

An interesting rare tylenchid species, *Antarctenchus urmiensis* n. sp. (Tylenchomorpha; Psilenchidae) from Urmia Lake islands, northwest Iran, with a discussion on the taxonomy of related genera

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Abstract

A population of an amphidelphic tylenchid nematode was recovered from Urmia Lake islands in association with native shrubs of the region. It had a Tylenchidae-type cloacal bursa in male and looked morphologically similar to the genera under the family Psilenchidae *sensu* Siddiqi, representing a new species, described and illustrated in present study as *Antarctenchus urmiensis* n. sp. It is characterized by the slender body at anterior region, gradually narrowing toward posterior end. Four lines in lateral fields in extremities, increasing to six at mid-body. Deirids at secretory-excretory pore (S-E pore) level and phasmids at 38–62% of the tail. Low-expanded cephalic region having a moderately sclerotized cephalic framework, continuous with body, and not flattened dorso-ventrally. Finely annulated cuticle. Amphidial apertures pore-like. Short (13–15 µm long) stylet, its conus 35–42% of the total length and three small tear-drop like knobs. Pharynx with ellipsoid metacarpus and small valve, small saccate pharyngeal bulb offset from intestine. Reproductive system didelphic-amphidelphic, vulva with no membrane or epyptigma. Tail conical, not elongate or filiform, usually slightly ventrally bent, with a sharp or blunt tip and a small indentation at dorsal side close to tip. Males with similar anterior body region and tail to those of female, tylenchoid spicules and small cloacal bursa. In molecular phylogenetic analyses using partial small and large subunit ribosomal DNA (SSU and LSU rDNA D2-D3) sequences, the new species appeared as an independent lineage between the clades of *Hoplolaimina sensu* Siddiqi and the clades of *Atetylenchus* spp. + *Psilenchus* spp. in the SSU phylogeny. In LSU phylogeny, it placed in a clade including representatives of *Atetylenchus*, *Psilenchus*, *Amplimerlinius*, *Pratylenchoides*, *Nagelus*, and *Geocenamus*.

The currently available phylogenies and taxonomic frameworks were re-evaluated for the placement of the didelphic Tylenchidae-like genera, the overall results showing the best resolution is achieved with the family concept of Psilenchidae under Dolichodoroidea.

Keywords

Atetylenchus, Morphology, Phylogeny, Psilenchidae, West Azarbaijan province.

The taxonomy and phylogeny of tylenchids have already been studied and revised by several authors (Bert et al., 2008; De Ley and Blaxter, 2002; Holterman et al., 2009; Panahandeh et al., 2019; Sturhan, 2012; Subbotin et al., 2006). Following the molecular phylogenetic study of De Ley and Blaxter (2002), the order Tylenchida Thorne, 1949 *sensu* Siddiqi (2000), together with the former order Aphelenchida Siddiqi, 1980, formed the infraorder Tylenchomorpha De Ley and Blaxter, 2002 within the order Rhabditida Chitwood, 1933. This infraorder now contains four superfamilies: Aphelenchoidea De Ley and Blaxter, 2002, Criconematoidea Taylor, 1936 Sphaerularioidea Lubbock, 1861, and Tylenchoidea (Örley, 1880) (Decraemer and Hunt, 2013). Six families, namely, Tylenchidae Örley, 1880, Dolichodoridae Chitwood, 1950, Hemicyclophoridae Skarbilovich, 1959, Hoplolaimidae Filipjev, 1934, Pratylenchidae Thorne, 1949, and Tylenchulidae Skarbilovich, 1947 are placed under the superfamily Tylenchoidea Örley, 1880 (Decraemer and Hunt, 2013). Currently, there are conflicting viewpoints about the taxonomic placement of didelphic genera attributed to Psilenchidae Paramonov, 1967 (Geraert, 2008; Hunt, Bert and Siddiqi, 2000; 2012); or no decisions were made on their position in the reference books, e.g. Plant Nematology (Decraemer and Hunt, 2013).

Based on available data, few researchers have discussed about the status of Psilenchidae. Subbotin et al. (2006) gave a historic overview on the case, and recently Hosseinvand et al. (2020) followed the framework used by Geraert (2008), stating that the followed taxonomic frame is supported by their resolved SSU phylogeny (also see Discussion section).

During present study, a didelphic tylenchid population was recovered from the soil samples obtained from the Urmia Lake islands. By its typological similarities, and having a Tylenchidae-type cloacal bursa in male, i.e. lacking a bursa enveloping the tail or a trilobed bursa, common in dolichodoids *sensu* Geraert (2019) and Decraemer and Hunt (2013), it looked similar to Psilenchidae *sensu* Siddiqi (2000) members (also see Discussion section). Thus, the present study aims to describe the recovered population as *Antarctenchus urmiensis* n. sp. using both traditional and phylogenetic approaches and discuss on the taxonomy of the genera placed under Psilenchidae *sensu* Siddiqi (2000).

Materials and methods

Nematode sampling and morphological identification

Several soil samples were collected from different parts of the Urmia Lake islands, northwest Iran.

The tray method (Whitehead and Hemming, 1965) was used to extract nematodes from soil samples. The nematodes of interest were handpicked under a Nikon SMZ1000 stereomicroscope, heat-killed by adding boiling 4% formalin solution, transferred to anhydrous glycerin according to De Gisse (1969), mounted in permanent slides and examined using a Nikon Eclipse E600 light microscope. Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope powered with differential interference contrast (DIC). Drawings were made using a drawing tube attached to the microscope and were redrawn using the CorelDRAW® software version 12.

Scanning Electron Microscopy (SEM)

Specimens preserved in glycerin were selected for observation under SEM according to Abolafia (2015). They were hydrated in distilled water, dehydrated in a graded ethanol-acetone series, critical point dried, coated with gold, and observed with a Zeiss Merlin microscope (5 kV) (Zeiss, Oberkochen, Germany).

