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Research Article

New information on insect nematodes from Iran: report of *Distolabrellus veechi* (Nematoda: Mesorhabditidae) as new genus and male of *Oscheius tipulae* (Nematoda: Rhabditidae)

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Abstract. In order to identify nematodes associated with insects in the forested areas of Guilan province, Iran, soil, wood, and insect samples were collected. Nematodes were extracted using the insect-baiting method, and the obtained specimens were analyzed based on both classic and molecular characteristics. This investigation led to the discovery populations belonging to the genera *Distolabrellus* and *Oscheius*, identified as *D. veechi* and *O. tipulae*, respectively. Molecular identification was carried out through molecular analysis, encompassing the D2-D3 expansion segment of 28S, ITS, and 18S genes of rRNA. The phylogenetic analyses placed these nematodes within their respective clades with high bootstrap support, confirming their identity. This survey marks the first discovery of *D. veechi* and male *O. tipulae* in Iran, introducing them as new records for the country.

Keywords: Insect nematology, Insect pathology, Biocontrol, Molecular Charzterization, Phylogeny

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Introduction

The genus *Distolabrellus* Anderson (1983) belongs to the family Mesorhabditidae, within the subfamily Mesorhabditinae, with *D. veechi* as its type species. This genus exhibits several distinctive features. The members of this genus have six separated lips with two alternating shapes. Coarse transverse annules and fine longitudinal striae with minute pores distinguish the body cuticle. Cheilostom is weakly cuticularised. The metastegostom is swollen, and the telostegostom is isoglottoid. Each metastegostomal swelling bears three denticles. Pharynx with distinct metacorpus. Female with monodelphic-prodelphic reproductive system, featuring a posteriorly located vulva and a conoid tail. The bursa is open and peloderan, featuring nine bursal papillae, including two precloacal papillae. Following its initial discovery, *D. veechi* has been recorded in various locations. Currently, there are four known species in this genus. In addition to *D. veechi*, three more species have been identified within this genus. The species include *D. pakistanensis* Tabassum *et al* (2005), *D. magnivulvatus* Abolafia and Pena-Santiago (2011), and *Distolabrellus vulvatus* Khatoon and Ahmad (2021). *D. pakistanensis* (now synonymized with *D. veechi*) was described as an entomopathogenic species by Tabassum *et al* (2005), who provided detailed information on its embryogenesis and life cycle.

The genus *Oscheius* Andrássy (1976) belongs to the family Rhabditidae Örley (1880) within the subfamily Rhabditinae Örley (1880). The type species *O. insectivorus* (originally described as *Rhabditis insectivora* by Körner in 1954) is distinguished by its short buccal tube and the lack of a median pharyngeal swelling, which are key characteristics defining the genus *Oscheius*. Later, Sudhaus and Hooper (1994) refined *Oscheius* classification, confirming it as a monophyletic taxon and dividing it into two species groups: Insectivora and Dolichura.

Differences in bursa morphology, rectum structure, and spicule features distinguish these groups. Several *Oscheius* species, especially those in the Insectivora group, have demonstrated entomopathogenic properties. Notably, *O. carolinensis* Ye *et al* (2010), *O. amsactae* Ali *et al* (2011), *O. niazii* and *O. siddiqii* Tabassum and Shahina (2010) are recognized as entomopathogenic nematodes. Rhabditid nematodes, including *Caenorhabditis elegans*, have become essential models in scientific research due to their experimental benefits, such as a brief life cycle, high reproductive capacity and ease of cultivation (Brenner, 1974). During a nematode survey of the eastern forests of Guilan province, located in northern Iran, two populations were recognized as *D. veechi* and *O. tipulae* Lam and Webster (1971). Detailed observations using light microscopy and molecular assays verified the accuracy of these identifications. This survey signifies the initial discovery of the genus *Distolabrellus* in Iran; furthermore, the presence of male *O. tipulae* in Iran marks a new record within the country. This publication provides a detailed description of *D. veechi* and *O. tipulae* based on morphological observations and molecular analysis, including partial sequences of the 18S rRNA, the D2-D3 expansion segment of the 28S rRNA, and ITS rRNA genes.

Materials and methods

Nematode populations

During 2021, wood, soil, and insect samples, mostly beetles belonging to the superfamily Scarabaeoidae, were obtained from diverse forested areas in Guilan province, northern Iran. The insect-baiting method described by Bedding and Akhurst (1975) was employed using last instar larvae of the greater wax moth, *Galleria mellonella* L., to extract nematodes from soil samples. The insects underwent dissection to remove nematodes from insect samples and were placed on Petri dishes containing agar. The soil samples were stored in plastic containers (300 ml) with lids; each containing with ten last instar larvae and kept in laboratory conditions for 5-7 days. The infected larvae, identified by their color and shape, were individually placed into the White trap (White, 1927). Finally, nematodes extracted from soil and insects were observed and selected under a stereomicroscope. Adult specimens intended for detailed microscopic analysis were gently euthanized using heat, then fixed in a 4:1:1 solution of formaldehyde, glycerin, and acetic acid, and subsequently processed into anhydrous glycerin (De Grisse, 1969). Permanent slides were prepared and examined with an Olympus BH2 light microscope. Morphometric measurements were made using a drawing tube attached to the microscope. Photomicrographs were taken with a digital camera mounted on the Olympus BH2 light microscope, and line drawings were created using the same microscope equipped with the drawing tube.

DNA extraction, PCR, and sequencing

Single nematode specimens were carefully selected and examined individually under a light microscope. Each specimen was then placed in 10 µl of distilled water on a glass microscope slide, crushed with a pipette tip, and transferred to 50 µl of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, Qiagen, Valencia, CA, USA) using a pipette. To ensure efficient extraction of genomic DNA (gDNA), the samples underwent additional processing, including incubation at 65°C for 1 hour to lyse the cells, followed by a brief centrifugation to eliminate debris. The extracted DNA was then stored at -20 ºC until required for PCR amplification.

