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# Description of *Aphelenchoides giblindavisi* n. sp. (Nematoda: Aphelenchoididae), and Proposal for a New Combination

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## Abstract

One new and one known species of the genus *Aphelenchoides* from Iran are studied. *Aphelenchoides giblindavisi* n. sp. is mainly characterized by having five lines in the lateral fields at mid-body, and a single mucro with several tiny nodular protuberances, giving a warty appearance to it, as revealed by detailed scanning electron microscopic (SEM) studies. The new species is further characterized by having a body length of 546 to 795 µm in females and 523 to 679 µm in males, rounded lip region separated from the rest body by a shallow depression, 10 to 11 µm long stylet with small basal swellings, its conus shorter than the shaft (*m* = 36–43), 52 to 69 µm long postvulval uterine sac (PUS), males with 16 to 18 µm long arcuate spicules, and three pairs of caudal papillae. The new species was morphologically compared with two species of the genus having five lines in the lateral fields namely *A. paramonovi* and *A. shamimi* and species having a warty-surfaced mucro at tail end and similar morphometric data ranges. The morphological features and morphometrics of the second studied species, *A. helicus*, agreed well with the data given for the type population. However, detailed study of fresh females revealed it has three drop-shaped stylet knobs and long PUS, making it typologically similar to the genus *Robustodorus*, meriting its taxonomic revision, i.e., transferring to it. In molecular phylogenetic analyses using partial small and large subunit ribosomal RNA gene (SSU and LSU rDNA) sequences, the new species formed a clade with *A. heidelbergi* in both SSU and LSU D2-D3 trees. The species *A. helicus*, however, clustered inside a well-supported clade of the genus *Robustodorus* in both trees, corroborating its newly proposed taxonomic placement as *Robustodorus helicus* n. comb.

### Key words

Molecular, morphology, morphometrics, phylogeny, *Robustodorus helicus* n. comb., rRNA gene, SEM, taxonomy.

The genus *Aphelenchoides* Fischer, 1894 (family Aphelenchoididae Skarbilovich, 1947), has around 175 nominal species (Mobasseri et al., 2018). This could also be an underestimate, and several sequences deposited at the GenBank database for example, are identified only at the genus level. This species-rich genus is the type genus of the family Aphelenchoididae, and is well known by prevalence of species lacking conspicuous apomorphies, helpful for its species delimitation. The term "foliar nematodes" is the common name of plant-parasitic forms commonly used by plant pathologists. Actually, some *Aphelenchoides* species are important plant parasites (Kanzaki and Giblin-Davis, 2012). They could be recovered from soil, mosses, mushrooms, decaying organic materials or in some cases, from plant tissues (Khusainov, 2013). Historically, several authors, e.g., Hunt (1993, 2008), Shahina (1996) and Andrássy (2007) have

provided list of valid species for the genus. The book of Baranovskaya (1981), however, is a useful resource, especially for some species with inaccessible descriptions. The Index to Organism Names website (www.organismnames.com), however, includes updated data of most of the newly described species. According to Shahina (1996), the species could be grouped based on the number of their lateral lines, i.e., the species usually have obscure, unknown, 2, 3 and 4 lateral lines, while species with 4 to 6, and 6 lateral lines do also rarely occur. Andrássy (2007), however, pointed out that six lines in lateral fields rarely occur. As far as our knowledge, until 1995 that the key of Shahina (1996) was published, *A. shamimi* Khera, 1970 was the only, having five lines in the lateral fields. Since 1995 until 2008-2009 that the checklist of Hunt (2008) was published, it was only *A. paramonovi* Eroshenko and Kruglik, 2004 that had five lines in the lateral fields. No species having five lines in lateral fields has been described since 2008 to 2009 till date. During recent years, revisions have been performed on taxonomic status and placement of some *Aphelenchoides* species. In a recent study, the species *Laimaphelenchus heidelbergi* Zhao et al., 2007 was transferred to the genus *Aphelenchoides* (Carta et al., 2016). In another study, the species *Tylaphelenchus christinae* Lieutier and Laumond, 1978 was transferred to the genus *Aphelenchoides* (Pedram et al., 2018a, 2018b) and finally, two species *A. subtenuis* (Cobb, 1926) Steiner and Buhrer, 1932 and *A. arachidis* Bos, 1977 have been transferred to the genus *Robustodorus* Andrássy, 2007 (Kanzaki et al., 2018). The latter action, in revising the taxonomic placement of those two species, was supported by both traditional and molecular criteria.

The history of the reported or described species of *Aphelenchoides* from Iran was given by Mobasseri et al. (2018). During our nematological surveys conducted in northern provinces of the country, two populations of the genus were recovered from Asalem forests in Gilan province, and a forest in Semnan province in north of Iran. Further morphological, morphometric and phylogenetic studies, especially detailed SEM studies, revealed the first species belongs to an unknown species, described herein as *Aphelenchoides giblindavisi* n. sp. The second species was identified as *A. helicus* Heyns, 1964, its detailed morphological studies using fresh females yielded new observations, meriting its transferring to the genus *Robustodorus*, an action that was supported by the molecular phylogenetic analyses using two genomic markers too.

Present study aims to (i) describe *Aphelenchoides giblindavisi* n. sp. and characterize it using morphological and molecular data and (ii) to revise the current taxonomic status of *A. helicus* using newly observed morphological traits and molecular phylogenetic criteria.

## Materials and methods

### Sampling, nematode extraction, and morphological observation

Several soil, moss, rotten wood, bark and insect cadavers were collected from the natural forests in Gilan and Semnan provinces, northwestern and northern Iran. The samples were placed in plastic bags, transferred to nematology laboratory of Tarbiat Modares University and maintained at 4 °C. Nematodes were extracted using the tray method (Whitehead and Hemming, 1965), heat killed by adding hot 4% formalin solution, transferred to anhydrous glycerin according to De Grisse (1969), and mounted on permanent slides. Both species were studied in detail in temporary slides using fresh females in water. Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope powered with differential interference contrast (DIC). For SEM studies, after their examination and identification, a few specimens preserved in glycerin were selected for observation under SEM following the protocol of Álvarez-Ortega and Peña-Santiago (2016). The nematodes were hydrated in distilled water, dehydrated in a graded ethanol and acetone series, critical point-dried, coated with gold, and observed with a Zeiss Merlin Scanning Electron Microscope.

