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Study of the genus *Plectus* Bastian, 1865 (Nematoda: Plectidae) from Iran

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Abstract: Three species of *Plectus* Bastian, 1865 *viz.*, *P. aquatilis* and *P. pusillus* from Kerman province and *P. velox* from Alborz province, Iran are described and illustrated. Partial sequences of 18S region of ribosomal DNA gene were amplified for *P. aquatilis* and *P. pusillus*. The Blast results of population of *P. aquatilis* from Iran showed 8–10 nucleotides differences with populations of the same species (AF036602; GQ892827; AY284700) reported from the UK, Belgium and The Netherlands, respectively. Whereas Iranian population of *P. pusillus* showed 14–16 nucleotides differences with *P.* cf. *pusillus* (AY284705; AY284704) reported from The Netherlands. Molecular analysis revealed close relationship of the Iranian plectids with *P.* cf. *parvus* (AY284699) reported from The Netherlands. Phylogenetic relationships with other related species in the genus *Plectus* and closely related genera that are available in the GenBank are given.

Key words: *Plectus*; morphometrics; phylogeny; 18S rDNA; Kerman; Alborz; Iran

Introduction

The order Plectida was established by Gadea in 1973. The members of this group have been reported from marine, fresh water and terrestrial habitats. The genus *Plectus* Bastian, 1865 is the most widely distributed within this order with 79 valid species and 24 species of uncertain status (Holovachov & Boström 2010). The first extensive study of the genus *Plectus* was presented by Maggenti (1961), then other scientists added more information about taxonomy of the genus (e.g., Andrássy 1985, 1998, 2005; Zell 1993; De Ley & Coomans 1994). Detailed phylogenetic analysis of this group was performed by Holovachov (2004), supplemented with new data on morphology and development of the superfamily Plectoidea Örley, 1880. Van Megen et al. (2009) confirmed the monophyletic origin of the family Plectidae based on SSU rDNA.

The present paper deals with three species of the genus *Plectus* Bastian, 1865, namely *P. aquatilis* Andrássy, 1985, *P. pusillus* Cobb, 1893 and *P. velox* Bastian, 1865 collected from natural areas of south and north of Iran. In addition, molecular analysis and phylogenetic position of the Iranian populations of *P. aquatilis* and *P. pusillus* are given.

Material and methods

Nematode materials
Nematodes were extracted from soil samples by Baermann's (1917) funnel technique. They 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 Olympus CH-2 microscope. LM observations were made using a Nikon Eclipse 80i microscope equipped with a Nikon Digital Sight DS–5M camera.

Phylogenetic analysis
DNA extraction was done using an *AccuPrep* Genomic DNA extraction kit (Bioneer Corporation, Korea, http://www.bioneer.com) according to the manufacturer's instructions. Ten specimen were picked into 1.5 ml tube containing 5 µl double distilled water. The tube was frozen in liquid nitrogen and the specimens crushed with a sterile needle for 5 min, vortexed and then 200 µl Tissue Lysis buffer (TL) and 20 µl proteinase K (20 mg ml^{-1}) was added. The homogenate was incubated at 60 °C for 2 hours. 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′-CATTCTTGGCA AATGCTTTCG-3′) (Blaxter et al. 1998) were used in the PCR reactions for amplification of the partial 18S region

Table 1. Nematode species and GenBank accession number used for phylogenetic study.

Species	GenBank accesion number	Reference	Origin
Anaplectus porosus	AY284696	Holterman et al. (2006)	The Netherlands
Anaplectus porosus	F040453	Holterman et al. (2008)	The Netherlands
Anaplectus grandepapillatus	AY284697	Holterman et al. (2006)	The Netherlands
Anaplectus grandepapillatus	AY284698	Holterman et al. (2006)	The Netherlands
Ceratoplectus armatus	AY284706	Holterman et al. (2006)	The Netherlands
Ceratoplectus cf. *armatus*	FJ474096	Holovachov et al. (2009)	Moldova
Ceratoplectus cf. *assimilis*	FJ474097	Holovachov et al. (2009)	Canada
Chronogaster boettgeri	AY593931	Holterman et al. (2006)	The Netherlands
Chronogaster typica	FJ040456	Holterman et al. (2008)	The Netherlands
Euteratocephalus palustri	AY284684	Holterman et al. (2006)	The Netherlands
Euteratocephalus sp.	AY284685	Holterman et al. (2006)	The Netherlands
Hemiplectus muscorum	FJ474098	Holovachov et al. (2009)	United States
Metateratocephalus crassidens	AY284686	Holterman et al. (2006)	The Netherlands
Pakira orae	FJ474099	Holovachov et al. (2009)	New Zealand
Plectus acuminatus	AF037628	Frisse et al., unpub.	United States
Plectus aquatilis	KC509902	**Present paper**	**Iran**
Plectus aquatilis	AY284700	Holterman et al. (2006)	The Netherlands
Plectus aquatilis	AF036602	Blaxter et al. (1998)	UK
Plectus aquatilis	GQ892827	Borgonie et al., unpub.	South Africa
Plectus cirratus	AY593930	Holterman et al. (2006)	The Netherlands
Plectus cf. *cirratus*	AY284701	Holterman et al. (2006)	The Netherlands
Plectus murrayi	AB649028	Kagoshima et al. (2012)	Japan
Plectus cf. *parvus*	AY284699	Holterman et al. (2006)	The Netherlands
Plectus cf. *parietinus*	AY284703	Holterman et al. (2006)	The Netherlands
Plectus cf. *parietinus*	AY284702	Holterman et al. (2006)	The Netherlands
Plectus pusillus	KC509903	**Present paper**	**Iran**
Plectus cf. *pusillus*	AY284704	Holterman et al. (2006)	The Netherlands
Plectus cf. *pusillus*	AY284705	Holterman et al. (2006)	The Netherlands
Plectus rhizophilus	AY593929	Holterman et al. (2006)	The Netherlands
Plectus rhizophilus	AY593928	Holterman et al. (2006)	The Netherlands
Plectus tenuis	FJ969135	van Megen et al. (2009)	Germany
Tylocephalus auriculatus	AY284707	Holterman et al. (2006)	The Netherlands
Tylocephalus auriculatus	AF202155	Felix et al. (2000)	France
Wilsonema otophorum	AY593927	Holterman et al. (2006)	The Netherlands
Wilsonema schuurmansstekhoveni	AJ966513	Meldal et al. (2007)	Belgium
Caenorhabditis elegans	EU196001	Kiontke et al. (2007)	United States
Caenorhabditis elegans	AY284652	Holterman et al. (2006)	The Netherlands

(~900 bp). 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^{-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), 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 visualized 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 same primers that were used for amplification step. Sequencing was performed in both directions. The DNA sequences were edited using Chromas version 1.45 (McCarthy 1997). Sequencing reactions were performed by Bioneer company (South Korea, http://eng.bioneer.com). Primers for the sequencing reaction were the same as the ones used in the amplification step. All sequences were confirmed in both directions and repeated. The ribosomal SSU sequences were analysed and aligned using the program ClustalW implemented in MEGA version 5.0 (Tamura et al. 2011). Sequences for the ingroups and outgroups were provided from NCBI GenBanks (Table 1). Phylogenetic trees were generated with the Bayesian inference method using the program Mr Bayes 3.1.2 (Ronquist & Huelsenbeck 2003). The analysis was run for 10^6 generations. Sequences of *Caenorhabditis elegans* (EU196001; AY284652) were chosen as outgroups for the phylogenic analysis of 18S rDNA based on De Ley & Blaxter (2004) study. The original partial 18S rDNA sequences of *P. aquatilis* and *P. pusillus* are deposited in the GenBank under accession numbers KC509902 and KC509903 respectively. The populations of Khabr and Rabor were used for molecular study in *P. aquatilis* and *P. pusillus*, respectively. The Bayesian tree was visualised with the TreeView program.

