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Description of *Aphelenchoides persicus* sp. n. (Nematoda: Aphelenchoididae) from Iran

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Research Paper

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Abstract

During a survey on the soil nematodes, a population of the genus *Aphelenchoides* was collected around the rhizosphere of persimmon in Guilan Province, Iran. The morphological and molecular characters confirmed the new species, namely *A. persicus* sp. n. The new species is characterized by a female body length (699–1068 µm), lip region offset from the rest of the body by a slight constriction, lateral fields with six incisures, stylet 12–13.5 µm long, with a clear basal swelling, excretory pore *ca* 1.5 metacorpal length posterior to base of the metacorpus, post uterine sac elongate, about 4–7 times than the vulval body diameter; conical female tail with a single centrally located mucron with tiny projection close to the tail tip, male body length (663–908 µm), and spicule well developed with rounded condylus, blunt conical rostrum, and a hook-like tip of dorsal limb. The new species belongs to the Group 2 category of *Aphelenchoides* species and was similar to seven known species with six lateral field incisures, including *A. allius*, *A. chinensis*, *A. meghalayensis*, *A. nechaleos*, *A. paranechaleos*, *A. parasexlineatus*, and *A. sexlineatus*. The molecular phylogeny based on 28S rDNA revealed that the new species stands close to *A. hamospiculatus* (MN931591; MN931592) and two unidentified *Aphelenchoides* (KY769057; LC583315). The measurements, line illustrations, LM photographs, and phylogenetic analysis are given for the new species.

Introduction

Family Aphelenchoididae Skarbilovich, 1947 contains several genera, including *Aphelenchoides*. Members of this family are primarily fungal feeders (Aliramaji *et al.* 2018). However, foliar nematodes are plant feeders (Subbotin *et al.* 2021), which could be economically significant due to the yield loss of crops (Shokoohi *et al.* 2022). *Aphelenchoides* was established by Fischer (1894) and comprised 175 nominal species (Aliramaji *et al.* 2018). This species-rich genus is the type genus of the family Aphelenchoididae. It is well known for the prevalence of species lacking conspicuous apomorphies, which is helpful for its species delimitation (Aliramaji *et al.* 2018). So far, *Aphelenchoides* species have been discovered in various habitats, including soil, mosses, mushrooms, decaying organic materials, and plant tissues (Subbotin *et al.* 2021). Morphologically, *Aphelenchoides* resembles genera, including *Basilaphelenchus* Pedram. Kanzaki, Giblin-Davis & Pourjam, 2018; *Robustodorus* Andrássy, 2007; *Schistonchus* (Cobb 1927) Fuchs, 1937; and *Tylaphelenchs* Rühm, 1956. However, it differs from the genera mentioned above in the stylet morphology. *Aphelenchoides* is distinguished from *Basilaphelenchus*, which bears stylet knobs elongated and posteriorly directed (Pedram *et al.* 2018a). Compared with *Robustodorus*, it differs in stylet knobs (small vs. robust and developed). It differs from *Schistonchus* in stylet (slender vs. robust). It also differs from *Tylaphelenchus* in stylet (slender with small knobs vs. robust with developed knobs (Kanzaki & Giblin-Davis 2012). Historically, several authors studied *Aphelenchoides*, emphasizing the feature to diagnose the species (Hunt 1993, 2008; Shahina 1996; Andrássy 2007). Among the characters, lateral filed incisures were the matter of debate among the nematologists. Shahina (1996) indicated that the lateral field with 4–6 incisures is rare. Similarly, Andrássy (2007) showed that species with six incisures of the lateral field are rare. The character was found to be six incisures in the present species of *Aphelenchoides* from Iran.

Additionally, several species have been transferred to *Aphelenchoides*. *Laimaphelenchus heidelbergi* Zhao, Davis, Riley and Nobbs, 2007 was transferred to the genus *Aphelenchoides* (Carta *et al.* 2016). *Tylaphelenchus christinae* Lieutier and Laumond, 1978 was transferred to the genus *Aphelenchoides* (Pedram *et al.* 2018a, 2018b). In contrast, *Aphelenchoides subtenuis* (Cobb 1926) Steiner and Buhrer, 1932 and *A. arachidis* Bos, 1977 have also been transferred to the genus *Robustodorus* Andrássy, 2007 (Kanzaki *et al.* 2018). Recently, *A. helicus* Heyns, 1964 was transferred to *Robustodorus* (Aliramaji *et al.* 2018).

During a survey on nematodes in northern provinces of Iran, a population of a new species of *Aphelenchoides* was recovered from a wild persimmon in Guilan Province. Therefore, the study's aims were 1) to describe the morphology and morphometrics of *Aphelenchoides persicus* sp. n. and 2) to study the phylogenetic position of the new species based on 28S rDNA.