1 µl of the extracted DNA was added to an Eppendorf tube containing 2.5 µl of 10X NH4 reaction buffer, 0.75 µl of MgCl2 (50 mM), 0.25 µl of a dNTPs mixture (10 mM each), 0.75 µl of each forward and reverse primer (10 mM), 0.2 µl of BIOTAQ DNA Polymerase (BIOLINE, UK), and 18.8 µl of ddH2O, adjusting the total volume to 25 µl. The D2/D3 expansion segment of the 28S rRNA gene was amplified using the forward primer D2A (5′–ACAAGTACCGTGAGGGAAAGTTG–3′) and the reverse primer D3B (5′–TCGGAAGGAACCAGCTACTA–3′) (Nunn, 1992). The ITS region was amplified with the forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer AB28 or internal primer 5.8SM5 (5'-GGCGCAATGTGCATTCGA-3') (Tanha Maafi *et al.*, 2003; Vovlas *et al.*, 2008). For the partial 18S rRNA gene, primers 1096F (5′-GGTAATTCTGGAGCTAATAC-3′) and 1912R (5'-TTTACGGTCAGAACTAGGG-3') (Holterman *et al.*, 2006) were used. The PCR cycling conditions were: an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, an annealing step at 55°C for 45 seconds, extension at 72°C for 3 minutes, and a final extension at 72°C for 10 minutes. The PCR products were subsequently sequenced in both directions using the aforementioned primers.

Phylogenetic analyses

Phylogenetic reconstructions were performed using newly obtained sequences of the D2-D3 expansion region of the 28S rRNA, ITS, and partial 18S rRNA, along with available rhabditid nematode sequences from GenBank. The new sequences were aligned with the Muscle algorithm (Edgar, 2004) using default settings in MEGA 5.0 (Tamura *et al*., 2011). The alignment was refined in MEGA 5.0. The most suitable model of sequence evolution was identified using the Bayesian Information Criterion (BIC) through the jModelTest program (Posada, 2008). Phylogenetic analyses were conducted using Bayesian inference (BI) with MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). A 50% majority rule consensus tree was generated, and posterior probabilities (PP) were assigned to relevant clades. The resulting trees were visualized using TreeView (Page, 1996).

Results

Iranian population of *Distolabrellus veechi* Anderson (1983)(Figs 1, 2)

Measurements, See Table 1. Descriptins

Female

Large nematodes ranging from 995 to 1159 µm in length, straight or slightly ventrally curved after fixation. Cuticle annulated, with fine longitudinal lines and transverse rows of fine cuticular punctations. Lip region continuous or slightly offset from the body, consisting of six separate lips, each bearing papillae. Lips elevated with shallow, grooved margins, displaying dimorphism. One subdorsal, one lateral, and one subventral lip relatively long, extending over the oral opening; the other three alternating lips slightly smaller and spheroid. Amphids small, located at base of lateral lips. Cheilostom weakly cuticularised. Gymnostom constituting over half of the stoma length. Metastegostom swollen; telostegostom conspicuous and isoglottoid. Each metastegostomal swelling bearing three simple or knobbed setose denticles. Neck 180 to 217 µm long, with a distinct metacorpus. Nerve ring located at the level of the isthmus, positioned 118 to 145 µm from the anterior end. Excretory pore opening at the level of the isthmus, 105 to 163 µm from the anterior end. Hemizonid slightly anterior to excretory pore. Reproductive system monodelphic-prodelphic. Vulval opening not protruding, located at the posterior region of the body. Vagina with thin walls, extending inward about one-fourth of the body width. Phasmids located 13-15 µm from the anus. Tail conical with a pointed tip.

Table 1. Morphometrics of Iranian population of *D. veechi*. All measurements are in µm and in the form average ± SD (range).

Character	Iranian population		Original description	
	Female	Male	Female	Male
N	10	8	20	15
L	1048.8 ± 76.3 (995.0-1159.0)	828.3 ± 62.5 (760.0-902.0)	1337 (1157-1471)	821 (724-993)
a	20.6 ± 2.0 (17.9-22.7)	19.7 ± 2.3 (17.0-21.1)	18 (16-21)	20 (17-21)
b	5.3 ± 0.4 (5.0-5.8)	4.8 ± 0.7 (4.1-5.4)	6.1 (5.2-6.9)	4.2 (3.7-5.3)
c	13.7 ± 1.0 (13.0-15.1)	25.8 ± 4.1 (22.1-30.1)	13 (11-21)	21 (18-26)
c'	3.3 ± 0.2 (3.1-3.5)	1.5 ± 0.1 (1.4-1.7)	3.9 (2.3-4.6)	1.7 (1.5-2.0)
V	85.5 ± 0.4 (85.1-86.1)	-	86 (84-89)	-
G1	57.6 ± 1.3 (56.6-59.5)	-	-	-
Stoma length	19.5 ± 1.3 (18.0-21.0)	17.5 ± 1.0 (16.0-18.0)	21 (19-24)	-
Neck length	200.0 ± 15.2 (180.0-217.0)	174.5 ± 13.9 (158.0-186.0)	220 (210-229)	-
Nerve ring	131.8 ± 13.7 (118.0-145.0)	120.3 ± 5.1 (115.0-126.0)	-	-
Excretory pore	128.5 ± 24.5 (105.0-163.0)	135.3 ± 15.6 (112.0-145.0)	183 (161-202)	148 (131-184)
Max. body diam.	51.3 ± 4.7 (48.0-58.0)	44.3 ± 8.5 (36.0-53.0)	74 (64-89)	-
Vulval body diam.	39.8 ± 3.5 (38.0-45.0)	-	-	-
Anal body diam.	23.5 ± 1.3 (22.0-25.0)	21.0 ± 1.0 (20.0-22.0)	-	-
Rectum length	37.8 ± 1.0 (37.0-39.0)	-	45 (39-50)	-
Tail length	76.8 ± 0.5 (76.0-77.0)	32.4 ± 2.6 (30.0-36.0)	101 (76-113)	-
Spicule length	-	61.2 ± 0.4 (61.0-62.0)	-	62 (57-73)
Gubernaculum length	-	36.8 ± 1.1 (35.0-38.0)	-	38 (33-43)

