

Research Paper

Cite this article: Hosseinvand M, Eskandari A, Azizi K, Palomares-Rius JE, Castillo P, Abolafia J, Ghaderi R, Helder J and Qing X (2023). Integrative taxonomy and phylogenetic relationships between representatives of genera within Merliniinae (Nematoda: Merliniidae), with new data on fourteen known and one unknown species. *Journal of Helminthology*, **97**, e89, 1–33 <https://doi.org/10.1017/S0022149X23000640>

Received: 17 June 2023
Revised: 26 September 2023
Accepted: 27 September 2023








Keywords:

Merlinius; *Nagelus*; *Paramerlinius*; *Scutylenchus*; *Telomerlinius*

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Integrative taxonomy and phylogenetic relationships between representatives of genera within Merliniinae (Nematoda: Merliniidae), with new data on fourteen known and one unknown species

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Abstract

The application of integrative taxonomic approaches is useful to species delineation based on a combination of distinct types of characters, here morphological features and ribosomal DNA sequences. In this study, we surveyed ectoparasitic nematodes of the subfamily Merliniinae in cultivated and natural environments in Iran. Results of morphological and morphometrical studies, light and scanning electron microscopic observations, and molecular analyses allowed us the identification of fourteen known and one unknown species including representatives of the genera *Amplimerlinius* (five species), *Geocenamus* (one species), *Merlinius* (three species), *Nagelus* (two species), *Paramerlinius* (one species), *Scutylenchus* (two species), and *Telomerlinius* (one species). The unknown species, *Scutylenchus* sp., characterized by having 35–50 incisures at mid-body; lateral field with 6 longitudinal incisures; lip region slightly offset by a constriction, flattened at front end; bearing 5–7 annuli; cephalic framework not refractive; stylet robust, 18.3–27 µm long; post anal intestinal sac absent; tail elongate conical, dorsally convex, with 24 (19–28) annuli in ventral side, ending to a smooth terminus and males common; spicules 24.5–31 µm long. The phylogenetic analyses were carried out using molecular data from nuclear ribosomal DNA (rDNA) genes *viz.* D2–D3 expansion segments of the large ribosomal subunit (28S rRNA), partial small ribosomal subunit (18S rRNA), and internal transcribed spacer (ITS). The molecular variability of D2–D3 expansion segments of the 28S rRNA and partial 18S rRNA was low in this family in comparison to the ITS region, which could be a more helpful molecular marker in species and genus identification.

Introduction

Considering congruent morphological and molecular data, Sturhan (2012) removed the subfamily Merliniinae Siddiqi, 1971 from Telotylenchidae *sensu* Siddiqi, 2000 and the genus *Pratylenchoides* Winslow, 1958 from Pratylenchidae Thorne, 1949, and amended the diagnosis of the family Merliniidae Siddiqi, 1971 (Ryss, 1993). According to Sturhan (2012), Merliniidae consists of two subfamilies: Merliniinae and Pratylenchoidinae Sturhan, 2012. The first subfamily comprises the genera *Geocenamus* Thorne & Malek, 1968 (= *Scutylenchus* Jairajpuri, 1971); *Merlinius* Siddiqi, 1970; *Paramerlinius* Sturhan, 2012; *Macrotylechus* Sturhan, 2012; *Amplimerlinius* Siddiqi, 1976; and *Nagelus* Thorne & Malek, 1968; whereas the subfamily Pratylenchoidinae is monogeneric with *Pratylenchoides* only. Another interesting genus described by Siddiqi & Sturhan, 2014, *Telomerlinius* Siddiqi & Sturhan, 2014, differs from all known genera in the subfamily Merliniinae by having four incisures in the lateral fields of females and males, having spicules with notch at tip, and sharing the absence of deirids with *Geocenamus*.

However, the taxonomic status of the genera included in the family Merliniidae has been subjected to a long controversial discussion; particularly, the exact taxonomic position of *Geocenamus*, *Merlinius*, and *Scutylenchus* remains as an unresolved problem. The genus *Geocenamus* was proposed by Thorne & Malek (1968), with *G. tenuidens* as its type species. Two years later, the genus *Merlinius* was proposed for 32 species of *Tylenchorhynchus* Cobb, 1913, which had six incisures in the lateral field, rather cylindroid spicules with prominently notched distal

end, a non-protruding gubernaculum, and a moderately developed bursa (Siddiqi 1970). One year later, the genus *Scutylechus* was proposed to accommodate *Tylenchorhynchus mamillatus* Tobar-Jiménez, 1966, mainly based on the mamillate tail shape and in having enlarged, scutella-like phasmids (Jairajpuri 1971).

Anderson (1977), Hooper (1978), and Fortuner & Luc (1987) considered *Scutylechus* as a junior synonym of *Merlinius*, and Brzeski (1991, 1998) regarded it as a junior synonym of *Geocenamus*. Siddiqi (1979, 2000) revalidated *Scutylechus* and listed the diagnostic characters as presence of longitudinal striae or grooves in the body cuticle and the absence of deirids. The validity of *Scutylechus* was subsequently accepted by some nematologists (Decraemer & Hunt 2006, 2013; Andrásy 2007; Hunt *et al.* 2013), but Geraert (2011) considered all three genera – *Geocenamus*, *Merlinius*, and *Scutylechus* – under *Geocenamus* (Table 1).

Sturhan (2012) considered *Merlinius* as a separate genus but synonymised *Scutylechus* with *Geocenamus*. In that work, certain species of *Merlinius* with a heavily sclerotised cephalic framework and a distinct refractive inner cuticle layer at tail terminus were transferred to *Paramerlinius* as a new genus. Also, he mentioned that *Scutylechus* and *Geocenamus* are similar in having four lateral incisures in all juvenile stages, refractive inner cuticle layer at tail terminus, presence of cephalic radial grooves, and a moderate cephalic framework. Sturhan (2011, 2012) concluded that the absence of longitudinal cuticular striae along the entire body appears to be the only essential character distinguishing *Geocenamus* species from *Scutylechus* and further noted that the presence of longitudinal striation should not be considered sufficient to discriminate between genera. Ghaderi *et al.* (2014) found that four species of *Scutylechus* form a distinct clade within Merliniinae, thus supporting the view of Siddiqi (1979, 2000) and Sturhan (2012) on *Scutylechus* as a distinct genus from *Merlinius*. However, they stated that the relationships of *Scutylechus* with other genera should be further studied and tested by inclusion of additional sequences of species of *Merlinius* and *Geocenamus*. The same authors also demonstrated phylogenetically the support for combination of *Pratylenchoidea* and Merliniinae into a single family, the Merliniidae using D2–D3 of 28S rRNA gene. Recently, other authors performed phylogenies with additional sequences using several ribosomal genes and regions (Carta *et al.* 2010; Alvani *et al.* 2017; Munawar *et al.* 2021) giving a view of the phylogenetic relationships among the different genera in Telotylenchidae.

However, additional genera and species are still necessary in order to clarify the position and validity of some genera in this family.

The present study aims to i) describe unknown species of *Scutylechus* under an integrative taxonomical approach; ii) add morphological and molecular data on several known species in the subfamily Merliniinae including members of *Amplimerlinius*, *Geocenamus*, *Merlinius*, *Nagelus*, *Scutylechus*, and *Telomerlinius*; and iii) infer phylogenetic relationships based on partial 18S rRNA, the ITS region, and the D2–D3 region of the 28S rRNA genes within subfamily Merliniinae with inclusion of several representatives of these genera within the subfamily in order to verify the status of the subfamily Merliniinae, preferably by combining morphological and DNA sequence information, if possible.

Material and methods

Nematode sampling and morphological identification

Soil samples were collected from the rhizosphere of different plants in the Khuzestan and Zanjan provinces, in southwestern and northwestern Iran, respectively. Additional samples were collected from an almond orchard in Valenzuela (Córdoba province), southern Spain, and topotype specimens on Arroyo Frio (Jaén province), southern Spain.

Nematodes were extracted by the tray method (Whitehead & Hemming 1965) and then killed and fixed by hot FP 4:1 and processed to anhydrous glycerol (De Grisse 1969). The nematodes were transferred to a drop of glycerol and a surrounding ring of paraffin wax on permanent slides and studied using a light microscope equipped with a Dino-eye microscope eyepiece camera in conjunction with its Dino Capture version 2.0 software. Drawings were made through a drawing tube attached to a light microscope and redrawn using Adobe Photoshop 7.0 ME software. Specimens were identified at species level using available identification keys (Geraert 2011; Ghaderi *et al.* 2017).

Scanning electron microscopy

For the scanning electron microscopy, specimens preserved in glycerine were selected for observation under SEM according to the Abolafia's (2015) protocol. The nematodes were hydrated in distilled water, dehydrated in a graded ethanol-acetone series,

Table 1. Classification of Merliniinae genera based on Siddiqi (2000), Geraert (2011), and Sturhan (2012)

Siddiqi 2000	Geraert 2011	Sturhan 2012	Present study–analysed taxa:
5 genera	3 genera	6 genera	
<i>Scutylechus</i>	Synonymised with <i>Geocenamus</i>	Synonymised with <i>Geocenamus</i>	2 species
<i>Geocenamus</i>	<i>Geocenamus</i> includes the following genera: <i>Scutylechus</i> , <i>Merlinius</i> , <i>Hexadorus</i> , <i>Pathotylenchus</i> and <i>Allentylenchus</i> All junior synonyms of <i>Geocenamus</i>	<i>Geocenamus</i>	1 species
<i>Merlinius</i>	Synonymised with <i>Geocenamus</i>	<i>Merlinius</i>	3 species
<i>Amplimerlinius</i>	<i>Amplimerlinius</i>	<i>Amplimerlinius</i>	5 species
<i>Nagelus</i>	<i>Nagelus</i>	<i>Nagelus</i>	2 species
–	–	<i>Paramerlinius</i>	1 species
–	–	<i>Macrotylenchus</i>	–
–	–	<i>Telomerlinius</i>	1 species

critical-point dried with liquid carbon dioxide, mounted on SEM stubs, coated with gold, and observed with a Zeiss Merlin microscope (5 kV) (Zeiss, Oberkochen, Germany).

3D modelling

To visualise important morphological characters in the lip region and to facilitate further zoological education, 3D models were manually reconstructed by Autodesk® Maya® based on SEM images following the procedure of Qing *et al.* (2015).

Nematode molecular identification

DNA extraction was performed from a single individual as described by Subbotin *et al.* (2000). Several sets of primers were used for PCR. A partial region of the 28S rRNA gene including the expansion domains D2 and D3 (D2–D3) was amplified by using the primers D2A (5′ – ACAAGTACCGTGAGGGAAAGTTG– 3′) and D3B (5′ – TCGGAAGGAACCAGCTACTA– 3′) (Nunn 1992). The portion of 18S rRNA was amplified using primers 988F (5′ –CTCAAAGAT-TAAGCCATGC– 3′), 1912R (5′ –TTTACGGTCAGAACTAGGG– 3′), 1813F (5′ –CTGCGTGAGAGGTGAAAT– 3′), and 2646R (5′ –GCTACCTTGTTACGACTTTT– 3′) (Holterman *et al.* 2006). The internal transcribed spacer region (ITS) separating the 18S rRNA and 28S rRNA genes from the 5.8S rRNA gene was amplified using forward primer 18S (5′ –TTGAT-TACGTCCCTGCCCTTT– 3′) and reverse primer 26S (5′ –TTTCACTCGCCGTTACTAAGG– 3′) (Vrain *et al.* 1992). All PCR assays were carried out according to the conditions described by Archidona-Yuste *et al.* (2016). 5x HOT FIREpol® Blend Master Mix (Solis Biodyne, Tartu, Estonia) was used in all PCR reactions. The PCR products were purified after amplification using ExoSAP-IT (Affimetrix, USB products, Kandel, Germany) and used for direct sequencing in both directions with the corresponding primers. The resulting products were purified and run in a DNA multicapillary sequencer (Model 3130XL Genetic Analyser; Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator Sequencing Kit v.3.1 (Applied Biosystems) at the Stab Vida sequencing facility (Caparica, Portugal). The sequence chromatograms of the two markers were analysed using DNASTAR LASERGENE SeqMan v. 7.1.0. The newly obtained sequences were deposited in the GenBank database under accession numbers indicated on the phylogenetic trees and in Table 2.

Scutylechus sp. (Semnan pop.)KX789703 (28S)KX789706 and KX789707 (18S)Semnan, Semnana province, Iran Apple tree.

Phylogenetic analyses

Sequenced genetic markers in the present study (after discarding primer sequences and ambiguously aligned regions) and sequences obtained from GenBank were used for phylogenetic reconstruction of family Merliniidae (Table 2). Outgroup taxa for each dataset were selected based on previous published studies (Carta *et al.* 2010; Alvani *et al.* 2017). Multiple sequence alignments of the newly obtained and published sequences were made using the FFT-NS-2 algorithm of MAFFT v. 7.450 (Katoh *et al.* 2019). Sequence alignments were visualized using BioEdit (Hall 1999) and manually edited and trimmed of the poorly aligned positions using a light filtering strategy (up to 20% of alignment positions), which has little impact on tree accuracy and may save some computation time as suggested by Tan *et al.* (2015), since methods for automated filtering of multiple sequence alignments frequently worsen single-gene phylogenetic inference (Tan *et al.* 2015).

Phylogenetic analyses of the sequence data sets were based on Bayesian inference (BI) using MrBayes 3.2.7a (Ronquist & Huel- senbeck 2003). The best-fitted model of DNA evolution was obtained using jModelTest v. 2.1.7 (Darriba *et al.* 2012) with the Akaike information criterion (AIC). The base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the AIC-supported model were then used in phylogenetic analyses. BI analyses were performed under a transitional model of invariable sites and a gamma-shaped distribution (TIM3 + I + G) for D2–D3 region and partial 18S, and a transitional and a gamma-shaped distribution (TIM2 + G) model for the ITS rRNA region. These BI analyses were run separately per dataset with four chains for 4×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples of 30% and evaluating convergence, the remaining samples were retained for more in-depth analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) were given on appropriate clades. Trees from all analyses were visualized using FigTree software version 1.4.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

In this study, we identified fourteen known species of the subfamily Merliniinae under morphological taxonomic characters and molecular criteria including *Amplimerlinius globigerus* Siddiqi, 1979; *A. longicauda* Castillo, Siddiqi & Gómez-Barcina, 1990; *A. macrurus* (Goodey, 1932) Siddiqi, 1976; *A. magnistylus* Castillo, Gómez-Barcina, Vovlas & Navas, 1991; *A. paraglobigerus* Castillo, Siddiqi & Gómez-Barcina, 1990; *Geocenamus tenuidens* Thorne & Malek, 1968; *Paramerlinius hexagrammus* (Sturhan, 1966) Sturhan, 2012; *Merlinius alboranensis* (Tobar-Jiménez, 1970) Tarjan, 1973; *M. brevidens* (Allen, 1955) Siddiqi, 1970; *M. nanus* (Allen, 1955) Siddiqi, 1970; *Nagelus obscurus* (Allen, 1955) Powers, Baldwin & Bell, 1983; *N. leptus* (Allen, 1955) Siddiqi, 1979; *Scutylechus rugosus* (Siddiqi, 1963) Siddiqi, 1979; *Telomerlinius teleosus* Siddiqi & Sturhan, 2014; and one unknown species of *Scutylechus* from Iran. Our *Scutylechus* sp. as well as *G. tenuidens*, *M. alboranensis*, and *T. teleosus* were measured, described, and illustrated herein, whereas brief description and morphometric values are given for the other eleven previously described species (Ghaderi & Karegar 2014; Ghaderi *et al.* 2014).

Subfamily Merliniinae Siddiqi, 1971

Diagnosis

Lateral fields each with six incisures (except *Telomerlinius*). Deirids present except in *Geocenamus*, *Scutylechus*, and *Telomerlinius*. Phasmids usually prominent, on tail. Lip region annulated; cephalic disc indistinct or distinct (*Geocenamus* and *Telomerlinius*). Stylet under 50 µm (except *Macrotylenchus*, up to 137 µm), with distinct basal knobs. Median and basal bulbs well developed. Vulva small, pore-like, transversely oval or slit-like, usually with epitygma. Ovaries paired. Spermathecae two- to four-lobed. Postrectal intestinal sac absent. Female tail conoid, subcylindroid, cylindroid, or subclavate, between two and six anal body widths long; terminal inner cuticle layer occasionally strongly thickened. Male tail conical, about as long as that of female. Bursa simple, moderately developed, enveloping tail. Hypoptygma (a pair of papillae on

Table 2. List of species, localities, and GenBank accession numbers of specimens obtained in this study for phylogenetic analysis based on 28S rRNA, 18S rRNA, and ITS genes

Species	GenBank Accession no.			Locality	Associated Plant
	28S rRNA	18S rRNA	ITS		
<i>Amplimerlinius globigerus</i>	KX789691	KX789712	–	Naghadeh, West Azerbaijan, Iran	Apricot
<i>Amplimerlinius globigerus</i>	KX789695 and KX789696	KX789714 and KX789715	–	Ahhar, East Azerbaijan, Iran	Apple and Willow, respectively
<i>Amplimerlinius longicauda</i> (topotypes)	OP382256–OP382260	–	OP382222–OP382226	Arroyo Frio, Jaén province, Spain	Portuguese oak
<i>Amplimerlinius macrurus</i>	OP382245–OP382247	–	OP382230 and OP382231	Aligudarz, Lorestan province, Iran	<i>Astragalus</i> sp.
<i>Amplimerlinius macrurus</i>	OP382248 and OP382249	–	OP382227–OP382229	Soltanieh in Zanjan province, Iran	<i>Poa trivialis</i>
<i>Amplimerlinius macrurus</i>	KX789692–KX789694	KX789710, KX789711 and KX789713	–	Mazandaran, Iran	Alder
<i>Amplimerlinius magnistylus</i>	OP382250	–	OP382232–OP382233	Valenzuela, Córdoba province, Spain	Almond
<i>Amplimerlinius paraglobigerus</i> (topotypes)	OP382251–OP38512255	–	OP382219–OP382221	Arroyo Frio, Jaén province	Portuguese oak
<i>Geocenamus tenuidens</i>	OP382273 and OP382274	OP382243–OP382244	–	Mongasht mountain, Dehdez, Khuzestan, Iran	<i>Astragalus</i> sp.
<i>Merlinius alboranensis</i>	OP382270 and OP382271	–	–	Dezful, Khuzestan province, Iran	<i>Crataegus aronia</i>
<i>Merlinius brevidens</i>	–	KX789708	–	Ardebil province, Iran	Wheat
<i>Merlinius nanus</i>	KX789700	KX789709	–	Abr forest, Semnan province, Iran	Willow
<i>Nagelus leptus</i>	KX789699	KX789718	–	Abr forest, Semnan province, Iran	Willow
<i>Nagelus obscurus</i>	KX789697 and KX789698	KX789716 and KX789717	–	Khalkhal, Ardabil province, Iran	Johnson grass, apple
<i>Paramerlinius hexagrammus</i>	KX789701	KX789719 and KX789720	–	Abr forest, Semnan province, Iran	Sloe
<i>Scutylenchus rugosus</i>	–	KX789704 and KX789705	–	Salmas, West Azerbaijan province, Iran	Peach and <i>Astragalus</i> sp., respectively
<i>S. rugosus</i> (pop. 272)	OP382272	OP382241	–	Tarom, Zanjan province, Iran	<i>Astragalus</i> sp.
<i>Scutylenchus</i> sp. (pop. 271)	OP382263 and OP382264	OP382239–OP382240	–	Sendan, Zanjan province, Iran	<i>Astragalus</i> sp
<i>Scutylenchus</i> sp. (pop. 222)	OP382267	OP382234	OP382216–OP382218	Damavand mountain, Mazandaran province, Iran	<i>Astragalus</i> sp
<i>Scutylenchus</i> sp. (pop. 286)	OP382265, OP382266, OP382268 and OP382269	OP382235–OP382238	OP382215	Meshkin Shahr, Ardabil province, Iran	<i>Astragalus</i> sp
<i>Telomerlinius teleosus</i>	OP382261 and OP382262	OP382242	–	Dezful, Khuzestan province, Iran	<i>Prosopis cineraria</i>

posterior lip of cloaca opening) always present. Spicules cylindroid in distal half, straight to slightly arcuate, with distal end broadly rounded, notched (as a main character) and devoid of ventro-lateral flanges or vela. Gubernaculum simple, trough-like, fixed.

