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# Molecular and Morphological Characterization of *Aphelenchoides fuchsi* sp. n. (Nematoda: Aphelenchoididae) Isolated from *Pinus eldarica* in Western Iran

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*Abstract: Aphelenchoides fuchsi* sp. n. is described and illustrated from bark and wood samples of a weakened Mondell pine in Kermanshah Province, western Iran. The new species has body length of 332 to 400 μm (females) and 365 to 395 μm (males). Lip region set off from body contour. The cuticle is weakly annulated, and there are four lines in the lateral field. The stylet is 8 to 10 μm long and has small basal swellings. The excretory pore is located ca one body diam. posterior to metacorpus valve or 51 to 62 μm from the head. The postuterine sac well developed (60–90 μm). Spicules are relatively short (15–16 μm in dorsal limb) with apex and rostrum rounded, well developed, and the end of the dorsal limb clearly curved ventrad like a hook. The male tail has usual three pairs of caudal papillae (2+2+2) and a well-developed mucro. The female tail is conical, terminating in a complicated step-like projection, usually with many tiny nodular protuberances. The new species belongs to the Group 2 sensu Shahina, category of *Aphelenchoides* species. Phylogenetic analysis based on small subunit (SSU) and partial large subunit (LSU) sequences of rRNA supported the morphological results.

*Key words: Aphelenchoides*, LSU, molecular, morphology, morphometrics, new species, phylogeny, SSU, taxonomy.

To find and prevent the spread of pests comprising pine wood nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970 in Iran, bark and wood samples must be sampled and inspected. So, during the past few years, a wide survey on dead or weakening pine trees in northern Iran was conducted and several species belonging to Aphelenchoididae Skarbilovich, 1947 were recovered and described: two new species of the genus *Ektaphelenchoides* Baujard, 1984 (Pedram et al., 2012; Aliramaji et al., 2014); a new species of the genus *Bursaphelenchus* Fuchs, 1937 (Pedram et al., 2011); and a new species of the genus *Laimaphelenchus* Fuchs, 1937 (Asghari et al., 2012). Up to now, *B. xylophilus*, which has caused serious damage to the coniferous forests, has not been found in Iran.

The genus *Aphelenchoides* Fischer, 1894 contains at present more than 150 nominal species (Hunt, 2008) and tends to be greatly conserved in gross morphology, making species identification a very difficult task. Also, many of the description fall below modern standards. Currently, species discrimination in *Aphelenchoides* is mainly based on morphology and morphometrics. In recent years, several species of the genus were described related to the pine trees in different countries, e.g., China (Cheng et al., 2009; Zhuo et al., 2010) and the United States (Kaisa, 2000), and some species isolated form packaging wood in South Korea (Cui et al., 2011; Fang et al., 2014b), India (Bina Chanu et al., 2013), South Africa (Wang et al., 2013), and Japan (Fang et al., 2014a). Recently a new species of *Aphelenchoides*, *Aphelenchoides huntensis* Esmaeili, Fang, Li, and Heydari, 2016 is described in association with pine trees in Iran. It would be likely to find more *Aphelenchoides* species if other substrates (e.g., bark beetles) were examined with such attention.

During 2013 and 2014, we conducted some inspections on wood and bark samples from dead or weakened pine trees in western Iran. As a result, a new species of *Aphelenchoides* was isolated from a weakened Mondell pine tree (*Pinus eldarica* L.), which is described and figured in this paper as *Aphelenchoides fuchsi* sp. n. It is the second new species of *Aphelenchoides* that is described from Iran.

## Materials and Methods

### *Sampling, extraction, mounting, and drawing*

Several bark and wood samples from weakened pine trees were collected from western Iran, which yielded an aphelenchid nematode belonging to the genus *Aphelenchoides*. The nematodes were recovered from the wood samples by soaking a small amount of wood in water for 48 hr and handpicked under a stereomicroscope model Olympus SZH (Japan). The nematodes were heat killed by adding boiling 4% formalin solution and then transferred to anhydrous glycerin and mounted in permanent slides according to the method by De Grisse (1969). Permanent slides were prepared and studied using a light microscope (Nikon E200). Drawings were made using a drawing tube attached to the same microscope, and photographs of live nematodes were taken by a digital camera attached to the microscope.

### *DNA extraction, polymerase chain reaction, and sequencing*

A single live nematode specimen of *A. fuchsi* sp. n. was picked out, examined on a temporary slide, and then transferred to a small drop of AE buffer (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0; Qiagen Inc., Valencia, CA) on a clean slide and squashed using a clean cover slip. The

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suspension was collected by adding 20 µl AE buffer. The DNA samples were stored at −20°C until used as polymerase chain reaction (PCR) templates. 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 primers 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). The D2-D3 expansion segments of 28S rRNA gene were amplified using forward primer D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and reverse primer D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) (Nunn, 1992). Polymerase chain reaction was performed in a final volume of 25 µl PCR mixture and contained 12.5 µl 2X GoTaq DNA polymerase mix (Promega Corporation, Madison, WI), each of a 1.2 µl forward and reverse primers solution (5 pM), 8 µl distilled water, and 2 µl of a 100 times-diluted crude DNA extract. The following PCR profile was used: 94°C for 5 min; 5 × (94°C, 30 sec; 45°C, 30 sec; and 72°C, 70 sec) followed by 35 × (94°C, 30 sec; 54°C, 30 sec; 72°C, 70 sec) and 72°C, 5 min. The PCR products were purified and sequenced directly for both strands using the same primers with an ABI 3730XL sequencer (Macrogen Corporation, Seoul, South Korea). The newly obtained sequences was submitted to GenBank database under accession numbers KT003986 (18S) and KT003987 (28S).