***Plectus aquatilis* Andrássy, 1985** (Figs 1, 2; measurements in Table 2)

Description. Population from Khabr, province of Kerman (7 ♀♀).

Female. Body small, cylindrical, ventrally arcuate upon heat fixation. Cuticle with fine transverse striations, annules 0.9 µm wide at mid-body. Lateral field with two ridges (alae); 6.6 µm wide at mid-body, occupying 15–19% of the corresponding body width.

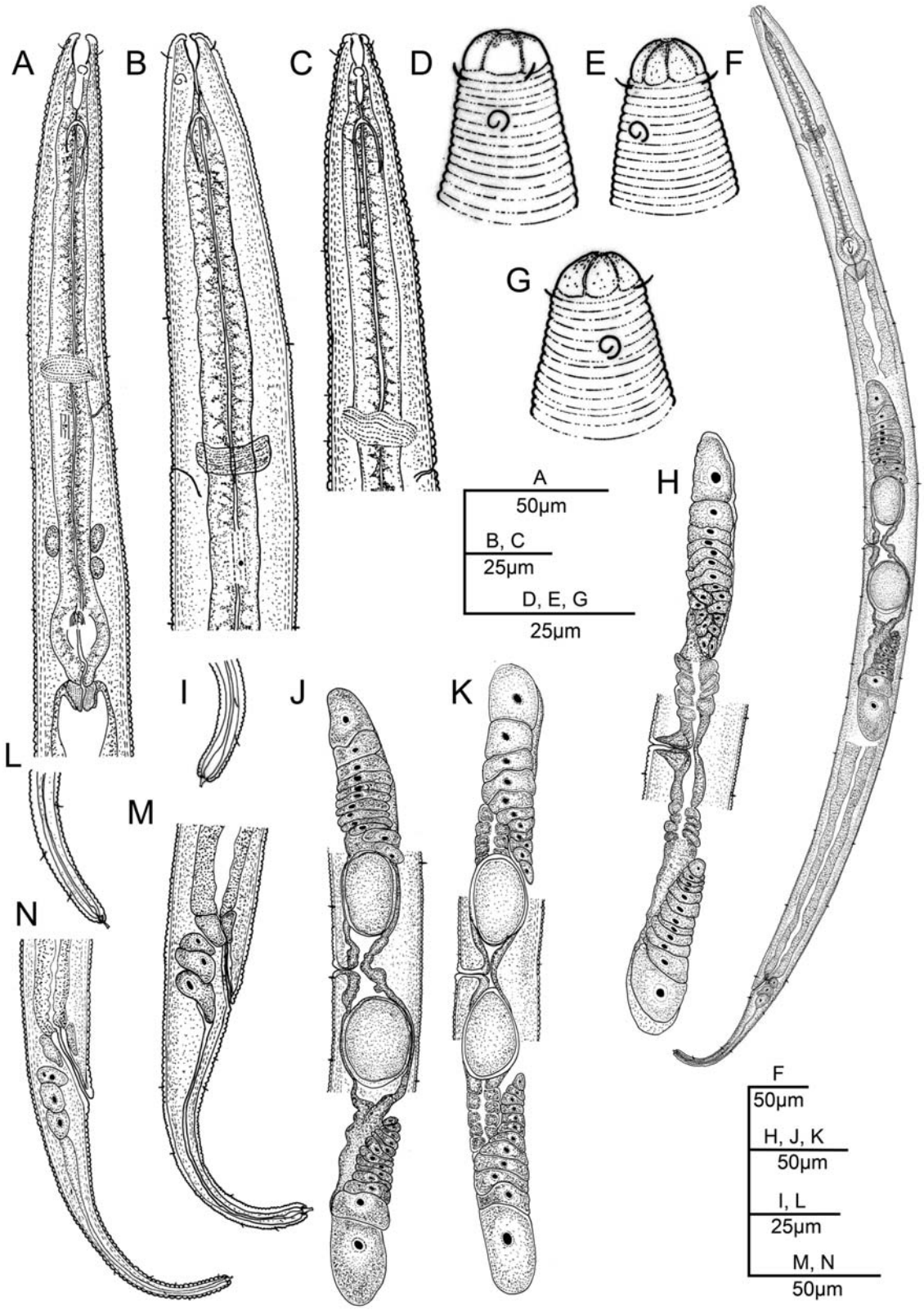

Fig. 1. *Plectus aquatilis* Andrássy, 1985. A: Neck; B, C: Anterior region; D, E, G: Anterior end showing lips and amphids (surface view); F: Entire female; H, J, K: Female reproductive system; I, L: Terminal part of tail; M, N: Female posterior end.

Head region narrow, continuous with body contour. Lip region rounded, twice as wide as high. Labial sensilla papilliform, visible under light microscope. Four cephalic setae, 0.2–0.5 times lip region diameter long, inserted on anteriormost body annule just posterior to lip base. Amphidial openings circular. Stoma plectoid, cylindrical, 2.2–2.8 times longer than lip region diameter, its broader part about 0.8 longer than nar-

