DOI:10.22034/ijon.2022.528225.1013 Research article

First report of *Aphelenchoides paramonovi*, and observations on three species of Aphelenchoidea (Rhabditida) from Iran

Parnaz Mortazavi, Fariba Heydari, Ebrahim Pourjam, Mehrdad Alizadeh, Majid Pedram✉
Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
✉Corresponding author E-mail: majid.pedram@modares.ac.ir

Received: 2021/04/16 Revised: 2022/02/17 Accepted: 2022/02/18

Abstract

This contribution provides data for four species of the superfamily Aphelenchoidea from Iran. *Aphelenchoides paramonovi* was recovered from decaying wood and bark samples collected from Golestan province, north Iran. It is characterized by 434-671 µm long females, five lines in the lateral fields, a finger-like mucron with a short bristle in tail terminus and males with 17-22 µm long spicules. *Paraphelenchus myceliophthorus* has 610-752 µm long females with 16-17 µm long stylet, two mucrons at tail tip and males with 23-26 µm long spicules. A conical tail, not ending to ventrally bent narrow tip was observed in a population of *Bursaphelenchus mazandaranense* recovered from Golestan province, as a new variation of tail morphology for the species. The two latter populations were sequenced for their partial large subunit ribosomal DNA (LSU rDNA D2-D3). *Cryptaphelenchus varicaudatus*, was isolated from haemocoel of the bark beetle *Orthotomicus erosus*, collected from Tehran province. The morphometric data and light microphotographs were provided for all the studied species.

Keywords: Golestan province, *Orthotomicus erosus*, *Paraphelenchus myceliophthorus*, taxonomy

How to cite: Mortazavi, P., Heydari, F., Pourjam E., Alizadeh, M. & Pedram, M. 2022. First report of *Aphelenchoides paramonovi*, and observations on three species of Aphelenchoidea (Rhabditida) from Iran. Iranian Journal of Nematology 1(1), 29-41.

اولین گزارش *Aphelenchoides paramonovi*، به همراه داده‌های تکمیلی ریخت‌شناسی و مولکولی سه گونه از بالاخانواده Aphelenchoidea (Rhabditida) از ایران

پرناز مرتضوی، فریبا حیدری، ابراهیم پورجم، مهرداد علیزاده، مجید پدرام✉
گروه بیماری‌شناسی گیاهی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران
✉پست الکترونیکی مسئول مکاتبات: majid.pedram@modares.ac.ir

دریافت: 1400/01/27 بازنگری: 1400/11/28 پذیرش : 1401/11/29

چکیده:

در این مطالعه داده‌های ریخت‌شناسی و مولکولی چهار گونه از بالاخانواده Aphelenchoidea ارائه شده است. در *Aphelenchoides paramonovi* که از نمونه‌های چوب و پوست در حال پوسیدن از استان گلستان جداسازی شده است، طول بدن افراد ماده 434 تا 671 میکرومتر، دارای پنج شیار طولی در سطوح جانبی، انتهای دم با یک زائده‌ی انگشت مانند و با زایده مویی کوتاه در انتها و آلت نرینه به طول 17 تا 22 میکرومتر است. *Paraphelenchus myceliophthorus* دارای ماده‌هایی به طول 610 تا 752 میکرومتر، استایلت به طول 16تا 17میکرومتر، انتهای دم دارای یک جفت زائده و آلت نرینه به طول 23 تا 26 میکرومتر می باشد. دم مخروطی با انتهای باریک نشده و خمیده نشده به سمت شکمی در یک جمعیت از *Bursaphelenchus mazandaranense* به دست آمده از استان گلستان مشاهده شد که تنوعی جدید برای گونه است. دو جمعیت اخیر برای بخشی از ژن رمزگردان RNA زیرواحد بزرگ ریبوزوم (LSU rDNA D2-D3) توالی‌یابی شدند. *Cryptaphelenchus varicaudatus* از هموسل سوسک پوست خوار *Orthotomicus erosus* به دست آمده از استان تهران جدا شد. داده‌های ریخت‌سنجی و تصاویر تهیه شده با میکروسکپ نوری برای تمام گونه‌های مورد مطالعه نیز ارائه می شود.

واژه‌های کلیدی: استان گلستان، تاکسونومی، *Orthotomicus erosus*، *Paraphelenchus myceliophthotus*

Introduction

The members of superfamily Aphelenchoidea Fuchs 1937 have a wide feeding habit ranging from mycetophagy, predatory, plant feeding and insect related or parasitism (Hunt 1993; Kanzaki & Giblin-Davis 2012). Typologically, almost all aphelenchoidid representatives share a well-developed metacorpus (Kanzaki & Giblin-Davis 2012). Exceptionally, a small metacorpus does also rarely occur (Pedram *et al.* 2018). The superfamily currently includes two families (Kanzaki & Giblin-Davis 2012). The family Aphelenchoididae Skarbilovich, 1947 includes seven subfamilies: Acugutturinae Hunt 1980, Aphelenchoidinae Skarbilovich 1947, Ektaphelenchinae Paramonov 1964, Entaphelenchinae Nickle 1970, Parasitaphelenchinae Rühm 1956, Seinurinae Husain & Khan 1967 and Tylaphelenchinae Kanzaki, Li, Lan & Giblin-Davis 2014. The family Aphelenchidae Fuchs 1937 includes two subfamilies Aphelenchinae Fuchs 1937 and Paraphelenchinae Goodey 1951 (Hunt 2008; Kanzaki *et al.* 2014). The genera *Paraphelenchus* Micoletzky 1922 (Micoletzky 1925), *Aphelenchus* Bastian 1865, *Cryptaphelenchus* Fuchs 1937, *Ektaphelenchoides* Baujard 1984, *Ektaphelenchus* Fuchs 1937, *Deviburs aphelenchus* Kakuliya 1967, *Seinura* Fuchs 1931, *Aprutides* Scognamiglio 1974, *Bursaphelenchus* Fuchs 1937, *Laimaphelenchus* Fuchs 1937, *Sheraphelenchus* Nickle 1970, *Robustodorus* Andrássy 2007, *Schistonchus* (Cobb 1927) Fuchs 1937, *Aphelenchoides* Fischer 1894 and *Basilaphelenchus* Pedram, Kanzaki, Giblin-Davis & Pourjam 2018, have been reported from Iran (Miraeiz *et al.* 2018; Pedram *et al.* 2018). In the present study, four populations of Aphelenchoidea were collected from Tehran and Golestan provinces. *Aphelenchoides paramonovi* Eroshenko & Kruglik 2004, represents a new record for nematode fauna of Iran. The other populations belonged to *Paraphelenchus myceliophthorus* Goodey 1958, *Bursaphelenchus mazandaranense* Pedram, Pourjam, Ye, Atighi, Robbins & Ryss 2011 and *Cryptaphelenchus varicaudatus* Pedram, 2017. New host associations or morphological variations are discussed for the latter species.