DNA extraction, PCR, and sequencing

A single nematode specimen of the new species was picked out and transferred to a small drop of TE buffer (10mM Tris-Cl, 0.5mM EDTA; pH 9.0, Qiagen) on a clean slide and squashed using a clean coverslip. The suspension was collected by adding 25 µl TE buffer. Two DNA samples were prepared in this manner. DNA samples were stored at -20°C until using as PCR templates. Primers for partial amplification of SSU rDNA were forward primer 22F (5'-TCCAAGGAAGGCAGCAGGC-3') and reverse primer 13R (5'-GGGCATCACAGACCTGTTA-3') (Dorris et al., 2002). Sometimes the reverse primer 1573R (5'-TA CAAAGGGCAGGGACGTAAT-3') (Mullin et al., 2005) was used interchangeably. The LSU rDNA D2-D3 expansion segments were amplified using the forward D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and reverse D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Nunn, 1992) primer pairs. The PCR products were sequenced in both directions using the same primers used in PCR with an ABI3730XL sequencer. The newly obtained sequences were deposited into the GenBank database under the accession numbers MW208950 and MW208951 for SSU and MW208952 for LSU rDNA.

The newly obtained sequences were compared with those of other nematode species available in GenBank using the BLAST homology search program. For reconstruction of the phylogenetic relationships, two

independent SSU and LSU datasets were prepared. The selected DNA sequences (representatives of most tylench genera were included) were aligned using Q-INS-i algorithm of the online version of MAFFT (version 0.91b) (<http://mafft.cbrc.jp/alignment/seaver/>) (Kato and Standley, 2013). The poorly aligned positions and divergent regions of SSU and LSU datasets were eliminated using all three less stringent options. The model of base substitution was selected using MrModeltest.2 (Nylander, 2004). The Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) running the chains for 5,000,000 generations for both datasets. After discarding burn-in samples, the remaining samples were retained for further analyses. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% consensus majority rule. Adequacy of the posterior sample size was evaluated using autocorrelation statistics as implemented in Tracer v1.6 (Rambaut and Drummond, 2009). *Aphelenchus avenae* Bastian, 1865, *Paraphelenchus pseudoparietinus* Micoletzky, 1922 and *P. acontioides* Taylor and Pillai, 1967 were used as outgroup taxa. The output files of the phylogenetic program used herein were visualized using Dendroscope V3.2.8 (Huson and Scornavacca, 2012) and redrawn in the CorelDRAW® software version 12.

Results

Description

Antarctenchus urmiensis n. sp.

Figures 1-3.

Measurements

See Table 1.

Female

Body ventrally curved, C shape after fixation, slender (mostly at neck region), gradually narrowing towards distal end. Cuticle finely annulated all over the body (from post cephalic plate to tail tip), the transverse striae sometimes not reaching the lateral fields. Cephalic region low, wide, with four annuli in SEM, its base ca. 3.4 times the height, or ca. 1.5 times wider than the width at apex. Lateral fields with four lines in the anterior and posterior body region, increasing to six at mid-body. Deirid at secretory-excretory pore

(S-E pore) level, phasmids at about mid-tail. Stylet short, its conus shorter than the shaft, about 37–46% of the total stylet, with three tear-drop like knobs. The stylet guiding apparatus complex, usually three rings were observed, forming two chambers at shaft region. Pharynx tylenchoid, procorpus slender, metacorpus small, oval, at 40.0–45.8% of the pharynx with weak valve, isthmus narrow, pharyngeal bulb small, with usually one visible nucleus. Cardia large. Intestine simple, rectum and anus functional. S-E pore position just anterior of pharyngeal bulb. Hemizonid distinct, just anterior to S-E pore. Nerve ring encircling isthmus. Reproductive system didelphic-amphidelphic, each branch composed of an ovary, with oocytes in a single row, oviduct tubular, spermatheca spherical, empty, crustaformera quadricolumellate, uterus tubular, vagina weakly sclerotized, vulva a transverse slit, its lips slightly protruding, vulval flap and epyptigma absent. Tail conical, not elongate-filiform, usually slightly ventrally bent, with a sharp or blunt tip and a small indentation at dorsal side close to tip.

Male

Similar to female in general morphology except for reproductive system. Testis single, elongate, spermatocytes at single row behind germinal zone. Spicules tylenchoid, moderately sclerotized, slightly ventrally curved. Gubernaculum well sclerotized, crescent shaped. Bursa small, cloacal Tail similar to that of female.

Etymology

The species name is named after the city of Urmia, from where the new species was recovered in one of the islands of the Urmia Lake.

Type locality and habitat

Recovered from a soil sample collected in one of the islands of Urmia Lake, the Kaboodan island, Urmia, West Azarbaijan province, northwest Iran, in September 2019, in association with wild shrubs. The GPS coordinates for the locality are: 37°29'27.378"N 45°40'1.620"E.

Type material

Holotype female, seven paratype females, eight paratype males and four paratype juveniles were deposited in the nematode collection at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

