Four new geographical records of rhabditid nematodes (Nematoda: Rhabditida: Rhabditomorpha) from Iran with a note on the phylogenetic position of *Pelodera*

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Summary. During a survey of soil nematodes in Iran, four new records of rhabditids, namely *Cuticularia oxycerca* (de Man, 1895) Andrássy, 1983; *Mesorhabditis acuticauda* Ahmad, Shah & Mahamood, 2010; *Pelodera pseudoteres* Schulte, 1989 and *Protorhabditis oxyuroides* Sudhaus, 1974 were recovered. Molecular analysis of an Iranian population of *P. pseudoteres* based on 18S rDNA showed three nucleotide differences compared with a population from the USA (strain SB116; 1648 bp). By contrast, a strain of phylogenetically close species, *P. teres*, also from the USA (strain EM437; 1646 bp), showed 19 nucleotide differences. Phylogenetic analysis also places the Iranian strain closest to *P. pseudoteres* from the USA and confirms that molecularly characterised *Pelodera* species form a monophyletic clade. In addition, illustrations and measurements for all species and SEM observations for *M. acuticauda* are provided. All species are recorded for the first time from Iran.

Key words: *Cuticularia*, Iran, *Mesorhabditis*, *Pelodera*, *Protorhabditis*, phylogeny, SEM, taxonomy, 18S rDNA.

The nematodes of Rhabditina Andrássy, 1974 are largely free-living but ecologically diverse, including multiple transitions to parasitism (Sudhaus, 2010). The latest revision of this family was provided by Andrássy (2005) and Sudhaus (2011), who listed all genera and their species.

The genus *Mesorhabditis* Osche, 1952 is distributed all over the world (Andrássy, 2005). The members of this genus are mainly terrestrial but could be found in other habitats (*e.g.* fallen leaves, plant residues and fresh water; Andrássy, 2005). Sudhaus (1976) considered sixteen species of this genus as valid. However, the first deep revision on this genus was provided by Sudhaus (1978), who revised six species and described three as new species. Andrássy (1983, 1984, 2005) assumed 21 species as valid within the genus *Mesorhabditis*. Finally, Sudhaus (2011) divided this genus into two groups including *Monhystera* and *Spiculigera*, having 34 valid species in total. The most recent species from this genus was described by Ahmad *et al*. (2010).

The genus *Pelodera* Schneider, 1866 has been studied by some scientists (Sudhaus, 1991; Andrássy, 2005; Shokoohi & Abolafia, 2011). Members of this genus are mainly terrestrial; however, some species may be found in decayed and freshwater habitats (Andrássy, 2005). One species of the genus *Pelodera* was transferred to the genus *Rhomborhabditis* Andrássy, 1983, as *Pelodera pseudoteres* Schulte, 1989. Later, Sudhaus (2011) put this species as valid within the *Teres*-group of the genus *Pelodera*.

The genus *Cuticularia* van der Linde, 1938 is less studied, being a rare taxon within the Rhabditina. The first revision of this genus was provided by Eroshenko (2002), describing *C. annulata* as a new species. This genus comprises seven species (Andrássy, 2005), with *C. oxycerca* being the species with the most distribution worldwide.

Finally, the genus *Protorhabditis* (Osche, 1952) Dougherty, 1953 has been revised by Sudhaus (1991) and Andrássy (2005). The last revision on this genus including morphometric table and key to species was provided by Abolafia & Peña-Santiago (2007), while Sudhaus (2011) provided a list of species, considering 15 valid species under this genus.

Concerning the Rhabditina in Iran, some species of this group have been reported previously (Shokoohi & Abolafia, 2011). This paper documents four new reports of rhabditid species from Iran, *Cuticularia oxycerca* (de Man, 1895) Andrássy, 1983, *Mesorhabditis acuticauda* Ahmad, Shah & Mahamood, 2010, *Pelodera pseudoteres* Schulte, 1989 and *Protorhabditis oxyuroides* Sudhaus, 1974. Phylogenetic placement of the Iranian *P. pseudoteres* is given based on 18S rDNA sequences. The classification of Andrássy (2005) is followed herein.

MATERIALS AND METHODS

Studies on Morphology. The nematodes were extracted from soil samples by the Baermann funnel technique (Baermann, 1917) and a centrifugation method according to Jenkins (1964). Worms were fixed with hot 4% formaldehyde solution and processed to anhydrous glycerin according to De Grisse (1969). Measurements were taken using an ocular micrometer and drawings were made using a drawing tube attached to the microscope (Olympus CH-2). For SEM, fixed specimens were hydrated (1 day), dehydrated in a graded ethanol series (25, 30, 50, 70, 95, 100%) and finally in acetone (100%), critical point dried, coated with gold and observed with a JEOL JSM-5800 microscope operating at 4kV. The terminology used for morphology of stoma and spicules follows the proposals by De Ley *et al.* (1995) and Abolafia & Peña-Santiago (2006), respectively.

Phylogenetic analysis. The sequences of several species belonging to the family Rhabditidae used for phylogenetic analysis were found in the GenBank. 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 of double distilled water. The tube was frozen in liquid nitrogen and was pulverized using a vortex; 200 µl Tissue Lysis buffer (TL) and 20 µl proteinase K (20mg ml^{-1}) were then 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'-GCTTGTCTCAAAGATTAAGCC-3') and the reverse primer SSU_R_26 (5'-CATTCTTGGCAAA TGCTTTCG-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 Co., Iran), 1 µl of each primer (10 pmol μl^{-1}) and ddH_2O to a final volume of 25 µl. The amplification was carried out using an Eppendorf master cycler gradient (Eppendorf, Hamburg, Germany), which was 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 1mM EDTA) to check the quality of the DNA product. 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 with the primers that were used for the amplification. Sequencing was performed in both directions. The DNA sequence was edited using Chromas version 1.45 (McCarthy, 1997). Sequencing reactions were performed by the Bioneer Co. (South Korea) (http://eng.bioneer.com). Primers for the sequencing reaction were those used in the amplification step. The sequence was confirmed in both directions and repeated. Available sequences for other Rhabditomorpha and outgroup were obtained from NCBI GenBank. The ribosomal SSU sequences were aligned using BioEdit (Hall, 1999). Phylogenetic trees were generated using the Bayesian inference method as implemented in the program Mr Bayes 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 10^6 generations. The distance matrix option of Mega 5 (Tamura *et al.*, 2011) was used to calculate genetic distances according to the Maximum composite Likelihood of sequence evolution. The Bayesian tree was visualised with the TreeView program.

For phylogenetic analysis of 18S rDNA, *Panagrolaimus detritophagus* (EU543176) was used as outgroup. This selection was based on earlier studies (De Ley & Blaxter, 2002; Kiontke & Fitch, 2005; van Megen *et al*., 2009). The original partial 18S sequence of *P. pseudoteres* is deposited in the GenBank under accession number KC509908.

Table 1. Measurements of *Cuticularia oxycerca* (de Man, 1895) Andrássy, 1983 and *Mesorhabditis acuticauda* Ahmad, Shih & Mahamood, 2010. All measurements in μm in the form: mean ± standard deviation (range).