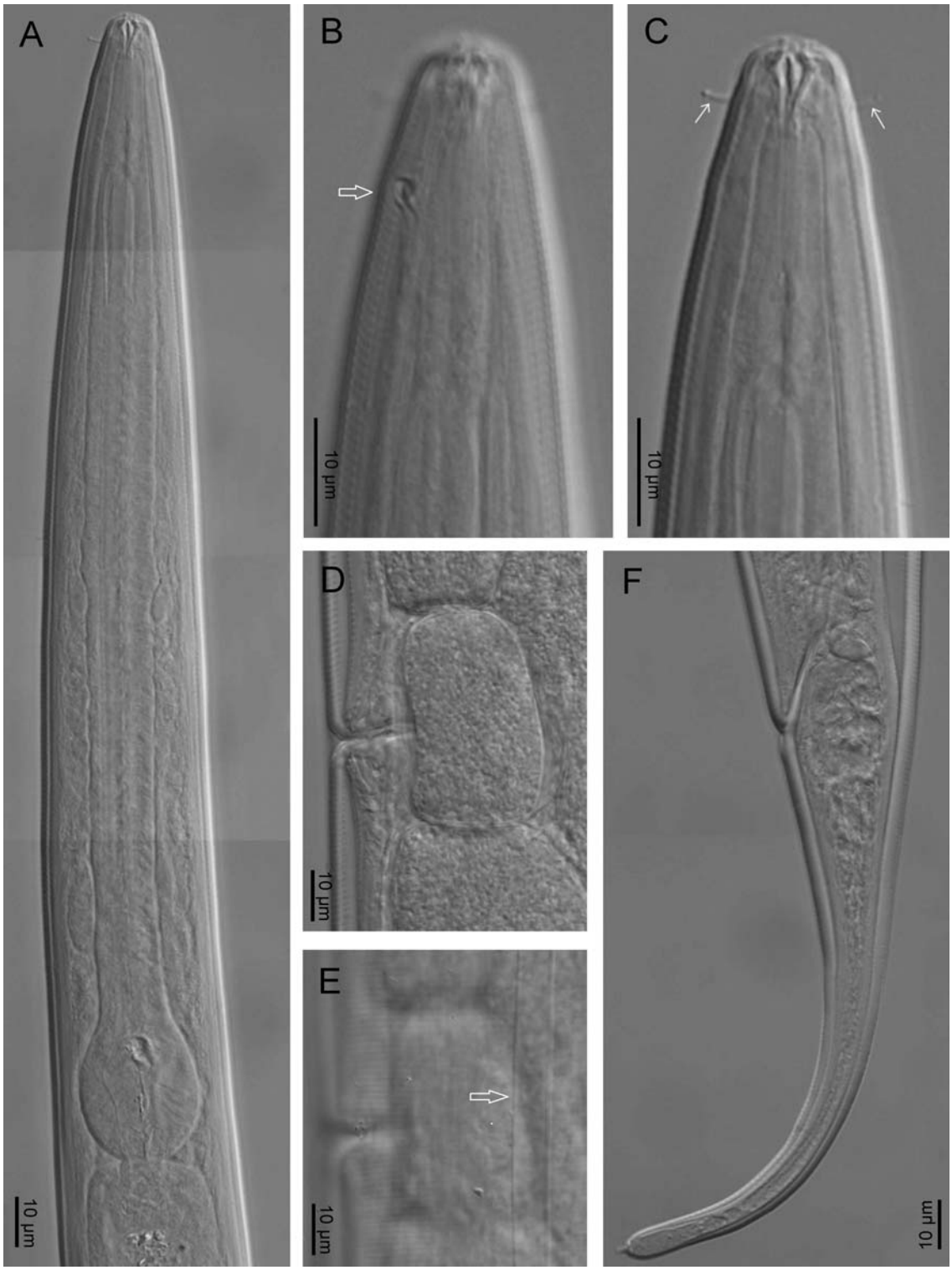

Fig. 2. *Plectus aquatilis* Andrássy, 1985. (LM). A: Neck; B: Anterior end (arrow indicates amphid); C: Anterior end (arrow indicates cephalic seta); D: Vagina; E: Lateral field (arrow); F: Female

rower part. Cheilostom short, gymnostom well cuticularised, wide, slightly arched, stegostom narrower. Pharynx about 170–196 µm long, largely cylindrical; basal bulb spheroid, 29 × 23 µm in size, 1.4–2.9 times cardia length. Anterior part of basal bulb having a *parietinus*-like grinder, with 8–10 pairs of denticulate ridges. Post-bulbar extension about 5–7 µm long. Haustrulum narrow. Cardia 11 µm long, with long process that is shorter than basal pharyngeal bulb. Intestine without granules. Rectum 0.9–1.3 anal body diameters long. Nerve ring at 42–61% of neck length. Secretory-excretory pore just posterior to nerve ring, located at 63–72% of neck length. Deirid at 65–74% of neck length. Reproductive system didelphic, amphidelphic with reflexed ovaries. Uterus tubular, equal to 1.9 of the corresponding body diameters long. Ovaries reflexed dor-

Table 2. Measurements of *Plectus aquatilis* [mean ± standard deviation (range)]. All measurements are in µm.

