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

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Abstract: Aphelenchoides macrospica n. sp. is described and illustrated from the West Azerbayjan province, northwestern Iran. The new species is characterized by its body length of 807 to 963 μm (males) and 792 to 1,094 μm (females), offset cephalic region, lateral fields with four incisures, long stylet with 15 to 16 μm length, and excretory pore situated opposite or behind the nerve ring. Pharyngeal glands overlapping intestine dorsally and extending for 90 to 121 μm, tail terminus mucronate in both sexes. Vagina directed anteriad, and spicules are relatively large (27–32 μm) with well-developed broadly rounded apex and condylus. The new species comes close to seven known species of the genus namely *A. arcticus*, *A. blastophthorus*, *A. haguei*, *A. huntensis*, *A. lucknowensis*, *A. parasaprophilus*, and *A. xui*, but it differs from them by the body size, stylet length, size of spicules, and length of postvulval uterine sac. The results of phylogenetic analyses based on sequences of D2-D3 expansion region of 28S and 18S rDNA, confirmed its status as a new species.

Key words: LSU, molecular, morphology, new species, phylogeny, SSU, taxonomy, West Azerbayjan province.

Aphelenchoides Fischer, 1894, is a highly diverse genus of the superfamily Aphelenchoididea Skarbilovich, 1947, with more than 153 nominal species that are morphologically very similar to each other (Hunt, 2008). *Aphelenchoides* spp. have a wide distribution and common habitats include soil, moss, insect frass, figs, decaying fruit, etc. Although most species of *Aphelenchoides* are fungivores (Kanzaki and Giblin-Davis, 2012), 13 species have been reported as plant parasitic in a wide variety of plants (Sánchez-Monge et al., 2015). The major plant-parasitic species, including *A. besseyi* Christie, 1942, *A. fragariae* (Ritzema Bos, 1890) Christie, 1932, and *A. ritzemabosi* (Schwartz, 1911) Steiner and Buhrer, 1932, have been extensively studied due to their economic impact and yield losses. Among them, *A. besseyi* is listed within the top 10 plant-parasitic nematodes (PPN) (Jones et al., 2013). Based on our current knowledge, the occurrence of *Aphelenchoides* species in Iran is well known, including 34 species till date (Ghaderi et al., 2012; Esmaeili et al., 2016a, 2016b; Golhasan et al., 2016). Recently, three new species of the genus, *A. huntensis* Esmaeili, Fang, Li, and Heydari, 2016, *A. fuchsi* Esmaeili, Heydari, Ziaie, and Gu, 2016, associated with pine trees and *A. iranicus* Golhasan, Heydari, Alvarez-Ortega, Esmaeili, Castillo, and Palomares-Rius, 2016, associated with oak trees have described for the first time from Iran.

In a general nematological survey in natural and cultivated areas of northwestern Iran during 2013-14, an unknown population of *Aphelenchoides* from Naqadeh city, northwestern Iran, was recovered from soil samples collected around the rhizosphere of rose plants. Infestations ranged from 1 to 10 nematodes/400 gram of soil. The population morphologically and molecularly did not match any of the species of the genus so far described. The species is illustrated and described herein as *Aphelenchoides macrospica* n. sp.

The objectives of the present study were: (i) to verify the taxonomic status of this species; and (ii) to determine the molecular phylogenetic affinities of *A. macrospica* n. sp. with closely related species using rRNA gene sequences (partial 28S and 18S ribosomal DNA).

Materials and Methods

Sampling, extraction, mounting, and drawing

Soil samples were randomly collected from some natural and cultivated areas of West Azerbayjan province during September 2013 to May 2014. The nematodes were extracted by sieving and centrifugation flotation (Jenkins, 1964) and tray (Whitehead and Hemming, 1965) methods. Nematodes of interest were handpicked, killed, fixed, and transferred to anhydrous glycerin using the method proposed by De Grisse (1969). The permanent slides were prepared and studied by a light microscope (Nikon E200). Drawings and measurements were made using a drawing tube attached to the microscope. Photographs were taken by a digital camera system connected to the same microscope.

DNA extraction, PCR, and sequencing

For DNA extraction, an adult nematode was handpicked and placed in a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, Qiagen) on a clean slide and crushed using a sterilized razor blade. The obtained suspension was collected by adding 20 μl AE buffer and 2 μl proteinase K (600 μg/ml) (Promega, Benelux, The Netherlands). The tubes were incubated at 65°C (1 h), then at 95°C (15 min), and finally at 80°C (15 min). One μl of extracted DNA was transferred to an Eppendorf tube containing: 2.5 μl 10 × NH_4 reaction buffer, 0.75 μl $MgCl_2$ (50 mM), 0.25 μl dNTPs mixture (10 mM each), 0.75 μl of each primer (10 mM), 0.2 μl BIOTAQ DNA Polymerase (BIOLINE, UK), and ddH_2O to a final volume of 25 μl. The D2/D3

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domain region of LSU was amplified with the forward primer D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and the reverse primer D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) (Nunn, 1992). For the first fragment of 18S, the forward primer 1096F (5′-GGT AAT TCT GGA GCT AAT AC-3′) was used in combination with the reverse primer 1912R (5′-TTT ACG GTC AGA ACT AGG G-3′) and the second fragment was amplified with forward primer 1813F (5′-CTG CGT GAG AGG TGA AAT-3′) and reverse primer 2646R (5′-GCT ACC TTG TTA CGA CTT TT-3′) (Holterman et al., 2006). PCR cycle conditions were as follows: one cycle of 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, annealing temperature of 55°C for 45 s, 72°C for 3 min, and finally one cycle of 72°C for 10 min. PCR products were purified after amplification using ExoSAP-IT (Affmetrix, USB products) and sequenced directly for both strands using the same primers with an ABI 3730XL sequencer (Macrogen Macrogen Corporation, South Korea).

DNA sequencing alignment and phylogenetic inference

Multiple sequence alignments of D2-D3 of 28S and partial 18S rDNA sequences were made using MUSCLE (Edgard, 2004) followed by postalignment trimming with G-Blocks as implemented in SeaView Version 4 (Gouy et al., 2010). Bayesian phylogenetic analysis was carried out in MrBayes v. 3.2.1 (Ronquist and Huelsenbeck, 2003) using the GTR + I + G model as selected by the Akaike information criterion using MEGA7 (Kumar et al., 2016). Analyses were run under default settings for 3×10^6 generations, 25% of the converged runs were regarded as burnin. Markov Chain Monte Carlo methods within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using 50% majority rule. The clades of the resulted trees were numerated according to Kanzaki and Giblin-Davis (2012).

Results

Aphelenchoides macrospica[*] n. sp.
(Figs. 1–6)

Measurements

See Table 1.

