

First Record of Three Known Species of the Family Tylenchidae Örley, 1880 (Nematoda: Tylenchina) from Iran with New Morphological and Molecular Data

Y. Panahandeh¹, E. Pourjam^{1*}, F. Aliramaji¹, M. R. Atighi¹, and M. Pedram¹

ABSTRACT

Three species belonging to three genera of the family Tylenchidae, namely, *Cephalenchus leptus*, *Eutylenchus excretorius*, and *Lelenchus leptosoma* were discovered as first reports for Iran's nematode fauna. They were characterized by morphological, morphometric and molecular phylogenetic studies. The two latter genera are new for Iran. Iranian population of *Cephalenchus leptus* is characterized by lateral field with six lines in females, stylet length of 16-20 μm and tail length of 153-290 μm with pointed tip. The recovered population of *Eutylenchus excretorius* is characterized by 848-1,038 μm long females' body, stylet length of 20.0-21.5 μm , having advulval flaps and absence of male. The Iranian population of *Lelenchus leptosoma* is defined by its narrow slender body, flattened lip region, sinuous amphidial opening and absence of lateral field. The phylogenetic relationships of the three recovered species were studied using the partial sequences of 28S rDNA D2/D3 segment and revealed the genera *Cephalenchus* and *Eutylenchus* forming a monophyletic clade, while, *Lelenchus* was placed inside the clade of currently sequenced species of *Malenchus* in Bayesian tree.

Keywords: Morphometric, Nematode, Northern Iran forests, Phylogeny, Sabalan region, Taxonomy.

INTRODUCTION

An overview on reported tylenchid species from Iran (till 2012) was presented by Ghaderi *et al.* (2012). Morphological and morphometric data of some subsequent studies on other members of the family Tylenchidae Örley, 1880 were recently given by Atighi *et al.* (2013), Ghaemi *et al.* (2012), Panahandeh *et al.* (2014) and Mirbabaei Karani *et al.* (2015).

During surveys in grasslands of the Sabalan region in northwestern Iran and some other locations like northern natural forests, three species belonging to three genera *Cephalenchus* Goodey, 1962, *Eutylenchus* Cobb, 1913 and *Lelenchus* Andrassy, 1954 were recovered and studied

in the present research. The aims of the present study were: (i) to perform a detailed study on morphology of the three recovered species, and (ii) to reconstruct their phylogenetic relationships with other species and genera using the partial sequences of 28S rDNA D2/D3.

MATERIALS AND METHODS

Nematodes were extracted from soil samples using the tray method (Whitehead and Hemming, 1965). For light microscopy (LM) studies, the specimens were heat killed by adding 4% formaldehyde solution and processed to anhydrous glycerine according to De Grisse (1969). Photographs were taken using an Olympus DP72 digital camera

¹ Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

*Corresponding author; e-mail: pourjame@modares.ac.ir



attached to an Olympus BX51 microscope equipped with differential interference contrast (DIC).

For DNA extraction, a single live nematode specimen of the recovered species was picked out, examined on temporary slide, and transferred to a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, QIAGEN Inc., Valencia CA, USA) on a clean slide and crushed using a cover slip. The suspension was collected by adding 20 μ L AE buffer. DNA samples were stored at -20°C until used. Primers for 28S rRNA gene D2/D3 amplification were forward primer D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse primer D3B (5'-TGCGAAGGAACCAGCTACTA-3') (Nunn, 1992). PCR was carried out in a total volume of 30 μ l (16.7 μ l distilled water, 3 μ l 10X PCR buffer, 0.6 μ l 10 mM dNTP mixture, 1.2 μ l 50 mM MgCl_2 , 1.5 μ l of each primer (10 pmol μl^{-1}), 0.6 μ l of Taq DNA polymerase (5 unit μl^{-1} , CinnaGen, Tehran, Iran) and 5 μ L of DNA template). The thermal cycling program was as follows: denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 80 seconds. A final extension was performed at 72°C for 10 minutes. The PCR products were sequenced in both directions using the same primers with an ABI 3730XL sequencer. Newly obtained sequences of the studied species were deposited into the GenBank (accession number KP730040 for *Cephalenchus leptus*, KP730041 for *Eutylenchus excretorius* and KP730042 for *Lelenchus leptosoma*).

For molecular phylogenetic studies, the sequences of D2/D3 fragment of 28S rDNA available in GenBank were obtained. The newly generated DNA sequences and the selected sequences from the GenBank database were aligned by ClustalX2 software using default parameters (<http://www.clustal.org/>). The model of base substitution was selected using MrModeltest 2 (Nylander, 2004). The Akaike-supported model, a general time reversible model,

including among-site rate heterogeneity and estimates of invariant sites (GTR+G+I) was used in both phylogenetic analyses. Bayesian analysis was used to infer a phylogenetic tree using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003), running the chains for 10^6 generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to determine equilibrium distribution and help estimate the posterior probabilities of the phylogenetic tree (Larget and Simon, 1999) using the 50% majority rule. The maximum likelihood (ML) tree was reconstructed using the software RaxML GUI version 1.1 (Silvestro and Michalak, 2012). The Bayesian posterior probability (BPP) and ML boot strap (BS) values higher than 50% were given on appropriate clades in the form: BPP/ML BS. The output files of the phylogenetic programs were visualised using Dendroscope version 3.2.8 (Huson and Scornavacca, 2012) and re-drawn in CorelDRAW[®] software version 16. For both Bayesian inference (BI) and ML analyses, the species *Aphelenchus avenae* Bastian, 1865 (accession number JQ348400) was used as outgroup taxon. The sequences/isolates of Tylenchidae was selected with exclusion of the genus *Psilenchus* de Man, 1921 with regarding its most close phylogenetic affinity with members of Merliniinae Siddiqi, 1971 (Carta et al., 2010; Subbotin et al., 2006).

RESULTS AND DISCUSSION

Descriptions

Iranian population of *Cephalenchus leptus* Siddiqi, 1963

(Figures 1 and 2, Table 1)

Female: Body straight to slightly curved ventrally after heat fixation. Cuticle 0.7-1.0 μm thick at mid-body, in stylet region varying to 1.0-1.2 μm , coarsely annulated,