Locality	Khabr	Dalfard	Ghariatolarab	Baft	Lalezar	Goghar
Province	Kerman	Kerman	Kerman	Kerman	Kerman	Kerman
Habitat	Soil	Soil	Soil	Almond	Soil	Soil
Character	Female	Female	Female	Female	Female	Female
n	7	42	1	1	28	22
Body length	860.8±82.6 (745.0–991.0)	979.7±116.0 (816.0–1224.0)	1013.2	986.8	935.2±90.9 (803.0–1132.0)	1013.2±103.4 (868.0–1237.0)
Body diam. (BD)	37.4±3.4 (35.0–44.0)	45.0±8.5 (29.0–59.0)	41.2	42.6	44.3±11.5 (29.0–71.0)	46.6±8.4 (32.0–60.0)
Pharynx length	181.6±11.4 (170.0–196.0)	212.1±24.9 (134.0–256.0)	209.2	210.7	201.4±23.0 (167.0–251.0)	218.3±17.8 (176.0–248.0)
Tail length	99.2±9.0 (88.0–111.0)	119.3±18.2 (93.0–176.0)	120.6	114.7	112.8±11.4 (93.0–138.0)	116.5±11.3 (94.0–138.0)
Anal body diam.	23.0±3.6 (20.0–30.0)	26.3±3.3 (21.0–34.0)	26.5	25.0	25.3±4.9 (21.0–41.0)	25.1±2.9 (21.0–32.0)
a	22.6±1.9 (20.4–25.9)	22.7±3.2 (16.2–30.1)	24.6	23.1	21.9±3.7 (15.9–27.7)	22.3±3.6 (16.5–27.7)
b	4.1±0.2 (3.9–4.5)	4.3±0.3 (3.6–5.2)	4.3	4.2	4.3±0.2 (3.8–4.8)	4.2±0.2 (3.8–4.5)
c	8.5±1.1 (7.6–10.8)	8.5±1.0 (6.4–10.7)	8.4	8.6	8.3±0.5 (7.1–9.4)	8.6±0.8 (7.5–10.2)
c’	4.4±0.7 (3.0–5.0)	4.6±0.5 (3.5–6.1)	4.6	4.6	4.6±0.7 (3.4–5.6)	4.7±0.4 (4.1–5.5)
Labial region diam.	10.2±2.3 (8.0–15.0)	12.4±1.4 (10.0–16.0)	11.8	13.2	11.1±0.7 (10.0–13.0)	11.6±1.0 (9.0–14.0)
Labial region high	3.3±0.6 (3.0–4.0)	2.3±0.6 (2.0–3.0)	3.0	3.0	2.7±0.6 (2.0–3.0)	2.3±0.6 (2.0–3.0)
Cephalic seta length	2.7±0.6 (2.0–3.0)	2.7±0.6 (2.0–3.0)	3.0	2.0	3.3±0.6 (3.0–4.0)	3.3±0.6 (3.0–4.0)
Stoma length (STL)	22.9±2.1 (20.0–25.0)	22.0±3.0 (15.0–26.0)	26.5	25.0	18.9±1.3 (16.0–21.0)	21.7±3.6 (18.0–31.0)
Stoma diam. (STD)	3.7±0.6 (3.0–5.0)	5.2±0.7 (4.0–6.0)	5.9	5.9	4.6±0.5 (4.0–6.0)	4.6±0.6 (4.0–6.0)
STL/STD	6.3±1.0 (4.6–7.6)	4.3±0.8 (3.3–6.0)	4.5	4.3	4.2±0.6 (3.0–6.1)	4.8±0.8 (3.5–6.6)
Amphid location	13.8±1.8 (11.0–16.0)	13.8±2.5 (11.0–26.0)	16.5	13.2	13.4±1.0 (12.0–16.0)	13.9±1.7 (11.0–16.0)
NR	107.9±10.5 (92.0–119.0)	115.1±10.4 (93.0–134.0)	183.8	119.1	109.6±8.0 (97.0–128.0)	117.8±9.5 (100.0–135.0)
Excretory pore to ant. end.	124±3.6 (120.0–127.0)	120.7±4.0 (117.0–125.0)	128.0	150.0	125.0±5.0 (120.0–130.0)	137.3±7.8 (131.0–146.0)
V	50.4±1.3 (48.2–52.4)	48.3±1.7 (45.5–51.9)	49.4	49.3	48.8±1.9 (45.0–54.3)	48.9±1.8 (46.2–55.1)
v’	57.2±2.1 (53.1–59.7)	54.8±2.0 (50.9–59.2)	56.0	55.8	55.5±2.5 (50.4–63.2)	55.4±2.2 (51.5–62.5)
G1	20.4±2.1 (17.5–22.8)	22.1±3.7 (16.0–30.0)	14.2	25.9	21.1±3.0 (13.9–27.5)	22.4±3.8 (14.4–27.9)
G2	20.9±3.8 (15.9–26.3)	21.7±3.2 (16.9–28.1)	15.7	26.8	21.3±3.1 (15.9–30.2)	22.2±3.4 (14.5–27.4)
Vagina length	14.8±1.4 (13.0–17.0)	16.9±3.5 (12.0–26.0)	16.5	17.6	17.7±3.1 (13.0–24.0)	17.5±2.7 (15.0–24.0)
Vagina/BD	39.6±3.2 (35.7–43.4)	37.8±5.3 (24.3–45.0)	40.0	41.4	40.9±5.7 (31.3–50.7)	38.7±6.0 (25.6–45.5)
Cardia length	13.8±2.6 (10.0–18.0)	14.5±2.1 (9.0–21.0)	12.6	13.2	14.8±2.4 (11.0–19.0)	16.5±3.0 (12.0–24.0)
Cardia width	14.2±2.3 (11.0–18.0)	14.8±2.6 (10.0–22.0)	14.7	13.5	17.9±2.5 (13.0–24.0)	18.2±3.5 (12.0–25.0)
Rectum length	27.4±2.0 (25.0–30.0)	24.5±7.3 (12.0–36.0)	32.4	33.8	27.2±3.7 (21.0–34.0)	26.3±6.1 (15.0–38.0)
Egg length	43.0	–	–	48.5	–	–
Egg width	25.0	–	–	23.5	–	–

sally, anterior ovary on right and posterior ovary on left side of intestine. Both genital branches equally developed; entire reproductive tract (reproductive branches plus reflexed ovaries) 8–10 times longer than mid-body diameter. Vulva a transverse slit, situated at 48–52% of body length from anterior end, vulval lips not protruding. No sperm nor spermatheca observed. Tail 88–111 µm long, conoid in its anterior part and cylindrical in its posterior part, ventrally curved in posterior half with two and four caudal setae on ventral and dorsal side respectively. Spinneret present. Three caudal glands present, arranged in a tandem.

Male. Not found.

Diagnosis of Iranian specimens. Iranian specimens of *P. aquatilis* are characterized by 0.75–1.24 mm long body in females, absence of hypodermal glands, 15–31 µm long stoma, lip region 8–16 µm in diameter, circular amphids located in middle part of stoma or 11–16 µm from anterior end, two lateral incisures, 134–256 µm long pharynx, amphidelphic female reproductive system, tail elongate-conoid (88–176 µm, c = 6.4–10.8, c' = 3.0–6.1 in females) with rounded terminus and functional spinneret, bearing two setae on the ventral and four setae on the dorsal side, including subterminal seta near tail terminus.

Locality and habitat. The specimens examined were found in six localities in the province of Kerman, associated with the rhizosphere of the almond (*Prunus dulcis* L.) and in soil (see Table 2).

Other materials examined. Lalezar, Goghar, Dalfard and Ghariatolarab populations of Kerman province associated with soil sediments (wet soil) and Baft population associated with almond conforms to Khabr population in all characteristics except for having longer body, pharynx and tail length (Table 2).

Remarks. The specimens studied here fit well with the original description of the *P. aquatilis* published by Andrássy (1985), however, they have wider ranges of variability of stoma length (15–31 *vs* 24–26 µm), pharynx length (134–256 *vs* 210–250 µm) and tail length (88–176 *vs* 134–144 µm). The Iranian specimens are similar to those examined by Zell (1993), except in having a relatively longer pharynx (134–256 *vs* 97–153 µm). Population described by Tahseen & Mustaqim (2011) is also very similar to Iranian specimens in measurements and morphology.

This species is reported for the first time from Iran.

***Plectus pusillus* Cobb, 1893** (Figs 3, 4; measurements in Table 3)

Description. Population from Rabor, province of Kerman (7 ♀♀).

Female. Body small, cylindrical, tapering towards both extremities but more pronounced posteriorly; ventrally arcuate upon heat fixation. Cuticle with fine transverse striations, more prominent in pharyngeal and tail regions; annules 0.8 µm wide at mid-body. Lateral field with two ridges (alae); 2.8 µm wide at mid-body, occupying 12.5% of the corresponding body width. Head region narrow, continuous with body contour. Lips rounded, lip region twice as wide as high. Labial sensilla papilliform, visible under light microscope. Four cephalic setae, less than half of lip region diameter, inserted on anteriormost body annule, just posterior to lip base. Amphidial openings circular. Stoma plectoid, 1.7–2.5 times longer than lip region diameter, its broader part about 1.8 longer than narrower part. Cheilostom short, gymnostom well cuticularised, stegostom cylindrical, slightly narrower. Pharynx about 86–112 µm long, largely cylindrical; basal bulb oval in shape, 14 × 11 µm in size. Anterior part of basal bulb having a simple grinder. Haustrulum narrow. Cardia 6 µm long, with long process that is shorter than basal pharyngeal bulb. Intestine without granules. Rectum 1–1.3 anal body diameters long. Nerve ring at 48–55% of neck length. Secretory-excretory pore just posterior to nerve ring, located at 61–76% of neck length. Deirid at 67–83% of neck length. Reproductive system didelphic, amphidelphic with reflexed ovaries. Ovaries reflexed dorsally, anterior ovary on right and posterior ovary on left side of intestine. Uterus tubular, equal to 1.1 of the corresponding body diameters long. Both genital branches equally developed; entire reproductive tract (reproductive branches plus reflexed ovaries) 7–10 times mid-body diameter. Vulva a transverse slit, situated at 50–53% of body length from anterior end, vulval lips not protruding. No sperm nor spermatheca observed. Tail 41–48 µm long, subcylindrical and ventrally curved in posterior half with two caudal setae on each side. Spinneret present. Three caudal glands present, arranged in a tandem.