Materials and methods

Sampling and nematode extraction

Several soil, wood and bark samples were collected from different regions of Golestan and Tehran provinces, Iran, during 2018-2020. The nematodes were extracted from the samples by the tray method (Whitehead & Hemming 1965). *Cryptaphelenchus varicaudatus* was isolated from the haemocoel of the bark beetle *O. erosus* Wollaston, 1857 specimens by dissecting under a Nikon SMZ1000 stereomicroscope. Nematodes were heat-killed by adding hot 4% formalin solution, transferred to anhydrous glycerin according to De Grisse (1969), mounted on permanent slides, and examined using a Nikon Eclipse E600 light microscope. Morphological characters were studied using an Olympus BX51 light microscope equipped with differential interference contrast optics (DIC). The light microphotographs were taken using an Olympus DP72 digital camera attached to the microscope.

DNA extraction, PCR, and sequencing

For molecular analyses, a single female specimen of *A. paramonovi*, *P. mycephothorus* and *B. mazandaranense* were picked out, examined in a drop of distilled water on separate temporary slides under the light microscope, transferred to 20 µl of TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) on a clean slide, and then crushed using a cover slip. The suspension was collected by adding 20 µl TE buffer. The DNA samples were stored at -20°C until used as PCR templates. Primers for LSU rDNA D2-D3 amplification were forward primer KK28S-1 (5'-AAGGATTCCCTTAGTAACGGCGAGTG-3') (Kiontke *et al.* 2004) and reverse primer 1006R (5'-GTTCGATTAGTCTTTCGCCCCT-3') (Holterman *et al.* 2008), and forward primer D2A (5'-ACAAGTACCGTGAGGGAAAGT-3') and reverse primer D3B (5'-TCGGAAGGAACCAGCTACTA-3') (Nunn 1992). The efficacy of several combinations of the aforementioned primers were also tested.

Phylogenetic analyses

The newly obtained sequences of the D2-D3 fragments of LSU rDNA were compared with other relevant sequences available in GenBank database using the BLAST homology search program. The selected sequences with including newly generated, and outgroup

sequences were aligned using Clustal X2 (http://www.clustal.org/). The resulting alignment was manually edited using MEGA6 (Tamura *et al.* 2013). The model of base substitution was selected using MrModeltest 2 (Nylander 2004). The Akaike-supported model, a general time reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I), was used in LSU phylogeny. The Bayesian analysis was performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) and a random starting tree, running the chains for 2×10^6 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 a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic tree (Larget & Simon 1999) using the 50% majority rule. The convergence of model parameters and topology was assessed based on the average standard deviation of split frequencies and potential scale reduction factor values. Adequacy of the posterior sample size was evaluated using autocorrelation statistics, as implemented in Tracer v.1.6 (Rambaut & Drummond 2009). The tree file was visualised using Dendroscope v.3.2.8 (Huson & Scornavacca 2012) and was digitally drawn in CorelDRAW software version 17.

Results and discussion

Aphelenchiodes paramonovi Eroshenko & Kruglik, 2004

(Fig. 1)

Measurements

See Table 1.

Description

Female

Body slightly ventrally curved after heat relaxation, slightly tapering towards both ends. Cuticle with fine transverse annuli. Lateral fields with five incisures, forming four equally distant bands at mid-body. Cephalic region separated from the rest body by a shallow constriction. Stylet short, its conus *ca* 30% of the total length, with small basal swellings. Procorpus cylindrical, metacorpus rounded to slightly ovate, its valve plates sclerotized, situated more or less post-centrally. Pharyngo-intestinal junction immediately behind metacorpus, pharyngeal glands lobe overlapping intestine dorsally for 9.8 times body width at metacorpus. Excretory pore slightly posterior to base of metacorpus. Hemizonid indistinct. Intestine simple, rectum and anus functional. Reproductive system consisting of an outstretched ovary with oocytes mostly in single row, oviduct, axial spermatheca usually with sperm, uterus, vagina, vulva a transverse slit, post-vulval uterine sac (PUS) *ca* 3.1 times vulval body width long and often containing sperm. Tail conical, dorsally convex, ventrally slightly concave, having a finger-like mucro with a short bristle at tip.

Male

General morphology similar to that of female, except for reproductive system and tail end morphology. Genital system with outstretched testis, spermatocytes in single line at distal region of testis. Spicules paired, condylus well-developed with rounded tip, rostrum small and the tip of the dorsal limb oblique. Caudal papillae comprised of three pairs (P1 lacking), their arrangement as follows: the cloacal pair (P2) immediately posterior to cloacal aperture, the second caudal pair (P3) at about middle of the tail and the third pair (P4) near the tail tip. Tail conical, dorsally convex, ventrally concave, having a simple sharp mucro at tip.

Remarks

During present study, our efforts to sequence the LSU and SSU fragments of this population were not successful. Four species of the genus namely *A. giblindavisi* Aliramaji, Pourjam, Álvarez-Ortega, Jahanshahi Afshar & Pedram 2018, *A. paramonovi*, *A. shamimi* Khera, 1970 and *A. hamospiculatus* Mortazavi & Pedram, 2021 have five lines in their lateral fields. The Iranian population of *A. paramonovi* differs from *A. giblindavisi* by a finger-like mucro with short bristle on the female tail tip (*vs* a warty mucro), male spicules shape (tip of dorsal limb oblique *vs* simple) and smaller *c* ratio (14.1 (11.7-15.4) *vs* 18.9 (17.2-20.4)). It differs from *A. shamimi* by the different female tail tip (having a finger-like mucro with a short bristle *vs* a simple mucro), male spicules shape (tip of dorsal limb oblique *vs* simple) and longer stylet (11.5 (10.5-12.5) *vs* 8-9 μm). It differs from *A. hamospiculatus* by the different female tail tip (having a finger-like mucro with a short bristle *vs* a warty mucro), male spicules shape (having a small and blunt rostrum *vs* slightly bent inwards), tip of the dorsal limb (oblique *vs*

hook-like) and slightly longer stylet (11.5 (10.5-12.5) *vs* 9.4 (9-10) µm).

