DOI: 10.22034/ijon.2023.710647 Research article

First record of *Metarhabditis blumi* (Rhabditida: Rhabditidae) and the male of *Diploscapter coronatus* (Rhabditida: Diploscapteridae) from Iran

Parisa Jalalinasab[1], Ramin Heydari[1], Reza Talaei-Hassanloui[1] and Javad Karimi[2]✉

1 Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

2 Department of Plant Protection, Ferdowsi University of Mashhad, Mashhad, Iran

✉Corresponding author E–mail: jkb@ferdowsi.um.ac.ir

Received: 2025/01/26 Revised: 2025/08/30 Accepted: 2025/09/02

Abstract

During a 2021 survey on insect-associated nematodes in forests of Guilan province, northern Iran, samples of soil, decaying wood, and insects were collected and examined. Analyses of morphological and morphometric characteristics of the isolated nematodes revealed that the populations belong to the genera *Metarhabditis* and *Diploscapter*. They were identified as *Metarhabditis blumi* and *Diploscapter coronatus*, respectively. To support this identification, DNA sequences were used for Nblast analysis and the phylogenetic trees reconstructed based on the sequences of the internal transcribed spacer (ITS) region of rRNA gene (for *M. blumi*) and the 18S rRNA gene (for *D. coronatus*.). Since the sequences of type populations were not available in public databases, comparisons were made with other identified and reliable populations of the same species, which confirmed the morphological identifications. This study represents the first record of *M. blumi* and the first report of the male of *D. coronatus* from Iran.

Keywords: Insect-associated nematodes, Molecular phylogenetics, Morphological identification, Nematode taxonomy, New record

How to cite: Jalalinasab, P., Heydari, R., Talaei-Hassanloui, R. & Karimi, J. 2022. First record of *Metarhabditis blumi* (Rhabditida: Rhabditidae) and the male of *Diploscapter coronatus* (Rhabditida: Diploscapteridae) from Iran. Iranian Journal of Nematology 1(2), 259-271.

اولین گزارش از *Metarhabditis blumi* (Rhabditida, Rhabditidae) و جنس نر *Diploscapter coronatus* (Rhabditida: Diploscapteridae) از ایران

پریسا جلالی نسب[۱]، رامین حیدری[۱]، رضا طلایی حسنلویی[۱]، جواد کریمی[۲]✉

۱- گروه گیاه‌پزشکی، دانشکده کشاورزی و منابع طبیعی، دانشگاه تهران، کرج، ایران

۲- دانشکده گیاه‌پزشکی، دانشگاه فردوسی مشهد، مشهد، ایران

✉پست الکترونیکی مسئول مکاتبات: jkb@ferdowsi.um.ac.ir

دریافت: ۱۴۰۳/۱۱/۰۷ بازنگری: ۱۴۰۴/۰۶/۰۸ پذیرش: ۱۴۰۴/۰۶/۱۱

چکیده

طی مطالعه‌ای در سال ۱۴۰۰ بر روی نماتدهای مرتبط با حشرات در جنگل‌های استان گیلان، شمال ایران، نمونه‌هایی از خاک، چوب پوسیده و حشرات جمع‌آوری و بررسی شدند. در این بررسی جمعیت‌هایی از جنس‌های *Metarhabditis* و *Diploscapter* جمع‌آوری و بر اساس ویژگی‌های ریخت‌شناختی و ریخت‌سنجی، به‌ترتیب به عنوان *Metarhabditis blumi* و *Diploscapter coronatus* شناسایی گردیدند. برای تأیید شناسایی، ناحیه ITS rRNA برای *M. blumi* و ژن 18S rRNA برای *D. coronatus* توالی‌یابی شدند. به دلیل در دسترس نبودن توالی‌های جمعیت‌های تیپ در پایگاه‌های داده عمومی، مقایسه با سایر جمعیت‌های شناسایی‌شده و معتبر همان گونه‌ها انجام شد و شناسایی ریخت‌شناختی را تأیید کرد. این بررسی نخستین گزارش از وجود *M. blumi* و نماتدهای نر *D. coronatus* در ایران را ارائه می‌کند.

واژه‌های کلیدی: آرایه‌بندی نماتدها، شجره‌شناسی مولکولی، شناسایی ریخت‌شناختی، گزارش جدید، نماتدهای مرتبط با حشرات

Introduction

The genus *Metarhabditis* Tahseen, Hussain, Tomar, Shah, & Jairajpuri, 2004, within the family Rhabditidae Örley, 1880, was established with *Metarhabditis andrassyana* Tahseen, Hussain, Tomar, Shah, & Jairajpuri, 2004, as the type species. This genus is characterized by six globular lips forming three doublets, metastegostom with a cluster of knobbed, setose denticles born on each plate, cylindrical pharyngeal corpus, a didelphic-amphidelphic reproductive system, and a short rectum (equal to or slightly longer than the anal body diameter). Additionally, the lips are fused in pairs. In males, the bursa is either pseudo-leptoderan or peloderan, bearing eight pairs of genital papillae, two of which are located anterior to the cloacal aperture. The last pair is significantly reduced or even absent. The genital papillae follow a 1+1+1/3+2+ph arrangement, and the spicules are free and not fused distally (Tahseen *et al*. 2004).

The genus was revised by Sudhaus (2011), who transferred five species from *Rhabditis* Dujardin, 1845, including *R. adenobia* Poinar, 1971, *R. blumi* Sudhaus, 1974, *R. costai* Martins, 1985, *R. freitasi* Martins, 1985, and *R. rainai* Carta & Osbrink, 2005, as well as *Oscheius amsactae* Ali, Pervez, Andrabi, Sharma & Verma, 2011, from *Oscheius* Andrássy, 1976 into *Metarhabditis*. Subsequently, *M. giennensis* Abolafia & Peña-Santiago, 2019 was described from Spain, along with a key for species identification (Abolafia & Peña-Santiago 2019). *Metarhabditis longicaudata* Khanum, Javed & Mehmood, 2019, was later described from Pakistan. To date, the genus *Metarhabditis* includes nine valid species.