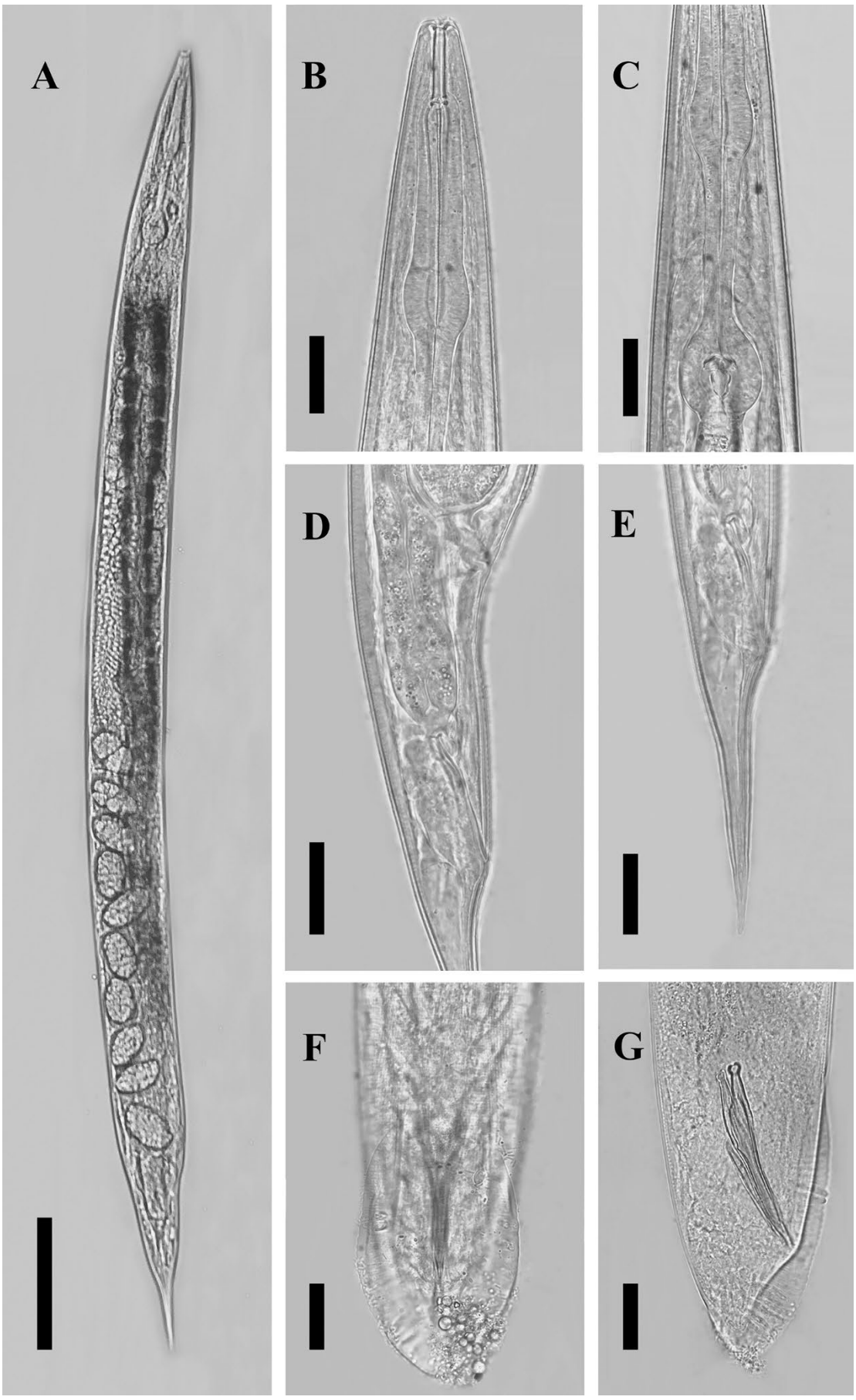

Fig. 1. Photomicrographs of Iranian population of *D. veechi*. A: Female entire body; B: Female head and anterior part of pharynx; C: Posterior part of pharynx; D: Vulva region and Anus; E: Female tail; F, G: Male tail and spicules. (Scale bars: A = 100 µm, B-G = 20 µm).

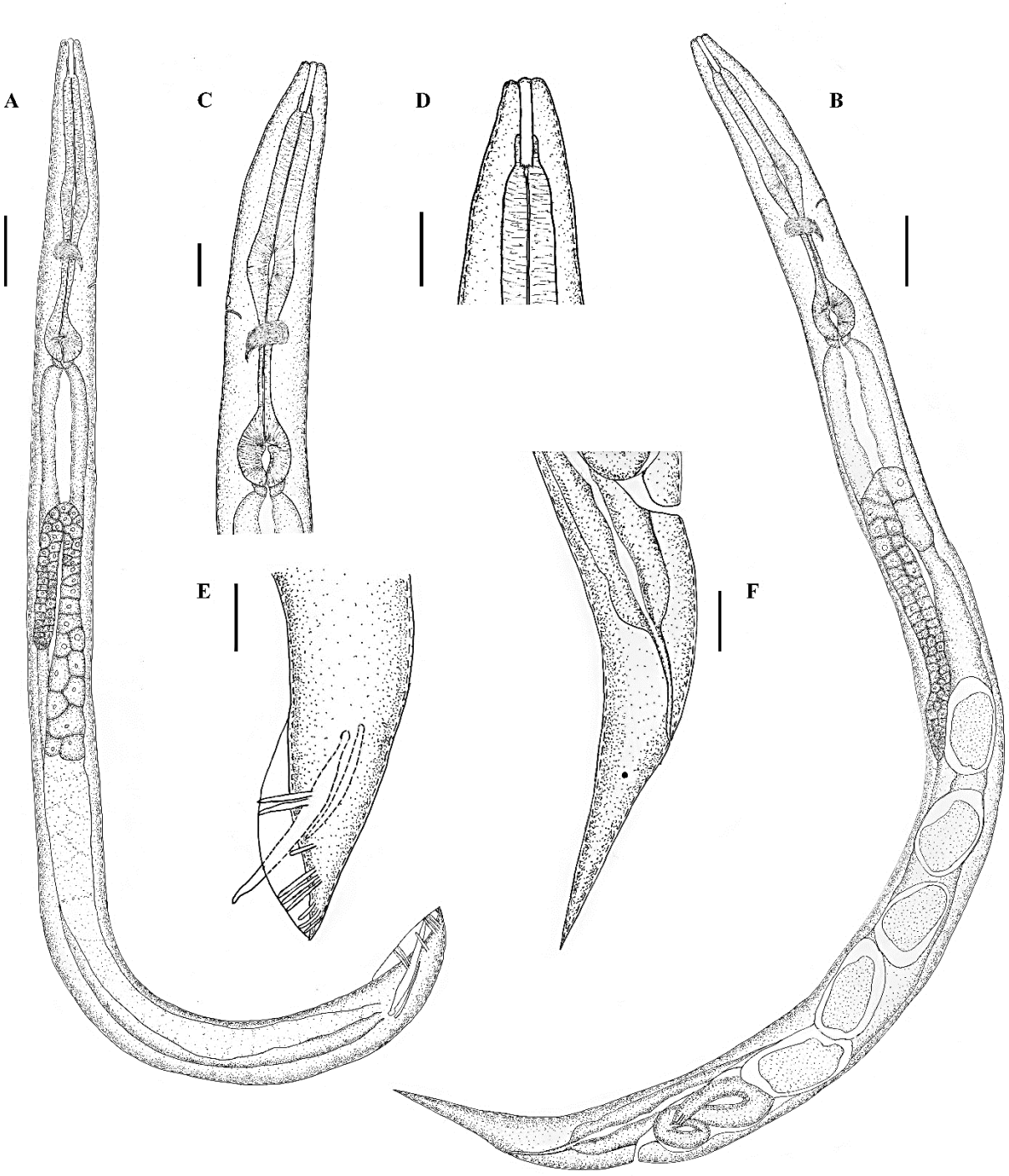

Fig. 2. Line drawings of Iranian population of *D. veechi*. A: Male entire body; B: Female entire body; C: Female anterior region; D: Female head; E: Male tail and spicules; F: Female tail (Scale bars: A, B = 40 µm, C-F = 20 µm).