### DNA Extraction, PCR and sequencing

DNA of two recovered species was extracted from one single female nematode. Each specimen was picked out, studied onto a temporary slide, transferred to a small drop of TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, 100 QIAGEN Inc., Valencia CA, USA) on a clean slide and squashed using a clean slide cover glass. The suspension was collected by adding 15 µl of the aforementioned buffer. The DNA samples were stored at –20 °C until using as PCR templates. PCR was carried out in a total volume of 30 µl (19.2 µl distilled water, 3 µl 10× PCR buffer, 0.6 µl 10 mM dNTP mixture, 1.2 µl 50 mM MgCl2, 1.2 µl of each primer (10 pmol/µl), 0.6 µl of *Taq* DNA polymerase (5 unit/µl, CinnaGen, Tehran, Iran) and 3 µl of DNA template). The thermal cycling program for amplifying

two genomic fragments (SSU and LSU rDNA D2-D3) was as follows: denaturation at 95 °C for 4 min, followed by 32 cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 40 sec, and extension at 72 °C for 80 sec. A final extension was performed at 72 °C for 10 min (Pedram, 2017; Mobasseri et al., 2017). Primers for 28S rDNA D2-D3 amplification were forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGT-3′) and reverse primer D3B (5′-TGCGAAGGAACCAGCTACTA-3′) (Nunn, 1992). Primers for amplification of 18S rDNA were forward primer SSU 1813F (5′-CTGCGTGAGAGGTGAAAT-3′) and reverse primer SSU 2646R (5′-GCTACCTTGTTACGACTTTT-3′) as used by Holterman et al. (2006).

The PCR products were sequenced in both directions using the same primers with an ABI 3730XL sequencer (Applied Biosystems) at Macrogen (Seoul, South Korea). Newly obtained sequences were deposited into the GenBank database (accession numbers: MG545999 for the partial SSU and MG546000 for the partial LSU rDNA D2-D3 of the new species, and KP264116 for the SSU and KP264117 for the partial LSU rDNA D2-D3 of *A. helicus*).

### Phylogenetic analyses

The newly obtained SSU and LSU rDNA D2-D3 sequences were compared with those of other nematode species available in GenBank using the BLAST homology search program. The selected sequences for reconstructing of each phylogenetic trees were aligned using the Q-INS-i algorithm of online version of MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013). The Gblocks program (version 0.91b) with all the three less stringent parameters, a server tool at the Castresana Lab (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), was used for post-editing of the alignments, i.e., to eliminate poorly aligned regions or divergent positions. The most appropriate model of nucleotide substitution was selected using the Akaike information criterion in MrModeltest 2 (Nylander, 2004). The general time reversible model, including a gamma distribution for rates across sites and a proportion of invariant sites (GTR + G + I) was selected for both datasets. Bayesian inference (BI) was performed using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) running the chains for five million generations. After discarding burn-in samples, the remaining samples were retained for further analyses. The Markov chain Monte Carlo method within a Bayesian framework was used to estimate Bayesian posterior probabilities (BPP) of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. Adequacy of the posterior sample size was evaluated using autocorrelation statistics as implemented in TRACER v.1.5 (Drummond and Rambaut, 2007). A maximum likelihood (ML) tree was reconstructed by using RaxmlGUI 1.1 (Silvestro and Michalak, 2012) software using the same nucleotide substitution model as in the BI including 1000 bootstrap (BS) pseudoreplicates. For SSU phylogeny, the species, *Panagrolaimus detritophagus* Fuchs, 1930, *Plectonchus* sp. and *Brevibucca saprophaga* Goodey, 1935 (accession numbers EU543176, AY593920 and EU196018 respectively) and for LSU rDNA D2-D3 dataset, the species *Panagrellus redivivus* (Linnaeus, 1767) Goodey, 1945 and *Poikilolaimus piniperdae* Fuchs, 1930 (accession numbers DQ408249 and DQ059060 respectively) were used as outgroup taxa (according to previous studies, e.g., van Megen et al., 2009; Ryss et al., 2013; Pedram, 2017). The inferred trees were visualized using Dendroscope V.3.2.8 (Huson and Scornavacca, 2012) and re-drawn in CorelDRAW software version 16.

## Results

### Systematics

### *Aphelenchoides giblindavisi* n. sp.

(Table 1; Figs. 1–4).

### *Description*

*Female*: Body slightly ventrally curved when heat-relaxed, very gently narrowing towards both ends. Body annules about one µm wide at mid-body. Lateral field with five incisures, occupying about 25% of corresponding body width, initiating from the median bulb region with two lines, extending to five at mid-body, and again reducing to two bands at middle of the tail, under SEM. Lip region rounded, finely annulated (Fig. 4) separated from the rest body by a shallow depression, ca. 2.5 µm high and 5 to 6 µm broad. The rounded cephalic disc, six inner labial papillae, vestigial outer labial papillae, four cephalic papillae, amphidial openings and the rounded oral aperture visible under SEM (Fig. 4), as schematically illustrated by Hooper and Clark (1980). Stylet short and weak, conus shorter than the shaft, the lumen well visible all over the stylet, having small swellings at base. Procorpus slender, median bulb rounded, 1.3 ± 0.2 (1.8–2.4) times longer than the wide, its valvular plates well sclerotized, central. Pharyngo-intestinal junction just posterior to metacorpus, pharyngeal glands lobe overlapping intestine dorsally for 63 to

**Table 1. Morphometrics of *Aphelenchoides giblindavisi* n. sp. from Iran. All measurements are in µm and in the form: mean ± S.D. (range).**

| | | Female | Male |
|---|---|---|---|
| Character | Holotype | Paratypes | Paratypes |
| n | – | 12 | 12 |
| L | 653 | 671 ± 72.4 (546–795) | 632 ± 41.4 (523–679) |
| a | 39.6 | 34.9 ± 2.2 (31.4–39.6) | 37.4 ± 2.2 (32.4–41.0) |
| b | 8.6 | 8.9 ± 0.8 (7.4–10.1) | 8.7 ± 0.4 (8.0–9.4) |
| b′ | 4.5 | 4.5 ± 0.4 (3.9–5.0) | 4.5 ± 0.2 (4.2–5.0) |
| c | 18.7 | 18.9 ± 1.0 (17.2–20.4) | 18.9 ± 1.7 (15.8–21.5) |
| c′ | 3.5 | 3.4 ± 0.2 (3.1–3.7) | 2.8 ± 0.2 (2.6–3.1) |
| V or T | 70.1 | 70.6 ± 1.6 (68.8–73.5) | 52.8 ± 3.4 (47.4–59.2) |
| Head height | 2.5 | 2.5 ± 0.0 (2.0–2.5) | 2.5 ± 0.0 (2.0–2.5) |
| Head diam. | 5.5 | 5.5 ± 0.4 (5–6) | 5.5 ± 0.3 (5–6) |
| Stylet | 10.5 | 10.5 ± 0.5 (10–11) | 10.0 ± 0.4 (10–11) |
| Stylet conus | 4.5 | 4.5 ± 0.2 (4.0–4.5) | 4.0 ± 0.2 (3.5–4.5) |
| m | 42.9 | 38.9 ± 2.3 (36.4–42.9) | 38.4 ± 2.1 (35–40) |
| Median bulb | 58 | 58.0 ± 3.2 (54–65) | 56.0 ± 1.4 (54–58) |
| Excretory pore | 69 | 69.0 ± 6.2 (60–84) | 66.5 ± 1.8 (64–69) |
| Hemizonid | 80 | 89.0 ± 10.6 (77–110) | 81.0 ± 2.6 (78–85) |
| Pharynx | 76 | 75.5 ± 2.8 (71–81) | 72.0 ± 3.4 (65–78) |
| Nerve ring | 75 | 75.0 ± 2.8 (71–81) | 73.0 ± 2.3 (70–78) |
| Median bulb length | 13.5 | 14 ± 1 (12–15) | 13.0 ± 0.9 (11–14) |
| Median bulb diam. | 10.5 | 11.0 ± 1.1 (9.0–12.5) | 10.0 ± 0.9 (8.0–11.5) |
| Median bulb length/diam. | 1.3 | 1.3 ± 0.1 (1.1–1.4) | 1.3 ± 0.1 (1.2–1.6) |
| Pharyngeal overlapping | 70 | 73.0 ± 6.7 (63–87) | 67.0 ± 6.4 (60–80) |
| Maximum body diam. | 16.5 | 19.0 ± 2.4 (15–23) | 17.0 ± 1.4 (14–19) |
| Vulval body diam. (VBD) | 16 | 18.0 ± 1.8 (14.5–20.0) | – |
| Body diam. at median bulb | 13.5 | 14.0 ± 1.4 (12.0–15.5) | 13.0 ± 0.8 (11–14) |
| Postvulval uterine sac (PUS) | 65 | 62.4 ± 6.0 (52–69) | – |
| PUS/VBD | 4.1 | 3.5 ± 0.4 (2.7–4.1) | – |
| Vulva–anus | 160 | 161 ± 21.6 (114–190) | – |
| Ovary or testis length | 295 | 257 ± 58.7 (183–356) | 334 ± 35 (248–394) |
| Anal (cloacal) body width | 10 | 10 ± 1 (9.0–12.5) | 12.0 ± 0.6 (10.5–13.0) |
| Tail length | 35 | 35.0 ± 2.5 (30–39) | 34.0 ± 1.8 (31–37) |
| Spicules length (arc line) | – | – | 17.0 ± 0.7 (16–18) |
| Capitulum | – | – | 7.0 ± 0.5 (6–8) |