The obtained sequences of the partial 18S and partial 28S D2-D3 region ribosomal DNA (rDNA) gene of *A. fuchsi* sp. n. were compared with those of other aphelenchids species available in GenBank (see Table 1 for selected sequences of SSU and Table 2 for LSU D2-D3) using BLAST homology search program. Outgroup was chosen according to previous published data (Fang et al., 2014a, 2014b; Esmaeili et al., 2016). The newly obtained and published sequences were aligned using MAFFT ver. 7 (Katoh et al., 2002) with default parameters. Sequence alignment was edited using BioEdit (Hall, 1999). The best fitted model of DNA evolution was obtained using jModelTest2 (Darriba et al., 2012) with the Akaike information criterion. The Akaike-supported model, the base frequency, the proportion of invariable sites, and the gamma distribution shape parameters and substitution rates in the Akaike information criterion were then used in phylogenetic analyses. The tree topology was confirmed using MrBayes 3.2.3 (Ronquist and Huelsenbeck, 2003) with four chains (three heated and one cold). The number of generations for the total analysis was set to 10 million, with the chain sampled every

Table 1. Species used for analysis of phylogenetic relationships and the accession numbers for SSU sequences deposited in GenBank.

| Species | Accession number | Authors | Species | Accession number | Authors |
|---|---|---|---|---|---|
| *Aphelenchoides fuchsi* sp. n. | KT003986 | Present paper | *Aphelenchoides* sp. | DQ901553 | Subbotin et al., 2006 |
| *Aphelenchoides besseyi* | AY508035 | Ye et al., 2007 | *Aphelenchoides* sp. | EU287591 | Zhao et al., 2008 |
| *Aphelenchoides bicaudatus* | HQ283351 | Huang et al., 2012 | *Aphelenchoides* sp. | GU337995 | Cui et al., 2011 |
| *Aphelenchoides blastophthorus* | JQ957879 | Rybarczyk-Mydłowska et al., 2012 | *Aphelenchoides* sp. | GU337994 | Cui et al., 2011 |
| *Aphelenchoides blastophthorus* | AY284646 | Holterman et al., 2006 | *Aphelenchus avenae* | AB368918 | Unpublished |
| *Aphelenchoides fragariae* | DQ901551 | Subbotin et al., 2006 | *Aprutides guidetti* | KJ636424 | Unpublished |
| *Aphelenchoides fragariae* | AB067755 | Unpublished | *Ficophagus aureus* | DQ912922 | Davies et al., 2015 |
| *Aphelenchoides fujianensis* | FJ520227 | Zhuo et al., 2010 | *Ficophagus benjamina* | KJ638352 | Davies et al., 2015 |
| *Aphelenchoides huntensis* | KR864863 | Esmaeili et al., 2016 | *Ficophagus centerae* | DQ912923 | Davies et al., 2015 |
| *Aphelenchoides macronucleatus* | FJ235883 | Unpublished | *Ficophagus* sp. | KJ638366 | Davies et al., 2015 |
| *Aphelenchoides paradaliansis* | GU337993 | Cui et al., 2011 | *Ficophagus* sp. | KJ638358 | Davies et al., 2015 |
| *Aphelenchoides ritzemabosi* | DQ901554 | Subbotin et al., 2006 | *Ficophagus* sp. | KJ638361 | Davies et al., 2015 |
| *Aphelenchoides rotundicaudatus* | KFF772858 | Fang et al., 2014b | *Ficophagus* sp. | KJ638364 | Davies et al., 2015 |
| *Aphelenchoides saprophilus* | FJ040408 | Holterman et al., 2006 | *Ficophagus* sp. | KJ638370 | Davies et al., 2015 |
| *Aphelenchoides subtenuis* | JQ957891 | Rybarczyk-Mydł;owska et al., 2012 | *Ficophagus* sp. | EU038299 | Davies et al., 2015 |
| *Aphelenchoides varicaudatus* | HQ283351 | Huang et al., 2012 | *Ficophagus* sp. | KJ638360 | Davies et al., 2015 |
| *Aphelenchoides xui* | FJ643487 | Wang et al., 2013 | *Ficophagus* sp. | KJ638357 | Davies et al., 2015 |
| *Aphelenchoides* sp. | JQ957884 | Rybarczyk-Mydł;owska et al., 2012 | *Ficophagus* sp. | KM817192 | Davies et al., 2015 |
| *Aphelenchoides* sp. | AY284646 | Holterman et al., 2006 | *Ficophagus* sp. | KC526928 | Davies et al., 2015 |
| *Aphelenchoides* sp. | FJ040409 | Holterman et al., 2006 | *Ficophagus* sp. | AB904757 | Davies et al., 2015 |
| *Aphelenchoides* sp. | FJ040410 | Holterman et al., 2006 | *Ficophagus* sp. | KJ638354 | Davies et al., 2015 |
| *Aphelenchoides* sp. | JQ957885 | Huang et al., 2012 | *Laimaphelenchus heidelbergi* | EU287587 | Zhao et al., 2008 |
| *Aphelenchoides* sp. | GU337997 | Cui et al., 2011 | *Laimaphelenchus belgradiensis* | KF881745 | Oro, 2015 |
| *Aphelenchoides* sp. | GU337999 | Cui et al., 2011 | *Laimaphelenchus penardi* | AY593918 | Holterman et al., 2006 |
| *Aphelenchoides* sp. | GU337998 | Cui et al., 2011 | *Laimaphelenchus perissii* | EU287590 | Zhao et al., 2008 |
| *Aphelenchoides* sp. | JQ957883 | Huang et al., 2012 | *Martininema guanzagzhouensis* | DQ912924 | Davies et al., 2015 |
| *Aphelenchoides* sp. | AB661626 | Unpublished | *Robustodorus megadorus* | KC687094 | Unpublished |
| *Aphelenchoides* sp. | GU337996 | Cui et al., 2011 | *Schistonchus caprifici* | GU190764 | Davies et al., 2015 |
| *Aphelenchoides* sp. | EU287589 | Zhao et al., 2008 | *Schistonchus* sp. | HM151003 | Davies et al., 2015 |
| *Aphelenchoides* sp. | DQ901550 | Subbotin et al., 2006 | – | – | – |

TABLE 2. Species used for analysis of phylogenetic relationships and the accession numbers for LSU D2-D3 sequences deposited in GenBank.