The presently recovered population was further compared with two similar species (mainly based on similar female tail end differentiation) with no data on their lateral lines, namely *A. emiliae* Romaniko 1966 and *A. macromucrons* Slankis, 1967. It differs from *A. emiliae* by male spicules shape (tip of dorsal limb oblique *vs* simple), shorter females (562 (434-671) *vs* 770-860 µm), a shorter stylet (11.5 (10.5-12.5) *vs* 15 µm), smaller *c* ratio (14.1 (11.7-15.4) *vs* 17.1) and anteriorly located vulva (*V* = 69 (66.8-72.8) *vs* 74.2), and from *A. macromucrons* by male spicules shape (tip of dorsal limb oblique *vs* simple), shorter females (562 (434-671) *vs* 720-740 µm) and smaller *a* (30.7 (27.1-35.3) *vs* 37.6-39.7), *b* (8.4 (6.8-10) *vs* 11-12.9) and *c* (14.1 (11.7-15.4) *vs* 19-19.8) ratios.

Aphelenchoides paramonovi was originally reported from Primorsky territory, Russia, in association with wood of *Pinus koraiensis* Siebold & Zuccarini. In the present study, it was isolated from the rhizospheric soil of an oak in Ramian County, Golestan province, north Iran (GPS coordinate: 36°55.329' N, 55°06.939' E). The Iranian population of this species was in morphological and morphometric agreement with the type population.

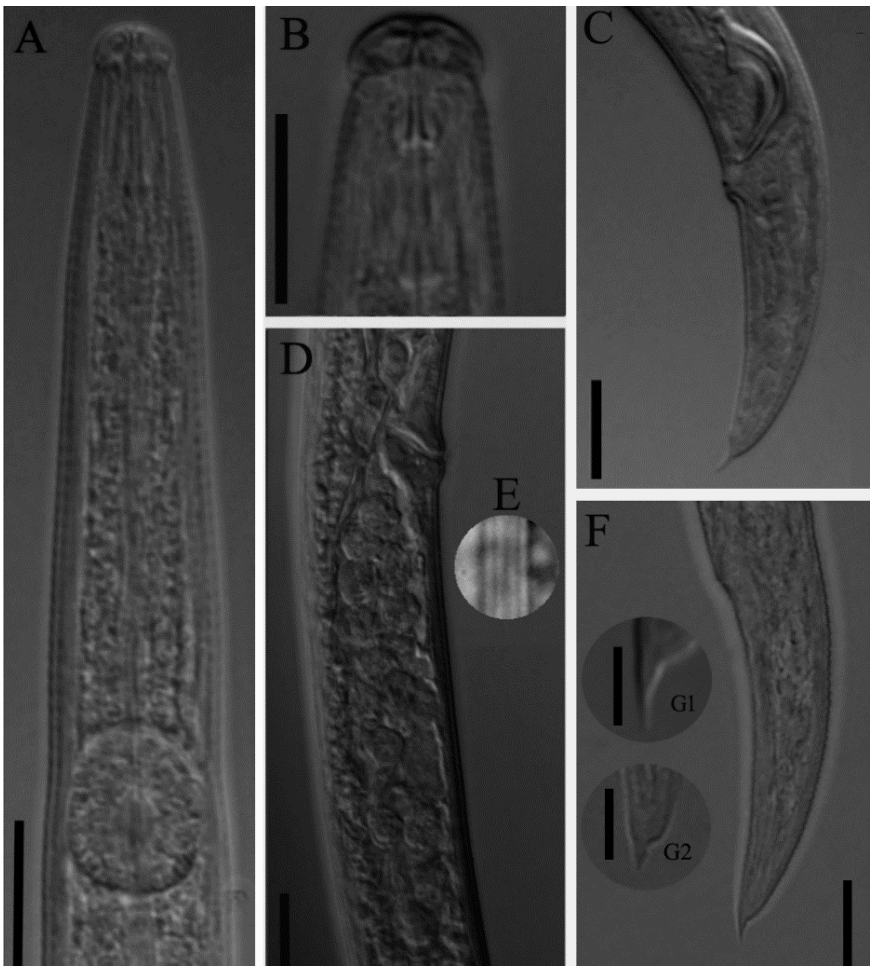

Fig. 1. Light microphotographs of Iranian population of *Aphelenchoides paramonovi* Eroshenko & Kruglik, 2004. A: Part of pharynx; B: Female cephalic region and stylet; C: Male tail in lateral view; D: Female post-vulval uterine sac; E: Enlarged view of part of lateral lines; F: Female tail in lateral view; G1 & G2: Female tails tip (Scale bars: A-F = 10 µm, G1 & G2 = 5 µm).

Table 1. Morphometric data of type and Iranian populations of *Aphelenchiodes paramonovi* Eroshenko & Kruglik, 2004 and *Paraphelenchus myceliophthorus* Goodey, 1958. All measurements are in µm and in the form: mean ± s.d. (range).