Description

Males: Body slender, J-shaped when heat-relaxed. Cuticle with fine transverse annulations about 1.2 to 1.5 μm apart at midbody. Lateral field marked by four incisures (i.e., three ridges), not areolate. Lip region hemispherical and annulated, 3 to 4 μm, set off by a shallow constriction from remainder of body. Stylet relatively robust with clear basal swellings, conus occupying *ca* 38% to 48% of its total length. Procorpus cylindrical, *ca* 3.5 stylet length long. Metacorpus (median bulb) rounded to oval, conspicuous valve plates situated centrally. Dorsal pharyngeal gland orifice opening into lumen of metacorpus midway between anterior end of metacorpal valve and anterior end of metacorpus. Pharyngo-intestinal junction immediately posterior to base of metacorpus. Nerve ring is situated at *ca* half stylet length posterior to metacorpus. Pharyngeal gland lobe slender, *ca* three to four body diam. long, overlapping intestine dorsally. Excretory pore located at 15 to 25 μm posterior to the base of median bulb; hemizonid invisible. Testis single, anteriorly outstretched, locating left of intestine, spermatocytes in one single column. Vas deferens composed of small rounded cells. Spicules paired and very robust, rosethorn-shaped with prominent and broadly rounded condylus; rostrum short, moderately developed with blunt tip and less developed than condylus. Dorsal and ventral limb of spicule with 38 to 45 μm and 18 to 22 μm long, respectively, arc line from distal to proximal end 27 to 32 μm long. Capitulum 11 to 14 μm long with slightly depression. Bursa and gubernaculum absent. No single precloacal papilla (P1) observed. Three pairs of subventral caudal papillae present with their arrangement as follows: first pair located just posterior to cloacal aperture (P2), second pair postcloacal subventral papillae (P3) located at about 59% of tail, the last pair postcloacal papillae just anterior to tail end

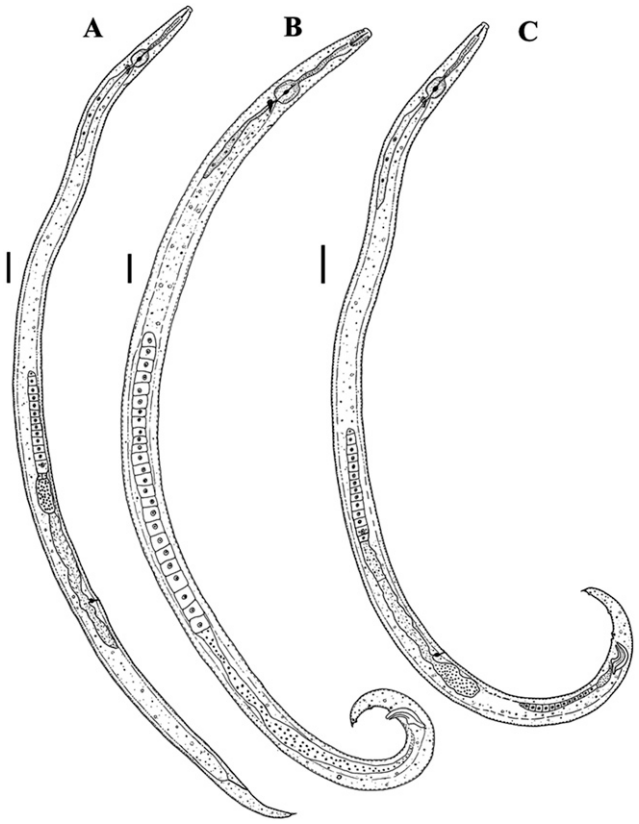

Fig. 1. Line drawing of entire body of *Aphelenchoides macrospica* n. sp. A: Female, B: Male, C: Intersex (scale bars: A and C = 40 μm; B = 20 μm).

[*] The new species refers to the spicule length ("macro" means long, "spica" means of spicule in Latin).

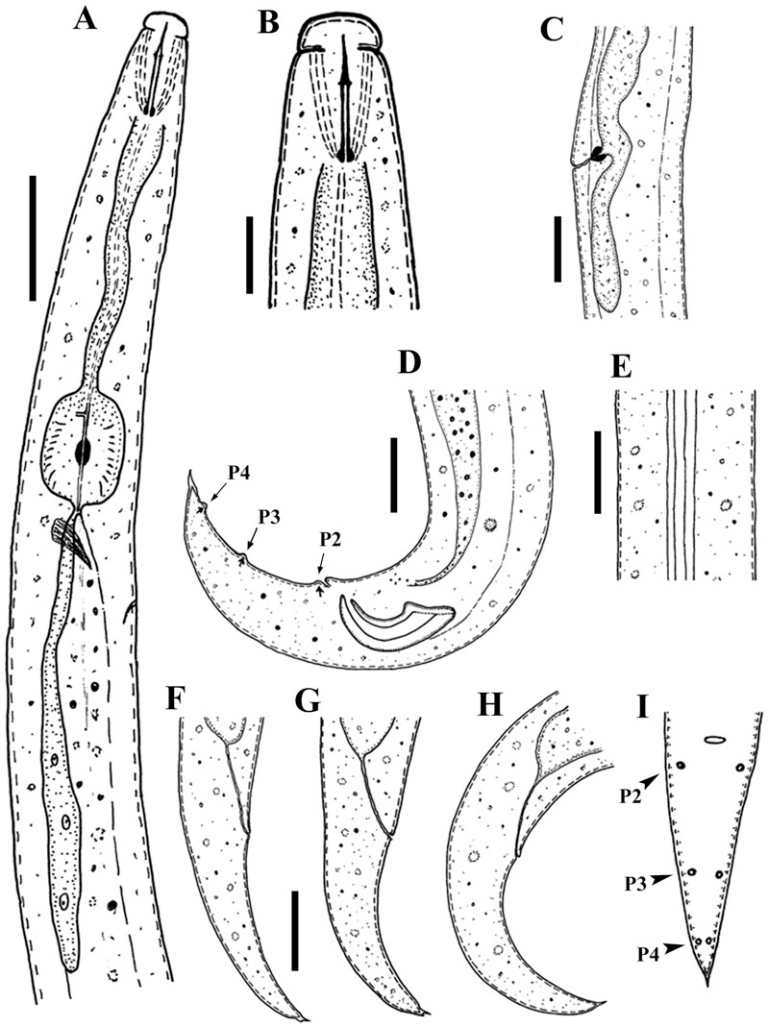

Fig. 2. Line drawing of *Aphelenchoides macrospica* n. sp. A: Female pharynx region; B: Female head in details; C: Vulval region showing post uterine sac; D: Male posterior body showing spicules and genital papillae (arrowheads, ventral view); E: Lateral field; F-H: Female tails; I: Male posterior body and showing caudal papillae in ventral view (arrowhead). (Scale bars: A and D–I = 20 μm; B, C = 10 μm).