The genus *Diploscapter* Cobb, 1913, in the family Diploscapteridae Micoletzky, 1922 is characterized by a bilaterally symmetrical cephalic region bearing six lips, each with a labial sensillum. These small nematodes possess a cuticle with fine annulations, and in some species with delicate longitudinal incisures. The lip region is set off from the neck. The subdorsal and subventral lips are hook-shaped and sclerotized, while the lateral lips are membranous and modified. Stoma rhabditoid and elongated, with a non-cuticularized cheilostom, parallel-walled gymnostom, and a metastegostom without denticles. The pharynx includes a swollen metacorpus. The female reproductive system is didelphic-amphidelphic, with the vulva located at or slightly behind the mid-body. Males possess free and well sclerotized spicules, and a peloderan or leptoderan bursa, open and moderately developed, with six to nine pairs of genital papillae (Shokoohi & Abolafia 2019).

During a nematode survey conducted in the eastern forests of Guilan province, northern Iran, two insect-associated nematode species were recovered from soil samples and identified as *Metarhabditis blumi* (Sudhaus 1974) Sudhaus, 2011 and *Diploscapter coronatus* (Cobb 1893) Cobb, 1913. Detailed morphological observations using light microscopy, combined with molecular analyses of the ITS region (*M. blumi*) or partial 18S rRNA gene (*D. coronatus*), confirmed the identifications. This study represents the first report of the genus *Metarhabditis* in Iran, and the first record of the male *D. coronatus* in Iran, although the female of *D. coronatus* was previously recorded from Iran by Eyualem *et al.* (1998). Here, we provide a detailed morphological redescription of the Iranian populations of both species, supported by molecular characterization, as well.

Materials and methods

Nematode populations

During 2021, several samples of soil, decaying wood, and insects (particularly beetles from the superfamily Scarabaeoidea) were collected across various forest regions in Guilan province, northern Iran. Nematodes were extracted from soil using the insect-baiting technique developed by Bedding and Akhurst (1975), employing final instar larvae of the greater wax moth, *Galleria mellonella* L. Soil samples were placed in 300 mL plastic containers with lids and inoculated with 10 larvae per container. The containers were maintained under laboratory conditions for 5-7 days. Infected larvae were identified based on color and morphological changes, then transferred individually to White traps (White 1927).

To extract the nematodes directly from insects, specimens were first washed to remove superficially attached nematodes and then dissected. Cadavers were placed on agar Petri dishes for 7–10 days to encourage nematode emergence. Nematodes from both soil and insect samples were observed under a stereomicroscope

and manually picked. Adult nematodes intended for morphological analysis were fixed using boiling FGA solution (formaldehyde, glycerin, and acetic acid in a 4:1:1 ratio), followed by processing into anhydrous glycerin (De Grisse 1969). Permanent slides were prepared and examined using an Olympus BH2 light microscope. Morphometric data were obtained using a drawing tube attached to the microscope. Photomicrographs were taken with a digital camera mounted on the same microscope, and line drawings prepared with the aid of the drawing tube.

DNA Extraction, PCR and Sequencing

Single nematode specimens were handpicked under a stereomicroscope, examined under light microscopy, and transferred to 10 µL distilled water on a glass slide. Each specimen was crushed with a pipette tip, and the lysate was collected into 50 µL TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, Qiagen, Valencia, CA, USA). DNA extracts were stored at -20°C until use.

For PCR amplification, 1 µL of DNA extract was added to a reaction mixture containing 2.5 µL 10X NH_4 buffer, 0.75 µL $MgCl_2$ (50 mM), 0.25 µL dNTPs mixture (10 mM each), 0.75 µL of each primer (10 mM), 0.2 µL BIOTAQ DNA Polymerase (BIOLINE, UK), and double-distilled water to a final volume of 25 µL.

The ITS rRNA region was amplified using the forward primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') pairs (Subbotin *et al*. 2006), along with the reverse primer 5.8SM5 (5'-GGCGCAATGTGCATTCGA-3') (Vovlas *et al*. 2008). The partial 18S rRNA gene was amplified using primers 1096F (5′-GGTAATTCTGGAGCTAATAC-3′), 1912R (5'-TTTACGGTCAGAACTAGGG-3') (Holterman *et al*. 2006).

PCR cycling conditions included an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 3 min, with a final extension at 72°C for 10 min. PCR products were sequenced in both directions using the same primers as used for amplification.

Phylogenetic analyses

Newly generated ITS and partial 18S rRNA sequences, along with related sequences of rhabditid nematodes retrieved from GenBank, were used for phylogenetic reconstructions. Sequence alignments were performed using MUSCLE (Edgar 2004) implemented in MEGA 5.0 (Tamura *et al*. 2011), using default parameters. Sequence alignments were manually checked and refined in MEGA 5.0.

The best-fitting nucleotide substitution model for each dataset was selected using the Bayesian Information Criterion (BIC) implemented in jModelTest (Posada 2008). Phylogenetic analyses were conducted using Bayesian inference (BI) in MrBayes.1.2 (Ronquist & Huelsenbeck 2003). A 50% majority-rule consensus tree was constructed from the retained topologies, and posterior probabilities (PP) values were shown at major clades. Resulting trees were visualized using TreeView (Page 1996).

Results

***Metarhabditis blumi* (Sudhaus, 1974) Sudhaus, 2011**
(Figs 1 and 2)

Measurements

See Table 1.

Description

Female: Large nematodes, body straight or slightly ventrally curved after fixation. Cephalic region continuous with the body contour, six lips distinct, amphidial openings not observed. Stoma contains setose denticles at the base, resembling bristle-like teeth, and nodes on metastegostom, indicating a complex structure. Pharynx composed of a cylindrical corpus, narrow isthmus, and spherical to oval basal bulb; cardia small, surrounded by intestinal tissue. Intestine simple, without distinct specializations. Rectum short, anus functional and distinct. Nerve ring encircles the isthmus. Excretory pore located at level of isthmus. Reproductive system didelphic-amphidelphic, both ovaries reflexed at distal ends. Uteri usually filled with developing eggs. Vulval lips non-protruding. Tail conical, gradually tapering to a pointed tip.