Type Genus

Merlinius Siddiqi, 1970

Other Genera

Amplimerlinius Siddiqi, 1976
Geocenamus Thorne & Malek, 1968
Macrotylenchus Sturhan, 2012
Nagelus Thorne & Malek, 1968
Paramerlinius Sturhan, 2012
Scutylenchus Jairajpuri, 1971
Telomerlinius Siddiqi & Sturhan, 2014

Key to identification of the genera of subfamily Merliniinae

1	Deirid present	2
	Deirid absent	6
2	Deirid situated in the four-incisures region of the lateral field	3
	Deirid situated in the six-incisures region of the lateral field	5
3	Stylet slender and more than 90 µm, tail terminus annulated, without refractive inner cuticle layer	<i>Macrotylenchus</i>
	Stylet short and usually under 50 µm, tail terminus usually smooth, with refractive inner cuticle layer surrounding the tail tip	4
4	Cephalic framework heavily sclerotized, stylet 20-50 µm	<i>Paramerlinius</i>
	Cephalic framework moderate to weak, stylet less than 20 µm	<i>Merlinius</i>
5	Cephalic region continuous with body contour, female tail approximately cylindrical, with refractive inner cuticle layer around its end	<i>Amplimerlinius</i>
	Lip region slightly offset by expansion, female tail elongate-conoid to subcylindrical without refractive inner cuticle layer around its end	<i>Nagelus</i>
6	Lateral field with four incisures, cephalic region distinctly offset, stylet slender, basal bulb overlap with intestina	<i>Telomerlinius</i>
	Lateral field usually with six incisures, cephalic region continues to slightly offset, stylet usually robust, basal bulb offset	7
7	Body cuticle marked by longitudinal striae or grooves at entire body	<i>Scutylenchus</i>
	Body cuticle without longitudinal striae or grooves (rarely at anterior or posterior end of body)	<i>Geocenamus</i>

Genus *Amplimerlinius* Siddiqi, 1976

Diagnosis

Body medium to large sized, arcuate to strongly curved. Cuticle with prominent annuli. Lateral field with six incisures in adults, fourth and third stage juveniles and four in second stage juveniles. Deirids located in six-incisures region of lateral field. Cephalic region continuous with body contour, annuli not broken by radial grooves or indentations, face view rounded, cephalic plate fused with first annulus. Amphidial apertures ovate, cephalic framework heavily sclerotised. Stylet robust, 20–47 µm long; conus about half of total stylet length; knobs large, rounded. Female tail cylindrical to subclavate, smooth or annulated terminus; with distinct hyaline but without refractive inner cuticle layer around its end. Spicules robust, slightly arcuate, blunt and notched at tip. Gubernaculum trough-shaped in lateral view.

Type species

Amplimerlinius amplius Siddiqi, 1976

Other species

A. globigerus Siddiqi, 1979
A. hornensis Bello, Mahajan & Zancada, 1987
A. icarus (Wallace & Greet, 1964) Siddiqi, 1976
A. intermedius (Bravo, 1976) Siddiqi, 1976
A. longicauda Castillo, Siddiqi & Gómez-Barcina, 1990
A. macrurus (Goodey, 1932) Siddiqi, 1976
A. magnistylus Castillo, Gómez-Barcina, Vovlas & Navas, 1991
A. nectolineatus Siddiqi, 1976
A. omentelus Kleynhans & Heyns, 1983
A. paraglobigerus Castillo, Siddiqi & Gómez-Barcina, 1990
A. parbati Zarina & Maqbool, 1990
A. planitierus (Eroshenko, 1984) Eroshenko & Volkova, 1988
A. quercinus Mahajan, 1996
A. siddiqii Mancini, Cotroneo & Moretti, 1982
A. sikkimensis Shaw & Khan, 1992
A. socialis (Andrássy, 1962) Siddiqi, 1976

A. truncatus (Poghossian, 1979) Geraert, 2011
A. umbonatus Ivanova, 1982
A. uramanatiensis Ghaderi & Karegar, 2014
A. viciae (Saltukoglu, 1973) Siddiqi, 1976

Amplimerlinius globigerus Siddiqi, 1979

The Iranian populations from Naghadeh and Ahar in West Azerbaijan and East Azerbaijan provinces were brecovered in the rhizosphere of apricot and apple trees, respectively. Females can be characterised by having a straight to slightly ventrally curved body posture, distinct cuticular annuli, lip region continuous, rounded with flattened anterior end, with 7–8 annuli, stylet with rounded to slightly posteriorly directed knobs, tail cylindrical. Males similar to females except for sexual characters, bursa encircling the entire tail. The morphology and morphometric characters of the Iranian populations are consistent with the other populations from Iran (Ghaderi & Karegar 2014).

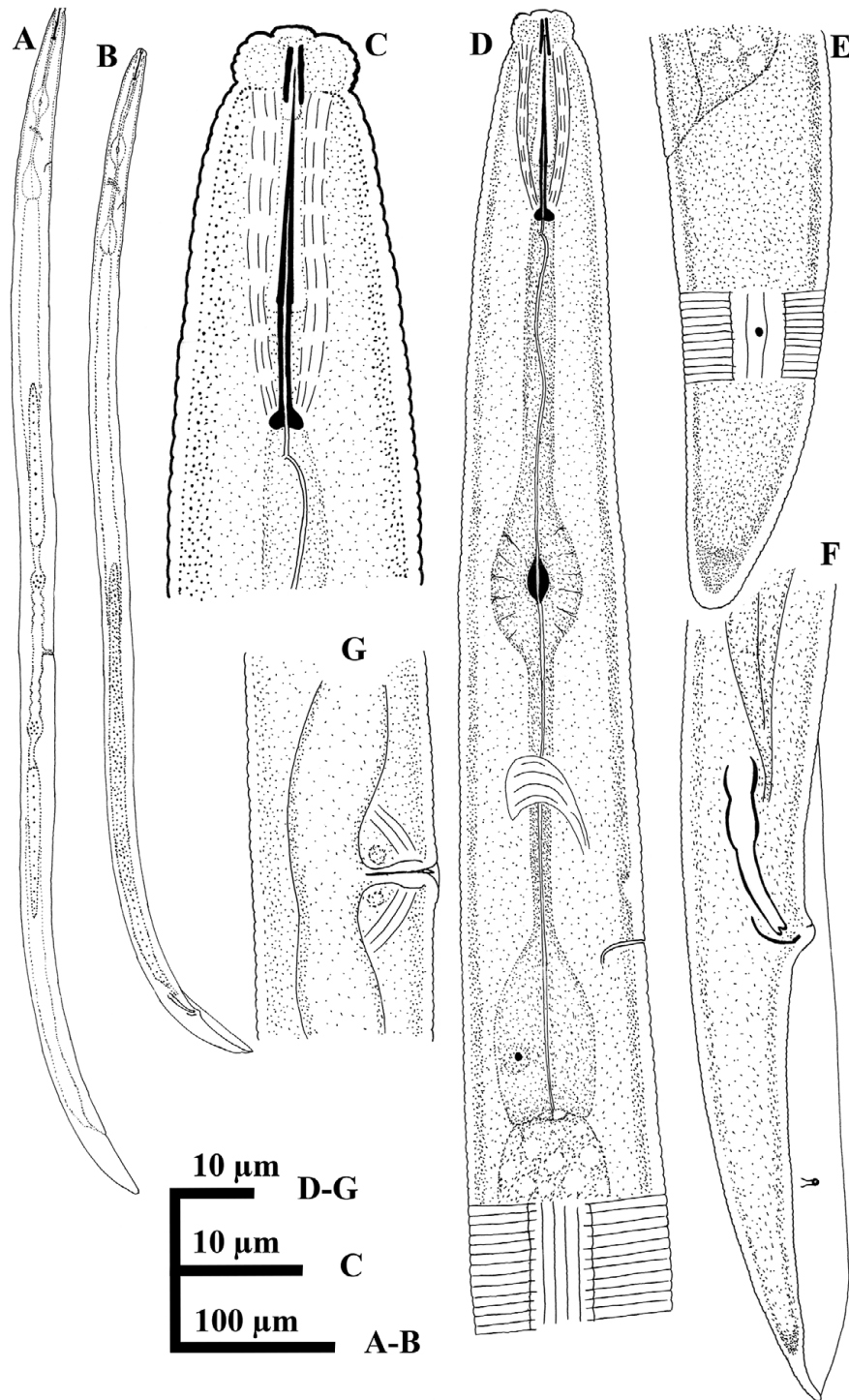


Figure 1. Line drawings of the Iranian population of *Geocenamus tenuidens*. A, C–E, female and B, F, male. A, B, entire body. C, anterior region. D, pharyngeal region. E, F, tail region. G, vulval region.

***Amplimerlinius longicauda* Castillo, Siddiqi & Gómez-Barcina, 1990**

(Figure S1, Table S2)

Morphology and morphometry of topotype specimens of this species agree with the original description (Castillo *et al.* 1990) and can be characterised by having an elongate female tail measuring 3.2–3.5 anal body widths long, a stout stylet measuring 33–37 μm, and outer bands of lateral fields with few scattered striae in pharyngeal and tail regions.

***Amplimerlinius macrurus* (Goodey, 1932) Siddiqi, 1976**

(Figures S2, S3; Table S3)

The Iranian populations of *A. macrurus* from the rhizosphere of alder trees in Mazandaran province and *Astragalus* sp. in Lorestan province were characterised by cylindrical and slightly ventrally curved body, rounded lip region continuous with flattened anterior end, robust stylet having rounded to posteriorly directed knobs, cylindrical tail, terminus with distinct annuli which are smaller than



Figure 2. Light micrographs of the Iranian population of *Geocenamus tenuidens*. A–E, female and F, male. A–C, anterior region. D–F, tail region. Scale bars = 10 μm .

or as wide as other tail annuli, bearing 43 (38–52) tail annuli, hyaline region 11.0 (9.5–12.5) μm in Mazandaran population but tail cylindrical to subclavate with smooth terminus or with large irregular annuli at terminus in Lorestan population (Figures S2 & S3). The morphological and morphometric characters of the Iranian populations are consistent with the other populations from Iran (Ghaderi & Karegar 2014).

***Amplimerlinius magnistylus* Castillo, Gómez-Barcina, Vovlas & Navas, 1991**

(Figure S1, Table S3)

The Spanish population of *A. magnistylus* from the rhizosphere of almond trees in Córdoba province was characterised by cylindrical and slightly ventrally curved body, lip region continuous anteriorly flattened, stylet long and robust (43–45 μm in females), knobs rounded with anterior surfaces backwardly directed, basal bulb elongate-saccate, slightly longer than isthmus,

vulva cavity with double sunken epiptygma, tail elongate-cylindroid; terminus hemispherical, with fine striae. The morphological and morphometric characters of the population from Valenzuela (Córdoba province) are consistent with the original population described at Bujalance (Córdoba province) (Castillo *et al.* 1991).

***Amplimerlinius paraglobigerus* Castillo, Siddiqi & Gómez-Barcina, 1990**

(Figure S1, Table S2)

Morphology and morphometry of topotype specimens of this species agree with the original description (Castillo *et al.* 1990) and can be characterised by a small body and stylet measuring under 1 mm and under 24 μm , respectively; lip region with 8–10 annuli and cephalic sclerotisation not appearing bead-like in optical section, and a slender isthmus about 1.5 times length of the basal bulb.

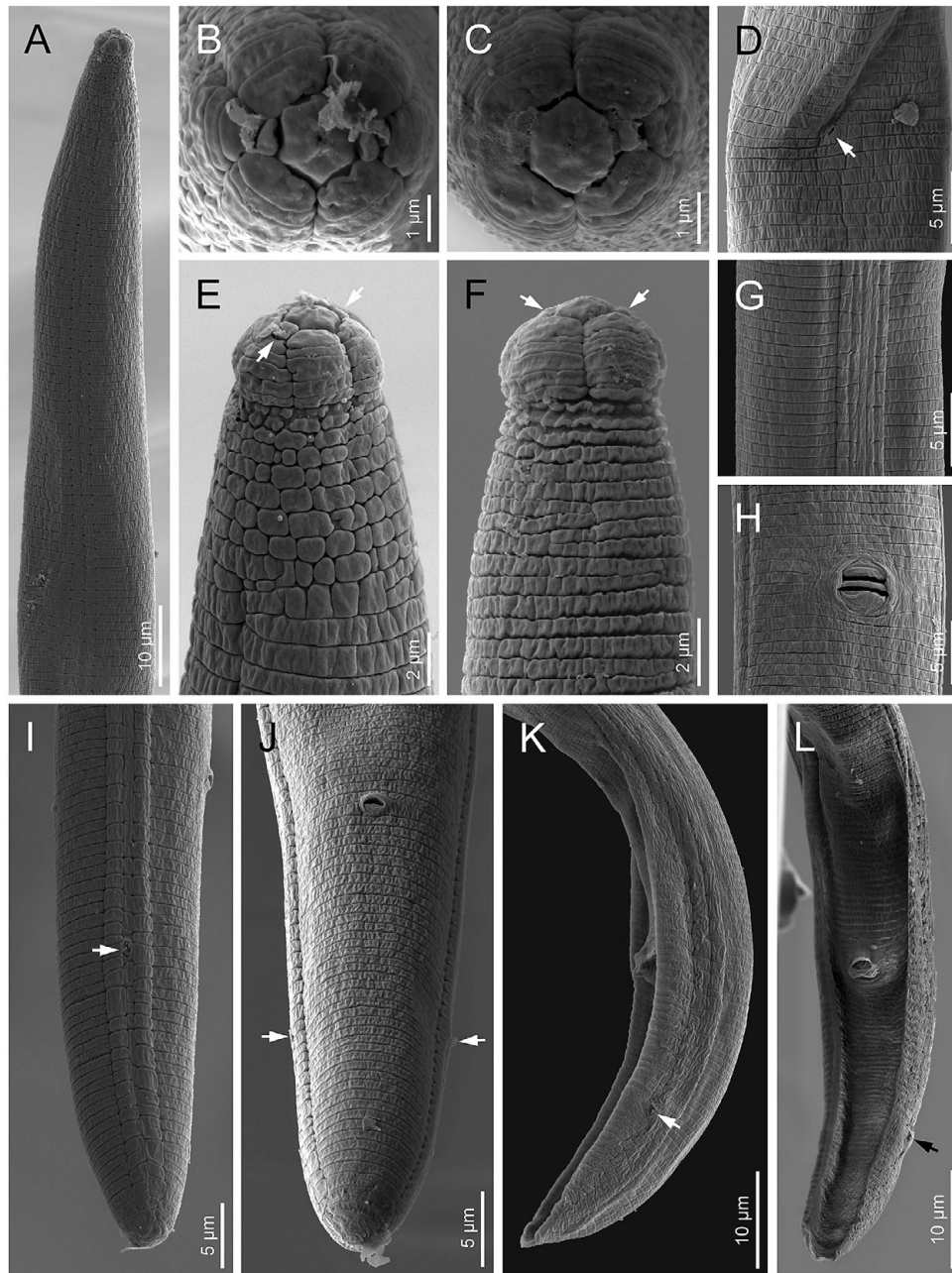


Figure 3. SEM micrographs of the Iranian population of *Geocenamus tenuidens*. A–J, female and K, L, male. A, E, F, anterior region (white arrows indicating the amphidial apertures). B, C, *en face* view of cephalic region. D, excretory pore in ventral view (white arrow indicating the excretory pore). G, lateral field. H, vulval region in ventral view showing small lateral flaps. I–L, tail region (white and black arrows indicating the phasmid).

Genus *Geocenamus* Thorne & Malek, 1968

Diagnosis

Body about 1 mm or longer. Body cuticle without longitudinal striae (except for the anterior body region in some species). Lateral field with six incisures in adults and four in all juvenile stages. Deirid absent. Cephalic region offset, with six radial grooves and conspicuous, perioral disc. Cephalic framework moderate. Stylet slender, 25–30 µm, conus longer than shaft. Female tail elongate-conoid to subcylindroid, terminus smooth or annulated, refractive inner cuticle layer at tail end present. Spicules slender, slightly arcuate and notched at tip. Gubernaculum crescent-shaped in lateral view.

Type species

Geocenamus tenuidens Thorne & Malek, 1968

Other species

- G. arcticus* (Mulvey, 1969) Tarjan, 1973
- G. angelescresti* Chitambar & Ferris, 2005
- G. deserticola* (Ivanova & Shagalina, 1983) Fortuner & Luc, 1987
- G. khashanicus* Volkova, 1995
- G. superbus* (Allen, 1955) Fortuner & Luc, 1990
- G. tokobaevi* (Sultanalieva, 1983) Fortuner & Luc, 1987

Geocenamus tenuidens Thorne & Malek, 1968
(Figures 1–3, Table 3)

Table 3. Morphometrics of *Geocenamus tenuidens* from Iran. All measurements are in μm and in the form: mean \pm s.d. (range)

	Present study				Geraert, 2011
	Female		Male		Female/Male
n	14	CV	10	CV	?
L	854 \pm 49.7 (746–919)	5.8	786 \pm 32.6 (740–845)	4.1	760–1390
a	38.0 \pm 3.3 (31.2–43.3)	8.6	44.0 \pm 4 (36.0–49.0)	9.1	30.0–50.0
b	6.0 \pm 0.4 (5.4–6.8)	6.4	5.0 \pm 0.2 (5.2–6.0)	3.9	–
c	18.0 \pm 1.4 (16.4–20.9)	7.9	15 \pm 0.8 (14.3–17.0)	5.4	12.0–24.0
c'	2.0 \pm 0.1 (2.4–3.0)	5.1	3.0 \pm 0.1 (2.8–3.3)	4.9	2.4–3.6
V or T	53.0 \pm 1.2 (51.0–55.5)	2.4	–	–	50–58
Stylet	22.0 \pm 0.6 (21.6–24)	2.6	22.0 \pm 0.5 (22.0–23.5)	2.3	21–31
Pharynx	136 \pm 3.2 (132–143)	2.4	139 \pm 7.2 (131–151)	5.1	134–181
Median bulb	68 \pm 2.1 (64–71)	3.1	72 \pm 4.6 (67–80)	6.3	–
MB	49 \pm 1.3 (47–52)	2.7	96 \pm 3.5 (91–102)	3.6	48–56
Excretory pore	116 \pm 4.2 (111–125)	3.6	110 \pm 4.9 (102–119)	4.4	–
Head–vulva	457 \pm 21.8 (414–501)	4.7	–	–	–
Head–anus	809 \pm 48.1 (705–874)	5.9	735 \pm 32.6 (690–793)	4.4	–
Body width	22 \pm 1.7 (19–26)	7.8	17 \pm 1.6 (16.3–21.0)	9.4	–
Vulva body width	22 \pm 1.6 (19–26)	7.4	–	–	–
Anal body width	17 \pm 1.0 (15.5–19.5)	6.1	16 \pm 0.6 (15–17)	3.8	–
Tail length	45 \pm 4.0 (41–55)	8.8	51 \pm 1.8 (49–54)	3.6	43–77
Tail annuli	48 \pm 4.5 (44–59)	9.3	–	–	45–80
Phasmid	17 \pm 2.1 (15.5–23.0)	12.2	26 \pm 1.5 (25–30)	5.6	–
Spicules	20 \pm 0.3 (20.5–21.6)	1.5	–	–	19–31
Gubernaculum	6.8 \pm 0.3 (6.2–7.0)	4.5	–	–	8–11

Description of female

Body slightly arcuate after heat fixation. Longitudinal striation or ridges indistinct except lateral fields. Body annuli distinct but fine, 1.2 (1.0–1.3) μm at mid-body. Lateral field originating at the level of the stylet and extending up to tail terminus, with six incisures, outer bands areolated, 7.8 (6–9) μm wide occupying 36 (30–39) % of the corresponding body diameter. Cephalic region distinctly offset by a constriction, bearing 5–6 annuli with distinct perioral disc. Cephalic framework not refractive. Stylet slender, conus 13.3 (12.5–14.0) μm or 58.5 (57.7–60.0) % of the total stylet length; basal knobs weak and posteriorly sloping, 2.7 (2.5–3.0) μm across. DGO 2.5 (2.3–2.8) μm behind stylet knobs. Median bulb oval, 10.8 (9–12) μm \times 18 (14.5–21.0) μm , occupying 57 (47–64) % of body wide at that level. Basal bulb pyriform 11.6 (9.5–13.0) μm \times 25 (20–30) μm . Nerve ring at 97 (92–104) μm from anterior end. Hemizonid 4 to 6 annuli anterior to excretory pore, at 117 (111–125) μm from anterior end. Deirids not seen. Reproductive system didelphic, vulva slightly sunken into the body, with small epiptygma. Vagina perpendicular, 8.3 (6.5–9.5) μm long occupying 22.5 (20–26) % of vulval body diameter, spermatheca rounded and filled with globular sperm cells. Post anal intestinal sac absent. Tail elongate, conical to subcylindrical, tail terminus coarsely striated. Phasmids located at 39 (35–45) % of tail.