	A. paramonovi				***P. myceliophthorus***			
	Iranian population		**Eroshenko & Kruglik (2004)**		**Iranian population**		**Goodey (1958)**	
Characters	Females	Males	Females	Males	Females	Males	Females	Males
n	7	5	14	4	3	4	50	50
L	562 ± 89.9 (434-671)	456 ± 45.2 (419-532)	606 ± 16.7 (515-707)	572 ± 21 (537-635)	640 ± 11 (610-752)	653 ± 17 (640-678)	669 (580-820)	580-820
a	30.7 ± 3.1 (27.1-35.3)	33.2 ± 4.5 (29.9-40.9)	34.0 ± 0.8 (28-40)	37.0 ± 2.7 (31-42)	33.6 ± 1.4 (27.1-34.3)	24.9 ± 1.0 (23.9-35.8)	22-34	23-32
b	8.4 ± 1.0 (6.8-10.0)	8.2 ± 1.0 (7.1-9.5)	10.2 ± 0.2 (8-11)	10.8 ± 0.8 (8-12)	4.5 ± 0.2 (4.3-4.7)	4.6 ± 0.2 (4.4-4.8)	4.1-6.6	3.9-5.9
c	14.1 ± 1.3 (11.7-15.4)	14.9 ± 2.0 (12.7-17.2)	16.0 ± 0.3 (14-18)	14.0 ± 1.6 (11-19)	19.4 ± 0.8 (18.5-19.9)	20.6 ± 0.5 (20.2-21.3)	13-24	14-30
c′	3.5 ± 0.3 (3.0-3.8)	2.6 ± 0.2 (2.3-2.8)	4.1 ± 0.1 (3.2-5.0)	3.5 ± 0.4 (2.5-4.3)	3.2 ± 0.2 (3.0-3.4)	2.0 ± 0.01 (1.9-2.1)	–	–
T or V	69 ± 2 (66.8-72.8)	56.8 ± 1.8 (53.7-58.3)	66.0 ± 0.4 (64-70)	60.0 ± 1.8 (57-67)	72.7 ± 0.3 (72.4-73.0)	46.7 ± 1.3 (45.0-47.7)	71-78	46-47
m	38.4 ± 3.3 (33.3-41.7)	30.0 ± 4.4 (25-37)	–	–	28.1 ± 4.0 (23.5-31.3)	31.9 ± 3.8 (29.4-37.5)	–	–
Cephalic region diam.	5.9 ± 0.7 (5-7)	5.4 ± 0.5 (5-6)	–	–	7.7 ± 1.5 (6-9)	7.5 ± 0.6 (7-8)	–	–
Cephalic region height	2.6 ± 0.6 (2.0-3.5)	2.1 ± 0.2 (2.0-2.5)	–	–	4 ± 1 (3-5)	3.5 ± 0.6 (3-4)	–	–
Stylet length	11.5 ± 0.8 (10.5-12.5)	11.7 ± 1.2 (10.5-13)	12-13	11-12	16.7 ± 0.6 (16-17)	16.5 ± 0.6 (16-17)	–	16
Stylet conus	4.5 ± 0.5 (4-5)	3.5 ± 0.5 (3-4)	–	–	4.7 ± 0.6 (4-5)	5.3 ± 0.5 (5-6)	–	–
Median bulb length/diam.	1.3 ± 0.1 (1.2-1.4)	1.3 ± 0.0 (1.3-1.4)	–	–	1.6 ± 0.1 (1.4-1.7)	1.7 ± 0.1 (1.6-1.9)	–	–
Pharynx	66.6 ± 6.2 (56-76)	56.2 ± 3.3 (53-60)	–	–	142 ± 7 (135-149)	141 ± 4.3 (135-144)	–	–
Max body width	18.3 ± 2.4 (16-22)	13.8 ± 0.4 (13-14)	–	–	18 ± 1 (17-19)	18.8 ± 1.0 (18-20)	–	–
Anal body width	11.6 ± 1.5 (10-14)	12.0 ± 0.7 (11-13)	–	–	10.3 ± 0.6 (10-11)	16.3 ± 1.0 (15-17)	–	–
Post-vulval uterine sac	53.7 ± 10.4 (42-73)	–	–	–	32.7 ± 2.5 (30-35)	–	–	–
Tail length	39.9 ± 4.7 (35-47)	30.8 ± 2.6 (26-34)	–	–	33.0 ± 1.0 (32-34)	31.8 ± 1.3 (30-33)	–	–
Spicules length	–	19.2 ± 2.6 (17-22)	–	22 ± 0.2 (22-23)	–	24.8 ± 1.3 (23-26)	–	28
Gubernaculum	–	–	–	–	–	13.3 ± 1.0 (12-14)	–	13.5

***Paraphelenchus myceliophthorus* Goodey, 1958**

(Fig. 2)

Measurements

See Table 1.

Female

Body straight to slightly ventrally bent after heat relaxation. Cuticle with fine striae, lateral fields with six incisures at vulval region. Cephalic region continuous with the body contour. Stylet with distinct lumen and no basal knobs. The pharynx with long and cylindrical procorpus, well developed elliptical median bulb with large central valve, narrow isthmus and small pharyngeal bulb. The nerve ring surrounding the isthmus at about its middle. Excretory pore slightly posterior to the nerve ring. Intestine simple, rectum and anus functional. The reproductive system composed of an outstretched ovary with oocytes mostly at multiple rows behind germinal zone, tubular oviduct, spermatheca elongate, visible crustaformeria, thick-walled uterus, vagina perpendicular to body axis, vulva a transverse slit and PUS *ca* 1.8 times vulval body width. Tail subcylindrical, with a widely rounded terminus and a pair of short ventro-lateral papillae.

Male

Generally similar to female in appearance, apart from sexual dimorphism. Testis single, outstretched. Spicules slender, tylenchid and slightly ventrally curved. Gubernaculum crescent shape. Bursa absent. Tail conical, its tip rounded and slightly ventrally bent with a central ventrally located small mucro. Caudal papillae four pairs, all ventro-lateral. The cloacal pair is followed by the second pair at about middle of the tail, and the third and fourth pairs close to tail tip.

.

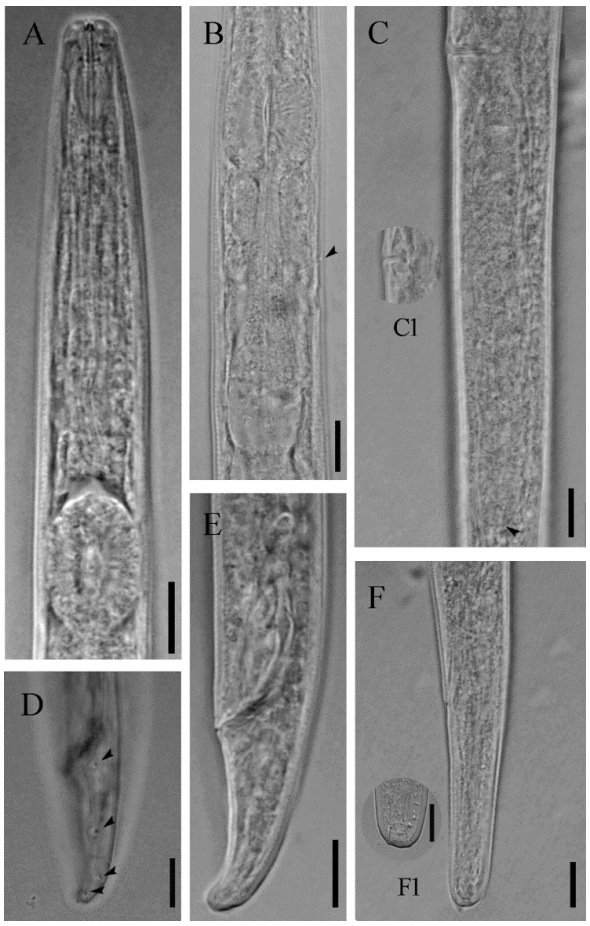

Fig. 2. Light microphotographs of Iranian population of *Paraphelenchus myceliophthorus* Goodey, 1958. A & B: Anterior and posterior parts of female pharynx (arrowhead showing excretory pore); C: Part of female reproductive system (arrowhead showing the end of post-vulval uterine sac); C1: Vulva; D: Papillae in lateral view; E: Male tail; F: Female tail in lateral view; F1: Female tail tip in ventral view (Scale bars = 10 µm).