Male. Not found.

Diagnosis of Iranian specimens. Iranian specimens of *P. pusillus* are characterized by 0.34–0.50 mm long body in females, absence of hypodermal glands, 12–17 µm long stoma, lip region 6–9 µm in diameter, circular amphids located in middle part of stoma or 9–12 µm from anterior end, two lateral incisures, 85–112 µm long pharynx, amphidelphic female reproductive system, tail subcylindrical (41–48 µm, c = 8.5–11, c' = 3.1–4.1 in females) with rounded terminus and functional spinneret, bearing two setae on the dorsal and two setae on the ventral side.

Locality and habitat. The specimens examined were from two localities collected from the province of Kerman, in Rabor, associated with *Juglans regia* L.; and in Lalezar, associated with soil sediments (wet soil) (Table 3).

Other materials examined. Lalezar population of Kerman province associated with soil sediments (wet soil) conforms to Rabor population in all characteristics except for having longer pharynx (Table 3).

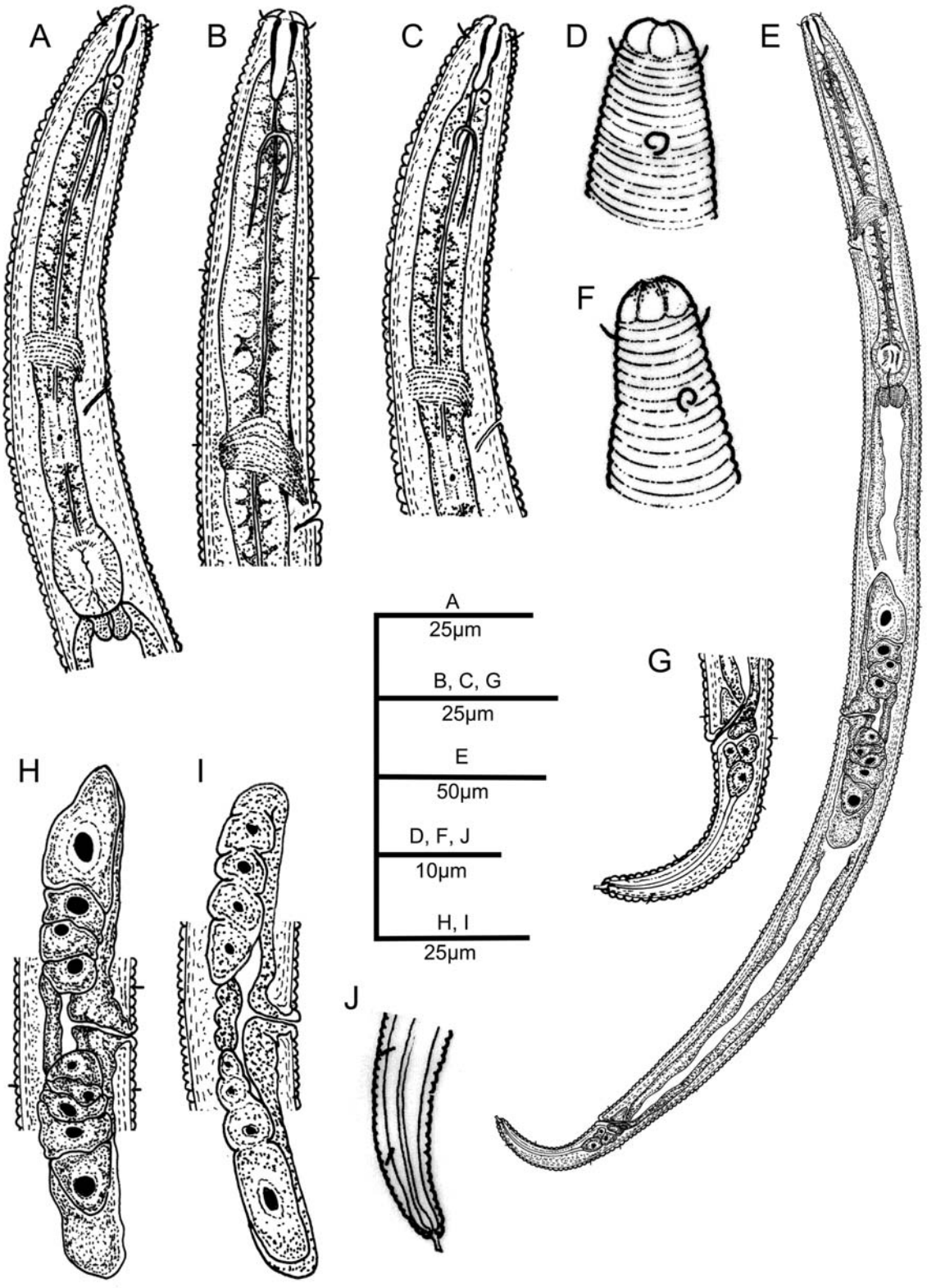

Fig. 3. *Plectus pusillus* Cobb, 1893. A: Neck; B, C: Anterior region; D, F: Anterior end showing lips and amphids (surface view); E: Entire female; G: Female posterior end; H, I: Female reproductive system; J: Terminal part of tail.

Remarks. The material examined agrees well with the morphometrics of populations reported previously (Zell 1993; Andrássy 2005), however, our specimens have somewhat longer body (0.34–0.50 *vs* 0.33–0.44 mm) and shorter pharynx (85–112 *vs* 100–115 µm).

This species is reported for the first time from Iran.

***Plectus velox* Bastian, 1865** (Fig. 5; measurements in Table 3)

Description. Population from Chenarak, province of Alborz (2 ♀♀).

Table 3. Measurements of *Plectus pusillus* and *P. velox* [mean ± standard deviation (range)]. All measurements are in μm.