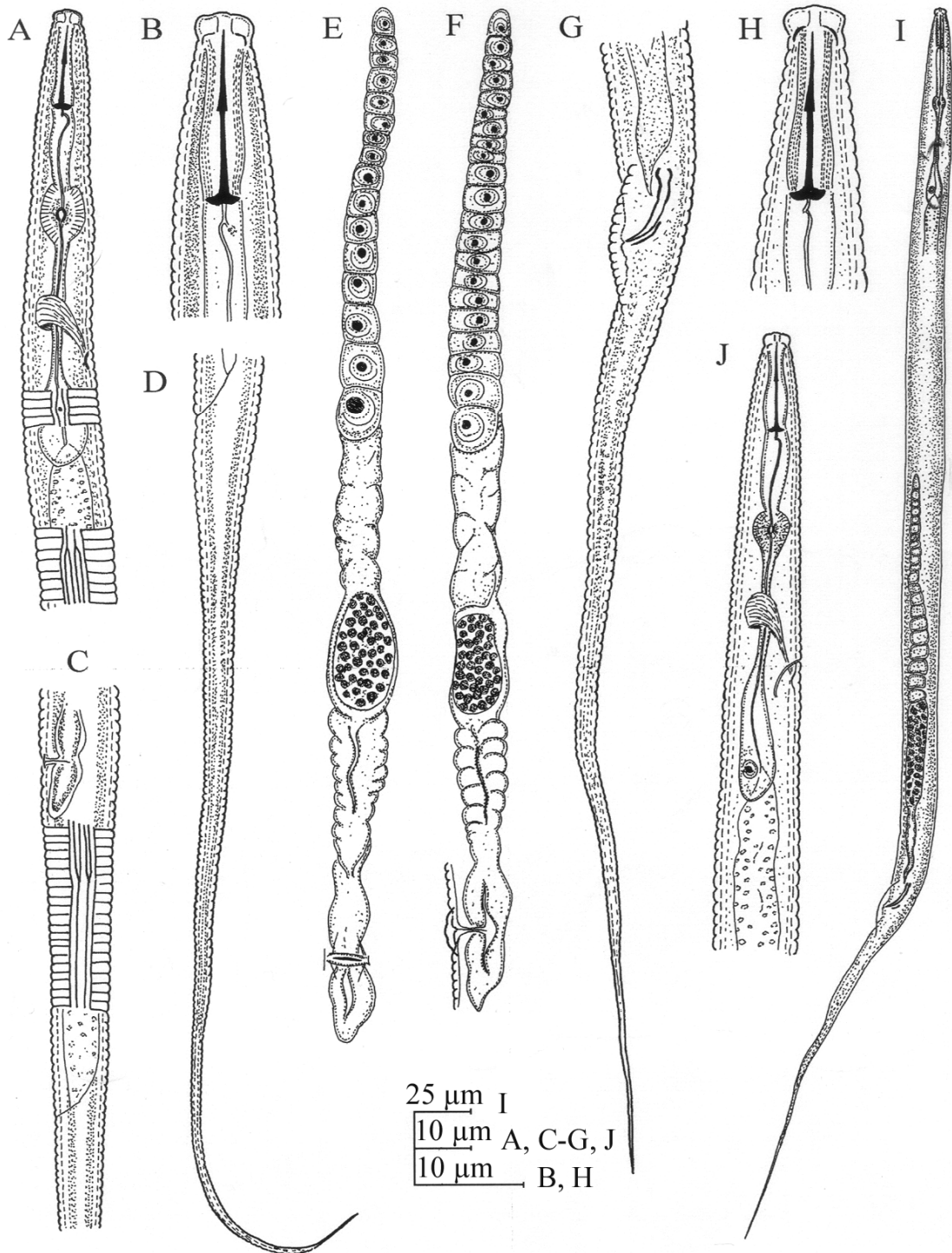


Figure 1. Iranian population of *Cephalenchus leptus*. (A and J) Female pharyngeal region; (B and H) Female anterior end; (C) Female vulva region; (D) Female tail; (E and F) Female reproductive system; (G) Male tail and cloacal region; (I) Male entire body.

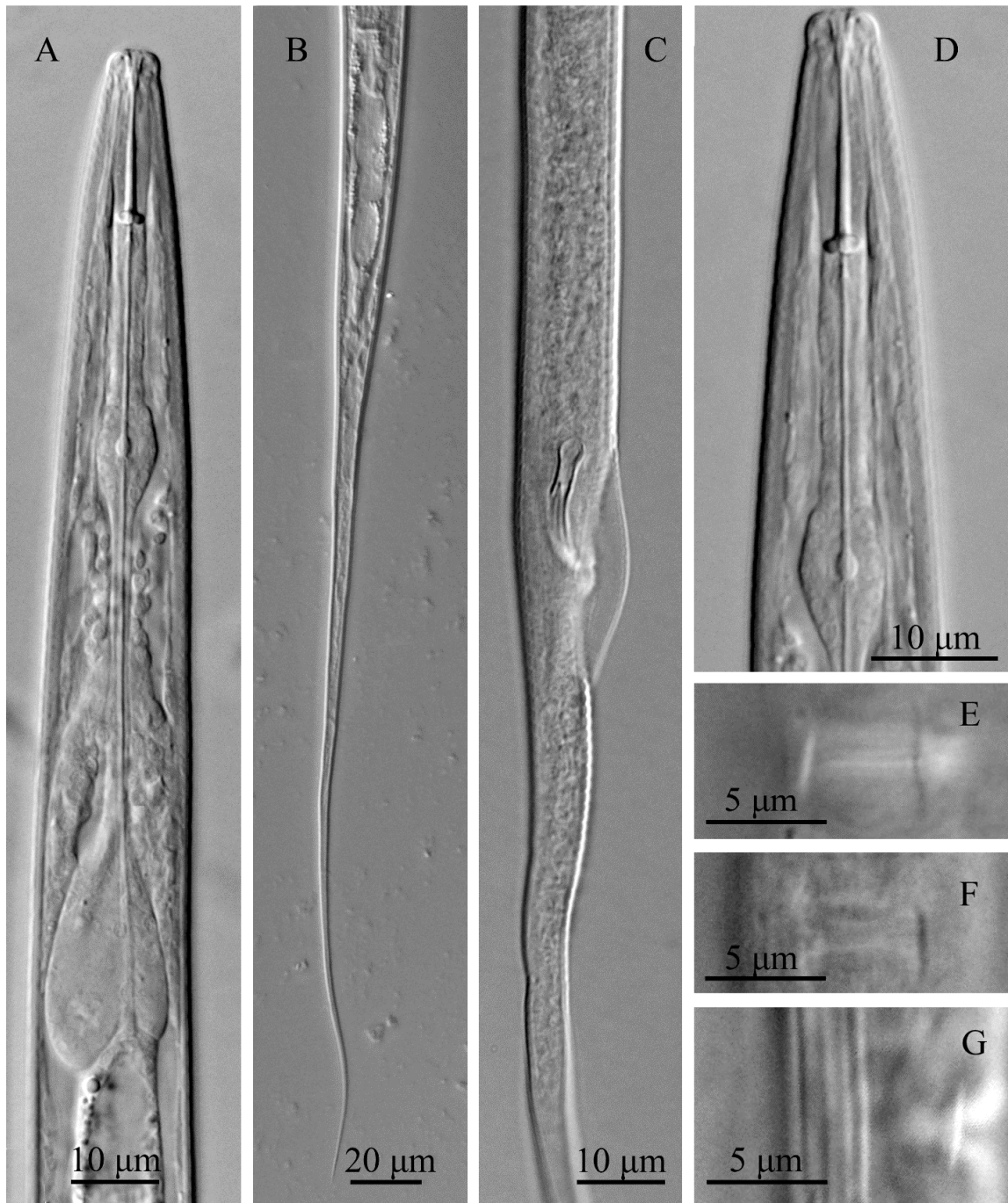


Figure 2. Iranian population of *Cephalenchus leptus*. (A) Female pharyngeal region; (B) Female tail; (C) Male cloacal region; (D) Female anterior end; (E and F) Female vulva region showing advulval flaps; (G) Female lateral field.

annuli 1.9-2.3 μm wide at mid-body. Lateral field with six lines, outer lines crenate. Lip region 1.5-1.8 times wider than high with rounded corners in lateral view, separated from the rest body by a shallow constriction,

bearing 3 annuli. Cephalic framework weak, with distinct vestibule. Stylet well developed, 3.8-4.2 times of body diam. at the level of cephalic region, cone about 37.5-51.5% of total stylet length,

Table 1. Morphometrics for six Iranian populations of *Cephalenchus leptus*. All measurements are in μm , and in the form: Mean \pm SD (range).

