

Integrative identification of *Scutylenchus tartuensis* (Tylenchoidea: Merliniidae) based on two populations from Iran

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Accepted for publication 10 September 2026

Summary. Two populations of *Scutylenchus tartuensis* recovered from the rhizosphere of camelthorn and mugwort in South Khorasan province, eastern Iran, are described based on morphological and molecular data. The phylogenetic relationships of the new populations with representatives of the family Merliniidae were reconstructed and discussed using D2-D3 expansion segments of the large subunit and internal transcribed spacer regions of ribosomal DNA (LSU D2-D3 and ITS rDNA) based on Bayesian inference. The first ITS sequences of the species, from the Iranian populations of *S. tartuensis*, were utilised to reconstruct and analyse the corresponding phylogenetic tree. In the LSU phylogenetic tree, the sequence of the new population formed a clade with other sequences of the species, and in the ITS rDNA phylogenetic tree, sequences of the new populations formed a clade with *S. quercinus* and two unidentified species of the genus *Scutylenchus* with different levels of relatedness.

Key words: Birjand, D2-D3 of LSU, ITS, morphology, morphometric data, phylogeny.

The Bagheran mountain range in Birjand city, South Khorasan province, is considered one of the most important natural regions in eastern Iran, and contains diverse vegetation. Some rangeland plants in this area have medicinal properties and are also a natural food source for honeybees. Due to the importance of the vegetation in this region, identifying pathogens, including plant-parasitic nematodes, is essential for its conservation and management.

Several nematode species have been reported from Birjand city to date. These include *Merlinius pistaciei* Fatema & Farooq, 1992 from pomegranate (Bajestani *et al.*, 2016); *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, from barberry (Behdani *et al.*, 2017); *Xiphinema persicum* Jahanshahi Afshar, Pedram & Mobasseri, 2021, and *X. babaii* Naghavi, Niknam & Vazifeh, 2022 from walnut; and *Ditylenchus persicus* Esmacili, Heydari, Castillo & Palomares-Rius, 2017 from saffron (Behdani *et al.*, 2024).

In the Bagheran mountain range, nematode diversity is largely unexplored. During recent surveys of the plant-parasitic nematodes in this

region, *Coslenchus iranicus* Noras Mofrad & Azimi, 2026, was obtained (Noras Mofrad & Azimi, 2026). In the present study, two populations of the genus *Scutylenchus* Jairajpuri, 1971, were recovered.

The genus *Scutylenchus* is particularly distinguished from other genera in Merliniinae Siddiqi, 1971 by the presence of longitudinal striae on the body cuticle and the absence of deirids (Siddiqi, 2000). The validity of this genus was accepted by some nematologists (Decraemer & Hunt, 2006; Andr  ssy, 2007; Bert & Hunt, 2025). Some nematologists considered the genera *Geocenamus* Thorne & Malek, 1968, *Merlinius* Siddiqi, 1970, and *Scutylenchus* under *Geocenamus* (Brzeski, 1998; Geraert, 2011). Sturhan (2012) considered *Merlinius* as a separate genus but synonymised *Scutylenchus* with *Geocenamus*.

The present study aims to provide morphological and morphometric data for the newly recovered populations of the genus *Scutylenchus* and to determine their molecular phylogenetic affinities with other populations and species of *Scutylenchus* and other taxa in the family Merliniidae Siddiqi, 1971 (Ryss, 1993).

MATERIALS AND METHODS

Nematode extraction and morphological observations. Several soil samples were collected from the rhizosphere of cameltorn (*Alhagi persarum* Boiss & Buhse.) and mugwort (*Artemisia sieberi* Waldst & Kit.) in Birjand, South Khorasan province, eastern Iran. The centrifugal-flotation technique (Jenkins, 1964) was used to extract the nematodes from the soil samples. The collected specimens were killed in a hot 4% formaldehyde solution and transferred to anhydrous glycerin, according to De Grisse (1969). Observations and measurements were conducted using a Leitz SM-LUX light microscope with a drawing tube. Some specimens were photographed using an Olympus BX51 light microscope with a Tucsen Michrome 20 digital camera.