Materials and methods

Nematode extraction and morphological observations

Several soil samples were collected randomly in Guilan Province, Iran (36°58'15.7"N; 50°16'48.8"E), during June and May 2021. The samples were collected using an auger and then transferred through the cooler box to the laboratory. The tray method (Whitehead & Hemming 1965) was used to extract nematodes. The nematodes were handpicked under an Olympus SZ16 stereomicroscope (Japan). The collected specimens were killed in a hot 4% formaldehyde solution, transferred to anhydrous glycerin according to De Grisse (1969), and mounted on permanent slides. Measurements and observations on morphology were made under an Olympus BH-2 Light microscope (Japan). Photographs were taken using a digital camera attached to an Olympus BX51 microscope (Japan). Drawings were made using a drawing tube attached to the microscope Olympus BX51.

DNA extraction, polymerase chain reaction (PCR), and sequencing

DNA was extracted from a single female. The nematode was squashed in TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, Qiagen) on a clean slide with a cover slip and the pressure of a plastic probe. The supernatant was extracted from the tube and stored at –20°C. Primers for LSU D2/D3 amplification were forward primer D2A (5'ACAAGTACCGTGAGGGAAAGT3') and reverse primer D3B (5'TGCGAAGGAACCAGCTACTA3') (Nunn 1992). The thermal cycling program for amplifying genomic fragment (LSU rDNA D2–D3) was as follows: denaturation as 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 40 sec, and extension at 72°C for 90 sec. A final extension was performed at 72°C for 10 min. Polymerase chain reaction was performed in a final volume of 30 ml PCR mixture and contained 15 ml 2X GoTaq DNA polymerase mix (Sina Clon), each of a 1.5 ml forward and reverse primers solution (5 pmol), 9 ml distilled water, and 3 ml of a 100 times-diluted crude DNA extract. PCR products were purified and sequenced directly for both strands using the same primers with an ABI 3730XL sequencer (Bioneer Corporation, South Korea).

Alignment and phylogenetic inference

The molecular sequences of D2/D3 expansion segments of 28S ribosomal RNA gene of the new species were compared with those of other nematode species available in GenBank using the BLAST homology search program. The sequences were aligned using the Q-INSi algorithm of the online version of MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley 2013). The Gblocks program (version 0.91b) has all three less stringent parameters (http://phylogeny.lirmm.fr/phylo_cgi/one_task.cgi?task_type=gblocks) and was used for post-editing of both alignments (i.e., to eliminate poorly aligned regions or divergent positions). 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 analyses. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003), running the chains for 3×10^6 generations for both datasets. After discarding burn-in samples, the remaining samples were retained for further analysis. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon 1999) using the 50% majority rule. Aphelenchid and classic rhabditid species, including *Steinernema carpocapsae* Weiser, 1955; *Panagrellus reivivus* (Linnaeus, 1767) Goodey, 1945; *Acrobeles singulus* Heyns, 1969; *Paraphelenchus acontioides* Taylor and Pillai, 1967; and *Aphelenchus avenae* Bastian, 1965 (accession numbers KJ950293, DQ145647, DQ145622, HQ218322, and KR527123, respectively) were used as outgroup taxa (according to previous studies (e.g., Mortazavi and Pedram 2020; Aliramaji *et al.* 2020a, 2020b)). The phylogenetic program output files were visualised using Dendroscope V.3.2.8 (Huson and Scornavacca 2012) and re-drawn in CorelDRAW v. 2017. The original partial 28S rDNA sequence of *A. persicus* sp. n. was deposited in GenBank under the accession number OR146497.

Results

***Aphelenchoides persicus* sp. n.**

(Figures 1 and 2, measurements in Table 1)

Female (Figs 1A–R; 2A–N): Body slightly ventrally curved when heat-relaxed, very gently narrowing towards both ends. Body annuli about one µm wide at mid-body. Cuticle weak, less than one µm at mid-body. Lateral field with six incisures, occupying about 22–32% of corresponding body width, initiating with two at anterior end, extending to six at mid-body, in some areas, five incisures are also observed (Figures 1H, I; 2F), and reducing to two bands at posterior end. Lip region rounded, finely annulated, offset from body contour, 2.0–2.4 µm height and 5.5–7.3 µm width. Stylet not robust, conus thin, shorter than the shaft, the lumen well visible all over the stylet, having small swellings at base. Procorpus slender, median bulb rounded or oval, 1.4 ± 0.1 (1.2–1.6) times longer than the width, its valvular plates well sclerotized, slightly posterior to the central. Nerve ring at isthmus level. Excretory pore *ca* 1.5 metacorpal length posterior to base of the metacorpus. Pharyngo-intestinal junction just posterior to metacorpus, pharyngeal glands lobe overlapping intestine dorsally for 53–87 µm. Hemizonid 2–5 µm posterior to excretory pore. Intestine simple, rectum and anus functional. Reproductive system monodelphic-prodelphic, ovary outstretched, oocytes in one or two rows in germinal zone, oviduct distinct, spermatheca rectangular to elongate oval, possessing relatively large cells with clearly confirmed cell nuclei at anterior end. Crustaformeria and uterus boarder, well developed, vagina straight to slightly anteriorly directed. Vulva a simple transverse slit without any vulval flap. Post uterine sac (PUS) elongate, about 4–7 times than the vulval body diameter or 40–77% of the vulva-anus distance, bearing sperm in some specimens. Tail conical, ventrally almost flat, ending to a single mucro with a tiny projection close to the tail tip.