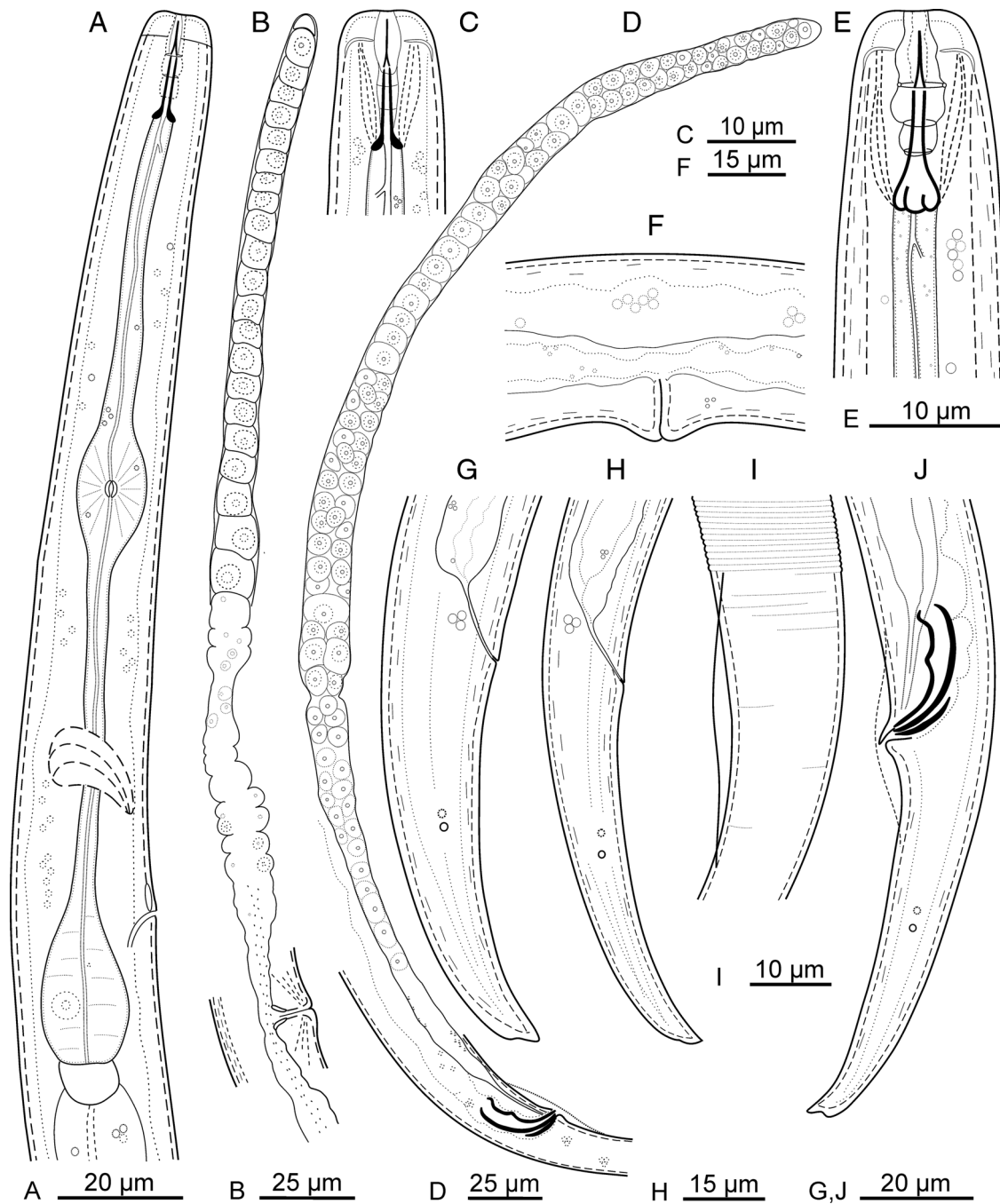


Figure 1: Line drawings of *Antarctenchus urmiensis* n. sp. (A-C, E-I: Female; D&J: Male) (A) Pharynx; (B) Anterior genital tract; (C&E) Anterior body end; (D) Male reproductive system; (F) Vulval region; (G&H) Female tail (phasmids are shown at two foci); (I) Bursa; (J) Male tail, spicule, and bursa.

(five slides) Five paratype females and four paratype males (four slides) were deposited in the USDA Nematode Collection, Beltsville, MD. The new species binomial has been registered in the ZooBank database

(zoobank.org) under the identifier: urn:lsid:zoobank.org:act:4FD480D0-4838-41E7-8AF3-708449E0B546. The LSID for the publication is: urn:lsid:zoobank.org:pub:5C029901-8785-47AB-9812-91C50F3B5E2A.