Male

Males generally similar to females. Testis monorchic, reflexed dorsally. Spicules long, expanded, and linear, measuring 61 to 62 µm in length, distally fused for about half their length. Gubernaculum linear, measuring 35 to 38 µm in length. Bursa peloderan, with 9 pairs of genital papillae. Two pairs of papillae precloacal, seven pairs postcloacal. Pairs 3 and 7 shorter, located on the dorsal side. Phasmid located in the tail region, between pairs 6 and 7. Tail conoid with a pointed tip.

Remark

The population studied exhibits a remarkable similarity in most morphological and morphometric details to the type population described by Anderson (1983). Although minor morphological differences were noted between this population and the original description, these variations were considered insignificant due to their continuum. Specifically, the body length in this population is slightly shorter, ranging from 995 to 1159 µm compared to 1157 to 1471 µm in the original description. Similarly, the tail length is slightly shorter, measuring between 76 to 77 µm versus 76 to 113 µm. Additionally, the rectum length in this population is shorter, spanning 37 to 39 µm compared to 39 to 50 µm.

These minor deviations are attributed to natural population variations and do not significantly alter the overall morphological integrity of the species. The lip region in the studied population may appear continuous or slightly offset, contrasting with the distinctly offset lip region described in the original population. It is important to note that body length and diameter are highly dependent on the physiological state of the individual and its habitat, thereby rendering them unreliable as definitive species characters. Therefore, these observed differences are considered part of the natural morphological variability within the species (Bongers, 1990; Ferris *et al.*, 1993).

Iranian population of *Oscheius tipulae* Lam and Webster (1971) (Figs 6, 7)

Measurements. See Table 2.

Female

Body length ranging from 754 to 929 µm, straight or slightly ventrally curved after fixation. Lip region continuous with the body, comprising six distinct lips with small papillae. Lateral body surfaces featuring four longitudinal ridges. Buccal cavity measuring 14 to 15 µm in length, with the gymnostom region longer than the well-cuticularized cheilostom region. Lumen of the stegostom containing a glottoid apparatus with two to three denticles. Pharynx with a distinct, swollen metacorpus. Isthmus clearly separated from the metacorpus. Pharyngeal bulb oval-shaped. Nerve ring located at the isthmus level, 92 to 104 µm from the anterior end. Excretory pore opening at the level of the isthmus, 107 to 127 µm from the anterior end. Deirid not visible. Reproductive system didelphic-amphidelphic. Vulval opening slightly protruding, located at the mid to posterior region of the body. Oviduct short, tubular uterus with a swollen lumen, about twice the corresponding body diameter in length. Vagina with thin walls, extending inward about one-third of the body width. Tail conical with a pointed tip.

Male

Males morphologically similar to females, with body length ranging from 600 to 682 µm. Reproductive system exhibiting a reflex at the anterior part. Spicules paired and symmetrical, with ventral curvature, measuring 24 to 25 µm in length, lacking hooked tips. Gubernaculum almost straight, slightly curved, thin, and elongated, measuring 9 to 10 µm in length. Bursa peloderan, with 9 pairs of papillae of varying lengths, arranged in a 1+1+1/3+3 pattern, including three precloacal and six postcloacal pairs. First precloacal pair located at the level of the spicule capitulum. Distance between p1 and p2 slightly greater than the distance between p2 and p3. Postcloacal papillae divided into two groups of three, equidistantly spaced. Pairs 5 and 8 bending dorsally, not extending to the edge of the bursa. Phasmids inconspicuous. Tail conical with a slight ventral indentation.

Remark

The nematodes examined in this study exhibit a notable resemblance to the original description of *O. tipulae* provided by Lam and Webster (1971). However, our specimens are characterized by longer body lengths, ranging from 754 to 929 µm, compared to the original 624 to 780 µm. Additionally, our specimens exhibit a slightly higher b index (5.6-6.3 vs. 4.5-5.6) and a higher c index (8.8-13 vs. 6.2-8.5), indicating some morphological variations.

Overall, the morphological characteristics of our nematodes closely align with those of *O. tipulae* as characterized by Lam and Webster (1971). No significant differences were found in other morphological traits when comparing the Iranian specimens to those studied by Lam and Webster (1971). It is important to note that minor variations among isolates are anticipated due to differences in geographical distribution and habitat, which can influence morphological traits. These minor differences in some cases can be attributed to various

environmental and ecological conditions that may impact the physical characteristics of the nematodes, leading to variations in certain morphological indices (Yeates *et al.*, 1993; Ferris and Bongers, 2006). In conclusion, given the overall similarities and the observed minor differences, it can be inferred that the examined specimens largely conform to the original description of *O. tipulae*, with the observed variations falling within the natural range of species variability. These findings suggest that minor morphological differences may arise due to environmental and geographical factors, which should be considered when interpreting morphological data.

Phylogenetic position of the Iranian population of *D. veechi* and *O. tipulae*

The amplification of the D2-D3 expansion segments of the 28S rRNA, ITS, and the partial 18S rRNA from the Iranian population of *D. veechi* produced fragments of 574 bp, 611 bp, and 726 bp, respectively. Molecular phylogenetic trees were constructed using Bayesian analysis under the GTR+I+G model (Tavare´, 1986) to determine the relative position of *D. veechi* among other *Distolabrellus* species and different genera within the Rhabditidae family. The trees, based on partial 18S, D2-D3 segments of 28S, and ITS rRNA, are presented in Figures 3, 4, and 5, respectively. In these figures, the Iranian population of *D. veechi* is highlighted in bold. The BlastN search of partial 18S rRNA gene sequence of the Iranian population of *D. veechi* (GenBank accession number PP780019) revealed the highest match with sequences of *D. veechi* (AF082999 and AF083011) with 99.45% identity. The 28S rRNA D2-D3 sequence of this population (GenBank accession number PP780021) showed the highest match with sequences of *D. veechi* (OK597215 and OM066808) with 99.27% identity. Also, the ITS rRNA sequence of this species (GenBank accession number PP785372) indicated the highest match with sequences of *D. veechi* (OQ067244) with 99.34% identity.