| Species | Accession number | Authors | Species | Accession number | Authors |
|---|---|---|---|---|---|
| *Aphelenchoides fuchsi* sp. n. | KT003987 | Present paper | *Ficophagus* sp. | AB535556 | Davies et al., 2015 |
| *Aphelenchoides besseyi* | EU325682 | Zhao et al., 2008 | *Ficophagus* sp. | AB535565 | Davies et al., 2015 |
| *Aphelenchoides fragariae* | EU325684 | Zhao et al., 2008 | *Ficophagus* sp. | KC526929 | Davies et al., 2015 |
| *Aphelenchoides huntensis* | KR864862 | Esmaeili et al., 2016 | *Ficophagus* sp. | KM817193 | Davies et al., 2015 |
| *Aphelenchoides rotundicaudatus* | KF772859 | Fang et al., 2014b | *Ficophagus* sp. | KJ638372 | Davies et al., 2015 |
| *Aphelenchoides stellatus* | KF638651 | Fang et al., 2014a | *Ficophagus* sp. | KJ638376 | Davies et al., 2015 |
| *Aphelenchoides stammeri* | AM396582 | Unpublished | *Ficophagus* sp. | KJ638377 | Davies et al., 2015 |
| *Aphelenchoides varicaudatus* | HQ283353 | Huang et al., 2012 | *Ficophagus* sp. | KJ638378 | Davies et al., 2015 |
| *Aphelenchoides xui* | FJ643488 | Wang et al., 2013 | *Ficophagus* sp. | KJ638384 | Davies et al., 2015 |
| *Aphelenchoides xylocopae* | AB434933 | Kanzaki et al., 2008 | *Laimaphelenchus australis* | EU287600 | Zhao et al., 2008 |
| *Aphelenchoides* sp. | DQ328682 | Subbotin et al., 2006 | *Laimaphelenchus belgradiensis* | KF881746 | Oro, 2015 |
| *Aphelenchoides* sp. | EU287597 | Zhao et al., 2008 | *Laimaphelenchus deconincki* | KF998578 | Unpublished |
| *Aphelenchoides* sp. | EU287599 | Zhao et al., 2008 | *Laimaphelenchus heidelbergi* | EU287595 | Zhao et al., 2008 |
| *Aphelenchoides* sp. | EU084037 | Unpublished | *Laimaphelenchus persicus* | JN006987 | Asghari et al., 2012 |
| *Aphelenchus avenae* | AB368536 | Kanzaki et al., 2008 | *Laimaphelenchus perissii* | EU287598 | Zhao et al., 2008 |
| *Ficophagus altermacrophylla* | AB535534 | Davies et al., 2015 | *Laimaphelenchus* sp. | AB368539 | Kanzaki et al., 2008 |
| *Ficophagus aureus* | DQ912926 | Davies et al., 2015 | *Laimaphelenchus* sp. | KJ472144 | Unpublished |
| *Ficophagus benjamina* | AB535558 | Davies et al., 2015 | *Laimaphelenchus* sp. | KJ567061 | Miraeiz et al., 2015 |
| *Ficophagus benjamina* | AB535553 | Davies et al., 2015 | *Martininema fistulosus* | KC250363 | Davies et al., 2015 |
| *Ficophagus centrae* | DQ912928 | Davies et al., 2015 | *Martininema guanzagzhouensis* | DQ912927 | Davies et al., 2015 |
| *Ficophagus microcapus* | GU392234 | Davies et al., 2015 | *Martininema* sp. | KM817190 | Davies et al., 2015 |
| *Ficophagus laevigatus* | DQ912926 | Davies et al., 2015 | *Schistonchus caprifici* | GU190765 | Davies et al., 2015 |
| *Ficophagus virens* | AB535565 | Davies et al., 2015 | *Schistonchus hirtus* | GQ849473 | Davies et al., 2015 |
| *Ficophagus* sp. | AB535555 | Davies et al., 2015 | *Schistonchus macrophylla* | AB535531 | Davies et al., 2015 |

1,000 generations and the burn-in value was 25%. The Markov Chain Monte Carlo method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees using 50% majority rule (Larget and Simon, 1999). The consensus trees were selected to represent the phylogenetic relationships with branch length and support level and visualized using TreeGraph 2 (Stöver and Müller, 2010).

## RESULTS AND DISCUSSION

### *Aphelenchoides fuchsi*[*] sp. n.
(Figs. 1,2).

#### *Measurements*

See Table 3.

#### *Description*

*Females:* Body is cylindrical, straight, somewhat ventrally arcuate when heat relaxed. Cuticle weakly annulated, lateral field with four incisures (i.e., three ridges). Lip region is rounded, offset, ca 3 to 3.5 μm high, and 6 to 7 μm broad. Stylet with small basal swellings, procorpus cylindrical. Median bulb is strongly developed, almost rectangular, with conspicuous valve situated more or less centrally. Nerve ring is situated at ca half metacarpus (median bulb) length posterior to it. Pharyngointestinal junction is immediately posterior to metacorpus. Pharyngeal gland lobe is slender, ca five to six body diam. long, overlapping intestine dorsally. Excretory pore is at level of nerve ring or opposite the posterior level of the nerve ring, the position varying from 1/2 to 2/3 metacorpus length behind metacorpus. Hemizonid is faint, situated ca two to three times metacorpus diam. posterior to excretory pore. Monodelphic, ovary is outstretched anteriorly, developing oocytes in single row. Oviduct connecting ovary and spermatheca. Spermatheca is elongate, containing compressed disklike or oblong sperm in single row. Vagina is directed anteriad. Vulva is transverse, with slightly raised lips, and vulval flap is absent. Postuterine sac is well developed, extending for about 71% to 90% of vulva to anus distance, often containing sperm. Rectum and anus are visible. Tail is conical, terminating in a complicated step-like projection, usually with many tiny nodular protuberances.

*Males:* They are much less common than females; body slender, cylindrical, and J-shaped when heat relaxed. Anterior region and cuticle are similar to female. Testis is single, anteriorly outstretched, locating left of intestine, occupying 53.4% to 66.2% of body length. Lips of cloacal opening protruding slightly. Spicules are arcuate, relatively short, and apex and rostrum are rounded and well developed, end of dorsal limb is clearly curved ventrally. Gubernaculum is absent. Tail is conical, bearing a short sharp mucro ca 1.5 to 2 μm long. Three pairs of subventral caudal papillae are present: first pair located just posterior to cloacal aperture, second pair in mid-tail region, and third pair just anterior to tail end. Bursa is absent.