Figure 1. Photomicrographs of the Iranian population of *Metarhabditis blumi*. A: Entire male; B: Entire female; C & D: Anterior end of female; E: Pharyngeal region of female showing corpus, isthmus, and terminal bulb; F: Female conical tail with pointed tip; G & H: Male tail showing bursa, spicules, and genital papillae. (Scale bars: A & B = 100 µm, C-H = 20 µm).

Male: Similar to females in general morphology, but smaller in size. Spicules curved at the distal end, well sclerotized; gubernaculum linear, simple. Bursa leptoderan, bearing eight pairs of genital papillae arranged in a 1+1+1/3+2+ph pattern: two pairs located anterior to the cloacal opening, one pair at the cloacal level, and five pairs posterior to it. The last pair often minute or occasionally absent. Tail conical, tapering to a pointed terminus, generally shorter than in females.

Remark: The present Iranian population of *M. blumi* closely resembles the original description in both morphological and morphometric characteristics (Sudhaus 1974). Minor variations, such as a slightly shorter female tail (140-162 *vs.* 156-221 µm) and slightly longer gubernaculum (21-24 *vs.* 16-22 µm) in males, were observed but are considered intraspecific variability and not taxonomically significant.

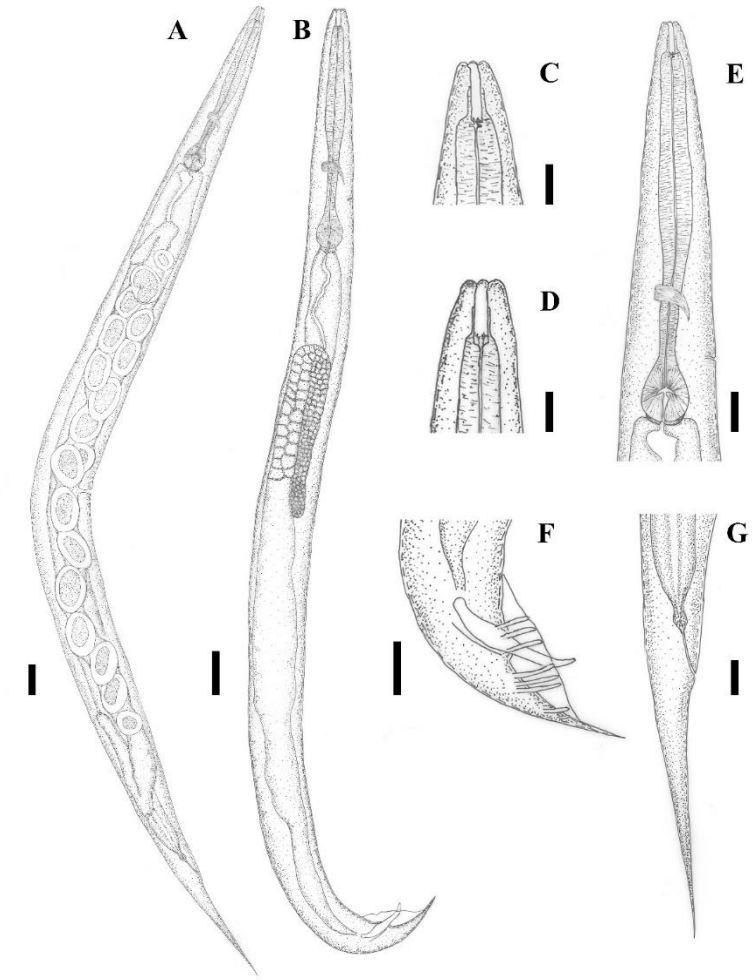

Figure 2. Line drawings of Iranian population of *Metarhabditis blumi*. A: Entire female; B: Entire male; C & D: Anterior region of female showing lip structure and stoma; E: Female anterior region including pharynx; F: Male tail with spicules and genital papillae; G: Female tail, conical with pointed tip. (Scale bars: A & B = 40 µm, C-G = 20 µm).

***Diploscapter coronatus* (Cobb, 1893) Cobb, 1913**
(Figs 4 and 5)

Measurements
See Table 2.

Description

Female: Body straight or slightly ventrally curved after fixation. Cuticle finely annulated with transverse striations. Cephalic region with well-sclerotized, hook-shaped subdorsal and subventral lips; lateral lips membranous and fan-shaped. Stoma rhabditoid; cheilostom weakly cuticularized, without glottoid apparatus. Pharynx composed of corpus, distinct isthmus, and basal bulb. The corpus is subdivided into longer procorpus and a shorter metacorpus. Terminal bulb spherical to oval. Cardia conical, surrounded by intestinal tissue. Nerve ring encircling isthmus. Excretory pore located at the level of the anterior part of the pharyngeal bulb. Deirids present at the level of the isthmus. Reproductive system didelphic-amphidelphic with two reflexed ovaries. Vulva at mid-body to slightly posterior, vulval lips not elevated. Rectum short, slightly exceeding the anal body diameter. Tail conical, uniformly narrowing toward a finely rounded or sharp tip. Phasmids located at the anterior one-fifth of tail length.

Table 1. Morphometrics of the Iranian population of *Metarhabditis blumi* (Sudhaus, 1974) Sudhaus, 2011 and the type population. All measurements are in μm and presented as mean ± SD (range).