Origin Character	Present study										Total range Females
	Hamedan-Ekbatan Females	Hamedan-Ganjnameh Females	Tonekabon Females	Ramsar Female	Kahnoj Females	Jiroft Females	Males	Jiroft Females	Males	Total range Females	
n	2	6	6	1	3	6		4		28	
L	(775, 778) (45.8, 51.7)	749.8 \pm 73.7 (632-835) 50.3 \pm 7.1 (45.0-64.2)	647.5 \pm 44.0 (609-727) 48.1 \pm 3.8 (44.3-53.9)	723 51.6	651.3 \pm 9.0 (641-657) 48.4 \pm 3.6 (45.8-52.6)	634.2 \pm 73.0 (545-724) 42.5 \pm 1.3 (40.3-44.3)	619.0 \pm 43.6 (569-660) 44.9 \pm 3.1 (40.8-47.4)				(545-835) (40.3-64.2)
a	(6.7, 7.0)	6.6 \pm 0.5 (5.6-6.9)	6.3 \pm 0.4 (5.8-6.7)	6.8	6.5 \pm 0.0 (6.4-6.5)	6.7 \pm 0.3 (6.4-7.2)	7.2 \pm 0.5 (6.6-7.8)				(5.6-7.2)
b	(3.1, 3.2)	3.1 \pm 0.1 (2.9-3.3)	3.1 \pm 0.3 (2.8-3.6)	3.1	3.1 \pm 0.1 (3.0-3.2)	3.6 \pm 0.1 (3.4-3.7)	3.5 \pm 0.4 (3.2-4.0)				(2.8-3.7)
c'	(21.8, 42.0)	22.5 \pm 4.8 (16.4-29.0)	24.4 \pm 3.5 (20.1-28.9)	23.1	24.9 \pm 1.6 (23-26)	18.5 \pm 1.9 (16.7-21.0)	17.5 \pm 2.0 (14.8-19.3)				(16.4-42.0)
V	(56.6, 58.3)	56.0 \pm 1.9 (54.0-58.9)	56.8 \pm 1.8 (55.0-60.3)	56.3	55.6 \pm 0.9 (54.6-56.3)	60.0 \pm 1.4 (58.3-61.6)	54.0 \pm 6.1 (16-20)				(54.0-61.6)
Stylet	(17, 19)	18.0 \pm 1.0 (17-20)	17 \pm 1 (16-18)	19	16.7 \pm 0.3 (16.5-17.0)	17.5 \pm 1.0 (16-19)	17.3 \pm 1.0 (16-18)				(16-20)
Pharynx	(110, 116)	114.3 \pm 4.7 (109-121)	102.2 \pm 5.0 (96-110)	107	100.7 \pm 1.5 (99-102)	94.0 \pm 7.7 (82-101)	86.3 \pm 8.8 (73-91)				(82-121)
Head-vulva	(440, 452)	420.0 \pm 43.8 (347-464)	367.2 \pm 20.2 (344-400)	407	362.0 \pm 4.5 (358-367)	380.7 \pm 43.2 (322-436)					(322-464)
Body width	(15, 17)	15.0 \pm 1.3 (13-16)	13.5 \pm 1.0 (12-15)	14	13.5 \pm 1.0 (12.5-14.0)	15.0 \pm 1.5 (13-17)	14.0 \pm 1.7 (12-16)				(12-17)
Vulva-anus	(83, 86)	80.5 \pm 7.3 (72-89)	70.7 \pm 5.0 (65-80)	85	78.3 \pm 10.0 (72-90)	74.7 \pm 10.3 (64-92)					(64-92)
Tail	(240, 252)	244.5 \pm 30.0 (213-290)	209.7 \pm 29.5 (171-262)	231	211 \pm 6 (207-218)	178.8 \pm 22.0 (153-210)	179.5 \pm 24.0 (148-202)				(153-290)
Tail/V-A	(3, 3)	3.0 \pm 0.1 (2.9-3.3)	3.0 \pm 0.5 (2.4-4.0)	2.7	2.7 \pm 0.5 (2.3-3.0)	2.5 \pm 0.1 (2.3-2.5)					(2.3-4.0)
PUS	(16, 19)	19.5 \pm 2.0 (17-22)	16.3 \pm 2.0 (14-20)	19	16.5 \pm 1.8 (15.0-18.5)	16.5 \pm 1.5 (14-18)					(14-22)



knobs anteriorly concave, posteriorly convex, not directed backward. Pharyngeal dorsal gland orifice 1-2 μm from stylet base. Procorpus short, as long as stylet length, posteriorly joining to a well-developed muscular metacarpus with a prominent valve 34-50 μm from anterior end (MB 40-47%), isthmus slender and narrow, posterior bulbular region pyriform, slightly dorsally overlapped in six examined females. Nerve ring encircling middle of isthmus. Deirids small, at level of anterior half of isthmus. Excretory pore slightly posterior to nerve ring position, 63-88 μm from lip region, duct moderately cuticularized. Hemizonid distinct, 2-3 annuli anterior to excretory pore. Genital tract single, straight, spermatheca oval, 10-14 μm long, filled with spheroid sperm (in Jiroft and Ganjnameh populations), postvulval uterine sac 1.0-1.5 times the corresponding body width long, vagina perpendicular to body axis, with a thin cuticular lining 42-48% of the corresponding body diam., vulva a transverse slit, advulval flaps present, varying in size from large (in Jiroft population) to small. Tail long and filiform with pointed terminus.

Male: Rare (found only in Jiroft population). General morphology similar to that of female, except in sexual organs. Straight from anterior end to cloaca and ventrally bent in tail, after fixation. Bursa small, 29-32 μm long with crenate margin, spicules tylenchoid, 15.0-16.5 μm long, gubernaculum simple, 5.5-6.5 μm long. Cloacal lips slightly raised.

Remarks

All six studied populations of *Cephalenchus leptus* corresponded well with the original description (Siddiqi, 1963). In six females from three populations (Ekbatan, Ganjnameh and Ramsar), the terminal bulb overlapped slightly the intestine dorsally and in two populations from Jiroft and Ganjnameh, spermatheca was filled with spheroid sperm. Females of all populations had advulval flaps

with different sizes and male was found in one population (Jiroft population). Compared to the original description and the data given by Geraert (2008), the examined populations showed no remarkable differences. Some individuals of the studied population were also deposited in the USDA Nematode Collection, Beltsville, MD, USA.

Iranian population *Eutylenchus excretorius* Ebsary and Eveleigh, 1981

(Figures 3 and 4, Table 2)

Female: Body cylindrical, tapering towards both ends, straight after fixation. Cuticle *ca* 1.2-1.5 μm thick, coarsely annulated, annuli 1.0-1.5 μm wide at mid-body, longitudinal ridges 12 at mid-body, divided by deep transverse grooves into separate blocks. Lip region flattened, without conspicuous annuli, distinctly separated from the rest of body by a deep constriction. Lip region with four separated lobes in en-face view, each lobe with elongated and flexible setae, 10-12 μm long. Oral disc prominent. Stylet long, 2.3-3.1 times as the corresponding lip region width, conus needle like, sclerotized, comprising 37-45% of entire stylet length, shaft slender, knobs well developed and slightly directed posteriorly. Dorsal pharyngeal gland orifice *ca*. 3 μm posterior to the knobs. Procorpus cylindrical, median bulb oval, muscular with refractive valves, located at anterior half of pharynx, isthmus slender, basal bulb elongated and saccate. Nerve ring encircling anterior portion of isthmus. Excretory pore at the level of anterior half of basal bulb. Reproductive system monodelphic, prodelphic, ovary with oocytes mostly in simple row, oviduct short, spermatheca indistinct and empty, crustaformeria distinct, uterus simple, postvulval uterine sac spacious, 1.0-1.6 times the corresponding body diameter, vulva a transverse slit, surrounded by advulval flaps. Tail long, conoid, gradually tapering to a finely rounded or pointed terminus. Longitudinal blocked ridges ending at middle of tail, the rest tail with fine annuli.