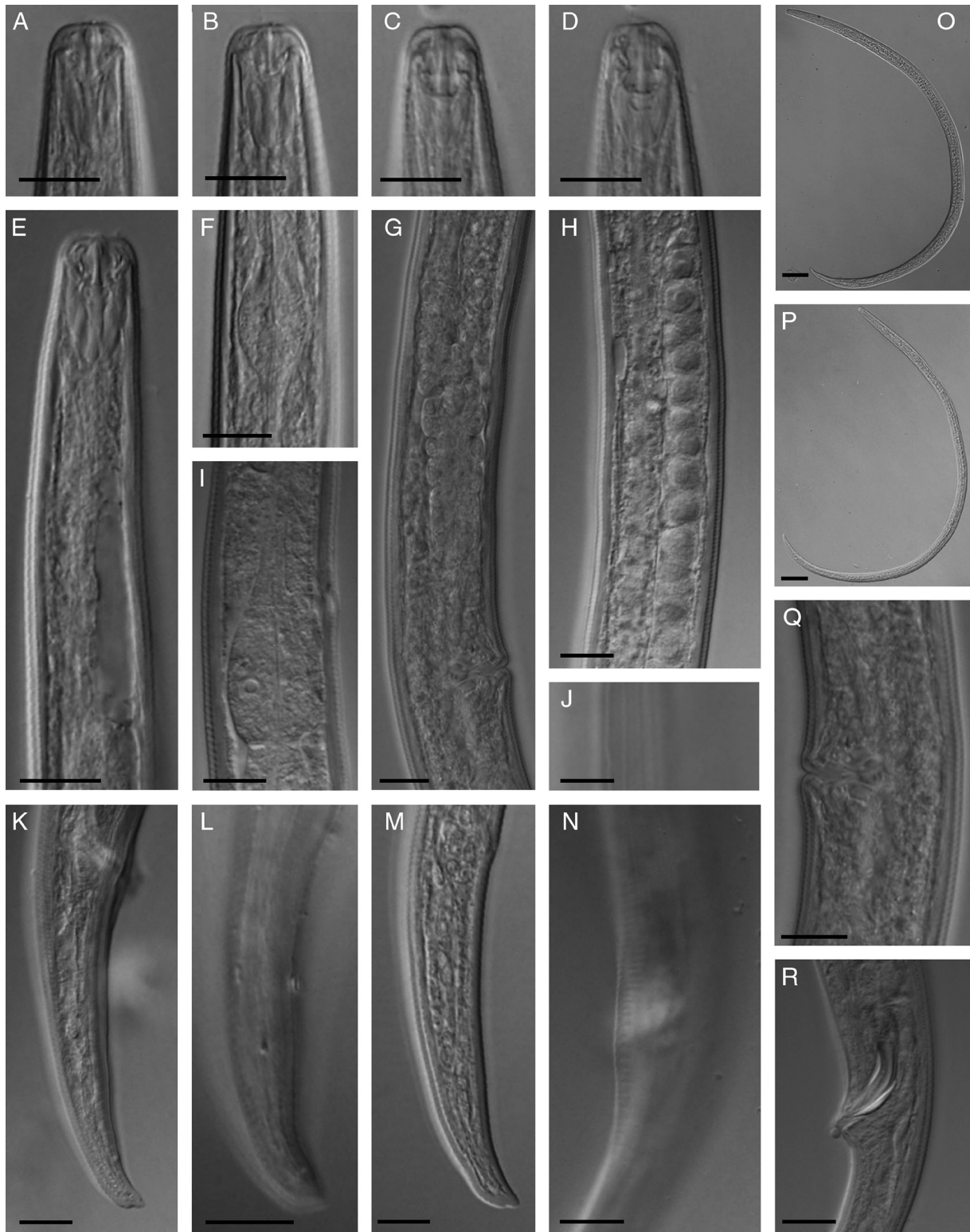


Figure 2: Light micrographs of *Antarctenchus urmiensis* n. sp. (A,B,E,F,G,H,I,J,M,P,Q: Female; C,D,K,L,N,O,R: Male) (A-D) Anterior body end; (E) Anterior body region; (F) Pharyngeal median bulb; (G) Part of female reproductive system; (H) Distal end of ovary; (I) Pharyngeal bulb; (J) Lateral lines; (K) Male tail; (L) Phasmid; (M) Female tail; (N) Bursa; (O&P) Entire body; (Q) Vulval region; (R) Spicule and gubernaculum. (Scale bars: A-N, Q&R = 10 μ m; O&P = 50 μ m).