DNA extraction, PCR and sequencing. For molecular analyses, single female specimens were picked out, examined in a drop of distilled water on a temporary slide under a light microscope, and transferred to 7 µl of worm lysis buffer (500 mM KCl, 100 mM Tris-Cl pH 8, 15 mM MgCl₂, 10 mM DTT, 4.5% Tween 20) on a clean slide, and then crushed with a coverslip. The suspension was collected by adding 7 µl of lysis buffer. Two microliters of proteinase K (600 µg ml⁻¹) were added and the tubes were frozen at -70°C for at least 10 min and then incubated at 65°C (1 h) and 95°C (10 min) consecutively. After incubation, the tubes were centrifuged for 2 min at 14,000 rpm. The DNA samples were stored at -20°C until used as a PCR template (Maafi *et al.*, 2003). Primers for LSU rDNA D2-D3 amplification were forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT-3') and reverse primer D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') (De Ley *et al.*, 1999). Primers for amplification of ITS rDNA were forward primer rDNA1 (5'-TTG ATT ACG TCC CTG CCC TTT-3') and reverse primer rDNA1.58S (5'-ACG AGC CGA GTG ATC CAC CG-3') (Subbotin *et al.*, 2000). To amplify the above-mentioned loci, polymerase chain reactions (PCR) were performed as described by Azimi and Abdolkhani (2023). Amplification success was evaluated by electrophoresis on a 1% agarose gel. The PCR products were subjected to sequencing using an Applied Biosystems 3500 (ABI) sequencer, Pishgam Corporation, Tehran, Iran. The newly obtained sequences of the new populations were deposited in the GenBank database (accession numbers PZ231290 for LSU rDNA D2-D3, and PZ231292, PZ231293 for ITS rDNA).

Phylogenetic analyses. The newly obtained sequences of the D2-D3 fragments of LSU and ITS

rDNA were compared with those of other nematode species available in the GenBank database using the BLAST homology search program, and the sequences selected to reconstruct each phylogeny were retrieved. The sequences were aligned by ClustalX version 2 using the default parameters (Larkin *et al.*, 2007). The editing of the two alignments was performed manually in the MEGA7 program (Kumar *et al.*, 2016). The base substitution model was selected using MrModeltest 2 (Nylander, 2004) based on the Akaike information criterion. A general time-reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I), was used in the two phylogenies.

Bayesian analyses were performed to infer the phylogenetic trees using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003), running the chains for four million generations. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analyses. The Markov chain Monte Carlo (MCMC) method within the Bayesian framework was used to determine the equilibrium distribution and help estimate the posterior probabilities of the phylogenetic trees (Larget & Simon, 1999) using the 50% majority rule. Bayesian posterior probability (BPP) values greater than 0.50 are given on appropriate clades. The output files of the phylogenetic program were visualised using Dendroscope v3.2.8 (Huson & Scornavacca, 2012), and trees were digitally drawn in CorelDRAW software version 23.

RESULTS

Scutylenchus tartuensis (Krall, 1959) Siddiqi, 1979 (Figs 1 & 2; Table 1)

Female. Body slightly arcuate ventrally when heat-relaxed. Cuticle annuli rounded, distinct, 1.5 to 2.5 µm wide at mid-body. Cuticle with longitudinal ridges except lateral field. Lateral field about 30.5-35.5% of the corresponding body diameter, with six incisures, irregularly areolated. Lip region slightly offset by a shallow depression, flattened at front end, 3.8-4.5 µm high and 8.0-8.5 µm wide, bearing mostly six annuli, occasionally five or seven annuli. Cephalic framework distinct, with retractive basal plate extending posteriorly for about two annuli. Stylet well developed, its conus 48-55% of total length, basal knobs rounded, posteriorly directed. Dorsal gland orifice near the stylet base. Pharyngeal median bulb oval with prominent valve, 12.0-13.5 µm wide and 18.5-23.5 µm long, terminal bulb elongated, 12.5-14.0 µm wide and 28-33 µm long. Nerve ring encircling middle of isthmus, 84-100 µm