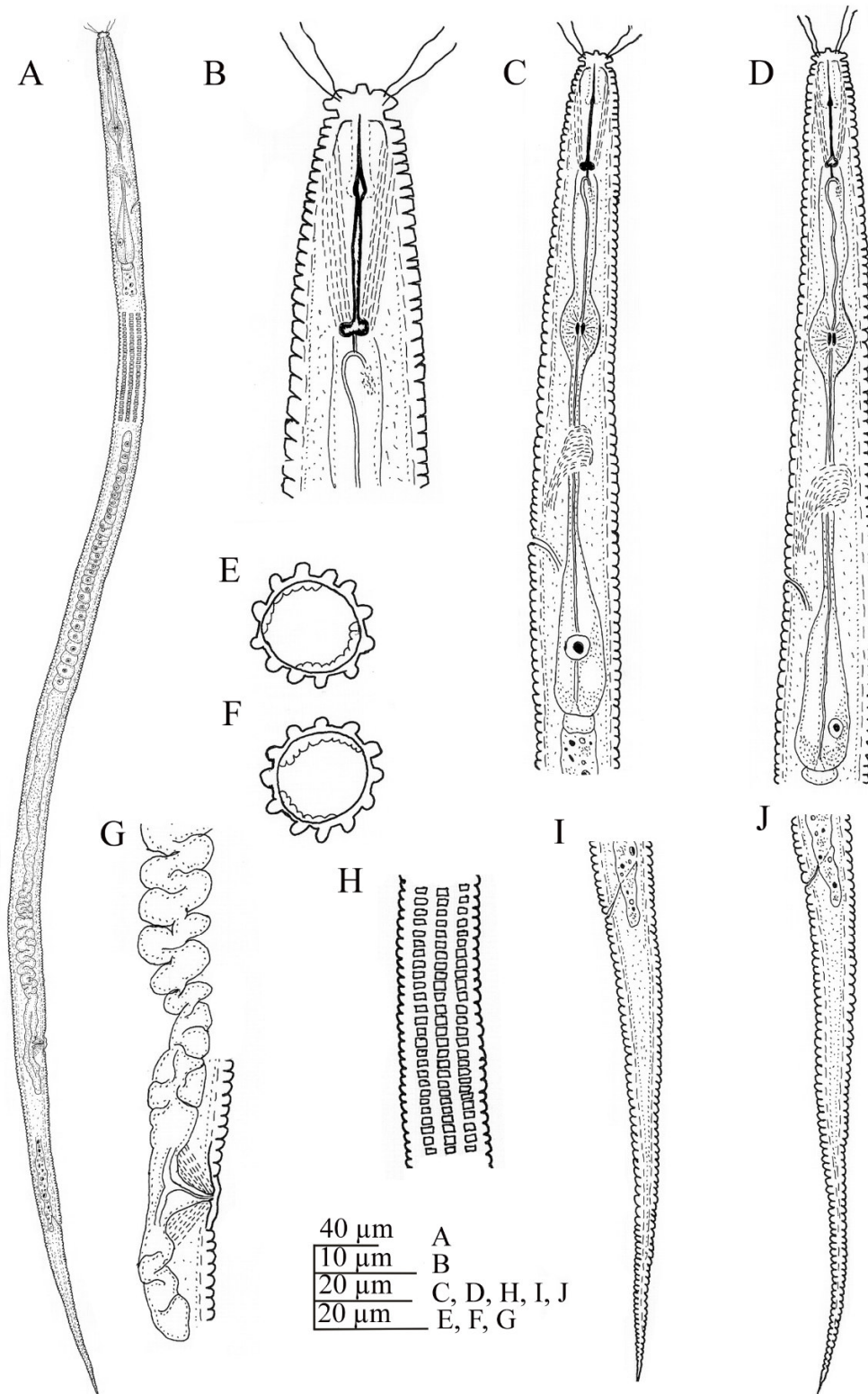


Figure 3. Iranian population of *Eutylenchus excretorius*. Female. (A) Entire body; (B) Anterior end; (C and D) Pharyngeal region; (E and F) Mid-body cross section; (G) Vulva; (H) Cuticular ornamentation; (I and J) Tail.

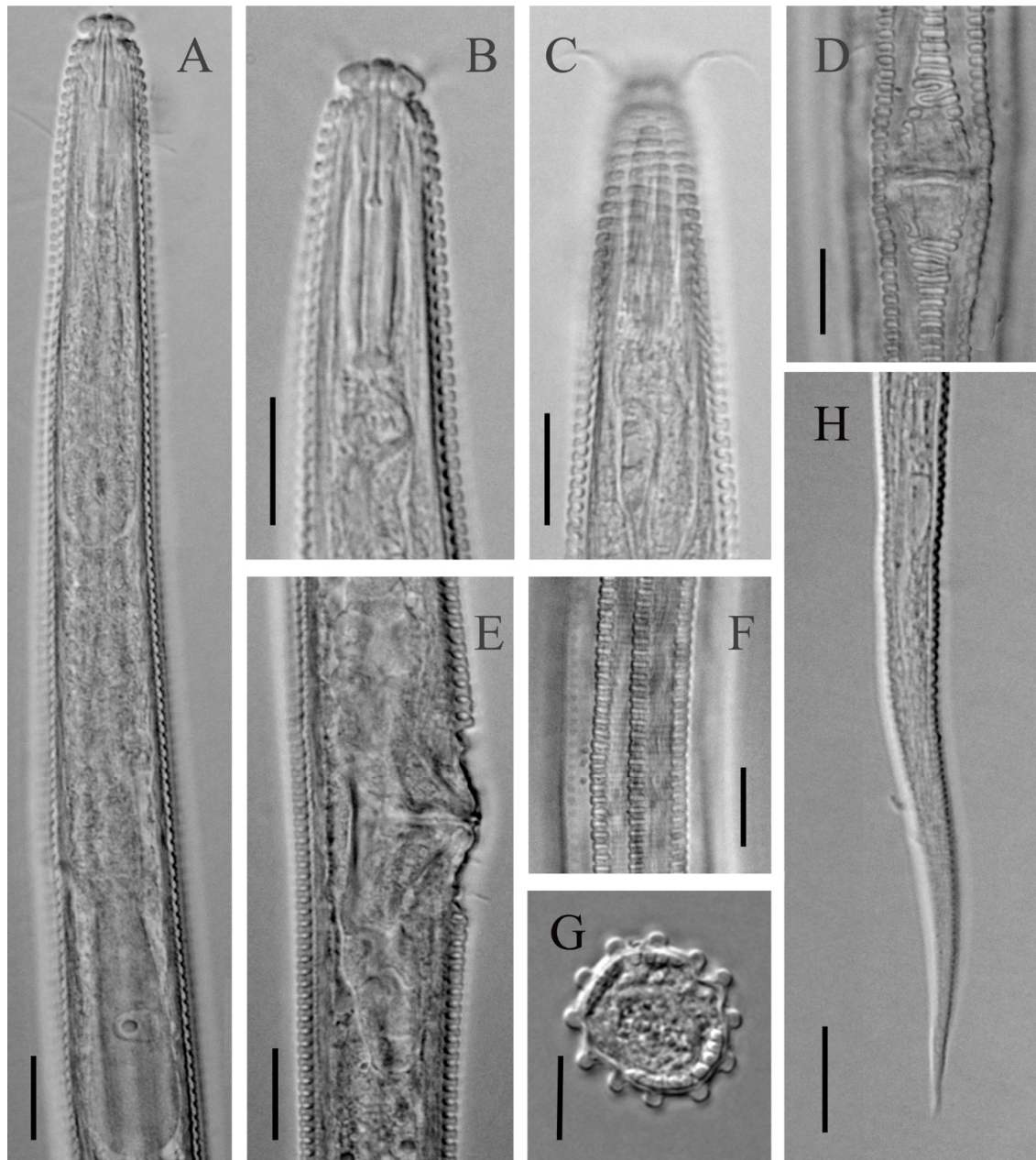


Figure 4. Iranian population of *Eutylenchus excretorius*: Female. (A) Pharyngeal region; (B) Anterior end; (C) Anterior end showing cephalic setae; (D) Vulva, ventral view; (E) Vulva, lateral view; (F) Cuticle surface; (G) Mid-body, cross section; (H) Tail end. All scale bars= 10 μ m.

Male: Unknown.

Remarks

Eutylenchus excretorius was reported from Germany, Russia, Poland (Geraert, 2008)

and Spain (Palomares-Rius *et al.*, 2009). This is the first representative of the genus occurring in Iran. It was recovered from the rhizosphere of unidentified grasses growing in a natural marsh in Sabalan region, northwest of Iran. Morphological characters and ranges of morphometric data of the studied population of this species are in full

Table 2. Morphometrics of Iranian population of *Eutylenchus excretorius* and the data from other reports. All measurements are in μm and in the form: Mean \pm SD (range).

