

New data on four plant parasitic and plant associated nematodes in Southern Khorasan province, eastern Iran

Mehdi Behdani¹, Esmat Mahdikhani-Moghadam¹ and Majid Pedram²

¹Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

²Department of Plant Pathology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
e-mail: mahdikhani-e@um.ac.ir

Accepted for publication 12 April 2024

Summary. Four species of plant parasitic and plant-associated nematodes were recovered from soil samples collected from Southern Khorasan province, eastern Iran. These species were characterised using both morphological and molecular approaches. The first species, *Xiphinema persicum*, has a wide distribution in Southern Khorasan province, and was recovered from different localities in association with barberry plants showing growth reduction, discoloration and general weakness syndrome with a relatively high population density. Newly recovered populations of this species were characterised by female body length 2036.0-3074.5 µm long having offset lip region, odontostyle 80.0-95.8 µm long, odontophore 38.5-49.5 µm long, and conical tail with a wide, less differentiated bulge at tip. The present results showed this species could be regarded as a potential pest in eastern Iran. The second species belonged to *X. babaii*, and had female body length of 2032-2224 µm, having offset lip region, odontostyle 83-88 µm long, odontophore 45-50 µm long, and conical tail 23.5-32.0 µm long. Its occurrence in eastern Iran is a new record, extending its geographical distribution area outside western Iran. The recovered population of this species was in morphological and morphometric agreement with the type population. The third species, *Aphelenchoides smolae*, was recovered from two localities and its importance as a potential threat to saffron production was discussed. The Iranian populations of this species were characterised by female body 503-895 µm long, stylet 11.0-13.8 µm long with small basal swellings, postvulval uterine sac relatively long, tail conical with a mucro having warty surface and spicules 22.5-28.7 µm long in median line, and had no remarkable differences compared to the type population. It was associated with saffron corms and is reported from Iran for the first time. The last species belongs to *Ditylenchus persicus*. The Southern Khorasan population of this species was characterised by its female body 622-770 µm long having continuous lip region, thin stylet 7.0-8.5 µm long, median bulb present, terminal bulb pyriform having short overlap with intestine, postvulval uterine sac 24-30 µm long and conical tail with rounded or dull end. It is in morphological and morphometric agreement with the type population and has recovered from the rhizosphere of saffron. The observed difference in the status of pharyngeal bulb of this population in comparison with the type population is discussed. The phylogenetic analyses of the recovered species were performed using D2-D3 sequences of 28S rDNA and the resulted topologies were discussed.

Key words: *Aphelenchoides smolae*, D2-D3, *Ditylenchus persicus*, new record, taxonomy, *Xiphinema persicum*, *X. babaii*.

Nematodes of the family Longidoridae Thorne, 1935 are ectoparasites of higher plants and besides direct damage, could transmit some species of plant pathogenic viruses and/or retain them in the field (Taylor & Brown, 1997; Decraemer & Robbins, 2007; Cai *et al.*, 2020a). During the last five years, several species of the family have been reported or

described from Iran (Gharibzadeh *et al.*, 2018; Mobasseri *et al.*, 2019, 2022; Bakhshi Amrei *et al.*, 2020, 2022; Jahanshahi Afshar *et al.*, 2021; Asghari *et al.*, 2023; Naghavi *et al.*, 2022; Pour Ehtesham *et al.*, 2023). In the last five years, the *Xiphinema americanum*-group of genus *Xiphinema* Cobb, 1913 has been enriched by adding three species *X.*

primum Mobasser *et al.*, 2019; *X. persicum* Jahanshahi Afshar *et al.*, 2021; and *X. babaii* Naghavi *et al.*, 2022, from Iran. Based on currently available data, these three species have only been reported from north and northwestern Iran.

The genus *Ditylenchus* Filipjev, 1936 has enriched in the last five years by adding the species *D. azarbaijanensis* Khakbaz *et al.*, 2021; *D. acantholimonis* Aliverdi *et al.*, 2022; *D. paraoncogenus* Hashemi *et al.*, 2022 and *D. pedrami* Azimi & Abdolkhani, 2022 originally described from Iran. Again, there are no data on the occurrence of these species in other localities except their type locality.

In the last five years, several species of the genus *Aphelenchoides* Fischer, 1894 have been added to the genus: *A. giblindavisi* Aliramaji *et al.*, 2018; *A. primadentus* Mobasser *et al.*, 2018, *A. kheirii* Golhasan *et al.*, 2018, *A. tabarestanensis* Golhasan *et al.*, 2019; *A. hamospiculatus* Mortazavi & Pedram, 2020 and *A. persicus* Aliramaji *et al.*, 2023. During the last decade, few studies have focused on identification of plant parasitic and plant associated nematodes in eastern Iran. *Ektaphelenchus berbericus* Alvani *et al.*, 2016 and *Basiria birjandiensis* Alvani *et al.*, 2016 are species that have been originally described from the region.

The Southern Khorasan and Razavi Khorasan provinces are located in eastern Iran. These regions have different climates, yielding on cultivation of various agricultural and horticultural crops. The most important agricultural products in this area include saffron, barberry, jujube, and pistachio. Recently, a project was carried out to identify nematodes associated with bulbous and other cultivated plants in these areas, and as the result, some species of plant parasitic and plant associated

nematodes were recovered. Thus, the aims of present study are to characterise four recently recovered nematode species from these areas using both traditional and molecular tools.

MATERIALS AND METHODS

Nematode samples. Several soil samples were collected from the saffron, garlic, narcissus, barberry, walnut and pistachio fields of two Southern and Razavi Khorasan provinces. The studied nematode populations were recovered from 10 soil samples with the information of the associated plants and GPS information presented in Table 1. Soil samples were transferred to the nematology laboratory of Ferdowsi University, and stored in cool condition until used to extract the nematode specimens. Nematodes were extracted using centrifuge (Jenkins, 1964) and tray (Whitehead & Hemming, 1965) methods. The long-body sized species e.g. longidorids were directly extracted using two 20 and 60 mesh (US standard mesh numbers, equal to 850 and 250 μm sized openings, respectively) sieves. The extracted specimens were transferred into the dry glycerin (De Grisse, 1969) and permanent slides were prepared for morphological study.

DNA extraction, PCR and sequencing. In order to extract genomic DNA, a single female nematode of each of the recovered populations was transferred to a drop of distilled water by a sterilised needle. After studying of the specimen under temporary slide, it was transferred to a small drop of TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0) on a clean glass slide and crushed using a clean cover slip and a plastic pipette tip. The suspension was collected by adding 20 μl TE buffer. Two DNA samples for

Table 1. Species names, locality, associated plants, GPS data and isolate code of studied taxa in present study.

No	Species	Locality	Isolate code	Associated plant	GPS coordinates
1	<i>Xiphinema persicum</i>	Khezri	PL91	Pistachio	34°0.894' N, 58°49.615' E
2	<i>X. persicum</i>	Qaen	PL83	Pistachio	33°52.4741' N, 59°11.6569' E
3	<i>X. persicum</i>	Sarayan	PL117	Pistachio	33°54.348' N, 57°28.005' E
4	<i>X. persicum</i>	Sarayan	PL121	Saffron	33°52.294' N, 58° 25.415' E
5	<i>X. persicum</i>	Darmian	X27	Barberry	32°55.965' N, 59°55.240' E
6	<i>X. persicum</i>	Birjand	X19	Walnut	32°47.3841' N, 59°15.9201' E
7	<i>X. babaii</i>	Birjand	X17	Walnut	32°49.7641' N, 59°09.1287' E
8	<i>Aphelenchoides smolae</i>	Sarayan	SS66	Saffron	33°41.169' N, 58°22.671' E
9	<i>A. smolae</i>	Ferdous	SS41	Saffron	34°13.931' N, 58°6.663' E
10	<i>Ditylenchus persicus</i>	Birjand	X16	Saffron	32°46.4415' N, 59°18.6771' E

each of the species *Aphelenchoides smolae* Cai *et al.*, 2020 and *Xiphinema babaii*, four DNA samples for *X. persicum* and one DNA sample for *Ditylenchus persicus* Esmaili *et al.*, 2017 were prepared in this way. The DNA samples were stored at -20°C until PCR. The primers for amplification of D2-D3 of 28S rDNA were forward D2A (5'-ACA AGT ACC GTG AGG GAA AGT - 3') and reverse D3B (5'-TCG GAA GGA ACC AGC TAC TA - 3') (Nunn, 1992). The PCR reaction to amplify this genomic locus was performed with the following cycles: one cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30s, annealing temperature of 52°C for 1 min, 72°C for 1 min, and finally one cycle of 72°C for 10 min. The successfully amplified fragments were sequenced using the primers used for PCR and deposited into the GenBank database under the accession numbers presented in the trees.