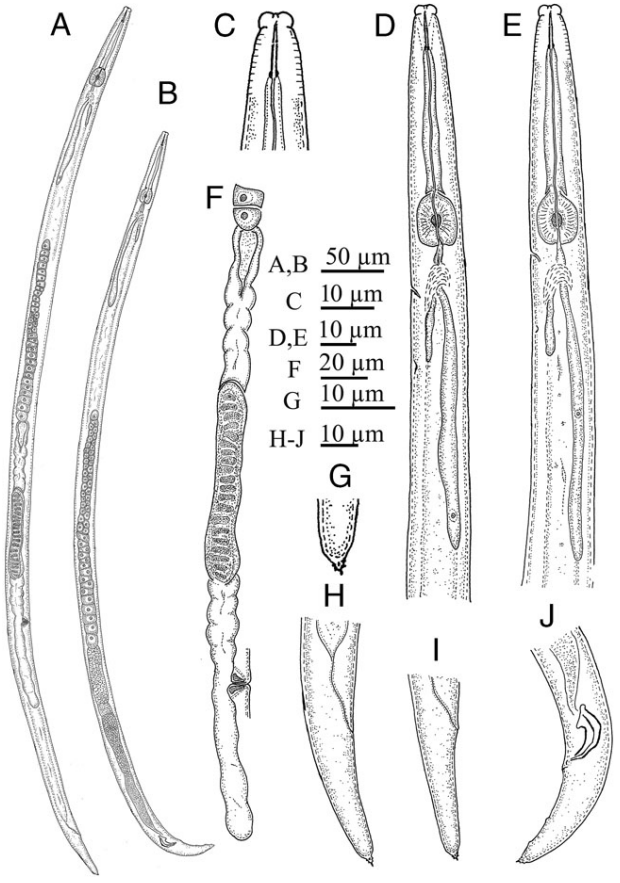


Figure 1: Line drawings of *Aphelenchoides giblindavisi* n. sp. (A, B) Entire body of female and male. (C) Anterior end. (D, E) Pharynx. (F) Part of female reproductive system. (G) Tail end. (H, I) Tail of female. (J) Posterior body region of male.

87 µm. Intestine simple, rectum and anus functional. Nerve ring surrounding pharyngeal glands at ca. 1.3 ± 0.1 (1.1–1.4) stylet length posterior to the base of metacorpus. Hemizonid at ca. 1.7 ± 0.6 (1.0–2.8) stylet length posterior to base of excretory pore. Reproductive system monodelphic-prodelphic, ovary outstretched, oocytes in multiple rows in germinal zone, developing oocytes in single row, oviduct sometimes with mature egg, crustaformeria and uterus boarder not well discernible, vagina straight to slightly anteriorly directed. Postvulval uterine sac (PUS) elongate, about 32 to 51% the vulva to anus distance long. Vulva a simple transverse slit. Tail conical, ventrally almost flat, ending to a single mucro having many tiny nodular protuberances, giving a warty appearance to it under SEM.

*Male*: Abundant, equal to females in number. General morphology similar to that of female, except for reproductive system and the posterior body end more ventrally bent after fixation. Genital system monorchic, testis outstretched with spermatocytes arranged at multiple and single rows at germination and growth zone, respectively. Spicules arcuate, condylus well-developed, rounded at end, rostrum small, rounded, the distal end of spicules simple, without any type of differentiation. Male caudal papillae composed of three pairs (single P1 papilla lacking), arranged as follows: the first pair (P2) at about two annules anterior to cloacal aperture (n = 1, as observed in detail under SEM), the second pair (P3) at about middle of the tail and the third pair (P4) vestigial, close to tail end (Fig. 3I). Tail similar to that of female, ending to a single warty mucro.

*Etymology*: The new species is named after Prof. Robin M. Giblin-Davis, an outstanding scientist in the systematics of aphelenchid nematodes.

*Type habitat and locality*: Recovered from soil samples collected about the rhizosphere of *Sambucus canadensis* L. in forests at Asalem-Khalkhal road, Gilan province, northwestern Iran, in July 2014. GPS coordinates: N 37°36′17.76″, E 48°43′51.43″.

*Type material*: Holotype female, nine paratype females and males were deposited at the Nematode Collection at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Three paratype females and three paratype males were deposited at each of the following collections: WANECO collection, Wageningen, The Netherlands (www.waneco.eu/) with the slide codes: WT3722 and WT3723, and Ghent University Museum, Zoology Collections, Ghent, Belgium with the slide codes: UGMD 104381 and UGMD 104382.

*Diagnosis and relationships*: *Aphelenchoides giblindavisi* n. sp. is an amphimictic species characterized mainly by having five lines in lateral fields and single warty mucro at tail end, as revealed by detailed SEM studies. It was further characterized by 546 to 795 µm long females and 523 to 679 µm long males, short 10 to 11 µm long stylet having small swellings at base and distinct lumen all over the stylet, 62.5 ± 6.0 (52–69) µm long PUS, common males with 16 to 18 µm long arcuate spicules having well-developed rounded condylus, small blunt rostrum, simple distal end and three pairs of caudal papillae (lacking single precloacal P1 papilla). By having a single mucro at the tail end, the new species could provisionally assigned to the group 2 of intraspecies grouping of species of the genus *sensu* Shahina (1996).