Origin	Present study	Brzeski (1996)	Palomares -Rius (2009)
Character	Females	Females	Females
n	10	20	20
L	945 \pm 51 (848-1038)	879 \pm 35.5 (821-932)	820 \pm 20.9 (791-858)
a	43.2 \pm 3.1 (36.5-46.5)	39.9 \pm 3.1(34-47)	39.7 \pm 1.4(37.5-41.9)
b	6.6 \pm 0.4 (6.0-7.2)	6.1 \pm 0.2 (5.5-6.3)	6.3 \pm 0.3(5.5-6.9)
c	7.9 \pm 0.5(7.3-9.0)	8.0 \pm 0.5 (7.3-9.0)	7.4 \pm 0.3(6.8-7.8)
c'	10.0 \pm 1.0 (8.8-11.8)	8.9 \pm 0.8 (7.6-10.6)	9.0 \pm 0.5 (8.3-9.8)
V	74.6 \pm 1.0 (72.9-75.8)	74.3 \pm 0.9(73-76)	73.4 \pm 0.8(72-74)
Stylet	21.0 \pm 0.4 (20.0-21.5)	21.2 \pm 0.9 (20-23)	21.0 \pm 0.6(20-22)
MB	41.2 \pm 1.1 (38.9-42.6)	38.7 \pm 1.5 (37-41)	41.3 \pm 1.6(39-44)
E. pore	111.1 \pm 4.7 (104-119)	102.0 \pm 7.3(94-116)	90.0 \pm 4.2 (83-98)
Pharynx	143.2 \pm 4.2 (136-149)	145.0 \pm 4.6(135-152)	–
Head-vulva	705.8 \pm 43.4 (618-786)	–	–
Body width	22.0 \pm 1.9 (20-26)	–	–
Vulva - anus	120.8 \pm 9.8 (109-137)	–	104.0 \pm 5.9(98-113)
Tail	119.8 \pm 5.9(112-129)	110 \pm 8.3 (98-127)	111.0 \pm 3.7 (107-118)
Tail/V-A	1.0 \pm 0.1 (0.8-1.2)	–	–
PUS	26.9 \pm 5.4 (20-33)	–	–

agreement with the data given in other reports (see Table 2). Some individuals of the studied population were also deposited in the USDA Nematode Collection (Beltsville, MD, USA).

Lelenchus leptosoma (de Man, 1880)
Andrássy, 1954

(Figures 5 and 6, Table 3)

Female: Body narrow, slender and slightly arcuate ventrally after heat relaxation. Cuticle thin, indistinctly striated. Lateral fields inconspicuous. Lip region continuous and smooth, 2-3 μm wide at base and 4-5 μm high. Amphidial aperture long and sinuate (see Figure 6). Stylet delicate, conus 33-43% of the total length, knobs small and rounded. Dorsal pharyngeal gland orifice 1.0-1.5 μm from stylet base. Procorpus narrow, cylindrical, metacarpus spindle shaped with faint valve apparatus, isthmus very slender and elongated, basal bulb pyriform. Nerve ring

enveloping isthmus at mid-point. Excretory pore slightly posterior to nerve ring level. Reproductive system monodelphic-prodelphic, ovary with single row of oocytes, composing 19.4-25.7% of total body length, spermatheca oval, filled with small spheroid sperm, postvulval uterine sac short, 5-9 μm long, vulva a transvers slit, vagina with thin walls, slightly directed anteriorly. Tail filiform with very finely pointed tip.

Male: General morphology similar to that of female, except in sexual organs. Testis single, anteriorly outstretched, 132-176 μm long. Spicules tylenchoid, slender and faintly curved. Gubernaculum simple, 3-4 μm long. Caudal alae adanal, with smooth margin, 24-29 μm long. Tail filiform as in female.

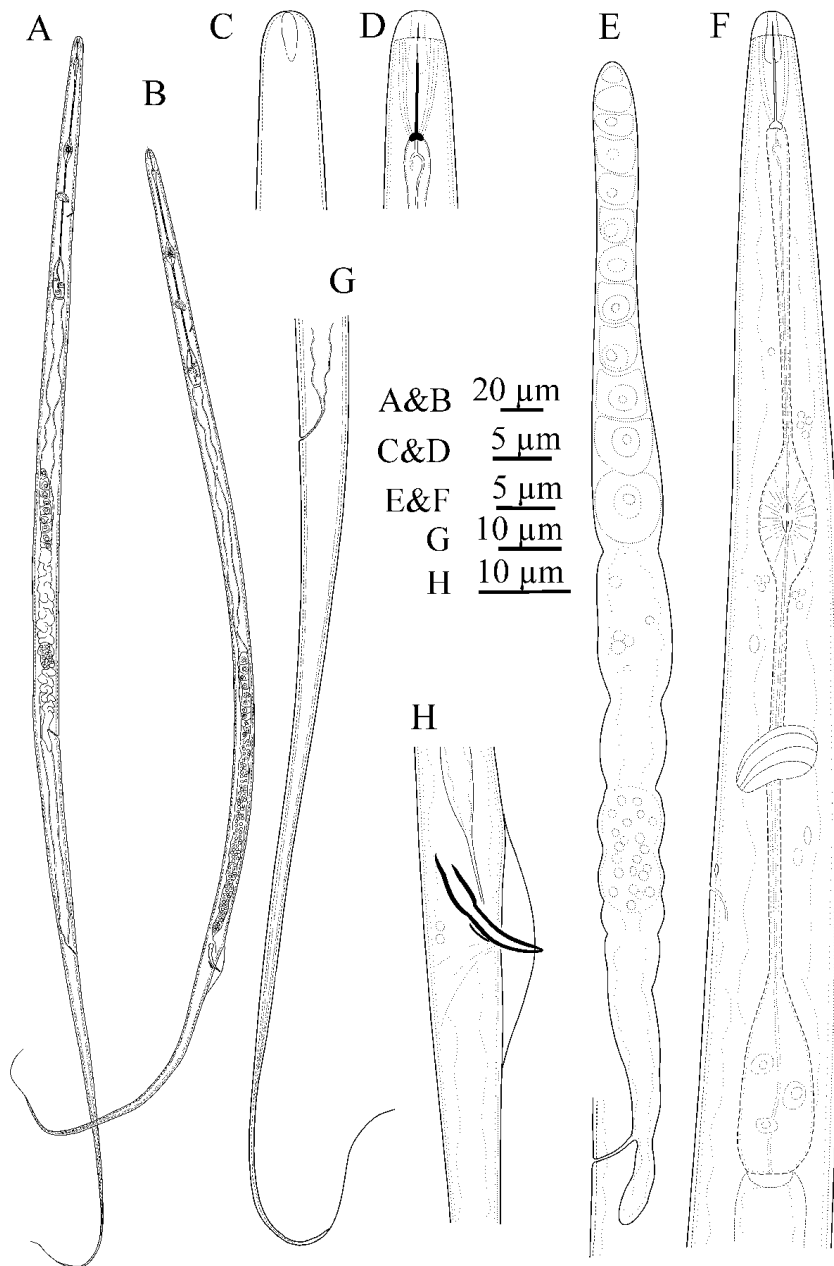


Figure 5. Iranian population of *Lelenchus leptosoma*. (A and B) Female and male entire body; (C) Amphidial pouch; (D) Details of anterior end; (E) Female reproductive system; (F) Pharynx; (G) Female tail; (H) Male cloacal region.

Remarks

The studied population of *Lelenchus leptosoma* was recovered from soil samples of grasslands of Aghamali Gunei, Sabalan region, northwestern Iran. The morphological and morphometric characters

are in agreement with other data reported for the species (see Table 3). Lacking of prominent lateral field is the main character for this species. Some individuals of the studied population were also deposited in the USDA Nematode Collection (Beltsville, MD, USA).