Description of male

Morphologically similar to female except for sexual characters. Body slightly shorter. Body annuli 1.1 (1.0–1.2) μm at mid-body.

Lateral field 6.5 (5.3–7.0) μm wide. Cephalic region offset, 6.8 (6.2–7.1) μm wide and 3.9 (3.7–4.5) μm high. Conus 58 (56–59) % of the total stylet length. DGO 2.5 (2–3) μm behind stylet knobs. Nerve ring at 96 (91–102) μm from anterior end. Hemizonid 3–5 annuli anterior to excretory pore and located at 114 (102–119) μm from anterior end. Bursa surrounded tail tip, 70 (65–81) μm in length. Spicules tylenchoid, notched at tip. Gubernaculum simple and ventrally arcuate.

Voucher specimens

Fourteen females and eight males were deposited in the nematode collection of the department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran.

Host and locality

Recovered from the rhizosphere of *Astragalus* sp. in Mongasht Mountain in Dehdez region, Khuzestan province, southwest of Iran. GPS coordinates: 31°47'16" N, 50°25'32" E.

Diagnosis and relationships

G. tenuidens is similar to *G. arcticus* (Mulvey, 1969) Tarjan, 1973.

G. tenuidens differs from *G. arcticus* by a shorter stylet (22.8 (21.6–24.0) vs. 32–38 μm), shorter tail (45.6 (41–55) vs. 65–80 μm) and shorter spicules (21.0 (20.5–21.6) vs. 26–28 μm) and in tail terminus (striated vs. smooth).

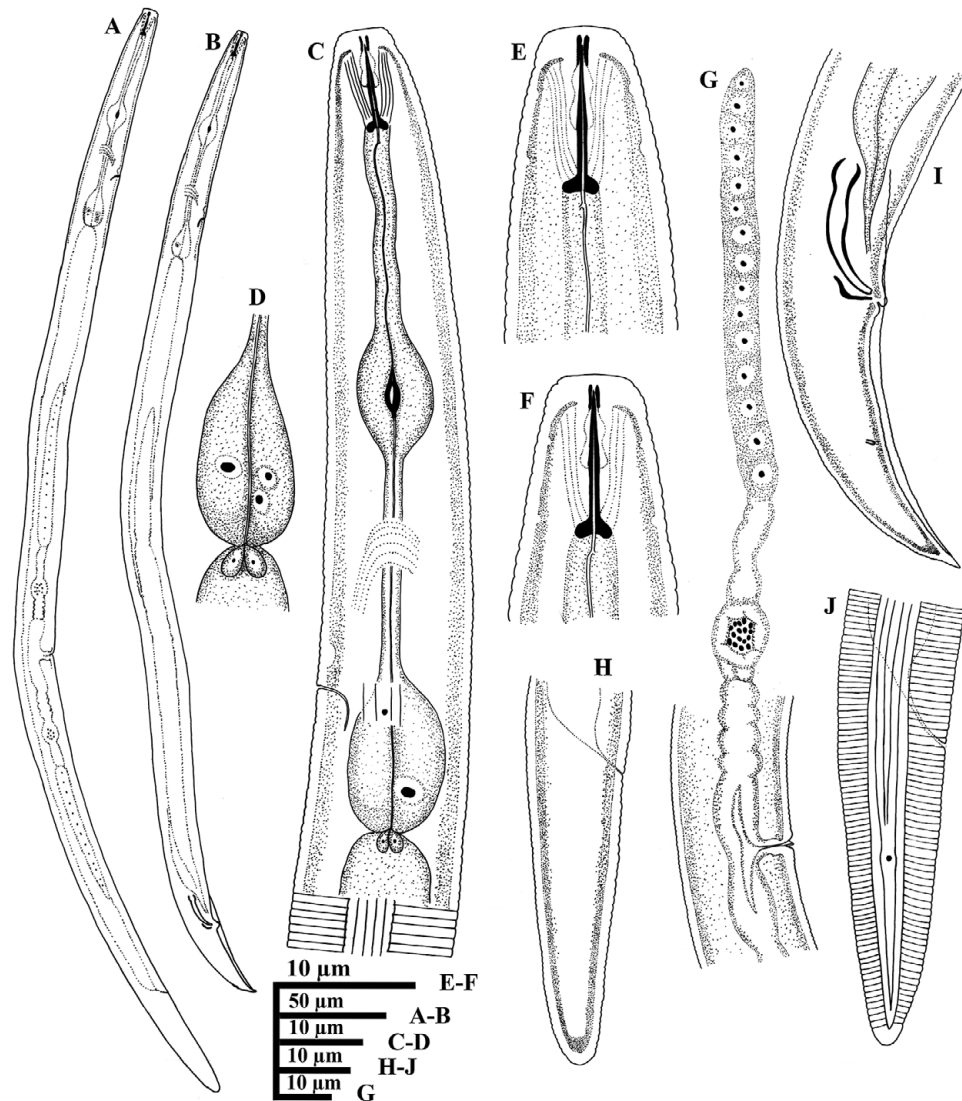


Figure 4. Line drawings of the Iranian population of *Merlinius alboranensis*. A, C, D, F–H, J, female and B, E, I, male. A, B, entire body. C, pharyngeal region. D, basal bulb of pharynx. E, F, anterior region. G, reproductive system. H–J, tail region.

Fadavi Khalajlo *et al.* (2013) described *G. tenuidens* from tomato plants in North Khorasan province, but the population was described without perioral disc, with strong stylet knobs, short conus, and 32–45 tail annuli, while *G. tenuidens* has been described originally with a distinct perioral disc, slender stylet with long conus and tail with 45–80 annuli. Morphological and morphometric characters of our population fit well with those of *G. tenuidens*, so we consider the present population as the first population of the species reported from Iran.

Genus *Merlinius* Siddiqi, 1970

Diagnosis

Body usually 1 mm or less. Body cuticle lacking longitudinal striae or grooves. Lateral field with six incisures in adults and four in all juvenile stages. Lip region continuous or slightly offset. Perioral disc and first annulus merged, lip region hexagonal but not separated from radial incisures, the lateral sectors demarcated from defective incisures, submedian sectors wider than lateral sectors, amphidial apertures nearly rounded in first annulus. Stylet usually under

20 µm long, conus half of stylet length or shorter. Deirids distinct and in four-incisure region of lateral field. Vulva with transverse slit, epitygma indistinct. Female tail conoid to subcylindroid, terminal cuticle normally thickened and with refractive inner cuticle layer around tail end. Spicules cylindroid, straight to slightly arcuate, tip bluntly notched. Gubernaculum crescent-shaped in lateral view.

Type species

Merlinius brevidens (Allen, 1955) Siddiqi, 1970

Other species

- M. acuminatus* Minagawa, 1985
- M. alboranensis* (Tobar-Jiménez, 1970) Tarjan, 1973
- M. bavaricus* (Sturhan, 1966) Siddiqi, 1970
- M. bijnorensis* Khan, 1971
- M. bilqeesae* Khan & Khan, 1995
- M. bogdanovikatjkovi* (Kirjanova, 1941) Siddiqi, 1970
- M. capitonis* Ivanova, 1983
- M. circellus* Anderson & Ebsary, 1982

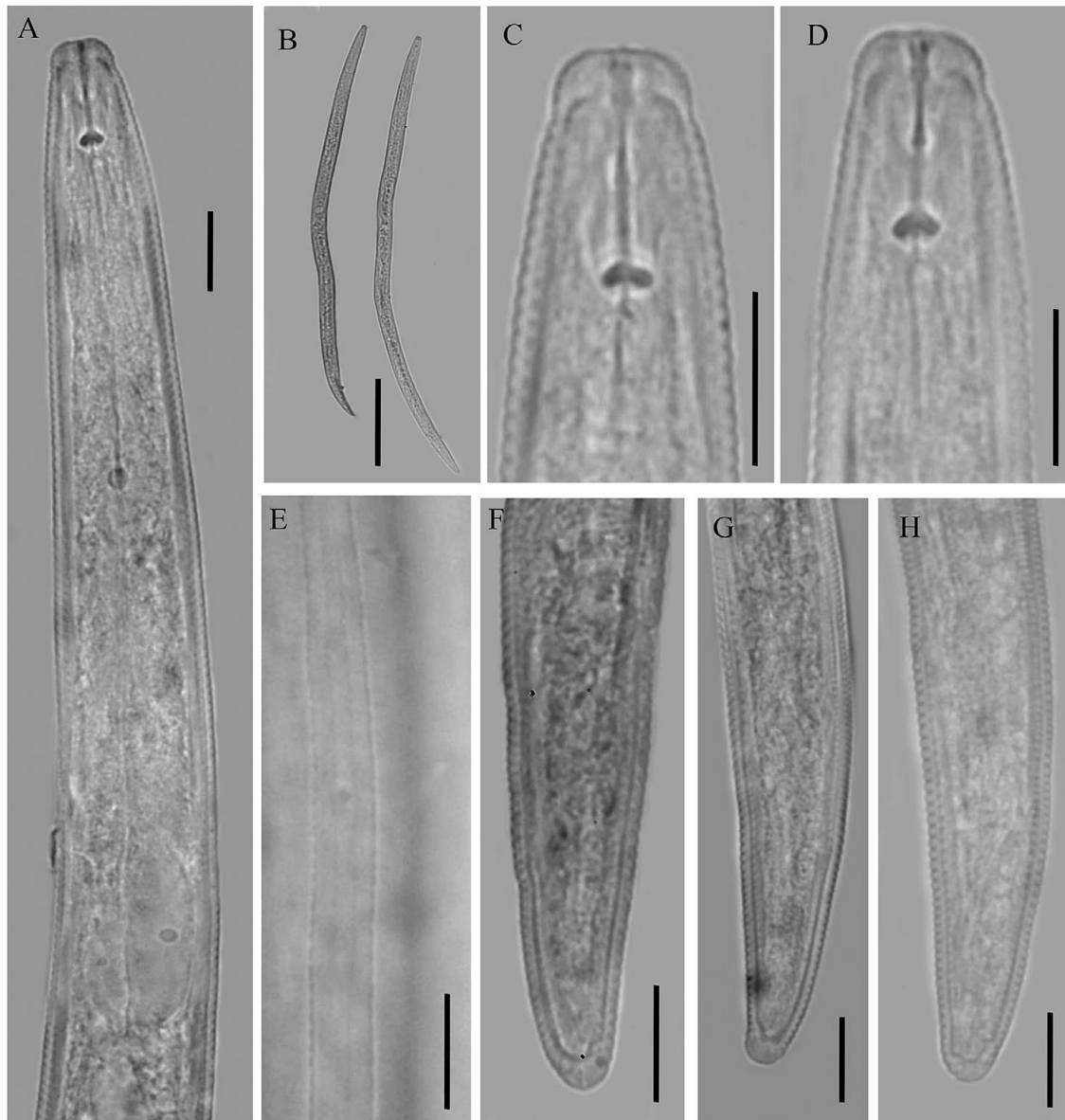


Figure 5. Light micrographs of the Iranian population of *Merlinius alboranensis*. A, B (right), C–H, female and B (left), male. A, pharyngeal region. B, entire body. C, D, anterior region. E, lateral field and deirid. F–H, tail region. Scale bars: A, C–H = 10 μ m; B = 100 μ m.

M. communicus Sultan, Singh & Sakhuja, 1989
M. gatevi Budurova, 1988
M. graminicola (Kirjanova, 1951) Siddiqi, 1976
M. indicus Zarina & Maqbool, 1995
M. joctus (Thorne, 1949) Sher, 1974
M. khuzdarensis Handoo, Khan & Islam, 2007
M. lineatus (Allen, 1955) Siddiqi, 1970
M. loofi Siddiqi, 1979
M. microdorus (Geraert, 1966) Siddiqi, 1970
M. mollicephalus Eroshenko & Volkova, 1988
M. montanus Maqbool & Shahina, 1987
M. nanus (Allen, 1955) Siddiqi, 1970
M. niazae Maqbool, Fatima & Hashmi, 1983
M. nothus (Allen, 1955) Siddiqi, 1970
M. obesus (Gagarin, 2004) Sturhan, 2012
M. pistaciei Fatema & Farooq, 1992
M. plerorbus Anderson & Ebsary, 1982
M. processus Siddiqi, 1979

M. productus (Thorne, 1949) Sher, 1974
M. pseudobavaricus Saltukoglu, Geraert & Coomans, 1976
M. pyri Fatema & Farooq, 1992
M. tetylus Anderson & Ebsary, 1982
M. tortilis Kazachenko, 1980

Species inquirendae

M. salechardicus Nesterov, 1985
M. kirjanovae (Karapetjan, 1979) Eroshenko & Volkova, 1987

Merlinius alboranensis (Tobar-Jiménez, 1970) Tarjan, 1973
 (Figures 4–6, Table 4)

Description of female

Body ventrally arcuate to C-shaped after heat fixation. Longitudinal striation or ridges except lateral fields, absent. Body annuli distinct, 1.1 (0.9–1.2) μ m at mid-body. Lateral field originating at the level of the procorpus and extending up to tail terminus, with six incisures

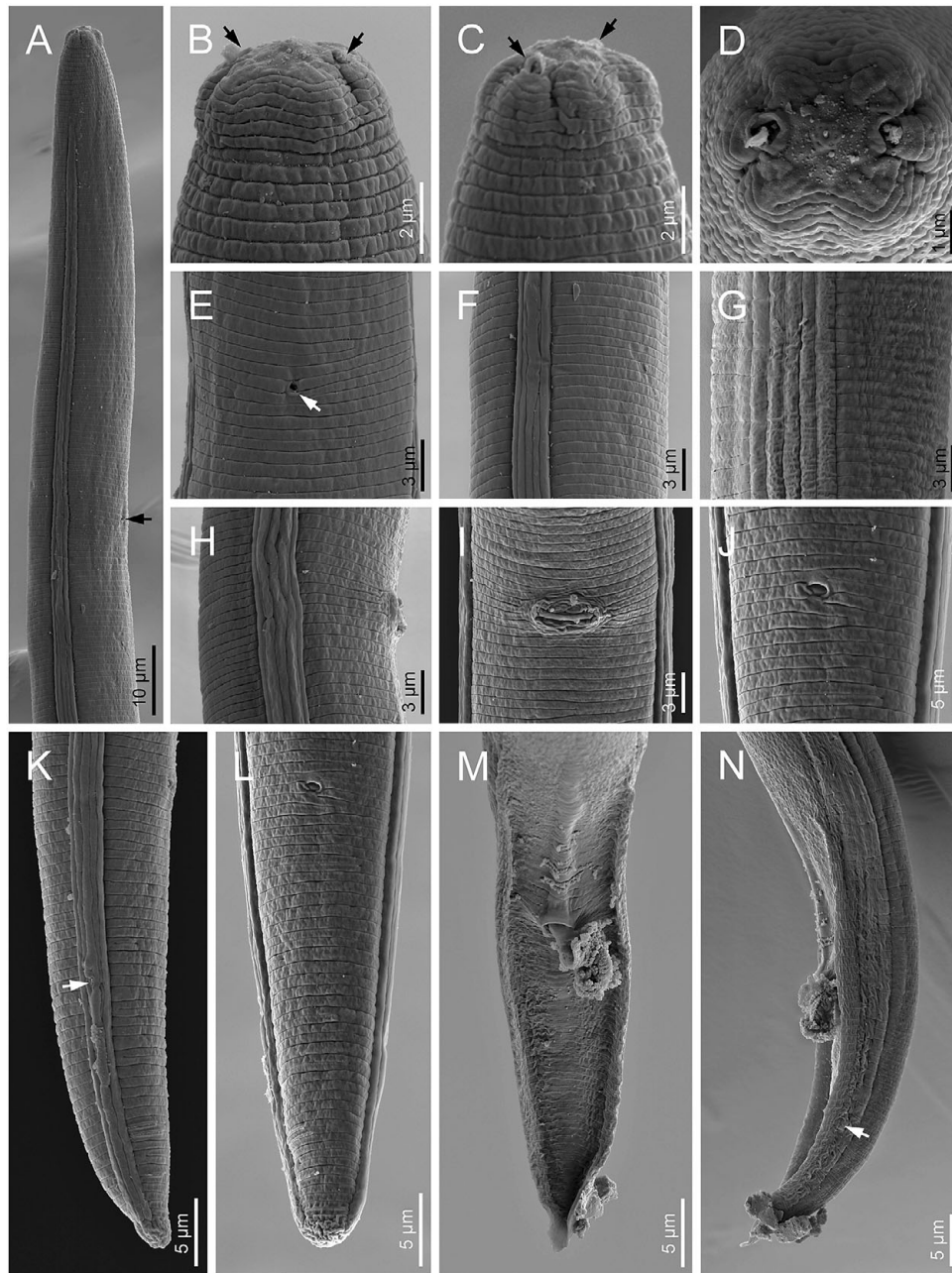


Figure 6. SEM micrographs of the Iranian population of *Merlinius alboranensis*. A–L, female and M, N, male. A, anterior region (black arrow indicating the excretory pore). B, C, lateral view of cephalic region (black arrows indicating the amphidial apertures). D, *en face* view of cephalic region. E, excretory pore in ventral view (white arrow indicating the excretory pore). F, lateral field at deirid region. G, lateral field at mid-body. H, Lateral field at vulval region. I, vulval region in ventral view. J, anus in ventral view. K–N, tail region (white arrows indicating the phasmid).

at mid-body, 6.3 (5.4–7.5) μm wide occupying 31 (28–34) % of body diameter. Lip region slightly offset by constriction, flattened at front, bearing 4–5 annuli. Cephalic framework not refractive. Stylet delicate, conus 5.3 (5.0–5.6) μm or 49 (48–52) % of total stylet length, basal knobs small and posteriorly directed, 2.8 (2.6–3.0) μm across. DGO 1.7 (1.5–2.0) μm behind stylet knobs. Median bulb oval, 10.4 (9.5–11.0) $\mu\text{m} \times 16$ (14–19) μm , occupying 64 (56–69) % of body wide at that level. Basal bulb pyriform, 11.1 (9.5–12.5) $\mu\text{m} \times 21$ (19–23) μm . Nerve ring at 72 (63–76) μm from anterior end. Hemizonid 1 to 2 annuli anterior to secretory-excretory pore, at 85 (68–95) μm from anterior end. Deirids in four-inscures region.

Reproductive system didelphic, with epitygma, vagina perpendicular, 7.9 (7.0–9.5) μm occupying 39 (33–43) % of vulval body diameter. Spermatheca slightly ovate and filled with globular sperm cells. Post anal intestinal sac absent. Tail elongate, conical to sub-cylindrical with 41 (32–50) annuli in ventral side, tail terminus smooth. Phasmids located at 45 (40–47) % of tail.