Species	*C. oxycerca*		*M. acuticauda*	
Locality	Shahrood		Northern Tehran	
Province	Semnan		Tehran	
Rhizosphere	Mallow		Grass	
n	6♀♀	9♂♂	10♀♀	10♂♂
L	613.7 ± 44.7 (574-677)	546 ± 61.4 (487-675)	404±35.2 (353-467)	288±75.1 (167-357)
a	14.5 ± 1.5 (12.1-15.9)	14.7 ± 0.8 (14-15.8)	15.6±1.8 (12.8-18.5)	13.9±3.9 (7.2-16.9)
b	3.5 ± 0.2 (3.1-3.8)	3.3 ± 0.2 (3-3.6)	4.6±0.4 (3.9-5.0)	3.6±1.0 (1.9-4.6)
c	37.3 ± 6.7 (29.2-45.8)	20.8 ± 2.2 (18-25)	10.4±1.2 (8.3-12.1)	18.5±6.4 (8.2-24.6)
c'	0.8 ± 0.1 (0.7-1)	1 ± 0.0 (1-1.1)	3.8±0.5 (3.2-4.7)	1.6±0.2 (1.1-1.9)
V	57.4 ± 1.7 (55-57)	–	77.8±5.8 (66-85)	–
Lip region diameter	11.8 ± 0.8 (11-13)	11 ± 0.7 (10-12)	6.0±0.8 (5-7)	5.3±0.6 (5-6)
Stoma	20.7 ± 1.2 (20-23)	20.8 ± 1.3 (19-23)	14.4±1.0 (13-16)	13.2±1.4 (11-15)
Pharyngeal corpus	97.2 ± 3.3 (93-103)	93.8 ± 7.5 (86-107)	44.3±2.7 (40-48)	41.0±4.2 (32-44)
Isthmus	45.7 ± 2.8 (43-51)	41.8 ± 3.2 (34-45)	21.0±2.8 (15-25)	21.0±3.5 (17-28)
Bulb	34 ± 1.7 (31-35)	31 ± 2.4 (28-36)	20.7±1.2 (19-23)	18.4±1.9 (15-20)
Pharynx length	176.7 ± 8.9 (165-187)	165.6 ± 12.4 (151-190)	87.6±3.9 (82-94)	81.7±5.3 (70-88)
Nerve ring – ant. end	110 ± 7.6 (102-120)	103 ± 11 (92-124)	69.5±4.4 (66-78)	63.2±5.8 (54-72)
Excretory pore – ant. end	113 ± 10.3 (103-128)	110 ± 9.2 (102-127)	80.9±5.8 (74-869	73.5±8.0 (65-84)
Deirid – ant. end	147 ± 9.9 (140-154)	135 ± 5.7 (131-139)	?	88.5±11.6 (80-97)
Annuli width	–	–	0.7±0.2 (0.7-1.3)	0.7±0.2 (0.7-1.3)
Cuticle thickness	6.5 ± 0.5 (6-7)	5.7 ± 0.5 (5-6)	0.7±0.2 (0.7-1.3)	0.7±0.2 (0.7-1.3)
Body diameter: neck base	19.3 ± 2.1 (17-23)	18 ± 1.3 (16-20)	7.6±0.7 (7-9)	6.5±0.7 (6-8)
Body diameter: midbody	42.7 ± 5.1 (36-49)	37 ± 3.9 (32-44)	26.1±2.1 (22-28)	20.9±1.3 (19-23)
Body diameter: anus	20 ± 1.1 (18-21)	25 ± 1.7 (22-28)	10.4±0.9 (9-11)	10.5±1.7 (9-13)
Anterior genital branch	211, 243	377.0 ±74.4 (319-461)	165, 142	212, 249
Posterior genital branch	200, 215	–	–	–
Rectum	27.3 ± 1.5 (25-29)	25.4 ± 2.4 (21-29)	19.6±2.8 (16-24)	?
Tail	16.8 ± 2.6 (13-20)	26.4 ± 1.3 (24-28)	39.7±4.5 (33-46)	16.2±2.4 (13-20)
Vulva–anterior end	352.7 ± 33.1 (321-396)	–	317.0±25.9 (283-360)	–
Spicules	–	31.9 ± 2.4 (30-35)	–	34.6±1.8 (33-37)
Gubernaculum	–	9 ± 0.5 (9-10)	–	14.9±1.3 (12-16)

RESULTS

Cuticularia oxycerca (de Man, 1895) Andrássy, 1983

(Figs 1 & 2)

Measurements. Table 1.

Population from province of Semnan (6 females, 9 males).

Female. Body almost straight, slightly curved ventrad after fixation. Cuticle loose, thick. Lateral field not visible. Lip region offset from the neck, having six rounded lips, bearing small papillae. Stoma rhabditoid, 20-23 μm long, with distinct cheilo-, gymno- and stegostom. Cheilostom finely cuticularised. Gymnostom longer than cheilostom, having well cuticularised walls. Stegostom having glottoid apparatus with three denticles. Pharyngeal collar present, covers less than half of the stoma. Pharyngeal corpus 1.8-2.2 times isthmus length, with procorpus longer than metacorpus. Metacorpus distinct, swollen. Isthmus robust and distinctly separated from metacorpus. Basal bulb ovoid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 49-61% of neck length. Excretory pore opening at isthmus level, at 50-62% of neck length. Deirid at isthmus level, at 73-78% of neck length. Intestine without distinct specialisation. Reproductive system didelphic-amphidelphic. Ovaries straight. Oviducts short. Uteri differentiated in a distal part swollen (spermatheca) and a proximal part tubular. Vagina with fine walls, extending inward one-third of the body width. Vulva not protruding, located slightly posterior to middle part of body. Rectum 1.2-1.5 times anal body diameter. Tail almost dome-shaped or cupola-shaped with short conoid, pointed distal part. Phasmid at 16-20% of tail length.

Male. General morphology similar to female. Body length 0.48-0.67 mm, curved ventrally after fixation. Genital system monorchic, with testis reflexed ventrad anteriorly. Tail cupola-shaped, with pointed tip and mucro. Bursa peloderan, opening anteriorly with eight pairs of papillae, three precloacal and five postcloacal, arranged in 1+2/5 pattern (according to Andrássy, 1983), the GP 7th is shorter. Spicules with rounded manubrium; calamus

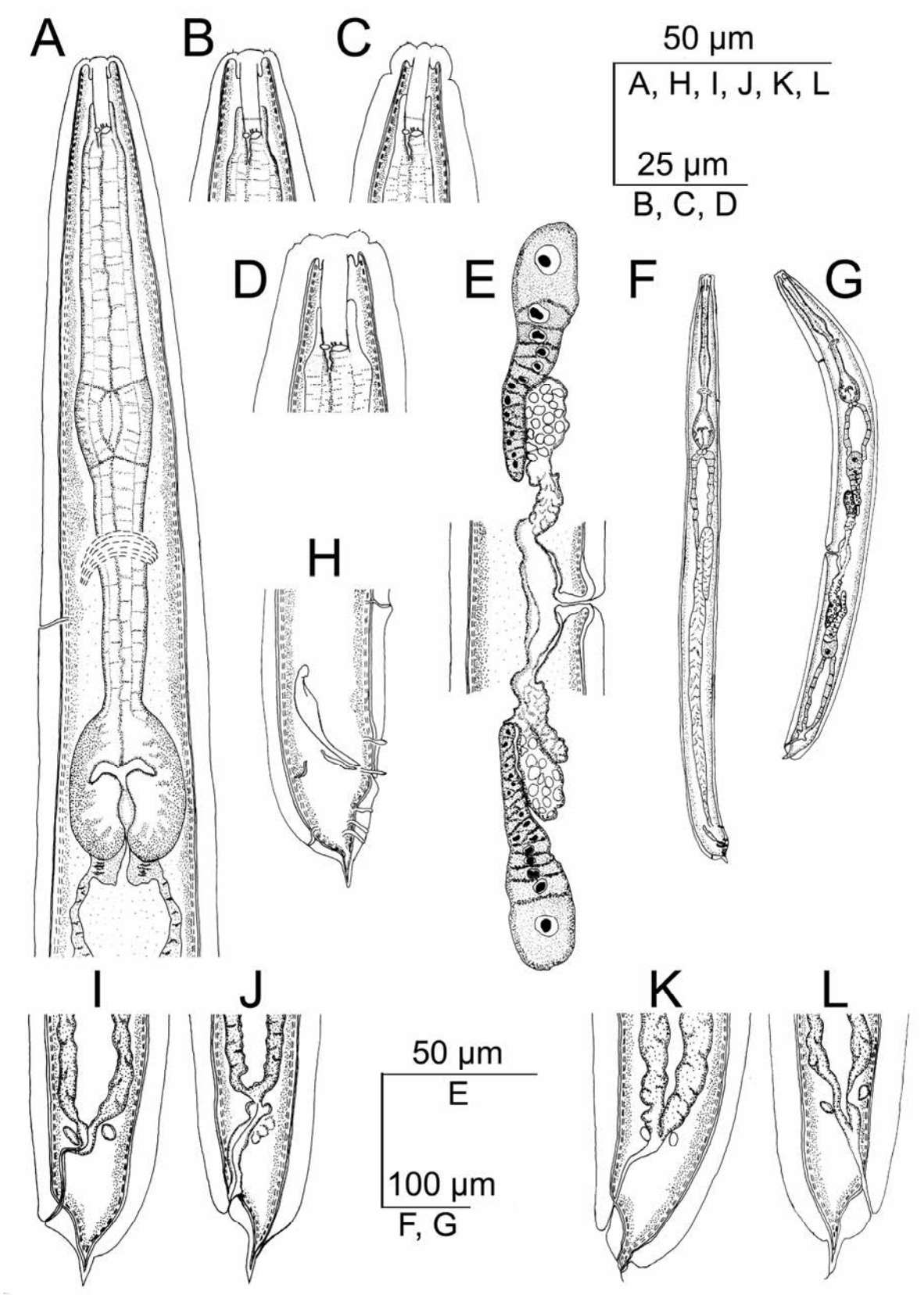

Fig. 1. *Cuticularia oxycerca* (de Man, 1895) Andrássy, 1983. A: Neck. B-D: Lip region. E: Female reproductive system. F: Entire male. G: Entire female. H: Male posterior end. I-L: Female posterior end.