Phylogenetic analyses. The newly generated sequences for four aforementioned species were checked/trimmed using Chromas (<http://www.technelysium.com.au/chromas.html>). The edited sequences were compared with other relevant sequences available in GenBank database using the BLAST homology search program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Several sequences with different degree of identity values were downloaded for phylogenetic analyses of each species. Three datasets were prepared for phylogeny of each genus and the newly generated sequences as well as outgroup sequences (according to previously published data) were included. The LSU datasets including Aphelenchoidea Fuchs, 1937 and Anguinidae Nicoll, 1935 sequences were aligned using Clustal X2 (<http://www.clustal.org/>) and manually edited using MEGA7 (Kumar *et al.*, 2016). The LSU dataset of *Xiphinema americanum*-group spp. was aligned using the online version of MAFFT version 7 (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley, 2013). The Gblocks program (http://phylogeny.lirmm.fr/phylo.cgi/one_task.cgi?task_type=gblocks) with all three less stringent parameters was used for post-editing of this alignment, *i.e.*, to eliminate poorly aligned regions or divergent positions. The best substitution model for datasets was selected using the Akaike information criterion (AIC) by using PAUP*/MrModeltest v2.2 (Nylander, 2004). The Akaike-supported model, a general time reversible model, including a gamma distribution for rates across sites and a proportion of invariant sites (GTR + G + I) was used in LSU phylogeny of three datasets. The Bayesian analysis was done using MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003)

and a random starting tree, running the chains for 2×10^6 generations for two Aphelenchoidea and Anguinidae datasets and 3×10^6 generations for the third dataset. After discarding burnin samples, the remaining samples were maintained for further analyses. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was applied to compute the posterior probabilities of the phylogenetic tree (Larget & Simon, 1999) using the 50% majority rule. The phylogenetic trees were visualised using Dendroscope v.3.2.8 (Huson & Scornavacca, 2012) and were digitally drawn in CorelDRAW software version 17.

RESULTS

Xiphinema persicum Jahanshahi Afshar, Pedram & Mobasseri, 2021 (Fig. 1 A-D)

Measurements. See Table 2.

Female. Body long, slender, C- or open G-shape after killing by heat. Cephalic region separated from the rest of the body by a sharp constriction. Amphidial fovea stirrup-like, its slit wide. Odontostyle 1.7 to 2.0 times longer than odontophore, the latter with moderately developed flanges. The guiding ring double, the fixed ring 66.5-85.0 μm distant from the anterior body end. Pharyngeal bulb about 3.5 times corresponding body diameter long with three nuclei, the dorsal nucleus (DN) larger, at 17.0-19.5% of bulb length and two ventrosublateral nuclei (S1N) at 51-59.5% of the bulb, cardia semicircular, intestine simple and prerectum hard to observe. Reproductive system didelphic-amphidelphic, both branches about equally developed, ovaries reflexed and contain symbiont bacteria, *pars dilatata oviductus* poorly developed, uteri short, vagina 16.0 to 17.5 μm long with a chamber-like structure in most specimens, *pars proximalis vaginae* 8.0 to 9.5 μm high and 10.0 to 12.5 μm wide and vulva a transverse slit. Tail conical, its dorsal side convex and ventral side straight to slightly concave, tip of the tail rounded, appearing as a wide bulge.

Male. Not found.

Juveniles. Not found.

Remarks. This species was described by Jahanshahi Afshar *et al.* (2021) from Semnan province in association with cypress and pistachio trees. The morphological and morphometric characters of the Southern Khorasan populations fit well with those given for the type and second population of the species reported in the description. During the present study, it was recovered from six

Table 2. Morphometrics of *Xiphinema persicum* females recovered from the rhizosphere of saffron, barberry and pistachio from Southern Khorasan province and data of the type population. All measurements are in μm and in the form: mean \pm S.D. (range).

Population Character	PL83	PL117	X19	X27	PL121	PL91	Type population (Jahanshahi Afshar <i>et al.</i> , 2021)
n	10	7	6	5	5	5	18
L	2601.1 \pm 126.4 (2382.2-2765.5)	2797.9 \pm 209.7 (2546.6-3074.5)	2480.0 \pm 292.3 (2036.2-2886)	2387.9 \pm 302.9 (2115.0-2796.6)	2740.7 \pm 238.6 (2367-2960.0)	2603 \pm 211 (2382-2888)	2494 \pm 153 (2233-2736)
a	94.5 \pm 4.3 (87.9-100.7)	86.7 \pm 11.6 (64.1-96.7)	70.8 \pm 11.1 (52.0-81.0)	71.4 \pm 9.2 (60.4-79.2)	76.3 \pm 10.3 (61.9-84.1)	90.7 \pm 7.0 (82.7-99.0)	91.7 \pm 6.3 (82.0-102.0)
b	7.4 \pm 0.3 (6.9-7.8)	8.2 \pm 1.0 (7.1-9.6)	9.3 \pm 1.7 (8.1-12.6)	7.1 \pm 0.8 (6.3-8.1)	7.9 \pm 0.8 (7.2-9.0)	7.2 \pm 0.6 (6.4-7.7)	7.6 \pm 0.8 (5.6-8.4)
c	82.8 \pm 2.1 (78.8-85.7)	86.8 \pm 12.1 (68.4-102.3)	76.2 \pm 6.4 (68.2-85.7)	70.3 \pm 13.1 (60.8-89.6)	81.4 \pm 6.7 (74.0-88.4)	85.9 \pm 4.8 (80.4-91.4)	87.2 \pm 6.0 (76.8-97.7)
c'	1.8 \pm 0.1 (1.6-1.9)	1.7 \pm 0.1 (1.6-1.8)	1.7 \pm 0.1 (1.5-1.9)	1.8 \pm 0.3 (1.4-2.1)	1.7 \pm 0.1 (1.5-1.8)	1.7 \pm 0.1 (1.6-1.8)	1.6 \pm 0.1 (1.5-1.8)
V	53.7 \pm 0.7 (52.4-54.5)	55.0 \pm 0.8 (54.3-56.3)	53.8 \pm 1.5 (51.8-55.8)	54.7 \pm 0.8 (54.2-55.9)	53.6 \pm 1.6 (51.9-55.8)	52.8 \pm 0.3 (52.5-53.2)	54.0 \pm 0.8 (52.8-55.5)
Lip region height	5.0 \pm 0.3 (4.5-5.5)	4.6 \pm 0.3 (4.4-5.1)	4.3 \pm 0.3 (4.1-4.8)	4.5 \pm 0.4 (4.0-4.9)	4.4 \pm 0.4 (4.1-4.9)	4.5 \pm 0.4 (4.0-4.9)	4.5 \pm 0.4 (3.5-5.0)
Lip region width	10.3 \pm 0.3 (9.7-10.6)	10.5 \pm 1.2 (9.5-13.2)	9.1 \pm 0.7 (8.2-10.3)	9.5 \pm 0.3 (9.1-9.8)	10.4 \pm 0.9 (9.7-11.6)	9.7 \pm 0.2 (9.4-9.8)	9.9 \pm 0.4 (9.5-11.0)
Odontostyle	90.7 \pm 1.8 (87.8-94.0)	88.5 \pm 4.2 (80.8-92.2)	84.9 \pm 2.5 (82.6-89.7)	84.0 \pm 2.7 (80.0-85.8)	90.1 \pm 3.9 (87.6-95.8)	90.4 \pm 1.1 (89.4-92.0)	84.3 \pm 1.3 (82-87)
Odontophore	47.6 \pm 1.2 (45.7-49.5)	47.6 \pm 1.1 (45.3-48.8)	44.6 \pm 1.3 (42.8-46.6)	41.2 \pm 3.1 (38.5-44.6)	46.0 \pm 2.9 (42.0-48.9)	46.0 \pm 2.9 (42.0-48.9)	46.2 \pm 1.8 (44-49)
Total stylet	138.3 \pm 1.4 (135.1-140.1)	136.2 \pm 3.9 (129.5-140.5)	129 \pm 2 (127.6-132.4)	125.2 \pm 5.0 (118.9-130.4)	136.0 \pm 5.7 (129.6-143.0)	138.3 \pm 1.4 (135-140)	130.6 \pm 2.3 (126-135)
Guiding ring from anterior end	81.7 \pm 2.9 (76.5-85.0)	77.2 \pm 4.0 (72.0-82.4)	72.9 \pm 1.8 (69.9-75.7)	67.9 \pm 1.0 (66.5-68.7)	75.8 \pm 5.2 (70.8-83.0)	80.7 \pm 3.0 (77.3-84.6)	73.3 \pm 2.0 (70.0-76.5)
Pharynx	351.3 \pm 12.0 (337.3-368.7)	343.8 \pm 34.6 (288.5-383.4)	274 \pm 31 (228.6-305.8)	336.6 \pm 8.3 (325.2-344.3)	349.0 \pm 23.5 (329-376)	362.3 \pm 28.5 (337.3-395.4)	327.6 \pm 41.6 (270-436)
Anterior end to vulva	1397.6 \pm 63.3 (1295.6-1481.3)	1538.7 \pm 122.8 (1394.4-1716.2)	1332 \pm 141.4 (1122.3-1549.8)	1306.8 \pm 185.7 (1148.5-1564.0)	1466.5 \pm 113.6 (1320-1589)	1375.0 \pm 112.5 (1251-1522)	1343 \pm 80 (1205-1464)
Body width at mid. body	27.5 \pm 0.8 (26.5-29.0)	32.8 \pm 5.3 (27.4-44.0)	35.3 \pm 3.6 (32.0-40.2)	34.2 \pm 7.8 (26.7-41.6)	36.6 \pm 7.2 (28.5-46.0)	28.7 \pm 1.6 (27.1-30.8)	27.3 \pm 1.9 (24-31)
Body width at anus	17.8 \pm 0.6 (17-19)	18.9 \pm 2.7 (16.4-24.7)	18.4 \pm 1.2 (17-20)	19.1 \pm 3.1 (15.9-22.1)	20.1 \pm 1.5 (18.3-21.5)	18.1 \pm 0.6 (17.3-18.7)	17.8 \pm 1.0 (16.5-20.0)
Body width at base of pharynx	23.9 \pm 0.3 (23.4-24.3)	26.2 \pm 6.6 (15.8-38.1)	27.9 \pm 2.8 (25.4-32.6)	29.3 \pm 6.1 (23.9-36.1)	24.4 \pm 0.7 (23.4-25.0)	23.8 \pm 0.8 (23.0-24.8)	24.3 \pm 1.3 (23-27)
Rectum	16.6 \pm 2.0 (14.2-18.4)	15.9 \pm 1.4 (14.6-17.7)	-	-	-	-	18.0 \pm 1.9 (15-21)
Tail	31.4 \pm 1.5 (28.4-32.7)	32.7 \pm 4.4 (26.3-41.2)	31.8 \pm 1.4 (29.9-33.7)	34.3 \pm 3.9 (31.2-40.0)	33.7 \pm 1.9 (32-36)	30.3 \pm 1.9 (28.4-32.3)	29.0 \pm 1.4 (27-32)
Hyaline region of tail	6.4 \pm 0.7 (5.3-7.2)	9.0 \pm 0.7 (8.2-9.8)	6.9 \pm 0.7 (5.9-7.8)	8.3 \pm 0.9 (7.3-9.5)	8.5 \pm 0.7 (7.8-9.4)	6.7 \pm 0.9 (5.5-7.4)	7.4 \pm 0.9 (5.5-8.5)