#### *Diagnosis and relationships*

*Aphelenchoides fuchsi* sp. n. is characterized by body length of 332 to 400 μm (females) and 365 to 395 μm (males). Lip region with distinct constriction from the

[*] The new species is named in honor of Prof. Gilbert Fuchs, a pioneering scientist in the systematics of aphelenchids.

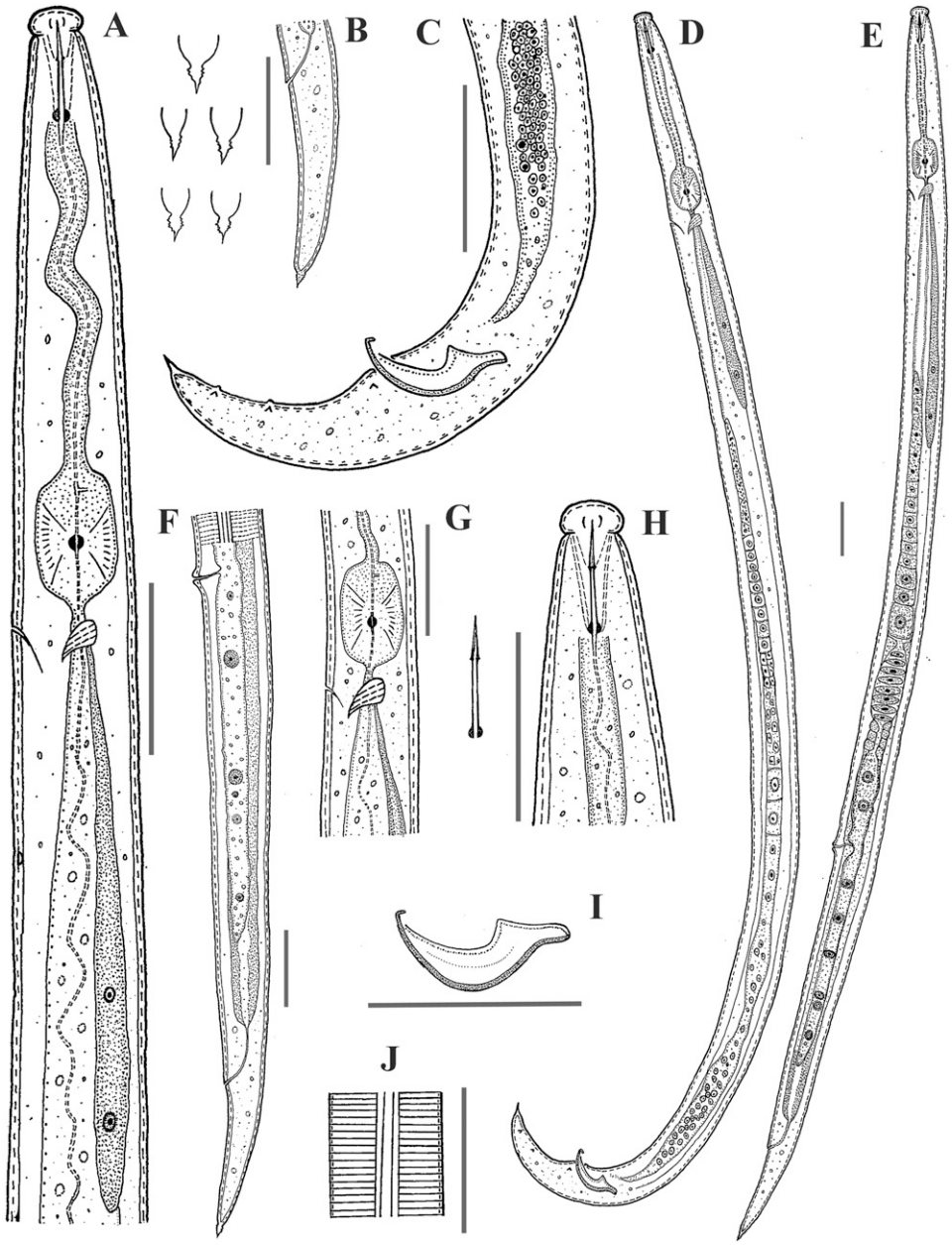


Fig. 1. Line drawing of *Aphelenchoides fuchsi* sp. n. A: Female anterior region; B: Female tail; C: Male posterior body showing caudal papillae; D: Male entire body; E: Female entire body; F: Female vulva to body-end; G: Metacorpus region and showing excretory pore; H: Female anterior head; I: Spicule in detail; J: Lateral field. (All scales in 15 μm.)

rest body. Cuticle with four lateral lines. Medium sized (8–10 μm) stylet with small basal swellings. The excretory pore is located ca one body diam. to posterior of metacorpus valve. The female tail is conical and terminates in a complicated step-like projection, usually with many tiny nodular protuberances. Spicules are relatively short (15–16 μm in dorsal limb) with the apex and rostrum rounded and well developed, the end of the dorsal limb is clearly curved ventrally like a hook (Fig. 2G,F).

*Aphelenchoides fuchsi* sp. n. has a tail terminus with tiny nodular protuberances in female. According to the category of *Aphelenchoides* species sensu Shahina (1996), the new species belongs to Group 2, which is defined as having the female tail terminus with "one or sometimes two mucronate structures." On the basis of the four lateral lines, stylet length, conical female tail, and shape of spicules, the new species appears morphologically most similar to four species from Group 2 including *Aphelenchoides arcticus* Sanwal, 1965, *Aphelenchoides blastophthorus* Franklin, 1952, *Aphelenchoides saprophilus* Franklin, 1957, and *Aphelenchoides xui* Wang, Wang, Gu, Wang, and Li, 2013. It is also similar to three species from Group 4 including *Aphelenchoides franklini* Singh, 1969, *Aphelenchoides gynotylurus* Timm and Franklin, 1969, and *Aphelenchoides marinus* Timm and Franklin, 1969.