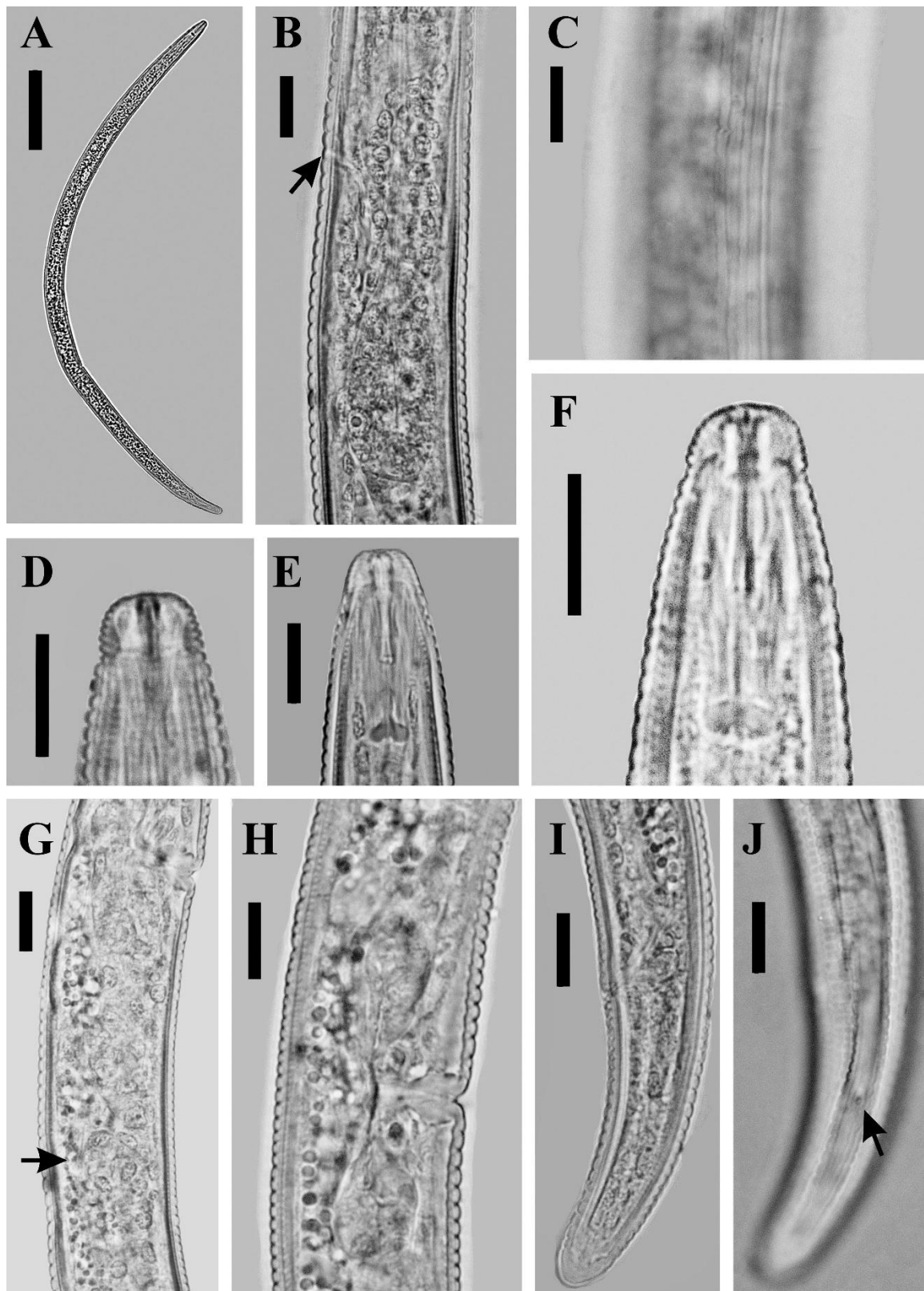


Fig. 1. Light photomicrographs of *Scutylenchus tartuensis* from Iran (population 1). A: Entire body; B: Terminal bulb (the arrow indicates the excretory pore); C: Lateral field at mid-body; D-F: Anterior body region; G: Part of reproductive system (the arrow indicates the spermatheca); H: Vulval region; I, J: Posterior body region (the arrow indicates the phasmid). Scale bars: A = 100 µm; B-J = 10 µm.