Description of male

Morphologically similar to female except for sexual characters. Body straight to ventrally curved. Body annuli 1.1 (0.9–1.2) μm at mid-body. Lateral field 6.5 (5.2–8.3) μm wide. Lip region offset, 7.3

Table 4. Morphometrics of *Merlinius alboranensis* from Iran. All measurements are in μm and in the form: mean \pm s.d. (range)

	Present study		Tobar-Jiménez (1970)
	Female	CV	Female/Male
n	12		10/10
L	523 \pm 34 (487–604)	6.5	430–470
a	25.6 \pm 2.2 (22.8–29.6)	8.6	24.6–29.6
b	4.7 \pm 0.3 (4.2–5.2)	7.2	4.5–5.3
c	12.9 \pm 1.3 (11.0–15.2)	10.2	12.4–15.1
c'	2.8 \pm 0.3 (2.4–3.6)	11.3	–
V or T	57.0 \pm 1.2 (54.6–58.7)	2.1	58–61
Stylet	10.7 \pm 0.4 (10.0–11.5)	3.9	11–11
Median bulb	51.2 \pm 2.8 (44–54)	5.5	–
MB	46.5 \pm 2.8 (44–55)	6.1	–
Excretory pore	88.8 \pm 8.1 (70–102)	9.1	–
Deirid	93.5 \pm 5.1 (86–105)	5.5	–
Pharynx	110 \pm 6.6 (97–117)	6.0	–
Head–vulva	298 \pm 19.4 (269–341)	6.5	–
Head–anus	482 \pm 31.7 (450–554)	6.5	–
Body width	20 \pm 1.6 (18–23)	7.9	–
Vulval body width	20 \pm 1.5 (18.5–23.5)	7.4	–
Anal body width	14 \pm 1.1 (12.5–16.5)	8.1	–
Tail length	40.5 \pm 4.7 (32–50)	11.7	–
Phasmid	18 \pm 1.4 (15–20)	7.9	–
Spicules	–	–	17–20
Gubernaculum	–	–	5–7

(6.8–7.5) μm wide and 2.3 (2.1–2.5) μm high. Conus 48 (47–49) % of total stylet length. DGO 1.5 μm behind stylet knobs. Nerve ring at 73 (63–80) μm from anterior end. Hemizonid 1–2 annuli anterior to excretory pore and located at 82 (76–87) μm from anterior end. Bursa enveloping entire tail, 61 (58–68) μm . Spicules tylenchoid, at tip with a notch. Gubernaculum simple, ventrally arcuate.

Voucher specimens

Seven females and five males were deposited in the collection of the department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran. Five females and a male were deposited in the collection of the Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran.

Host and locality

Recovered from the rhizosphere of Hawthorn (*Crateagus aronia* L.), collected in Dezfule Region in Khuzestan province, southwest of Iran. GPS coordinates: 32°90'42" N, 48°57'06" E; altitude: 1107 m. a.s.l.

Relationships

M. alboranensis comes close to *M. brevidens* (Allen, 1955) Siddiqi, 1970; *M. capitonis* Ivanova, 1983; *M. loofi* Siddiqi, 1979; *M. microdorus* (Geraert, 1966) Siddiqi, 1970; and *M. nanus* (Allen, 1955) Siddiqi, 1970 according to stylet length, tail shape,

body length, tail annuli, and cephalic region shape. It differs from *M. brevidens* by a shorter stylet (10.8 (10.0–11.5) vs. 13.0–16.5 μm) and a weakly developed cephalic framework (vs. distinctly refractive). From *M. capitonis*, it differs by a shorter body (523 (487–604) vs. 680–810 μm) and a slightly offset cephalic region (vs. offset). From *M. loofi*, it differs by a shorter tail (40.6 (32–50) vs. 62 μm), smaller c' ratio (2.9 (2.5–3.6) vs. 4.3–5.7), and lower number of tail annuli (42 (34–52) vs. 50–60). From *M. microdorus*, it differs by a shorter stylet (10.8 (10.0–11.5) vs. 12.0–15.5 μm) and a slightly but visible lower lip region. Finally, it differs from *M. nanus* only in tail terminus (smooth vs. annulated) and cuticular annulations (shallow vs. deep).

The Iranian population of *M. alboranensis* is very similar to the original description (Tobar-Jiménez, 1970) but differs only by a slightly longer body (523 (487–604) vs. 450 (430–470) μm). Ghaderi *et al.* (2018) proposed that *M. alboranensis* may be synonymised with *M. microdorus*. This species has been reported several times from Iran (Seraji *et al.* 2000; Jahanshahi Afshar *et al.* 2006; Palashi *et al.* 2012; and Hatami *et al.* 2014) but not described or illustrated in those works. In the present paper, it was described and illustrated for the first time from Iran.

Merlinius brevidens (Allen, 1955) Brzeski, 1991

The Iranian population of *M. brevidens* from the rhizosphere of wheat in Ardabil province could be characterised by having a

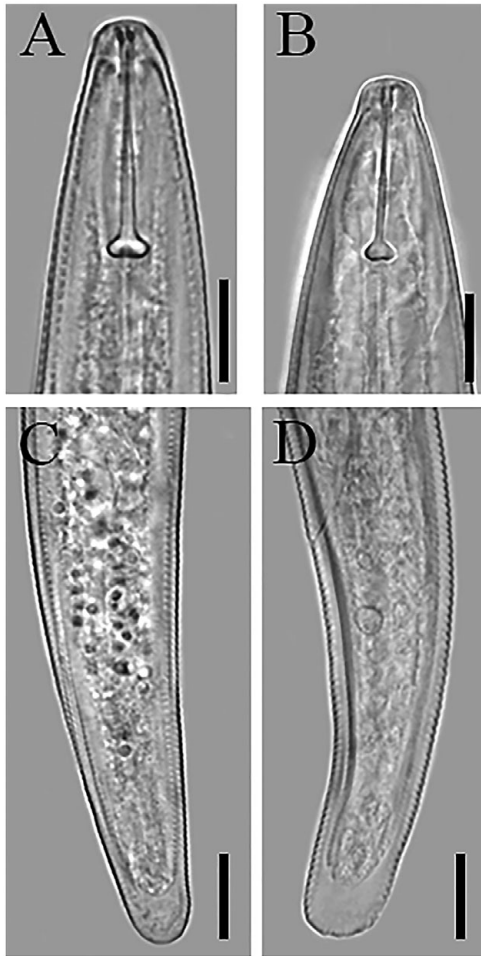


Figure 7. Light micrographs of the Iranian population of *Nagelus leptus*. A–D, female. A, B, anterior region. C, D, tail region. Scale bars = 10 μ m.

slightly ventrally arcuate to C-shaped body, lip region slightly set off by constriction, with 5–7 annuli, and a distinct refractive basal plate of framework. Stylet delicate, knobs rounded and posteriorly directed. Tail conoid with rounded terminus, tail tip smooth. The morphological and morphometric characters of this population fit well with those of the original description (Allen, 1955) and other Iranian populations (Ghaderi *et al.* 2014).

Merlinius nanus (Allen, 1955) Brzeski, 1991

The Iranian population of *M. nanus* from the rhizosphere of barley in East Azerbaijan can be characterised by body ventrally curved to C-shaped, lip region with 5–6 annuli, cephalic framework lightly sclerotised and tail subcylindrical with 45 (38–56) annuli, ending to an annulated terminus. The Iranian population of *M. nanus* is consistent with the original description (Allen 1955) and other Iranian populations (Naseri *et al.* 2008; Ghaderi *et al.* 2014).

Genus Nagelus Thorne & Malek, 1968

Diagnosis

Body about 1 mm or longer. Lateral fields with six incisures in adults, fourth and third stage juveniles and four in second stage juveniles. Cephalic region slightly offset by expansion, annuli not broken by radial grooves or indentations, perioral disc merged with first annulus, cephalic region hexagonal but not separated from

radial incisures, amphidial apertures nearly rounded, located at labial disc. Lip region continuous or slightly offset by a constriction. Cephalic framework lightly sclerotised. Deirids conspicuous in six-incisure region of lateral field. Stylet robust, conus about as long as shaft, knobs posteriorly sloping. Basal bulb saccate. Vulval slit with epiptygma. Female tail elongate-conoid to subcylindrical, terminus with distinct hyaline region but without refractive inner cuticle layer around its end. Spicules robust. Gubernaculum simple and curved in lateral view.

Type species

Nagelus leptus (Allen, 1955) Siddiqi, 1979

Other species

N. borealis Powers, Baldwin & Bell, 1983

N. exacutus Volkova, 1993

N. gerriae Khan & Singh, 1999

N. jamalensis (Nesterov, 1973) Siddiqi, 1979

N. macrophasmidus (Khan & Darekar, 1979) Siddiqi, 1986

N. obscurus (Allen, 1955) Powers, Baldwin & Bell, 1983

N. parobscurus (Mulvey, 1969) Siddiqi, 1986

N. sobaekensis (Choi & Geraert, 1993) Siddiqi, 2000

N. varians (Thorne & Malek, 1968) Siddiqi, 1986

Nagelus leptus (Allen, 1955) Siddiqi, 1979

(Figure 7, Table 5)

Description of female

Body ventrally arcuate to C-shaped after heat fixation. Body annuli distinct, 0.9–1.0 μ m at mid-body. Lateral field originating at the level of the precorpus and extending up to tail terminus, with six incisures, 6.5 (6.0–7.0) μ m wide that occupied 27 (28–34) % of body diameter. Cephalic region narrower than adjective of body and slightly marked by a depression, rounded, 8.5 (8.0–9.0) μ m in width, bearing 8 annuli. Cephalic framework lightly sclerotised. Stylet robust, conus 12 (11–13) μ m or 48 (47–50) % of total stylet length, basal knobs small and posteriorly directed, 6.0–6.5 μ m across. DGO 3.0–3.5 μ m behind stylet knobs. Median bulb oval, 18.5 (18–19) μ m \times 12.5 (12–13) μ m. Basal bulb pyriform, 13–15 μ m \times 20–24 μ m. Nerve ring at anterior half of isthmus. Hemizonid 1 to 2 annuli anterior to excretory pore and located at 124 (121–128) μ m from anterior end. Deirids located in six-incisures region. Reproductive system didelphic, with small epiptygma, vagina perpendicular, 10–11 μ m occupying 37 (34–41) % of vulval body diameter. Spermatheca rounded, offset, without sperm cell. Post anal intestinal sac absent. Tail tapering, with 61 (58–66) annuli in ventral side, tail terminus annulated. Hyaline 9.0 (8.5–9.5) μ m. Phasmids located at 48 (46–53) % of tail.

Voucher specimens

Five females were deposited in the collection of the department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran.

Host and locality

Recovered from the rhizosphere of Willow, collected in Abr forest, Semnan province, northwest of Iran. GPS coordinates: 36°04'59" N, 53°30'20" E.

Table 5. Morphometrics of *Nagelus leptus* from Iran. All measurements are in μm and in the form: mean \pm s.d. (range)

Nagelus leptus	
Female	
n	5
L	686 \pm 80.3 (605–802)
a	27.0 \pm 3.1 (24.8–32.0)
b	4.0 \pm 0.4 (4.1–5.1)
c	11.0 \pm 1.2 (10.0–13.0)
c'	3.5 \pm 0.3 (3.1–3.9)
V	59 \pm 3.6 (56.5–64.3)
Stylet	25 \pm 1.6 (24–28)
Pharynx	157 \pm 20.1 (136–178)
Median bulb	80 \pm 7.1 (74–88)
MB	51.0 \pm 2.4 (49.0–54.4)
Head–vulva	416 \pm 47.2 (360–457)
Head–anus	628 \pm 76.9 (546–739)
Vulva–anus	232 \pm 43.9 (189–285)
Tail length	57 \pm 6.3 (50–64)
Body width	25 \pm 1.7 (24–28)
Vulval body width	24 \pm 0.5 (23–25)
Anal body width	16 \pm 0.7 (15–17)

Diagnosis and relationships

N. leptus is similar to *N. obscurus* and differs from it by a higher number of lip region annuli (8–9 annuli vs. 5–6 annuli) and a slightly wider tail tip.

Nagelus obscurus (Allen, 1955) Powers, Baldwin & Bell, 1983

The Iranian population of *N. obscurus* from rhizosphere of Johnson grass, apple in Ardabil province can be characterised by lip region continuous from body contour to slightly offset by a depression, cephalic framework lightly sclerotised, deirids located at six incisures region, basal bulb pyriform, spermatheca filled with sperm, tail conical, tail tip annulated, and hyaline 10–11 μm . This population corresponds well with the original description of *N. camelliae* (Kheiri, 1972). Brzeski (1998) synonymised *N. camelliae* Kheiri, 1972 with *N. obscurus*. This population morphologically and morphometrically fits well with the original description and other Iranian populations (Kheiri 1972; Ghaderi et al. 2014).

Genus *Paramerlinius* Sturhan, 2012

Diagnosis

Body medium or large. Lateral field with six incisures in adults, fourth and third stage juveniles and four in second stage juveniles. Deirids situated in four-incisures region of lateral fields. Lip region continuous or slightly offset by constriction, 5–9 annuli, without longitudinal grooves, occasionally with indentations or grooves, prioral disc mostly indistinct. Framework heavily sclerotised. Stylet robust, 20–50 μm long, conus as long as shaft. Median bulb well developed. Basal bulb pyriform to saccate. Reproductive system

amphidelphic. Tail broadly conoid or subcylindrical with smooth terminus, with refractive inner cuticle layer surrounding the tail tip. Phasmid mostly prominent. Second-stage juveniles with four incisures in each lateral field. Spicules robust, with blunt and notched tip. Gubernaculum simple, curved in lateral view.

Type species

Paramerlinius hexagrammus (Sturhan, 1966) Sturhan, 2012

Other species

P. adakensis (Bernard, 1984) Sturhan, 2012

P. affinis (Allen, 1955) Sturhan, 2012

P. alpinus (Allen, 1955) Sturhan, 2012

P. arenosus (Ivanova & Shagalina, 1983) Sturhan, 2012

P. clavicaudatus (Choi & Geraert, 1975) Ghaderi, 2019

P. ekbali (Khan & Singh, 1999) Sturhan, 2012

P. elongatus (Ivanova & Shagalina, 1983) Sturhan, 2012

P. falcatus (Eroshenko, 1981) Sturhan, 2012

P. grandis (Allen, 1955) Sturhan, 2012

P. macrodens (Allen, 1955) Sturhan, 2012

P. neohexagrammus (Ivanova, 1978) Sturhan, 2012

Paramerlinius hexagrammus (Sturhan, 1966) Siddiqi, 1979

The Iranian population of *P. hexagrammus* from the rhizosphere of Sloe (*Prunus divaricata*) in Abr forest of Semnan province can be characterised by body almost straight to slightly curved, cylindrical. Cephalic region continuous from body to slightly offset by constriction, with 5–6 annuli, tail subcylindrical, with smooth terminus, and 46 (40–54) annuli. Males similar to female except for sexual characters, bursa encircling the entire tail, spicules and gubernaculum measuring 32–37 μm and 9.0–10.0 μm , respectively. The morphological and morphometric characters of the Iranian population are consistent with the other populations from Iran (Ghaderi & Karegar 2014).

Genus *Scutylenchus* Jairajpuri, 1971

Diagnosis

Body medium-sized, about 1 mm. Body cuticle marked by longitudinal striae or grooves. Lateral field with six incisures in adults and four in all juvenile stages. Deirid absent. Lip region continuous or slightly offset, with six radial grooves (six lobes) and conspicuous, oral disc rounded and offset from first annuli. Vulva usually in a cavity or depression, with epiptygma. Female tail conoid to subcylindrical with bluntly to finely rounded tip, terminal cuticle normally thickened and with refractive inner cuticle layer around tail end. Phasmid conspicuous. Spicules robust with notched tip. Gubernaculum crescent-shape in lateral view.

Type species

Scutylenchus mamillatus (Tobar-Jiménez, 1966) Jairajpuri, 1971

Other species

S. baluchiensis Maqbool, Ghazala, Fatima & Qasim, 1985

S. boghiae (Choi & Geraert, 1993) Siddiqi, 2000

S. brevicaudatus Peng & Hunt, 1995

S. chengi (Munawar, Miao, Castillo and Zheng, 2020) comb. nov.

= *Geocenamus chengi* Munawar, Miao, Castillo and Zheng, 2020

S. conicaudatus (Ghaderi & Karegar, 2016) comb. nov.

= *Geocenamus conicaudatus* Ghaderi & Karegar, 2016

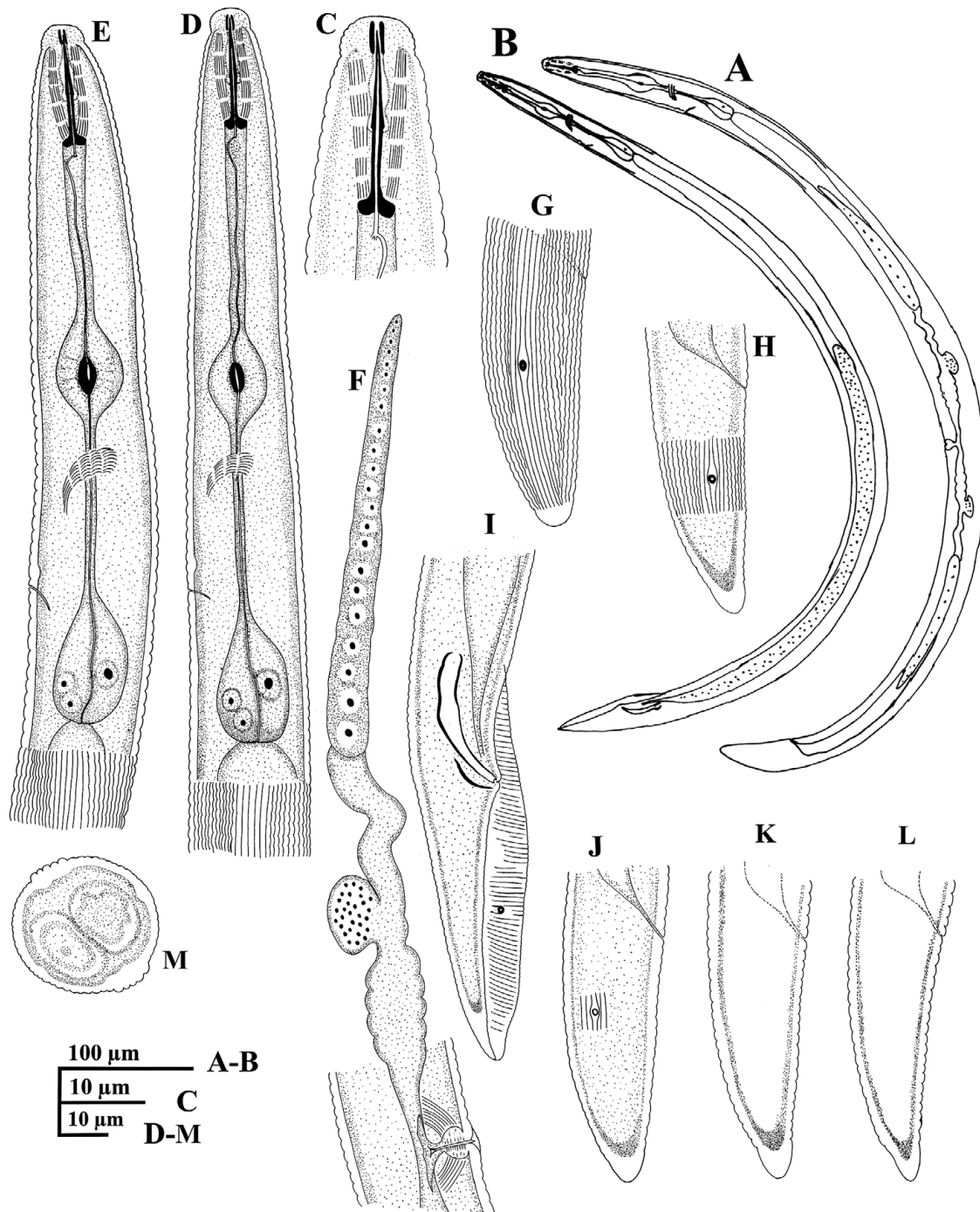


Figure 8. Line drawings of the Iranian (Sendan) population of *Scutylenchus* sp. A, C, D, F–H, J–M, female and B, E, I, male. A, B, entire body. C, anterior region. D, E, pharyngeal region. F, reproductive system; G–L, tail region. M, cross section at mid-body.