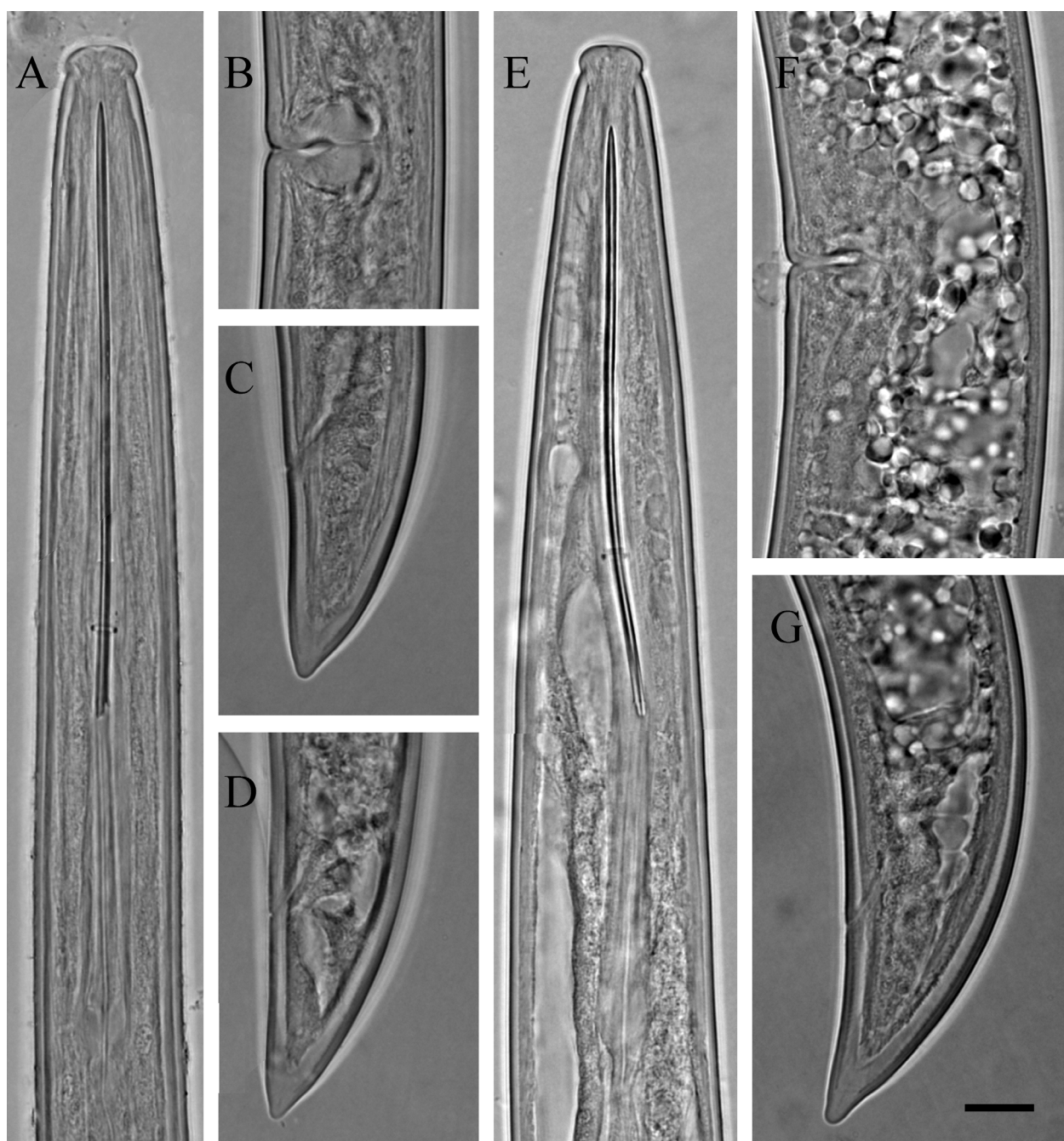


Fig. 1. Light microphotographs of Southern Khorasan population of *Xiphinema persicum* and *X. babaii* (female). A-D: *X. persicum*; E-G: *X. babaii*; A & E: Anterior body region; B & F: Vulval region; C, D & G: Tail (Scale bar =10 μ m).

localities in both provinces in association with different plants (Table 1). The species is more prevalent in barberry fields, and most shrubs in infested fields showed general weakness, chlorosis, leaf fall, white fruits, fruit drop and in some cases, slow decay. This species could be a potential threat to barberry production in this region, and this is an open field for future researches.

***Xiphinema babaii* Naghavi, Niknam & Vazifeh, 2022
(Fig. 1 E-G)**

Measurements. See Table 3.

Female. Medium-sized, C- to open G-shaped after heat killing. Lip region relatively flattened at frontal end, separated from the rest body by a

Table 3. Morphometrics of *Xiphinema babaii* females recovered from the rhizosphere of walnut from Southern Khorasan province and data of the type population. All measurements are in μm and in the form: mean \pm S.D. (range).

Character	Population	Southern Khorasan	Type population (Naghavi <i>et al.</i> , 2022)
n		10	6
L		2166 \pm 69.3 (2032-2224)	2100 \pm 100 (2000-2100)
a		63.5 \pm 8.6 (45.6-71.2)	60.6 \pm 0.2 (56-64)
b		7.1 \pm 0.8 (6.2-8.1)	7.1 \pm 0.2 (6.2-8.7)
c		73 \pm 6 (64.9-85.7)	62.6 \pm 0.4 (61-65)
c'		1.5 \pm 0.2 (1.1-1.7)	1.7 \pm 0.1 (1.6-1.8)
V		55.4 \pm 1.7 (52.1-57.7)	53.4 \pm 0.3 (52-54)
Lip region height		4.3 \pm 0.3 (3.9-4.7)	-
Lip region width		9.5 \pm 0.4 (8.9-10.3)	9.3 \pm 0.1 (8.7-10.0)
Odontostyle		85.0 \pm 1.5 (83-88)	84.3 \pm 0.8 (82.5-88.0)
Odontophore		48.0 \pm 1.5 (45-50)	49.0 \pm 0.1 (50.0-55.5)
Total stylet		133 \pm 2 (129.7-135.1)	134.8 \pm 1.5 (133-137)
Guiding ring from anterior end		73.0 \pm 2.3 (68.8-76.3)	73.7 \pm 0.5 (70-78)
Pharynx		308.0 \pm 30.8 (264.5-349.7)	308.7 \pm 16.5 (281-342)
Anterior end to vulva		1199.4 \pm 38.8 (1148.5-1273.3)	-
Body width at mid. body		34.9 \pm 6.0 (28.9-48.3)	34.6 \pm 0.5 (31.5-37.0)
Body width at anus		20.3 \pm 3.7 (17.9-29.4)	20.6 \pm 1.3 (20-25)
Body width at base of pharynx		25.9 \pm 1.2 (24.1-26.9)	29.7 \pm 1.3 (27-32)
Rectum		21.0 \pm 3.2 (16.9-23.9)	-
Tail		29.9 \pm 2.7 (23.5-32.0)	43.2 \pm 0.1 (42.0-43.5)
Hyaline region of tail		9.0 \pm 0.3 (8.3-9.4)	-

constriction. Amphidial fovea cup-shaped. Odontostyle 1.7 times longer than odontophore, guiding sheath 5-6 μm long depending on the resting status of odontostyle. Pharyngeal bulb three times of corresponding body width, occupying about 24% of the pharynx with three nuclei, the larger dorsal nucleus (DN) at 9.5 to 12.0% and two smaller ventrosublateral nuclei (SIN) at 59.5 to 62% of the bulb, cardia 2.5 \times 7.4-6.4 \times 11.6 μm in size, prerectum about 6-11 times, and rectum about 1.03-1.5 times anal body width long. Reproductive system didelphic-amphidelphic, each branch composed of a reflexed ovary containing endosymbiont bacteria, less developed oviductus, sphincter, short uterus, ovejector, vagina about 17 μm long and vulva a transverse slit. Tail conical, dorsally convex, ventrally straight to slightly concave, its tip rounded or sometimes with a less developed mucro-like differentiation with rounded tip.

Male. Not found.

Juveniles. Not found.

Remarks. *Xiphinema babaii* was isolated and described from the rhizosphere of rose in Eastern Azerbaijan province of Iran (Naghavi *et al.*, 2022). It is only currently known from the type locality. Present data extends the geographical distribution of the species into eastern Iran. For the associated plant

and GPS data of occurring point, see Table 1. The Southern Khorasan population of the species had greater c (64.9-85.7 vs 61-65) and shorter tail (23.5-32.0 vs 42-45 μm) while comparing with the type population. The new range in tail length of this species is herein reported.