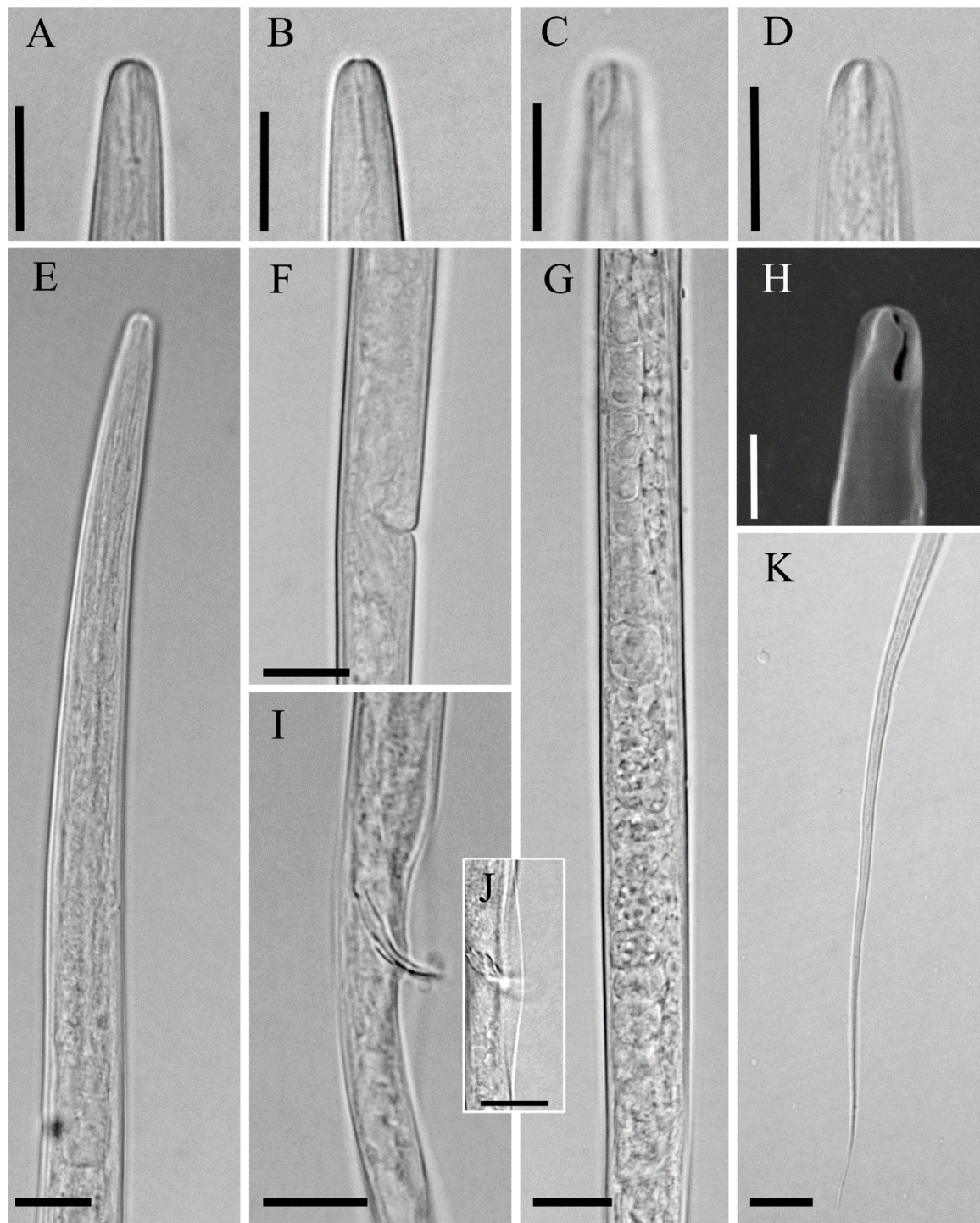


Figure 6. Iranian population of *Lelenchus leptosoma*. (A and B) Female anterior end; (C) Female amphidial aperture; (D) Female amphidial pouch; (E) Female pharyngeal region; (F) Female vulva region; (G) Female reproductive system; (H) Scanning electron microscopy of amphidial aperture (female); (I) Male cloacal region, (J) Male caudal alae; (K) Female tail tip. All scale bars= 10 µm, H= 4 µm.

Table 3. Morphometrics of Iranian population of *Lelenchus leptosoma* and the data from other reports. All measurements are in μm and in the form: Mean \pm S.D (range).

Origin	Present study		Raski and Geraert, 1986		Mizukubo, 1989	
	Females	Males	Females	Male	Females	Females
n	10	5	5	1	20	
L	550 \pm 27 (510-585)	535 \pm 31 (485-570)	571 \pm 126 (475-776)	536	506 \pm 21.2(457-540)	
a	48.3 \pm 4.8 (42.5-58.0)	50.7 \pm 5.0 (44.1-57.0)	57 \pm 7.85 (50-67)	-	47.1 \pm 3.0 (39.4-54.0)	
b	5.1 \pm 0.2 (4.7-5.4)	4.8 \pm 0.2 (4.6-5.0)	5.5 \pm 0.59 (4.9-6.3)	5.7	5.3 \pm 0.23 (4.8-5.9)	
c	3.4 \pm 0.1 (3.1-3.6)	3.2 \pm 0.1 (3.1-3.3)	2.9 \pm 0.29 (2.6-3.3)	2.7	2.9 \pm 0.21 (2.7-3.4)	
c'	21.8 \pm 2.4 (18.1-25.0)	22.2 \pm 2.3 (19.1-24.6)	35 \pm 10.08 (23-41)	27.9	27.0 \pm 2.6 (20.2-29.9)	
V or T	54.5 \pm 1.4 (51.9-56.4)	28.9 \pm 1.9(26.5-30.9)	50 \pm 2.67 (47-53)		50.6 \pm 1.7(48.8-54.8)	
Stylet	7.7 \pm 0.4 (7.0-8.0)	7.8 \pm 0.4 (7.0-8.0)	7.0-8.0	7	8.1 \pm 0.25 (7.6-8.6)	
MB	42.7 \pm 1.4 (41-45)	44.4 \pm 1.6 (42.9-46.8)	-	-	42.0 \pm 1.1 (40.1-45.3)	
E. pore	80.3 \pm 4.2 (75-86)	79.6 \pm 4.4 (72-83)	68 \pm 0.87(67-69)	75	65.2 \pm 1.5 62-68)	
Pharynx	107.1 \pm 7.3 (98-120)	110.4 \pm 0.3 (106-114)	97 \pm 3.75 (92-101)	94	94.6 \pm 2.0 (90-98)	
Head-vulva	299 \pm 15 (279-318)	-	-	-	-	
Body width	11.4 \pm 0.9 (10-12)	10.6 \pm 0.5 (10-11)	-	-	-	
Vulva - anus	86.7 \pm 4.3 (80-94)	-	85 \pm 7.25 (82-89)	-	74.9 \pm 3.0 (69-81)	
Tail	164 \pm 12 (145-177)	167 \pm 9 (151-172)	184 \pm 43.35 (145-231)	195	175 \pm 17.1 (133-193)	
Tail/V-A	1.9 \pm 0.1 (1.7-2.0)	-	-	-	2.3 \pm 0.26(1.7-2.7)	
Spicules	-	14.5 \pm 1.0 (14-16)	-	14	-	
Gubernaculum	-	3.4 \pm 0.4 (3-4)	-	4	-	
Bursa	-	26.6 \pm 1.9 (24-29)	-	-	-	

Molecular Phylogenetic Studies

The selected 28S rDNA D2/D3 sequences of the species/isolates of 31 tylenchids (including sequences of three studied species) and one aphelenchid outgroup were used for analyses. The dataset was composed of 736 total characters from which 483 characters were variable. The average nucleotide composition was as follows: 20.9% A, 21.2% C, 32.3% G and 24.7% T. In the inferred tree (Figure 7), the Iranian population of *E. excretorius* formed a clade with the European isolate of the

same species and, as expected, the Iranian population of *C. leptus*, was in close phylogenetic relation with the only other sequenced species of the genus, *C. hexalineatus* (Geraert, 1962) Geraert and Goodey, 1964. Both genera are members of the subfamily Tyldorinae Paramonov, 1968 (*Sensu* Geraert, 2008), also occupying the same clade in our tree with 0.94 BPP and 58% ML BS values. In the present tree, the phylogenetic relation of three major clades A, B, and C is not well resolved because of polytomy, but, with the exclusion of the genus *Malenchus* Andrassy, 1968, the position of members of Tylenchinae Skarbilovich, 1959 and Boleodorinae Khan, 1964 inside the corresponding subfamilies