*Aphelenchoides fuchsi* sp. n. differs from *A. arcticus*, *A. blastophthorus*, *A. franklini*, *A. gynotylurus*, *A. marinus*, and

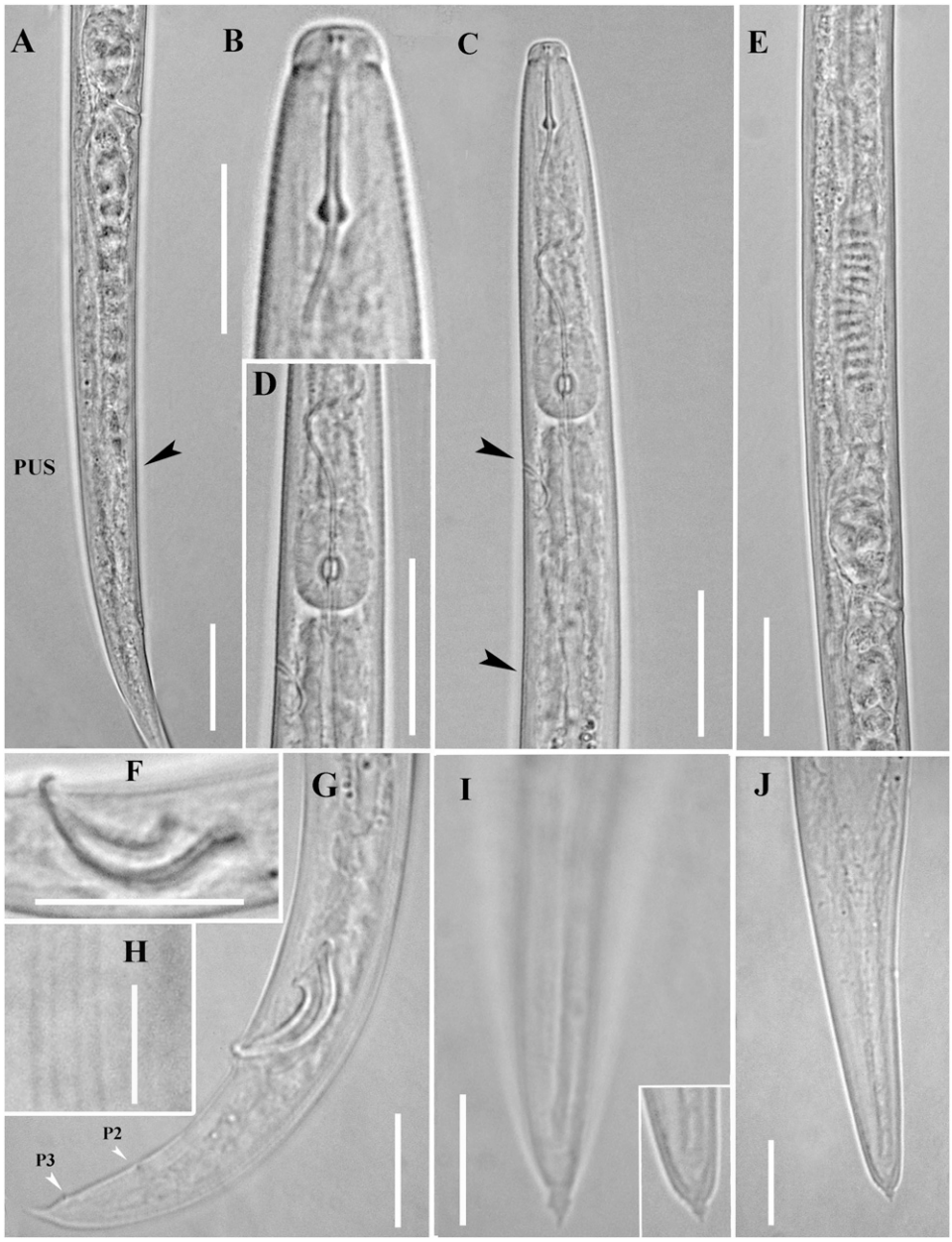


Fig. 2. Light micrographs of *Aphelenchoides fuchsi* sp. n. A: Female posterior body showing post-uterine sac by arrow; B: Female head and stylet in detail; C: Female pharynx region showing excretory pore and hemizonoid by arrow; D: Metacorpus and position of excretory system; E: Female genital tract; F: Spicules in detail; G: Male posterior body showing spicules and papillae arrangement by arrows; H: Lateral field; I,J: Female tail. (A,C–E = 20; B,F–J = 10 μm.)

*A. saprophilus* by the female tail terminus, i.e., ending of a step-like projection/or offset mucro with many tiny nodular protuberances in *A. fuchsi* sp. n. vs. a shallow constriction narrowed sharply with a very fine mucro at the tip (*A. arcticus*), tapering to a simple conspicuous mucro (*A. blastophthorus*), a simple pointed ventral mucro (*A. franklini*), ending in a digitate to blunt/or sometimes sickle-shaped mucro (*A. gynotylurus*), acutely conical tapering to a point that is sometimes truncate/or subacute (*A. marinus*), and a short ventral mucro (*A. saprophilus*).

Moreover, *A. fuchsi* sp. n. differs from *A. arcticus* by the male spicule shape (dorsal limb with a hook-like tip vs. smoothly rounded tip) and the offset vs. nonoffset lip region. It differ from *A. blastophthorus* by the shorter body length of female (320–400 vs. 680–900 μm), the shorter stylet length (8–10 vs. 14–19 μm), lower a ratio (25–32 vs. 40–41), and the male spicule length in dorsal limb (15–16 vs. 24–31 μm). It differs from *A. franklini* by having a longer postuterine sac (5.5 vs. 2.5 times the body width), the shorter body length of female (320–400 vs. 556 μm), the male spicule length and shape in dorsal limb (15–16 μm with clearly curved ventrad like a hook vs. 23 μm long, with slightly curved and rounded tip). It differs from *A. gynotylurus* by having the shorter stylet length (8–10 vs. 15–16 μm), shorter body length of female (332–400 vs. 490–650 μm), and the male

TABLE 3. Morphometrics of *Aphelenchoides fuchsi* sp. n.