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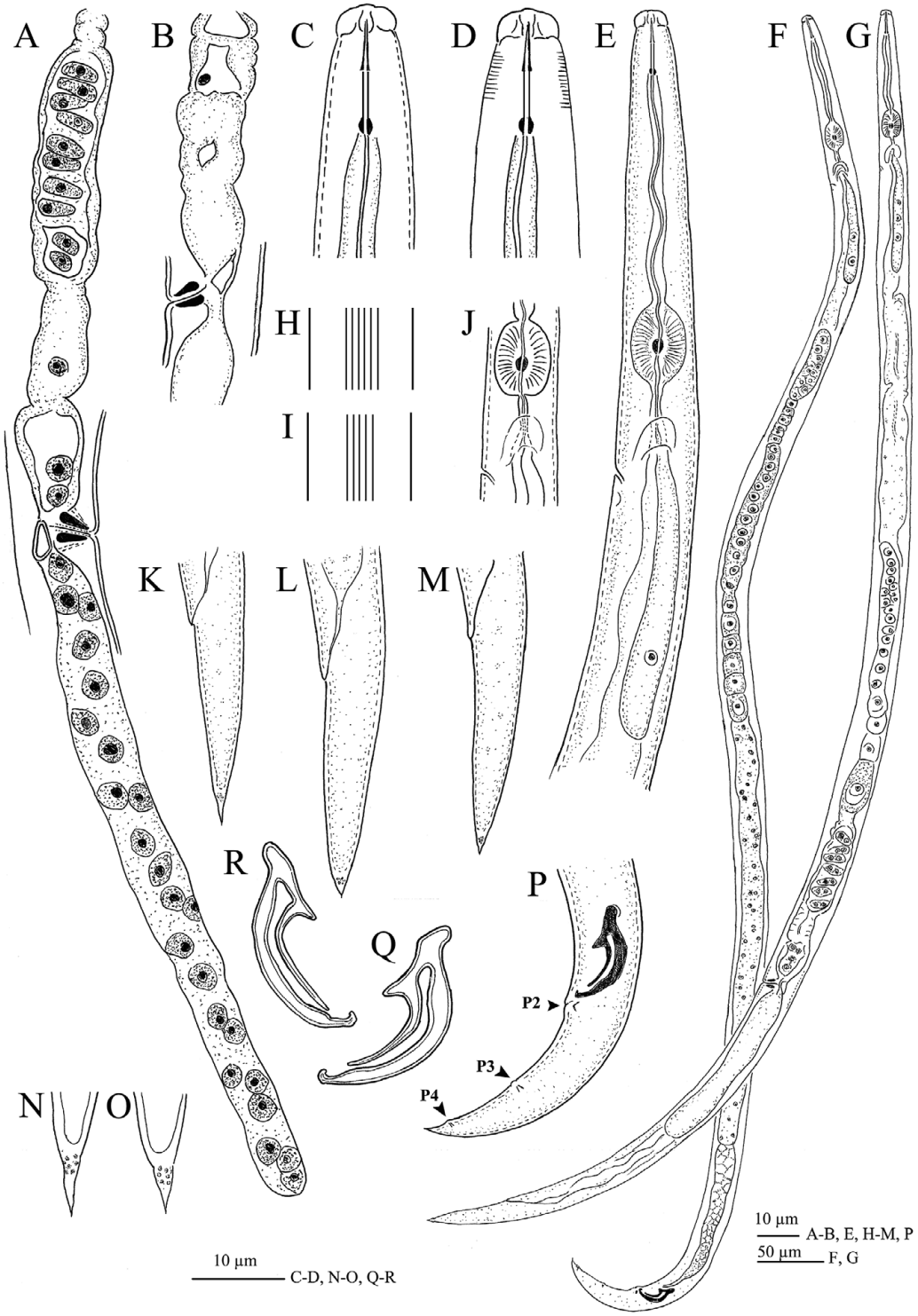

Figure 1 . *Aphelenchoides persicus* sp. n. A: female reproductive system with post uterine sac (PUS); B: vagina; C, D: anterior end; E: pharynx; F: entire male; G: entire female; H, I: lateral field; J: metacorpus and excretory pore; K–M: female posterior end; N, O: female tail tip; P: male posterior end; Q, R: spicules.

Male (Figures 1F, P–R; 2L–N): Abundant, equal to females in number. General morphology similar to that of female, except for reproductive system and the posterior end more ventrally bent after fixation. Genital system monorchic, testis outstretched with spermatocytes arranged in single to two and single row at germination and growth zone, respectively. Spicules arcuate, condylus well developed, rounded, slightly dorsally at the end, rostrum small, blunt conical, tip of dorsal limb hook-like. Male caudal papillae composed of three pairs (single P1 papilla lacking), arranged as follows: the first pair (P2) at cloacal level or slightly posterior, the second pair (P3) at about middle of the tail, and the third pair (P4) vestigial, close to the tail end. Tail similar to that of female, ending with a single mucro with tiny protuberance.