	Present study		Type population (Sudhaus, 1974) Sudhaus, 2011	
Character	Females	Males	Females	Males
n	10	5	-	-
L	1309 ± 127 (1180-1500)	1051 ± 18.4 (1026-1070)	1324-1819	995–1415
a	21.0 ± 3.2 (15.6-25.2)	21.5 ± 4.2 (18.5-24.4)	18.0-23.3	17.3–22.3
b	5.4 ± 0.7 (4.7-6.5)	4.4 ± 0.1 (4.3-4.5)	4.8-6.1	4.2–5.9
c	8.5 ± 0.9 (7.6-10.1)	19.0 ± 3.0 (15.3-21.9)	7.0-9.7	17–27
V	49.9 ± 1.7 (46.8-51.5)	-	48-52	-
G1	25.1 ± 3.1 (23.0-27.3)	-	-	-
G2	26.5 ± 8.0 (20.8-32.1)	-	-	-
Stoma length	29.5 ± 0.7 (29.0-30.0)	26.0 ± 1.4 (25.0-27.0)	29-35	25–27
Pharynx length	242 ± 14.0 (220-261)	238 ± 2.1 (236-240)	244-298	226–276
Nerve ring from anterior	171 ± 5.7 (167-175)	169 ± 19.8 (155-183)	-	-
Excretory pore from anterior	201 ± 7.1 (196-206)	187 ± 2.1 (185-188)	208-227	-
Max. body diameter	63.8 ± 11.9 (50.0-80.0)	49.5 ± 10.6 (42.0-57.0)	59-85	49–68
Anal body diameter	25.0 ± 4.2 (22.0-28.0)	17.8 ± 2.5 (16.0-22.0)	-	-
Testis length	-	606 ± 32.7 (576-640)	-	-
Tail length	151 ± 9.5 (140-162)	57.4 ± 8.4 (48.0-69.0)	156-221	50–66
Spicules length	-	50.0 ± 3.9 (44.0-54.0)	-	45–51
Gubernaculum length	-	22.2 ± 1.1 (21.0-24.0)	-	16–22

Male: Similar to female in general morphology, except for the reproductive system. Testis reflexed ventrally at the tip. Spicules separate, well cuticularized, distally curved. The gubernaculum is simple and nearly straight with a slight proximal curve. Bursa peloderan, open, bearing seven pairs of genital papillae; two precloacal pairs, and five postcloacal pairs, arranged in a 1+1/1+2+1+1 pattern. Tail short and conical.

Remark: The Iranian population of *D. coronatus* closely matches the morphological and morphometric features of both the type population (Cobb, 1893; 1913) and the previously reported Iranian population described by Eyualem *et al.* (1998) as well. No significant differences were observed, and the population is considered conspecific with both the type material and the previous Iranian specimens. Compared to the previously reported Iranian population, minor variations such as a slightly shorter stoma length (17-19 *vs.* 19-23 μm) and a more slender body in females (*a* = 12.6-14.7 *vs.* 15.5-17), were observed, but these are regarded as intraspecific variability and not taxonomically significant.

Phylogenetic position of Iranian populations of *M. blumi* and *D. coronatus*

Sequencing of the internal transcribed spacer (ITS) region from the Iranian population of *M. blumi* yielded a 684 bp fragment (GenBank accession number: PP780170). A BLASTN search revealed 91.5% identity with *M. blumi* (DQ121436), 88.8% identity with *M. amsactae* (MH392568), and 86.1% identity with another sequence of *M. amsactae* (MT873044).