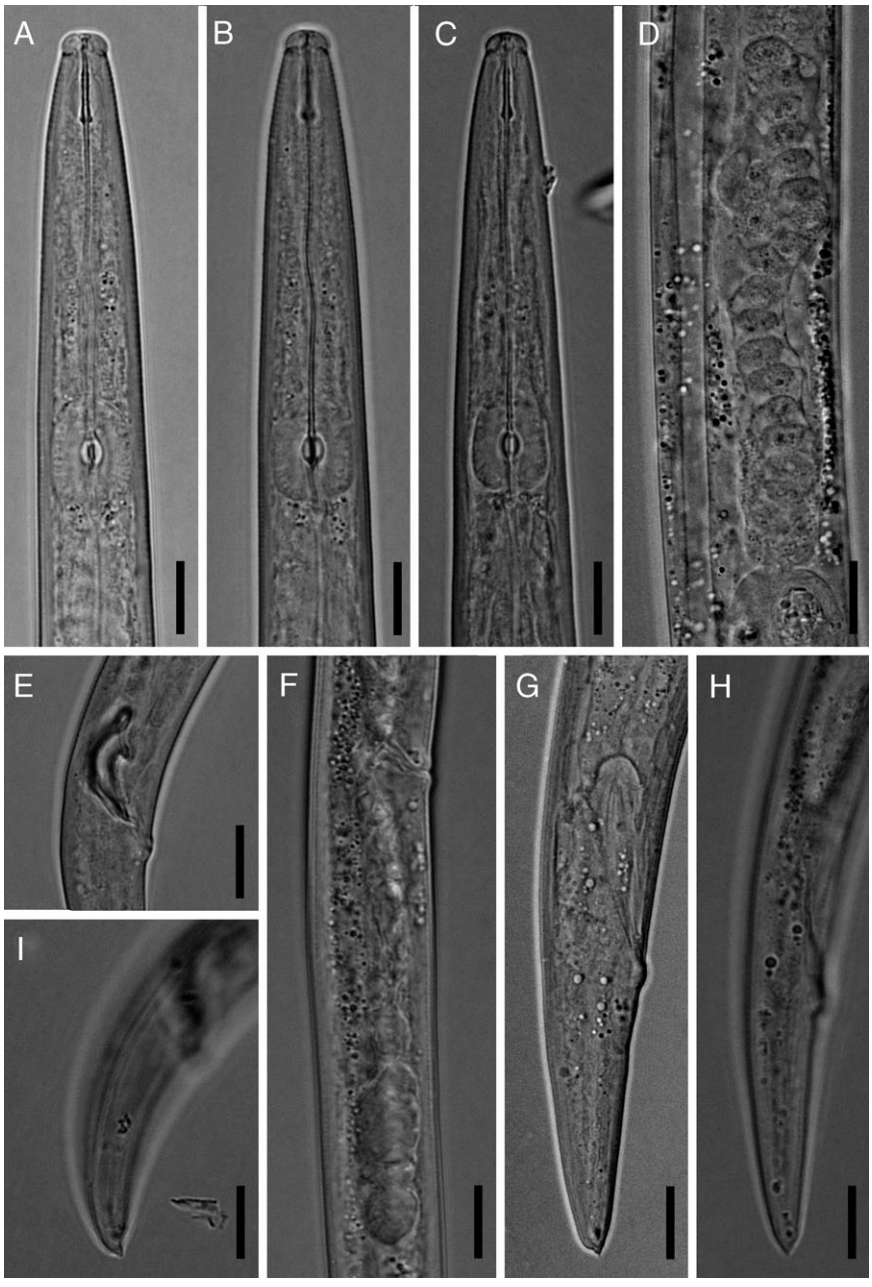


Figure 2: Light microphotographs of *Aphelenchoides giblindavisi* n. sp. (A–C) Anterior region. (D) Part of female reproductive system (junction of oviduct and spermatheca). (E) Spicule. (F) Vulva region and postvulval uterine sac. (G, H) Posterior body region of female. (I) Tail end of male (All scale bars = 10 µm).

By having five lines in lateral fields, the new species comes close to two species sharing the same feature namely *A. paramonovi* and *A. shamimi*. By having a warty mucro at tail end and similar ranges of morphometric data, the new species comes close to *A. ensete* Swart, Bogale & Tiedt, 2000, *A. fuchsi* Esmaeili et al., 2016a, *A. haguei* Maslen, 1979, *A. heidelbergi* (Zhao et al., 2007) Carta et al., 2016, *A. huntensis* Esmaeili et al., 2016b, *A. paraxui* Esmaeili et al., 2017a and *A. xui* Wang et al., 2013. The comparisons with the aforementioned species are as follows:

Compared to *A. paramonovi*, the new species differs by its centrally located valve of median bulb (vs