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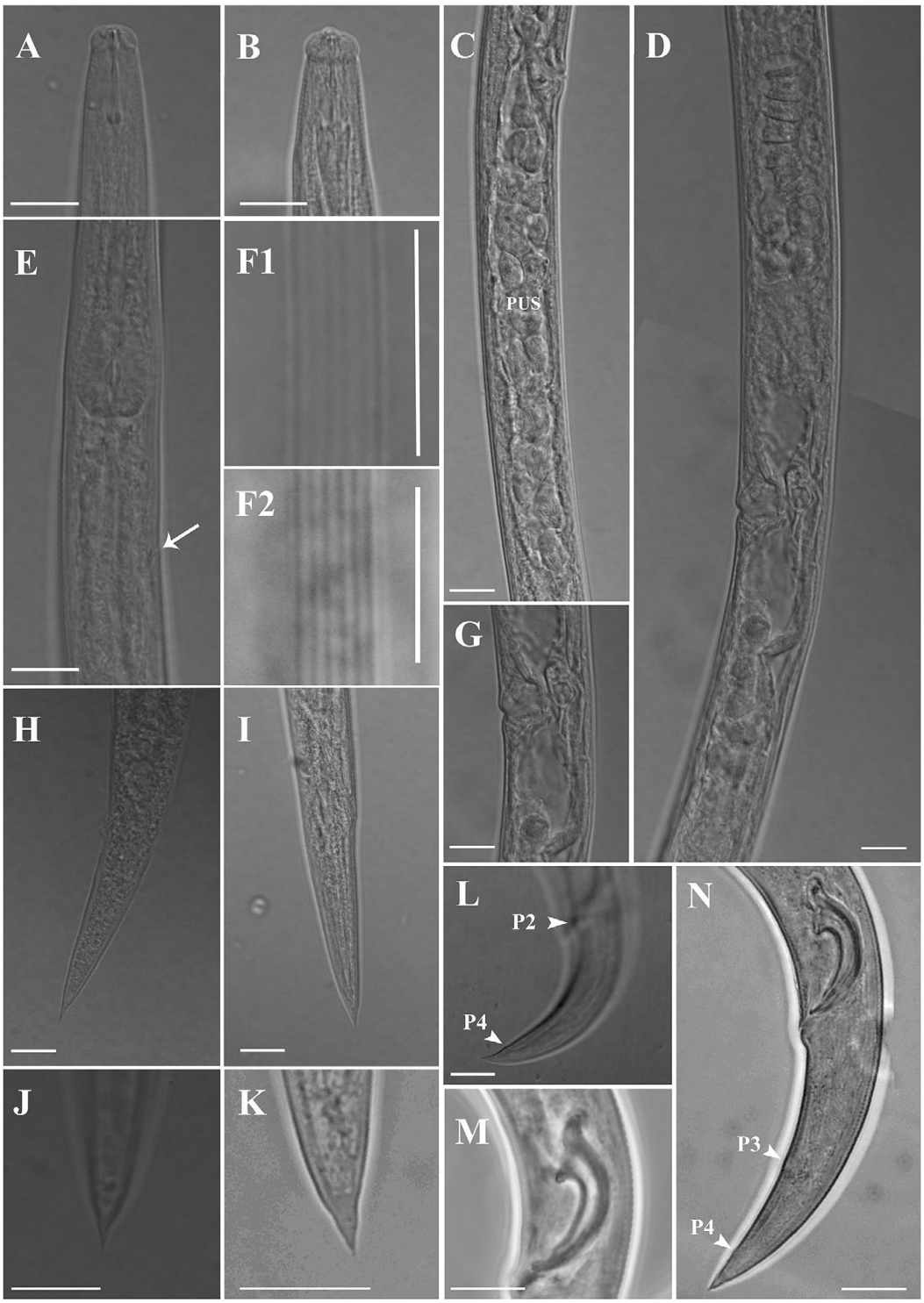

Figure 2 . *Aphelenchoides persicus* sp. n. (LM). Female. A, B: anterior end; C: post uterine sac; D: female reproductive system; E: metacorpus and excretory pore (arrowhead showing excretory pore); F: lateral field (F1, F2); G: vagina; H–K: female posterior end. Male. L: genital papillae; M: lateral view of spicule region; N: lateral view of tail region. (P2 = subventral precloacal, P3, P4 = postcloacal pairs) Scale bar: all measurements = 10 μm.

Diagnosis

Aphelenchoides persicus sp. n. is characterized by having a long body length (699–1068 μm in females and 663–908 μm in males), stylet 12.0–13.5 μm, with a distinct basal swelling, a long PUS, a little more than half the distance between vulva to anus, six incisures of lateral field, female tail with a simple mucro with tiny projection close to the tail tip, male with spicules 20–24 μm long with round condyles, conical rostrum and tip of dorsal limb hook-like and male tail with three pairs of caudal papillae (2 + 2 + 2).