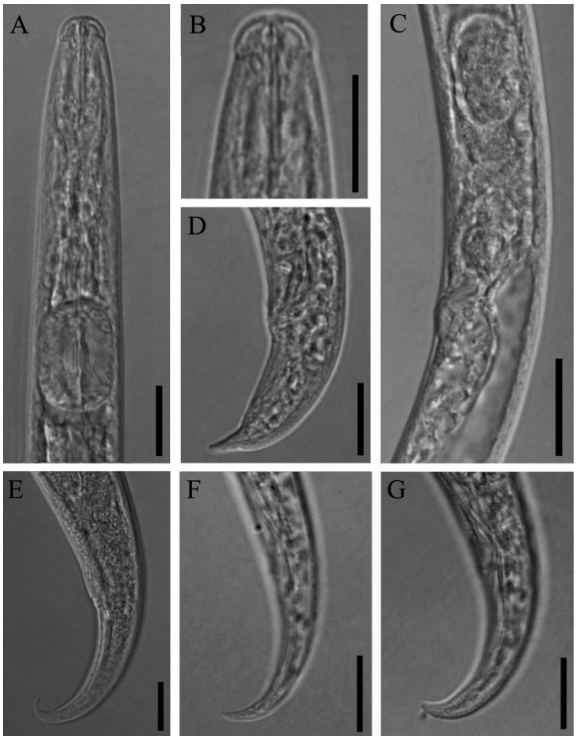

Fig. 3. Light microphotographs of *Bursaphelenchus mazandaranense* Pedram, Pourjam, Ye, Atighi, Robbins & Ryss, 2011 (A-D, F & G: Minudasht population; E: Paratype female). A: Part of female pharynx; B: Cephalic region and stylet; C: Part of female reproductive system; D: Male tail in lateral view; E-G: Female tail (Scale bars = 10 μm).

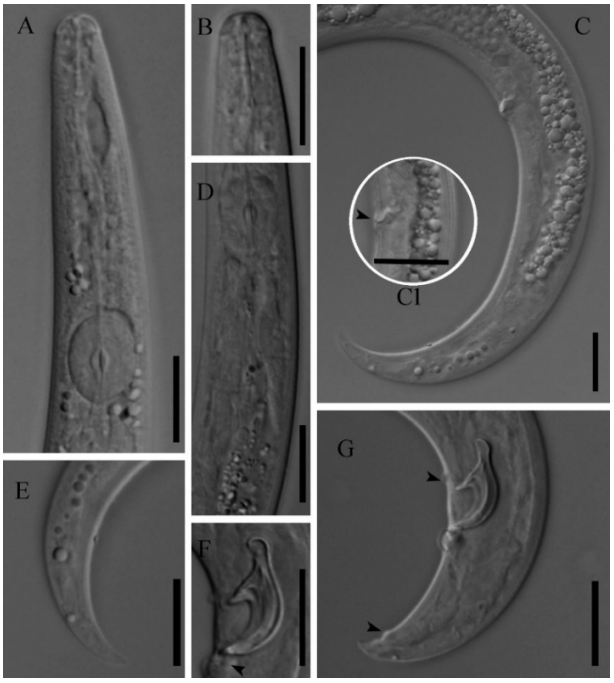

Fig. 4. Light microphotographs of *Cryptaphelenchus varicaudatus* Pedram, 2017 isolated from the bark beetle *Orthotomicus erosus*. A & D: Anterior and posterior part of female pharynx; B: Cephalic region and stylet; C: Female posterior body region; C1: Sclerotized vagina; E: Female posterior body end; F: Spicule and P2 papillae (arrowhead); G: Male tail (the upper arrowhead showing P1, the lower arrowhead showing P3 papillae) (Scale bars = 10 μm).

Table 2. Morphometric data of type and presently studied populations of *Bursaphelenchus mazandaranense* Pedram, Pourjam, Ye, Atighi, Robbins & Ryss, 2011 and *Cryptaphelenchus varicaudatus* Pedram, 2017. All measurements are in µm and in the form: mean ± s.d. (range).

	B. mazandaranense				***C. varicaudatus***			
	Present population (Minudasht)		**Pedram *et al.* (2011)**		**Population isolated from *Orthotomicus erosus***		**Pedram (2017)**	
Characters	Females	Males	Females	Males	Females	Males	Females	Males
n	5	6	20	15	2	2	19	9
L	400 ± 55 (326-453)	430 ± 7.1 (358-435)	558 ± 55.5 (457-701)	496 ± 44 (415.5-581.5)	315, 279	219, 205	315 ± 25.3 (275-367)	256 ± 15.4 (235-278)
a	27.4 ± 1.7 (25.6-30.2)	27.9 ± 2.5 (23.9-30.4)	25.0 ± 1.5 (22.3-28.0)	26 ± 1 (24-27)	26.3, 25.4	27.4, 20.5	34.0 ± 1.4 (21.7-26.5)	23.5 ± 1.7 (21-26)
b	7.2 ± 0.7 (6.4-8.0)	7.1 ± 0.3 (6.8-7.5)	7.8 ± 0.8 (7-10)	6.8 ± 0.5 (6.2-7.2)	7.7, 8.0	5.5, 6.8	6.8 ± 0.6 (5.9-8.0)	6.5 ± 0.3 (5.9-6.7)
c	13.9 ± 2.1 (11.6-16.7)	14.8 ± 0.6 (14.3-15.7)	15.3 ± 2.0 (12.0-19.3)	15.0 ± 1.3 (12.5-17.5)	–	16.8, 14.6	–	15.1 ± 1.0 (14.0-16.4)
c´	3.5 ± 0.5 (2.9-4.1)	2.1 ± 0.2 (1.9-2.3)	3.5 ± 0.3 (2.5-4.0)	2.3 ± 0.1 (2.1-2.5)	–	1.6, 1.4	–	1.9 ± 0.1 (1.8-2.0)
T or V	72.7 ± 0.8 (71.9-73.5)	41.2 ± 4.7 (37.2-49.4)	75 ± 1 (72.5-77.0)	58.0 ± 3.5 (51.5-63.0)	75.4, 80.6	84.5, 87.8	79.5 ± 1.6 (75.1-81.8)	–
m	41.8 ± 0.2 (40-43)	42.4 ± 5.1 (36.4-47.6)	–	–	44.9, 52.5	38.5, 30.8	–	–
Cephalic region diam.	6.8 ± 0.6 (6.0-7.5)	5.7 ± 0.6 (5.0-6.5)	8.0 ± 0.2 (8-9)	7.5 ± 0.5 (7-8)	8, 7	4, 4	–	–
Cephalic region height	2.8 ± 0.3 (2.5-3.0)	2.4 ± 0.3 (2.0-2.8)	3.0 ± 0.1 (3.0-3.5)	3.0 ± 0.0 (3-3)	3, 3	3, 2	–	–
Stylet length	10.7 ± 1.0 (10-12)	10.8 ± 0.6 (10.0-11.5)	12.0 ± 0.5 (11.5-13.0)	11.0 ± 0.5 (11.5-12.0)	7.8, 8.0	7.8, 7.3	7.6 ± 0.4 (7.0-8.5)	7.0 ± 0.7 (6-8)
Stylet conus	4.5 ± 0.5 (4-5)	4.6 ± 0.4 (4-5)	4.0 ± 0.3 (3.5-4.5)	3.7 ± 0.3 (3.5-4.0)	3.5, 4.2	3, 2	–	–
Median bulb length/diam.	1.4 ± 0.1 (1.3-1.5)	1.5 ± 0.1 (1.4-1.6)	1.2 ± 0.1 (0.9-1.3)	1.2 ± 0.1 (1.0-1.4)	1.0, 1.1	1.3, 1.0	–	–
Pharynx	55.6 ± 3.0 (51-59)	57.2 ± 3.3 (53-61)	72 ± 2 (69.0-76.5)	71.0 ± 2.5 (68.5-75.0)	41, 55	40, 30	46.8 ± 3.0 (41-53)	41.5 ± 2.3 (38-44)
Max body width	14.6 ± 1.7 (14-16)	14.6 ± 0.5 (14-15)	10.7 ± 1.0 (10-13)	14.0 ± 0.7 (13-15)	12, 11	8, 10	13 ± 1 (12-15)	–
Anal body width	8.3 ± 1.1 (6.5-9.0)	12.8 ± 0.8 (12-14)	8.3 ± 1.1 (6.5-9.0)	12.8 ± 0.8 (12-14)	10, 10	8, 10	–	–
Post-vulval uterine sac	33.5 ± 4.2 (30-38)	–	44.5 ± 4.5 (38-57)	–	8, 7	–	8.4 ± 0.6 (8-9)	–
Tail length	29.0 ± 4.6 (26-37)	27.4 ± 2.3 (24-30)	36.5 ± 4.0 (30-44)	32.5 ± 2.0 (30-36)	–	13, 15	–	16.8 ± 1.2 (16-17)
Spicules length	–	12.4 ± 1.1 (11-14)	–	12.5 ± 0.5 (12.0-13.5)	–	7, 11	–	10.5 ± 0.5 (8.5-11.0)