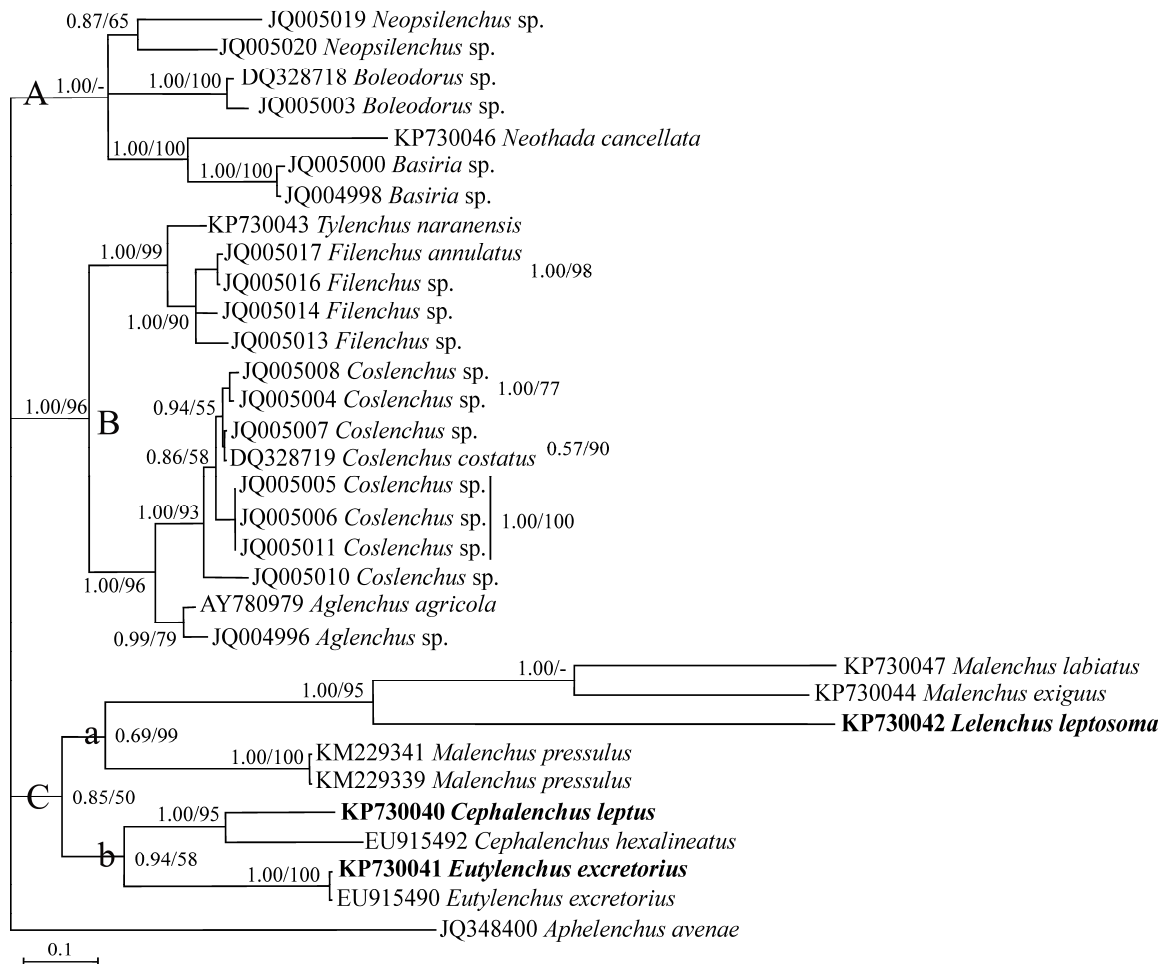


Figure 7. Bayesian 50% majority rule consensus tree inferred from 32 sequences of the D2/D3 domains of the 28S rDNA under the GTR+I+G model. BPP and ML BS values are given for each appropriate clade in the shape BPP/ML BS. The newly sequenced taxa/isolates are in bold.



(*sensu* Geraert, 2008) is supported. The position of *Lelenchus* in subclade C,a and the relation of the latter subclade with the subclade C,b is not in accordance with currently accepted taxonomic schedules of tylenchids and needs further phylogenetic studies. The phylogenetic position of the genus *Lelenchus* in our tree is also surprising. It formed a well-supported clade (1.00/95) with two isolates of *Malenchus*. A similar phylogenetic affinity of both genera, *Malenchus* and *Lelenchus*, was previously published by Ashrafi *et al.* (2012) using SSU sequences, too. Currently, there are not enough molecular data to explain such a phylogenetic position for *Lelenchus* and other members of Ecpthyadophorinae Skarbilovich, 1959 (*sensu* Siddiqi, 2000; Geraert, 2008) and discuss their phylogenetic relationships.

ACKNOWLEDGEMENTS

The authors thank the Iranian National Science Foundation (INSF) and Tarbiat Modares University (Iran) for financial supports. The kind help of Dr. Svetlana Malysheva (Centre of Parasitology, Russian Academy of Sciences) in providing the SEM image of one of the studied isolates is deeply appreciated.

REFERENCES

1. Andrassy, I. 1954. Revision der Gattung *Tylenchus* Bastian, 1865 (Tylenchidae, Nematoda). *Acta Zool. Hung.*, **1**: 5-42.
2. Andrassy, I. 1968. Fauna Paraguayensis. 2. Nematoden aus den Galeriewaldern des Acaray-Flusses. *Opusc. Zool. Budapest*, **8**: 167-315.
3. Andrassy, I. 1981. The Genera and Species of the Family Tylenchidae Örley, 1880 (Nematoda). The Genus *Malenchus* Andrassy, 1968. *Acta Zool. Acad. Sci. Hung.*, **27**: 1-47.
4. Ashrafi, S., Mugniéry, D., van Heese, E. Y. J., van Aelst, A. C., Helder, J. and Karssen, G. 2012. Description of *Meloidoderita salina* sp. n. (Nematoda, Sphaeronematidae) from a Microtidal Salt Marsh at Mont-Saint-Michel Bay in France. *ZooKeys*, **249**:1-26.
5. Atighi, M. R., Pourjam, E., Pereira, T. J., Okhovaat, S. M., Alizadah, B. A., Mundo-Ocampo, M. and Baldwin, J. G. 2013. Redescription of *Filenchus annulatus* (Siddiqui and Khan, 1983) Siddiqui, 1986 Based on Specimens from Iran with Contributions to the Molecular Phylogeny of the Tylenchidae. *Nematol.*, **15**: 129-141.
6. Bastian, H. C. 1865. Monograph on the Anguillulidae or Free Nematoides, Marine, Land, and Freshwater; with Descriptions of 100 New Species. *Trans. Linn. Soc. London*, **25**:73-184.
7. Brzeski, M. 1996. On the Genus *Eutylenchus* Cobb, 1913 (Nematoda: Tylenchidae). *Nematologica*, **42**: 1-8.
8. Carta, L., Skantar, A. and Handoo, Z. 2010. Molecular rDNA Phylogeny of Telotylenchidae Siddiqui, 1960 and Evaluation of Tail Termini. *J. Nematol.*, **42**: 359.
9. Cobb, N. A. 1913. New Nematode Genera Found Inhabiting Fresh water and Non-Brackish Soils. *J. Wash. Acad. Sci.*, **3**: 432-444.
10. De Grisse, A. T. 1969. Redescription et Modification de Quelques Techniques Utilisées dans L'étude des Nematodes Phytoparasitaires. *Meded. Rijksfac. Gent*, **34**: 351-369.
11. De Man, J. G. 1880. Die Einheimischen, Frei in der Reinen Erde und im Süßen Wasser Lebenden Nematoden. Vorläufiger Bericht und Descriptiv-systematischer Theil. *Tijdschr. Nederl. Dierk. Ver.*, **5**: 1-104.
12. Ebsary, B. A. and Eveleigh, E. S. 1981. *Eutylenchus excretorius* n. sp. (Nematoda: Atylenchidae) from Quebec, Canada. *Can. J. Zool.*, **59**: 1973-1975.
13. Geraert, E. 1962. Bijdragen tot de Kennis der Plantenparasitaire en der Vrijlevende Nematoden van Kongo. II. De Nematodenfauna in en om de Wortels van Musa Parasidiaca Normalis. *Ganda-Congo Rijksuniversiteit Gent*, **3**: 1-73.
14. Geraert, E. and Raski, D. 1986. Unusual *Malenchus* Species (Nematoda: Tylenchidae). *Nematologica*, **32**: 27-55.
15. Geraert, E. 2008. The Tylenchidae of the World: Identification of the Family Tylenchidae (Nematoda). Academia Press, 540 PP.