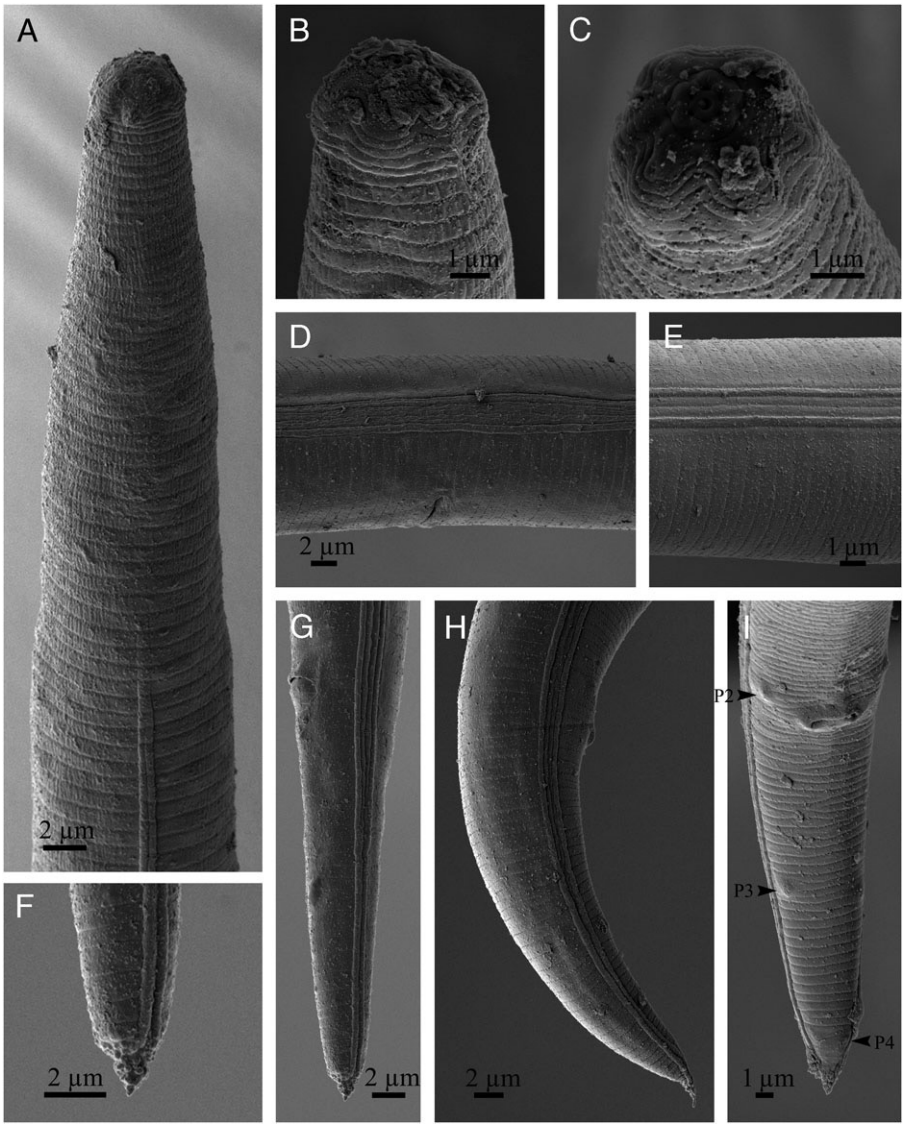


Figure 3: Scanning electron microphotographs of *Aphelenchoides giblindavisi* n. sp. (A) Details of anterior region. (B, C) Lip region. (D, E) Lateral lines in different parts of mid-body. (F) Details of tail end. (G) Female posterior body region. (H, I) Male caudal region.

remarkably posterior), warty mucro at tail end (vs having a finger-like mucro with a short bristle), and slightly greater c (17–20 vs 14–18).

Compared to *A. shamimi*, by longer body (546–795 vs 490–540 µm), greater a (31–40 vs 26–29), smaller b´ (3.9–5.0 vs 7.0–7.7), greater c (17–20 vs 12–14) and having a warty mucro at tail end (vs having a ventrally located single mucro).

Compared to *A. ensete, A. fuchsi, A. haguei, A. huntensis, A. paraxui* and *A. xui,* species having warty mucro at tail end and similar morphometrics, the new species has basic differences in number of lines in lateral field (five vs four). Besides, it can be distinguished from *A. ensete*, by its shorter stylet (10–11 vs 12–14 µm) and conical ventrally flat tail (vs conical, uniformly narrowing toward tip). From *A. fuchsi,* mainly by longer body of females (546–795 vs 332–400 µm) and greater c (17–20 vs 12–14). From *A. haguei*, by greater c (17–20 vs 11–16) and shorter tail (30–39 vs 42–54 µm). From *A. huntensis*, in its remarkable differences in partial sequences of LSU rDNA D2-D3 and distant positions in corresponding phylogenetic tree, greater c (17–20 vs 13–18), longer PUS (52–69 vs 27–40 µm) and shorter spicules (16–18 vs 24–25 µm). From *A. paraxui*, in its remarkable differences in partial sequences of LSU rDNA D2-D3 and distant positions in corresponding phylogenetic tree, greater c (17–20 vs 13–17), lower c′

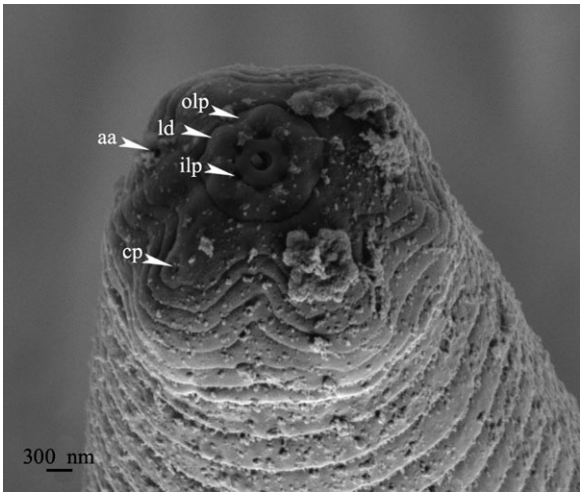


Figure 4: Scanning electron microphotograph of *Aphelenchoides giblindavisi* n. sp. Details of lip region. Abbreviations: ilp–inner lip papillae, aa–amphidial openings, olp–outer labial papillae, cp–cephalic papillae, ld–labial disc.

(3.1–3.7 vs 3.5–4.5), longer stylet (10–11 vs 8–9 µm), longer PUS (52–69 vs 11–15 µm) and shorter tail (30–39 vs 44–47 µm). From *A. xui*, in its remarkable differences in partial sequences of LSU rDNA D2-D3 and distant positions in corresponding phylogenetic tree, shorter PUS (52–69 vs 68–132 µm), stylet (10–11 vs 11–13 µm) and spicules (16-18 vs 18-23 µm), and no differentiation at distal end of spicules (vs end of the dorsal limb clearly curved ventrally like a hook).

Finally, compared to *A. heidelbergi*, a species having warty mucro at tail end and close phylogenetic affinities in both inferred trees, the new species has remarkable mismatches/gaps in alignment of both SSU and LSU rDNA D2-D3. Furthermore, the new species could be separated from *A. heidelbergi* by basic differences in number of lateral lines (five in new species vs three) and simple ventral side of distal end of spicules (vs having two small protrusions).

## Iranian population of *Aphelenchoides helices*

(Table 2; Figs. 5–7).

The presently studied population of the species was in full morphological and morphometric agreement with the type population described by Heyns (1964). It was however almost identical to two populations reported by Rashid et al. (1986) and Adeldoost et al. (2017). New observations of fresh females in temporary slides using DIC microscopy revealed that lip region is well offset by constriction, three lips in lateral view are equally sized and tulip-shaped, vestibule is developed and sclerotized, stylet conus is

**Table 2. Morphometrics of Iranian population of *Robustodorus helicus* n. comb. All measurements are in µm and in the form: mean ± S.D. (range).**

| | Females |
|---|---|
| n | 15 |
| L | 464 ± 37.3 (395–547) |
| a | 29.2 ± 1.8 (26.7–33.5) |
| b | 7.8 ± 0.6 (6.7–8.8) |
| b′ | 4.6 ± 0.4 (3.8–5.4) |
| c | 19.7 ± 1.7 (16.8–22.8) |
| c′ | 2.8 ± 0.3 (2.4–3.1) |
| V | 69.3 ± 1.6 (64.4–71.1) |
| Head height | 2.0 ± 0.3 (2.0–2.5) |
| Head diam. | 5.0 ± 0.4 (4.5–5.5) |
| Stylet | 10.0 ± 0.4 (10–11) |
| Stylet conus | 4.0 ± 0.3 (4.0–4.5) |
| m | 41.3 ± 2.8 (36.4–45.0) |
| Median bulb | 47.5 ± 2.9 (39–51) |
| Excretory pore | 53.0 ± 4.8 (46–62) |
| Hemizonid | 63.0 ± 5.6 (53–69) |
| Pharynx | 60 ± 4 (49–65) |
| Nerve ring | 58 ± 4 (45–62) |
| Median bulb length | 11.5 ± 0.9 (10.0–13.5) |
| Median bulb diam. | 10.0 ± 0.8 (8–11) |
| Median bulb length/diam. | 1.2 ± 0.1 (1.1–1.3) |
| Pharyngeal overlapping | 40 ± 5 (31–49) |
| Maximum body diam. | 16.0 ± 1.6 (13–20) |
| Vulval body diam. (VBD) | 15.0 ± 1.4 (12–19) |
| Body diam. at median bulb | 13.0 ±1.1 (11.5–15.0) |
| Postvulval uterine sac (PUS) | 38 ± 8 (28–55) |
| PUS/VBD | 2.5 ± 0.5 (2.0–3.7) |
| Vulva-anus | 119 ± 13 (101–147) |
| Ovary length | 161 ± 20 (130–193) |
| Anal body diam. | 8.0 ± 0.9 (7–10) |
| Tail length | 24.0 ± 1.4 (21–26) |