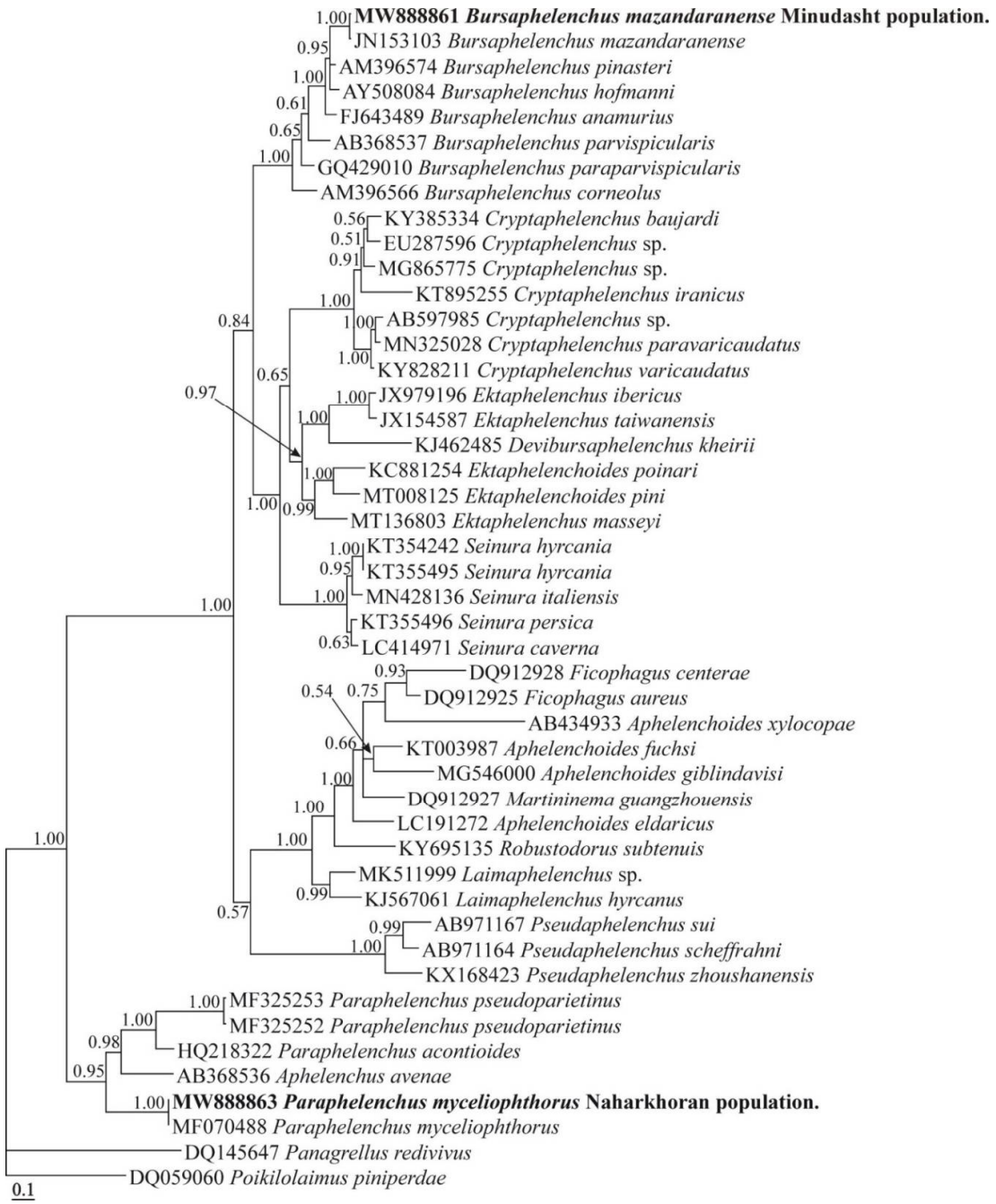

Fig. 5. Bayesian 50% majority rule consensus tree inferred using LSU rDNA D2-D3 sequences of recently recovered *Paraphelenchus myceliophthorus* Goodey, 1958 and *Bursaphelenchus mazandaranense* Pedram, Pourjam, Ye, Atighi, Robbins & Ryss, 2011 populations from Iran, under the GTR + G + I model. Bayesian posterior probabilities (BPP) more than 50% are given for appropriate clades. New sequences are in bold font.