S. dongtingensis Xu, Xie, Zhao, Zhang & Su, 2012
S. hexincisus (Jairajpuri & Baqri, 1968) Siddiqi, 1979
S. koreanus (Choi & Geraert, 1971) Siddiqi, 1979
S. laminatus (Wu, 1969) Anderson & Ebsary, 1982
S. lenorus (Brown, 1956) Siddiqi, 1979
S. longus (Wu, 1969) Skwiercz, 1984
S. myungsugae (Choi & Geraert, 1993) Siddiqi, 2000
S. ordinarius (Volkova, 1993) comb. nov.

= *Geocenamus ordinarius* Volkova, 1993

S. paniculoides (Vovlas & Esser, 1990) Siddiqi, 2000
S. patternus (Eroshenko & Volkova, 1987) Xu, Xie, Zhao, Zhang & Su, 2012
S. persici (Zhang, Munawar, Castillo, Han & Zheng, 2022) comb. nov.

= *Geocenamus persici* Zhang, Munawar, Castillo, Han & Zheng, 2022

S. quadrifer (Andrássy, 1954) Siddiqi, 1979
S. quercinus Sheikhzadeh, Mobasseri, Valizadeh & Pedram, 2022

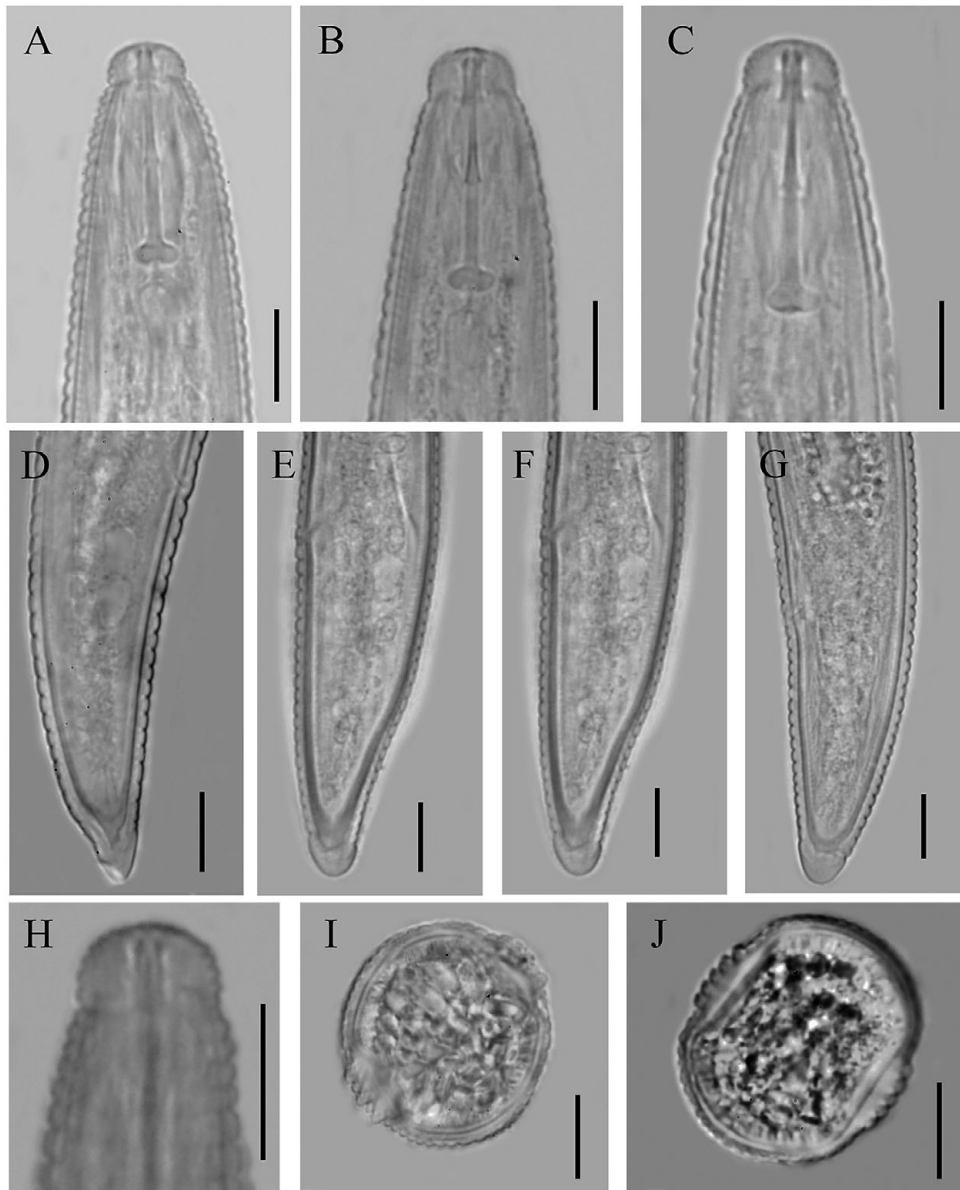


Figure 9. Light micrographs of the Iranian (Sendan) population of *Scutylechus* sp. A–J, female. A–C, H, anterior region. D–G, tail region. I, J, cross section at mid-body. Scale bars = 10 μm.

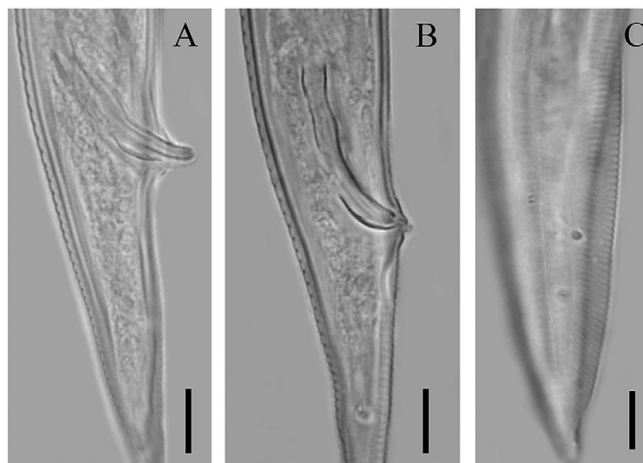


Figure 10. Light micrographs of the Iranian (Sendan) population of *Scutylechus* sp. A–C, male. A–C, tail region. Scale bars = 10 μm.

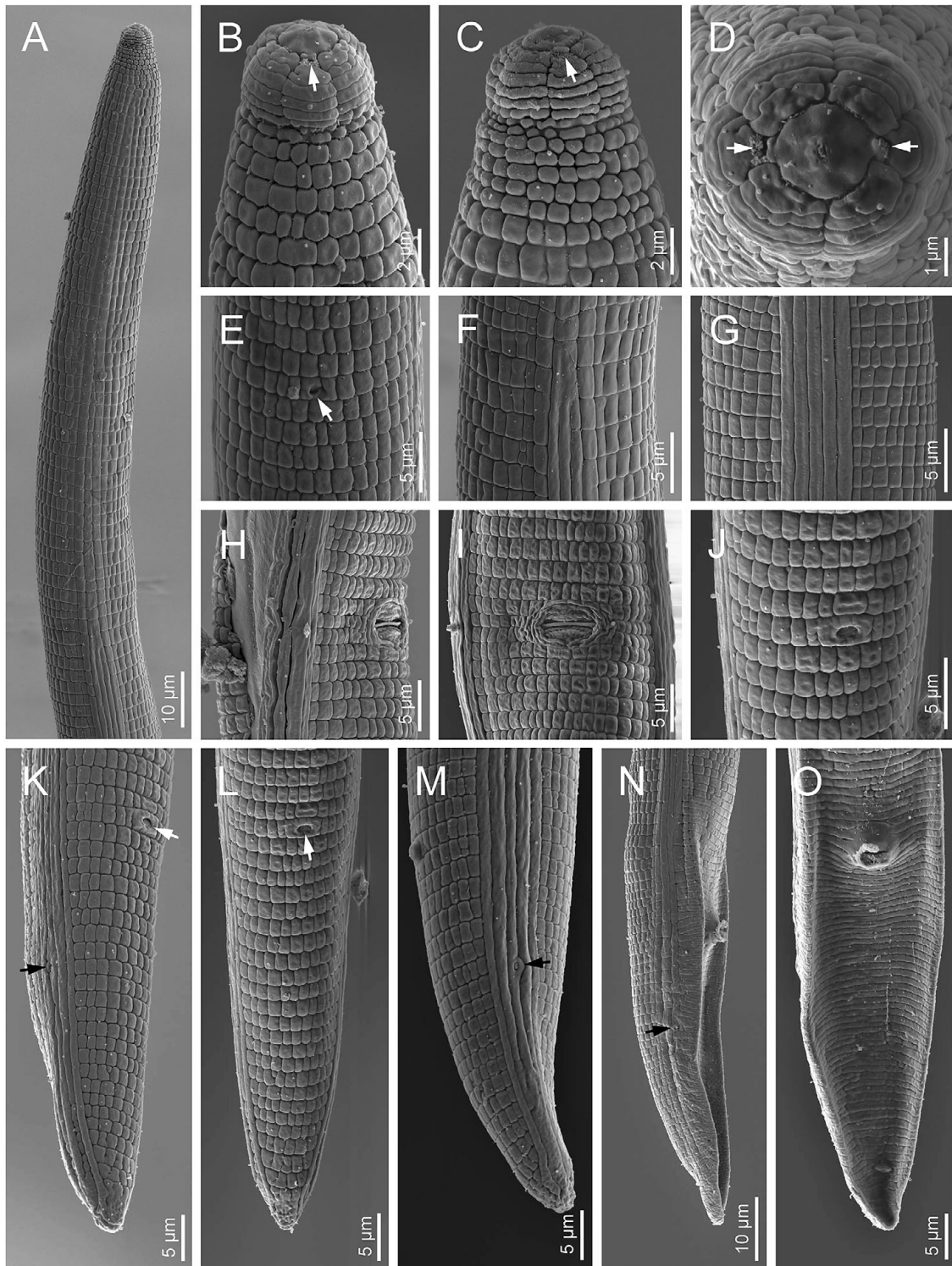


Figure 11. SEM micrographs of the Iranian population of *Scutylenchus* sp. A–M, female and N, O, male. A, anterior region. B, C, lateral view of cephalic region (white arrows indicating the amphidial apertures). D, *en face* view of cephalic region (white arrows indicating the amphidial apertures). E, excretory pore in ventral view (white arrow indicating the excretory pore). F, lateral field at pharyngeal region. G, lateral field at mid-body. H, I, vulval region. J, anus. K–O, tail region (white arrows indicating the anus and black arrows indicating the phasmid).

S. rugosus (Siddiqi, 1963) Siddiqi, 1979
S. siddiqii (Mulk, 1978) Skwiercz, 1984
S. seonunensis (Choi & Kim, 2001) comb. nov.
 = *Geocenamus seonunensis* Choi & Kim, 2001

S. sobolevi (Mukhina, 1970) Siddiqi, 1979
S. sphaerocephalus Ivanova, 1982
S. squamatus (Eroshenko & Volkova, 1988) Xu, Xie, Zhao,
 Zhang & Su, 2012

Table 6. Morphometrics of *Scutylemchus* sp. from Iran. All measurements are in μm and in the form: mean \pm s.d. (range)

Slid name	Sendan mountain				Sabalan mountain				Semnan province				Azizi-SP1
	Female		Male		Female		Female		Male		Female		
	21	cv	16	cv	10	cv	14	cv	4		5		
L	756 \pm 42.3 (698–811)	5.5	705 \pm 55.3 (598–805)	7.8	730 \pm 7 (725–735)	0.9	721 \pm 58 (618–822)	8.0	739 \pm 57.3 (672–806)		705 \pm 117.3 (622–788)		
a	28.3 \pm 3.4 (14.7–32.5)	12.2	30 \pm 1.9 (26.4–33)	6.4	28.3 \pm 0.8 (27.7–29)	3.1	27.4 \pm 2.8 (24–31.8)	10.5	29.8 \pm 2.4 (26.5–31.9)		26.6 \pm 1 (25.9–27.4)		
b	5.2 \pm 0.6 (2.7–6)	12.5	5 \pm 0.4 (4.3–5.9)	7.7	5.2 \pm 0.1 (5.1–5.3)	2.4	4.9 \pm 0.3 (4.3–5.8)	7.5	4.8 \pm 0.3 (4.5–5.2)		4.8 \pm 0.6 (4.3–5.2)		
c	15.7 \pm 2.2 (8.5–19.3)	14.0	13 \pm 1.1 (12.3–16.8)	8.6	15.6 \pm 0 (15.6–15.7)	0.5	16.8 \pm 1.2 (15.2–19)	7.6	14 \pm 0.8 (12.9–14.9)		17.8 \pm 0 (17.7–17.9)		
c'	2.3 \pm 0.2 (1.8–2.9)	12.5	2 \pm 0.2 (2.3–3.2)	10.3	3.1 \pm 0.3 (2.8–3.3)	10.9	2.1 \pm 0.2 (1.9–2.5)	9.6	2.9 \pm 0.2 (2.6–3)		1.9 \pm 0.3 (1.7–2.2)		
V or T	55.2 \pm 2.2 (49.3–58)	4.0	49 \pm 4.1 (42.4–54.6)	8.2	55.5 \pm 0.2 (55.3–55.7)	0.4	56.3 \pm 0.9 (55.3–58.3)	1.7	–		58.2 \pm 1.4 (57.2–59.3)		
Stylet	21.3 \pm 0.7 (20.3–23)	3.3	21 \pm 1.6 (20.2–27.3)	7.8	21.2 \pm 1 (20.5–22)	4.9	20.2 \pm 1.3 (18.3–23.5)	6.8	20.2 \pm 0.6 (19.8–21)		19.7 \pm 0.4 (19.4–20)		
Conus	11 \pm 0.3 (10.4–11.6)	3.2	10 \pm 0.5 (10.2–12.5)	5.5	10.6 \pm 0.1 (10.5–10.7)	1.3	9.9 \pm 0.9 (8.4–11.5)	9.0	9.9 \pm 0.1 (9.8–10)		10.5 \pm 0.7 (10–11)		
m (conus/stylet %)	51.6 \pm 1.3 (49.7–55.6)	2.6	51 \pm 2.1 (45.7–54.7)	4.2	50 \pm 1.8 (49–51)	3.6	49 \pm 2.1 (44.2–52.2)	4.3	49.7 \pm 0.3 (49.4–50)		53.2 \pm 2.4 (51.5–55)		
Median bulb	68 \pm 4.3 (59–75)	6.3	66 \pm 5.8 (56–77)	8.8	68.5 \pm 2.1 (67–70)	3.0	67 \pm 5.1 (62–80)	7.6	66 \pm 1.5 (65–68)		67 \pm 1.4 (66–68)		
MB	49 \pm 2.3 (43–52)	4.8	49 \pm 3 (41.1–52.5)	6.3	49 \pm 2.2 (48–51)	4.6	46 \pm 1.9 (42–49)	4.2	44.5 \pm 0.2 (44–45)		45.5 \pm 0.1 (45–46)		
Excretory pore	104 \pm 12 (80–118)	11.6	112 \pm 8.6 (95–127)	7.7	115.5 \pm 0.7 (115–116)	0.6	121 \pm 10.9 (113–142)	9.0	116 \pm 0 (116–116)		109 \pm 2.2 (109–109)		
Pharynx	140 \pm 7.9 (126–156)	5.6	134 \pm 8.2 (120–148)	6.1	139.5 \pm 2.1 (138–141)	1.5	146 \pm 10.1 (133–171)	6.9	148 \pm 2.6 (146–151)		146 \pm 3.5 (144–149)		
Head–vulva	417 \pm 28.4 (358–460)	6.8	–	–	406 \pm 2.1 (404–407)	0.5	399 \pm 30.9 (351–452)	7.7	–		410 \pm 57.9 (369–451)		
Head–anus	709 \pm 40.4 (653–785)	5.7	653 \pm 51 (552–742)	7.8	683 \pm 6.3 (679–688)	0.9	687 \pm 57.2 (618–792)	8.3	686.2 \pm 55.9 (620–750)		665 \pm 111 (587–744)		
Body width	26 \pm 1.5 (24–29)	5.7	23 \pm 1.6 (21–27)	6.9	25.7 \pm 1 (25–26.5)	4.1	26 \pm 3.1 (21–32)	11.9	25 \pm 1.7 (23–27)		26.3 \pm 3.3 (24–28.7)		
Vulva body width	26 \pm 1.5 (24–29)	5.9	–	–	25.7 \pm 1 (25–26.5)	4.1	26.5 \pm 3.2 (21–32)	12.2	–		26.3 \pm 3.3 (24–28.7)		
Anal body width	20 \pm 1.3 (17.3–23)	6.5	18 \pm 1.3 (16–20)	7.1	15 \pm 1.4 (14–16)	9.4	20 \pm 1.8 (17.5–23)	9.1	18 \pm 1.5 (17–20)		20		
Tail length	47.3 \pm 4.8 (41–60)	10.2	51 \pm 6.1 (43–63)	11.9	46.5 \pm 0.7 (46–47)	1.5	43 \pm 5.2 (36.5–54)	12.1	53 \pm 2.2 (51–56)		39 \pm 6.3 (35–44)		
Tail annuli	22.5 \pm 1.7 (20–26)	7.8	–	–	29.5 \pm 4.9 (26–33)	16.7	22.7 \pm 2.2 (20–26)	10.0	–		19 \pm 4.2 (16–22)		
Testis	–	–	349 \pm 45.8 (289–440)	13.1	–	–	–	–	–		–		
Spicules	–	–	27 \pm 1.3 (25.5–31)	4.8	–	3.1	–	–	26 \pm 1.4 (24.5–28)		–		
Gubernaculum	–	–	9.0 \pm 1.2 (7.0–11)	13.3	–	–	–	–	8.8 \pm 0.3 (8.4–9.0)		–		

Table 7. Morphometrics of Damavand mountain population of *Scutylechus sp.* from Iran. All measurements are in μm and in the form: mean \pm s.d. (range)

	Female		Male	
n	15	CV	12	CV
L	771 \pm 50.4 (655–838)	6.5	719 \pm 55 (600–758)	7.6
a	30 \pm 1.6 (27–33)	5.3	31 \pm 1.6 (28–33)	5.3
b	5.6 \pm 0.4 (4.8–6.3)	7.9	5.2 \pm 0.4 (4.4–5.7)	7.9
c	16.4 \pm 1.4 (12.8–19.3)	9.1	13.4 \pm 1.0 (12.3–15.4)	7.5
c'	2.4 \pm 0.2 (1.8–2.7)	9.1	3 \pm 0.1 (2.8–3.2)	3.8
V or T	53 \pm 1.3 (50.5–54.5)	2.4	–	–
Stylet	21.1 \pm 0.4 (20.3–21.8)	2.1	21 \pm 0.3 (20.5–21.8)	1.7
Median bulb	67 \pm 4.2 (59–75)	6.2	68 \pm 6.7 (56–77)	9.8
MB	49 \pm 2.8 (43–53)	5.8	49 \pm 3.6 (41–52)	7.3
Excretory pore	116 \pm 9.7 (91–131)	8.3	115 \pm 8 (103–128)	6.9
Pharynx	138 \pm 8.4 (126–156)	6.1	137 \pm 6.7 (128–148)	4.9
Head–vulva	412 \pm 30.3 (357–451)	7.3	–	–
Head–anus	723 \pm 50.6 (604–790)	7.1	665 \pm 52.2 (553–701)	7.8
Body width	25.5 \pm 1 (24–27)	4.2	23 \pm 0.9 (22–24)	3.9
Vulva body width	25 \pm 0.7 (24–27)	3.1	–	–
Anal body width	19 \pm 1 (18–22)	5.5	18 \pm 1.3 (16–20)	7.6
Tail length	47 \pm 2.9 (41–51)	6.2	52.3 \pm 4.1 (47–61)	7.8
Phasmid	19 \pm 3 (15–24)	15.8	22 \pm 3.2 (16–28)	14.7
Tail annuli	23 \pm 2.4 (19–26)	10.7	–	–
Spicules	–	–	26.5 \pm 0.9 (25.5–28)	3.4
Gubernaculum	–	–	8.0 \pm 0.6 (7.0–9.0)	8.2

S. stegus (Thorne & Malek, 1968) Siddiqi, 1979

S. tartuensis (Krall, 1959) Siddiqi, 1979

S. tessellatus (Goodey, 1952) Siddiqi, 1979

S. thomasi Skwiercz, 1984

S. tumensis Skwiercz, 1984

S. variabilis Ivanova & Shagalina, 1983

S. vietnamensis (Nguyen, Linh, Nguyen, Liebanas, Nguyen & Trinh, 2019) comb. nov.

= *Geocenamus vietnamensis* Nguyen, Linh, Nguyen, Liebanas, Nguyen & Trinh, 2019

Remark

All six new combinations transferred from the genus *Geocenamus* to *Scutylechus* due to having longitudinal incisures in entire body with lateral field.