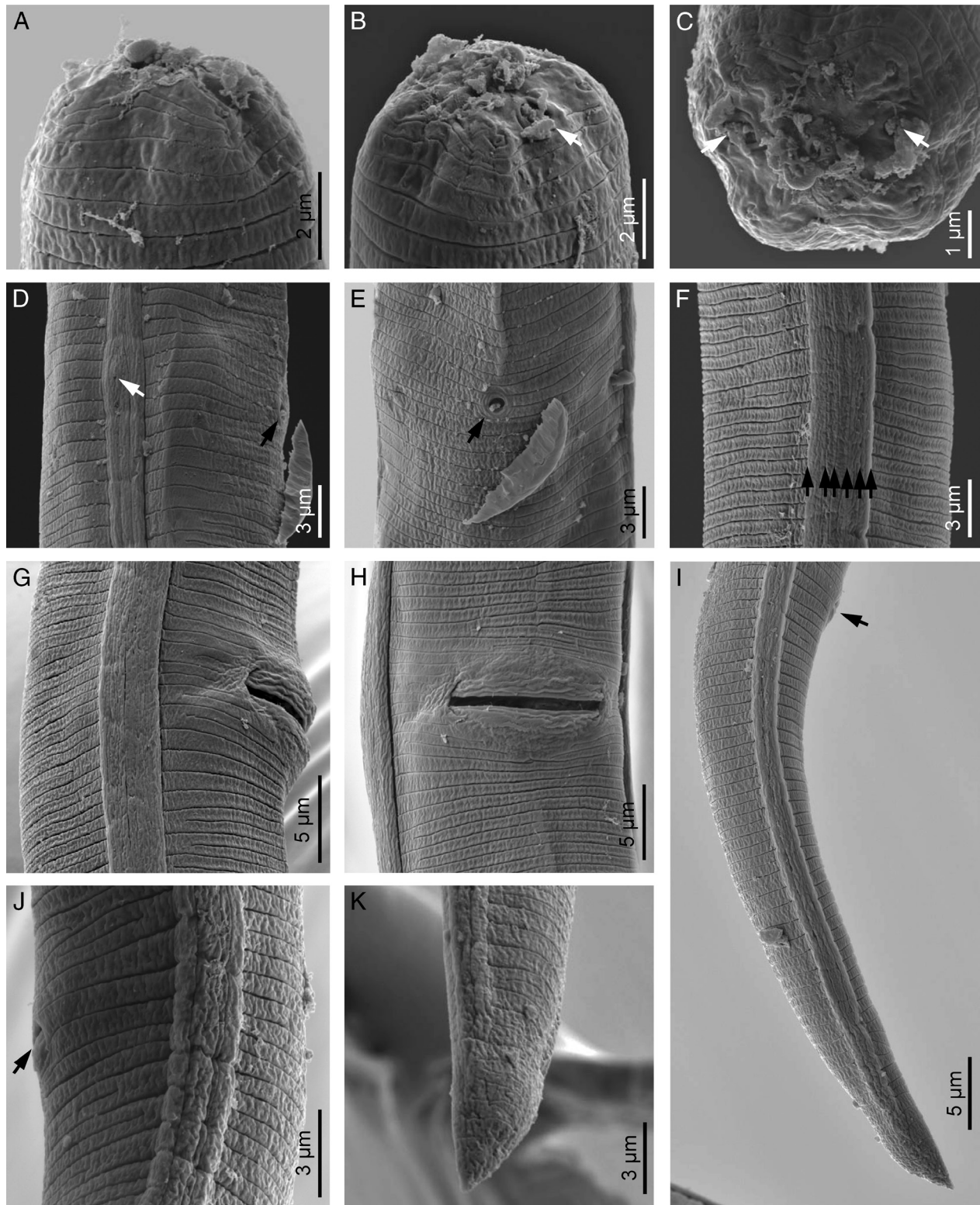


Figure 3: Scanning electron microscopic (SEM) images of *Antarctenchus urmiensis* n. sp. (Female) (A-C) Anterior end in ventral, sublateral and frontal views, respectively (arrows pointing the amphidial openings); (D) Deirid (with arrow) and excretory pore in lateral view (black arrow); (E) Secretory-excretory pore (arrow); (F) Lateral field; (G&H) Vulva in lateral and ventral views, respectively; (I) Posterior end in lateral view (arrow pointing the anus);(J) Anus (arrow) in lateral view; (K) Tail tip.

Table 1. Morphometrics of *Antarctenchus urmiensis* n. sp. All measurement are in μm and in the form: mean \pm s.d. (range).

	Holotype	Paratypes	
	Female	Females	Males
n	1	8	8
L	1,095	1,022 \pm 96 (875–1,162)	895 \pm 40 (819–942)
a	45.6	41.7 \pm 2.4 (38.5–45.6)	38.9 \pm 2.3 (35.6–42.8)
b	6.7	6.5 \pm 0.8 (5.5–8.2)	5.7 \pm 0.3 (5.1–5.9)
c	15.2	15.5 \pm 1.3 (14.2–18.2)	14.1 \pm 0.6 (13.5–15.5)
c'	5.5	5.1 \pm 0.5 (4.0–5.8)	3.7 \pm 0.2 (3.4–4.0)
V	54	55.0 \pm 0.0 (54–58)	–
Cephalic region width at apex	6	6.4 \pm 0.6 (5.5–7.0)	5.7 \pm 0.5 (5.0–6.5)
Cephalic region width at base	9.5	9.5 \pm 0.4 (9.0–10.4)	9.1 \pm 0.3 (8.5–9.5)
Cephalic region height	2.8	2.8 \pm 0.3 (2.3–3.2)	2.6 \pm 0.3 (2.2–3.0)
Stylet conus length	5.5	5.5 \pm 0.4 (5–6)	5.7 \pm 0.7 (5–7)
Stylet total length	13	13.9 \pm 0.4 (13.0–14.5)	14.0 \pm 0.5 (13.5–15.0)
Dorsal gland orifice (DGO)	2.5	2.2 \pm 0.3 (2.0–2.8)	2.4 \pm 0.5 (2.0–3.2)
Anterior end to median bulb distance	69	68.6 \pm 5.7 (58–77)	69.8 \pm 1.8 (67–72)
Median bulb length	19	19.8 \pm 1.3 (18–21)	20.8 \pm 0.7 (20–22)
Median bulb width	11	11.1 \pm 0.6 (10–12)	10.0 \pm 0.5 (9–11)
Anterior end to nerve ring distance	101	109.3 \pm 8.6 (94–122)	100.3 \pm 7.0 (92–115)
Anterior end to hemizonid distance	117	121.6 \pm 6.7 (112–131)	115.4 \pm 4.9 (110–126)

Anterior end to excretory pore distance	121	127.4±6.5 (119–135)	122.0±4.6 (117–132)
Neck (stoma+pharynx)	162	158.3±11.4 (132–168)	157.1±3.0 (151–160)
Anterior end to vulva distance	592	568.4±45.3 (511–644)	–
Body width at vulva or cloaca	24	24.6±2.4 (21–29)	23.0±0.8 (22–24)
Body width at anus	13	13.1±2.2 (11–18)	17.4±1.1 (16–19)
Anterior genital branch/testis length	254	261.6±37.4 (207–317)	475.0±35.8 (421–530)
Posterior genital branch length	250	254.1±37.3 (208–332)	–
Tail length	72	66.3±6.4 (60–73)	63.4±2.9 (59–67)
Anus to phasmid distance	30	33.8±4.1 (27–42)	34.4±4.7 (27–43)
Spicules length	–	–	25.6±1.6 (24–29)
Gubernaculum length	–	–	10.1±1.0 (8–11)

Diagnosis and relationships

Antarctenchus urmiensis n. sp. (family Psilenchidae, subfamily Antarctenchinae Spaul, 1972 *sensu* Siddiqi (2000)) is characterized by its slender anterior body region, four lines in lateral fields in extremities, increasing to six at mid-body, deirids at S-E pore level and phasmids at 38–62% of the tail, low expanded cephalic region having a moderately sclerotized cephalic framework, continuous with body, not flattened dorso-ventrally, amphidial apertures pore-like, short stylet with three small tear-drop like knobs, didelphic-amphidelphic reproductive system, vulva with no membrane or epyptigma, conical tail, males with tylenchoid spicules, small cloacal bursa, no hypoptigma and similar tail to that in female.

The new species was morphologically compared with the type and the only one representative species of the genus, *Antarctenchus hooperi* Spaul, 1972. It was further compared with two morphologically similar species *Atetylenchus amiri* (Maqbool and Shahina, 1984) Geraert and Raski, 1987 and *Meiodorus hyalacus* (Anderson and Ebsary, 1982) Siddiqi, 1986 (also see Discussion section).