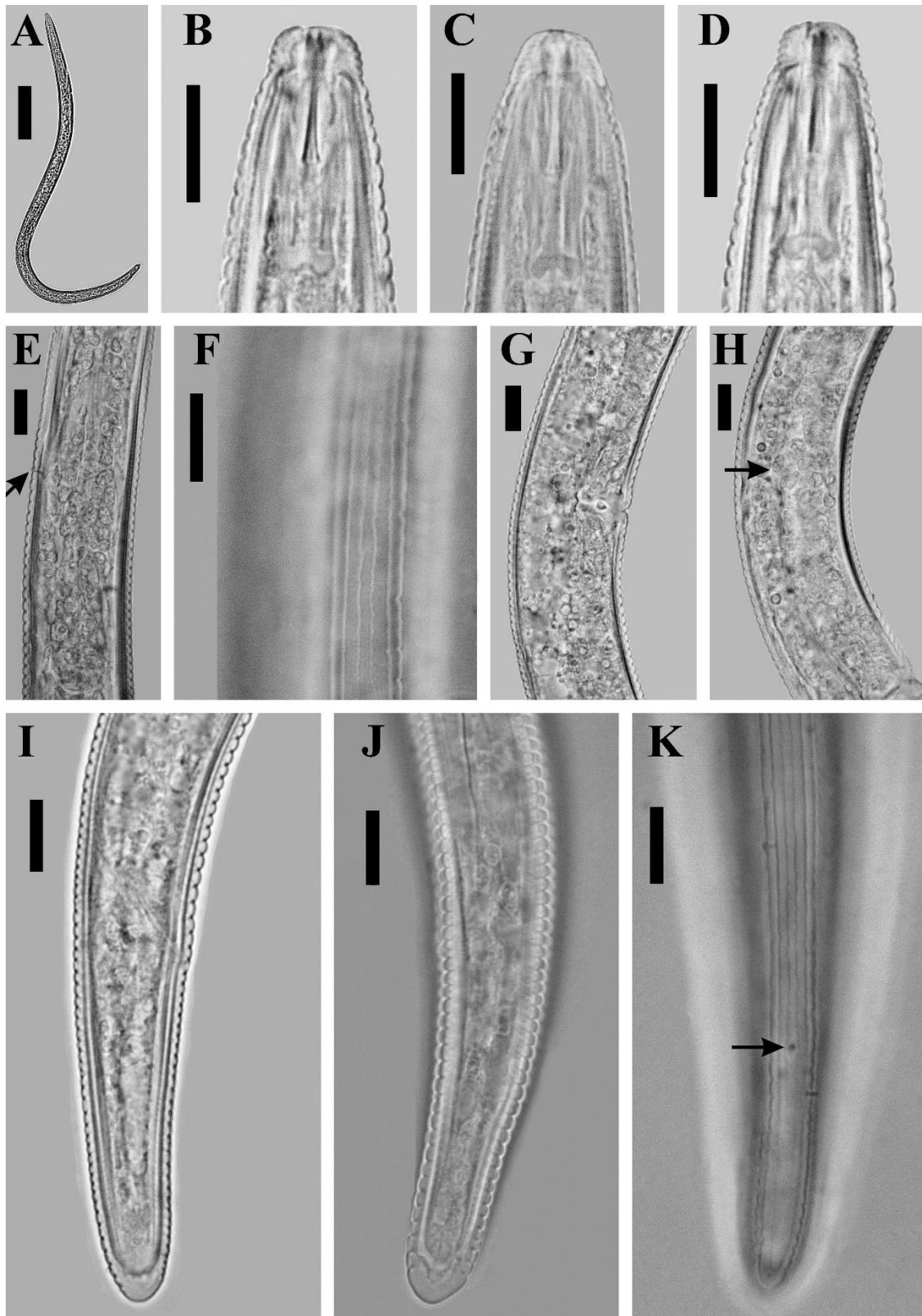


Fig. 2. Light photomicrographs of *Scutylenchus tartuensis* from Iran (population 2). A: Entire body; B-D: Anterior body region; E: Terminal bulb (the arrow indicates the excretory pore); F: Lateral field at mid-body; G: Vulval region; H: Part of reproductive system (the arrow indicates the spermatheca); I-K: Posterior body region (the arrow indicates the phasmid). Scale bars: A = 100 μ m; B-K = 10 μ m.

Table 1. Morphometrics of two populations of *Scutylenchus tartuensis* (Krall, 1959) Siddiqi, 1979 from South Khorasan province, Iran. All measurements are in μm and in the form: mean $\pm$ s.d. (range).