Scutylechus sp.

(Figures 8–11, S4–S6; Tables 6, 7)

Description of female

Body ventrally arcuate to C-shaped after heat fixation. Cuticular annuli distinct, 2.2 (1.6–2.7) μm across at mid-body. Cuticle with 35–50 incisures (35–42 in Damavand population, 40–42 in Semnan population, 36–40 in Zanjan population, 40–46 in Sabalan population, and 40–50 in Sendan population) excluding lateral fields (200 cross sections from 70 specimens: 35 incisures = 11.5%;

36 incisures = 20%; 37 incisures = 9.5%; 38 incisures = 4.5%; 39 incisures = 1.5%; 40 incisures = 3%; 41 incisures = 1%; 42 incisures = 14%; 44 incisures = 5%; 45 incisures = 3.5%; 47 incisures = 7.5%; 48 incisures = 18.5%; 49 incisures = 5.5%; 50 incisures = 6.5%). Lateral field prominent, with six longitudinal incisures, 7.8 (6.5–9.6) μm wide or 30 (20–35) % of the corresponding body diameter. Lip region slightly offset by a constriction, flattened at front end, bearing 5–7 annuli, 8.3 (7.4–9.4) μm wide and 4.2 (3.8–5.1) μm high; in SEM images, with six radial grooves and a conspicuous offset, rounded oral disc. Amphidial apertures small and slightly ovate, located at lateral view of oral disc in SEM images. Cephalic framework not refractive. Stylet robust, conus 10.0 (8.5–12.5) μm or 51 (49–53) % of total stylet length, basal knobs round and slightly posteriorly directed, 4.3 (3.9–4.7) μm wide. DGO 2.1 (1.5–2.6) μm behind stylet knobs. Pharyngeal median bulb oval with prominent valve, 12.2 (10.0–13.5) μm \times 17.4 (16–20) μm , occupying 59 (57–69) % of body wide at that level. Basal bulb pyriform 13.5 (11.5–15.0) μm \times 25 (23–27) μm . Nerve ring at 91 (80–104) μm from anterior end. Hemizonid 1 to 2 annuli anterior to excretory pore and located at 112 (90–131) μm from anterior end. Deirids absent. Reproductive system amphidelphic-didelphic, vulva with small lateral flaps and epipygma. Vagina perpendicular, 11.0 (8.0–12.0) μm occupying 37 (31–41) % of the vulval body diameter; spermatheca round and filled with globular sperm cells. Post anal intestinal sac absent. Tail elongate conical, dorsally convex, with 24 (19–28) annuli in ventral side, ending to a smooth terminus. Phasmids located at 38 (33–44) % of tail length.

Description of male

Generally similar to female except for sexual characters. Body straight to ventrally curved. Body annuli 2.1 (1.7–2.7) μm at mid-body. Lateral field 7.5 (6.5–8.5) μm wide. Cephalic region offset, 7.6 (7.2–8.3) μm wide and 4.3 (3.8–4.6) μm high. Conus 50 (49–51) % of total stylet length. DGO 2.2 (1.7–2.6) μm behind stylet knobs. Nerve ring at 90 (83–101) μm from anterior end. Hemizonid 1–2 annuli anterior to excretory pore and located at 105 (90–115) μm from anterior end. Bursa surrounded tail tip, 74 (65–87) μm in length. Spicules tylenchoid, notched at tip. Gubernaculum simple, crescent-shaped.

Host and locality

Recovered from the rhizosphere of *Astragalus* sp. in five localities at Alborz mountains: Sendan mountain, Zanjan province (GPS coordinates 36°23'45" N, 49°08'09"E); Sabalan mountain, Ardabil province (GPS coordinates 38°29'57" N, 47°70'03"E), Damavand mountain, Mazandaran province (GPS coordinates 35°59'20" N, 52°07'16"E) in October 2019, and Semnan population (GPS coordinates 36°02'40" N, 53°28'16"E) and Zanjan population (GPS coordinates 36°41'42" N, 48°44'13"E).

Diagnosis and relationships

Scutylumchus sp. characterised by having 35–50 longitudinal incisures at mid-body (except lateral fields); cephalic region slightly offset by a constriction with 5–7 annuli; cephalic framework not refractive; stylet 18.3–27.3 μm ; tail 32–63 μm and 19–28 annuli in ventral side with smooth tail tip and spicules 24.5–31 μm .

Scutylumchus sp. in regard to morphological and morphometric characters comes close to *S. siddiqii* (Mulk, 1978) Skwiercz, 1984, *S. laminatus*, *S. tessellatus* (Goodey, 1952) Siddiqi, 1979, and *S. mamillatus* (Tobar-Jiménez, 1966) Jairajpuri, 1971. Our population differs from *S. siddiqii* by a shorter body (598–838 vs. 820–1220 μm), shorter spicules (24.5–31.0 vs. 30–36 μm), and tail characteristics (dorsally convex, smooth at tip vs. regularly tapering, annulated at tip). From *S. laminatus*, it differs by a shorter body (598–838 vs. 800–1200 μm), the number of longitudinal striae excluding lateral field (35–50 vs. 56–58), and tail characteristics (smooth at tip, bearing 16–33 annuli and 35–63 μm long vs. annulated at tip, bearing about 50 annuli and 62–88 μm long). The Iranian population of *Scutylumchus* sp. can be distinguished from *mamillatus* by a shorter body (598–833 vs. 890–990 μm), lower number of lip region annuli (5–6 vs. 6–7), tail characteristics (elongate conical, with rounded tip vs. subcylindrical with digitate tip), and longer spicules (24.5–31.0 vs. 21–24 μm). Finally, it differs from *S. tessellatus* by lip region (slightly offset by a constriction vs. offset by a deep groove), slightly stylet length (18.3–27.0 vs. 16.5–20.5 μm), tail shape (dorsally convex in posterior half vs. straight), and tail tip (smooth vs. annulated). Our population of *Scutylumchus* sp. differs from *S. quercinus* by number of longitudinal striae without lateral fields (35–50 vs. 22–25), number of lateral fields (six vs. four), number of tail annuli (19–28 vs. 14–18), and tail shape (elongate conical, dorsally convex vs. subcylindrical).

Scutylumchus rugosus (Siddiqi, 1963) Brzeski, 1991 (Figure S7, Table S6)

The Iranian population of *S. rugosus* from the rhizosphere of *Astragalus* sp. in Zanjan province can be characterised by straight to slightly ventrally curved body, having 29–34 longitudinal striae excluding lateral field, lip region slightly offset by a constriction, with 5–6 annuli, and tail with 29 (23–35) annuli, with annulated rarely smooth terminus. This population corresponds well with the

original description (Siddiqi 1963) and other populations from Iran (Hasanzadeh *et al.* 2005; Ghaderi & Karegar 2016) except for tail terminus striation (usually annulated vs. usually smooth).

Genus *Telomerlinius* Siddiqi & Sturhan, 2014

Diagnosis

Body about 1 mm or less, straight or slightly ventrally curved after relaxed. Cuticle distinctly annulated, without longitudinal striae excluding lateral field. Lateral field with four incisures forming three longitudinal bands. Cephalic region distinctly offset from the body, perioral disc present and prominent, offset from cephalic region annuli, with six radial conspicuous grooves, framework slightly sclerotised. Amphidial apertures ovate, located in first annulus. Stylet delicate slender, conus being longer than shaft; basal knobs small rounded, posteriorly directed. Deirid absent. Median bulb muscular with distinct valve. Basal bulb large, sac-like, usually slightly overlapping intestine in dorsal side. Female genital system didelphic-amphidelphic. Vulva at mid-body, with transverse slit, equipped with prominent epitygma. Spermatheca axial, rounded or oval, with small rounded sperm. Post anal sac lacking. Female tail conoid with smooth rounded terminus and without refractive inner cuticle layer around its end. Male similar to female. Spicules thick, slightly curved ventrally, with notched tip. Gubernaculum small, saucer-shaped. Cloaca opening with distinct pair of hypopygma on the posterior lip.

Type species

Telomerlinius mellumensis Siddiqi & Sturhan, 2014

Other species

Telomerlinius teleosus Siddiqi & Sturhan, 2014

Telomerlinius teleosus Siddiqi & Sturhan, 2014

(Figures 12–14, Table 8)

Description of female

Body elongate, straight to slightly ventrally curved when killed by heat. Cuticle annuli fine, 2.3 (2.2–2.4) μm at pharyngeal region and 1.6 (1.5–1.8) μm at mid-body. Lateral field with four distinct incisures, 8.4 (8.0–9.4) μm wide occupying 41 (39–45) % of body diameter. Cephalic region rounded, offset from body by a sharp constriction, with usually 5–6 annuli, showing six longitudinal grooves and perioral disc. Cephalic framework lightly sclerotised. Stylet delicate and slender, 3.0 (2.9–3.2) times head wide long, conus needle-like, 13.8 (13.8–14.0) μm or 62.2 (60.9–65.2) % of total stylet length. Basal knobs rounded, posteriorly sloping 3.0 (2.5–3.3) μm across. DGO 1.2 (1.0–1.5) μm behind stylet knobs. Median bulb well developed and oval, 18.8 (18.0–19.0) μm long and 11.7 (11.0–12.0) μm wide that occupied 61 (55–63) % of body wide at adjacent level, with distinct valvular aperture. Basal bulb elongate-cylindrical, with 9.5 (5.0–13.0) μm overlapping intestine. Cardia distinct and rounded. Nerve ring located at 86 (78–90) μm from anterior end. Hemizonid 1–2 annuli anterior to excretory pore and located at 98 (95–103) μm from anterior end. Deirid absent. Reproductive system didelphic. Vulva a transverse slit with anterior flap. Vagina not sclerotised, perpendicular to body axis. Spermatheca spherical, not offset, 8–11 μm long and filled with globular sperm. Rectum slightly curved. Anus distinct. Tail elongate-conoid with smooth rounded terminus, with 23 (21–25) large annuli at ventral side. Phasmid not so large, just anterior to middle of tail, 48 (42–51) % of tail.

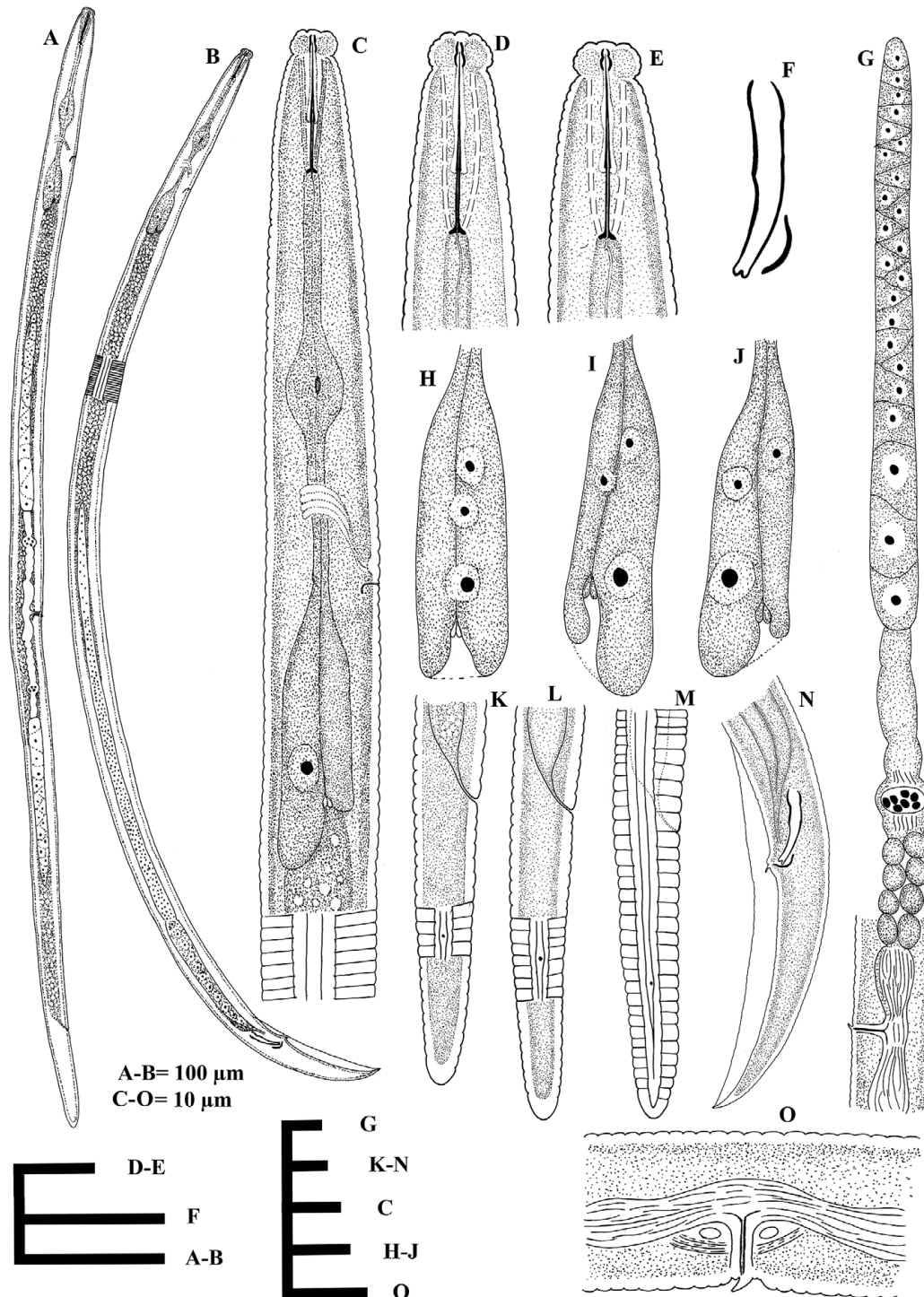


Figure 12. Line drawings of the Iranian population of *Telomerlinius teleosus*. A, C, E, G, I–M, O, female and B, D, F, H, N, male. A, B, entire body. C, pharyngeal region. D, E, anterior region. F, spicules and gubernaculum. G, reproductive system. H–J, basal bulb of pharynx. K–N, tail region. O, vulval region.

Description of male

Similar to female in general morphology. Body annuli finer than female, 1.4 (1.3–1.6) μm at mid-body. Lateral field with four incisures that occupied 40 (37–46) % of body wide in diameter. Lip region with 7.2 (6.6–7.8) μm wide and 4.7 (4.0–5.3) μm high. Basal bulb 7 (5.0–10) μm overlapping intestine. Testis single, out-stretched, occupied about half or 49 (43–55) % of whole body length. Spicules tylenchid-shape with notch at tip. Gubernaculum

very curved and saucer-shape. Hypoptygma present and slightly prominent. Bursa enveloped tail, 100 (90–110) μm long.

Voucher specimens

Five females and six males were deposited in the collection of the Department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran.

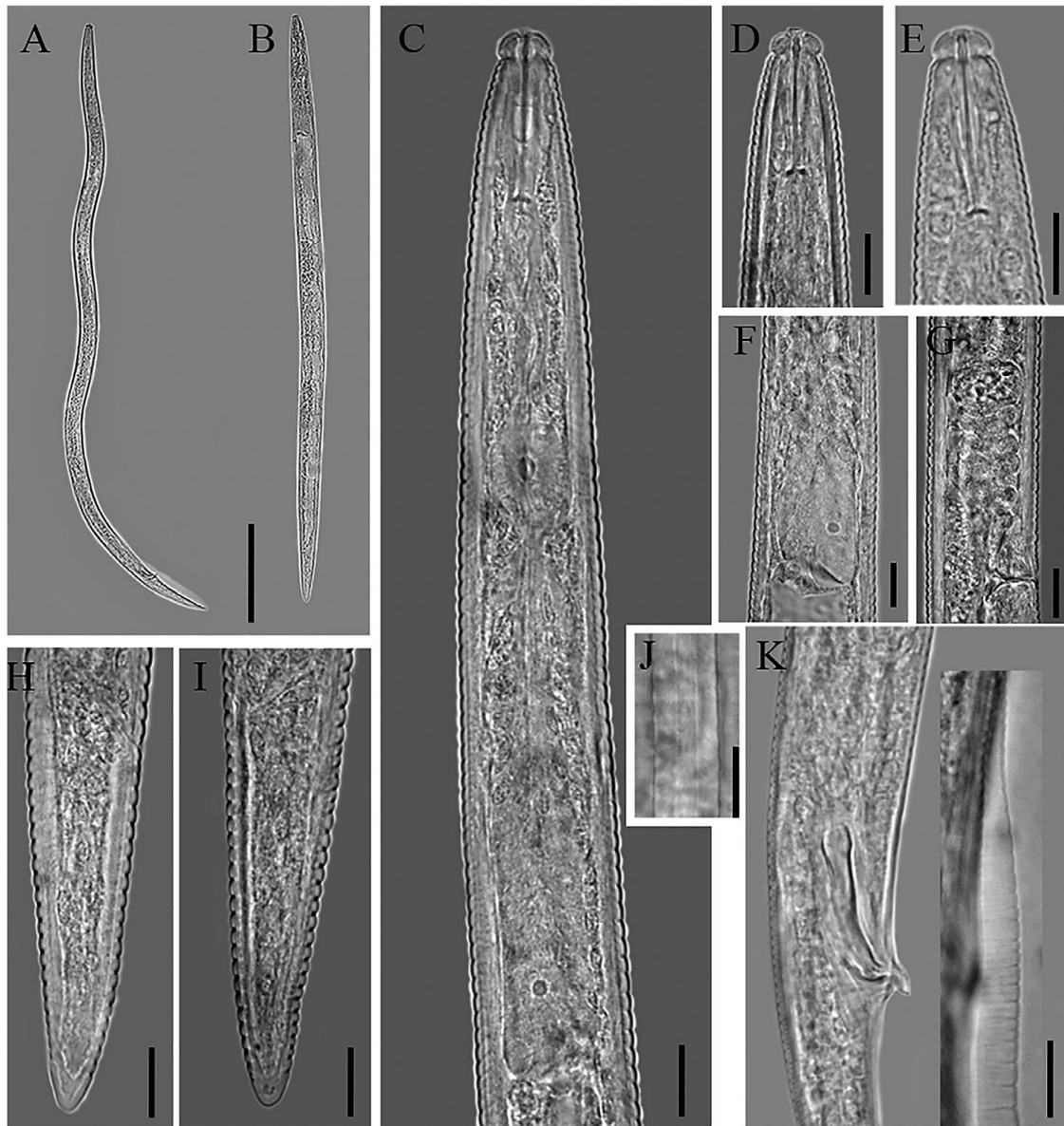


Figure 13. Light micrographs of the Iranian population of *Telomerlinius teleosus*. B, C, E, F–J, female and A, D, K, male. A, B, entire body. C, pharyngeal region. D, E, anterior region. F, basal bulb of pharynx. G, vulval region. H, I, K, tail region. J, lateral field at mid-body. Scale bars: A–B = 100 μm ; C–K = 10 μm .

Host and locality

Recovered from the rhizosphere of Khejri tree (*Prosopis cineraria* (L.) Druce), collected in Dezful region of Khuzestan province, southwest of Iran, in September 2019. GPS coordinates: 32°06'50" N, 48°27'16" E.