| Character | Male | | Female |
|---|---|---|---|
| | Holotype | Paratypes | Paratypes |
| n | – | 5 | 15 |
| L | 367 | 377.8 ± 12.2 (365–395) | 382.3 ± 20.3 (332–400) |
| a | 30.6 | 30.5 ± 0.8 (29.4–31.7) | 28.3 ± 2.3 (25.1–32.9) |
| b | 6.6 | 6.6 ± 0.2 (6.5–6.9) | 6.8 ± 0.3 (6.4–7.3) |
| b' | 2.8 | 2.8 ± 0.1 (2.7–3.0) | 3.4 ± 0.3 (3.0–3.8) |
| c | 15.3 | 13.7 ± 1.1 (12.7–15.3) | 12.8 ± 0.8 (11.8–14.3) |
| c' | 1 | 1.1 ± 0.1 (1.0–1.2) | 4.6 ± 0.5 (3.8–5.3) |
| V or T | 66.2 | 53.9 ± 5.4 (53.4–66.2) | 68.1 ± 2.6 (64.4–74.5) |
| Lip region height | 3 | 3.1 ± 0.2 (3.0–3.5) | 3.1 ± 0.2 (3.0–3.5) |
| Lip region width | 5 | 4.2 ± 0.4 (4.0–5.0) | 6.2 ± 0.4 (6.0–7.0) |
| Stylet length | 9 | 9.0 ± 0.4 (8.5–9.5) | 9.2 ± 0.7 (8.0–10.0) |
| Conus length | 4 | 3.9 ± 0.2 (3.5–4.0) | 3.9 ± 0.3 (3.5–4.5) |
| m[a] | 44.4 | 43.3 ± 1.6 (41.2–44.4) | 42.7 ± 1.9 (40–45) |
| Body diam. | 12 | 12.4 ± 0.5 (12.0–13.0) | 13.6 ± 1.3 (11.0–15.0) |
| Body diam. at median bulb | 11 | 11.1 ± 0.2 (11.5–11.5) | 11.7 ± 1.0 (10.0–13.0) |
| Median bulb width | 6 | 6.5 ± 0.9 (6.0–8.0) | 7.7 ± 0.6 (7.0–8.0) |
| Median bulb length | 12 | 11.2 ± 0.4 (11.0–12.0) | 11.8 ± 0.8 ( 11.0–12.5) |
| Median bulb length/diam. ratio | 2 | 1.7 ± 0.2 (1.4–2.0) | 1.5 ± 0.0 (1.5–1.6) |
| Excretory pore from anterior end | 57 | 58.8 ± 3.4 (55–64) | 58.5 ± 3.6 (51–62) |
| Hemizonid from anterior end | 95 | 88 ± 6.1 (84–95) | 76 ± 1.6 (74–78) |
| Ovary length or testis | 243 | 224 ± 19.2 (195–243) | 162.6 ± 25.1 (128–210) |
| Postuterine sac | – | – | 74 ± 8.6 (60–90) |
| Vulva to anus distance | – | – | 91.8 ± 8.8 (69–100) |
| Postuterine sac length/vulva to anus (%) | – | – | 80.7 ± 6.9 (74.7–90.0) |
| Anal (cloacal) body diameter | 8 | 9.0 ± 0.7 (8.0–10.0) | 5.5 ± 0.5 (6.0–7.0) |
| Tail length | 24 | 27.8 ± 2.3 (24–30) | 29.9 ± 2.1 (25.0–33.0) |
| Spicule (dorsal limb) | 16 | 15.6 ± 0.5 (15.0–16.0) | – |
| Spicule (ventral limb) | 9 | 8.7 ± 0.4 (8.0–9.0) | – |
| Spicule (curved median line) | 11 | 10.7 ± 0.4 (10.0–11.0) | – |
| Spicule (chord) | 10 | 9.4 ± 0.4 (9.0–10.0) | – |
| Mucro | – | – | 1.8 ± 0.4 (1.5–2.0) |

All measurements are in μm and in the form: mean ± SD (range).
[a] Length of conus as percentage of total stylet length.

spicule length in dorsal limb (15–16 μm long, with clearly curved ventrad like a hook vs. 21–24 μm, smoothly rounded tip). It differs from *A. marinus* by having the shorter body length of female (332–400 vs. 570–860 μm), the shorter stylet length (8–10 vs. 13–14 μm), and the male spicule length in dorsal limb (15–16 vs. 24–25 μm). It differs from *A. saprophilus* by the shorter body length of female (332–400 vs. 454–623 μm) and the male spicule length in dorsal limb (15–16 vs. 23 μm). Also, it can be distinguished from *A. xui* by the shorter body length of female (332–400 vs. 548–882 μm), the shorter stylet length (8–10 vs. 11.1–13.2 μm), and the male spicule length in dorsal limb (15–16 vs. 21.7–33.4 μm).

The female tail of *A. fuchsi* sp. n. is also similar to some *Laimaphelenchus* species. *Laimaphelenchus* contains two groups of species: one with a vulval flap and one without (Hunt, 1993). Moreover, *Laimaphelenchus* is also characterized by the vagina either having a cuticular annulus at the point where it joins the uterus or is surrounded by a relatively thick refractile tube or strong musculature (Zhao et al., 2007). Considering these two morphological characters (absence of vulval flap and vagina structure in new species), the new species was far from *Laimaphelenchus* and therefore placed in *Aphelenchoides*. In addition, the entire *Aphelenchoides* and *Laimaphelenchus* groups are in urgent need of revision based on sequences of full-length SSU rDNA sequences or other informative loci combined with detailed morphological diagnostic characters.

*Type habitat and locality*

The type population was recovered from bark and wood samples of a weakened Mondell pine tree (*P. eldarica*) in vicinity of Cheshmeh-e-Nezamei, city of Gilan-e-Gharb, Kermanshah Province, western Iran (GPS coordinates: N 33°59′, E 46°12′; 1,248 m above sea level).

*Remark*

*Aphelenchoides fuchsi* sp. n. was successfully cultured on *Botryotinia fuckeliana* growing on Potato Dextrose Agar. The nematode was not culturable on *Botrytis cinerea*.