***Aphelenchoides smolae* Cai, Gu, Wang, Fang & Li, 2020
(Fig. 2)**

Measurements. See Table 4.

Female. Body slender, slightly tapering towards both extremities, more so towards distal end by having a uniformly narrowing tail, slightly ventrally arcuate after heat-killing. Lip region separated from the rest body by a depression. Cuticle annuli fine, lateral fields with four longitudinal incisures. Stylet fine, its conus about 47% of the total length. Procorpus cylindrical, median bulb well-developed, spherical to slightly oval with developed valve plates, the pharyngeal junction with intestine immediately after the median bulb, the dorsal pharyngeal gland's lobe three to four times body width long, overlapping intestine dorsally, intestine simple, rectum and anus functional. Nerve ring encircling anterior end of intestine and pharyngeal glands. Secretory-excretory pore posterior to

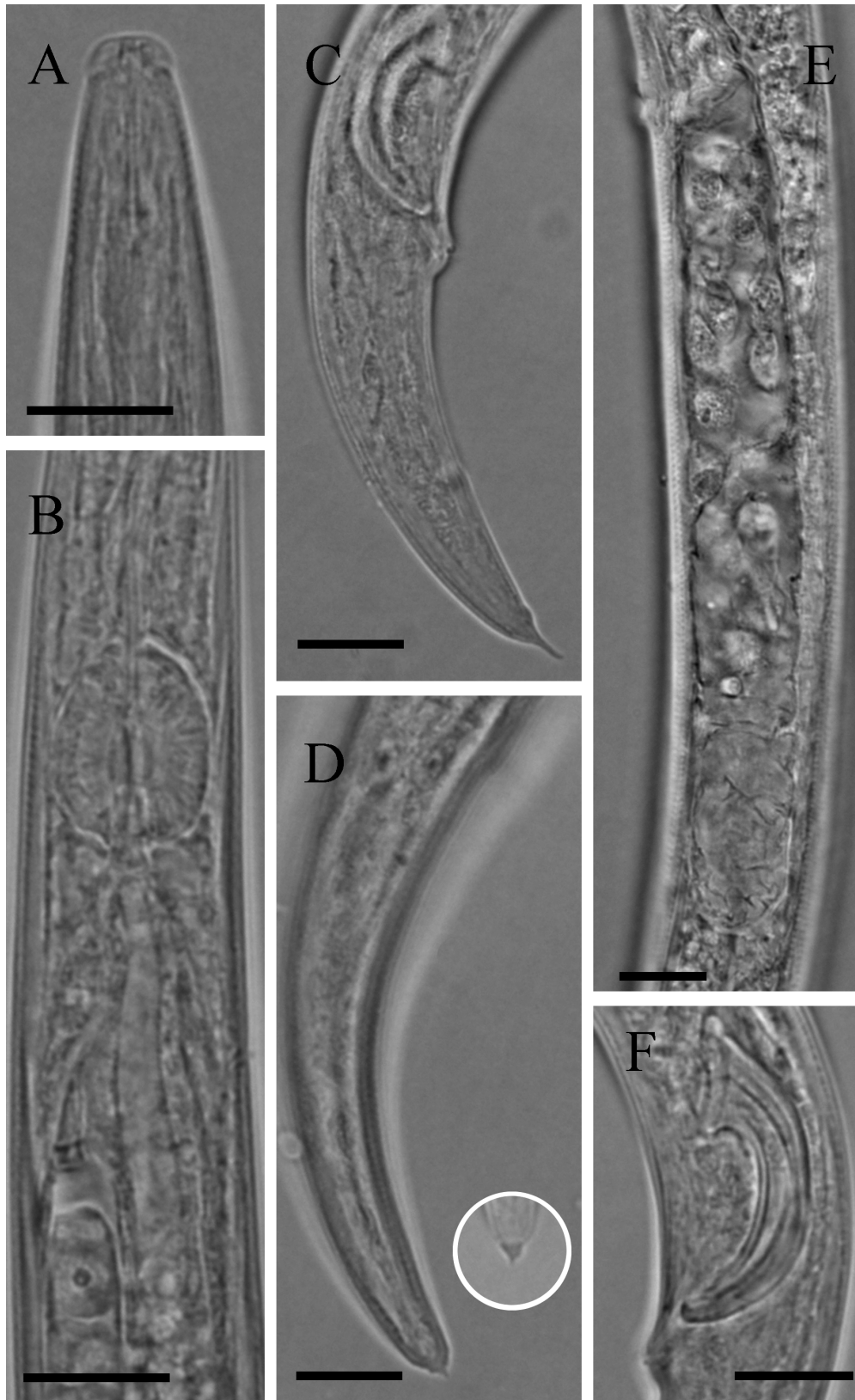


Fig. 2. Light microphotographs of Southern Khorasan population of *Aphelenchoides smolae*. (A, B, D, E, female; C, F: male). A: Anterior body region; B: Part of pharynx showing metacarpus; C & D: Tail (indication shows tail tip); E: Postvulval uterine sac; F: Spicules (Scale bar =10 μ m).

Table 4. Morphometrics of *Aphelenchoides smolae* recovered from the rhizosphere of saffron from Southern Khorasan province and data of the type population. All measurements are in μm and in the form: mean \pm S.D. (range).

Character	Southern Khorasan populations				Type population (Cai <i>et al.</i> , 2020b)	
	Females (SS66)	Males (SS66)	Females (SS41)	Males (SS41)	Females	Males
n	9	7	5	4	20	20
L	731.1 \pm 84.1 (599-895)	627 \pm 129 (505-840)	687.3 \pm 149.7 (503-878)	602 \pm 65 (528-670)	826 \pm 74 (672-1002)	785.0 \pm 43.3 (683-844)
a	28.7 \pm 3.1 (23.4-33.9)	29.0 \pm 4.7 (24.0-37.0)	30.9 \pm 2.1 (28.4-34.3)	28.1 \pm 3.0 (25.2-32.1)	31 \pm 2 (28.3-37.8)	31.8 \pm 1.5 (30.0-36.0)
b	9.4 \pm 0.7 (8.4-10.4)	9.2 \pm 1.3 (7.8-11.4)	9.8 \pm 1.7 (8.2-12.2)	9.1 \pm 0.5 (8.3-9.5)	10.4 \pm 0.7 (9.2-11.7)	10.1 \pm 0.5 (9.2-11.0)
b'	4.4 \pm 0.5 (4.1-5.0)	4.4 \pm 0.2 (4.0-4.5)	4.6 \pm 0.3 (4.3-4.9)	4.4 \pm 0.1 (4.4-4.5)	4.9 \pm 0.4 (4.2-5.8)	4.7 \pm 0.4 (4.2-5.8)
c	15.6 \pm 1.3 (14.2-18.2)	13.8 \pm 1.8 (10.9-16.4)	15.9 \pm 1.3 (14.6-17.9)	15.3 \pm 3.3 (11.8-19.3)	16.0 \pm 1.4 (14.7-21.2)	16.2 \pm 1.1 (14.4-18.6)
c'	3.6 \pm 0.5 (3.1-4.7)	3.0 \pm 0.4 (2.3-3.6)	3.5 \pm 0.2 (3.1-3.7)	2.7 \pm 0.5 (2.3-3.3)	3.7 \pm 0.3 (3.1-4.1)	2.9 \pm 0.2 (2.6-3.4)
V/T	69.0 \pm 0.7 (60.0-70.0)	61.1 \pm 4.2 (55.0-65.7)	69.3 \pm 0.8 (68.7-70.6)	63.2 \pm 1.1 (61.9-64.4)	69.3 \pm 1.4 (67.4-73.9)	58.2 \pm 2.8 (54.1-63.4)
Stylet	13.5 \pm 0.2 (13.3-13.8)	13.3 \pm 0.6 (12.4-14.2)	12.3 \pm 0.9 (11.0-13.5)	13.0 \pm 0.6 (12.3-13.7)	13.6 \pm 0.5 (13.0-14.9)	13.2 \pm 0.4 (12.4-14.0)
Conus	6.1 \pm 0.2 (5.9-6.4)	6.3 \pm 0.4 (5.9-7.9)	5.2 \pm 0.4 (4.7-5.7)	6.1 \pm 0.2 (5.8-6.2)	-	-
m (conus as percent of total stylet)	45.1 \pm 2.3 (42.9-47.9)	47.3 \pm 1.6 (45.4-49.2)	42.2 \pm 1.6 (39.3-43.2)	46.3 \pm 1.3 (45.3-47.7)	-	-
Pharynx	78.1 \pm 4.5 (71.2-85.7)	67.8 \pm 6.6 (56.2-73.7)	69.7 \pm 5.1 (60.7-73.2)	66.3 \pm 6.5 (57.1-72.3)	-	-
Median bulb	69.1 \pm 4.2 (63.8-76.2)	61.7 \pm 7.2 (51.1-73.6)	62.9 \pm 4.3 (55.3-65.4)	59.6 \pm 5.3 (52.1-64.0)	-	-
MB	88.7 \pm 1.7 (84.4-89.7)	91.1 \pm 4.2 (87.5-100.0)	90.3 \pm 0.9 (88.8-91.2)	90.0 \pm 2.5 (87.5-92.9)	-	-
Median bulb width	15.0 \pm 1.1 (13.1-16.5)	12.4 \pm 0.4 (11.9-13.0)	-	12.3 \pm 0.2 (12.2-12.5)	16.6 \pm 1.3 (15.0-21.5)	15.8 \pm 0.6 (14.3-16.7)
Secretory-excretory pore	88.2 \pm 6.7 (78.7-101.1)	82.9 \pm 8.6 (73.9-92.7)	85.1 \pm 11.1 (67.3-96.3)	80.7 \pm 8.4 (73.9-92.7)	96.0 \pm 6.7 (88-120)	97.0 \pm 4.4 (90-105)
Anterior end to vulva	505 \pm 60 (407.2-623.2)	-	475.5 \pm 100.2 (355-605)	-	-	-
Anterior end to anus	684 \pm 81 (557.6-845.4)	-	-	-	-	-
Vulva to anus distance	178.9 \pm 21.3 (150.4-222.2)	-	-	-	-	-
Lip region height	3.5 \pm 0.3 (3.3-3.9)	3.1 \pm 0.1 (3.0-3.2)	3.3 \pm 0.2 (3.1-3.6)	3.2 \pm 0.3 (2.9-3.6)	3.3 \pm 0.2 (3.1-3.6)	3.3 \pm 0.2 (2.8-3.9)
Lip region width	7.5 \pm 0.3 (7.3-7.9)	6.7 \pm 0.3 (6.5-7.0)	7.4 \pm 0.2 (7.1-7.6)	6.8 \pm 0.3 (6.5-7.1)	7.7 \pm 0.5 (7.2-9.4)	7.3 \pm 0.3 (6.8-7.8)
Body width	24.8 \pm 3.3 (20.8-30.4)	21.6 \pm 2.9 (18.1-25.2)	22.3 \pm 4.6 (16.3-28.4)	21.5 \pm 3.0 (18.5-25.2)	-	-
Vulval body width	23.6 \pm 2.4 (20.0-27.4)	-	20.7 \pm 4.2 (15.2-26.0)	-	-	-
Anal body width	13.0 \pm 1.2 (11.5-14.9)	15.4 \pm 2.3 (13.2-18.1)	12.4 \pm 2.4 (9.6-15.7)	15.1 \pm 2.0 (13.7-18.1)	14.1 \pm 1.3 (11.9-16.8)	16.7 \pm 0.6 (15.8-17.4)
Postvulval uterine sac (PUS)	76.8 \pm 12.7 (59.5-97.7)	-	83.0 \pm 18.9 (59.8-106)	-	104 \pm 18 (74-135)	-
PUS/vulval body width	3.3 \pm 0.7 (2.4-4.4)	-	3.8 \pm 0.7 (3.2-4.8)	-	-	-
PUS/vulva to anus distance (%)	43.9 \pm 11.0 (29.0-65.0)	-	46.3 \pm 11.4 (37.3-63.0)	-	50.8 \pm 5.9 (35.7-62.4)	-
Tail length	47.0 \pm 4.8 (41.5-55.1)	45.5 \pm 7.7 (35-56)	42.9 \pm 6.8 (32.2-49.1)	40.1 \pm 4.9 (33.2-44.8)	51.0 \pm 3.5 (45-58)	50.0 \pm 2.7 (44-55)
Spicules (arc)	-	25.9 \pm 2.3 (23.1-28.7)	-	24.5 \pm 2.1 (22.5-27.3)	-	26.0 \pm 1.0 (24.3-27.7)