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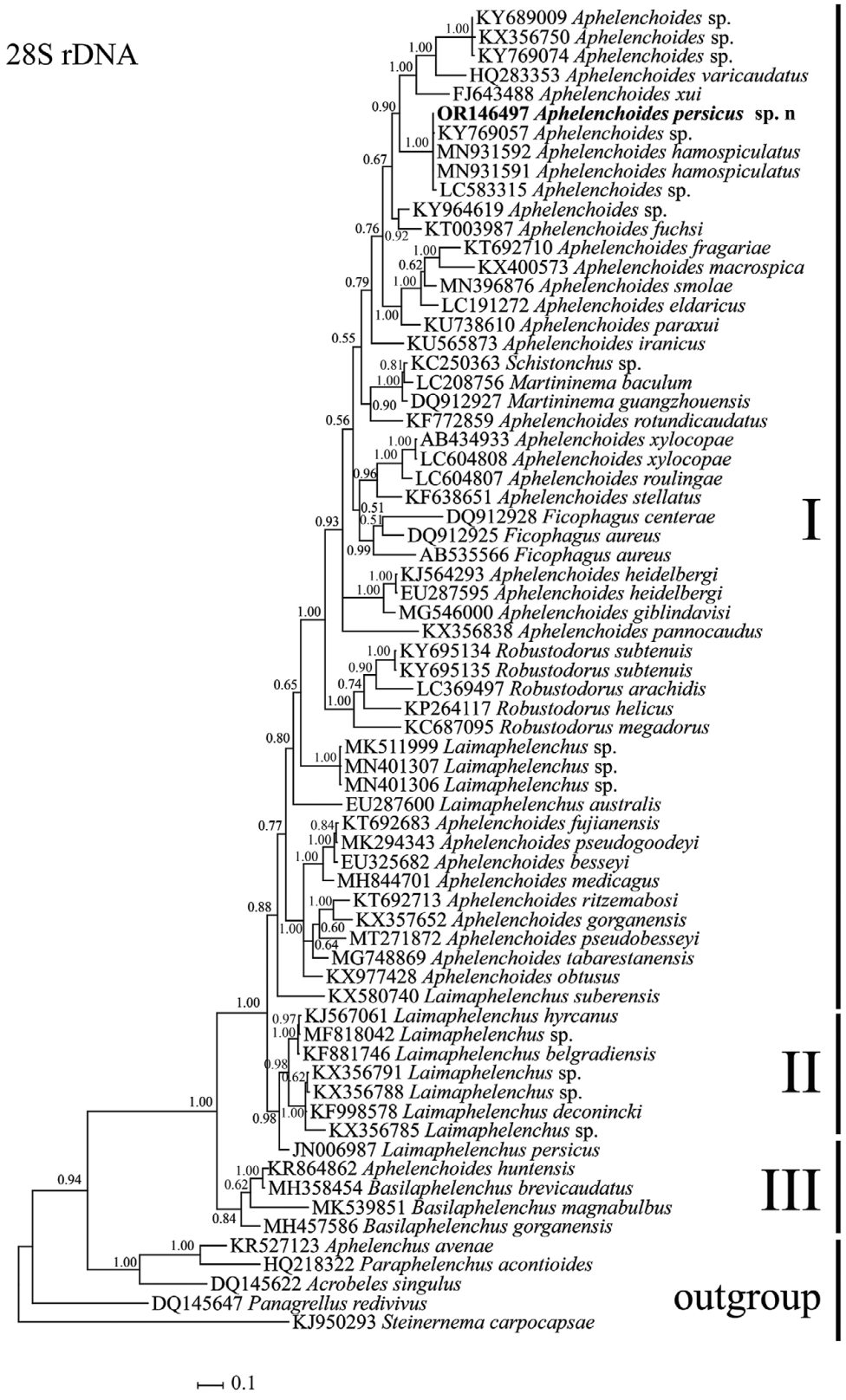

Figure 3 . Bayesian 50% majority rule consensus tree of *Aphelenchoides persicus* sp. n. based on large subunit (LSU) rDNA (D2–D3 segment) sequences under GTR + I + G model. Bayesian posterior probability values of more than 0.50 are given for appropriate clades. The new sequence is indicated in bold.

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Table 1. Morphometrics of *Aphelenchoides persicus* sp. n. All measurements in μm and in the form: mean ± s.d. (range), except for ratio