(P4). Tail conoid, slightly ventrally arcuate, three times anal body diam. Tail terminus with a simple mucron.

Females: Body cylindrical, habitus straight to slightly curved when heat killed. Cuticle and anterior body region similar to male. Reproductive tract consisting of ovary, oviduct, spermatheca, crustaformeria, uterus, vagina + vulva, and postvulval uterine sac (PUS). Ovary is outstretched anteriorly, developing oocytes in single row. Oviduct, visible in few specimens, comprising two cells and connected with spermatheca, a sac *ca* one vulval body diam. long, formed by small rounded cells, 2 to 3 μm in diameter. Containing sperm cells in some individuals; Crustaformeria not clearly differentiated from other parts of genital tube. Uterus roundish, with thick wall. Vagina sloping anteriorly, with one pair oval sclerotizations as it joins the uterus. Vulva a simple slit in ventral view, without vulval flap. PUS occupying 19% to 30% of distance from vulva to anus and *ca* 1.6 times the corresponding body diam. long, often containing sperm. Rectum and anus visible. Tail conoid, slightly ventrally arcuate, 3.4 times anal body diam., tail terminus varied from bearing a simple mucro to a short trunk bearing one or two unequal processes.

Intersex: Rare, resembling female in body shape and size, but with certain secondary male characters. Body slender, J-shaped or C-shaped after heat fixation. Cuticle and anterior body region similar to females and males. Reproductive systems of both sexes were observed in single nematodes; ovary outstretched with oocytes in single file; conspicuous spermatheca filled with round sperm cells. Vulval flap absent, vagina sloping anteriorly, with one pair of oval sclerotizations as it joins the uterus. Postuterine sac well developed. Testis anteriorly outstretched, locating left of intestine, spermatocytes in one single column. Gonads slightly less developed especially for the male gonad. Tail resembling in shape more the male. Form of head, stylet, esophagus, number of incisures in lateral field, position of nerve ring, excretory pore, spicule, and position of caudal papillae as those of females and males.

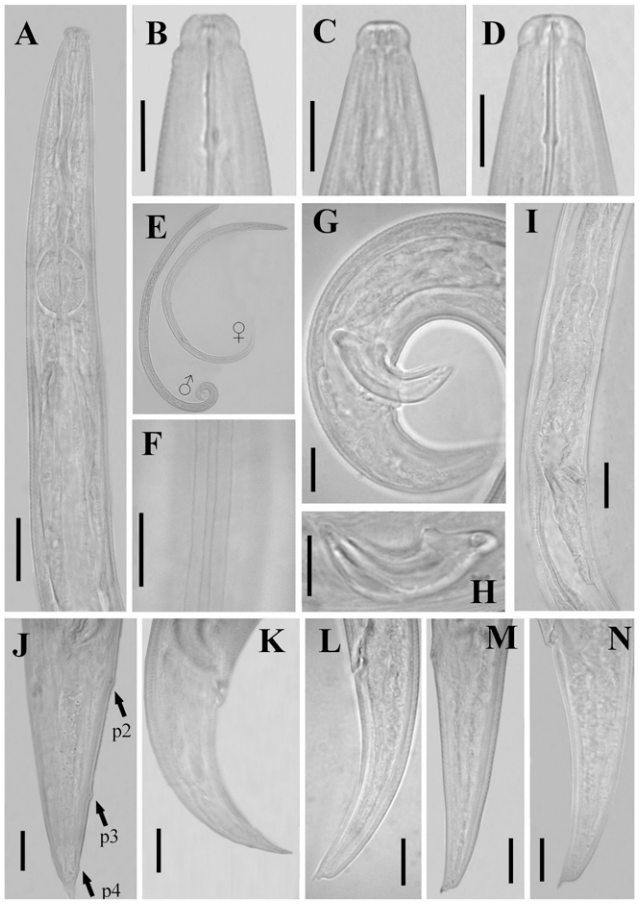

Fig. 3. Light micrographs of *Aphelenchoides macrospica* n. sp. A: Anterior end; B-D: Female head in details; E: Female and male entire body; F: Lateral field; G, H: Male posterior body showing spicules; I: Vulval region showing post uterine sac; J: Male posterior body showing genital papillae (arrowheads, ventral view); K: Male tail; L-N: Female tail. (Scale bars: A, I = 20 μm; B–H and J–N = 10 μm).

Diagnosis and Relationships

Aphelenchoides macrospica n. sp. is characterized by its body lengths of 729 to 1,094 μm (females) and 807 to 963 μm (males). Cephalic region slightly offset, four lines in lateral field. The robust stylet with 15 to 16 μm long and clear basal swellings; posteriorly position of the excretory pore related to the base of metacorpus. The male has a long spicule (dorsal limb 38 to 45 μm long), with broadly rounded apex and well-developed rounded condylus and rounded rostrum. Post-vulval uterine sac (PUS) occupying about one-fourth of distance from vulva to anus, and tail with mucronate terminus in both sexes.