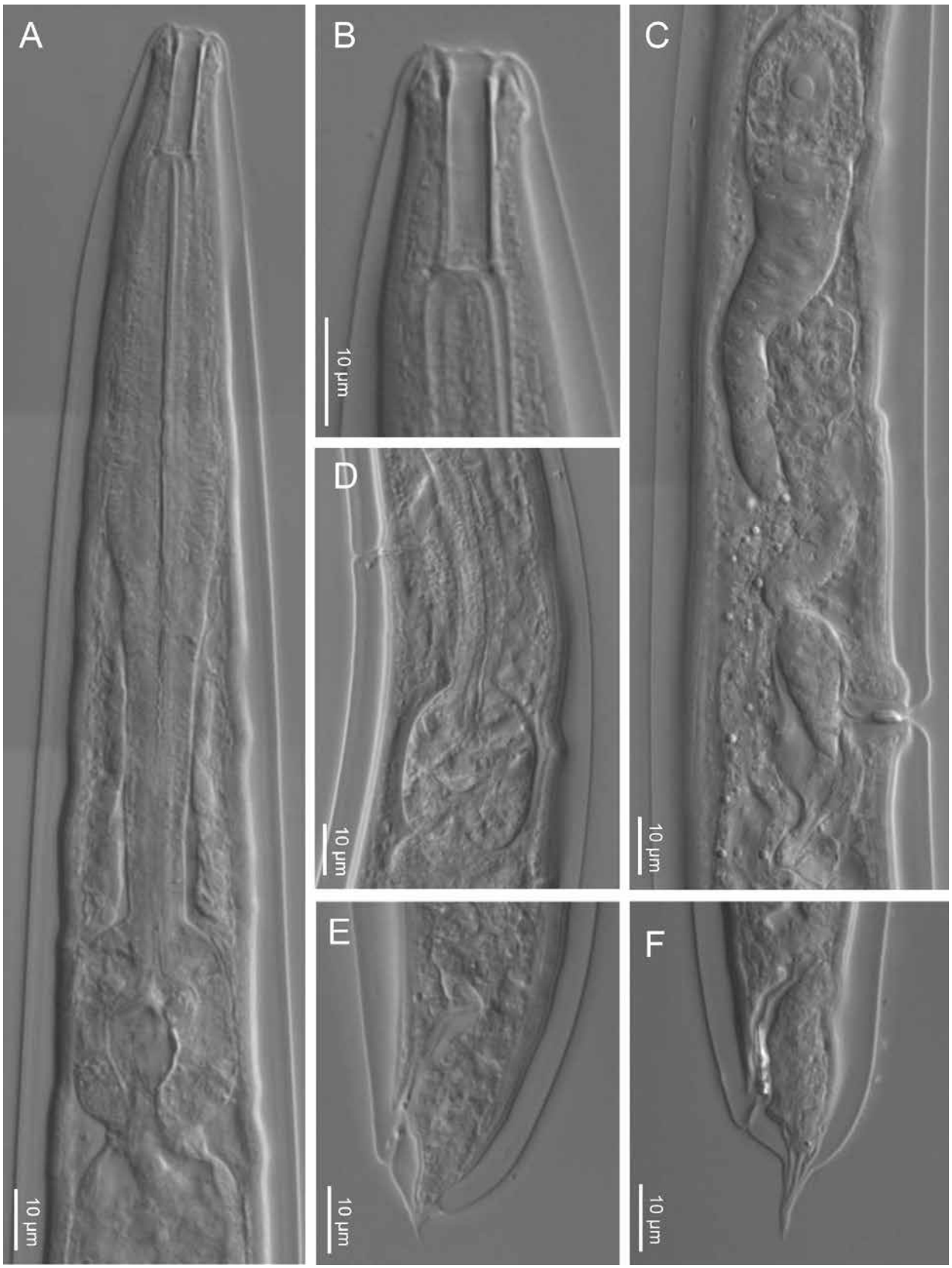

Fig. 2. *Cuticularia oxycerca* (de Man, 1895) Andrássy, 1983 (LM). A: Neck. B: Lip region. C: Female reproductive system. D: Excretory pore. E, F: Female posterior end.

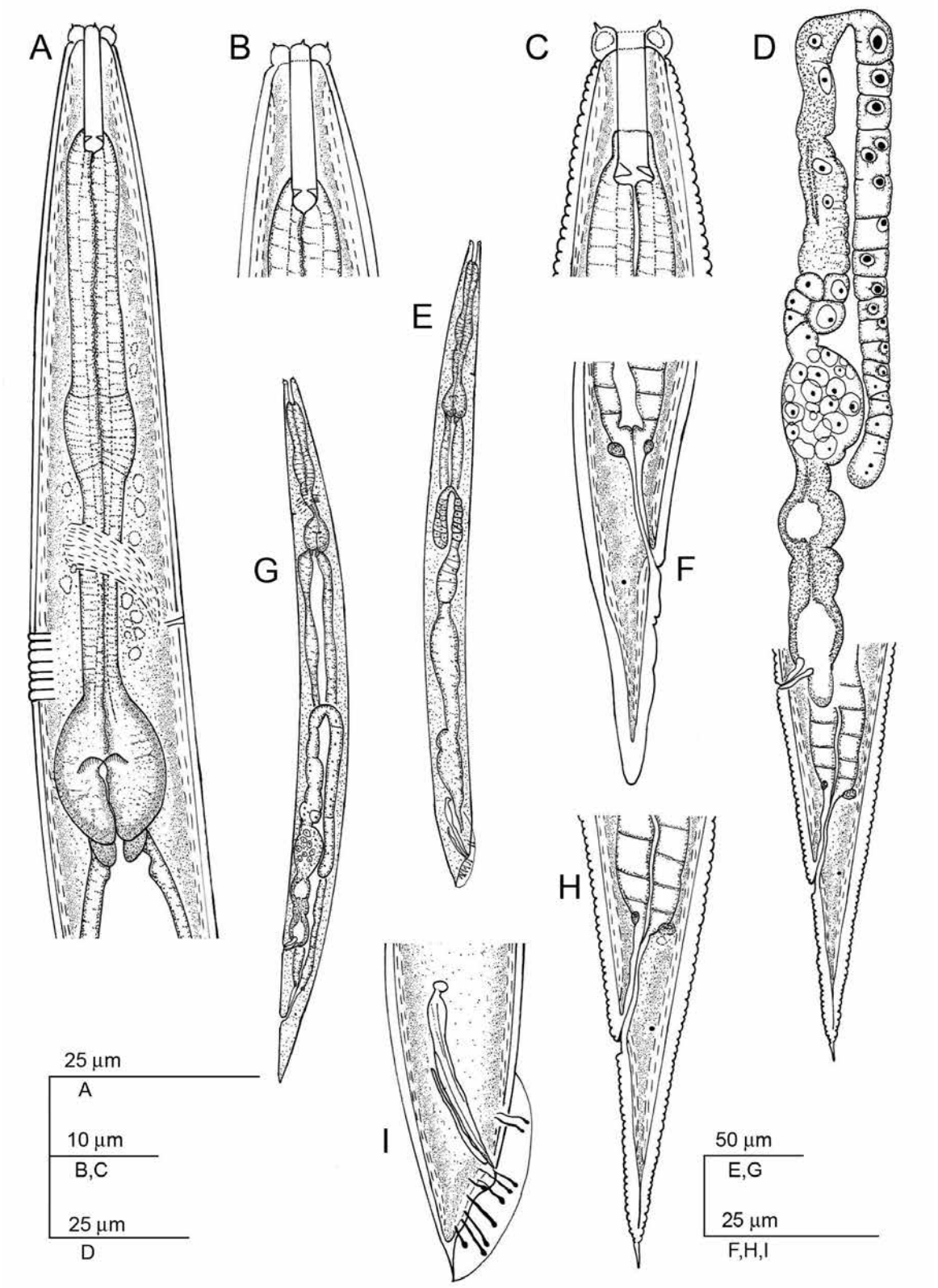

Fig. 3. *Mesorhabditis acuticauda* Ahmad, Shah & Mahamood, 2010. A: Neck. B, C: Lip region. D: Female reproductive system. E: Entire male. F, H: Female tail. G: Entire female. I: Male tail.

short and offset; and lamina curved ventrad and thinner distally. Gubernaculum curved ventrad.

Locality and habitat. The specimens were found in Shahrood (province of Semnan), in association with common mallow (*Malva sylvestris* L.).