Characters	Female		Male
	Holotype	Paratype	
n	–	9	10
L	853	855 ± 109.2 (699–1068)	825 ± 81.7 (663–908)
a	36.3	39.4 ± 2.1 (36.3–42.0)	46.2 ± 1.8 (43.3–49.1)
b	8.9	9.1 ± 0.8 (7.9–10.3)	8.5 ± 0.7 (7.1–9.2)
b′	4.9	5.4 ± 0.5 (4.9–6.2)	5.0 ± 0.5 (4.1–5.7)
c	17.1	18.0 ± 1.2 (16.6–20.2)	17.0 ± 1.3 (15.6–19.0)
c′	3.8	3.9 ± 0.3 (3.3–4.3)	3.6 ± 0.2 (3.3–3.9)
V or T	70.2	69.3 ± 0.8 (67.6–70.2)	58.5 ± 9.2 (45.3–71.1)
Head height	3.2	3.0 ± 0.3 (2.5–3.5)	3.0 ± 0.3 (2.5–3.5)
Head diameter	7.3	6.5 ± 0.6 (5.5–7.3)	6.3 ± 0.3 (6.0–6.5)
Stylet length	12.5	12.5 ± 0.6 (12.0–13.5)	12.3 ± 0.5 (11.5–13.0)
Stylet conus	6.2	5.9 ± 0.3 (5.5–6.5)	5.8 ± 0.5 (5.0–6.5)
M (conus/stylet length)	49.6	47.3 ± 1.3 (45.8–49.6)	46.9 ± 2.8 (43.5–50.0)
Median bulb from anterior end	73.0	73.0 ± 4.5 (66.0–82.0)	76.0 ± 3.3 (71.0–81.0)
Secretory-excretory pore from anterior end	102	104 ± 10.1 (92.0–124)	105 ± 7.5 (93.0–117)
Hemizonid from anterior end	100	101.5 ± 4.1 (98.0–106)	106 ± 5.5 (99.0–114)
Pharyngeal base from anterior end	96.0	94.0 ± 7.7 (81.0–106)	97.0 ± 3.4 (92.0–104)
Nerve ring from anterior end	91.0	94.0 ± 7.9 (80.0–107)	96.0 ± 3.9 (91.0–105)
Median bulb length	14.0	14.8 ± 1.3 (13.0–17.0)	14.6 ± 0.7 (13.0–15.0)
Median bulb diam.	10.0	10.7 ± 1.1 (9.5–13.5)	9.8 ± 1.4 (7.5–12.0)
Median bulb length/diam.	1.4	1.4 ± 0.1 (1.2–1.6)	1.5 ± 0.2 (1.3–1.9)
Pharyngeal overlap	78.0	65.0 ± 8.5 (53.0–78.0)	68.0 ± 6.9 (61.0–83.0)
Maximum body diam.	23.5	21.7 ± 2.4 (17.5–25.5)	17.9 ± 2.1 (13.5–20.0)
Vulval body diameter (VBD)	21.0	20.3 ± 2.1 (16.5–24.0)	–
Body diameter at median bulb	15.5	15.0 ± 1.5 (12.5–18.0)	14.0 ± 1.1 (12.0–15.5)
Post-vulval uterine sac (PVUS)	125	115.5 ± 22.9 (78.0–157)	–
PVUS/VBD	6.0	5.7 ± 1.0 (4.4–7.2)	–
Vulva body end	254	262 ± 31.3 (220–321)	–
Vulva–anus distance	204	215 ± 28.0 (180–268)	–
Ovary/testis length	264	293 ± 114.4 (166–502)	468 ± 111.8 (343–634)
Anal (cloacal) body width	13.0	12.0 ± 1.2 (11.0–14.0)	13.6 ± 1.1 (11.5–15.5)
Tail length	50.0	47.5 ± 4.1 (40.0–53.0)	48.6 ± 4.1 (41.0–57.0)
Spicule length (arc line)	–	–	22.3 ± 1.3 (20.0–24.0)
Capitulum	–	–	9.6 ± 1.1 (7.5–11.0)

Relationships

The most extensive work on *Aphelenchoides* was done by Shahina (1996). Based on the key provided by Shahina (1996), the new species belongs to Group 2, which is defined as having the female tail terminus with 'one or sometimes two mucronate structure'. In having six lines in the lateral fields, the new species is most similar to five species from Group 2 including *A. chinensis* Husain and Khan, 1967; *A. meghalayensis* Bina and Mohilal, 2017; *A. nechaleos* Hooper and Ibrahim, 1994; *A. paranechaleos* Hooper and Ibrahim, 1994; and *A. parasexlineatus* Kulinich, 1984. It is also similar to two species from Group 4, including *A. allius* Feng, 2012 and *A. sexlineatus* Eroshenko, 1967.

Compared with *A. allius*, it differs in the tail end (a simple mucro (Figures 1N–O; 2J–K, 3D) vs. finger-like projection with midline (see Feng 2012)), and stylet (with distinct basal swelling vs. lacking basal swelling) and longer PUS (5.7 vs. 3.8 times vulva body