According to grouping of *Aphelenchoides* species *sensu* Shahina (1996), *A. macrospica* n. sp. is within Group 2: "tail with one or sometimes two mucronate structure." Based on four lateral lines, tail terminus shape and spicule features, the new species is close to seven species from Group 2, i.e., *A. arcticus* Sanwal, 1965, *A. blastophthorus* Franklin, 1952, *A. haguei* Maslen, 1979, *A. huntensis*, *A. lucknowensis* Tandon and Singh, 1973, *A. parasaprophilus* Sanwal, 1965, and *A. xui* Wang, Wang, Gu, Wang, and Li, 2013. Furthermore, some relationships were also detected with species of Group 4 *sensu* Shahina (1996), such as *A. parietinus* (Bastian, 1865) Steiner, 1932, and *A. gynotylurus* Timm and Franklin, 1969. The new species differs from *A. arcticus* by having slightly offset lip region vs. nonoffset, longer stylet (15–16 μm vs. 12–13 μm and mean = 15 μm vs. 12 μm in female and male, respectively), greater median bulb length (19–24 μm vs. 12.5–13.8 μm), greater spicule length (mean = 41 μm vs. 21 μm and 19.6 μm vs. 13 μm for dorsal and ventral limbs, respectively). From *A. blastophthorus*, it differs by having slightly shorter stylet (mean = 15.4 μm vs. 17 μm and 15.1 μm vs. 17.1 μm in female and male, respectively), longer spicule (dorsal limb = 41 μm vs. 28 μm), different spicule tip (smoothly rounded vs. hooked), shorter PUS (occupying 19%–30% of distance between vulva to anus vs. 50%). Compared to *A. haguei*, the new species has longer stylet (15–16 vs. 11.5–13 μm), vagina angled obliquely forward vs. at 90° to the body axis and longer spicule (dorsal limb = 38–45 μm vs. 16–25 μm). It can be distinguished from *A. huntensis* by longer body length (729–1,094 μm vs. 507–683 μm and 807–963 μm vs. 636–640 μm in female and male, respectively), longer stylet (15–16 μm vs. 9–10 μm) and slightly greater median bulb length (19–24 μm vs. 16–17 μm and 20–24 μm vs. 17–17.5 μm in female and male, respectively). The new species differs from *A. lucknowensis* by longer body length (729–1,094 μm vs. 560–700 μm and 807–963 μm vs. 550–650 μm in female and male, respectively), greater a-ratio (23.5–35 vs. 21–26), longer tail (3.2–3.8 times anal body diam. long vs. 2.5–3 anal body diam. long), and greater spicule length (dorsal limb = 38–45 μm vs. 18–24 μm). *Aphelenchoides macrospica* n. sp. also differs from *A. parasaprophilus* by having longer stylet (mean = 15.4 vs. 11.7 μm) and spicule (41 μm vs. 21 μm and 19.6 μm vs. 10 μm dorsal and ventral limbs, respectively). From *A. xui*, the new species can be distinguished by having longer body length (729–1,094 μm vs. 548–882 μm and 807–963 μm vs. 546–819 μm in female and male, respectively), longer stylet (15–16 μm vs. 11.1–13.2 μm), different spicule shape (dorsal limb with smoothly rounded tip vs. a hooked tip), slightly greater median bulb length (19–24 μm vs. 14.6–19 μm and 20–24 μm vs. 11.8–15.3 μm in female and male, respectively), and shorter PUS (40–60 μm vs. 68–132 μm). It differs from *A. parietinus* by having longer body length (mean = 900 μm vs. 450 μm), longer stylet (15–16 μm vs. 11–13 μm), and presence of male in population. It can be distinguished from *A. gynotylurus* by having longer body length (729–1,094 μm vs. 490–650 μm and 807–963 μm vs. 430–560 μm in female and male, respectively), greater spicule length (dorsal limb = 38–45 μm vs. 21–24 μm), and shape of the tail terminus (varied from simple mucron

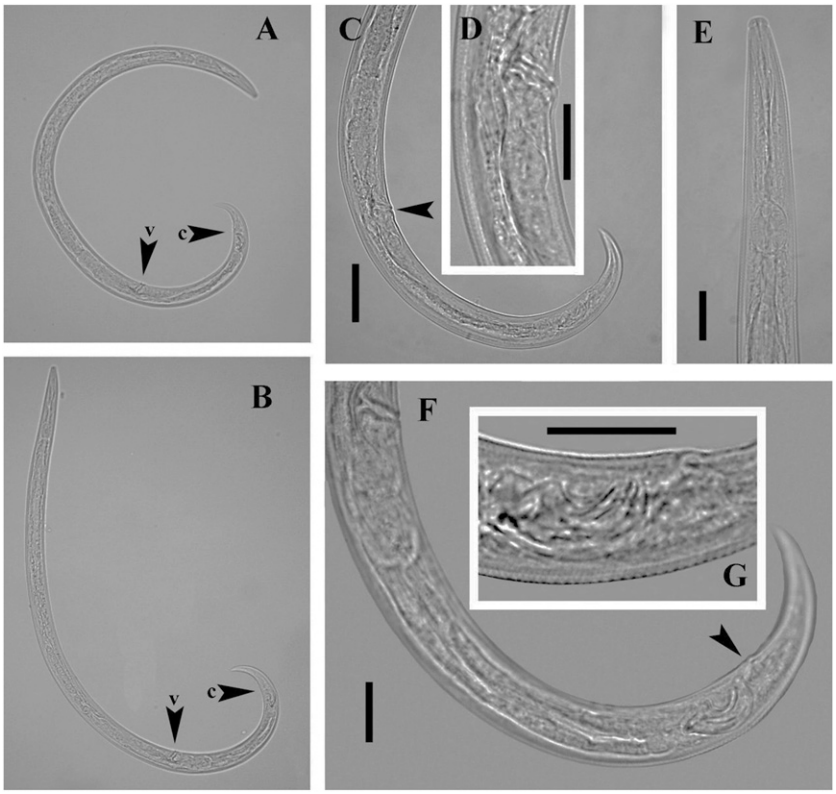

Fig. 4. Light micrographs of *Aphelenchoides macrospica* n. sp. Intersex specimens. A, B: Entire body showing vulva and cloacal region (arrowheads, ventral view); C, F: Posterior end of body; D: Vulval region showing post uterine sac; E: Anterior end; G: Cloacal region (Scale bars: A–G = 20 μm).

to a short trunk bearing one or two processes vs. swollen knob on the tip). It is also similar to *Robustodorus megadorus* (Allen, 1941) Andrássy, 2007, but it can be distinguished by having slightly shorter stylet (mean = 15.4 μm vs. 17 μm), number of incisures in lateral field (four vs. three lines), and female tail shape (mucronate tail terminus vs. without mucron).

Furthermore, due to molecular analysis, the new species should be compared with *A. fragariae* and *A. saprophilus* Franklin, 1957, as well. The new species differs from *A. fragariae* by having longer stylet (mean = 15.4 μm vs. 10 μm), number of incisures in the lateral fields (four vs. two), and longer spicule length in dorsal limb (38–45 vs. 24–31 μm). It differs from *A. saprophilus* by having longer body length (729–1,094 μm vs. 454–623 μm and 807–963 μm vs. 476–627 μm in female and male, respectively), longer stylet (mean = 15.4 μm vs. 11 μm), longer spicule (dorsal limb = 41 μm vs. 23 μm), and different spicule tip (smoothly rounded vs. hooked).

Type Habitat and Locality

The new species recovered from soil samples associated with the rhizosphere of rose plants (*Rosa persica* Michx. ex Juss.) in Hasanlou old hill, Naqadeh (GPS coordinates: N 37° 09′, E 45° 27′), West Azerbayjan province, northwestern Iran.

Type Material

Holotype male (slide AAM004) together with nine paratype specimens (six females, three males, one intersex; slides AAM001, AAM002, AAM003) deposited in the Nematode Collection of the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran. Two paratype females and two paratype males (UGMD 104317) deposited at the Museum voor Dierkunde, Ghent University, Ghent, Belgium, and two paratype females, two paratype males and one paratype intersex deposited in the National Nematode Collection of the Department of Nematology, Iranian Research Institute of Plant Protection, Tehran, Iran.