Remarks. Morphology and measurements of the Iranian population agree with those of previous material examined (de Man, 1895; Andrássy, 1983; Eroshenko, 2002; Tahseen *et al*., 2009); however, it differs in female tail length (13-20 *vs* 30-60 µm), and gubernaculum length (9-10 *vs* 10-16 µm). Especially from Eroshenko's (2002) population, our material differs slightly in body length ratio (*vs* 600-1800 µm in females and 500-1500 µm in males). From Tahseen *et al.* (2009) population it differs by having slightly shorter female (574-677 *vs* 602-815 µm).

This genus and species is reported for the first time from Iran.

Mesorhabditis acuticauda Ahmad, Shah & Mahamood, 2010

(Figs 3-5)

Measurements. Table 1.

Population from Tehran, province of Tehran (10 females, 6 males).

Female. Body slightly curved ventrad after fixation. Cuticle annulated; annuli 0.7-1.3 µm. Lateral field with four grooves. Six rounded lips, offset, each ending in a setiform papilla with acute terminus. Stoma rhabditoid, 13-16 µm long, with distinct cheilo-, gymno- and stegostom. Cheilostom finely cuticularised. Gymnostom longer than cheilostom, having well cuticularised lumen. Stegostom having glottoid apparatus without denticles. Pharyngeal collar present, but short. Pharyngeal corpus 1.9-2.7 times isthmus length, with procorpus longer than metacorpus. Pharyngeal corpus lumen zipper-like. Metacorpus distinct, swollen. Isthmus robust, distinctly separated from metacorpus. Basal bulb ovoid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 80-83% of neck length. Excretory pore opening at isthmus level, at 90-91% of neck length. Deirid not visible. Intestine without distinct specialisation. Reproductive system monodelphic-prodelphic, located at the right side of intestine. Ovary straight. Oviduct long, tubular. Uterus differentiated in a distal part swollen (spermatheca) having sperm and a proximal part more or less tubular. Two small postvulval lateral sacs present, sometimes not clearly visible. Vagina with fine walls, extending inward one-third of the body width. Vulva not protruding, posteriorly located (at approx. 80% of body length). Rectum 1.8-2.1 times anal body diameter. Vulva-anus/tail distance is 1.1-1.3 anal body diameters. Tail conical, with acute terminus. Phasmid at anus level.

Male. General morphology similar to female. Body curved ventrally after fixation. Genital system monorchic. Tail conical, curved ventrad. Bursa peloderan, opening anteriorly with ten pairs of papillae, two precloacal and eight postcloacal, arranged in 2/4+4 pattern (according to Andrássy, 1983), the GP 8th and GP 9th are joined at their base in some specimens. Spicules fused at about one third of their length; manubrium rounded; calamus short and offset; lamina curved ventrad with hump and zigzag-shaped at its tip. Gubernaculum curved ventrad, well expanded in its anterior part.

Locality and habitat. The specimens were found in Tehran (province of Tehran), in association with carpet grass (*Axonopus* sp.).

Remarks. This population from Iran is very similar to the original description of *M. acuticauda*. However, two morphological characters appear different: the presence of two postvulval lateral sacs (*vs* lacking sacs), and the shape of the spicule tip (zigzag-shaped *vs* straight). Although these features were not described by Ahmad *et al.* (2010), it may depend on the fact that the postvulval lateral sacs are sometimes not clearly visible, while only the median part between both sacs can be seen; in this case the genital system seems to lack sacs. The other character, the presence of spicule with zigzag-shaped tip is not always apparent or it could be a geographical variability.

This genus and species is reported for the first time from Iran.

Pelodera pseudoteres Schulte, 1989

(Fig. 6)

Measurements. Table 2.

Material from province of Yazd (one female and one male).

Female. Body slightly curved ventrad after fixation. Cuticle annulated; annuli 1.7 µm. Lateral field not visible. Lip region offset, having six rounded lips, bearing small papillae. Stoma rhabditoid, 25 µm long, with distinct cheilo-, gymno- and stegostom. Cheilostom finely cuticularised. Gymnostom longer than cheilostom, having well cuticularised lumen. Stegostom having glottoid apparatus with three denticles. Pharyngeal collar present, covers half of the stoma. Pharyngeal corpus 7 times isthmus length, with procorpus longer than metacorpus. Metacorpus distinct, swollen. Isthmus robust, distinctly separated from metacorpus. Basal bulb ovoid, with valvular apparatus.

Table 2. Measurements of *Pelodera pseudoteres* Schulte, 1989 and *Protorhabditis oxyuroides* Sudhaus, 1974. All measurements in µm in the form: mean ± standard deviation (range).

Species	*P. pseudoteres*			*P. oxyuroides*	
Locality	Meybod			Damghan	
Province	Yazd			Semnan	
Rhizosphere	Grape			Willow	
n	♀	♂	Juvenile	11♀♀	6♂♂
L	1179	934	659	587.9 ± 64.4 (452-668)	511.8 ± 36 (454-541)
a	15.7	18.9	16.3	24.4 ± 2.7 (19.7-27.7)	21.7 ± 2.7 (19.1-26.8)
b	5.5	4.9	3.9	4.5 ± 0.5 (3.3-4.9)	4 ± 0.2 (3.6-4.2)
c	23.5	25.2	15.6	8.6 ± 1.7 (6.5-12.6)	17.6 ± 0.8 (17.1-19.1)
c'	1.77	1.1	1.9	5.1 ± 0.8 (3.4-6.1)	1.6 ± 0.1 (1.3-1.7)
V	56	–	–	57.2 ± 5.9 (53-73)	–
Lip region diameter	20	19	13	7.2 ± 0.8 (6-8)	7.7 ± 0.5 (7-8)
Stoma	25	21	23	21.2 ± 1.7 (19-24)	22 ± 1.7 (19-23)
Pharyngeal corpus	129	104	69	75.4 ± 4 (68-83)	82.3 ± 16.5 (74-116)
Isthmus	19	21	43	35.2 ± 2.9 (31-40)	33.5 ± 1.6 (31-35)
Bulb	37	36	34	21.3 ± 1.5 (20-25)	21 ± 1.8 (19-23)
Pharynx length	186	162	147	131.2 ± 7.6 (118-141)	128.2 ± 6.3 (116-133)
Nerve ring-ant. end	160	143	121	96.5 ± 5.9 (87-105)	94 ± 3.7 (87-97)
Excretory pore-ant. end	197	152	141	95.3 ± 11 (73-111)	97.4 ± 5.1 (92-103)
Deirid-ant. end	?	?	?	112 (n = 1)	112 (n = 1)
Annuli width	1.7	1.3	1.0	1	1
Cuticle thickness	4	3	2	1	1
Body diameter: neck base	23	19	35	10.3 ± 1 (9-12)	10.3 ± 0.5 (10-11)
Body diameter: midbody	75	49	40	24.2 ± 2.3 (22-30)	23.2 ± 3.1 (20-28)
Body diameter: anus	28	36	22	13.9 ± 1.8 (12-16)	17.8 ± 0.8 (17-19)
Anterior genital branch	575	725	–	157.0 ± 18.4 (144-178)	354
Posterior genital branch	591	–	–	157.3±21.6 (137-180)	–
Rectum	37	–	23	21.5 ± 2.2 (19-26)	22 ± 3.6 (18-25)
Tail	50	37	42	70.5 ± 14.7 (44-94)	28.3 ± 2.3 (24-30)
Vulva-anterior end	660	–	–	333 ± 19.2 (292-357)	–
Spicules	–	55	–	–	25 ± 2 (22-28)
Gubernaculum	–	29	–	–	14.3 ± 3.1 (10-17)

Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 74% of neck length. Excretory pore opening at isthmus level, at 91% of neck length. Deirid not visible. Intestine without distinct specialisation. Reproductive system didelphic-amphidelphic. Ovaries straight. Oviducts long, tubular. Uteri differentiated in a distal part swollen (spermatheca) having sperm, a middle part tubular with narrow lumen and a proximal part more or less swollen, these two later parts separated by a sphincter-like structure. Vagina with fine walls, extending inwards one-third of the body width. Vulva protruding, located posterior to middle part of body. Rectum 1.3 times anal body diameter. Tail conical, with acute end; it tapers more or less suddenly but distinctly in its distal part. Phasmid at 34% of tail length from the anus.

Male. General morphology similar to female. Body curved ventrally after fixation. Genital system monorchic, with testis reflexed ventrad anteriorly; genital tract lacking needle-like crystalline fibres. Tail conical, curved ventrad, with pointed tip. Bursa peloderan, opened anteriorly with ten pairs of papillae, three precloacal and seven postcloacal, arranged in 1+2/7 pattern (according to Andrássy, 1983), the GP 9th and GP 10th shorter. All papillae swollen at tip. Spicules fused at about two-thirds of their length; manubrium rounded; calamus short and offset; lamina curved ventrad and expanded with rounded tip. Gubernaculum curved ventrad, expanded in its anterior part.