Compared to *Antarctenchus hooperi*, it has a low cephalic region (vs higher, after the original drawings)

not remarkably flattened dorso-ventrally (vs flattened), remarkably smaller amphidial openings (vs larger), stylet with three tear-drop like knobs (vs flange like), no epyptigma and hypoptigma (vs present), shorter spicules (24–29 vs 36–41 μm) and gubernaculum (8–11 vs 13–15 μm), and conical, slightly ventrally bent tail not elongate or filiform (vs conical, gradually narrowing to the distal part, terminating in a conoid to acute tip).

Compared to *Atetylenchus amiri*, by absence of vulval flap (vs presence), greater V (54–58 vs 49–51), slightly ventrally bent conical tail not elongate or filiform (vs conical, tapering to a narrow tip but becoming broader just before end).

Compared to *Meiodorus hyalacus*, by fine (vs coarse) body annuli, six lines in lateral fields (vs three), longer stylet (13.5–15.0 vs 10.5–11.0 μm) and tail characters (annulated all over the tail vs terminal 24% of tail smooth).

Molecular phylogenetic status

Sequencing of SSU and LSU rDNA D2-D3 fragments of the new species yielded two 939 and 1076 nucleotide long partial SSU, and one 671 nucleotide long LSU D2-D3 sequences. The BLAST search using

the longest SSU sequence (MW208950, used in the tree) revealed it has a 97.0–98.7% identity with several isolates of *Psilenchus* de Man, 1921 and *Atetylenchus* Khan, 1973. The BLAST search using LSU sequence revealed its identity with currently available LSU sequences of Tylenchoidea was less than 93%.

A number of 96 sequences (including the newly generated sequences and the sequences of outgroup taxa, for accession numbers see the SSU tree) were selected for SSU phylogeny. Their alignment included 1,544 characters of which 657 characters were variable. Fig. 4 represents the phylogenetic tree

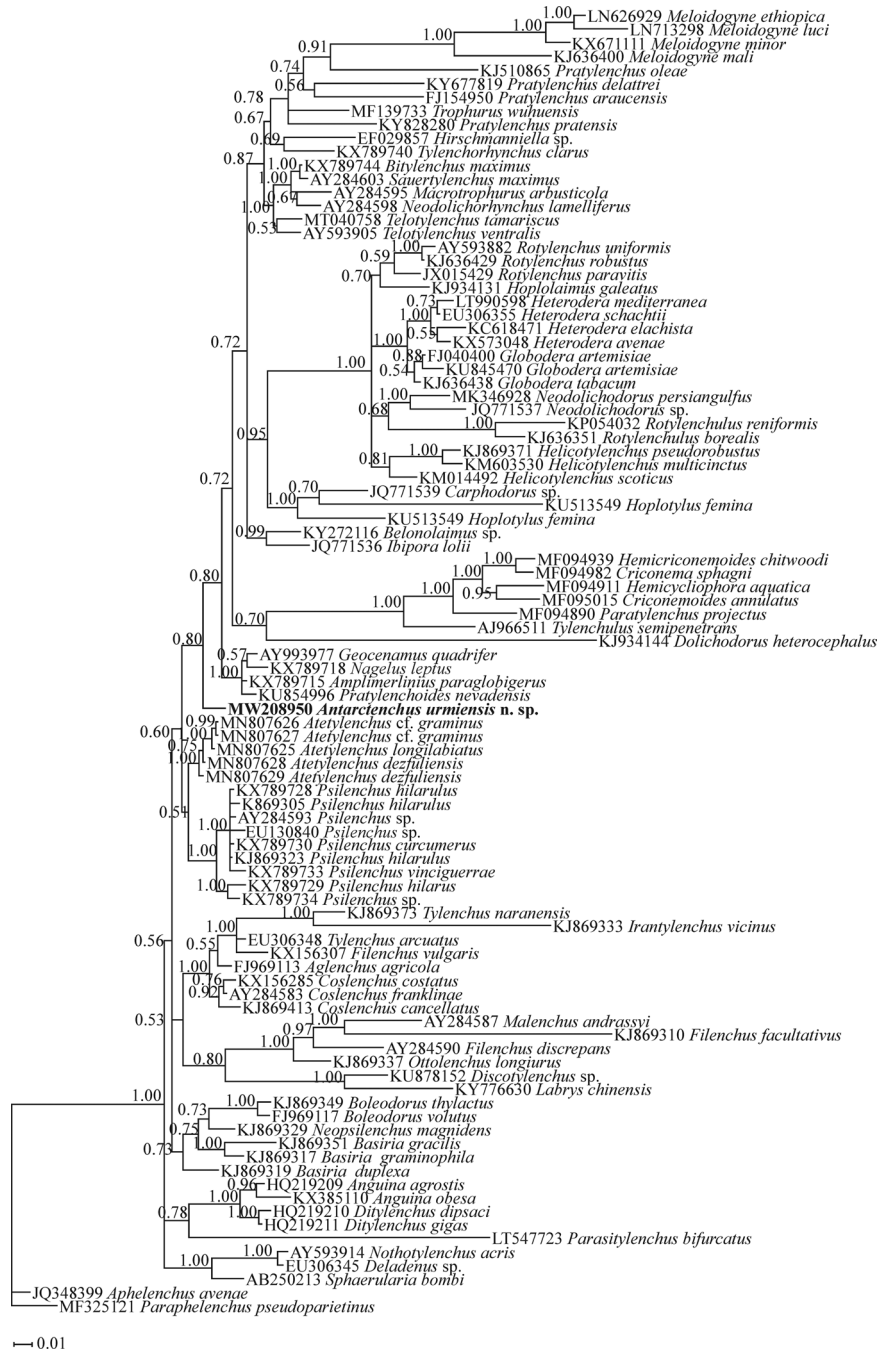


Figure 4: Bayesian 50% majority rule consensus tree of *Antarctenchenchus urmiensis* n. sp. based on SSU rDNA sequences under GTR+I+G model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. The new sequence is indicated in bold.

reconstructed using this dataset. In this tree the new species has appeared as an independent lineage in a basal position to the *Hoplolaimina sensu* Siddiqi (2000) excluding *Psilenchinae* Paramonov, 1967 *sensu* Siddiqi (2000).