In the molecular phylogenetic tree of the Iranian population of *D. veechi*, generated from the D2–D3 expansion segments of 28S rRNA, which included 24 in-group and two outgroup taxa, the Iranian population of *D. veechi* placed near *D. veechi* (EF990725 and MK942407). The molecular phylogenetic tree of the Iranian population of *D. veechi*, constructed from ITS rRNA, comprised 19 in-group and one outgroup taxa. In this tree, the Iranian population of *D. veechi*, was placed near the sequence of *D. veechi* (MN046389). The molecular phylogenetic tree of the Iranian population of *D. veechi*, constructed from 18S rRNA, comprised 22 in-group and three outgroup taxa. In this tree, the Iranian population of *D. veechi* was placed near the sequences of *D. veechi* (MK418957 and KX023405).

Table 2. Morphometrics of Iranian population of *O. tipulae*. All measurements are in µm and in the form average ± SD (range).

Character	Iranian population		Original description	
	Female	Male	Female	Male
N	10	6	-	-
L	847.8 ± 85.6 (754-929)	647.7 ± 42.6 (600-682)	730 (624-780)	676
a	19.8 ± 1.5 (17.7-21.2)	20.9 ± 1.0 (20-22)	19.8 (17.7-21.9)	17.2
b	6.0 ± 0.3 (5.6-6.3)	5.3 ± 0.3 (5-5.5)	5 (4.5-5.6)	5.4
c	10.8 ± 2.0 (8.8-13)	18.2 ± 4.7 (14.6-23.5)	8.6 (7.5-9.7)	24
c'	5.6 ± 0.3 (5.2-5.9)	2.0 ± 0.5 (1.5-2.5)	4.8 (4.3-5.8)	-
V	47.7 ± 1.5 (45.7-49.3)	-	49 (45.3-52.5)	-
G1	17.9 ± 1.8 (17.0-20.7)	-	37.3 (31.3-44.5)	-
G2	18.8 ± 0.6 (17.9-19.2)	-	34.4 (29.2-39.2)	-
Lip height	2.0 ± 0.0 (2-2)	2.0 ± 0.0 (2-2)	-	
Lip diam.	9.8 ± 0.5 (9-10)	9.0 ± 0.0 (9-9)	-	
Stoma length	14.5 ± 0.6 (14-15)	14.3 ± 0.6 (14-15)	-	
Neck length	140.3 ± 12.4 (126-153)	122.0 ± 2.0 (120-124)	140 (129-151)	126
Nerve ring	98.7 ± 6.1 (92-104)	81.0 ± 4.2 (78-84)	-	
Excretory pore	118.0 ± 9.2 (107-127)	102.7 ± 5.9 (96-107)	100 (81-109)	
Max. body diam.	43.0 ± 4.3 (37-47)	31.0 ± 1.0 (30-32)	33 (29-42)	39
Anal body diam.	14.5 ± 3.9 (10-19)	18.7 ± 2.3 (16-20)	-	
Rectum length	77.5 ± 9.9 (64-88)	-	-	
Tail length	81.0 ± 19.8 (58-106)	36.7 ± 6.7 (29-41)	105 (84-120)	28
Spicule length	-	24.7 ± 0.6 (24-25)	-	25
Gubernaculum length	-	9.3 ± 0.6 (9-10)	-	-

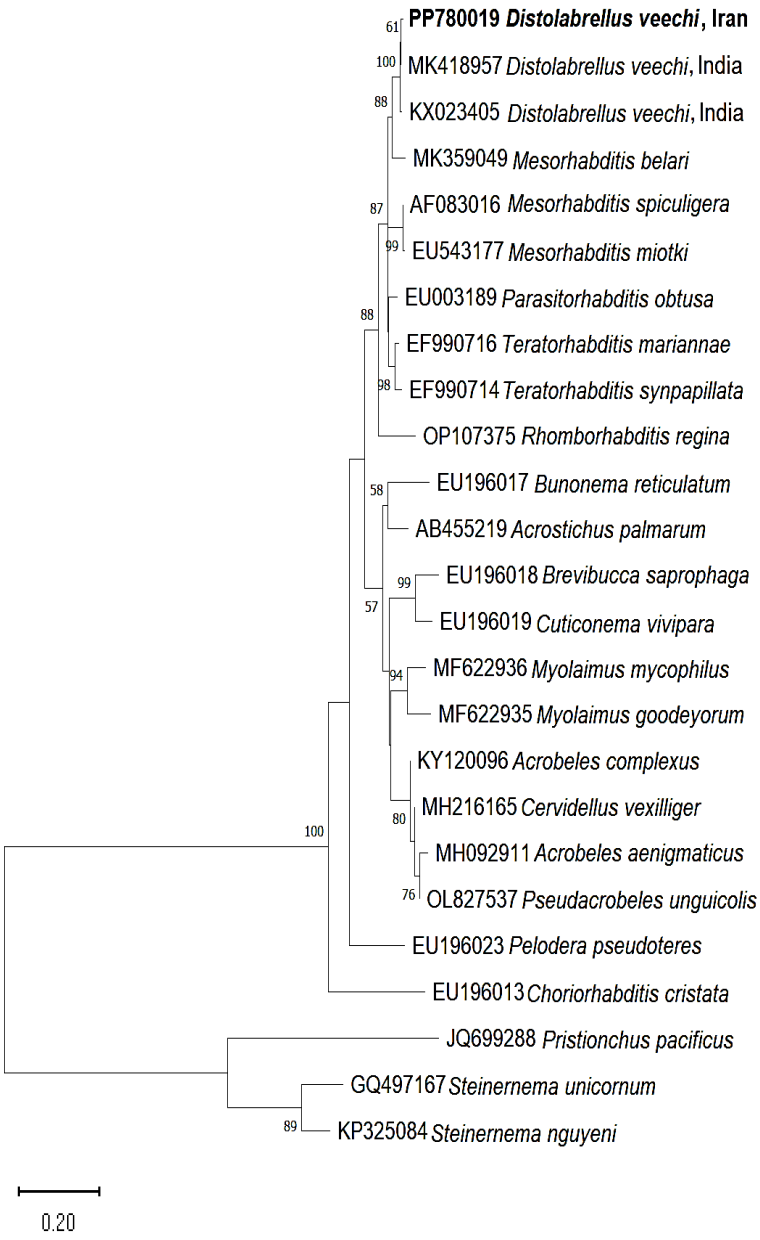

Fig. 3. The molecular phylogenetic tree of Iranian population of *D. veechi* generated from the partial 18S rRNA inferred from Bayesian analyses under GTR+G+I model. Posterior probability values exceeding 50% are given on appropriate clades. The studied species in this research are in bold font.