nerve ring. Genital system monodelphic-prodelphic, consisting of outstretched ovary, in some specimens reflexed at tip, tubular oviduct, rectangular spermatheca full of spheroid sperm in some specimens, crustaformeria, uterus, vagina, transverse vulval slit and developed postvulval uterine sac (PUS) 29-65% of vulva to anus distance, usually full of sperm. Tail conical, dorsally slightly more convex with a warty mucro at tip.

Male. Generally similar to female, except in distal body region shape that is more ventrally bent and genital system. Testis outstretched, the spermatocytes organised in two rows. Spicules paired, typical aphelenchoid, their condylus bluntly rounded, rostrum small, tip of dorsal limb hook-like. The caudal papillae three pairs. The precloacal single papilla (P1) absent, the cloacal pair (P2) at about the same level as cloacal opening, the second pair (P3) posterior to mid-tail and the third pair (P4) close to tail tip. Tail ventrally bent, conical, dorsally more convex, with a sharp mucro.

Remarks. *Aphelenchoides smolae* was isolated and described from medium soil of *Lilium orientalis* bulbs imported from the Netherlands to China (Cai *et al.*, 2020b). The Iranian populations of the species were prevalent in several saffron fields of Southern and Razavi Khorasan provinces (See Table 1 for the information of the associated plants and GPS of the occurring points) and represent the first report of the species from Iran. Iranian populations look very similar to the type population in morphological and morphometric characteristics. However, their PUS is slightly shorter (59.5-106.0 vs 74-135 μm).

***Ditylenchus persicus* Esmaeili, Heydari,
Castillo & Palomares-Rius, 2017
(Fig. 3)**

Measurements. See Table 5.

Female. Body cylindrical, gradually tapering toward both ends, more so toward posterior end by having elongate conical tail. Cuticle with fine transverse annuli, lateral fields with six longitudinal incisures. Lip region low, flat at frontal end, along with the rest of the body. Stylet delicate, its conus about 38% of the total length, knobs rounded. Dorsal pharyngeal gland orifice close to knobs. Procorpus slender, median bulb fusiform with small valve, isthmus narrower than procorpus, basal pharyngeal bulb shortly overlapping intestine, sometimes appearing as a short triangle-like structure projecting into intestine depending on rotation of the bulb. Secretory-excretory pore at level with the anterior end of pharyngeal bulb.

Reproductive system monodelphic, outstretched, extended towards the front of the body, ovary elongated and oocytes arranged in a single row, oviduct tubular, spermatheca elongate-oval, 2.0-2.5 times corresponding body diameter long, crustaformeria consists of four rows, each with four cells, uterus thin-walled, vulva a transverse slit, PUS 0.6 to 1.7 body diameters at the vulva region long. Tail elongate-conical, its distal end slightly bent towards the ventral side, with rounded or dull tip.

Male. Similar to female in general morphology, slightly shorter. Testis outstretched. Spermatocytes arranged in a single row. Spicules tylenchoid, gubernaculum 4-6 μm long. Bursa covering about 80% of the tail. Tail conical, with finely rounded tip.

Remarks. *Ditylenchus persicus* was originally described in association with grape. A stem-like extension projecting into the intestine was illustrated for the species in its original description (Esmaeili *et al.*, 2017a). A closer study of the Figs 2F & G in the original description and observation of the paratype specimens at the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, revealed the so-called stem-like extension is an artefact resulted from the rotation of the pharyngeal bulb having a short overlap over intestine. The information of distribution region and associated plant of presently studied population of the species are given in Table 1. The Southern Khorasan population is in accordance with the type population in most morphological traits, but, has a 24-30 μm long PUS (vs 14-18 μm) compared with the type population.

Molecular analyses and phylogenetic relationships. The amplification of D2-D3 expansion segments of 28S rRNA gene and its sequencing for four isolates of *Xiphinema persicum* yielded four 653-750 nt long amplicons, which were submitted in the GenBank with accession numbers PP051275, PP051276, PP051277, PP051278. These four sequences were identical while aligning. The BLAST search using the longest sequence revealed it has 99.86% identity with the D2-D3 sequence of the type population of *X. persicum* (MT073110). Figure 4 shows the phylogenetic tree reconstructed using the selected sequences of *Xiphinema americanum*-group species. In this tree, the clade of *X. persicum*, including the newly generated sequences from Southern Khorasan and those of the type population, have formed a maximally supported sister clade with several sequences of *X. simile* Lamberti, Choleva & Agostinelli, 1983. The amplification of D2-D3 of 28S rDNA and its sequencing for recovered population of *X. babaii*



Fig. 3. Light microphotographs of Southern Khorasan population of *Ditylenchus persicus*. (A-D, female; E: male). A: Anterior body region; B: Postvulval uterine sac; C: Short overlapping of pharyngeal bulb over intestine; D & E: Tail (Scale bar =10 μ m).

Table 5. Morphometrics of *Ditylenchus persicus* recovered from the rhizosphere of saffron from Southern Khorasan province. All measurements in μm and in the form: mean \pm S.D. (range).