Diagnosis and relationships

Telomerlinius teleosus differs from the other species of the genus, *T. mellumensis* Siddiqi and Sturhan, 2014, by a shorter stylet (21–23 vs. 25–30 μm), shorter spicules (18.5–21.0 vs. 22–25.5 μm) and cuticular annuli width (1.2–1.8 vs. 2.4–3.4 μm). Our population is in an intermediate position but more comes close to *T. teleosus*; however, it differs from the original description by a wider lateral field (41 (39–45) vs. about 26% of the corresponding body diameter), longer body (803 (743–856) vs. 530–740 μm), longer tail (63 (57–69) vs. 45–55 μm), slightly longer spicules (22 (20–24) vs. 18.5–21.0 μm), and longer gubernaculum (8.5 (7.5–9.5) vs. 6.0–7.5 μm).

3D modelling of lip region in the genera of Merliniinae

3D modelling in the genera of Merliniidae (except *Macrotylenchus*) showed four types of lip regions (Figure 15). However, the SEM images of the genus *Macrotylenchus* not available.

Amplimerlinius

Labial radial grooves absent; the lip region is not divided into sectors, and all lips are completely fused and merged with perioral disc (Type-1).

Nagelus

Similar to *Amplimerlinius*, but lip region is broadly oval and compressed dorso-ventrally (vs. spherical in *Amplimerlinius*) (Type-1).

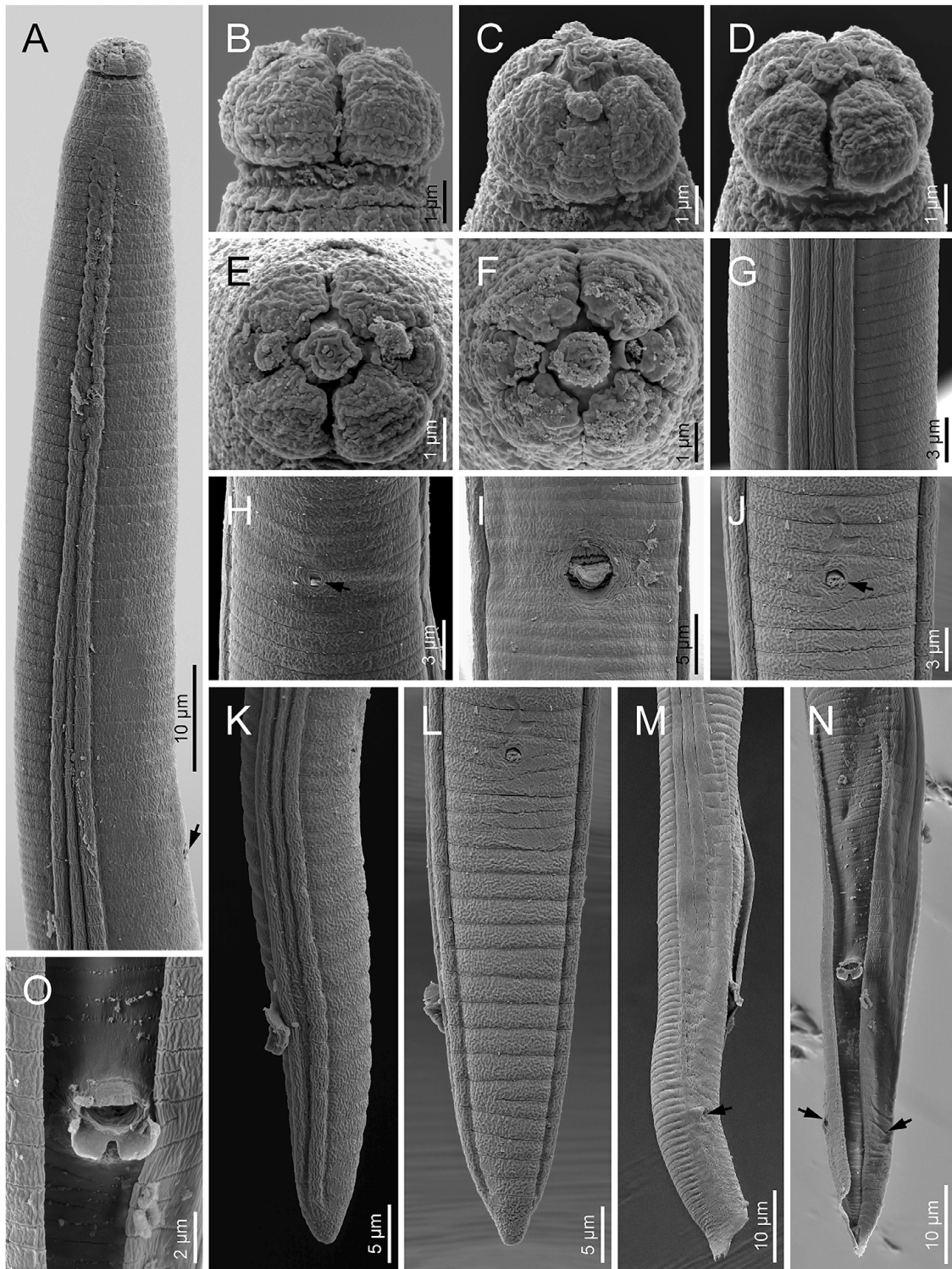


Figure 14. SEM micrographs of the Iranian population of *Telomerlinius telesosus*. A–L, female and M–O, male. A, anterior region (black arrow indicating the excretory pore). B–D, lateral view of cephalic region. E, F, *en face* view of cephalic region. G, lateral field at mid-body. H, excretory pore in ventral view (black arrow indicating the excretory pore). I, vulval region. J, anus (black arrow indicating the anus). K–N, tail region (black arrows indicating the phasmid). O, cloacal region.

Merlinius

Lip region with two separated lateral lips, reduced in size; two sectors of each subdorsal and subventral lips are fused. Perioral disc not distinct and merged with the first annulus of lip region (Type-2).

Scutylenchus and Geocenamus

Lip region distinctly separated into six sectors by radial grooves; lateral lips are slightly reduced in size. Perioral disc distinct and separated but not elevated from the lip region (Type-3).

Table 8. Morphometrics of *Telomerlinius teleosus* from Iran. All measurements are in μm and in the form: mean \pm s.d. (range)

	Present study		Siddiqi & Sturhan (2014)	
	Dezful region		Iranian population	Libyan population
	Female	Male	Female/Male	Female/Male
n	7	8	8/8	8/3
L	803 \pm 50.4 (743–856)	737 \pm 71.8 (625–845)	640 (530–740)	640 (600–680)
a	39.0 \pm 3.3 (35.0–43.0)	37.0 \pm 2.5 (34.0–42.0)	34.7 (29–40)	34.6 (28–39)
b	5.9 \pm 0.3 (5.3–6.3)	4.9 \pm 0.3 (4.4–5.3)	5.2 (4.5–6.7)	5.1 (4.7–6.7)
c	12.7 \pm 0.8 (11.6–14.0)	10.7 \pm 0.9 (9.0–11.6)	12.6 (10–14)	12.7 (10.5–13)
c'	4.1 \pm 0.3 (3.5–4.6)	4.1 \pm 0.3 (3.5–4.7)	3.6 (3.3–4.0)	3.5 (3.0–3.7)
V or T	53.0 \pm 0.7 (52.0–54.0)	–	55 (53–57)	54 (50–56)
Stylet	22.2 \pm 0.7 (21–23)	22.7 \pm 1.1 (21–24.7)	22.5 (21–23)	22.5 (21.5–23)
Median bulb	62 \pm 3.2 (57–65)	67 \pm 5.9 (60–76)	–	–
MB	45.0 \pm 2.4 (42.0–48.0)	45.0 \pm 3.7 (42.0–53.0)	–	–
Excretory pore	101 \pm 2.7 (98–106)	111 \pm 13.7 (98–131)	–	–
Pharynx	136 \pm 1.6 (134–139)	150 \pm 13.1 (130–165)	122 (112–133)	126 (115–135)
Head–vulva	426 \pm 21.3 (402–450)	–	–	–
Head–anus	739 \pm 48 (679–788)	669 \pm 68.6 (560–769)	–	–
Body width	20 \pm 0.5 (19–21)	19.7 \pm 2.2 (17–23)	–	–
Vulva body width	20 \pm 0.8 (19–21)	–	–	–
Anal body width	15 \pm 0.4 (14.5–16.0)	16.5 \pm 1.4 (14–19)	–	–
Tail length	63 \pm 4.4 (57–69)	69 \pm 6.4 (60–77)	49 (45–51)	50 (47–55)
Phasmid	30 \pm 3.3 (25–33)	–	–	–
Tail annuli	23 \pm 1.6 (21–25)	–	–	–
Spicules	–	22 \pm 1.4 (20–24)	20 (18.5–21)	19.4 (19–20)
Gubernaculum	–	8.5 \pm 0.7 (7.5–9.5)	7.0 (6.0–7.5)	7.0

Paramerlinius

More similar to *Scutylenchus* and *Geocenamus* than *Amplimerlinius*, as having six obscure sectors divided by radial grooves; perioral disc is hexagonal (Type-3).

Telomerlinius

Similar to *Scutylenchus* and *Geocenamus*, but perioral disc is distinctly elevated from the lip region (Type-4).

Molecular characterisation and phylogenetic relationships between representatives of family Merliniidae

The sequences obtained for the described species in this paper are listed in Table 2. The majority are from D2–D3 region of 28S rRNA and partial 18S rRNA; in some cases, the ITS region was also provided.

The D2–D3 domains of the 28S rRNA gene alignment after manual edition (754 bp long) included 109 sequences from the family Merliniidae and two outgroup species [*Psilenchus* sp. (DQ328716) and *Psilenchus hilarulus* (EU915489)]. Forty new sequences with their morphological characterisation were included in this analysis. The Bayesian 50% majority rule consensus tree inferred from the D2–D3 alignment is given in Figure 16. Smaller clades are coincident with other recent studies with related phylogenetic families (Alvani *et al.* 2017; Munawar *et al.* 2021). This tree

showed that many species from GenBank are difficult to distinguish using only this marker as a separated clade in the phylogenetic tree (i.e., *Pratylenchoides crenicauda*-*P. erzurumensis*-*P. variabilis*, *Scutylenchus tartuensis*-*S. rugosus*, and others). However, it seems fine for separating the majority of the genera in this family, but with low clade support in some genera clades (i.e., *Amplimerlinius*) or, in some cases, consisting of several clades distributed in the phylogenetic tree, but not clearly supported and phylogenetically relationship not well resolved between them and other genera (i.e., *Scutylenchus*).

The partial 18S rRNA alignment after manual edition (1693 bp long) included 59 sequences from the family Merliniidae and two outgroup species [*Psilenchus hilarulus* de Man, 1921 (KX789728) and *Psilenchus vinciguerrae* Brzeski, 1991 (KX789733)]. Twenty-six new sequences with their morphological characterisations were included in this analysis. The Bayesian 50% majority rule consensus tree inferred from the 18S rRNA sequence alignment is given in Figure 17. The tree contained two highly supported major clades (one with 0.96 PP support and the another with 1.00 PP support). These clades were partially coincident with another study (Carta *et al.* 2010), but in our case with a higher number of sequences and additional genera. Similarly, to the D2–D3 domains of the 28S rRNA the use of this marker for species separation is complicated due to the low variability associated with this marker. At the genus level, only some genera are clearly defined in the phylogenetic tree (i.e., *Pratylenchoides* and *Nagelus*).

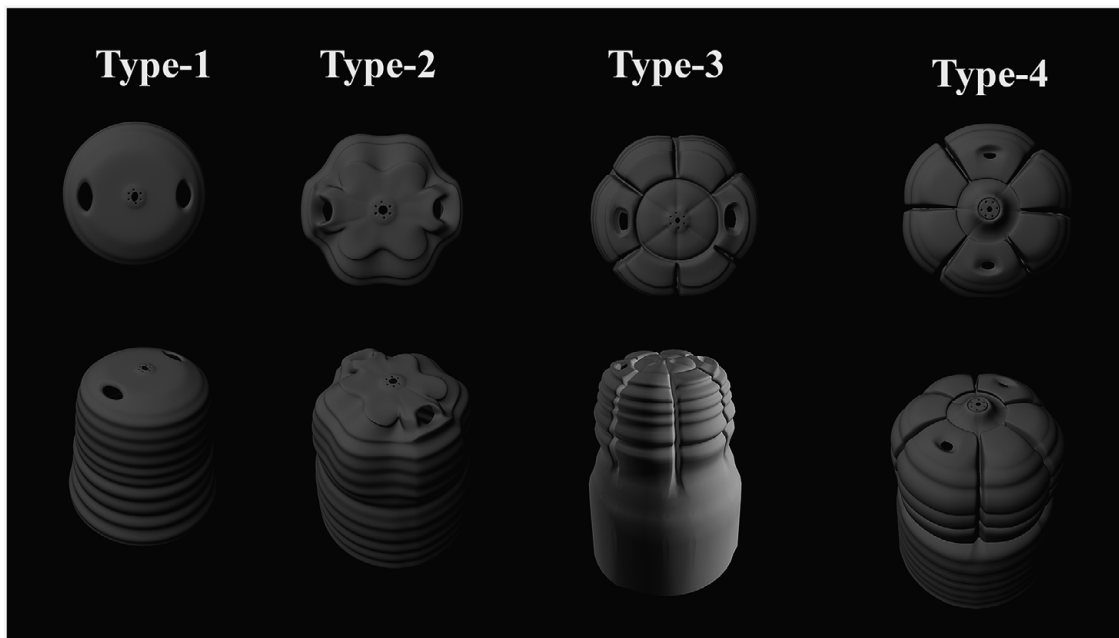


Figure 15. 3D modelling of the genera in Merliniinae. Type 1: Cephalic radial grooves absent; the lip region is not divided into sectors, and all lips are completely fused and merged with perioral disc, lip region continuous from body. Type 2: Lip region with two separated lateral lips, reduced in size; two sectors of each subdorsal and subventral lips are fused. Perioral disc not distinct and merged with the first annulus of lip region, lip region with slightly offset from body. Type 3: Lip region distinctly separated into six sectors by radial grooves; lateral lips are slightly reduced in size. Perioral disc distinct and separated but not elevated from the lip region, lip region offset from body. Type 4: Similar to Type 3, but perioral disc is distinctly elevated from the lip region, lip region distinctly offset from body.

The ITS rRNA gene alignment (778 bp long) included 41 sequences from the family Merliniidae and two outgroup species [*Coslenchus rhombus* Andr ssy, 1982 (MK874506) and *Filenchus* sp. (MH842880)]. Eighteen new sequences with their morphological characterisations were included in this analysis. The Bayesian 50% majority rule consensus tree inferred from the ITS alignment is given in Figure 18. The tree contained two low-supported major clades. In this case, this marker has an important amount of differences between species, clustering the majority of them in individual clades with the exception of *A. paraglobigeru* and *P. leiocauda*-*P. ritteri*. Genera are well separated with the exception of *Geocenamus* and *Scutylechus* with different clades distributed in the phylogenetic tree. However, the amount of available sequences is minimal in comparison to the other ribosomal markers used in this study.

Scutylechus sp. is clustered in a low-supported clade in the D2–D3 domains of the 28S rRNA phylogeny with *S. quercinus* (MZ437434), an unidentified *Scutylechus* species (KX789703) that could be considered as our *Scutylechus* sp. by the authors, and *S. paniculoides* (KJ585422). For partial 18S rRNA, this species occupies a clade with other *Scutylechus* spp. (including *S. quercinus*, MZ437435 and *S. quadrifer*, AY284599, AY993977) and other genera as *Geocenamus* spp. and *Merlinius* spp. Some of these sequences formerly described as an unidentified *Scutylechus* species (KX789706 and KX789707) could be considered as *Scutylechus* sp. *Scutylechus* sp. (OP382215–OP382218) in the ITS region is grouped in a highly supported clade with *S. quercinus* (MZ437433). The three populations of *Scutylechus* sp. sequenced in this study show 0, 5–6 nucleotides differences (99.1–99.3% similarity) and 13 nucleotides differences (3 were indels and 98.2% similarity) for the partial 18S rRNA, D2–D3 expansion segments of the 28S rRNA and ITS region, respectively. Intra-population variability (5 populations) was only 1 nucleotide difference for the

Alborz mountains population in the D2–D3 expansion segments of the 28S rRNA, and no intra-population was found for the ITS region. Our studied species (*Scutylechus* sp.) was identical for partial 18S rRNA with *S. quercinus* (MZ437435, 974 bp long) and was 99.8% similar to several species including an unidentified *Scutylechus* species in the GenBank found in Iran (KX89706 and KX89707) and in this study assumed to be *Scutylechus* sp. by the authors, *S. rugosus* (KX89704 and KX89705), *Merlinius brevidens* (KX89708), and *M. nanus* (KX89709) for partial 18S rRNA. Also, high similarities were found for the D2–D3 region marker. In this case, the marker for this species was 99.4% similar (4 nucleotides in difference) to *S. quercinus* (MZ437434) and by an unidentified *Scutylechus* species in the GenBank found in Iran (KX89703) and in this study assumed to be *Scutylechus* sp. by the authors, followed by *S. tartuensis* with 98.6–98.6% similarity (KP313853 and MT857723), and another unidentified *Scutylechus* species with 98.9% similarity (KJ585419). The ITS region showed the closest similarity to *S. quercinus* with similarity of 96.8% (MZ437433, shorter than our sequence, with 414 bp, 12 nucleotide difference including 3 indels), followed by *Merlinius* sp. with 87.8% similarity (MK981336) and *Geocenamus* sp. PQT-2018 with 86.7% similarity (MH191362). The closest *Scutylechus* species is an undescribed species with 85.1% similarity (JQ069956).

Amplimerlinius globigerus and *A. paraglobigerus* (topotype specimens) could be separated molecularly using D2–D3 domains of the 28S rRNA (KX789691, KX789695, KX789696, KJ585428, KP313840, and OP382251–OP38512255, respectively) with 97.4% (18 nucleotide differences) and the ITS region (MN262449, MN262450, MK874507, MK874504, annotated in GenBank as *A. paraglobigerus*, and OP382219–OP382221, respectively) with 82.8% (125 nucleotide differences) similarity. *Geocenamus tenuidens* showed a no-resolved phylogenetic relationship with *Merlinius* and other related genera for the partial 18S rRNA. For the D2–D3 domains of the 28S rRNA,

28S

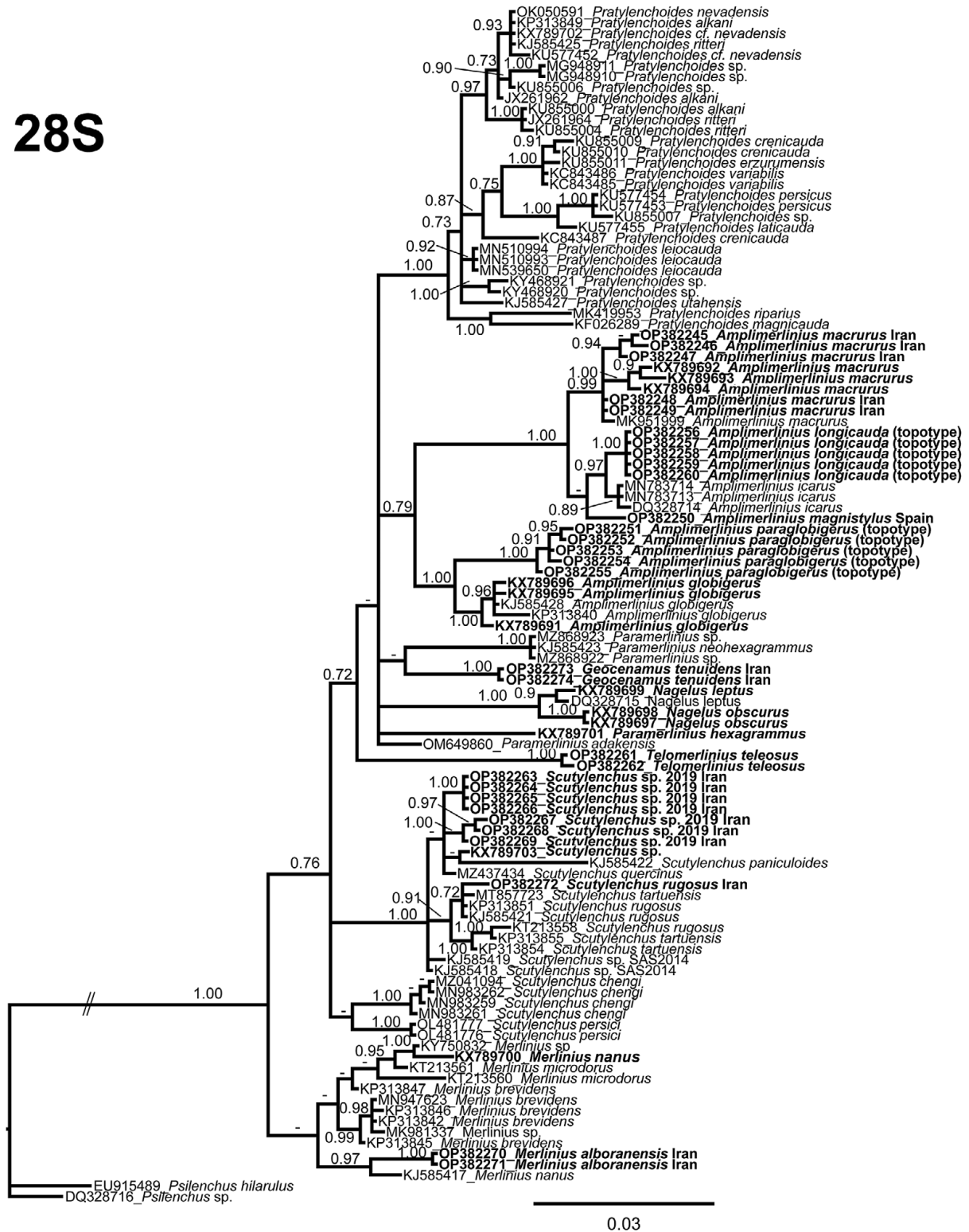


Figure 16. Phylogenetic relationships among Merliniidae species. Bayesian 50% majority rule consensus tree as inferred from D2–D3 expansion segments of 28S rRNA sequence alignment under a transitional model of invariable sites and a gamma-shaped distribution (TIM3 + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).