Species	*P. pusillus*		*P. velox*
Locality	Rabor	Lalezar	Chenarak
Province	Kerman	Kerman	Alborz
Habitat	Walnut	Soil	Cherry
Character	Female	Female	Female
n	7	3	2
Body length	430.4±37.3 (344.0–465.0)	464.9±30.4 (447.0–500.0)	1079.0, 864.0
Body diam. (BD)	21.7±1.7 (18.0–24.0)	20.2±3.5 (16.0–24.0)	66.0, 55.0
Pharynx length	101.5±8.8 (86.0–112.0)	91.5±6.5 (85.0–97.0)	215.0, 183.0
Tail length	44.9±2.6 (41.0–48.0)	45.1±2.2 (43.0–47.0)	79.0, 60.0
Anal body diam.	12.6±1.3 (11.0–15.0)	12.5±2.2 (10.0–15.0)	34.0, 26.0
a	19.6±0.7 (18.7–20.5)	23.4±3.3 (21.3–27.2)	16.3, 15.8
b	3.6±0.1 (3.4–3.8)	4.4±0.4 (4.0–4.7)	4.6, 4.2
c	9.5±0.7 (8.5–10.3)	10.3±0.7 (9.5–11.0)	13.6, 14.3
c'	3.6±0.2 (3.2–3.8)	3.7±0.5 (3.1–4.1)	2.3, 2.3
Labial region diam.	7.7±1.1 (6.0–9.0)	7.8±0.8 (7.0–9.0)	15.0, 14.0
Labial region high	3±1.0 (2.0–4.0)	4±1.0 (3.0.–5.0)	5.0, 6.0
Cephalic seta length	2.1±0.3 (1.8–2.4)	2.1±0.4 (1.7–2.4)	4.0, 5.0
Stoma length (STL)	15.8±0.9 (14.0–17.0)	13.2±1.5 (12.0–15.0)	21.0, 21.0
Stoma diam. (STD)	2.6±0.6 (2.0–4.0)	3.4±0.8 (3.0–4.0)	6.0, 4.0
STL/STD	6.2±1.3 (4.3–8.5)	3.9±0.6 (3.3–4.5)	3.5, 5.6
Amphid location	11.5±0.7 (11.0–12.0)	10.4±1.5 (9.0–12.0)	15.0, 13.0
NR	63.4±3.8 (57.0–69.0)	58.8±2.9 (56.0–62.0)	126.5, 116.0
Excretory pore to ant. end	72±2.6 (70.0–75.0)	62.0±2.6 (60.0–65.0)	132.0, 127.0
V	52.3±0.9 (50.8–53.6)	48.2±3.1 (44.7–50.0)	51.0, 50.0
v'	58.5±1.1 (56.3–59.4)	53.4±3.0 (50.0–55.3)	55.0, 53.0
G1	21.4±2.8 (18.3–26.3)	15.0±0.5 (14.4–15.4)	25.0, 28.0
G2	23.1±4.8 (16.9–30.5)	19.8±1.2 (19.1–21.2)	26.0, 26.0
Vagina length	10.4±1.6 (8.0–12.0)	9.4±3.9 (6.0–14.0)	25.0, 17.0
Vagina/BD	47.9±4.6 (41.0–53.2)	45.4±11.1 (35.7–57.5)	38.0, 31.0
Cardia length	5.6±0.5 (5.0–6.0)	7.3±0.6 (7.0–8.0)	18.0, 17.0
Cardia width	7.5±1.2 (6.0–9.0)	7.8±0.8 (7.0–9.0)	24.0, 17.0
Rectum length	13.4±1.5 (12.0–15.0)	15.2±2.2 (13.0–18.0)	32.0, 31.0

Female. Body cylindrical, tapering towards both extremities but more pronounced posteriorly; ventrally arcuate after fixation. Cuticle with transverse striations; annules 1.1 μm wide at mid-body. Lateral field with three ridges (alae); 9–10 μm wide at mid-body, occupying 14.5% of the corresponding body width. Head region narrow, offset from the body contour. Lips rounded, lip region twice as wide as high. Labial sensilla papilliform, visible under light microscope. Four cephalic setae, less than half of (0.3–0.4) lip region diameter in length, inserted on anteriormost body annule just posterior to lip base. Amphidial openings circular. Stoma plectoid, cylindrical, 1.4–1.5 times longer than lip region diameter, its broader part about 1.2 longer than narrower part. Cheilostom short, slightly cuticularised, gymnostom well cuticularised, stegostom cylindrical. Pharynx about 183–215 μm long, largely cylindrical; basal bulb spheroid (31–35 × 28–31 μm). Postbulbar extension very short. Anterior part of basal bulb with *parietinus*-like grinder. Haustrulum narrow. Cardia almost 0.5–0.6 times longer than basal pharyngeal bulb. Nerve ring at 54–57% of neck length. Secretory-excretory pore just posterior to nerve ring, located at 58% of neck length. Deirid at 64% of neck length. Intestine without granules; rectum, 0.9–1.2 times corresponding body diameter. Reproductive system didelphic, amphidephic with reflexed ovaries. Ovaries reflexed dorsally, anterior ovary on right and posterior ovary on left side of intestine. Uterus tubular, about one corresponding body diameter. Both genital branches equally developed; entire reproductive tract (reproductive branches plus reflexed ovaries) 8.2–8.5 times mid-body diameter. Vulva a transverse slit, situated at 50–51% of body length from anterior end, vulval lips not protruding, with epipthygmata. No sperm nor spermatheca observed. Hypodermal gland present in large number: 61 along ventral and 59 along dorsal side. Tail 60–79 μm long, conoid and ventrally curved in posterior half with two and three caudal setae on ventral and dorsal side respectively (Fig. 5 I). Spinneret present. Three caudal glands present, arranged in a tandem.

Male. Not found.

Diagnosis of Iranian specimens. Iranian specimens of *P. velox* are characterized by 0.86–1.1 mm long body in females, large number of hypodermal glands (61 in ventral and 59 in dorsal side), 21 μm long stoma, lip region 14–15 μm in diameter, circular amphids located at level of middle part of stoma or 13–15 μm from anterior end, three lateral incisures, 183–215 μm long pharynx, amphidelphic female reproductive system, tail conical (60–79 μm, c = 13.6–14.3, c' = 2.3 in females) with functional spinneret, bearing two ventral and three dorsal setae.