Character	Population		Southern Khorasan population		Type population (Esmaceli <i>et al.</i> , 2017a)	
	Females	Males	Females	Males	Females	Males
n	11	7	15	5		
L	694.4 \pm 47.4 (622-770)	601 \pm 21 (581-639)	784 \pm 84 (635-928)	689.0 \pm 21.5 (670-715)		
a	33.7 \pm 5.4 (28.3-41.6)	37.3 \pm 2.7 (34.3-42.1)	36.4 \pm 4.5 (30.0-46.3)	43.1 \pm 2.3 (39.6-45.0)		
b	5.5 \pm 0.6 (4.3-6.6)	4.7 \pm 0.5 (3.9-5.3)	6.3 \pm 0.7 (5.2-7.9)	5.9 \pm 0.3 (5.5-6.1)		
c	13.7 \pm 0.6 (12.8-14.4)	11.8 \pm 0.9 (10.8-13.0)	13.6 \pm 1.9 (10.6-17.1)	12.1 \pm 1.0 (11.2-13.2)		
c'	3.8 \pm 0.4 (3.2-4.5)	4.4 \pm 0.4 (3.8-5.0)	4.3 \pm 0.6 (3.2-5.3)	5.4 \pm 0.4 (5-6)		
V/T	78.5 \pm 1.7 (77.1-82.2)	42.0 \pm 2.8 (38.1-46.2)	80.5 \pm 2.2 (75.6-83.3)	43 \pm 3 (39.4-47.0)		
Stylet	7.5 \pm 0.5 (7.0-8.5)	7.2 \pm 0.2 (6.8-7.6)	6.2 \pm 0.6 (5-7)	5.7 \pm 0.4 (5-6)		
Conus	2.8 \pm 0.3 (2.3-3.4)	2.5 \pm 0.2 (2.1-2.6)	2.7 \pm 0.3 (2-3)	2.4 \pm 0.4 (2.0-2.8)		
m (conus as percent of total stylet)	38.0 \pm 3.5 (31.1-42.3)	34.6 \pm 2.2 (30.4-36.8)	43.1 \pm 2.6 (40-50)	41.9 \pm 4.1 (36.4-46.7)		
Pharynx	121.6 \pm 5.9 (110.9-129.2)	128.5 \pm 15.4 (110.6-152.3)	124 \pm 12.9 (100-145)	117 \pm 9.5 (109-130)		
Median bulb	47.8 \pm 2.5 (44.0-51.5)	45 \pm 4 (41.0-51.3)	-	-		
MB	39.3 \pm 1.6 (37.4-42.1)	35.6 \pm 5.2 (26.9-39.4)	-	-		
Secretory-excretory pore	99.6 \pm 5.2 (95.4-110.6)	95.3 \pm 6.8 (84.3-104.9)	110.0 \pm 15.4 (83-138)	94 \pm 15 (83-120)		
Anterior end to vulva	564.6 \pm 52.2 (488.2-645.2)	-	-	-		
Anterior end to anus	545.5 \pm 41.9 (488-601)	549 \pm 21 (526-589)	-	-		
Vulva to anus distance	92.7 \pm 9.5 (79.4-104.5)	51.1 \pm 3.5 (47.2-57.5)	94.0 \pm 11.3 (74-115)	-		
Lip region height	2.2 \pm 0.2 (2.0-2.5)	2.2 \pm 0.3 (1.8-2.6)	2.5 \pm 0.4 (2-3)	3.2 \pm 0.2 (3.0-3.5)		
Lip region width	5.8 \pm 0.1 (5.6-6.0)	5.5 \pm 0.3 (5.1-5.9)	5.5 \pm 0.5 (5-6)	5.6 \pm 0.4 (5-6)		
Body width	21.0 \pm 3.6 (13.7-26.7)	16.2 \pm 1.4 (14.7-18.6)	21.6 \pm 1.2 (20-23)	16.0 \pm 0.7 (15-17)		
Vulval body width	19.4 \pm 3.2 (15.8-23.5)	16.8 \pm 1.5 (15.2-18.8)	-	-		
Anal body width	13.6 \pm 2.0 (10.6-16.3)	11.8 \pm 0.9 (10.8-12.9)	13.7 \pm 1.0 (12-15)	10.6 \pm 0.5 (10-11)		
Postvulval-uterine sac (PUS)	27.1 \pm 1.9 (24.0-30.0)	-	16.3 \pm 1.5 (14-18)	-		
PUS/VBW	1.4 \pm 0.2 (1.2-1.7)	-	0.8 \pm 0.1 (0.6-0.9)	-		
PUS/vulva to anus distance (%)	29.5 \pm 3.9 (22.9-35.1)	-	17.6 \pm 3 (12.2-22.5)	-		
Tail length	51.4 \pm 3.3 (46.8-57.5)	51.1 \pm 3.5 (47.2-57.5)	58 \pm 7 (45-68)	57.0 \pm 2.8 (54-60)		
Spicules (arc)	-	16.8 \pm 1.5 (15.2-18.8)	-	16.0 \pm 0.7 (15-17)		
Gubernaculum	-	5.0 \pm 0.7 (4-6)	-	4.3 \pm 0.4 (4-5)		



Fig. 4. Bayesian 50% majority rule consensus tree inferred using the D2-D3 domains of 28S rDNA recently recovered *Xiphinema americanum*-group species from Southern Khorasan and other *Xiphinema* species under the GTR + G + I model. Bayesian posterior probability values of more than 0.50 are given for appropriate clades. New sequences are indicated in bold.

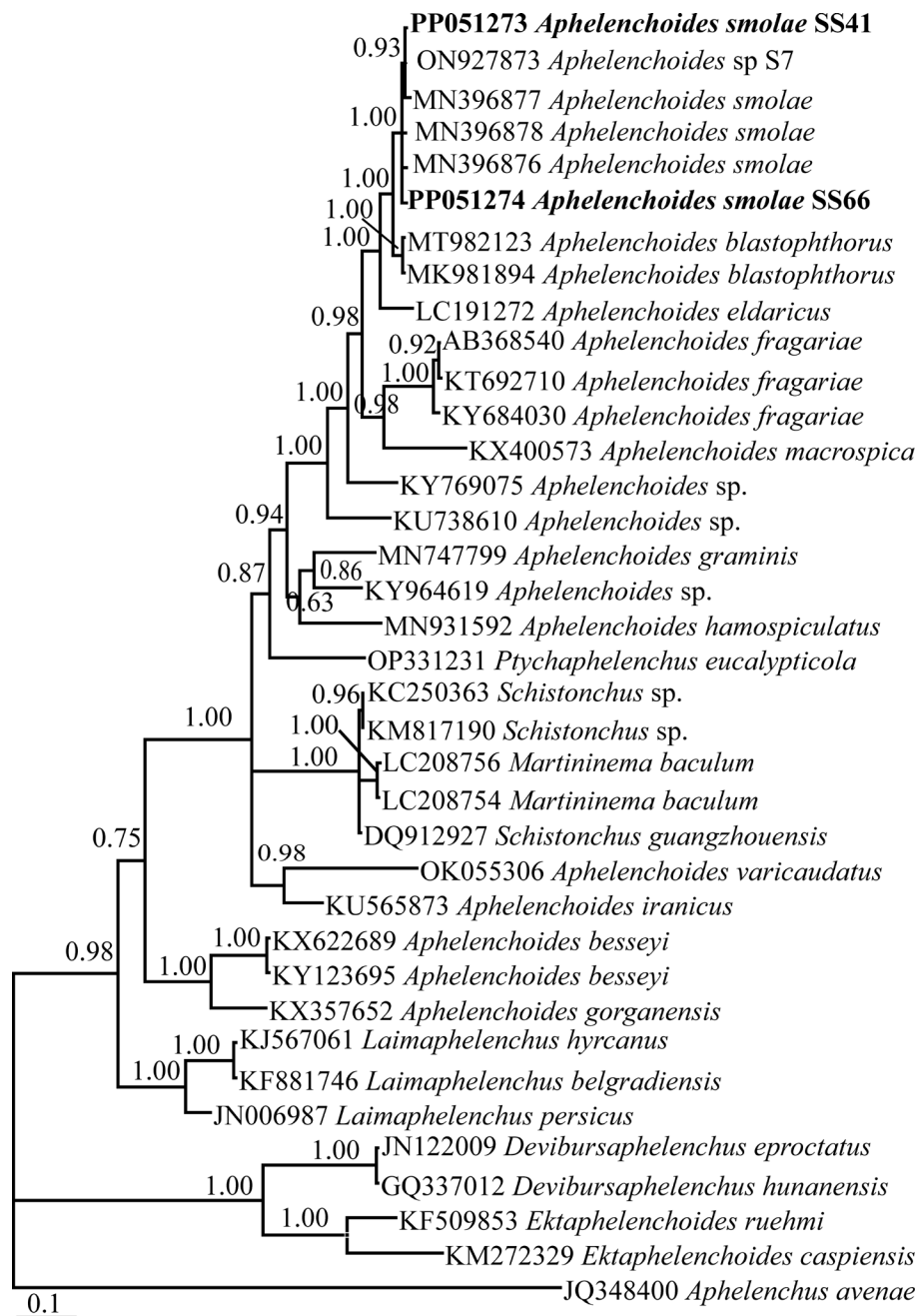


Fig. 5. Bayesian 50% majority rule consensus tree inferred from analysis of the D2-D3 domains of the 28S rDNA sequences of *Aphelenchoides smolae* and other Aphelenchoidea under the GTR + G + I model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. New sequences are indicated in bold.

yielded a 686 nt long sequence (PP051280). The BLAST search using this sequence revealed it has 99.42% identity with a sequence assigned to *X. pachtaicum* (Tulaganov, 1938) Kirjanova, 1951 (AY601607) and 99.13% identity with the original sequence of *X. babaii* (MK957226). The newly generated sequence has occupied a placement inside the clade that includes the original sequence of *X. babaii* (MK957226). This clade further

includes two accession numbers KX263186 assigned to *Xiphinema* sp. and AY601607 assigned to *X. pachtaicum*. Several other sequences of *X. pachtaicum* have distantly located.