Character	Population 1	Population 2
n	8	10
L	802.8 $\pm$ 41.4 (725-853)	739.2 $\pm$ 41.1 (675-803)
a	27.9 $\pm$ 1.4 (25.5-30.3)	29.7 $\pm$ 1.2 (28.2-31.5)
b	4.5 $\pm$ 0.2 (4.1-4.8)	4.9 $\pm$ 0.4 (4.4-5.7)
c	17.1 $\pm$ 1.1 (14.8-19.0)	15.7 $\pm$ 0.9 (14.4-17.6)
c'	2.7 $\pm$ 0.3 (2.3-3.1)	2.9 $\pm$ 0.2 (2.5-3.2)
V	56.3 $\pm$ 1.7 (53.3-59.4)	56.0 $\pm$ 1.2 (54.0-58.4)
Stylet length	20.1 $\pm$ 1.4 (18.4-23.1)	20.9 $\pm$ 1.0 (19.4-22.5)
Stylet conus length	9.1 $\pm$ 1.7 (8.2-13.6)	10.8 $\pm$ 0.7 (9.8-12.2)
Stylet knob width	5.7 $\pm$ 0.7 (4.7-6.8)	5.5 $\pm$ 0.5 (4.6-6.5)
m	44.9 $\pm$ 5.7 (40.2-58.8)	51.7 $\pm$ 1.2 (50.1-53.8)
DGO	2.4 $\pm$ 0.2 (2.2-2.7)	2.8 $\pm$ 0.5 (2.2-3.4)
MB	47.9 $\pm$ 1.6 (45.1-50.6)	46.8 $\pm$ 2.1 (44.5-51.3)
Anterior end to excretory pore	135.7 $\pm$ 5.2 (129-145)	115.8 $\pm$ 6.8 (106-123)
Pharynx length	178 $\pm$ 6 (169-188)	150.5 $\pm$ 10.2 (138-163)
Maximum body width	28.8 $\pm$ 2.0 (25.2-32.0)	24.9 $\pm$ 1.7 (22.8-28.5)
Vulval body width	28.8 $\pm$ 2.0 (25.2-32.0)	24.6 $\pm$ 1.5 (23-28)
Anal body width	17.9 $\pm$ 2.0 (14-21)	16.2 $\pm$ 0.9 (14.7-18.0)
Tail length	47.2 $\pm$ 3.6 (40.2-53.7)	47.3 $\pm$ 3.6 (41-52)
Tail annuli	24.5 $\pm$ 2.7 (21-28)	25.2 $\pm$ 2.1 (22-30)
T/vulva-anus	0.2 $\pm$ 0.0 (0.2)	0.2 $\pm$ 0.0 (0.1-0.2)

from anterior end. Excretory pore at the level of anterior end of terminal bulb. Hemizonid one to two annuli anterior to excretory pore. Reproductive system didelphic-amphidelphic, vulva with double epiptygma sunken in vulval cavity, vagina with swollen walls, 9-15 μm long, occupying 40-50% of the vulval body diameter, spermatheca offset, with rounded sperm. Tail regularly tapering to a smooth terminus, hyaline terminal part of tail 3-5 μm long. Phasmids located at 26.5-33.5% of tail length.

Male. Not found.

Host and locality. Population 1 was recovered from the rhizosphere of camelthorn collected from Birjand in South Khorasan province, eastern Iran. The GPS information of the sampling site is (32°42.6180'N, 59°21.1930'E). Population 2 was recovered from the rhizosphere of mugwort collected from Birjand in South Khorasan province, eastern Iran. The GPS information of the sampling site is (32°42'36.5"N, 59°21'11.1"E).

Remarks. Compared with the original description of the species by Krall (1959), the number of annuli from stylet knob base to lip constriction is lower (12-14 vs 18-21), and the stylet length is shorter (18.4-23.1 vs 24 μm). Compared to the general morphology and morphometric data ranges given by Geraert (2011), no considerable differences were observed. Compared with the Iranian populations of the species

associated with rapeseed, fruit tree seedlings, and tobacco in Kurdistan province and from potato in Khorasan Razavi province, no significant differences were observed (Ghaderi *et al.*, 2014). Compared with the Iranian populations of the species associated with barberry, jujube, and saffron in South Khorasan province by Alvani *et al.* (2017), the a ratio is lower (25.5-31.5 vs 31.6-41.4), the b ratio is lower (4.1-5.7 vs 5.9-6.8), and the distance from the anterior end to the excretory pore is higher (106-145 vs 74-99). *Scutylenchus tartuensis* was also reported from chickpea, lentil, and faba bean in Kermanshah province of Iran, but morphological and morphometric data were not provided for the species (Mohammadi Zameleh *et al.*, 2018).

Although *S. tartuensis* bears a strong resemblance to *S. paniculoides* (Vovlas & Esser, 1990) Siddiqi, 2000, it can be distinguished from it based on morphological differences. The lip region in *S. tartuensis* is conoid-truncate, continuous to slightly set off from the body by a shallow depression, and the cephalic framework is more developed, showing long outer extensions; whereas it is hemispherical, distinctly set off from the body by a constriction, and the cephalic framework has shorter outer extensions in *S. paniculoides* (Ghaderi & Karegar, 2016).