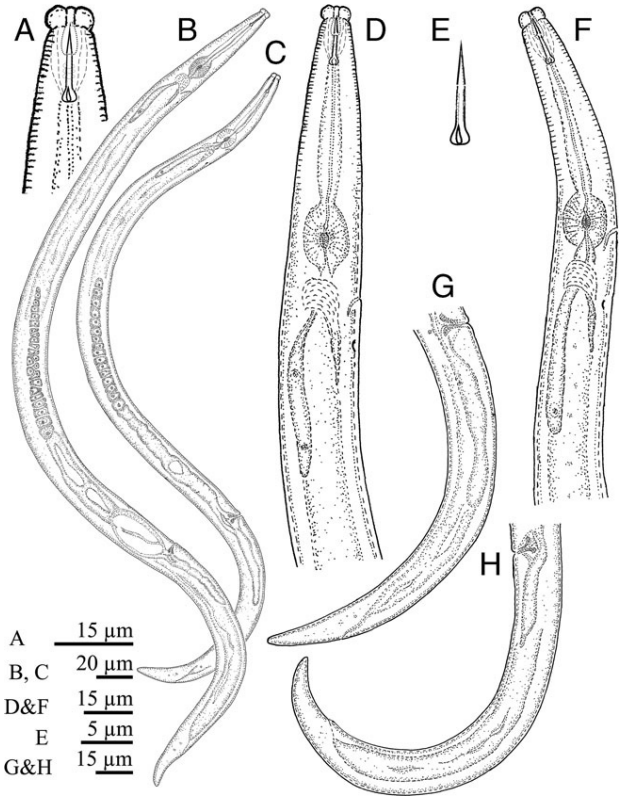


Figure 5: Line drawings of Iranian population of *Robustodorus helicus* n. comb. (A) Anterior end. (B, C) Female total body. (D, F) Pharynx. (E) Stylet. (G, H) Posterior body region.

inside a drop-shaped chamber, sheath surrounds the shaft and the knobs are three, well-developed and teardrop-shaped and procorpus is well muscular in anterior region, narrows in junction with the median bulb. The species however has a variably long PUS, three lines in the lateral field and a conical tail, lacking any type of mucro at the tip.

The observed minor intraspecific morphometric variations between newly recovered population and abovementioned populations are discussed as follows: in comparison with the type population, our population has greater c value (17–23 vs 12–15). In comparison with the data given by Rashid et al. (1986), our V value is slightly smaller (64–71 vs 70–74) and the c value is slightly greater (17–23 vs 14–18). And finally, in comparison with another Iranian population reported by Adeldoost et al. (2017), it has smaller c (17–23 vs 22–28) and b (6.7–8.8 vs 9–12.6) values.

The species was recovered from decaying wood samples of a dead forest tree collected from forests of Khar Turan National Park, Semnan province, Iran, during May 2013 (GPS coordinates: N 36°28′357″, E 54°59′893″) and successfully reared on *Botrytis cinerea* Pers. fungal plates at 23 to 25 °C, within 25 to 30 days. No males were observed in wild type population or inside the plates.

## *Molecular phylogenetic analyses*

Sequencings of SSU and LSU rDNA D2-D3 fragments of the new species yielded single sequences of 838 and 751 nt long (accession numbers MG545999 and MG546000, respectively). The length of the same genomic fragments for *Aphelenchoides helicus* was

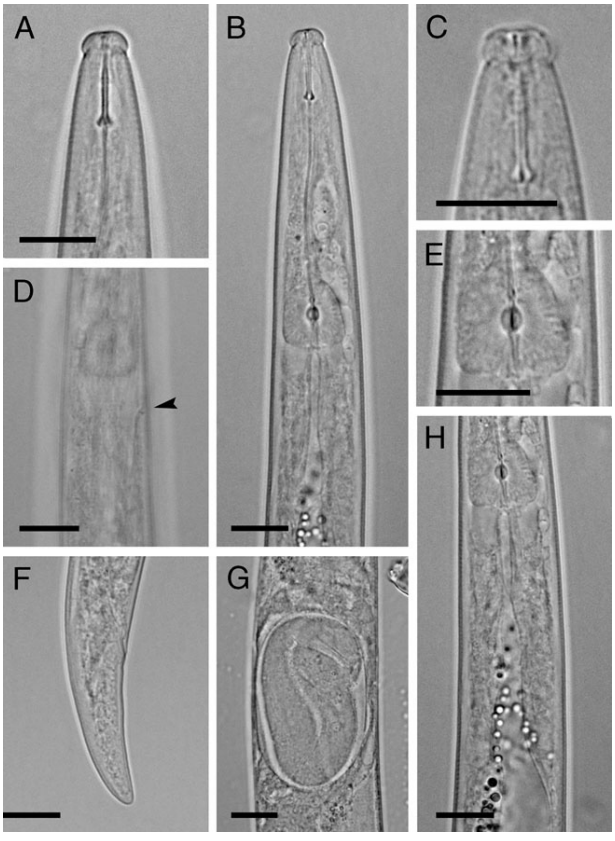


Figure 6: Light microphotographs of fresh females of Iranian population of *Robustodorus helicus* n. comb. (A) Anterior region. (B, H) Part of pharynx. (C) Anterior end. (D) Excretory pore. (E) Metacorpus. (F) Tail. (G) Embryo inside the mature egg inside the body of female. (All scale bars = 10 µm).