Molecular Phylogenetic Status

Amplification of D2/D3 expansion segment of 28S rDNA and the partial 18S rDNA from *A. macrospica* n. sp. yielded in PCR products of *ca* 641 and 774 bp, respectively. The dataset for partial LSU rDNA phylogenetic tree was composed of 851 total characters from which 530 characters were variable and for partial 18S rDNA phylogenetic tree was composed of 1,660 total characters from which 787 characters were variable. The average nucleotide composition of dataset was as follows: 24% A, 19.4% C, 29.4% G, and 27.2% T for

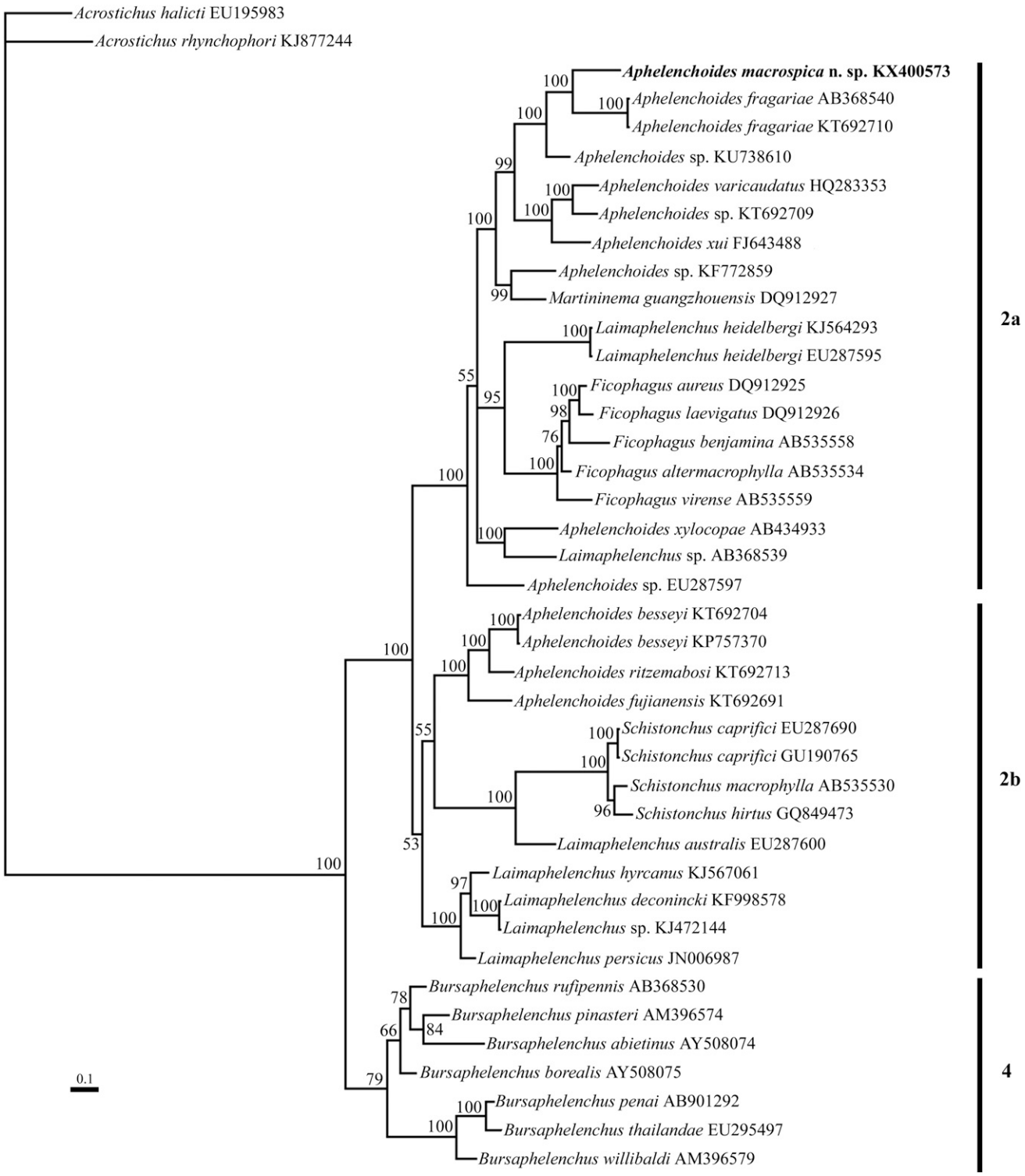

Fig. 5. Bayesian tree inferred from LSU gene DNA sequences. Posterior probabilities exceeding 50% are given on appropriate clades. Nematode species and GenBank accession numbers are listed for each taxon.

D2/D3 28S rDNA and 26.1% A, 20.3% C, 26.8% G, and 26.9% T for partial 18S rDNA. Figures 4 and 5 present phylogenetic trees based on the D2/D3 of 28S and partial 18S rDNA of 41 and 50 taxa, respectively.

According to our molecular analysis for D2/D3 28S rDNA, *A. macrospica* n. sp. was clustered in a clade with posterior probability (PP = 99) comprising *A. fragariae* (AB368540 and KT692710), *Aphelenchoides* sp. (KU738610 and KT692709), *A. varicaudatus* (HQ283353) and *A. xui* (FJ643488). However, *A. macospica* n. sp. is morphologically clearly different from the known species of the clade. Although there were no morphological data for