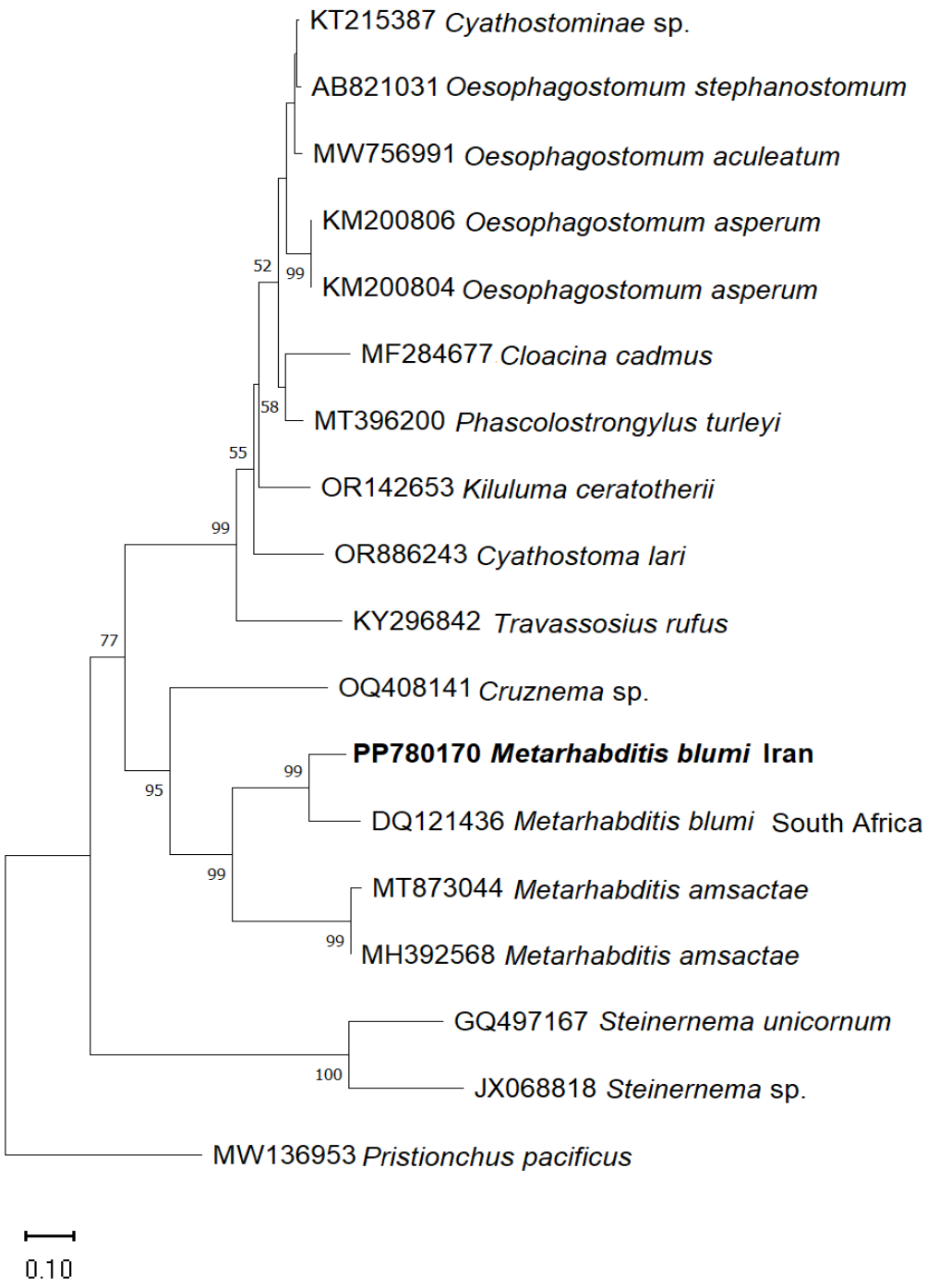

Figure 3. Bayesian phylogenetic tree of the Iranian population of *Metarhabditis blumi* inferred from ITS rRNA sequences using the GTR+G+I model. Posterior probability values >50% are shown on relevant clades. The Iranian population from this study is shown in bold.

Table 2. Morphometrics of the Iranian population of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913. All measurements are in µm and presented as mean ± SD (range).

	Present study		**Iranian population Eyualem *et al*. (1998)**	**Ethiopian population Eyualem *et al*. (1998)**
Character	Females	Males	Females	Females
n	10	6	14	20
L	319 ± 17.2 (300-338)	292 ± 26.7 (256-322)	350.9 ± 23.4 (317-403)	427.5 ± 25 (395-480)
a	13.2 ± 0.8 (12.6-14.7)	13.1 ± 1.6 (11.0-14.6)	16.2 ± 0.4 (15.5-17)	17.7 ± 1.2 (I5.8-19.8)
b	3.8 ± 0.2 (3.4-3.9)	3.6 ± 0.2 (3.3-3.9)	4.0 ± 0.2 (3.7-4.4)	4.0 ± 0.2 (3.6-4.1)
c	6.7 ± 0.6 (5.7-7.3)	14.3 ± 1.4 (12.8-16.1)	7 ± 1.1 (5.8-9.6)	7.8 ± 0.7 (6.3-9.2)
c′	4.3 ± 0.6 (3.5-4.8)	1.1 ± 0.1 (1.0-1.3)	5.3 ± 0.8 (3.5-6.1)	4.9 ± 0.7 (4.1-6.5)
V	52.6 ± 1.9 (49.5-54.4)	-	53.7 ± 2.1 (50.1-58.4)	53.2 ± 1.5 (50-55.7)
G1	15.4 ± 2.2 (12.3-17.8)	-	51.8 ± 7.2 (43-65)	60.7 ± 11.3 (39-94)
G2	13.8 ± 1.4 (12.2-15.4)	-	48.7 ± 9.1 (31-65)	55.9 ± 11.1 (38-72)
Stoma length	18 ± 1.0 (17.0-19.0)	17.0 ± 0.8 (16.0-18.0)	20.9 ± 1.2 (19-23)	21.8 ± 0.9 (20-23)
Pharynx length	85.0 ± 4.1 (80.0-89.0)	80.6 ± 8.2 (66.0-85.0)	67.5 ± 2.7 (64-73)	108.5 ± 5.2 (95-114)
Nerve ring from anterior	60.3 ± 2.1 (58.0-63.0)	56.0 ± 7.5 (43.0-62.0)	66.5 ± 3.1 (60-71)	70 ± 3 (65-77)
Excretory pore from anterior	55.5 ± 5.7 (49.0-63.0)	49.8 ± 4.9 (45.0-58.0)	75.1 ± 3.9 (67-82)	79 ± 4.9 (70-89)
Vulval body diameter	24.2 ± 1.5 (23.0-26.0)	-	-	-
Anal body diameter	11.2 ± 1.0 (10.0-12.0)	18.5 ± 2.1 (15.0-21.0)	9.6 ± 0.8 (9-12)	11.3 ± 1 (10-14)
Rectum length	15.0 ± 1.7 (13.0-17.0)	-	11.5 ± 1.6 (8-14)	19.6 ± 1.2 (17-22)
Testis length	-	139 ± 16.3 (120-160)	-	-
Tail length	48.0 ± 5.2 (42.0-57.0)	21.9 ± 2.2 (20.0-25.0)	51 ± 9 (36-67)	55.5 ± 6.3 (45-71)
Spicule length	-	26.3 ± 1.3 (24.0-28.0)	-	-
Gubernaculum length	-	11.5 ± 1.2 (10.0-13.0)	-	-

Alignment with the closest match (*M. blumi*, DQ121436) revealed 34 nucleotide differences across the ITS region. This level of divergence may reflect intraspecific variability or geographical differentiation. Given that the ITS region is known for its high variability and rapid evolution, such differences are not unexpected. As a results, the identification of the Iranian population as *M. blumi* is considered tentative and is supported by a combination of morphological characteristics and molecular data.

Despite this variation, phylogenetic analysis placed the Iranian population in a strongly supported clade with *M. blumi* (DQ121436), confirming its affiliation within the genus *Metarhabditis* (Figure 3).

A 796 bp sequence of the 18S rRNA gene was obtained from the Iranian population of *D. coronatus* (GenBank accession number: PP780016). A BLASTN

analysis showed 99.4% identity with *D. coronatus* (OZ038422), indicating a close genetic relationship. High similarity was also observed with other *D. coronatus* sequences, including KJ636377 and AY593921.