*Type material*

Holotype female, two paratype females, and two paratype males (Slides AAF002 and AAF003) were deposited in nematode collection of the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Iran. Three paratype females were deposited at each of the following collections: CABI, Egham, United Kingdom; USDA

Nematode Collection, Beltsville, MD, and Department of Nematology, WANECO Collection, Wageningen, the Netherlands.

*Molecular phylogenetic status*

Amplification of the D2-D3 expansion segment of 28S rDNA and partial 18S sequences from *A. fuchsi* sp. n. specimens yielded a single fragment of 750 and 1,700 bp based on gel size, respectively. Alignment of the sequences with MAFFT resulted in datasets of 1,752 characters for 18S and 869 characters for 28S D2-D3. The partial 18S alignment contained 59 in-group and 1 out-group taxon and was 1,685 bp in length after removing ambiguously aligned regions. The 50% majority rule consensus phylogenetic tree generated from the partial 18S rRNA alignment using Bayesian inference analysis under the GTR + I + G model is presented in Figure 3. The D2-D3 expansion segment of 28S rDNA alignment contained 47 in-group and 1 out-group taxon and was 771 bp in length after removing ambiguously aligned regions. The 50% majority rule consensus phylogenetic tree generated from the D2-D3 alignment using Bayesian inference analysis under the TIM3 + I + G model is presented in Figure 4. According to our phylogenetic trees, *A. fuchsi* sp. n. occupied a basal position in a strongly supported clade with a posterior probabilities support of 100% (D2-D3) and 64% (18S) with some other *Aphelenchoides* spp. as well as several species of other Aphelenchoididae such as *Schistonchus* s.l. and *Laimaphelenchus*. It clearly differs genetically from all members of Aphelenchoididae with available LSU and SSU sequence in GeneBank.

In the phylograms, *Aphelenchoides* appears to be polyphyletic, 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; Esmaeili et al., 2016). *Aphelenchoides*, *Laimaphelenchus*, and *Schistonchus* s.l. (see clades 1–3 given in the work of Davies et al., 2015) cannot be clearly separated, possibly implying that all three genera share a recent common ancestor (Zeng et al., 2007).

## Discussion

The genus *Aphelenchoides* is rich in species and has a world-wide distribution. Correct identification of *Aphelenchoides* species is not easy due to the inaccessibility of some references, poor literatures, inadequate description of several species, and lack of reliable detail.

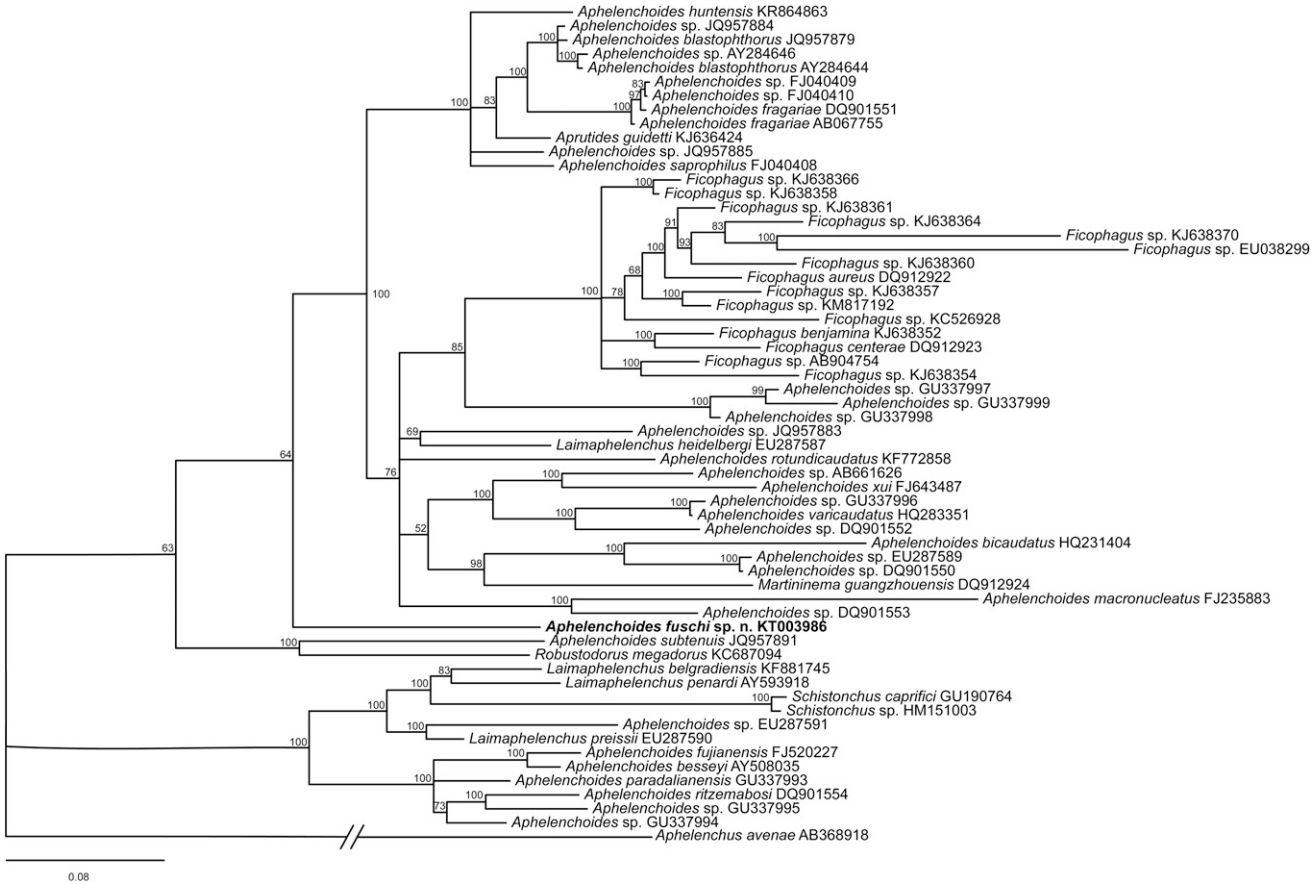


Fig. 3. Phylogenetic relationships of *Aphelenchoides fuchsi* sp. n. and aphelenchid nematodes based on partial 18S rDNA gene. The 10001st Bayesian tree inferred from 18S rDNA gene under GTR + I + G model (ln *L* = −18546.0367; freqA = 0.2644; freqC = 0.2061; freqG = 0.2611; freqT = 0.2684; R(a) = 1.0625; R(b) = 2.4425; R(c) = 0.9638; R(d) = 1.4607; R(e) = 4.2849; R(f) = 1.0000; Pinva = 0.2370; Shape = 0.4970). *Aphelenchus avenae* served as the outgroup species. Posterior probability values exceeding 50% are given on appropriate clades.