This species also bears similarities to *S. lenorus* (Brown, 1956) Siddiqi, 1979, but the lip region in *S.*

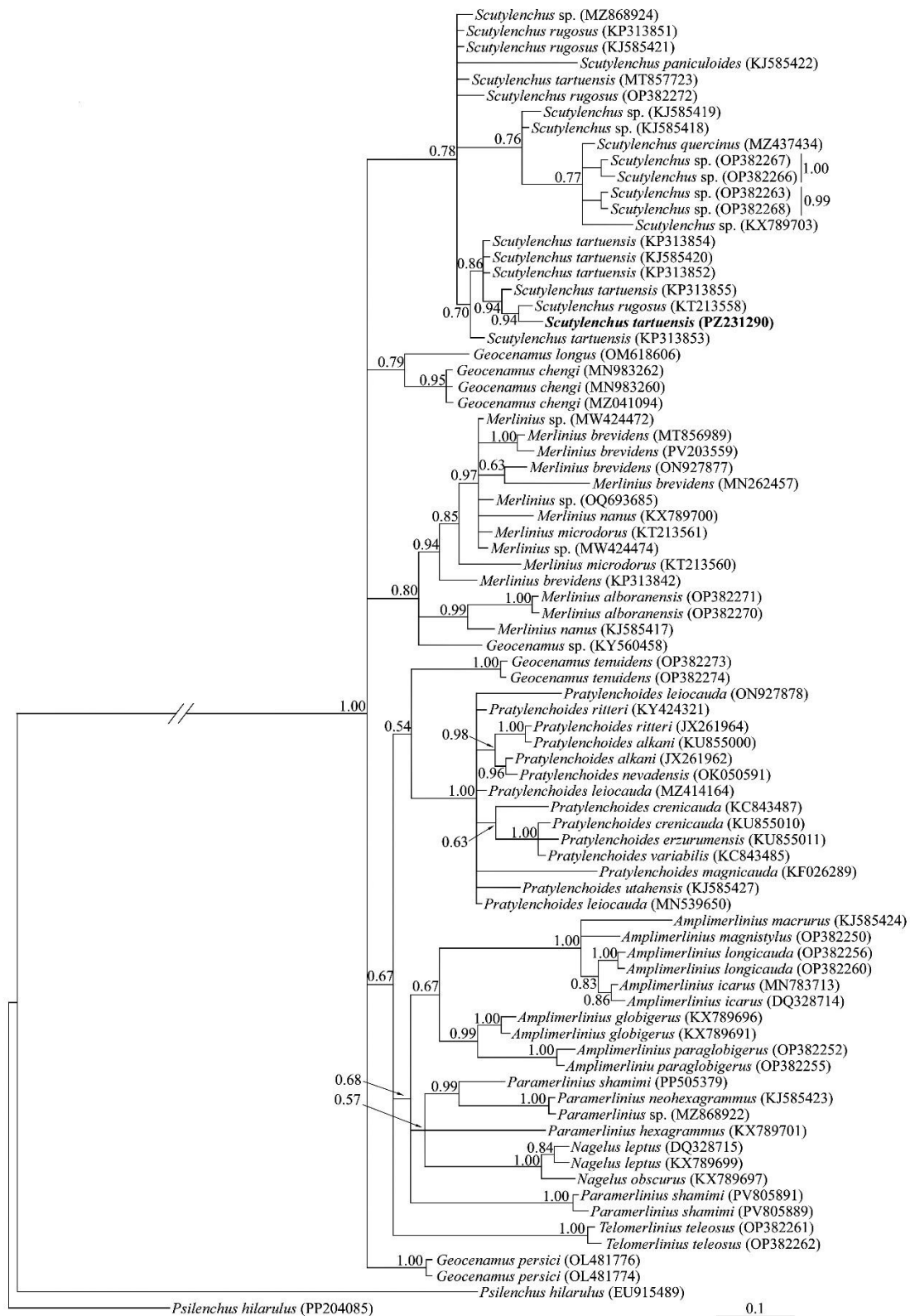


Fig. 3. Bayesian 50% majority rule consensus tree inferred from analysis of the D2-D3 expansion segments of the LSU rDNA sequences of *Scutylenchus tartuensis* under the GTR + G + I model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. A new sequence is indicated in bold.

lenorus is distinctly set off from the body by a constriction, the cephalic framework is not refractive, and the spermatheca is bilobed (Geraert, 2011). In addition to morphological characteristics, molecular data can also support species identification. The 28S sequence of *S. tartuensis* in the present study differed from that of *S. paniculoides* by 23 nucleotides and is clearly separated in the phylogenetic tree.