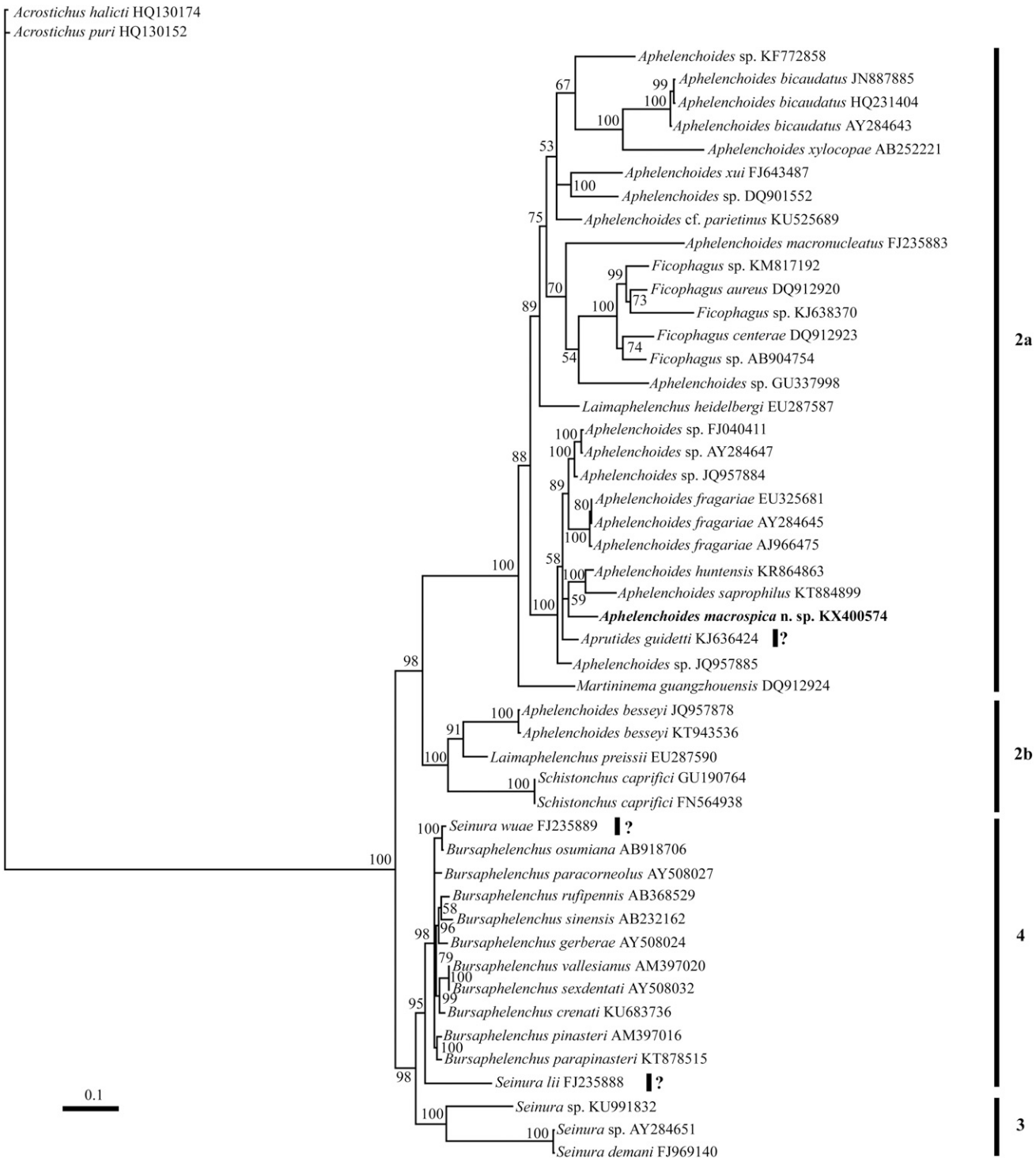

FIG. 6. Bayesian tree inferred from SSU gene DNA sequences. Posterior probabilities exceeding 50% are given on appropriate clades. Nematode species and GenBank accession numbers are listed for each taxon.

Aphelenchoides sp. (KU738610 and KT692709), a separate alignment between these species and the new species showed a major difference, i.e., 92 (16%) and 139 (21%) nucleotide differences based on 564 and 656 nucleotides, respectively. The new species in the phylogenetic tree inferred from partial 18S rDNA gene was clustered in a clade with maximal support with *A. huntensis* (KR864863), *A. saprophilus* (KT884899), *Aphelenchoides* sp. (JQ957884, JQ957885, FJ040411, and AY284647), *A. fragariae* (AJ966475), (AY284645 and EU325681), and *Aprutides guidetti* (KJ636424). The new species has been easily distinguishable from the known species of the genus from this clade, i.e., *A. huntensis*, *A. saprophilus*, and *A. fragariae* as discussed earlier, but there were not any special morphological data for the rest members of the clade, i.e., *Aphelenchoides* sp. (JQ957884, JQ957885,

TABLE 1. Morphometric data for *Aphelenchoides macrospica* sp. n. (measurements μm; mean ± s.d. and (range) for paratypes).

Character	Male		Female	Intersex
	Holotype	Paratypes	Paratypes	Paratypes
n	-	7	11	2
L	877	890 ± 59 (807–963)	900 ± 91 (729–1,094)	853, 890
a	32	30 ± 2.4 (27–32.5)	28.3 ± 3.7 (23.5–35)	28.4, 32
b	9.2	9.4 ± 0.3 (9–10)	9.7 ± 1 (8.7–11. 5)	8.4, 9
b′	4.4	4.6 ± 0.3 (4.3–5)	4.4 ± 0.4 (3.5–5)	4.7, 5
c	17.2	15.5 ± 2 (13–18)	15.6 ± 1.3 (14–18)	14, 16
c′	2.3	3 ± 0.3 (2.3–3.1)	3.4 ± 0.3 (3.1–3.8)	3, 3.1
V	-	-	69.5 ± 2.2 (65–72.3)	68, 71.6
T	50	55 ± 3.2 (49.5–59)	-	11.6, 20.4
Lip region height	4	3.9 ± 0.4 (3–4)	3.4 ± 0.5 (3–4)	3, 4
Lip region width	8	7.6 ± 0.5 (7–8)	7.2 ± 0.6 (7–8)	6, 7
Stylet length	15	15 ± 0.4 (14.5–16)	15.4 ± 0.5 (15–16)	15, 16
Conus length	7	6.4 ± 0.5 (6–7)	7.1 ± 0.7 (6–8)	6, 7
m[a]	47	42.7 ± 4.3 (38–48)	46 ± 2.6 (40–50)	40, 44
Maximum body diam.	27	30 ± 3 (27–35)	32 ± 3.5 (26–40)	28, 30
MB[b]	88	87.5 ± 6 (83–95)	91 ± 7 (80–98)	85, 88
Median bulb width	15	15 ± 0.4 (14–15)	15 ± 1.1 (14–17)	14, 15
Median bulb length	21	22.3 ± 1.5 (20–24)	21.6 ± 1.4 (19–24)	21, 22
Median bulb length/diam. ratio	1.4	1.5 ± 0.1 (1.3–1.7)	1.4 ± 0.1 (1.3–1.6)	1.5
Nerve ring from anterior body	95	95 ± 6.2 (85–101)	98 ± 5.9 (88–108)	100, 109
Excretory pore from anterior end	110	106 ± 8.5 (95–115)	104.9 ± 6.5 (93–110)	108, 115
Ovary length	-	-	235 ± 22 (210–274)	174, 235
Testis length	438	488 ± 37 (434–541)	-	103, 174
Postuterine sac	-	-	49.5 ± 6.5 (41–60)	50, 51
Vulva to anus distance	-	-	213 ± 27 (172–265)	-
Postuterine sac length/vulva to anus (%)	-	-	23.5 ± 4 (19–30)	-
Pharynx	90	92 ± 5.6 (84–100)	92 ± 6.9 (83–100)	98, 102
Overlapping	107	102 ± 14.6 (85–128)	106 ± 11.5 (90–127)	78, 90
Anal (cloacal) body diam.	22	20.5 ± 0.8 (20–22)	17 ± 1.3 (15–19)	18, 20
Tail length	51	58 ± 5.7 (50–65)	58 ± 4.4 (52–63)	56, 60
Spicule (dorsal limb)	40	41 ± 2.7 (38–45)	-	38, 39
Spicule (ventral limb)	19	19.6 ± 1.4 (18–22)	-	17, 18
Spicule (curved median line)	29	29.5 ± 2 (27–32)	-	27, 28
Capitulum	1.4	12.3 ± 1.1 (11–14)	-	12, 13

[a] Length of conus as percentage of total stylet length.
[b] Distance between anterior end of body and centre of median pharyngeal bulb as percentage of pharyngeal length.