Amplification of ITS rRNA of the Iranian population of *O. tipulae* yielded a fragment of 771 bp. The molecular phylogenetic tree was generated using Bayesian analysis with the GTR+I+G model (Tavaré, 1986) to determine the relative position of this species among various *Oscheius* species and other genera within the Rhabditidae family. The tree inferred by ITS rRNA is shown in Figure 5. The BlastN search of ITS rRNA sequence of the Iranian population of *O. tipulae* (GenBank accession number PP785371) indicated the closest similarity to sequences of *O. tipulae* (KJ938579) with 99.74% identity. The molecular phylogenetic tree of the Iranian population of *O. tipulae*, constructed from ITS rRNA, comprised 19 in-group and one outgroup taxa. In this tree, the Iranian population of *O. tipulae* was placed near the sequences of *O. tipulae* (KT728760 and KJ938579).

Discussion

Three isolates of rhabditid nematodes belonging to the genus *Distolabrellus* were collected from the forests of eastern Guilan province in 2021. These isolates were identified as *D. veechi*. Despite the different geographical origins of the three collected populations, analysis of the D2-D3 expansion segment of the 28S, ITS, and 18S genes of rRNA, along with morphological and morphometric data, revealed a high degree of similarity among them. Consequently, one population was selected to provide representative data. Table 1 presents the morphological traits of both females and males. The nematode isolate was confirmed as *D. veechi*, with its morphological characteristics closely matching the original description by Anderson, 1983.

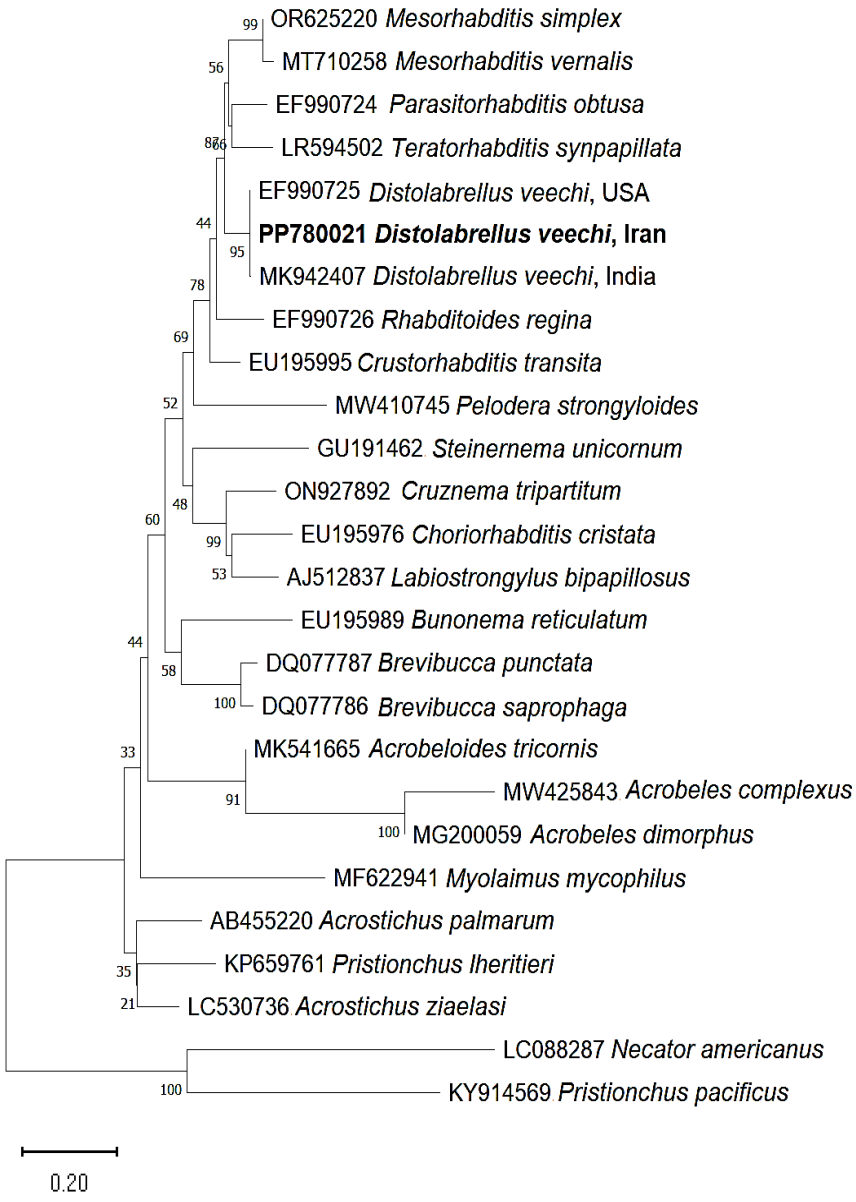

Fig. 4. The molecular phylogenetic tree of Iranian population of *D. veechi* generated from the D2–D3 of 28S rRNA inferred from Bayesian analyses under GTR+G+I model. Posterior probability values exceeding 50% are given on appropriate clades. The studied species in this research are in bold font.