Locality and habitat. The specimens examined were

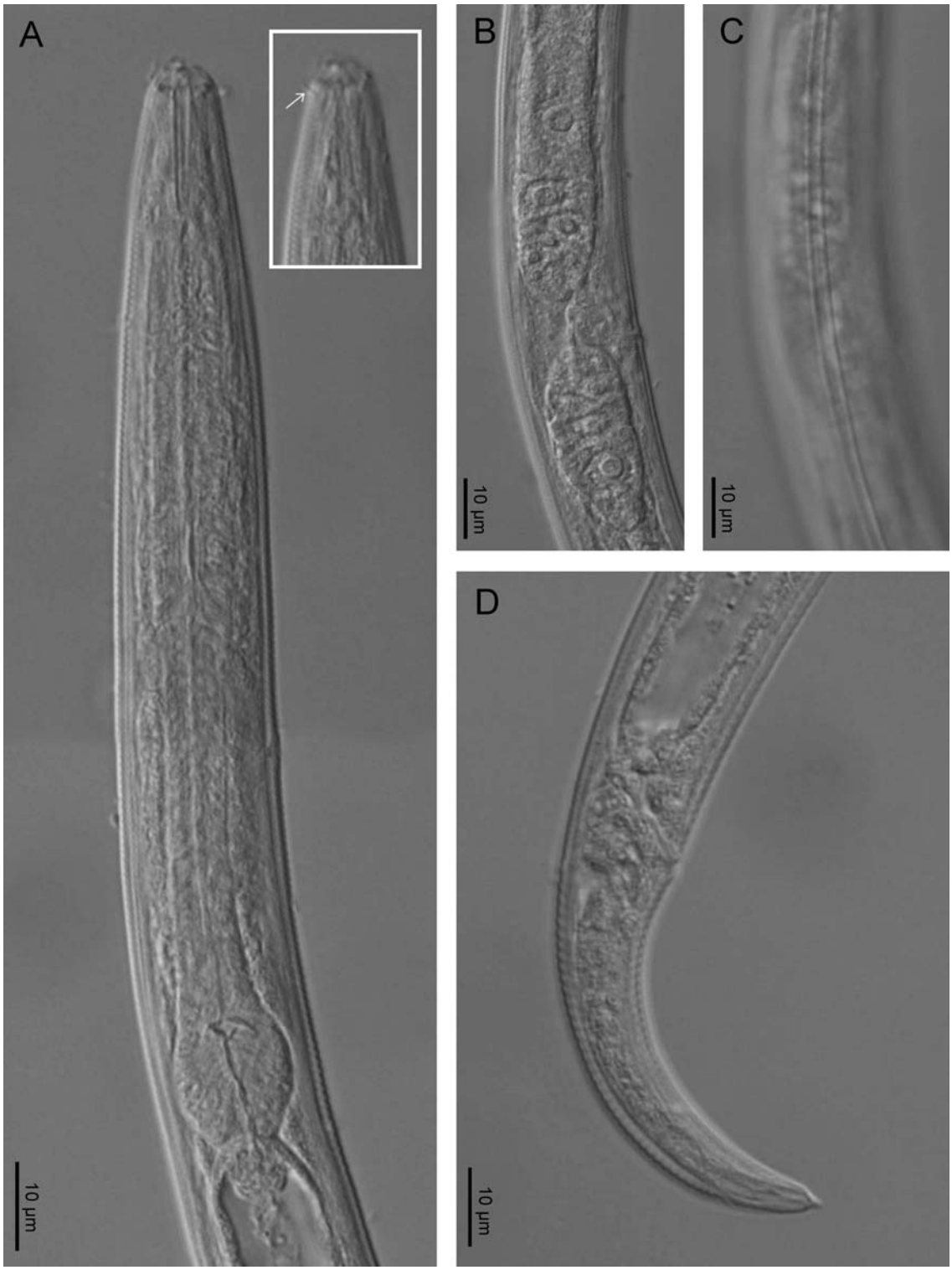

Fig. 4. *Plectus pusillus* Cobb, 1893. (LM). A: Neck (arrow indicates cephalic seta); B: Female reproductive system; C: Lateral field; D: Female posterior end.

found in one population in province of Alborz, associated with *Prunus avium* L. (Table 3).

Remarks. Iranian specimens of *P. velox* fit well with the material examined by Zell (1993), differing only in having shorter pharynx (183–215 *vs* 205–304 µm). In comparison with the material examined by Andrássy (2005), our specimens have somewhat shorter body (*vs* 0.95–1.4 mm) and pharynx (220–270 µm). Furthermore, current specimens are identical morphologically to *P. glandulatus* Tahseen, Baniyamuddin, Hussain & Ahmad 2004 described from India, but, differ in stoma length (21 *vs* 25–40 µm), amphid shape (circular *vs* oval shaped), and arrangement of setae on tail (2 on ventral and 3 on dorsal *vs* 1 on ventral and 3 on dorsal side).

This species is reported for the first time from Iran.

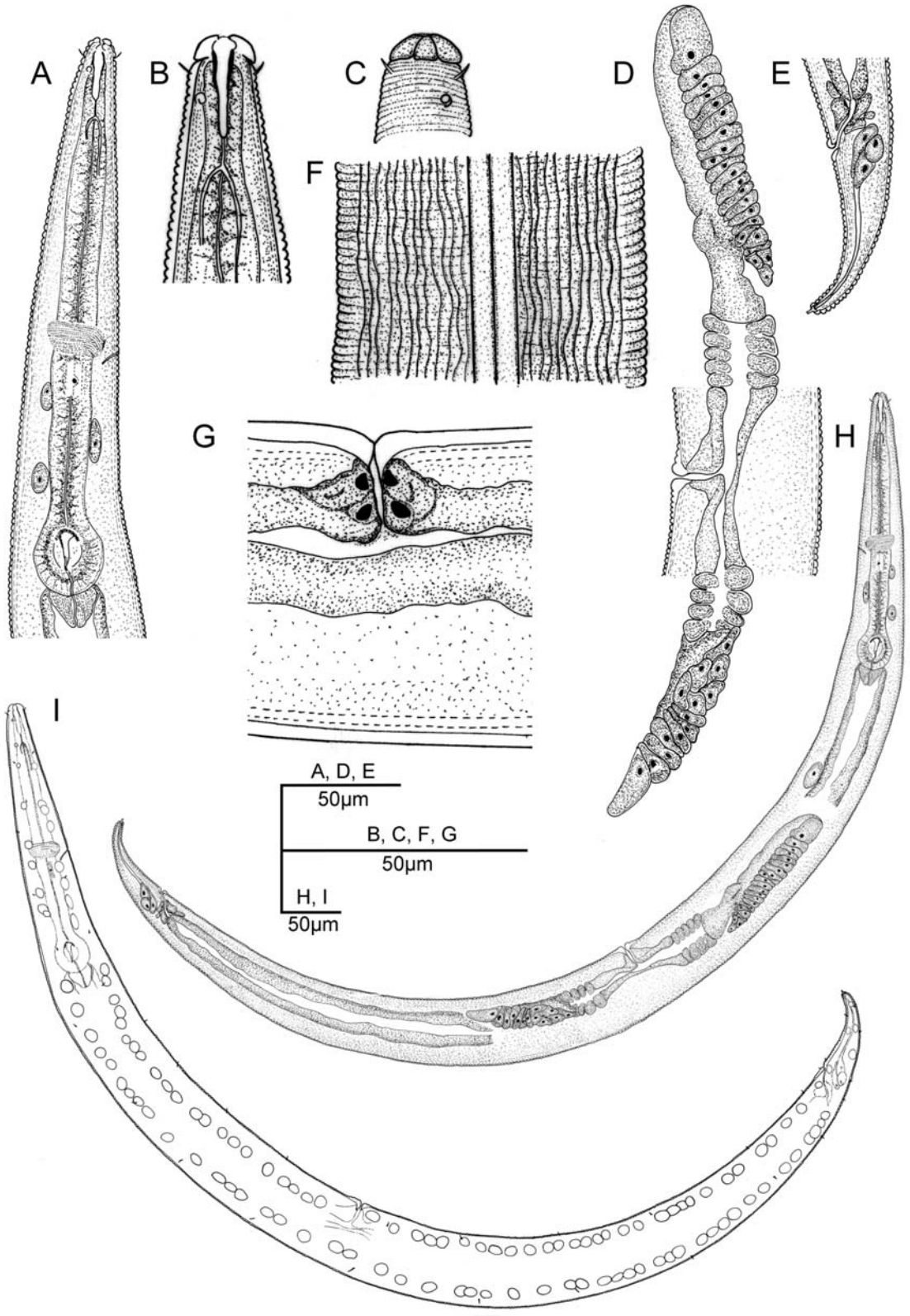

Fig. 5. *Plectus velox* Bastian, 1865. A: Neck; B: Anterior end; C: Anterior end showing lips and amphids (surface view); D: Female reproductive system; E: Female posterior end; F: Cuticle with lateral alae; G: Vagina; H: Entire female; I: Hypodermal glands.

