al.* (2005; ITS and D3 genes), van Megen *et al.* (2009; SSU rDNA gene) and Lewis *et al.* (2009; 18S rDNA, 28S rDNA, ND5 genes). Within the family Panagrolaimidae, a well-supported clade was

formed by members of the genus *Panagrolaimus* with weak bootstrap support (64%) in ML tree, and 1.00 posterior probability in Bayesian Inference tree, while the rest of *Panagrolaimus* species including *P. paetzoldi* (FJ590979) and *P. detritophagus* (FJ590980) groups with *Halicephalobus* species. In addition, *P. paetzoldi* (FJ040414) groups with *Panagrellus* species and *Baujardia mirabilis*. The Bayesian tree analysis of 18S rDNA sequence places *P. facetus* (KF011488) close to an unidentified *Panagrolaimus* (KC52709) and to *P. davidi* (HQ270131) and an unidentified *Panagrolaimus* (EU040129) in ML tree. The unidentified *Panagrolaimus* (U81579) and *P. subelongatus* (AY284681) places close to *P. trilabiatus* (KF011487) in ML and Bayesian tree, respectively. *P. trilabiatus* and *P. subelongatus*; both species have three lips. Finally, *P. cf. papillosus* places close to *P. davidi* (HQ270131) in Bayesian tree and two unidentified *Panagrolaimus* (KC522708; FJ590961) in ML tree. The ML and Bayesian tree topology for *Panagrolaimus* species obtained from 18S ribosomal DNA is consistent with the topology given by Shannon *et al.* (2005), van Megen *et al.* (2009) and Lewis *et al.* (2009). Although the results indicate a single well-supported monophyletic clade (referred to herein as Clade I, see Figs 10 & 11) that exclusively contained most of the *Panagrolaimus* species, the rest of *Panagrolaimus* species (*P. paetzoldi*, FJ040414, FJ590979; *P. detritophagus*, FJ590980; *Panagrolaimus*



**Fig. 10.** The Maximum Likelihood tree of new sequences of *Panagrolaimus* species from Iran and closely related species belonging to the family Panagrolaimidae based on 18S rDNA region from GenBank.





**Fig. 11.** The Bayesian Inference tree of new sequences of *Panagrolaimus* species from Iran and closely related species belonging to the family Panagrolaimidae based on 18S rDNA region from GenBank.

sp., FJ590956, FJ590959) were included within a separate clade, referred to herein as Clade III, IV. Although our analyses agrees with the previously published 18S rDNA tree of Panagrolaimomorpha (van Megen *et al.*, 2009; Lewis *et al.*, 2009), in the fact that the 18S rDNA gene does provide resolution within the panagrolaimids for the phylogenetic analysis within this group of nematodes, more rDNA genes are needed to understand more fully the relationships of the described species of *Panagrolaimus*.

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**Mehdizadeh, S., Shokoohi, E. and Abolafia, J.** Морфологическая и молекулярная характеристика видов *Panagrolaimus* Fuchs, 1930 (Nematoda, Rhabditida, Panagrolaimidae) из Ирана.

**Резюме.** Представители семи видов семейства Panagrolaimidae описаны из природных экосистем провинции Керман, Иран: *Panagrolaimus concolor*; *P. facetus*; *P. cf. labiatus*; *P. cf. papillosus*; *P. cf. subelongatus*; *P. superbis* и *P. trilabiatus*. Приведены описания, измерения, рисунки и светомикроскопические фотографии для всех видов. Проведен молекулярный анализ трех из семи выявленных видов. Лишь филогенетический анализ последовательностей 18S rDNA методом максимального правдоподобия (ML) показал близость *P. facetus* к *P. davidi* (HQ270131) и не определенному до вида *Panagrolaimus* (EU040129). В деревьях, построенных по результатам Байесова анализа (BI), этот вид объединяется с *Panagrolaimus* sp. (KC522709). *Panagrolaimus trilabiatus* близок у *P. subelongatus* (AY284681) и к *Panagrolaimus* sp. (FJ590963). В этом же древе *Panagrolaimus cf. papillosus* объединяется с *P. davidi* (HQ270131) и показывает родство к *P. facetus* (KF011488). В ML-дереве этот вид объединяется с двумя не определенными *Panagrolaimus* (KC522708; FJ590961). Нуклеотидные последовательности 18S rDNA для *P. facetus*, *P. cf. papillosus* и *P. trilabiatus* приводятся впервые. Как метод максимального правдоподобия (ML), так и Байесов анализ (BI) показали парафилию рода *Panagrolaimus*.

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