The amplification of D2-D3 of 28S rDNA and its sequencing of two isolates of *Aphelenchoides smolae* yielded two 358-657 nt long amplicons with accession numbers PP051273 and PP051274. The BLAST search using PP051273, the longest

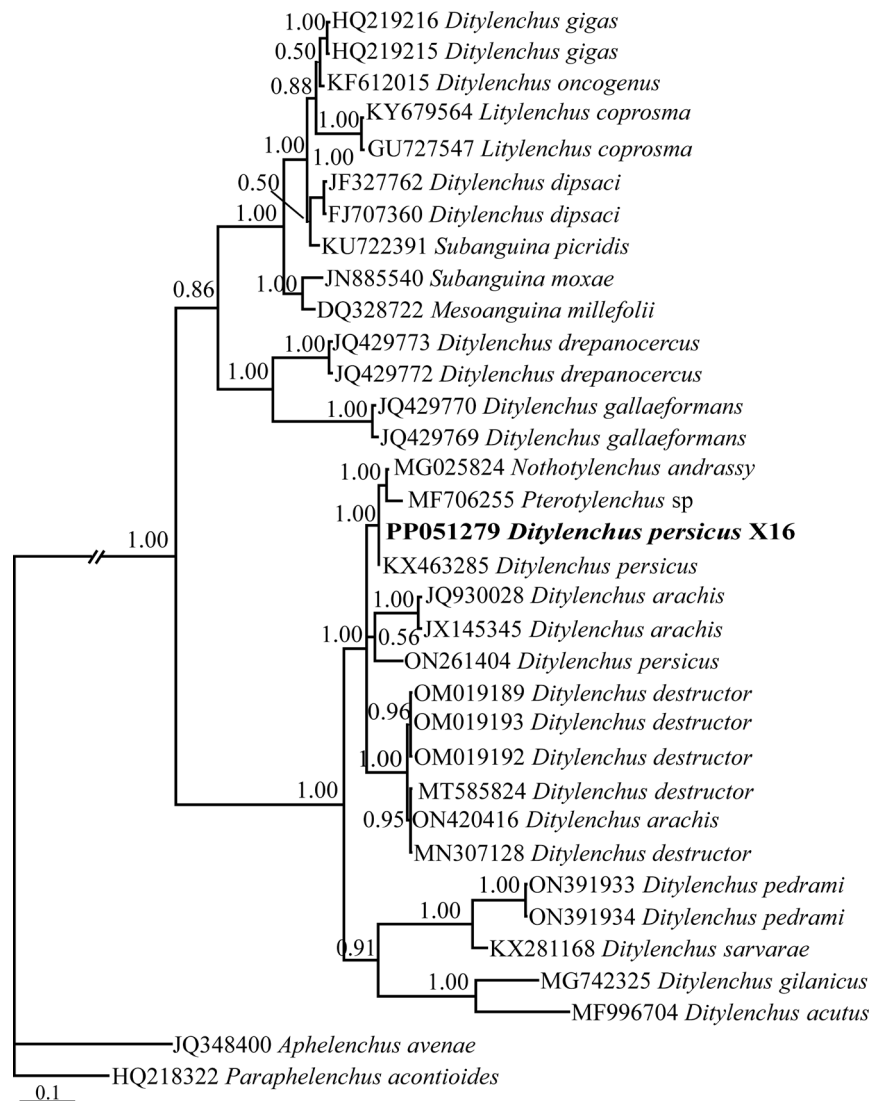


Fig. 6. Bayesian 50% majority rule consensus tree inferred from analysis of the D2-D3 domains of the 28S rDNA sequences of *Ditylenchus persicus* and other Anguinidae under the GTR + G + I model. Bayesian posterior probability values more than 0.50 are given for appropriate clades. New sequence is indicated in bold.

sequence, revealed it has 99.23% identity with original D2-D3 sequence of *A. smolae* (MN396878). In the reconstructed 28S tree using selected sequences of Aphelenchoidea (Fig. 5), the newly generated sequences for Iranian populations of *A. smolae* have occupied a placement inside the clade that included original sequences of the species (MN396876, MN396877, MN396878) and the sequence ON927873 assigned to *Aphelenchoides* sp., most probably belongs to this species.

The amplification of 28S rDNA D2-D3 and its sequencing for the recovered population of *Ditylenchus persicus* yielded a 754 nt long amplicon (PP051279). The BLAST search using this sequence revealed it has 100% identity with the original sequence of *D. persicus* (KX463285). This sequence

has occupied a placement inside the clade that includes the original sequence of the species (KX463285) in the LSU tree (Fig. 6). This clade further includes two accession numbers MG025824 and MF706255 assigned to *Nothotylenchus andrassyi* Jalalinasab *et al.*, 2018 and *Pterotylenchus* sp.

DISCUSSION

This research provides new data on diversity of plant-parasitic and plant associated nematodes in eastern Iran. The two ectoparasitic species, *Xiphinema persicum* and *X. babaii*, originally described from northern and northwestern Iran, were newly recovered in eastern Iran. The first species was collected from six regions in Southern

Khorasan in association with pistachio, barberry, walnut and saffron. The second population of this species (in its original description) was also associated with pistachio, and with regarding stunted growth, delayed bud opening, yellowing and leaf fall of barberry trees as observed in present study, it might be concluded that the species could be a causal agent, and could be regarded a potential threat to woody plants. In a previous study, a longidorid species originally described from western Iran (Esmaeili *et al.*, 2017b), was recovered from southern Iran (Monemi *et al.*, 2022, 2024) and further taxonomic studies, may yield on extending the distribution regions of native species. The new morphometric data ranges (tail length and the index c) were also observed and reported for the second species, *X. babaii*, as already discussed. Variation in tail length within this group has already documented for species inside this group (Gutiérrez-Gutiérrez *et al.*, 2012; Archidona-Yuste *et al.*, 2016) and also some other group of nematodes (e.g. aphelenchoidid members, see Miraeiz *et al.*, 2015). *Aphelenchoides smolae* was reported for the first time from Iran during present study. It was associated with saffron corms. The type population of this species was isolated and described from the rhizosphere and tissue of the bulbs of *Lilium orientalis* imported from the Netherlands to China (Cai *et al.*, 2020b). Most species of *Aphelenchoides* sp. are mycetophagous, but 13 species have been identified as plant parasites (Sánchez-Monge *et al.*, 2015). As the result, the tentative damage of *A. smolae* to the saffron, after the economic importance of this crop in Iran (Ghorbani, 2007), needs further studies. As already presented, occurrence of *Ditylenchus persicus* in Southern Khorasan province is a new record and new observations related to this species were already presented.

In presently resolved phylogenies using *Xiphinema americanum*-group spp. sequences, distant placement for the sequence AY601607 assigned to *X. pachtaicum*, compared to clade of sequences of different isolates of *X. pachtaicum* was observed, and by close relation of this sequence with the sequence of *X. persicum*, it most probably belongs to *X. persicum*. Similar case has reported by Lazarova *et al.* (2019). The similar argument could be applied to the sequence ON261404 assigned to *Ditylenchus persicus* occupying distant placement compared to the sequence of the type and presently provided sequence. As already discussed, the identity of some sequences/species deposited into the GenBank database needs further validations (Lazarova *et al.*, 2019; Aliverdi *et al.*, 2022; Monemi *et al.*, 2023) and sequences from topotype

populations could shed light on the identity of sequenced specimens (Jahanshahi Afshar, 2019; Lazarova *et al.*, 2019).

ACKNOWLEDGEMENTS

Financial supports of Ferdowsi University of Mashhad and Tarbiat Modares University are appreciated. We appreciate the kind help of Eng. Fatemeh Bazeghi for her critically reading the manuscript draft.

REFERENCES

- ALIVERDI, S., POURJAM, E. & PEDRAM, M. 2022. Description of *Ditylenchus acantholimonis* n. sp. (Rhabditida: Anguinidae) from Iran, a morphological and molecular phylogenetic study. *Nematology* 24: 109-118. DOI: 10.1163/15685411-bja10115
- ARCHIDONA-YUSTE, A., NAVAS-CORTÉS, J.A., CANTALAPIEDRA-NAVARRETE, C., PALOMARES-RIUS, J.E. & CASTILLO, P. 2016. Cryptic diversity and species delimitation in the *Xiphinema americanum*-group complex (Nematoda: Longidoridae) as inferred from morphometrics and molecular markers. *Zoological Journal of the Linnean Society* 176: 231-265. DOI: 10.1111/zoj.12316
- ASGHARI, M., ESKANDARI, A., CASTILLO, P. & PALOMARES-RIUS, J.E. 2023. A new needle nematode, *Longidorus zanjanensis* sp. nov. (Nematoda: Longidoridae) from north-western Iran. *Journal of Helminthology* 97: 1-12. DOI: 10.1017/S0022149X23000664
- BAKHSI AMREI, S., PENEVA, V., RAKHSHANDEHROO, F. & PEDRAM, M. 2020. Molecular and morphological description of *Longidorus behshahrensensis* n. sp. (Nematoda: Longidoridae) in natural forests of Abbas Abad, northern Iran. *European Journal of Plant Pathology* 156: 387-398. DOI: 10.1007/s10658-019-01884-y
- BAKHSI AMREI, S., PENEVA, V., RAKHSHANDEHROO, F. & PEDRAM, M. 2022. Description of *Longidorus armeniacae* n. sp. (Nematoda: Longidoridae), associated with *Prunus armeniaca* L. in Semnan province, Iran. *European Journal of Plant Pathology* 162: 739-750. DOI: 10.1007/s10658-021-02435-0
- CAI, R., ARCHIDONA-YUSTE, A., CANTALAPIEDRA-NAVARRETE, C., PALOMARES-RIUS, J.E. & CASTILLO, P. 2020a. New evidence of cryptic speciation in the family Longidoridae (Nematoda: Dorylaimida). *Journal of Zoological Systematics and Evolutionary Research* 58: 869-899. DOI: 10.1111/jzs.12393
- CAI, J., JU, J., WANG, X., FANG, Y. & LI, H. 2020b. *Aphelenchoides smolae* n. sp. (Tylenchina: Aphelenchoididae) found in *Lilium orientalis* imported into China from The Netherlands.