FJ040411, and AY284647). However, a separated alignment and nucleotide comparison of these species with the new species indicated that *A. macrospica* n. sp. is a unique species for the genus with 38 (5%) of 777 (*Aphelenchoides* sp. JQ957884), 53 (7%) of 777 (*Aphelenchoides* sp. JQ957885), 42 (5%) of 777 (*Aphelenchoides* sp. FJ040411), and 45 (6%) of 780 (*Aphelenchoides* sp. AY284647) nucleotide differences. Molecular profiles and phylogenetic analyses by using the two molecular markers of D2/D3 expansion segment of 28S rDNA and partial 18S revealed that the new species belongs to *Aphelenchoides*, and strongly supports the status of *A. macrospica* n. sp. as a new species. The new species can be clearly separated from all other sequenced *Aphelenchoides* spp.

DISCUSSION

Occurrence of intersexes in nematode life cycle are just known in relatively few genera. An intersex is an individual which exhibits a blending of male and female characters. Intersexes in nematodes have been observed most frequently in the insect parasitizing mermithids, where they occur normally (Hirschmann and Sasser, 1955), but in PPN turn out to be rare and there are some published reports of intersexes; Chitwood (1949) reported intersexual specimens in *Meloidogyne javanica* and Hirschmann and Sasser (1955) observed intersexuality in the genera *Ditylenchus*. Bajaj (1987) also observed intersexes in *Paratylenchus obtusicaudatus* Raski, 1975. Intersexuality was also observed in *Aphelenchoides composticola* Franklin, 1957 (Zhuo et al., 2009).

The family Aphelenchoididae has been divided into four clades based on LSU and SSU rDNA genes by Kanzaki and Giblin-Davis (2012). The topology of the inferred trees from these genes in the present study is in general agreement with that study except for some minor differences. Although three accessions of the genera *Seinura*, i.e., *S. demani* (FJ969140), *Seinura* sp. (AY284651, KU991832) are clustered together in a maximal support in clade 3 based on SSU rDNA gene, surprisingly two other accessions of this genera including *S. wuae* (FJ235889) and *S. lii* (FJ235888)

clustered in clade 4 next to *Bursaphelenchus* spp., which cannot be explained based on morphological data. Furthermore, *Aprotides*, the other genera of the subfamily Seinurinae that was not analyzed by Kanzaki and Giblin-Davis (2012), was also included in SSU rDNA gene analyses in the present study. Interestingly, it forms a sister clade with the clade comprising the new species, *A. saprophilus* (KT884899) and *A. huntensis* (KR864863) among subclade 2a. The only common character among them is the position of excretory pore posteriorly. Few molecular data of the subfamily Seinurinae are available in the GenBank, but it can be predicted that the subfamily and species of the genera *Seinura* probably are not monophyletic and their classification may need to be revised.

Based on partial LSU and SSU rDNA sequences, *A. macrospica* n. sp. is grouped with other genera of Aphelenchoidinae, i.e., *Ficophagus* Davies, Ye, Kanzaki, Bartholomaeus, Zeng, and Giblin-Davis, 2015, *Martininema* Davies, Ye, Kanzaki, Bartholomaeus, Zeng, and Giblin-Davis, 2015, *Laimaphelenchus* Fuchs, 1937, and *Schistonchus* s.s. Davies, Ye, Kanzaki, Bartholomaeus, Zeng, and Giblin-Davis, 2015, in clade 2. According to our molecular analysis, the members of the genus *Aphelenchoides* appears to be paraphyletic, a finding that agrees with previous studies (Zhao et al., 2008; Rybarczyk-Mydłowska et al., 2012; Wang et al., 2013; Fang et al., 2014a, 2014b; Miraeiz et al., 2015; Esmaeili et al., 2016a, 2016b; Golhasan et al., 2016). Furthermore *Aphelenchoides*, *Laimaphelenchus*, and *Schistonchus* s. l cannot be clearly separated, possibly implying that all the genera share a recent common ancestor (Zeng et al., 2007). More DNA sequences and morphological data will be necessary to revise the generic level and clarify their evolutionary relationships with other genera and within their species themselves.

Literature Cited

Andrássy, I. 2007. Free-living nematodes of Hungary (Nematoda errantia). Vol. II *in* C. Csuzdi and S. Mahunka, eds. Pedozoologica Hungarica No. 4. Budapest, Hungary: Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Sciences.

Bajaj, K. H. 1987. On the species of *Paratylenchus micoletzky* (Nematoda: Criconematina) from Haryana, India. Indian Journal of Nematology 17(2):318–326.

Chitwood, B. G. 1949. Root-knot nematodes. Part I. A revision of the genus *Meloidogyne* Goeldi, 1887. Proceedings of the Helminthological Society of Washington 16(2):90–104.

Christie, J. R. 1932. Recent observations on the strawberry dwarf nematode in Massachusetts. Plant Disease Reporter 16:113–114.

Christie, J. R. 1942. A description of *Aphelenchoides besseyi* n.sp., the summer-dwarf nematode on strawberries, with comments on the identity of *Aphelenchoides subtenuis* (Cobb, 1926) and *Aphelenchoides hodsoni* Goodey, 1935. Proceedings of the Helminthological Society of Washington 9:82–84.

Davies, K. A., Ye, W., Kanzaki, N., Bartholomaeus, F., Zeng, Y., and Giblin-Davis, R. M. 2015. A review of the taxonomy, phylogeny, distribution and co-evolution of *Schistonchus* Cobb, 1927 with proposal of *Ficophagus* n. gen. and *Martininema* n. gen. (Nematoda: Aphelenchoididae). Nematology 17:761–829.

De Grisse, A. T. 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 34:351–369.

Edgard, R. C. 2004. MUSCLE: Multiple sequence alignement with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Esmaeili, M., Fang, Y., Li, H., and Heydari, R. 2016a. Description of *Aphelenchoides huntensis* sp. n. (Nematoda: Aphelenchoididae) isolated from *Pinus sylvestris* in western Iran. Nematology 18:357–366.

Esmaeili, M., Heydari, R., Ziaie, M., and Gu, F. 2016b. Molecular and morphological characterization of *Aphelenchoides fuchsi* sp. n. (Nematoda: Aphelenchoididae) isolated from *Pinus eldarica* in western Iran. Journal of Nematology 48(1):34–42.

Fang, Y., Gu, J., Wang, X., and Li, H. 2014a. Description of *Aphelenchoides stellatus* n. sp. (Nematoda: Aphelenchoididae) found in packaging wood from Japan. Nematology 16:621–630.