Remarks

Paraphelenchus myceliophthotus was originally reported from southeast England in association with mushroom compost. Its occurrence in Iran was reported by Kheiri (1972). The morphological and morphometric data were however not available for us. In the present study, it was isolated from dead/rotten wood samples collected from natural Naharkhoran forest in Golestan province, north Iran. (GPS coordinate: 36°45.565' N, 54°28.231'E). The Iranian population of this species was in morphological and morphometric agreement with the type population.

***Bursaphelenchus mazandaranense* Pedram, Pourjam, Ye, Atighi, Robbins & Ryss, 2011**

(Fig. 3)

Measurements

See Table 2.

This species has described from Iran and is prevalent in north and northwest forests of the country (data not shown). In the present study, a population of the species was isolated from Minudasht county (GPS coordinates: 37°11.159' N, 55°26.152'E) in association with dead/rotten wood samples of an unidentified tree. The morphological, morphometric and molecular data of the recovered population are in agreement with the type population. The type population of the species has a conical tail, ending to a narrow ventrally bent tip. The presently recovered population however has a conical tail, not ending to a vernally bent narrow tip. The characters of tail in the type and presently studied population are given in Fig. 3E.

***Cryptaphelenchus varicaudatus* Pedram, 2017**

(Fig. 4)

Measurements

See Table 2.

The species has originally described from Iran (Pedram, 2017). It was isolated from bark samples of coniferous trees having galleries of the bark beetle, *Orthotomicus erosus*. The insect association of the species was however not examined/confirmed for the type population. Recently, it was isolated from the body and frass of the same beetle in Israel (Xue *et al.* 2019). In this study, it was isolated from the body of the *O. erosus* specimens, collected from the Chitgar park, city of Tehran (GPS coordinate: 35°43.43' N, 51°14.37' E). The recovered population is in morphological and morphometric agreement with the type population.

Molecular characterization and phylogenetic relationships

Two 982 and 1017 nt long D2-D3 expansion segments of LSU rDNA sequences were obtained in the present study for *P. myceliophthorus* and *B. mazandaranense* using the KK28S-1/1006R and D2A/D3B pairs, respectively. The efforts to amplify this region for *A. paramonovi*, as already mentioned, failed. The BLAST search using the newly generated sequence for *P. myceliophthorus* revealed it has 99.86% identity with the LSU sequence of another isolate of the species already deposited in GenBank database (MF070488), and the BLAST search using the newly generated sequence for *B. mazandaranense* revealed it has 99.68% identity with the LSU sequence of the type population of the species (JN153103).

A total of 45 sequences of Aphelenchoididae species and two sequences of classic rhabditids as outgroup sequences were selected for the LSU phylogeny and the phylogenetic tree was presented (Fig. 5). The newly generated sequence of Iranian population of *P. myceliophthorus* and the previously deposited sequence of the species fell into the clade of Aphelenchidae, and the newly generated sequence for *B. mazandaranense* and the sequence of the type population occupied a placement inside the *Bursaphelenchus* clade in this tree. The general topology of the herein inferred tree is consistent with the previously resolved topologies using ribosomal markers (*e.g.* Gu *et al.* 2020; Kanzaki *et al.* 2018).

Conflict of interest

All the authors certify that they do not have any conflict of interest, the presented data are original and the described species is not published, and is not under evaluation for publication elsewhere, all prevailing local, national and international regulations and conventions, and normal scientific ethical practices, have been respected.

Acknowledgements

The kind helps of Dr. Yousef Panahandeh by sampling is appreciated. This project was financially supported by Tarbiat Modares University.

References

Aliramaji F., Pourjam E., Álvarez-Ortega S., Jahanshahi Afshar F. & Pedram M. 2018. Description of *Aphelenchoides giblindavisi* n. sp. (Nematoda: Aphelenchoididae), and proposal for a new combination. *Journal of Nematology* 50(3), 437-452.

Andrássy I. 2007. *Free-living nematodes of Hungary (Nematoda errantia)*. Budapest, Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences.

Bastian H.C. 1865. Monograph on the Anguillulidae, or free nematoids, marine, land, and freshwater; with descriptions of 100 new species. *Transactions of the Linnean Society of London* 25, 73-184.

Baujard P. 1984. Remarques sur la sous-famille des Ektaphelenchinae Paramonov, 1964 et proposition d'*Ektaphelenchoides* n. gen. (Nematoda: Aphelenchoididae). *Revue de Nématologie* 7(2), 147-171.

Cobb N.A. 1927. Note on a new nema, *Aphelenchus retusus*, with a proposed division of *Aphelenchus* into three subgenera. *Journal of Parasitology* 14, 57-58.

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

Eroshenko A.S. & Kruglik I.A. 2004. *Aphelenchoides paramonovi* sp. n. (Nematoda: Aphelenchoididae) – a new wood pine inhabiting nematode species in the Primorsky Territory. In Sonin M.D. (Ed.), *Parasitic Nematodes of Plants and Insects* (pp. 46-49). Moscow, Nauka (In Russian).

Fischer M. 1894. Über eine Clematis-krankheit. *Bericht aus dem Physiolischen Laboratorium des Landwirthschaftlichen, Instituts der Universitat Halle* (11), 3, 1-11.

Fuchs A.G. 1931. *Seinura* gen. nov. *Zoologischer Anzeiger* 94, 226-228.

Fuchs A.G. 1937. Neue parasitische undhalbparasitische Nematoden bei Borkenkäfern und einige andere Nematoden. I. Teil. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* 70, 291-380.

Goodey T. 1951. *Soil and freshwater nematodes*. London. Methuen & Co., Ltd.

Goodey J.B. 1958. *Paraphelenchus myceliophthorus* n. sp. *Nematologica* 3(1), 1-5.

Gu J., Maria M., Liu L & Pedram M. 2020. Description of *Seinura italiensis* n. sp. (Tylenchomorpha: Aphelenchoididae) found in the medium soil imported from Italy. *Journal of Nematology* 52, 1-11.

Holterman M., Rybarczyk K., Van den Elsen S., Van Megen H., Mooyman P., Santiago R. P., Bongers T., Bakker J. & Helder J. 2008. A ribosomal DNA-based framework for the detection and quantification of stress-sensitive nematode families in terrestrial habitats. *Molecular Ecology Resources* 8, 23-34.