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diameter). Compared with *A. chinensis*, it differs in longer female body length (699–1068 vs. 380–550 μm), stylet (with distinct basal swelling vs. lacking basal swelling), tail mucro (terminal vs. ventral), and longer PUS (six vs. three times vulva body diameter). Compared with *A. meghalayensis*, it differs in a longer female body (699–1068 vs. 493–681 μm), longer stylet (12.0–13.5 vs. 8.6–10.3 μm), a longer PUS (40–77% vs. 27–35% of the vulva-anus distance), and tail mucro (short vs. long). Compared with *A. sexlineatus*, it differs in the longer female body (699–1068 vs. 600–640 μm), longer stylet (12.0–13.5 vs. 9.0 μm), and tail end (a single and simple mucro vs. finger like mucro). Compared with *A. parasexlineatus*, it differs in the longer female body (699–1068 vs. 450–790 μm), longer PUS (5.7 vs. 1.5 times vulva body diameter), and female tail (conical with a single mucro vs. conical with a finger-like mucro). Compared with *A. nechaleos*, it differs in longer females (699–1068 vs. 600–930 μm), longer stylet (12.0–13.5 vs. 10.5–11.5 μm), and spicule length (20–24 vs. 15–20 μm). Compared with *A. paranechaleos*, it differs in longer females (699–1068 vs. 630–860 μm), longer stylet (12.0–13.5 vs. 9.5–10.5 μm), spicule length (20–24 vs. 15–18 μm), and female tail length (40–53 vs. 35–40 μm).

Additionally, the tail end appendage is a significant character to distinguish the *Aphelenchoides* species. Hence, the new species, compared with *A. homospiculatus* Mortazavi and Pedram, 2020, differs in the longer female body (699–1068 vs. 467–666 μm), longer stylet (12.0–13.5 vs. 8–10 μm), and number of lateral field incisures (6 vs. 5). Compared with *A. kheirii* Golhasan, Heydari, Esmaeili, and Kanzaki, 2018, it differs in the longer female body (699–1068 vs. 448–520 μm), longer stylet (12.0–13.5 vs. 10–11 μm), number of lateral field incisures (6 vs. 4), and longer PUS (78–157 vs. 28–45 μm). Compared with *A. xui* Wang, Wang, Gu, Wang, and Li, 2013, it differs in number of lateral field incisures (6 vs. 5) and spicule (rostrum conical vs. rostrum rounded). Compared with *A. paraxui* Esmaeili, Heydari, Fang, and Li, 2017, it differs in the longer female body (699–1068 vs. 500–660 μm), longer stylet (12.0–13.5 vs. 8–9 μm), and number of lateral field incisures (6 vs. 4). Compared with *A. smolae* Wang, Wang, Gu, Wang, and Li, 2013, it differs in shorter stylet (12.0–13.5 vs. 13.0–14.9 μm) and number of lateral field incisures (6 vs. 4).

Molecular phylogenetical status

The phylogenetic tree based on the sequences of 28S rDNA formed three clades, including I) *Aphelenchoides* spp., *Ficofagus* spp., *Laimaphelenchus* spp., *Martininema* spp., *Robustodorus* spp., and *Schistonchus* spp., with 1.00 posterior probabilities support, II) *Laimaphelenchus* spp., including unidentified *Laimaphelenchus*; *L. hyrcanus* Miraeiz, Heydari, Tanha Maafi, and Bert 2015; *L. belgeradiensis* Oro, 2015; and *L. deconincki* Elmiligy and Geraert, 1972 with 0.98 posterior probabilities support, and III) *A. huntensis* Esmaeili, Fang, Li, and Heydari 2016; *Basilaphelenchus brevicaudatus* Mirzaie Fouladvand, Pourjam, Kanzaki, Giblin-Davis, and Pedram, 2019; *B. magnabulbus* Aliramaji, Mirzaie Fouladvand, Pourjam, Mortazavi, Jahanshahi Afshar, Kanzaki, Giblin-Davis, and Pedram, 2020; and *B. gorganensis* Mirzaie Fouladvand, Pourjam, Kanzaki, Giblin-Davis, and Pedram, 2019 with 0.84 posterior probabilities support. Additionally, within clade I, the phylogenetic result of 28S rDNA placed *Aphelenchoides persicus* sp. n. close to *A. hamospiculatus* Mortazavi and Pedram, 2020, and unidentified *Aphelenchoides* with a 1.00 posterior probabilities support. Besides, *A. persicus* sp. n. along with *A. varicaudatus* Ibrahim and Hooper, 1994; *A. xui*, *A. fragariae* (Ritzema Bos, 1890) Christie, 1932; *A. macrospica* Golhasan, Heydari, Esmaeili, and Miraeiz, 2017; *A. eldaricus* Esmaeili, Heydari, Golhasan, and Kanzaki 2017; *A. paraxui,* and *A. iranicus* Golhasan, Heydari, Álvarez-Ortega, Esmaeili, Castillo, and Palomares-Rius, 2016; and several unidentified *Aphelenchoides* formed a clade with a 0.79 posterior probability support values.

Type host and locality

The nematodes were recovered from the soil around the rhizosphere of wild persimmon (*Diospyros* sp.) in Guilan Province, northern Iran. The first author collected the samples on 1 June 2021.

Type material

A female holotype (slide number: GU-1A), two female paratypes (slide numbers: GU-2A and GU-3A), and three male paratypes (slide numbers: GU-2A and GU-3A) were deposited in the nematode collection of the Plant Protection Department, College of Plant Production, Gorgan University, Iran. In addition, two paratypes of females and males (slide numbers: GU-4A and GU-5A) were deposited at the Nematology collection of the Aquaculture Research Unit of the University of Limpopo, South Africa. Two paratypes of females and males (slide numbers: GU-6A and GU-7A) were deposited in WANECO collection, Wageningen, The Netherlands (http://www.waneco.eu).