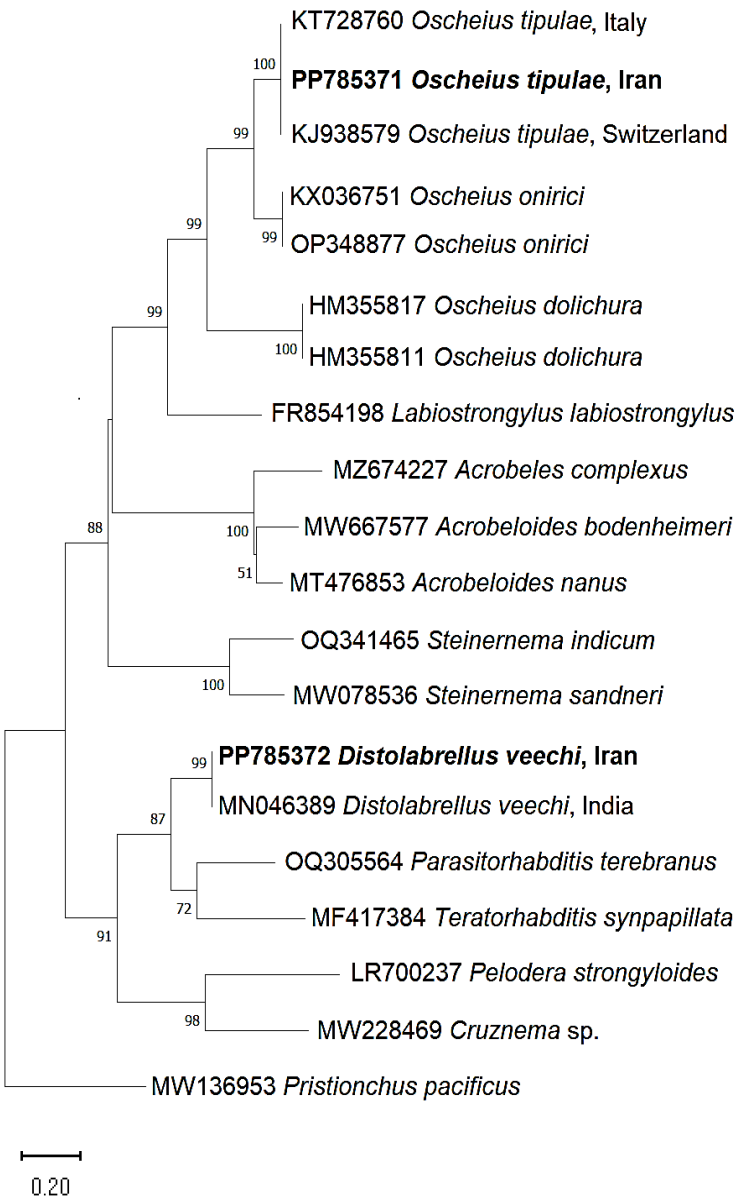

Fig. 5. The molecular phylogenetic tree of Iranian population of *D. veechi* and *O. tipulae* generated from the ITS rRNA inferred from Bayesian analyses under GTR+G+I model. Posterior probability values exceeding 50% are given on appropriate clades. The studied species in this research are in bold font.

Two isolates of rhabditid nematodes belonging to the genus *Oscheius* were collected from the forests of eastern Guilan province in 2021. To identify these isolates as *O. tipulae*, a comprehensive approach was employed. Despite the different geographical origins of the collected populations, analysis of the ITS rRNA, along with morphological and morphometric data, revealed a high degree of similarity among them. As a result, one of the populations was chosen to provide representative data. Table 2 outlines the morphological traits of both females and males. The nematode isolate was confirmed as *O. tipulae*, with its morphological features closely matching the original description by Lam and Webster (1971). This species first reported by Karimi et al (2018) from the country.

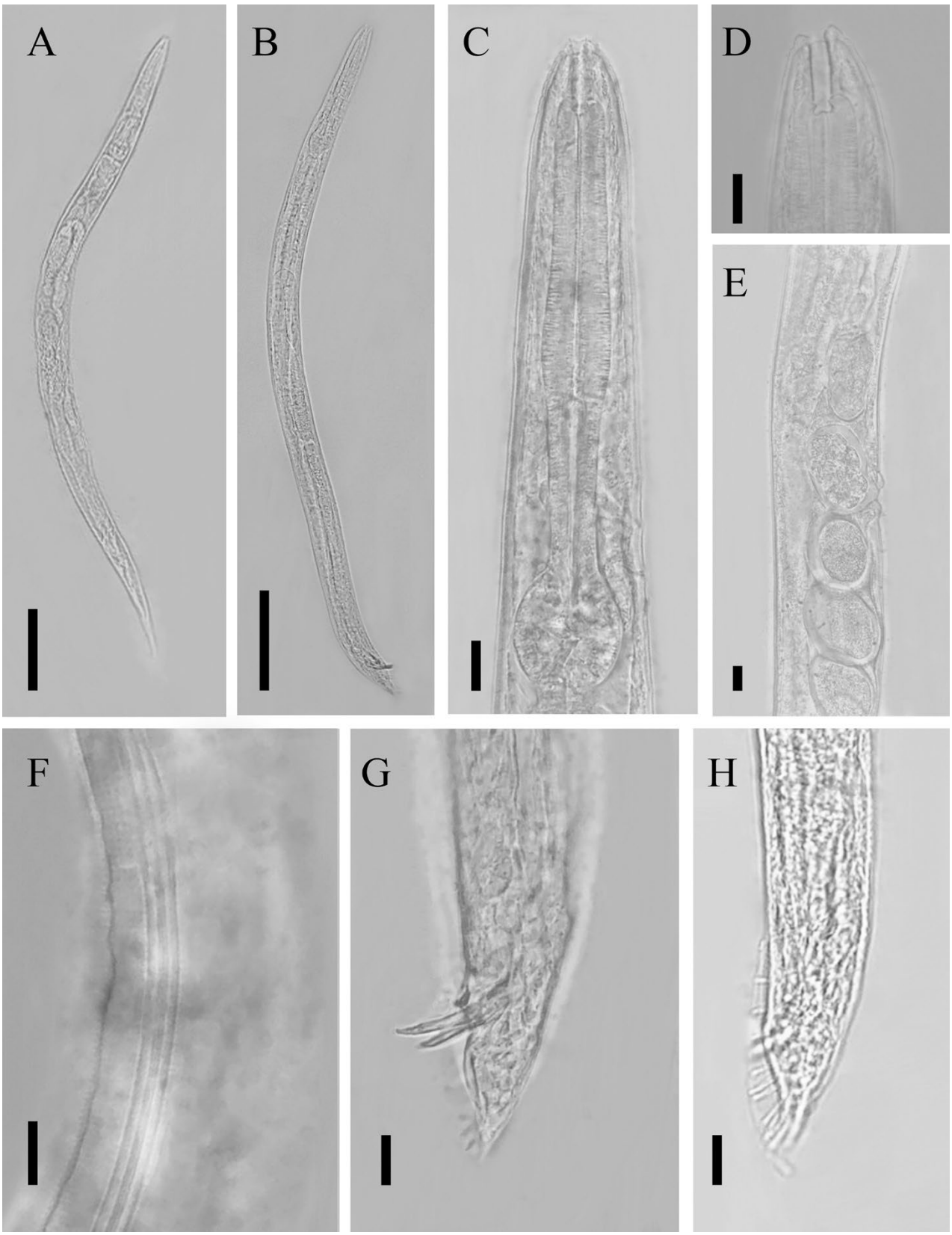

Fig. 6. Photomicrographs of the Iranian population of *O. tipulae.* A: Female entire body; B: Male entire body; C: Female anterior body; D: Female head; E: Vulva region, F: Lateral lines, G: Male Posterior body (tail and spicules); H: Bursa. (Scale bars: A, B = 40 µm, C-H = 10 µm).