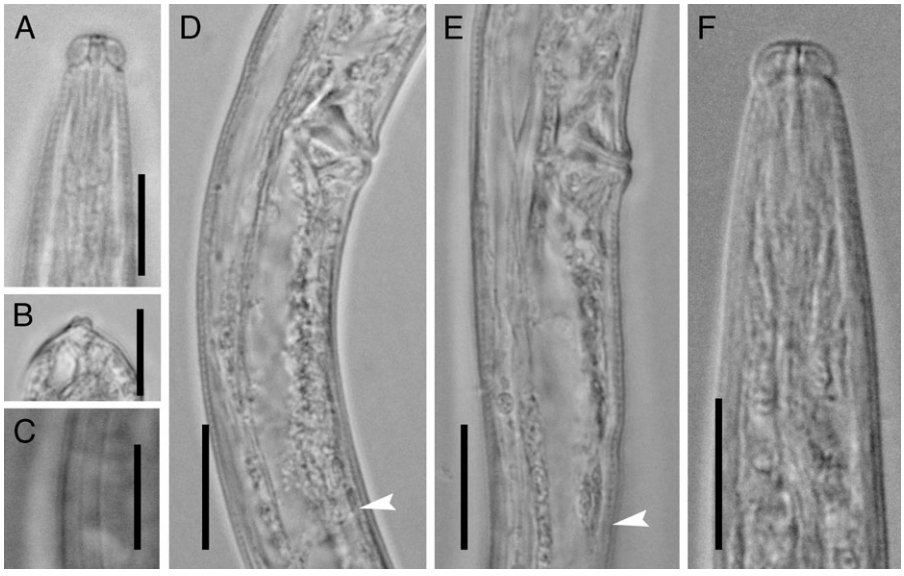


Figure 7: Light microphotographs of Iranian population of *Robustodorus helicus* n. comb. (A) Three tulip-shaped lips in lateral view. (B, C) Three lines in the lateral field forming two bands. (D, E) Postvulval uterine sac. (F) Anterior region. (All scale bars = 10 µm).

560 and 797 nt long (accession numbers KP264116 and KP264117 respectively). The attempts to get longer sequences of SSU sequences were unfortunately failed. The BLAST search using the SSU sequence of the new species revealed it has the highest identity with an isolate of *A. heidelbergi* (accession number EU287587, 99% identity: 2 gaps and 6 indels). Its partial LSU rDNA sequence had the highest identity with an unidentified isolate of *Aphelenchoides* sp. (accession number KX356818, 99% identity: 7 indels and 2 gaps) and an isolate of *A. heidelbergi* (accession number KJ564293, 90% identity: further than 70 indels). The BLAST search using partial SSU sequence of *A. helicus* revealed it has the highest identity (97-98%) with several isolates of *Robustodorus subtenuis* (JQ957886-JQ957893, KX356710-KX356713, KY695128). The results of the abovementioned search for the partial LSU sequence, revealed there are only two sequences of *R. subtenuis* (KY695134, KY695135) having the highest identity (83%).

Several available SSU and LSU rDNA sequences of *Aphelenchoides* spp. having the highest coverage with each other while aligning, were selected for reconstructing the both trees. Two separate datasets were prepared (for species names and accession numbers see Figs. 8,9). The 18S dataset was composed of 71 SSU sequences of aphelenchid/aphelenchoidid species/isolates, including two newly generated sequences for the new species and *A. helicus* and three sequences of classic rhabditids as outgroup taxa. The alignment had 1,437 total characters having 724 variable and 713 conserved characters. Fig. 8 represents the phylogenetic tree inferred using this dataset. In this tree, the new species has formed a clade with *A. heidelbergi* (EU287587) with 0.91 BPP and 85% ML BS value. Their clade is in sister relation with three unidentified isolates of *Aphelenchoides* sp. (KX356722, JQ957883 and KX356743) with 0.84 BPP and 56% ML BS. *A. helicus* was also clustered inside a well-supported clade of *Robustodorus* (BPP/ML BS values 1.00/99), including its all three currently sequenced species for their SSU.

The 28S dataset included 67 sequences of aphelenchid/aphelenchoidid species/isolates, including two newly generated sequences for *Aphelenchoides giblindavisi* n. sp. and *A. helicus* and two sequences of classic rhabditids as outgroup taxa. The alignment had 537 total characters having 338 variable and 199 conserved characters. Fig. 9 represents the phylogenetic tree inferred using this dataset. In this tree, the new species has formed a clade with *A. heidelbergi* (KJ564293) with 0.77 BPP and 63% ML BS value. Their clade is in a fully supported sister relation with an unidentified isolate of *Aphelenchoides* sp. (KX356818). *A. helicus* has also felt into the fully supported clade of *Robustodorus*, including its all three sequenced species for their LSU D2-D3.

## Discussion

Several aspects of the nematode genus *Aphelenchoides* are attractive for biologists, zoologists and