16. Geraert, E. and Goodey, J. B. 1964. The Priority of *Tylenchus hexalineatus* over *T. megacephalus*. *Nematologica*, **9**(1963): 471.
17. Ghaderi, R., Kashi, L. and Karegar, A. 2012. *The Nematodes of Iran* (Based on the Published Reports until 2011). Agricultural Education and Extension Publication, Tehran, Iran, 371 PP.
18. Ghaemi, R., Pourjam, E., Atighi, M. R., Pedram, M. and Karssen, G. 2012. First Record of the Genus *Discotylenchus* Siddiqi, 1980 (Nematoda: Tylenchidae) from Iran, with Description of One New and Data on Two Known Species. *Zootaxa*, **3493**: 72-82.
19. Goodey, J. B. 1962. *Tylenchus* (Cephalenchus) *megacephalus* n. sbg., n. sp. *Nematologica*, **7**: 331-333.
20. Huson, D. H. and Scornavacca, C. 2012. Dendroscope 3: An Interactive Tool for Rooted Phylogenetic Trees and Networks. *Syst. Biol.*, **61**: 1061-1067.
21. Khan, E. 1964. *Boleodorus mirus* n. sp. (Tylenchidae: Boleodorinae N. Sub-family) from Kufri, Simla (HP), India, with a Key to the Species of the Genus *Boleodorus* Thone, 1941. *Zool. Anz.*, **173**: 336-341.
22. Larget, B. and Simon, D. L. 1999. Markov Chain Monte Carlo Algorithms for the Bayesian Analysis of Phylogenetic Trees. *Mol. Biol. Evol.*, **16**: 750-759.
23. Mirbabaee Karani, H., Kashi, L., Ghaderi, R. and Karegar, A. 2015. Five Species of Tylenchidae and Dolichodoridae (Nematoda: Tylenchoidea) from Iran. *J. Agr. Sci. Tech.*, **15**: 227-240.
24. Mizukubo, T. 1989. *Aglenchus ainakamurae* n. sp. and Records of *Cephalenchus leptusand* *Lelenchus leptosoma* from Japan (Tylenchida: Tylenchidae). *Japan. J. Nematol.*, **19**: 18-31.
25. Nunn, G. B. 1992. Nematode Molecular Evolution. PhD. Dissertation, University of Nottingham, UK, 192 PP.
26. Nylander, J. A. A. 2004. *MrModeltest*. Program Distributed by the Author, Evolutionary Biology Centre, Uppsala University.
27. Örley, L. 1880. Monograph of the Anguillulids. *Term. Füz.*, **4**: 16-150. (in Magyar and German)
28. Palomares-Rius, J. E., Subbotin, S. A., Liebanas, G., Landa, B. B. and Castillo, P. 2009. *Eutylenchus excretorius* Ebsary and Eveleigh, 1981 (Nematoda: Tyloporinae) from Spain with Approaches to Molecular Phylogeny of Related Genera. *Nematol.*, **11**: 343-354.
29. Panahandeh, Y., Pourjam, E. and Pedram, M., 2014. Some new tylenchids (Tylenchina: Nematoda) for nematode fauna of Iran. *J. Agri. Sci. Tech.*, **16**: 461-477.
30. Paramonov, A. A. 1968. Principles of Ecological and Morphological Analysis of the Classification of Tylenchida. *Izv. Acad. Nauk SSSR Ser. Biol.*, **6**: 793-801. (in Russian)
31. Raski, D. J. and Geraert, E. 1986. New Species of *Lelenchus* Andrassy, 1954 and *Ecphyadophora* de Man, 1921 (Ecphyadophrinae: Tylenchidae) from Southern Chile. *Nematologica*, **31**: 244-265.
32. Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. *Bioinformatic.*, **19**(12): 1572-1574.
33. Siddiqi, M. R. 1963. Four New Species of the Genus *Tylenchus* Bastian, 1865 (Nematoda) from North India. *Zeit. F. Parasit.*, **23**: 170-180.
34. Siddiqi, M. R. 1971. On the Plant-parasitic Nematode Genera *Histotylenchus* gen. n. and *Telotylenchoides* gen. n. (Telotylenchinae), with Observations on the Genus *Paratrophurus* Arias (Trophurinae). *Nematologica*, **17**: 190-200.
35. Siddiqi, M. R. 1979. Seven New Species in a New Nematode Subfamily Duosulciinae (Tylenchidae), with Proposals for *Duosulcius* gen. n., *Zanenchus* gen. n. and *Neomalenchus* gen. n. *Nematologica*, **25**: 215-236.
36. Siddiqi, M. R. 2000. *Tylenchida Parasites of Plants and Insects*. 2nd Edition, CABI Publishing, Wallingford, UK, 833 PP.
37. Silvestro, D. and Michalak, I. 2012. RaxMLGUI: A Graphical Front-end for RAXML. *Org. Divers. Evol.* DOI: 10.1007/s13127-011-0056-0
38. Skarbilovich, T. S. 1959. On the Structure of Systematics of Nematodes Order Tylenchida Thorne, 1949. *Act. Parasitol. Polon.*, **7**: 117-132.
39. Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N. and Baldwin, J. G. 2006. Phylogenetic Analysis of Tylenchida Thorne, 1949 as Inferred from D2 and D3 Expansion Fragments of the 28S rRNA Gene Sequences. *Nematol.*, **8**: 455-474.
40. Whitehead A. G. and Hemming J. R. 1965. A Comparison of Some Quantitative Methods for Extracting Small Vermiform Nematodes from Soil. *Ann. Appl. Biol.*, **55**: 25-38.



مطالعه ریخت شناسی و مولکولی سه گونه شناخته شده از خانواده
Tylenchidae Örley, 1880 (Nematoda: Tylenchina) از ایران

ی. پناهنده، ا. پورجم، ف. علی رمجی، م. ر. عتیقی، و م. پدرام

چکیده

سه گونه متعلق به سه جنس از خانواده Tylenchidae به نام‌های *Cephalenchus leptus*، *Eutylenchus excretorius* و *Lelenchus leptosoma* براساس شاخص‌های ریخت‌سنجی، ریخت‌شناسی و تبارزایی مولکولی مورد مطالعه قرار گرفتند و برای نخستین بار برای فون‌نماتدهای ایران گزارش می‌گردند. از میان این سه جنس، دو جنس اخیر برای اولین بار از ایران گزارش می‌شوند. جمعیت ایرانی گونه *Cephalenchus leptus* با داشتن سطوح جانبی با شش شیار، استایلت به طول ۲۰-۱۶ میکرومتر و دم به طول ۲۹۰-۱۵۳ میکرومتر با انتهای تیز مشخص می‌گردد. دومین گونه با داشتن بدن به طول ۱۰۳۸-۸۴۸ میکرومتر، استایلت به طول ۲۱/۵-۲۰ میکرومتر، وجود پرده فرج و عدم وجود جس نر مشخص می‌گردد. جمعیت مورد مطالعه از گونه *Lelenchus leptosoma* با داشتن بدن باریک، سر باریک شده در دو جانب، خروجی آمفید سینوسی شکل و عدم وجود باند جانبی مشخص می‌گردد. روابط تبارزایی این سه جنس با استفاده از توالی نوکلئوتیدی ناحیه 28S rDNA D2/D3 مورد مطالعه قرار گرفت و مشخص شد که دو جنس اولی در یک گروه تک‌تبار قرار می‌گیرند در حالی که جنس *Lelenchus* به همراه گونه‌های توالی یابی شده برای ناحیه ژنومی نام‌برده گونه‌های جنس *Malenchus* تشکیل یک گروه تک‌تبار را در درخت تبارزایی بیس (Bayesian tree) می‌دهد.