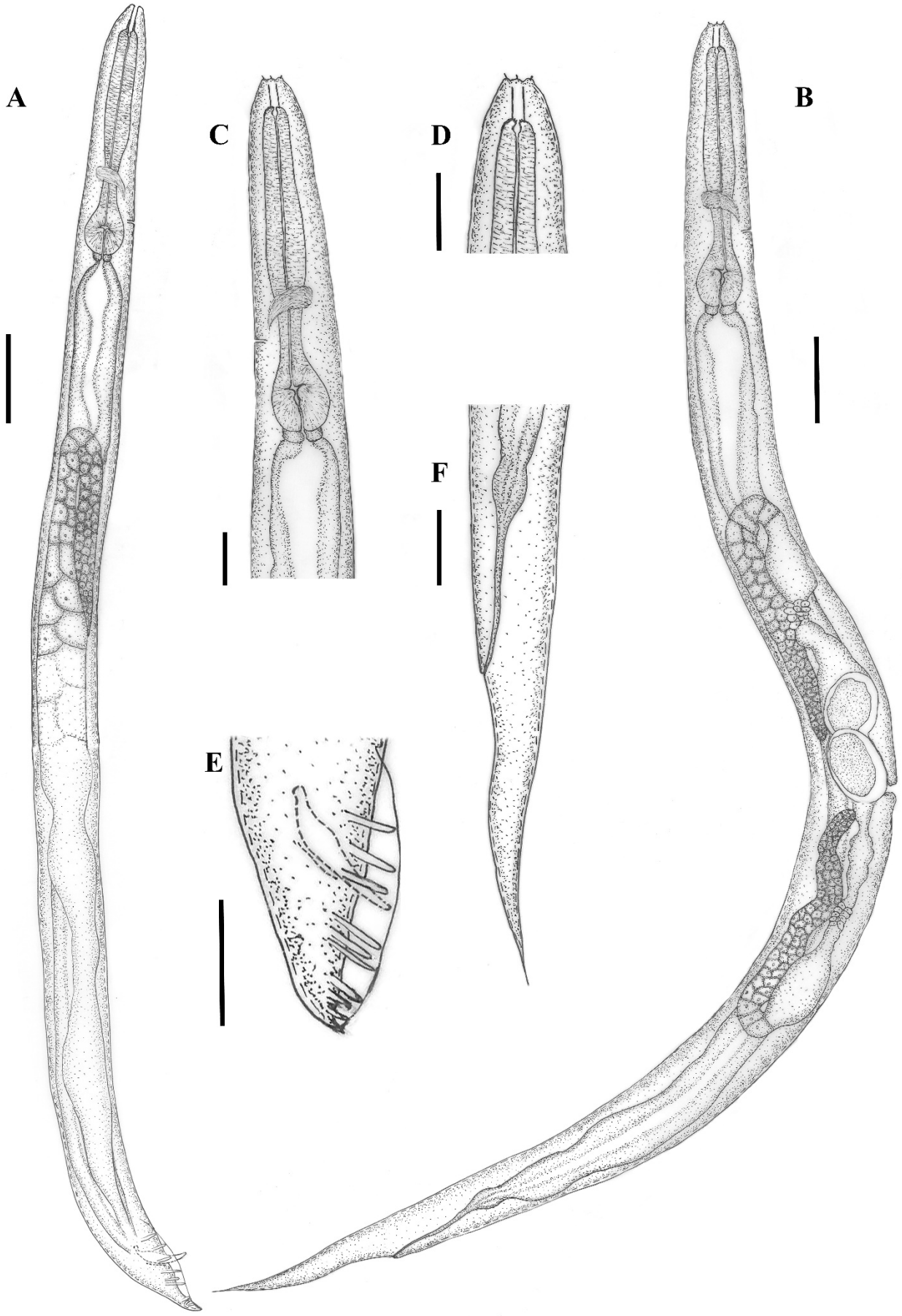

Fig. 7. Line drawings of the Iranian population of *O. tipulae.* A: Male entire body; B: Female entire body; C: Female anterior body; D: Female head; E: Male posterior body (tail and spicules); F: Female posterior body (tail). (Scale bars: A, B = 40 µm, C-F = 20 µm).

Author's Contributions

Parisa Jalalinasab: conceptualization, methodology, formal analysis, investigation, draft preparation, final review and edit; **Ramin Heydari**: Visualization, conceptualization, supervision, review and edit; **Reza Talaei-Hassanloui**: Visualization, conceptualization, final review and edit; **Ebrahim Shokoohi**: Visualization, review and edit; **Javad Karimi**: Visualization, conceptualization, supervision, project administration, formal analysis, final review and edit.

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Data Availability Statement

The specimens examined in this study are deposited in the nematode collection of the Department of Plant Protection, College of Agricultural and Natural Resources, University of Tehran, Karaj, Iran, and are available from the curator upon request.

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Ethics Approval

Insects and nematodes were used in this study. All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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اطلاعاتی نوین درباره نماتودهای حشرات در ایران: گزارش جنس *Distolabrellus veechi* (Nematoda: Mesorhabditidae) و جنس نر گونه *Oscheius tipulae* (Nematoda: Rhabditidae)

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چکیده: به منظور شناسایی نماتودهای مرتبط با حشرات در مناطق جنگلی استان گیلان، ایران، نمونه های خاک، چوب و حشره جمع آوری شد. نماتودها با استفاده از روش طعمه گذاری حشرات استخراج شده و نمونه های به دست آمده بر اساس ویژگی های مورفولوژیکی و مورفومتری تحلیل شدند. این بررسی به شناسایی جمعیت هایی متعلق به جنس‌های *Distolabrellus* و *Oscheius* منجر شد که به ترتیب به عنوان *D. veechi* و *O. tipulae* شناسایی شدند. شناسایی مولکولی از طریق تحلیل مولکولی شامل ناحیه گسترش یافته D2-D3 ژن 28S، ITS و 18S ژن‌های rRNA انجام شد. تحلیل‌های فیلوژنتیکی این نماتودها را با پشتیبانی بالا در کلادهای مربوطه شان قرار داده و هویت آنها را تأیید کرد. این بررسی برای اولین بار منجر به شناسایی *D. veechi* و نر *O. tipulae* در ایران شد و آنها را به عنوان رکوردهای جدید برای کشور معرفی کرد.

کلمات کلیدی: نماتودشناسی حشرات، بیماری‌شناسی حشرات، کنترل بیولوژیک، شناسایی مولکولی، فیلوژنی

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