Etymology

The species' name refers to the country of origin (Iran, old name: Persia) where the samples were collected.

Discussion

Aphelenchoides with detail and a key to species is provided by Shahina (1996), and it is a highly diverse genus with more than 153 species (Hunt 2008). Although several species of *Aphelenchides* have been recently described worldwide, they should be added to the available species list. The genus has few morphologically diagnostic taxonomic characters, and it is challenging to compare new species with older ones, often with incomplete descriptions (Kanzaki 2006). This study described a new species using molecular characterization. Molecular sequences and phylogenetic analyses strongly supported the status of *A. persicus* n. sp. as a new species. Although the features have been revised by Shahina (1996), the new species from Iran, *A. persicus* sp. n., belongs to a rare group of *Aphelenchoides* characterised by having six incisures in the lateral field.

The 28S phylogenetic result placed the new species, *A. persicus* sp. n., close to *A. hamospiculatus*, *A. varicaudatus*, *A. xui*, *A. fragariae*, *A. macrospica*, *A. eldaricus*, *A. paraxui*, and *A. iranicus*. The relationships between *A. persicus* sp. n., *A. hamospiculatus*, *A. xui*, and *A. paraxui* have been discussed already. The result obtained in the present study is in agreement with Aliramaji *et al.* (2018) and Golhasan *et al.* (2016), whose results categorized *Aphelenchoides* with simple or two mucronate appendages at the tail tip in the same group.

However, compared with *A. varicaudatus*, it differs in body length (699–1068 vs. 580–710 μm) and number of lateral field incisures (6 vs. 4). Compared with *A. fragariae*, it differs in female body length (699–1068 vs. 450–800 μm) and number of lateral field

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incisures (6 vs. 2). Compared with *A. macrospica*, it differs in the female tail (40–53 vs. 52–63 μm), spicule length (20–24 vs. 27–32 μm), stylet length (12.0–13.5 vs. 15–16 μm), PUS (78–157 vs. 41–60 μm), mucro (a single and straightforward vs. a single with unequal bifurcate), and number of lateral field incisures (6 vs. 4). Compared with *A. eldaricus*, it differs in the female body (699–1068 vs. 507–700 μm), spicule length (20–24 vs. 24–29 μm), stylet length (12.0–13.5 vs. 9–10 μm), PUS (78–157 vs. 27–40 μm), and number of lateral field incisures (6 vs. 3). Compared with *A. iranicus*, it differs in the female body (699–1068 vs. 330–383 μm), spicule length (20–24 vs. 10–11 μm), stylet length (12.0–13.5 vs. 7–9 μm), PUS (78–157 vs. 21–34 μm), and number of lateral field incisures (6 vs. 3).

Overall, *Aphelenchoides* were placed in several groups within the 28S phylogenetic tree, revealing *Aphelenchoides* as paraphyletic. This is in agreement with the finding of several nematologists (Zhao *et al.* 2007; Rybarczyk-Mydłowska *et al.* 2012; Wang *et al.* 2013; Fang *et al.* 2014; Miraeiz *et al.* 2015; Esmaeili *et al.* 2016, Golhasan *et al.* 2016; Aliramaji *et al.* 2018).

However, *Aphelenchoides*, with the sequences included in the present study, stands close with *Basilaphelenchus* (Pedram *et al.* 2018a); *Ficofagus* Davies, Ye, Kanzaki, Bartholomaeus, Zeng, and Giblin-Davis, 2015; *Laimaphelenchus* Fuchs, 1937; *Martininema* Davies, Ye, Kanzaki, Bartholomaeus, Zeng, and Giblin-Davis, 2015; *Robustodorus*; and *Schistonchus.* The mentioned above genera bear a high rate of morphological homoplasy, as indicated by Zeng *et al.* (2007), Kanzaki *et al.* (2018), and Pedram *et al.* (2018b). Thus, they can be separated via stylet morphology and molecular characters of rDNA. Additionally, the new species, *A. persicus* sp. n., was isolated from the soil, and its ecological role is yet to be investigated. The 28S rDNA phylogenetic tree showed that members of the superfamily Aphelenchoidea, especially *Aphelenchoides* and *Laimaphelenchus*, placed in several groups. Therefore, more information on the mentioned taxa is critical for their study. In contrast, *Basilaphelenchus*, *Schistonchus*, *Robustodorus*, *Ficofagus*, and *Maritinema* are placed together, despite the few sequences available and studied. Hence, for the mentioned taxa, more sequences belonging to various species reveal the actual position of the species. The main feature that distinguishes the members of the Aphelenchoidea is stylet morphology. In addition, posterior morphology also becomes essential to describe the new species, pointing to the importance of SEM photographs. In conclusion, the new species was confirmed by the morphological and molecular characters. However, using other rDNA and mtDNA might reveal the actual position of the aphelenchid nematodes.

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