Discussion

DNA characterization

The length of sequences of the 18S regions of *P. aquatilis* and *P. pusillus* was limited by the two primers used (SSU-F-04 and SSU-R-26) and are about 900 base pairs (bps) each. The Blast test demonstrated that Iranian population of *P. aquatilis* has 8 base pairs differences from the UK population of *P. aquatilis* (AF036602; 99% identity), 9 base pairs difference from

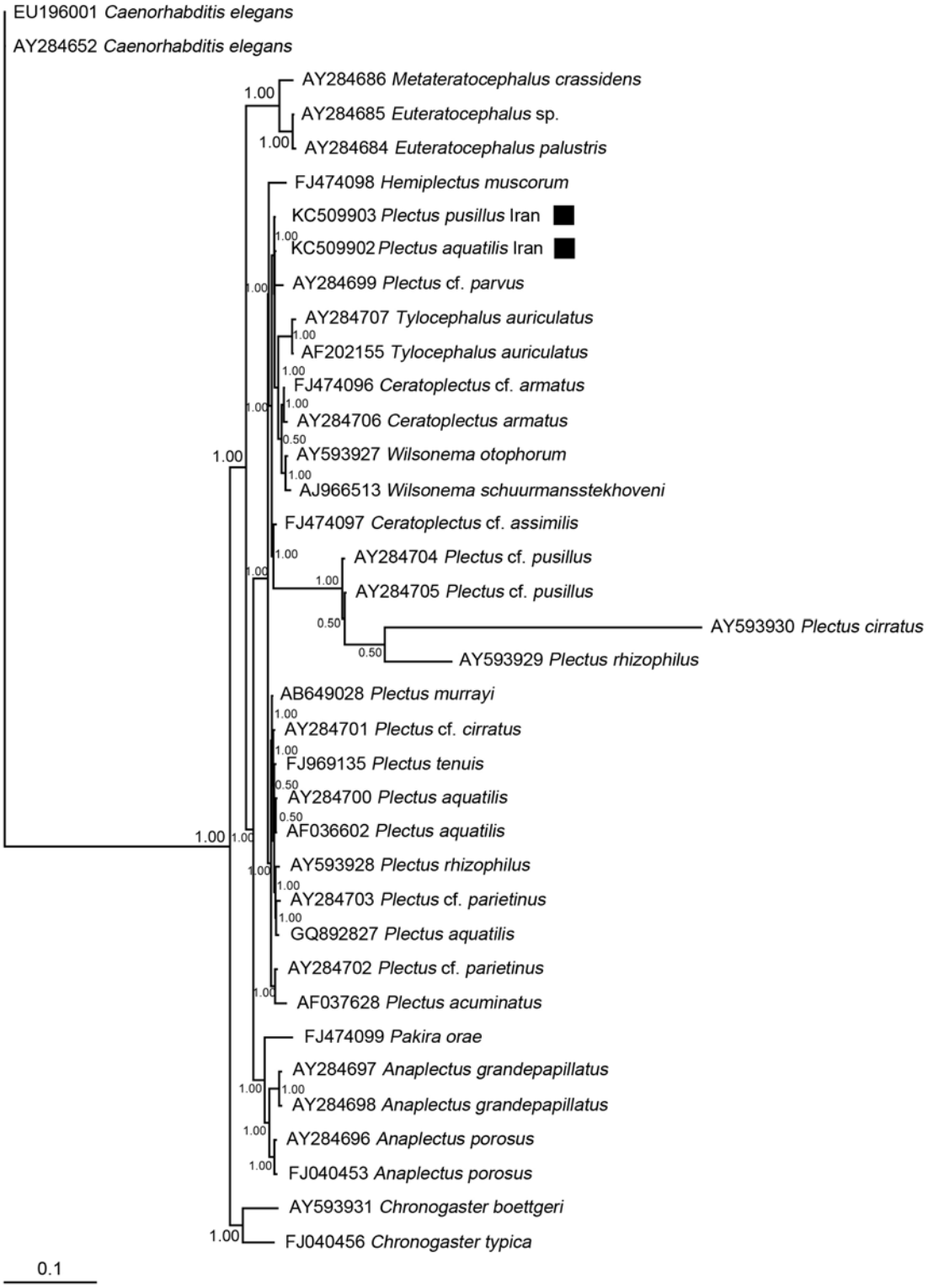

Fig. 6. Bayesian tree inferred from 18S rDNA sequences in the superfamily Plectoidea including *Plectus aquatilis* and *P. pusillus* (square mark) from Iran and sequences of closely related species (NCBI GenBank).

the Belgian population (GQ892827; 99% identity) and 10 base pairs difference from the population from the Netherlands (AY284700; 99% identity). Iranian population of *P. pusillus* showed 14–16 base pairs difference from the population of *P. pusillus* from The Netherlands (AY284705, AY284704; 98% identity, respectively). Thus, the length of the newly obtained SSU rDNA sequences of *P. aquatilis* and *P. pusillus* from Iran is sufficient for comparative and identification purposes.

On the phylogenetic position of the Iranian plectids

The consensus tree inferred from 18S rDNA (Fig. 6) revealed that the superfamily Plectoidea Örley, 1880 is a monophyletic group, agreeing this with the results published by van Megen et al. (2009) and Holovachov (2004). It is divided into four well supported clades: 1) *Chronogaster* spp. (Chronogasteridae) is a sister clade for Plectidae+Metateratocephalidae; 2) *Euteratocephalus* spp. and *Metateratocephalus crassidens* (Metateratocephalidae) comprise a sister clade for Plectidae; 3) *Pakira orae* and *Anaplectus* spp.; 4) *Plectus* spp., *Wilsonema* spp., *Tylocephalus auriculatus* and *Hemiplectus muscorum.*

The present analysis of 18S rDNA sequence indicates close relationship of Iranian plectids with *P.* cf. *parvus* (AY284699) from The Netherlands in partial agreement with Zell (1993), who put *P. pusillus* and *P. parvus* in the *parvus*-group. However, our analyses agrees with the previously published 18S rDNA trees of Plectida in the fact that the 18S rDNA gene alone does not provide any resolution within the genus *Plectus* and that other genes (e.g. LSU rDNA and mitochondrial DNA) need to be explored for the phylogenetic analysis within this group of nematodes.

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