Juvenile. Body slightly curved ventrad after fixation. Cuticle clearly annulated, with annuli 1 µm. Lateral field not visible. Lip region offset, having six rounded lips, bearing small papillae. Stoma rhabditoid, 23 µm long, with distinct cheilo-, gymno- and stegostom. Cheilostom finely cuticularised. Gymnostom longer than cheilostom, having well cuticularised lumen. Stegostom having glottoid apparatus with three denticles. Pharyngeal collar present, wraps around half of the stoma. Pharyngeal corpus 1.5 times isthmus length, with

procorpus longer than metacorpus. Metacorpus distinct, swollen, 25 µm long. Isthmus robust, distinctly separated from metacorpus. Basal bulb ovoid, 34 µm long, with valvular apparatus. Cardia conoid, 3 µm long, surrounded by intestinal tissue. Nerve ring at isthmus level, at 72% of neck length. Excretory pore opening at bulb level, at 84% of neck length. Deirid not visible. Intestine without distinct specialization. Rectum about one anal body diameter long. Tail conical, with acute end. Phasmid at 21% of tail length.

Locality and habitat. The specimens were found in Meybod (province of Yazd), in association with grape (*Vitis vinifera* L.).

Remark. The Iranian specimens of *P. pseudoteres* are almost identical to the original description of this species presented by Schulte (1989). According to the original description, the male lacks needle-like crystalline fibers in the genital tract, a character differentiating *P. pseudoteres* from *P. teres* Schneider, 1866; such structures are also lacking in the Iranian specimens. Other characters defined by Schulte (1989) are clearly visible in the Iranian population (*e.g.* offset lips, pharyngeal collar, conical tail and arrangement of bursal rays on male tail). However, the Iranian specimens differ in having a somewhat shorter stoma (21-25 *vs* 23-31 µm), and longer spicules (55 *vs* 36-48 µm).

This species is reported for the first time from Iran.

DNA Characterisation. The 18S rRNA gene sequence of *P. pseudoteres* amplified by the two primers SSU-F-04 and SSU-R-26 is 808 base pairs (bp) long. Within this fragment, only three base pair differences distinguish the Iranian population from the American population of *P. pseudoteres* (accession nr. EU196023; 99% identity) and 19 bp differences in comparison to *P. teres* (accession number AF083002; 97% identity).

Pairwise Maximum Composite Likelihood distance among the 18S rDNA region of *Pelodera* species showed that *P. pseudoteres* populations from Iran and USA have identical sequences at this genetic marker (see Table 3) where, among the molecularly characterised species of *Pelodera*, *P. pseudoteres* was closest to *P. teres*.

Protorhabditis oxyuroides Sudhaus, 1974
(Figs 7 & 8)

Measurements. Table 2.

Population from province of Semnan (11 females, 6 males).

Female. Body slightly curved ventrad after fixation. Cuticle annulated; annuli 1 µm. Lateral field not visible. Lip region continuous with body, having six rounded to conoid lips, bearing small papillae. Stoma rhabditoid, 19-24 µm long, with distinct cheilo-, gymno- and stegostom, the latter lacking glottoid apparatus. Cheilostom with weakly refractive walls. Buccal prism with straight walls, very long and narrow. Pharyngeal collar present, very short. Pharyngeal corpus 2.0-2.1 times isthmus length, with procorpus longer than metacorpus. Metacorpus distinct, swollen. Isthmus robust and distinctly separated from metacorpus. Basal bulb ovoid to spheroid, with valvular apparatus. Cardia conoid, surrounded by intestinal tissue. Nerve ring at isthmus level, at 73-74% of neck length. Excretory pore opening at isthmus level, at 62-79% of neck length. Deirid not visible. Intestine without distinct specialisation. Reproductive system didelphic-amphidelphic. Ovaries reflexed and continued with their corresponding oviducts. Uteri differentiated in a distal part swollen (spermatheca) and a proximal part more or less tubular. Vagina with thin walls, extending inwards less than half of the corresponding body diameter. Vulva protruding, located posterior to middle part of body. Rectum 1.5-1.6 times anal body diameter. Tail conical elongate. Phasmid at 34-48% of tail length.

Male. General morphology similar to female. Body curved ventrally after fixation. Genital system monorchic, with testis reflexed ventrad anteriorly. Tail elongate-conoid, curved ventrad, with pointed tip. Bursa peloderan, opening anteriorly with nine pairs of papillae, two precloacal and seven postcloacal, arranged in 1+1/4+3 pattern (according Andrássy, 1983), the GP 1st anterior to bursa, and GP 7th, GP 8th and GP 9th are shorter. Spicules with rounded manubrium, calamus short and offset, and lamina curved ventrad and expanded with pointed tip. Gubernaculum curved ventrad, expanded in its anterior part.

Locality and habitat. The specimens were found in Damghan (province of Semnan), in association with willow (*Salix* sp.).

Remarks. The specimens examined fit well with those studied by Sudhaus (1974), but the females are shorter (452-668 *vs* 595-872 µm) and the gubernaculum longer (10-17 *vs* 4-12 µm). Compared to the material examined by Andrássy (1983), the Iranian specimens have a smaller range in the body size (452-668 µm *vs* 590-870 µm in females and 454-541 µm *vs* 320-720 µm in males). For key to species identification see Abolafia & Peña-Santiago (2007).

This genus and species is recorded for the first time from Iran.

On the phylogenetic position of *Pelodera*. According to Sudhaus (2011), the genus *Pelodera* is divided into three monophyletic groups based on

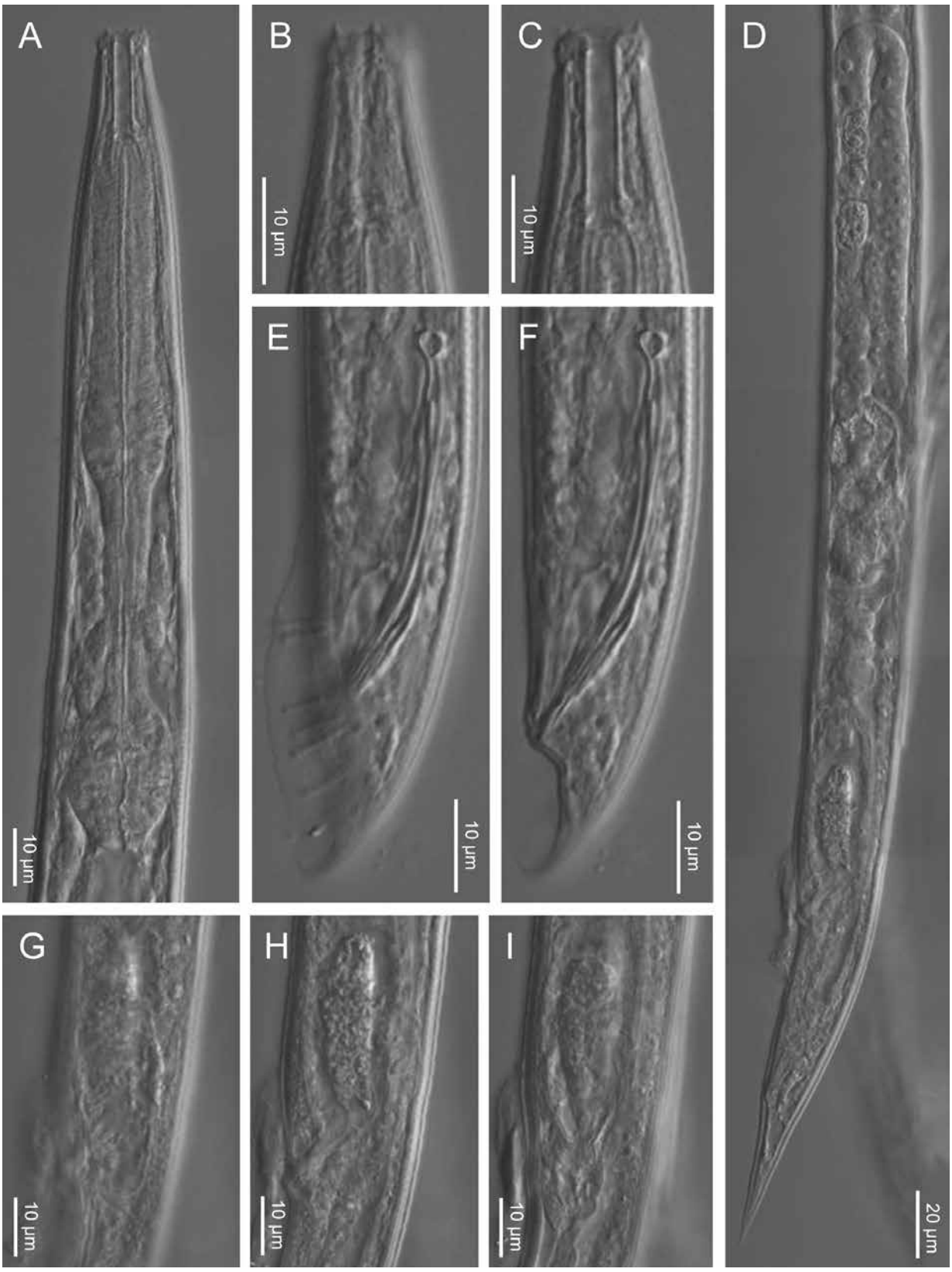

Fig. 4. *Mesorhabditis acuticauda* Ahmad, Shah & Mahamood, 2010 (LM). A: Neck. B, C: Lip region. D: Female reproductive system. E, F: Male posterior end. G: Postvulval sac. H: Vagina. I: Uterus.