- Nematology* 22: 799-813. DOI: 10.1163/15685411-00003341
- DE GRISSE, A.T. 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. *Mededelingen Rijksfakulteit Landbouwwetenschappen, Gent* 34: 351-369.
- DECRAEMER, W. & ROBBINS, R.T. 2007. The who, what and where of Longidoridae and Trichodoridae. *Journal of Nematology* 39: 295-297.
- ESMAEILI, M., HEYDARI, R., CASTILLO, P. & PALOMARES-RIUS, J.E. 2017a. Molecular and morphological characterisation of *Ditylenchus persicus* n. sp. (Nematoda: Anguinidae) from Kermanshah province, western Iran. *Nematology* 19: 211-223. DOI: 10.1163/15685411-00003041
- ESMAEILI, M., HEYDARI, R., ARCHIDONA-YUSTE, A., CASTILLO, P. & PALOMARES-RIUS, J.E. 2017b. A new needle nematode, *Longidorus persicus* n. sp. (Nematoda: Longidoridae), from Kermanshah province, western Iran. *European Journal of Plant Pathology* 147: 27-41. DOI: 10.21307/jofnem-2018-009
- GHARIBZADEH, F., POURJAM, E. & PEDRAM, M. 2018. Description of *Longidorus azarbaijanensis* n. sp. (Dorylaimida: Longidoridae) from Iran. *Journal of Nematology* 50: 207-218. DOI: 10.21307/jofnem-2018-009
- GHOORBANI, M. 2007. The economics of saffron in Iran. *Acta Horticulturae* 739: 321-331. DOI: 10.17660/ActaHortic.2007.739.42
- GUTIÉRREZ-GUTIÉRREZ, C., CANTALAPIEDRA-NAVARRETE, C., DECRAEMER, W., VOVLAS, N., PRIOR, T., PALOMARES RIUS, J.E.P. & CASTILLO, P. 2012. Phylogeny, diversity, and species delimitation in some species of the *Xiphinema americanum*-group complex (Nematoda: Longidoridae), as inferred from nuclear and mitochondrial DNA sequences and morphology. *European Journal of Plant Pathology* 134: 561-597. DOI: 10.1007/s10658-012-0039-9
- HUSON, D.H. & SCORNAVACCA, C. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Systematic Biology* 61: 1061-1067. DOI: 10.1093/sysbio/sys062
- JAHANSHAHI AFSHAR, F., PEDRAM, M. & MOBASSERI, M. 2021. Description of *Xiphinema persicum* n. sp. (Nematoda: Longidoridae), a *X. americanum*-group species from Iran. *European Journal of Plant Pathology* 159: 783-797. DOI: 10.1007/s10658-021-02201-2
- JENKINS, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. *Plant Disease Reporter* 48: 692.
- KATO, K. & STANDLEY, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772-780. DOI: 10.1093/molbev/mst010
- KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874. DOI: 10.1093/molbev/msw054
- LARGET, B. & SIMON, D.L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750-759. DOI: 10.1093/oxfordjournals.molbev.a026160
- LAZAROVA, S., OLIVEIRA, C.M., PRIOR, T., PENEVA, V. & KUMARI, S. 2019. An integrative approach to the study of *Xiphinema brevicolle* Lordello and Da Costa 1961, supports its limited distribution worldwide (Nematoda: Longidoridae). *European Journal of Plant Pathology* 153: 441-464. DOI: 10.1007/s10658-018-1571-z
- MIRAEIZ, E., HEYDARI, R., ALIRAMAJI, F., POURJAM, E., PEDRAM, M. & ATIGHI, M.R. 2015. New morphometric ranges for *Bursaphelenchus willibaldi* Schönfeld *et al.*, 2006 (Nematoda: Parasitaphelenchidae) from Iran. *Forest Pathology* 46: 29-34. DOI: 10.1111/efp.12210
- MOBASSERI, M., HUTCHINSON, M.C., JAHANSHAHI AFSHAR, F. & PEDRAM, M. 2019. New evidence of nematode endosymbiont bacteria coevolution based on one new and one known dagger nematode species of *Xiphinema americanum*-group (Nematoda, Longidoridae). *PLoS ONE*, 14, 6: 0217506. DOI: 10.1371/journal.pone.0217506
- MOBASSERI, M., POURJAM, E., FARASHIANI, M.E. & PEDRAM, M. 2022. Morpho-molecular characterisation of a new nematode species of the genus *Longidorus* Micoletzky, 1922 (Nematoda: Longidoridae) from northern Iran. *European Journal of Plant Pathology* 165: 1-14. DOI: 10.1007/s10658-022-02603-w
- MONEMI, S., ATIGHI, M.R. & PEDRAM, M. 2022. Occurrence of four species of *Longidorus* Micoletzky, 1922 (Nematoda: Longidoridae) in date palm gardens of the east Bushehr province. *Second Congress of Plant Pathology of Iran, Tehran, Iran*. 2022 September 3. Page 161.
- MONEMI, S., ATIGHI, M.R., CASTILLO, P. & PEDRAM, M. 2023. Morphological and molecular characterization of *Filenchus multistriatus* n. sp. (Tylenchomorpha: Tylenchidae) and data on a known species of the genus from Bushehr province, Southern Iran. *Journal of Nematology* 55: e2023-1. DOI: 10.2478/jofnem-2023-0008
- MONEMI, S., BEHDANI, M., MAHDIKHANI-MOGHADAM, E., AMIRZADI, N., ATIGHI, M.R., YE, W., JAHANSHAHI AFSHAR, F. & PEDRAM, M. 2024. Data on four species of *Longidorus* Micoletzky, 1922 (Nematoda:

- Longidoridae) from southern and southeastern Iran, including description of a new species. *Journal of Helminthology* 98: (in press). DOI: 10.1017/S0022149X24000282
- NAGHAVI, A., NIKNAM, G.R. & VASIFEH, N. 2022. A new species of *Xiphinema americanum* group (Nematoda: Longidoridae) from Iran, with additional data on three known species. *Systematic Parasitology* 99: 545-555. DOI: 10.1007/s11230-022-10044-6
- 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.
- POUR EHTESHAM, N., PEDRAM, M., ATIGHI, M.R. & JAHANSHANI AFSHAR, F. 2023. Morphological and molecular phylogenetic study of *Longidorus soosanae* n. sp. (Nematoda: Longidoridae) from Golestan province, northern Iran. *European Journal of Plant Pathology* 166: 227-239. DOI: 10.1007/s10658-023-02657-4
- RONQUIST, F. & HUELSENBECK, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574. DOI: 10.1093/bioinformatics/btg180
- SÁNCHEZ-MONGE, A., FLORES, L., SALAZAR, L., HOCKLAND, S. & BERT, W. 2015. An updated list of the plants associated with plant-parasitic *Aphelenchoides* (Nematoda: Aphelenchoididae) and its implications for plant parasitism within this genus. *Zootaxa* 4013: 207-224. DOI: 10.11646/zootaxa.4013.2.3
- TAYLOR, C.A. & BROWN, D.J.F. 1997. *Nematode vectors of plant viruses*. Wallingford, UK: CAB International.
- 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: 25-38.

M. Behdani, E. Mahdikhani-Moghadam and M. Pedram. Новые данные о четырех нематодах, паразитирующих на растениях и нематодах, ассоциированных с растениями, в провинции Южный Хорасан, восточный Иран.

Резюме. Четыре вида фитопаразитических и фитоассоциированных нематод были обнаружены в образцах почвы, собранных в провинции Южный Хорасан на востоке Ирана. Эти виды были охарактеризованы с использованием как морфологического, так и молекулярного подходов. Первый вид, *Xiphinema persicum*, широко распространен в провинции Южный Хорасан и был обнаружен в различных местах на растениях барбариса с признаками замедленного роста, обесцвечиванием и синдромом общего угнетения при относительно высокой плотности популяции. Новые популяции этого вида характеризовались длиной тела самки 2036,0-3074,5 мкм, обособленной губной областью, длиной одонтостилиа 80,0-95,8 мкм, длиной одонтофора 38,5-49,5 мкм и коническим хвостом с широким, менее дифференцированным утолщением на конце. Полученные результаты показали, что этот вид можно рассматривать как потенциального вредителя на востоке Ирана. Второй вид принадлежал к *X. babaii* и имел длину тела самки 2032-2224 мкм, обособленную область губ, длину одонтостилиа 83-88 мкм, длину одонтофора 45-50 мкм и конический хвост длиной 23,5-32,0 мкм. Его находка в восточном Иране является новым обнаружением, расширяющим его географическое распространение за пределы западного Ирана. Третий вид, *Aphelenchoides smolae*, был обнаружен в двух местах. Для иранских популяций этого вида характерны тело самки длиной 503-895 мкм, стилет длиной 11,0-14,2 мкм с короткими базальными вздутиями, относительно длинный поствувальварный маточный мешок, хвост конической формы с бородавчатой поверхностью и спикулами длиной 22,5-28,7 мкм. Заметных различий по сравнению с типовой популяцией не обнаружено. Этот вид выделен из луковец шафрана и впервые зарегистрирован в Иране. Последний вид принадлежал к *Ditylenchus persicus*. Южнохорасанская популяция этого вида характеризовалась длиной тела самки 622-770 мкм, слитной губной областью, тонким стилетом длиной 7,0-8,5 мкм, наличием медиального бульбуса и заднего бульбуса грушевидной формы с коротким перекрытием с кишкой, поствувальварным маточным мешком 24-30 мкм, длинным коническим хвостом с закругленным или тупым концом. Этот вид морфологически и морфометрически был сходен с типовой популяцией. Обсуждается обнаруженная разница в строении пищеводного бульбуса этой популяции по сравнению с типовой популяцией. Филогенетический анализ обнаруженных видов был проведен с использованием последовательностей D2-D3 28S рДНК, и полученные топологии были обсуждены.