Fang, Y., Gu, J., Wang, X., and Li, H. 2014b. Description of *Aphelenchoides rotundicaudatus* n. sp. (Nematoda: Aphelenchoididae) found in packaging wood from South Korea. Nematology 16:751–760.

Fischer, M. 1894. Ubereine Clematis-Krankheit. Bericht us dem Physiolischen Laboratorium des Landwirthschaftlichen. Instituts der Universitat Halle 3:1–11.

Franklin, M. T. 1952. A disease of *Scabiosa caucasica* caused by the nematode *Aphelenchoides blastophthorus* n. sp. Annals of Applied Biology 39:54–60.

Franklin, M. T. 1957. *Aphelenchoides composticola* n. sp. and *A. saprophilus* n. sp. from mushroom compost and rotting plant tissues. Nematologica 2:306–313.

Ghaderi, R., Kashi, L., and Karegar, A. 2012. The Nematodes of Iran (based on the published reports until 2011). Tehran, Iran: Agricultural Education and Extension Publication, p. 371.

Golhasan, B., Heydari, R., Alvarez-Ortega, S., Esmaeili, M., Castillo, P., and Palomares–Rius, J. E. 2016. *Aphelenchoides iranicus* n. sp. (Nematoda: Aphelenchoididae) from West Azerbaijan province. Iran. Nematology (In press).

Gouy, M., Guindon, S., and Gascuel, O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Molecular Biology and Evolution 27:221–224.

Hirschmann, H., and Sasser, N. 1995. On the occurrence of an intersexual form in *Ditylenchus triformis*, n. sp. (Nematoda, Tylenchida). Proceedings of the Helminthological Society of Washington 22:115–131.

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

Hunt, D. J. 1993. Aphelenchida, Longidoridae and Trichodoridae: Their systematics and bionomics. CAB International Wallingford, 352 pp.

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

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Jones, J., Haegeman, A., Danchin, E., Gaur, H., Helder, J., Jones, M., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J., Wesemael, W., and Perry, R. 2013. Review: Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology 14:946–961.

Kanzaki, N., and Giblin-Davis, R. 2012. Aphelenchoidea. Pp. 161–208 *in* R. Manzanilla-Lopez and N. Mendoza, Eds. Practical plant nematology. Guadalajara, México: Biblioteca Básica de Agricultura.

Kanzaki, N. 2006. Description of *Aphelenchoides xylocopae* n. sp. (Nematoda: Aphelenchoididae), the first observed association between nematodes and carpenter bees. Nematology 8:555–562.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.

Larget, B., and Simon, D. L. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16:750–759.

Maslen, N. R. 1979. Six new nematode species from the maritime Antarctic. Nematologica 25:288–308.

Miraeiz, E., Heydari, R., Tanha Maafi, Z., and Bert, W. 2015. *Laimaphelenchus hyrcanus* n. sp. (Nematoda: Aphelenchoididae), a new species from northern Iran. Zootaxa 3915(4):591–600.

Nickle, W. R., and Hooper, D. J.1991. The Aphelenchina: Bud, leaf, and insect nematodes. Pp. 465–507 *in* W. R. Nickle, ed. Manual of agricultural nematology. New York: Marcel Dekker.

Nunn, G. B. 1992. Nematode molecular evolution. Ph.D. dissertation, University of Nottingham, UK.

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

Rybarczyk-Mydłowska, K., Mooyman, P., van Megen, H., van den Elsen, S., Vervoort, M., Veenhuizen, P., van Doorn, J., Dees, R., Karssen, G., Bakker, J., and Helder, J. 2012. Small subunit ribosomal DNA-based phylogenetic analysis of foliar nematodes (*Aphelenchoides* spp.) and their quantitative detection in complex DNA backgrounds. Phytopathology 102:1153–1160.

Sánchez-Monge, A., Flores, L., Salazar, L., Hockland, S., and 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(2):207–224.

Sanwal, K. C. 1965. Two new species of the genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchoididae) from the Canadian Arctic. Canadian Journal of Zoology 43:933–940.

Shahina, F. 1996. A diagnostic compendium of the genus *Aphelenchoides* Fischer, 1894 (Nematoda: Aphelenchida) with some new records of the group from Pakistan. Pakistan Journal of Nematology 14:1–32.

Skarbilovich, T. S. 1947. Revision of the systematics of the family Anguillulinidae Baylis and Daubney, 1962. Doklady Akademii Nauk Sssr 57:307–308.

Steiner, G. 1932. Annotations on the nomenclature of some plant parasitic nematodes. Journal of the Washington Academy of Science 22:517–518.

Steiner, G., and Buhrer, E. M. 1932. Miscellaneous notes on nemic diseases. Plant Disease Reporter 16:137.

Tandon, R. S., and Singh, S. P. 1973. Two plant parasites of two different families of nematodes parasitizing lady finger (*Abelmoschus esculentus*) at Lucknow. Zoologischer Anzeiger 191:139–150.

Timm, R. W., and Franklin, M. T. 1969. Two marine species of *Aphelenchoides*. Nematologica 15:370–375.

Wang, X., Wang, P., Gu, J., Wang, J., and Li, H. 2013. Description of *Aphelenchoides xui* sp. n. (Nematoda: Aphelenchoididae) in packaging wood from South Africa. Nematology 15:279–289.

Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology 55:25–38.

Zeng, Y., Giblin-Davis, R. M., and Ye, W. 2007. Two new species of *Schistonchus* (Nematoda: Aphelenchoididae) associated with *Ficus hispida* in China. Nematology 9:169–187.

Zhao, Z. Q., Davies, K. A., Riley, I. T., and Nobbs, J. M. 2006. *Laimaphelenchus preissii* sp. nov. (Nematoda: Aphelenchina) from native *Callitris preissii* in South Australia. Transactions of the Royal Society of South Australia 130:10–16.

Zhao, Z. Q., Davies, K. A., Riley, I. T., and Nobbs, J. M. 2007. *Laimaphelenchus heidelbergi* sp. nov. (Nematoda: Aphelenchina) from Victoria, Australia, and emendment of the diagnosis of the genus. Transactions of the Royal Society of South Australia 132:185–195.

Zhao, Z. Q., Ye, W. M., Giblin-Davis, R. M., Li, D. M., Thomas, W. K., Davies, K. A., and Riley, I. T. 2008. Morphological and molecular analysis of six *Aphelenchoidoids* from Australian conifers and their relationship to *Bursaphelenchus* (Fuchs, 1937). Nematology 10:663–678.

Zhuo, K., Liao, J., Cui, R., and Li, Y. 2009. First record of female intersex in *Hirschmanniella shamimi* Ahmad, 1972 (Nematoda: Pratylenchidae), with a checklist of intersexes in plant nematodes. Zootaxa 1973:61–68.