Geocenamus tenuidens is related to *Paramerlinius neohexagrammus* (KJ585423) in a low-supported clade. Sequences of *M. alboranensis* (OP382270 and OP382271, D2–D3 region), *M. brevidens* (KX789708, partial 18S rRNA), and *M. nanus* (KX789700 and KX789709, 28S

rRNA and partial 18S rRNA, respectively) grouped with other species of *Geocenamus* in the partial 18S rRNA, and they formed a low-supported clade with other species of *Merlinius* in the case of D2–D3 domains of the 28S rRNA. In the case of D2–D3 domains of the 28S

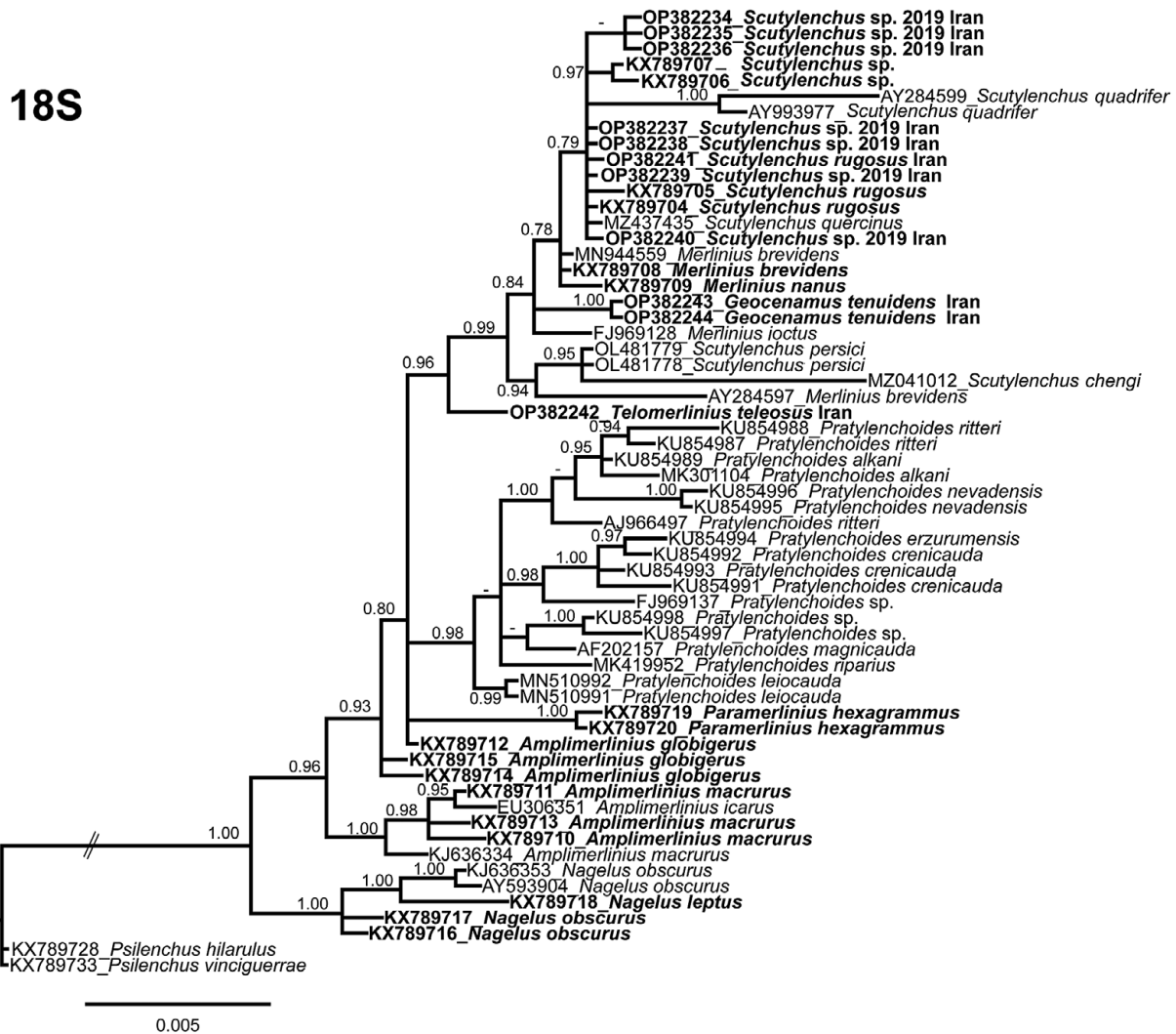


Figure 17. Phylogenetic relationships among Merliniidae species. Bayesian 50% majority rule consensus tree as inferred from partial 18S rRNA gene sequence alignment under a transitional model of invariable sites and a gamma-shaped distribution (TIM3 + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).

rRNA, *M. nanus* is closely related phylogenetically with *M. alboranensis* (KJ585417) in a well-supported clade (PP = 0.97) and is not clearly related with other sequences of *M. nanus* (KX789700). The sequences provided in this study for the genus *Nagelus* (*N. leptus* [KX789699 and KX789718 for 18S rRNA and 28S rRNA, respectively] and *N. obscurus* [KX789697-KX789698 and KX789716- KX789717 for 18S rRNA and 28S rRNA, respectively]) clustered together in a unique clade for partial 18S rRNA and D2–D3 domains of the 28S rRNA in high-supported clades in both molecular markers (PP = 1.00).

In this study, we provided for the first time molecular data for the genus *Telomerlinius* with only one species (*T. teleosus*). This genus is located phylogenetically in the same clade with the genera *Scutylechus* and *Geocenamus* but is clearly separated molecularly from them for the partial 18S rRNA in a moderately-supported clade (PP = 0.98). For the D2–D3 domains of the 28S rRNA phylogeny, the position of this genus is clearly separated from the other genera of the family Merliniidae, but the relationship with other genera is not clearly defined inside a major clade with a low support (PP = 0.72).

Discussion

In the present work, we provided an outline on the taxonomic position of the genera and species in the subfamily Merliniinae. We included representatives from all known genera in Merliniinae (except *Macrotylenchus*) and provided morphological and molecular data for recovered members. Genera in the subfamily as well as species in each genus could be distinguished based on previously published works (Geraert 2011; Sturhan 2012; Ghaderi & Karegar 2014; 2016; Ghaderi et al. 2017).

In this study, integrative taxonomy of fourteen known species including *Amplimerlinius globigerus*, *A. longicauda*, *A. macrurus*, *A. magnistylus*, *A. paraglobigerus*, *Geocenamus tenuidens*, *Merlinius alboranensis*, *M. brevidens*, *M. nanus*, *Nagelus leptus*, *N. obscurus*, *Paramerlinius hexagrammus*, *Scutylechus rugosus*, *Telomerlinius teleosus*, and one species described herein as *Scutylechus* sp. are performed. However, only two of these species described were collected from the type localities (*A. longicauda* and *A. paraglobigerus*), and future molecular analyses of type material of the studied species might clarify the species

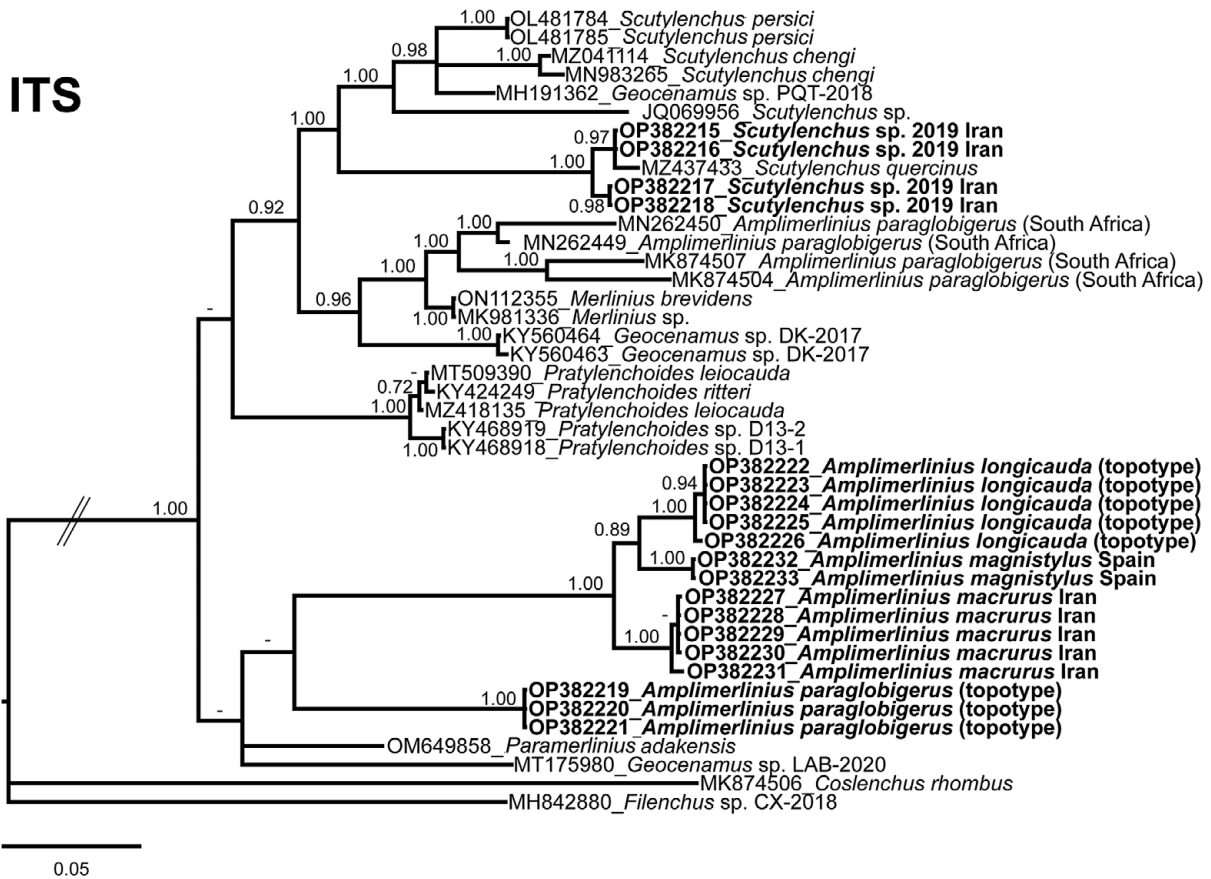


Figure 18. Phylogenetic relationships among Merliniidae species. Bayesian 50% majority rule consensus tree as inferred from ITS rRNA sequence alignment under transitional and a gamma-shaped distribution (TIM2 + G) model. Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).

identification or prevent possible cases of cryptic speciation in this complicated family of nematodes. Partial 18S rRNA does not have enough resolution for species separation as shown with the mixture of species in different clades, and in some cases, even genera (i.e., *Merlinius*, *Scutylenchus* vs. *Geocenamus*) (Figure 17), but only a few genera could be separated phylogenetically using this molecular marker (*Paramerlinius*, *Pratylenchoides*, *Nagelus*, and *Telomerlinius*). However, the D2–D3 domains of the 28S rRNA have a better resolution for all genera studied (*Amplimerlinius*, *Pratylenchoides*, *Merlinius*, *Nagelus*, *Scutylenchus*, and *Telomerlinius*), but *Geocenamus* and *Paramerlinius* occupied polyphyletic positions in the majority of the cases with low-medium support. This confirms the hypothesis about the validity of the genera even with the low differences found in both of these markers. In this sense, Ghaderi *et al.* (2014) supported the grouping of *Pratylenchoides* and Merliniinae into a single family – Merliniidae as proposed by Sturhan (2012) – but these authors did not accept the monophyly of the genus *Amplimerlinius* using the Shimodaira–Hasegawa (SH) test using D2–D3 domains of the 28S rRNA. The ITS region has a good differentiation among genera, but our analysis is very restricted and needs additional effort to get more species sequenced. However, this marker could have a potential problem with additional taxa. The sequences could have less similarity, increasing the alignment problems that occurred with other groups of nematodes (i.e., Longidoridae) being the only accepted phylogenies for closely related species (Palomares-Rius *et al.* 2017). Munawar *et al.* (2021) explored this marker within selected genera and a restricted

selection of sequences from the subfamilies Telotylenchinae and Merliniinae.

Nematode identification using molecular markers for this family with the sequencing data deposited in GenBank could be difficult for partial 18S rRNA and D2–D3 domains of the 28S rRNA, as many of the species are mixed between the different genera clades and interspecies differentiation is low (see branches length in trees). Additional markers as COI or ITS regions are needed in the future for a clear molecular separation between closely related species. Additionally, some of the species deposited in the GenBank are not published, or they need an integrative taxonomical study including morphological-morphometrical data with molecular markers.

Depending on the absence or presence of deirids, the subfamily Merliniinae is divided into two groups: the genera *Amplimerlinius*, *Nagelus*, *Merlinius*, and *Paramerlinius* with deirid and *Geocenamus*, *Telomerlinius*, and *Scutylenchus* without deirid. In our phylogenetic trees, the genera with deirid are not clearly separated from those without deirid. According to the lip region, we can divide the family Merliniidae into three groups; group 1: lip region with offset perioral disc (may be elevated or not elevated) and head annuli with six sectors (four in *Merlinius*), including genera *Geocenamus*, *Scutylenchus*, *Merlinius*, and *Telomerlinius*; group 2: perioral disc simple and fused with the first annulus of lip region without radial longitudinal grooves, including genera *Amplimerlinius*, *Nagelus*, and *Paramerlinius*; and group 3: lip region fused with the first annulus and without radial longitudinal grooves but the first annulus with six sectors, including *Pratylenchoides*. Our partial D2–D3

of 28S rRNA and 18S rRNA partially supported this grouping. The 18S rRNA tree supported Geraert's (2011) decision on the synonymisation of *Scutylenchus* and *Merlinius* with *Geocenamus*, but this marker can not separate the valid species. Our 18S rRNA phylogeny tree supported the validity of the genus *Paramerlinius* proposed by Sturhan (2012) and separated it from *Nagelus* spp.

In three markers used in our phylogeny, the genus *Amplimerlinius* formed two separated clades, one clade including *A. globigerus* and *A. paraglobigerus* with an average of less than 25 µm for stylet length and the latter including *A. macrurus*, *A. icarus*, *A. longicauda*, and *A. magnistylus* with an average of more than 25 µm for stylet length. Phylogenetic position in relation to stylet length was also confirmed for *Paramerlinius hexagrammus* and *P. neohexagrammus* in the 28S rRNA tree. In the genus *Pratylenchoides*, the Iranian population of *P. riparius* (Andrássy, 1985) Luc, 1986 (Hosseinvand *et al.* 2019) and *P. magnicauda* (Thorne, 1935) Baldwin, Luc & Bell, 1983, with an average of stylet 25 µm or more, formed a separated clade from other species of *Pratylenchoides* spp. bearing a stylet less than 25 µm.

The Lorestan population of *A. macrurus* [OP382245–OP382247 (D2–D3 region), OP382230 and OP382231 (ITS region)] slightly differs from our other populations of *A. macrurus* [KX789693, KX789694, OP382248 and OP382249, OP382227–OP382229 (D2–D3 region)] by its tail (Figures 2, 3), which is subclavate and smooth with wide annuli at the tip vs. cylindrical with fine annuli at the tip. However, this population is in other populations of *A. macrurus* phylogenetically and cannot be considered as a separate species.

Brzeski (1997) synonymised *N. camelliae* with *N. obscurus*; in this paper, we follow his action, but according to the 18S rRNA tree, the Iranian population of *N. obscurus* (identified as *N. camelliae*) (KX789716 and KX789717) occupied different phylogenetic positions from two other isolates/populations of *N. obscurus* deposited in GenBank (KJ636353 and AY593904). More sequences are needed to clarify inter-species variability in this genus.

This study provides for the first time molecular markers for *Telomerlinius*, and our phylogenetic analysis in both the markers studied (18S and 28S rRNA) supports the genus validity. The genus *Telomerlinius* with four lateral fields and the absence of deirids is similar to the family Telotylenchidae, but according to spicules shape, it comes close to Merliniinae. Our phylogenetic trees supported placing this genus in the family Merliniidae as proposed by Siddiqi & Sturhan (2014). The lip region of *Telomerlinius* is similar to *Geocenamus* and *Scutylenchus* but with an elevated perioral disc.

The taxonomic position of certain species in Merliniinae is yet unclear and under question. Eight species including *G. angelescresti* Chitambar & Ferris, 2005; *N. conicus* (Allen, 1955) Siddiqi, 1979; *N. djungaricus* (Razzhivin, 1974) Kapoor, 1983; *G. dobroticus* Budurova, Baicheva & Milkova, 1996; *G. kirjanovae* (Sagitov, 1973) Fortuner & Luc, 1987; *M. salechardicus* Nesterov, 1985; *G. superbus* (Allen, 1955) Fortuner & Luc, 1990; and *G. tokobaevi* (Sultanalieva, 1983) Fortuner & Luc, 1987 were listed by Sturhan (2012) under species of uncertain generic position. Unfortunately, there is neither morphological nor molecular evidence sufficient to assign them to one of the known genera. However, we believe that deirids in *G. angelescresti* in the original description (Chitambar & Ferris 2005) are misidentified, as under light or scanning microscopes, a real deirid can be seen as a smaller dot than represented in Fig. 3D and 4J of the original description. Moreover, other morphological characteristics (particularly typical stylet bearing long conus) as well as its cephalic region pattern (similar to type 3 in our study) confirm its position under the genus *Geocenamus*.

In summary, this study provides for the first time an integrative taxonomy for several species and in some cases for certain genera of the family Merliniidae. The complexity of the family, the possible cryptic nature of some species, and the possible misidentification of species in the sequences deposited in GenBank necessitate the use of an integrative taxonomy. The low diversity in partial 18S rRNA and D2–D3 domains of 28S rRNA genes urges the use of a multi-locus approach involving additional markers such as the ITS region and/or COI.

Supplementary material. To view supplementary material for this article, please visit <http://doi.org/10.1017/S0022149X23000640>.

Acknowledgements. None.

Financial support. This project was supported by the Iranian National Science Foundation (INSF) for financial support (Grant no. 99025922) and by the University of Jaén, Spain, through the Research Support Plan "PAIUIJA 2019/2020: EI_RNM02_2019".

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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