Hunt D.J. 1980. *Acugutturus parasiticus* n. g., n. sp., a remarkable ectoparasitic aphelenchoid nematode from *Periplaneta americana* (L.) with proposal of Acugutturinae n. subf. *Systematic Parasitology* 1, 167-170.

Hunt D.J. 1993. Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics. Wallingford, CABI Publishing.

Hunt D.J. 2008. A checklist of the Aphelenchoidea (Nematoda: Tylenchina). *Journal of Nematode Morphology and Systematics* 10(2), 99-135.

Husain S.I. & Khan A.M. 1967. On the status of the genera of the superfamily Aphelenchoidea (Fuchs, 1937) Thorne, 1949 with the descriptions of six new species of nematodes from India. *Proceedings of the Helminthological Society of Washington* 34(2), 167-174.

Huson D.H. & Scornavacca C. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Systematic Biology* 61(6), 1061-1067.

Kakuliya G.A. 1967. [New nematode genus *Deviburs aphelenchus* gen. n. (Nematoda: Aphelenchoididae).] *Bulletin of the Academy of Sciences of the Georgian SSR* 47, 439-443.

Kanzaki N. & Giblin-Davis R.M. 2012. Aphelenchoidea. In Manzanilla-López, R.H. & Marbán-Mendoza, N. (Ed.), *Practical plant nematology* (pp. 161-208). Jalisco, Biblioteca Básica de Agricultura.

Kanzaki N., Li H.F., Lan Y.C. & Giblin-Davis R.M. 2014. Description of two *Pseudaphelenchus* species (Tylenchomorpha: Aphelenchoididae) associated with Asian termites and proposal of new subfamily Tylaphelenchinae n. subfam. *Nematology* 16, 963-978.

Kanzaki N., Ekino T. & Masuya H. 2018. *Seinura caverna* n. sp. (Tylenchomorpha: Aphelenchoididae), an androdioecious species isolated from bat guano in a calcareous cave. *Nematology* 21, 207–225.

Kheiri A. 1972. Plant parasitic nematodes (Tylenchida) from Iran. *Biologisch Jaurboek Dodonaea* 40, 224-239.

Khera S. 1970. Nematodes from the banks of still and running waters. 8. Order Tylenchida. *Proceedings of the Zoological Society* 23, 53-65.

Kiontke K., Gavin N.P., Raynes Y., Roehrig C., Piano F. & Fitch D.H. 2004. *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. *Proceedings of the National Academy of Sciences* 101(24), 9003-9008.

Larget B. & Simon D.L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16(6), 750-759.

Micoletzky H. 1922. Die freilebenden Erd-Nematoden mit besonderer Berucksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision

sämtlicher nicht mariner, freilebender Nematoden in Form von Genus –Beschreibunger und Bestimmungsschlüsseln. *Archiv für Naturgeschichte*, Berlin, 87: 1-650.

Micoletzky H. 1925. Die Freilebenden Süsswasserund Moornematoden Dänemarks. *Mémoires de L'Académie Royale des Sciences et des Lettres de Danemark, Copenhague*, 10, 57-310.

Miraeiz, E. 2018. *Aphelenchoidea*. In Ghaderi, R., Kashi, L. & Karegar, A. (Ed.), *Plant-parasitic nematodes in Iran* (pp. 39-112). Shiraz. Marjaeelm & Iranian Society of Nematology.

Mortazavi P. & Pedram M. 2021. Description of *Aphelenchoides hamospiculatus* n. sp. (Aphelenchoidea: Aphelenchoididae) from Golestan province, north Iran. *Nematology*, 23, 201-213.

Nickle W.R. 1970. Description of Entaphelenchidae fam. n., *Roveaphelenchus jonesi* gen. n., sp. n., and *Sheraphelenchus entomophagus* gen. n., sp. n. (Nematoda: Aphelenchoidea). *Proceedings of the Helminthological Society of Washington* 37(1), 105-109.

Nunn G.B. 1992. *Nematode molecular evolution: an investigation of evolutionary patterns among nematodes based upon DNA sequences*. (Doctoral dissertation, University of Nottingham, UK).

Nylander J.A. 2004. *MrModeltest v2*. Evolutionary Biology Centre, Uppsala University, Uppsala. Sweden.

Paramonov A. A. 1964. *Fundamentals of phytohelminthology. Vol. II. Taxonomy of phytonematodes*. Moscow, Nauka (In Russian).

Pedram M. 2017. *Cryptaphelenchus varicaudatus* n. sp. (Rhabditida: Ektaphelenchinae) from Tehran province, Iran. *Journal of Nematology* 49(2), 223-230.

Pedram M., Pourjam E., Ye Y., Atighi M.R., Robbins R.T. & Ryss A. 2011. Description of *Bursaphelenchus mazandaranense* sp. n. (Nematoda: Parasitaphelenchidae) from Iran. *Russian Journal of Nematology* 19, 121-129.

Pedram M., Kanzaki N., Giblin-Davis R.M. & Pourjam E. 2018. A molecular phylogenetic approach for unravelling the taxonomic status of *Basilaphelenchus persicus* n. gen. n. sp. (Aphelenchoididae: Tylaphelenchinae). *Nematology* 20(6), 567-582.

Rambaut A. & Drummond A.J. 2009. *Tracer version 1.5*. UK.

Romaniko, V.I. 1966. Two new species of plant nematodes from wheat. *Zoologichesky Zhurnal*, 45, 929-931.

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

Rühm W. 1956. Die Nematoden der Ipiden. *Parasitologische SchriftenReihe* 6, 1-435.

Scognamiglio A. 1974. *Aprutides guidettii* n. sp. (Nematoda: Aphelenchoididae). *Bolletino del Laboratorio di Entomologia Agraria 'Filippov Silvestri'* 31, 17-21.

Skarbilovich T.S. 1947. Revision of the systematics of the family Anguillulinidae Baylis and Daubney, 1962. *Doklady Akademii Nauk SSSR* 57, 307-308.

Slankis, A. 1967. [*Aphelenchoides macromucrons* n. sp. (Tylenchida) from *Ips typographus* L.]. *Materialy nauchnoi Konferentsii Vsesoyuznogo Obshchestra Gel'mintologov* (1966) 5, 279-282. (In Russian)

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30(12), 2725-2729.

Whitehead A.G. & Hemming J.R. 1965. A comparison of some quantitative methods for extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55(1), 25-38.

Xue Q., Slonim O., Bucki P., Mendel Z., Protasov A., Golan O., Vieira P. & Miyara S.B. 2019. Diversity and distribution of nematodes associated with bark beetles in Israel. *Nematology* 21, 875-8.