Molecular characterisation and phylogenetic relationships. D2-D3 fragments of LSU rDNA phylogeny. To reconstruct the LSU rDNA tree, a newly obtained 707 bp long partial sequence of the D2-D3 region with accession number PZ231290 from population 1 was used. The BLAST search using this sequence revealed it had 99.71% identity (yielded from two mismatches) with another sequence of the same species (KP313855) from Iran (Alvani *et al.*, 2017). Previous studies based on the LSU rDNA gene had been conducted on this species. Six sequences of this species were available in GenBank, five of which are from Iran (KJ585420 and KP313852-KP313855) and one sequence from China (MT857723). The difference between the existing sequences is 0-8%. The difference between our sequence and KP313852 is four nucleotides; with KP313853, it is six nucleotides; with KP313854, it is four nucleotides; with KJ585420, it is four nucleotides; and with MT857723, it is nine nucleotides. Seventy-nine LSU sequences of the family Merliniidae were used in LSU phylogeny. This dataset comprised 777 characters. The phylogenetic tree inferred using this dataset is presented in Figure 3. The newly generated LSU sequence of the species formed a clade with other sequences of the species with relatively high support (BPP = 0.86).

Partial ITS rDNA phylogeny. Two partial sequences of 936 (population 1) and 820 (population 2) bp long of ITS rDNA (PZ231292 and PZ231293) were used to determine the phylogenetic relationships of the Iranian populations of *S. tartuensis*. A BLAST search using these sequences revealed they have 95.31% and 95.70% identity, respectively, with one unidentified species of the genus *Scutylechus* (OP382215) from Iran (Hosseinvand *et al.*, 2023). Sequence variations between these sequences were 25-30 mismatches and six to seven gaps. Thirty sequences of the family Merliniidae were used for ITS phylogeny. The phylogenetic tree inferred using this dataset is presented in Figure 4. In this tree, the generated sequences of the Iranian populations of *S. tartuensis* formed a clade with *S. quercinus* Sheikhzadeh, Mobasser, Valizadeh & Pedram, 2022, and two unidentified species of the genus *Scutylechus* with

maximum support (BPP = 1.00). This study provides the ITS sequences of the species for the first time.

DISCUSSION

The objectives of the present study were the morphological and molecular characterisation of the recovered populations of *Scutylechus tartuensis* from Iran. This species was first described from a potato field in Estonia (Krall, 1959). Then it subsequently was reported from Poland, Italy, and Germany (Brzeski, 1991). As previously mentioned, this species has been reported from various plants in different regions of Iran and has a wide distribution. Also, it has been reported from the rhizosphere of leguminous fields in Turkey (Kepenekçi & Ökten, 2000) and Jerusalem artichoke (*Helianthus tuberosus* L.) in Polish plantations (Zapałowska & Skwiercz, 2018). More sampling is needed to determine the habitat and geographic distribution of the species worldwide.

In the LSU tree, all sequences of this species are placed in the same clade except for MT857723, which is placed in a separate clade. KP313853 was also placed in a separate subclade. The morphological and morphometric data of these populations were not provided by the authors, which indicates that these populations require re-evaluation based on integrative taxonomy. Sequences from *S. tartuensis* were also placed in the same clade with sequences from *S. rugosus*. Distinction of these species using only this marker is difficult, and similar results have been obtained in previous studies (Azimi *et al.*, 2016; Munawar *et al.*, 2021; Hosseinvand *et al.*, 2023).

The number of sequences available from the family Merliniidae in the ITS rDNA region is much lower than in the LSU rDNA. Although the ITS region can often distinguish closely related species from each other, due to the very small number of sequences available in this region, the status of closely related species and genera is still unclear. A similar situation has been observed in another study (Hosseinvand *et al.*, 2023). The sequences of the present study are the first sequences of the ITS region of *S. tartuensis*. No sequences are available for *S. rugosus*, so the phylogenetic relationships of these two species cannot be stated with certainty.

The lack of morphological and morphometric data for some sequences, the possible misidentification of some species in the sequences deposited in GenBank, the possibility of cryptic species, and the lack of molecular investigation of many described species have created many challenges in clarifying the phylogenetic position of some genera and species of