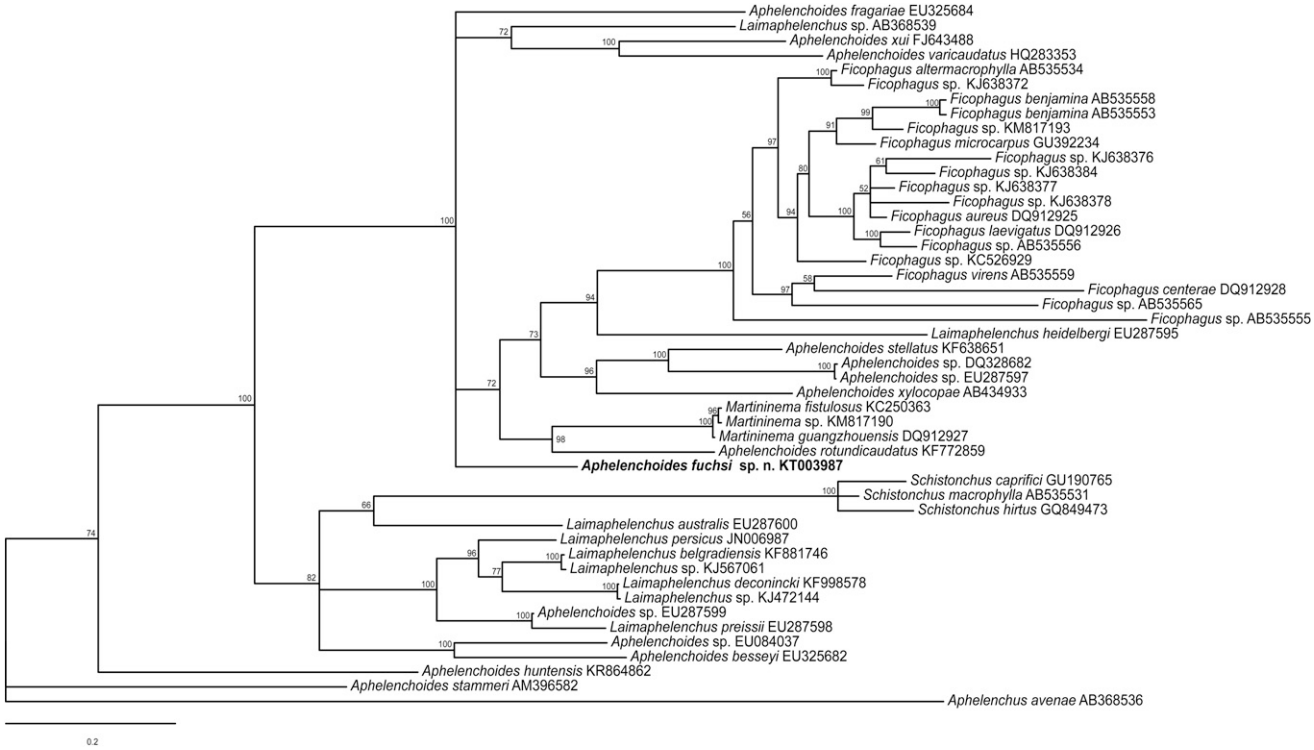


Fig. 4. Phylogenetic relationships of *Aphelenchoides fuchsi* sp. n. and aphelenchid nematodes based on partial 28S D2-D3 region rDNA gene. The 10001st Bayesian tree inferred from 28S D2-D3 region rDNA gene under TIM3 + I + G model (ln $L = -14298.9428$; freqA = 0.2097; freqC = 0.1828; freqG = 0.3009; freqT = 0.3065; R(a) = 0.8529; R(b) = 3.2742; R(c) = 1.0000; R(d) = 0.8529; R(e) = 4.4607; R(f) = 1.0000; Pinva = 0.1440; Shape = 0.6640). *Aphelenchus avenae* served as the outgroup species. Posterior probability values exceeding 50% are given on appropriate clades.

Moreover, this genus has few morphologically diagnostic taxonomic characters (Kanzaki, 2006).

In general, the morphology of the tail tip of *A. fuchsi* sp. n. is similar to some described species of the genus in Group 2, according to the category of *Aphelenchoides* species sensu Shahina (1996), which is defined as having the female tail terminus with "one or sometimes two mucronate structures" but this structure, in the new species, is not simple and the female tail terminus have a step-like projection with many tiny nodular protuberances. Moreover, the male spicule shape (dorsal limb with a hook-like tip) is a diagnostic character between similar species of the genus.

Recently, several species of the genus were described and molecularly studied (Cui et al., 2011; Wang et al., 2013; Fang et al., 2014a, 2014b; Esmaeili et al., 2016). Molecular characterization and phylogenetic analyses based on rDNA region sequences including 18S, internal transcribed spacer regions, and the D2-D3 expansion segments of 28S can assist in accurate identification of the species, although with the important proviso that most nominal *Aphelenchoides* species lack such reliable information.

Small subunit of rDNA contains sufficient phylogenetic signal for the identification of *Aphelenchoides* species (De Ley et al., 2006; Zhao et al., 2008; Van Megen et al., 2009) and have been shown to be more useful for species identification compared to D2-D3 expansion segments of 28S rRNA and internal transcribed spacer rRNA, as both of these markers showed more species variability than did partial 18S rDNA (Zhao et al 2008; Esmaeili et al., 2016). This is the second new species of the genus from Iran to be described and sequenced. *Aphelenchoides fuchsi* sp. n. was found in bark of a weakened pine tree but not in its wood. Therefore, the new species appears to be feeding on fungi or lichens growing on the bark of the tree.

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