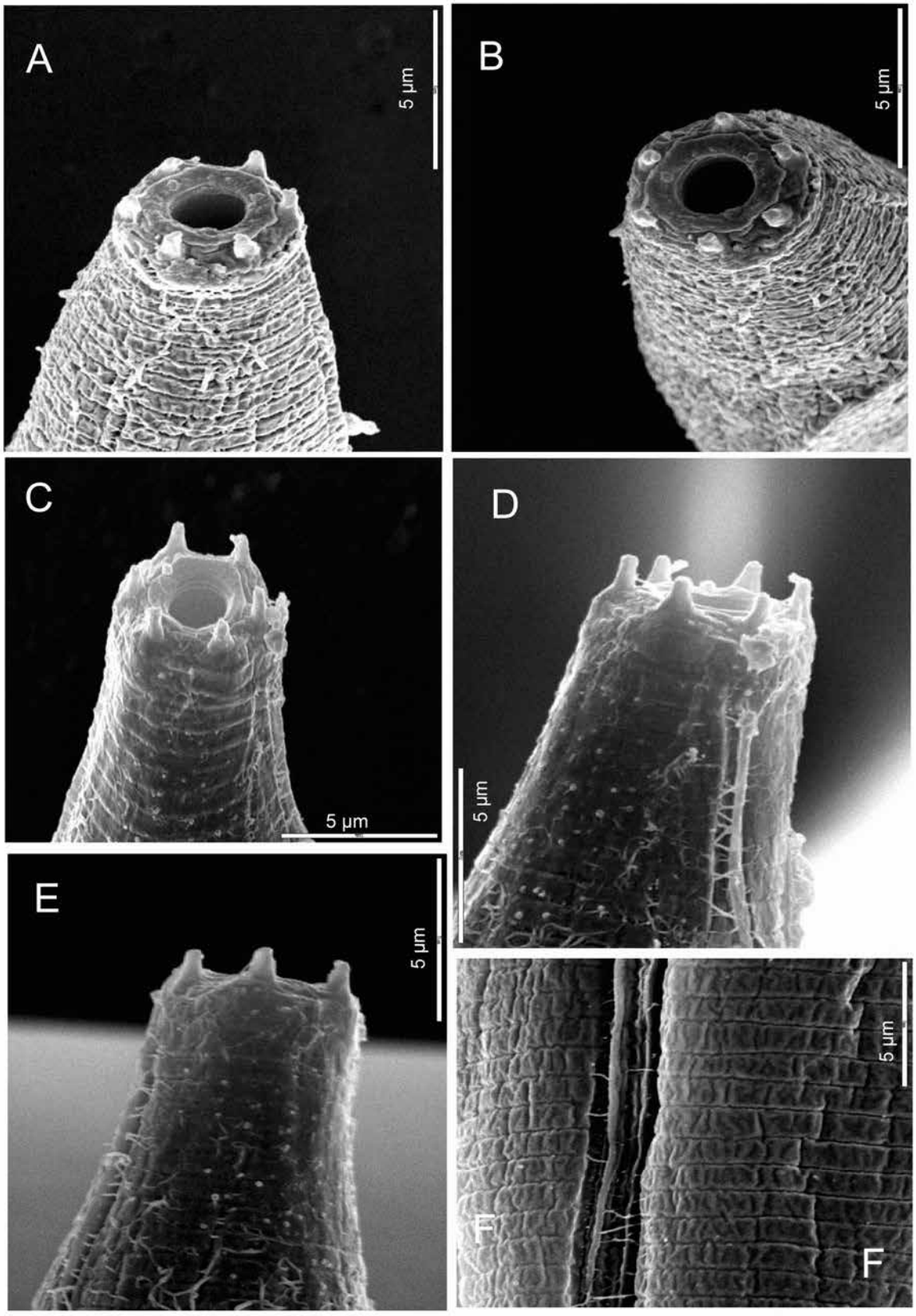

Fig. 5. *Mesorhabditis acuticauda* Ahmad, Shah & Mahamood, 2010 (SEM). A, B, C: Lip region (Frontal view). D, E: Lip region (Ventral view). F: Lateral field.

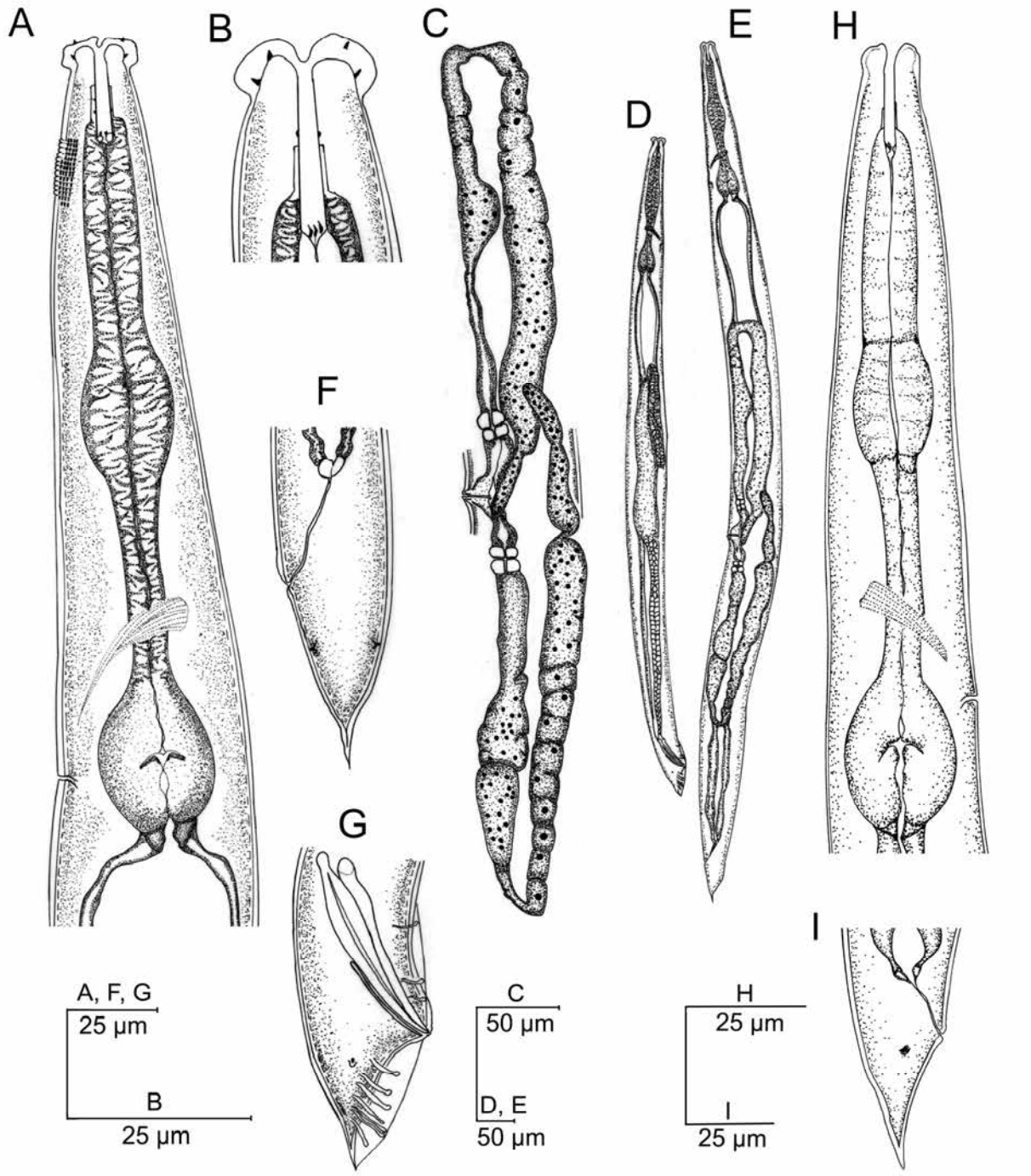

Fig. 6. *Pelodera pseudoteres* Schulte, 1989. A: Neck. B: Lip region. C: Female reproductive system. D: Entire male. E: Entire female. F: Female tail. G: Male tail. H: Anterior end of juvenile. I: Tail of juvenile.