A number of 136 sequences (including the newly generated sequences and the sequences of outgroup taxa, for accession numbers see the LSU tree) were selected for LSU phylogeny. Their alignment included 506 characters of which 336 characters were variable. Fig. 5 represents the phylogenetic tree reconstructed using this dataset. In this tree, the new species is included in a clade encompassing representatives of *Psilenchus*, *Atetylenchus*, and four sequences of *Merliniinae* Siddiqi, 1971, and its relationship with them was not resolved due to polytomy.

Discussion

During the present study, a tylenchid nematode with didelphic-amphidelphic type of female reproduction system was recovered from undisturbed regions of one of Urmia Lake islands, northwest Iran. It had a Tylenchidae-type cloacal bursa and looked similar to the *Psilenchidae* members *sensu* Siddiqi (2000) and was assigned to the genus *Antarctenchus* Spaul, 1972. Although some typological differences were observed between the new species and the type and the only one known species of the genus *Antarctenchus*, e.g. absence of a vulval flap and hypostigma, but we believe enough data supporting its placement in an independent genus are still lacking. On the other hand, there are several cases of which species of a genus could have or lack vulval flaps, epiptygma, and hypostigma in Tylenchomorpha. A future sequencing of the type species of *Antarctenchus* may help better determining the taxonomic placement of the new species.

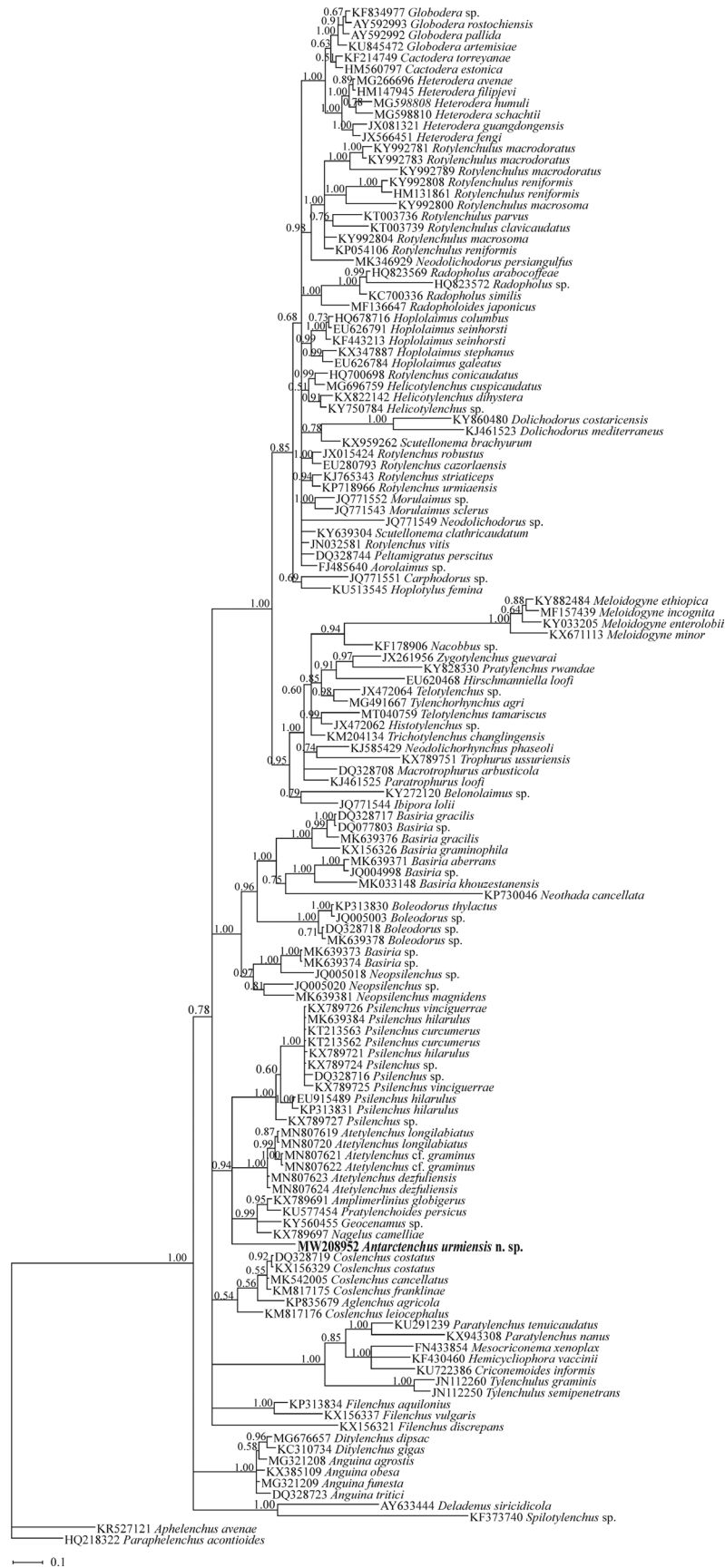
The new species has similarities with *Meiodorus hyalacus* and *Atetylenchus amiri* (see below) too. The first species has established based on female specimens, and its taxonomic placement could not be determined in the absence of males (by its unknown nature of bursa). By similarities in general morphology, i.e. similar cephalic region and stylet, *Meiodorus hyalacus* could probably belong to *Antarctenchus*, or at least, could be a member of the subfamily *Antarctenchinae*, but unless enough pieces of evidence become available, it is better to be regarded as *species incertae sedis*. The second species, *Atetylenchus amiri*, has a knobbed stylet and better fits the subfamily *Antarctenchinae*. Again, this species is regarded as *species incertae sedis* until enough pieces

of evidence helpful in determining a better taxonomic placement becomes available for it.