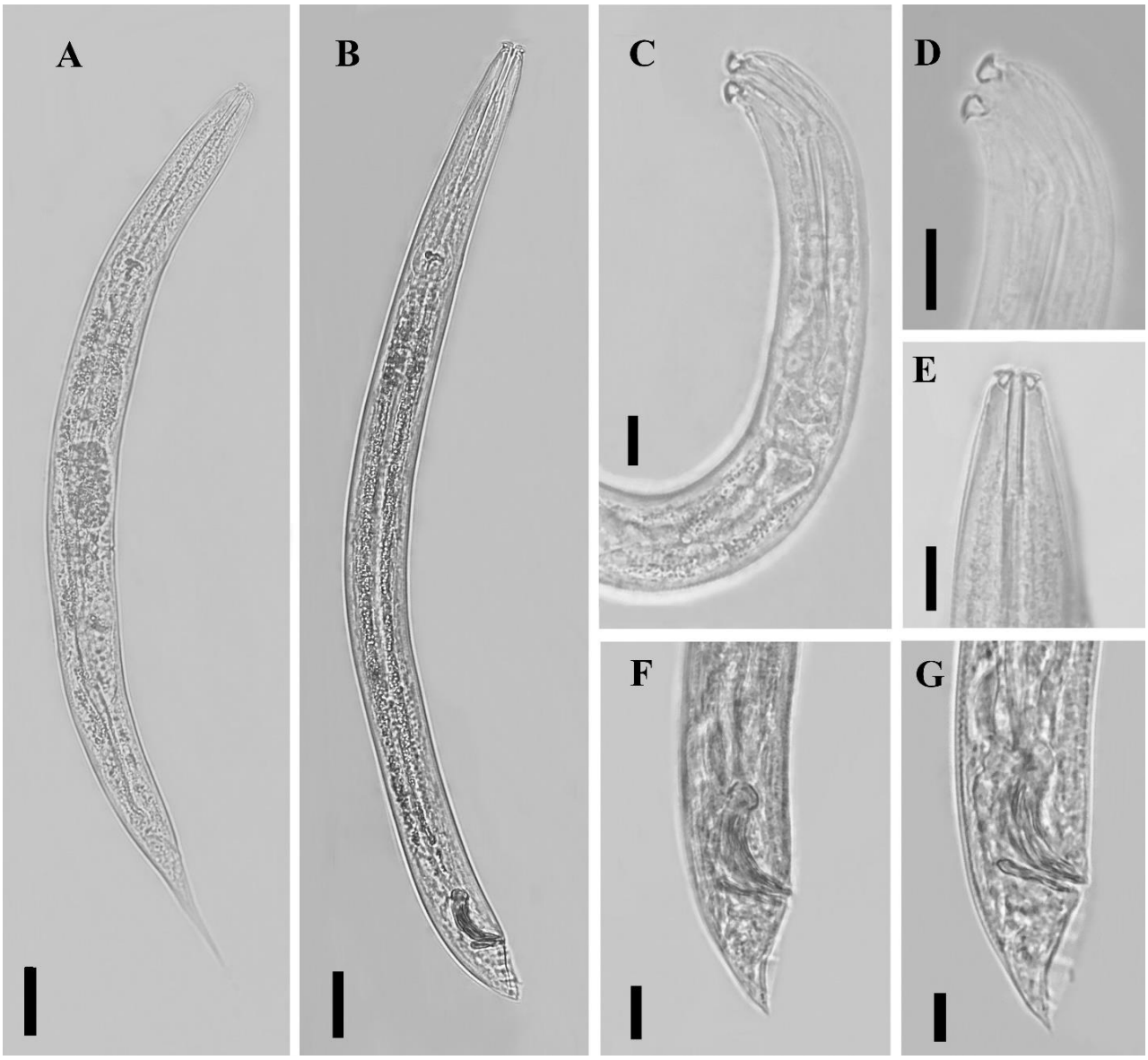

Figure 4. Photomicrographs of the Iranian population of *Diploscapter coronatus*. A: Entire female; B: Entire male; C: Anterior region of female showing pharyngeal structures; D & E: Female head showing lip region and stoma; F & G: Male posterior body with detailed view of tail and spicules. (Scale bars: A & B = 20 µm, C-G = 10 µm).

In the phylogenetic tree based on 18S rRNA sequences (Figure 6), the Iranian population clustered with strong support among *D. coronatus* sequences, further supporting its identification.

Discussion

The nematode populations identified in this study as *M. blumi* and *D. coronatus* exhibit strong morphological and molecular congruence with previously described populations. The Iranian population of *M. blumi* was largely consistent with the original description (Sudhaus, 1974), though the ITS sequence differed by 34 nucleotides from its closest match GenBank (*M. blumi*, DQ121436). Given the rapid evolution and inherent variability of the ITS region, this level of divergence likely reflects intraspecific variation

or geographical differentiation. Therefore, the identification of the Iranian population as *M. blumi* remains tentative and is supported by both morphological features and partial molecular evidence.