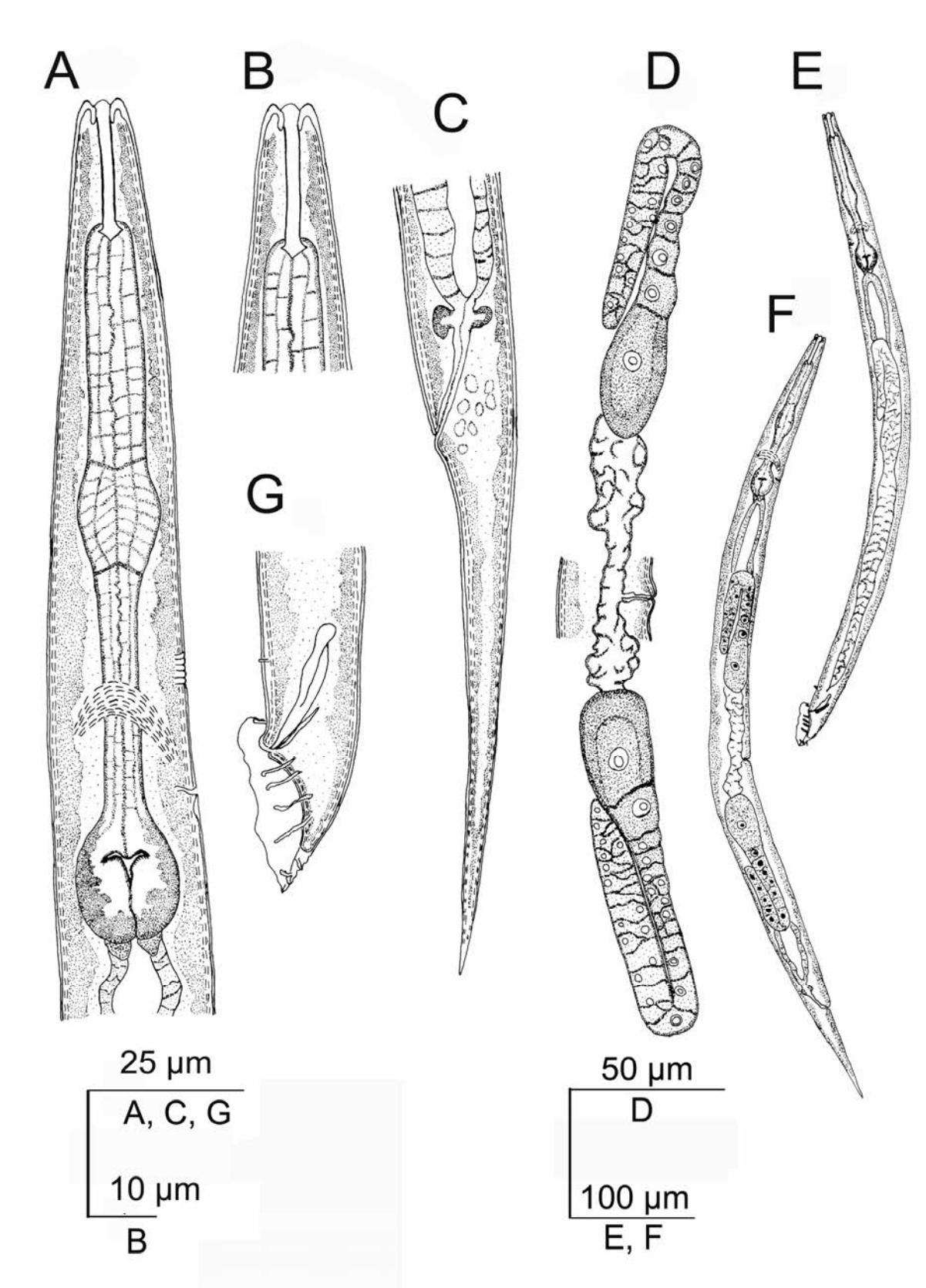

Fig. 7. *Protorhabditis oxyuroides* Sudhaus, 1974. A: Neck. B: Lip region. C: Female posterior end. D: Female reproductive system. E: Entire male. F: Entire female. G: Male posterior end.

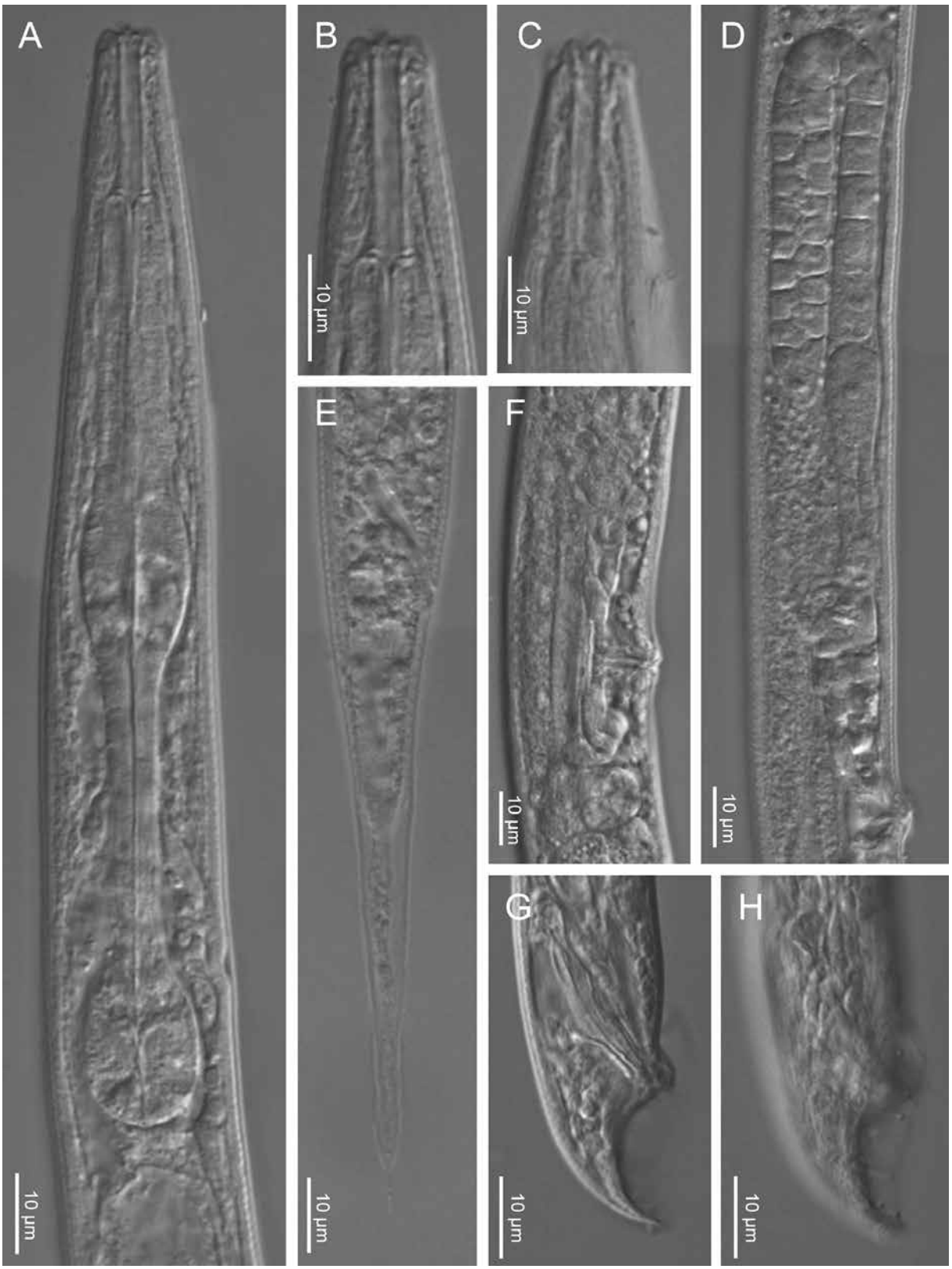

Fig. 8. *Protorhabditis oxyuroides* Sudhaus, 1974 (LM). A: Neck. B: Stoma. C: Lip region. D: Female reproductive system. E: Female posterior end. F: Vagina and postvulval sac. G: Male posterior end. H: Bursa and papillae.