There is currently no general agreement on the taxonomy of *Psilenchidae*. The family concept as proposed by Siddiqi (2000) looks however more applicable in taxonomy of the didelphic Tylenchidae-like genera (named herein as *psilenchs*), as, they have phasmid, a key feature differentiating them from Tylenchidae. On the other hand, the phylogenetic inferences using SSU and LSU markers, further corroborate the affinity of the *Psilenchidae* members with *Dolichodoroidea* Chitwood and Chitwood in Chitwood, 1950 *sensu* Siddiqi (2000) (Bert et al., 2008; Fadakar et al., 2020; Gharahkhani et al., 2019). In a recent study, *psilenchs* are assigned to the family Tylenchidae after the results of a SSU phylogeny (Hosseinvand et al., 2020). A recalculation of the inferred SSU tree in the latter study using the same sequences, alignment, postediting and inference methods, but using *aphelenchs* as outgroup taxa (as generally used for this purpose (e.g. Panahandeh et al., 2019; Pedram et al., 2018)), yielded a basically different topology, showing the affinity of the *psilenchs* to the *Dolichodoroidea* (Fig. S1). The importance of the outgroup in a reliable phylogenetic inference is already known and discussed (e.g. Jamil et al., 2019; Kirchberger et al., 2014).

In presently inferred SSU phylogeny, *psilenchs* occupied basal position in relation to included *Hoplolaimina*, reminding '*Psilenchus*-like forms may be considered as ancestors of *Hoplolaimina*' (Siddiqi, 2000) (also see Subbotin et al., 2006). In LSU phylogeny, the new species is in the clade including *Psilenchidae* genera and representative of *Merliniinae*, a similar topology to the topologies inferred by Gharahkhani et al. (2019), Bert et al. (2008), Subbotin et al. (2006), Fadakar et al. (2020), and Carta et al. (2010); and in conclusion, the results of SSU and LSU phylogenies using currently available data, are more congruent with the taxonomic framework of Siddiqi (2000); and the family concept of *Psilenchidae* for harboring the *psilenchs*. The future morphological and molecular phylogenetic data using further representatives and

Figure 5: Bayesian 50% majority rule consensus tree of *Antarctenchus urmiensis* n. sp. based on LSU rDNA D2-D3 sequences under GTR+I+G model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. The new sequence is indicated in bold.



different markers, may shed light on taxonomy of this group of nematodes, and help deciding a more fixed taxonomic place for this group of tylenchomorphs.

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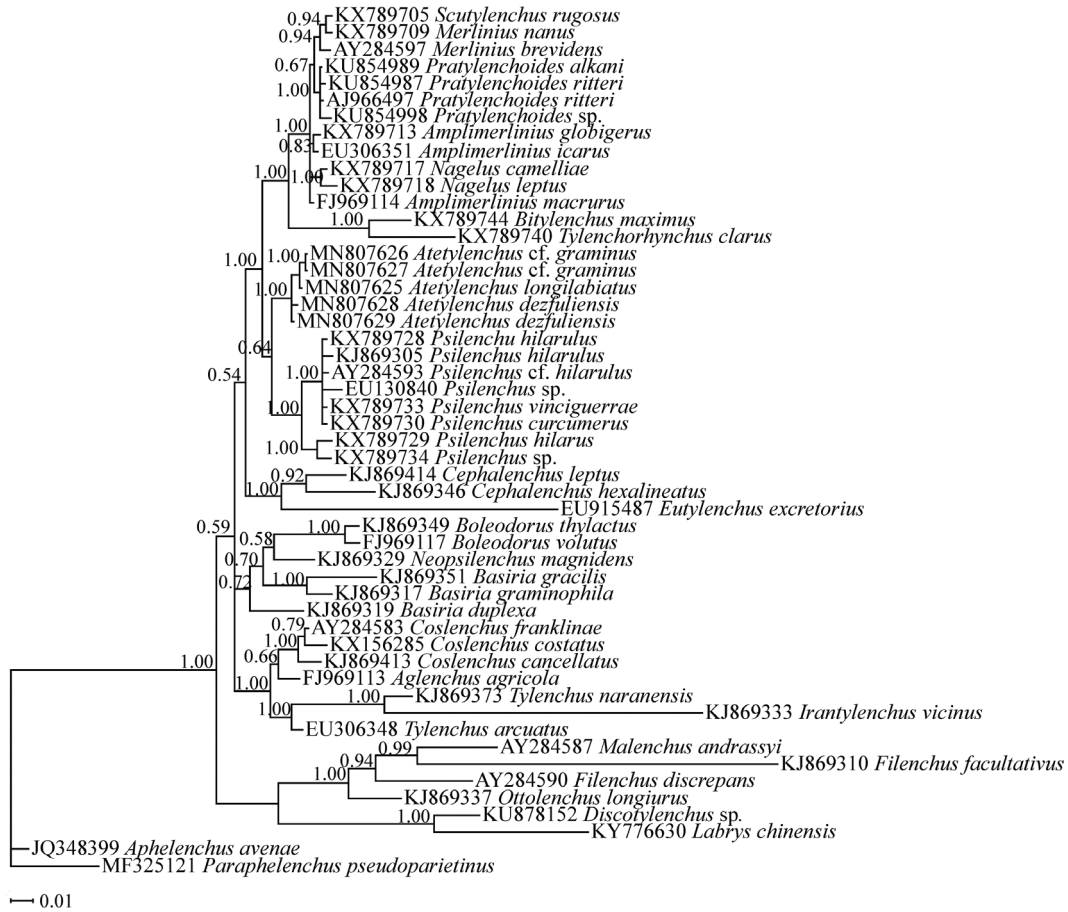


Figure S1: The SSU tree inferred using the original data by Hosseinvand et al. (2020), the same alignment, postediting and inference methods; but using aphelenchs as outgroup taxa.