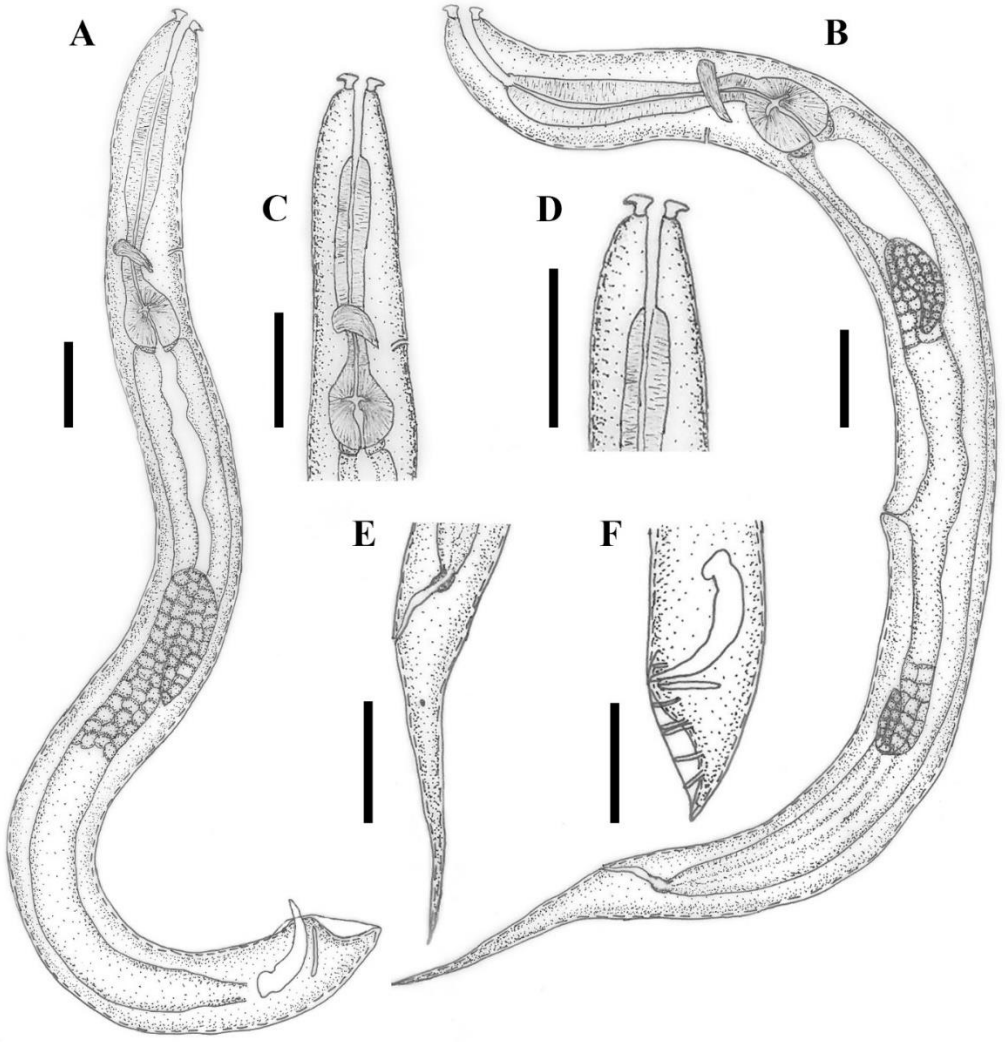

Figure 5. Line drawings of the Iranian population of *Diploscapter coronatus*. A: Entire male ; B: Entire female; C: Anterior region of female; D: Female head showing lip region; E: Posterior region of female showing tail morphology; F: Male posterior region showing spicules and bursa. (Scale bars: A-F = 20 µm).

In the case of *D. coronatus*, the Iranian population showed general agreement with the original descriptions and clustered phylogenetically with other well-characterized *D. coronatus* sequences based on the 18S rRNA data. The high degree of sequence similarity (99.37%) and congruent morphological characteristics support its identification with confidence.

Conflict of interest

All authors declare that they have no conflicts of interest related to this research.

Acknowledgments

The authors express their sincere gratitude to the University of Tehran and Ferdowsi University of Mashhad for their support and collaboration during this research.

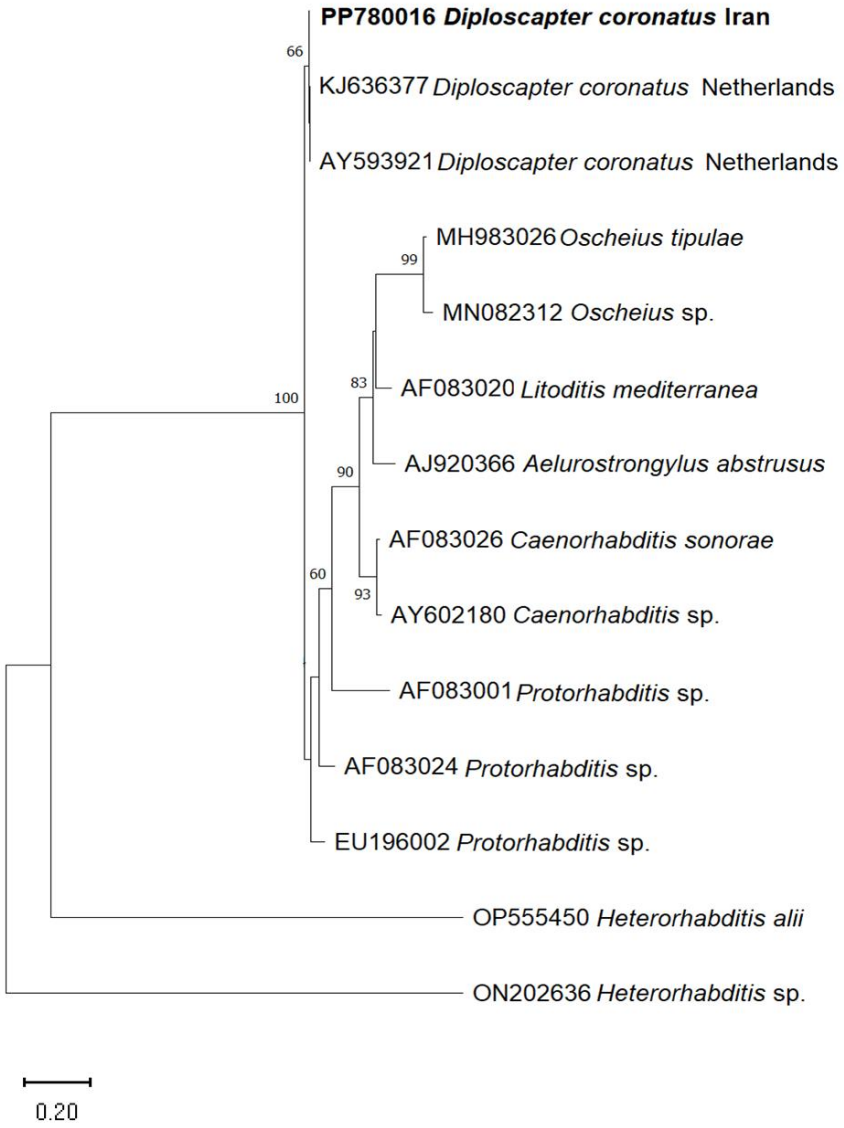

Figure 6. Bayesian phylogenetic tree of the Iranian population of *Diploscapter coronatus* inferred from partial 18S rRNA sequences using the GTR+G+I model. Posterior probability values > 50% are shown on relevant clades. The Iranian population from this study is shown in bold.