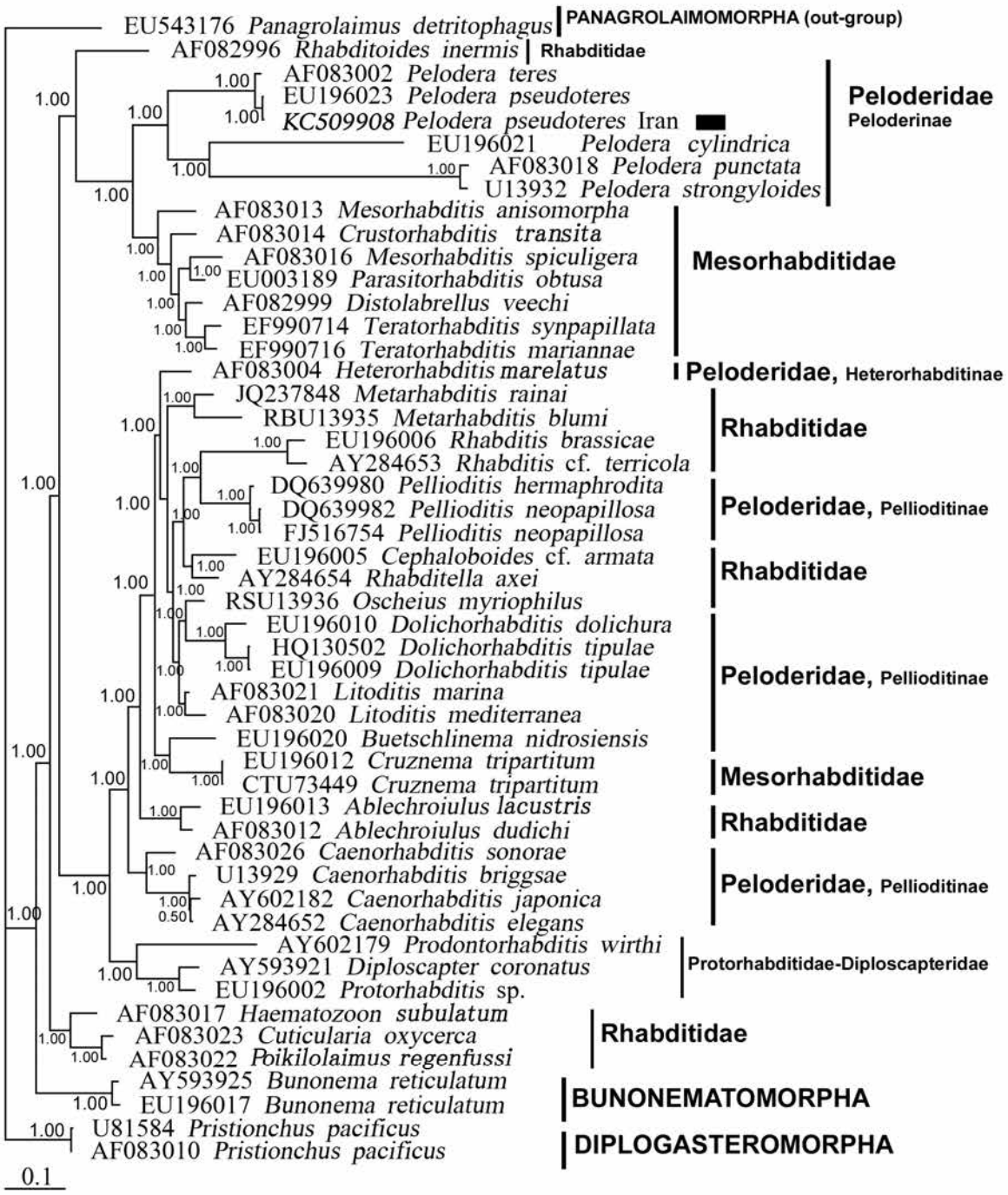

Fig. 9. The Bayesian tree inferred from the analysis of 18S rRNA gene sequence alignment of known and newly sequenced *Pelodera pseudoteres* (square marked) from Iran and other related taxa.

Table 3. Pairwise genetic distance of the 18S rDNA region among *Pelodera* and closely species studied formed a clade.

	Species	Fragment length	1	2	3	4	5	6	7	8	9	10	11	12
1	*Pelodera teres* AF083002	1646												
2	*Pelodera pseudoteres* EU196023	1648	0.027											
3	*Pelodera cylindrica* EU196021	1606	0.925	0.984										
4	*Pelodera punctata* AF083018	1649	0.941	0.998	1.287									
5	*Mesorhabditis spiculigera* AF083016	1362	0.340	0.343	0.779	0.843								
6	*Mesorhabditis anisomorpha* AF083013	1647	0.371	0.386	0.770	0.908	0.110							
7	*Teratorhabditis synpapillata* EF990714	1635	0.329	0.352	0.759	0.822	0.079	0.120						
8	*Teratorhabditis mariannae* EF990716	1683	0.325	0.345	0.768	0.837	0.078	0.125	0.027					
9	*Parasitorhabditis obtusa* EU003189	1685	0.325	0.333	0.795	0.853	0.054	0.115	0.055	0.043				
10	***Pelodera pseudoteres* KC509908**	**614**	**0.027**	**0.000**	**0.984**	**0.998**	**0.343**	**0.386**	**0.352**	**0.345**	**0.333**			
11	*Pelodera strongyloides* U13932	1613	0.920	0.975	1.308	0.011	0.832	0.908	0.819	0.827	0.842	0.975		
12	*Crustorhabditis transita* AF083014	1670	0.318	0.324	0.800	0.873	0.073	0.117	0.065	0.057	0.049	0.324	0.862	
13	*Distolabrellus veechi* AF082999	1697	0.328	0.340	0.767	0.827	0.055	0.125	0.047	0.039	0.045	0.340	0.816	0.050

several phenotypic traits derived from evolution, mainly tail morphology: the *Teres*-group is distinguished by having cupola-shaped or short conoid female tail and bursa with three precloacal papillae; the *Coarctata*-group is distinguished by having cupola-shaped female tail and bursa with two precloacal papillae; and the *Strongyloides*-group is distinguished by having cupola-shaped or conical female tail and bursa with two precloacal papillae. These groups correspond to different genera according to Andrássy (2005): *Rhomborhabditis* Andrássy, 1983, *Coarctadera* Dougherty, 1953 and *Pelodera* Schneider, 1866 *sensu stricto*, respectively.

In our phylogenetic analysis using 18S rDNA (Fig. 9), this morphological classification scheme is supported by molecular data. Thus, the molecularly characterised species of *Pelodera* form a monophyletic group with 100% posterior probability and are divided into the three groups indicated by Sudhaus (2011). This result suggests that the phenotypic characters are also informative for phylogenetic relationships. However, more taxon sampling within *Pelodera* is needed to understand the full phylogenetic status of the species within this genus.

In addition, our analysis places the *Pelodera* species in a clade together with *Distolabrellus* Anderson, 1983, *Mesorhabditis*, *Parasitorhabditis* Fuchs, 1937 and *Teratorhabditis* Osche, 1952, agreeing with Kiontke & Fitch (2005) and van Megen *et al*. (2009). Fitch (1997) demonstrated with the analysis of the SSU rDNA that *Pelodera* (using *P. strongyloides* Schneider, 1860) and *Teratorhabditis* (using *T. palmarum* Gerber & Giblin-Davis, 1990) are within the same monophyletic group, this being consistent with the current study.

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E. Shokoohi, S. Mehdizadeh, N. Amirzadi, J. Abolafia. Четыре новых сообщения о находках рабдитид (Nematoda: Rhabditida: Rhabditomorpha) в Иране и оценка филогенетических связей рода *Pelodera*.

Резюме. Обнаружены четыре новых для фауны Ирана вида почвенных рабдитид: *Cuticularia oxycerca* (de Man, 1895) Andrássy, 1983; *Mesorhabditis acuticauda* Ahmad, Shah & Mahamood, 2010; *Pelodera pseudoteres* Schulte, 1989 и *Protorhabditis oxyuroides* Sudhaus, 1974. Проведенный по последовательностям 18S рДНК филогенетический анализ положения иранской популяции *P. pseudoteres* показал различия в трех позициях по сравнению с популяцией этого вида из США (изолят SB116; 1648 п.н.). От филогенетически близкого вида *P. teres* (также из США, изолят EM437; 1646 п.н.) отличия составили 19 нуклеотидов. Филогенетический анализ показывает близость иранской популяции *P. pseudoteres* к представителям этого вида из США и подтверждает монофилетический характер линии, составленной видами *Pelodera*. Приводятся иллюстрации и измерения для вида *M. acuticauda*.