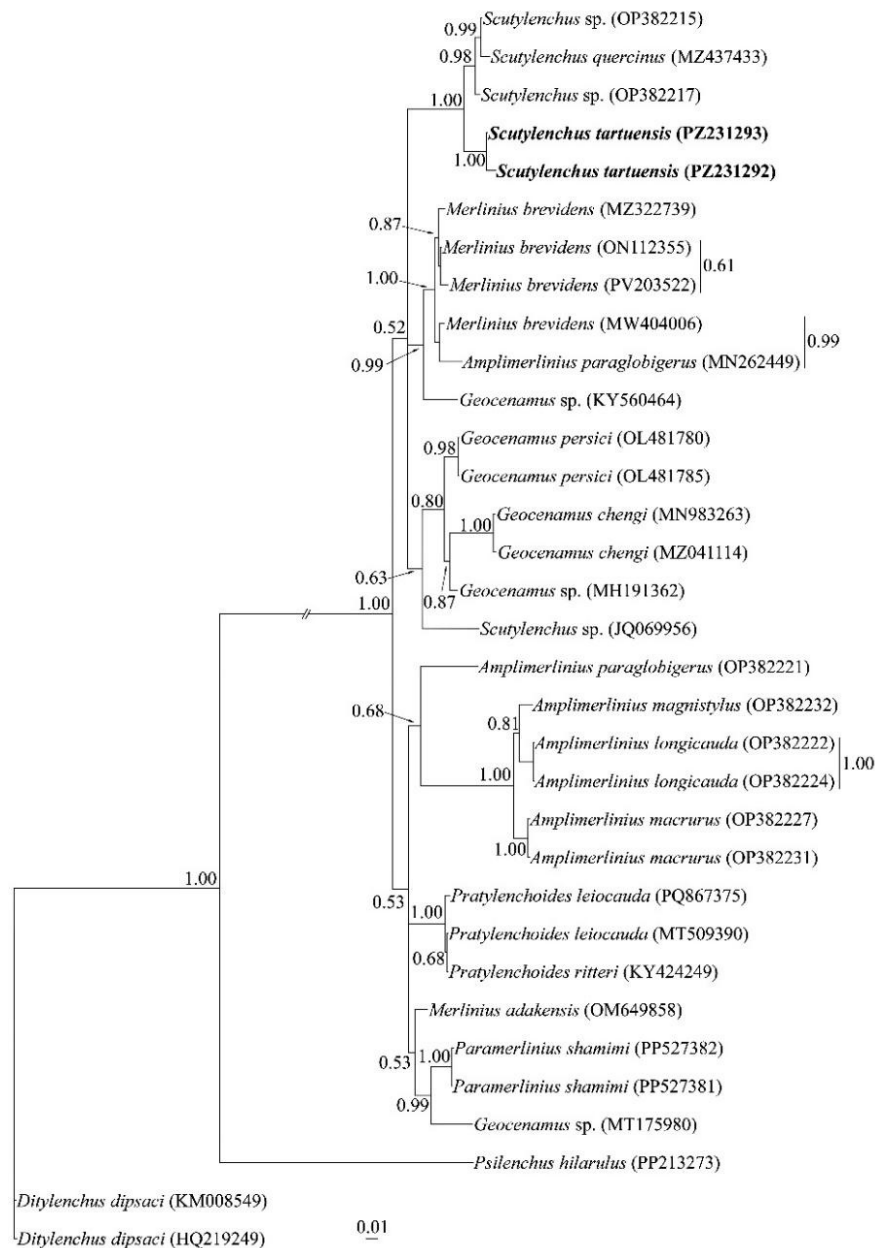


Fig. 4. Bayesian 50% majority rule consensus tree inferred from analysis of the ITS rDNA sequence of *Scutylenchus tartuensis* under the GTR + G + I model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. New sequences are indicated in bold.

the family Merliniidae (Munawar *et al.*, 2021; Hosseinvand *et al.*, 2023). Therefore, re-examination based on integrative taxonomy is necessary for the accurate recognition of genera and species, as well as for determining their phylogenetic positions.

ACKNOWLEDGEMENTS

The authors thank the Research Council of Shahid Chamran University of Ahvaz, Iran (Grant no. SCU.AP1404.638) for financial support.

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N. Noras Mofrad and S. Azimi. Интегративная идентификация *Scutylechus tartuensis* (Tylenchoidea: Merliniidae) на основе исследования двух популяций из Ирана.

Резюме. Две популяции *Scutylechus tartuensis*, выделенные из ризосферы верблюжьей колючки и полыни в провинции Южный Хорасан, на востоке Ирана, были описаны на основании морфологических и молекулярных данных. Филогенетические взаимоотношения новых популяций с представителями семейства Merliniidae были реконструированы методом Байесовского анализа на основе секвенсов генов D2-D3 участка большой субъединицы и внутренних транскрибируемых спейсерных областей рибосомной ДНК (LSU D2-D3 и ITS рДНК). Впервые полученные ITS последовательности для иранских популяций *S. tartuensis* были использованы для построения и анализа филогенетических деревьев. На филогенетическом LSU дереве, последовательность новой популяции образовала кладу с другими последовательностями этого вида. На филогенетическом ITS рДНК дереве последовательности новых популяций сформировали кладу вместе с *S. quercinus* и двумя неидентифицированными видами рода *Scutylechus*, демонстрируя различные уровни родства.