References

Abolafia J. & Peña-Santiago R. 2019. Description of *Metarhabditis giennensis* sp. n. (Nematoda, Rhabditida, Rhabditidae) from decaying wood of a riverbank forest in the southern Iberian Peninsula. *Zootaxa* 4652 (1), 145–154.

Ali S.S., Pervez R., Andrabi R., Sharma R. & Verma V. 2011. *Oscheius amsactae* n. sp. (Nematoda: Rhabditida), a necromenic associate of red-hairy caterpillar, *Amsacta moori* (Lepidoptera: Arctiidae) from Kanpur district, India. *Archives of Phytopathology and Plant Protection* 44, 871-881.

Andrássy I. 1976. *Evolution as a basis for the systematization of nematodes*. London, Pitman Publishing.

Bedding R.A. & Akhurst R.J. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21, 109-110.

Carta L.K. & Osbrink W. 2005. *Rhabditis rainai* n. sp. (Nematoda: Rhabditida) associated with the

Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Nematology* 7, 863-879.

Cobb N.A. 1893. Nematodes, mostly Australian and Fijian. *Department of Agriculture, New South Wales, Miscellaneous Publications (Sydney)* 13, 1-59.

Cobb N.A. 1913. New nematode genera found inhabiting fresh water and non-brackish soils. *Journal of Washington Academy of Sciences* 3, 333-432.

De Grisse A.T. 1969. Redescription ou modification de quelques techniques utilisées dans l'etude des nematodes phytoparasitaires. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* 34, 351-369.

Dujardin F. 1845. *Histoire naturelle des helminthes ou vers intestinaux*. Librairie Encyclopedique de Roret, Paris.

Edgar R.C. 2004. Muscle: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32(5), 1792-1797.

Eyualem, A., Karegar, A., Nabil, H. & De Ley, P. 1998. A redescription and ultrastructural study of *Diploscapter coronatus* (Cobb, 1893) Cobb, 1913 from Ethiopia and Iran. *Russian Journal of Nematology* 6, 17–22.

Holterman M., van der Wurff A., van den Elsen S., van Megen H., Bongers T., Holovachov O., Bakker J. & Helder J. 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* 23, 1792-1800.

Khanum T.A., Javed S. & Mehmood N. 2019. Description of new species of *Metarhabditis longicaudata* (Nematoda: Rhabditidae) with three new records from Sindh, Pakistan. *Plant Protection* 3(03), 131-139.

Martins W. 1985. *Rhabditis* (*Rhabditis*) *freitasi* sp. n. e *Rhabditis (Rhabditis) costai* sp. n. (Nematoda—Rhabditidae) isolados de otite bovina. *Memórias do Instituto Oswaldo Cruz* 80, 11-16.

Micoletzky H. 1922. Die freilebenden Erd-Nematoden mit besonderer Berücksichtigung der Steiermark und der Bukowina, zugleich met einer Revision sämtlicher nicht mariner, freilebender Nematoden in Form von Genus-Beschreibungen und Bestimmungsschlüsseln. *Archiv für Naturgeschichte* 87(1921), 1-650.

Örley L. 1880. Az Anguillulidák magánrajza. (Monographie der Anguilluliden) *Természetrajzi Füzetek* 4, 16–150.

Page R.D.M. 1996. TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* 12, 357-358.

Poinar G.O. Jr. 1971. *Rhabditis adenobia* sp. n. (Nematoda: Rhabditidae) from the colleterial glands of *Oryctes monoceros* L. and other tropical dynastid beetles (Coleoptera: Scarabaeidae). *Proceedings of the Helminthological Society of Washington* 38, 99–108.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25(7), 1253-1256.

Ronquist F. & Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19(12), 1572-1574.

Shokoohi E. & Abolafia J. 2019. *Soil and freshwater rhabditid nematodes (Nematoda, Rhabditida) from Iran*: *A compendium*. University of Jaen (UJA) Publishing, Jaen.

Subbotin S.A., Sturhan D., Chizhov V.N., Vovlas N. & Baldwin J.G. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. *Nematology* 8(3), 455-474.

Sudhaus W. 1974. Zur systematik, verbreitung, okologie und biologie neuer und wenigbekannter Rhabditiden (Nematoda) 2. Teil. *Zoologische Jahrbücher. Abteilung für Systematik, Geographie und Biologie der Tiere* 101, 417-465.

Sudhaus W. 2011. Phylogenetic systematisation and catalogue of paraphyletic "Rhabditidae" (Secernentea, Nematoda). *Journal of Nematode Morphology and Systematics* 14, 113-178.

Tahseen Q., Hussain A., Tomar V., Shah A.A. & Jairajpuri M.S. 2004. Description of *Metarhabditis andrassyana* gen. n., sp. n. (Nematoda: Rhabditidae) from India. *International Journal of Nematology* 14, 163-168.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10), 2731-2739.

Vovlas N., Subbotin S.A., Troccoli A., Liébanas G. & Castillo P. 2008. Molecular phylogeny of the genus *Rotylenchus* (Nematoda, Tylenchida) and description of a new species. *Zoologica Scripta* 37(5), 521-537.

White G. 1927. A method for obtaining infective nematode larvae from cultures. *Science* 66, 302-303.