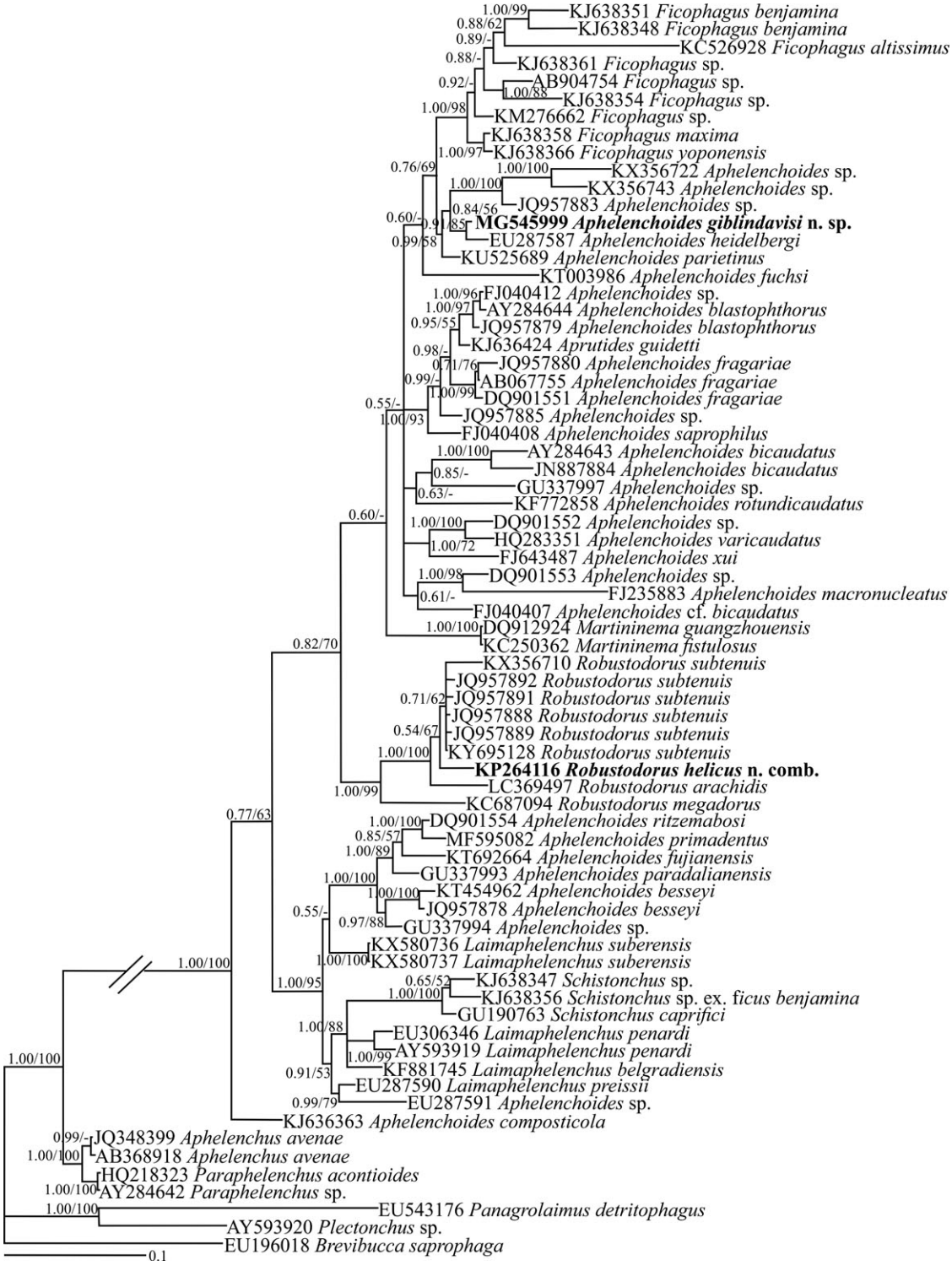


Figure 8: Bayesian tree inferred under the GTR + G + I model using SSU rDNA sequences of *Aphelenchoides giblindavisi* n. sp. and *Robustodorus helicus* n. comb. Posterior probability and bootstrap values exceeding 50% are given on appropriate clades in the form Bayesian posterior probability/maximum likelihood bootstrap value (BPP/BS). The new sequences are in bold font.

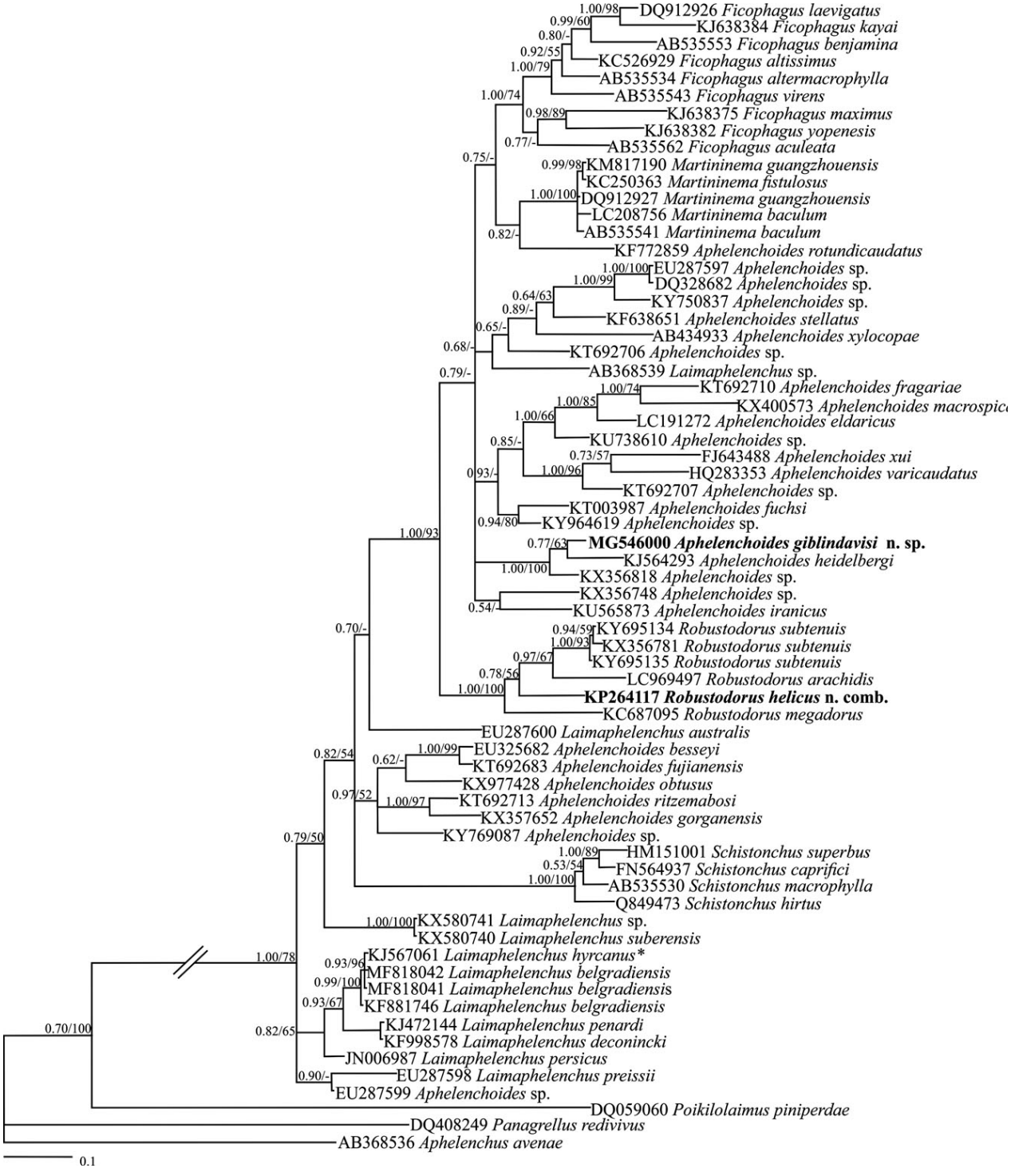


Figure 9: Bayesian tree inferred under the GTR + G + I model using partial LSU rDNA sequence of *Aphelenchoides giblindavisi* n. sp. and *Robustodorus helicus* n. comb. Posterior probability and bootstrap values exceeding 50% are given on appropriate clades in the form Bayesian posterior probability/maximum likelihood bootstrap value (BPP/BS). The new sequences are in bold font. The asterisk on *Laimaphelenchus hyrcanus* Miraeiz et al. (2015) refers to the possible synonymy of the species with *L. belgradiensis* Oro, 2015 as discussed by Pedram et al. (2018a, 2018b).

plant pathologists. In their ranking of top 10 plant-parasitic nematodes, Jones et al. (2013) included *A. besseyi* Christie, 1942, as one of the most important plant parasites. Again, Sanchez-Monge et al. (2015) focused on plant parasitic forms, introduced 13 plant parasites and their hosts, three of which being most important. The plant parasitic forms of the genus almost all feed ecto- or endoparasitically on aboveground parts of the host plants, however, an atypical root parasite, the species *A. subtenuis* does also occur (Mor and Spiegel, 1993). This species is just recently transferred to the genus *Robustodorus* (Kanzaki et al., 2018). The species *Aphelenchoides arachidis,* however, is already known as a facultative endoparasite of testa, pod shell, roots and hypocotyl of groundnuts and also feeds ectoparasitically on their roots. It feeds also on two fungi species in plates and probably in soil (Bridge et al., 1977). This species was also recently transferred to *Robustodorus* in aforementioned study. In the phylogenetic study using SSU rDNA sequences, Rybarczyk-Mydłowska et al. (2012) showed the phylogenetic relation of some plant parasitic forms. With taking into account of predatory and fungivorous feeding habits (Kanzaki and Giblin-Davis, 2012), besides plant parasitic forms, the genus could be regarded as one of the rare polyphagous nematode genera.

By having five lines in the lateral field, *A. giblindavisi* n. sp. belong to the rarest morphospecies groups of *Aphelenchoides* spp. *sensu* Shahina (1996). In future, another morphospecies group might be needed to establish and include the species having a warty mucro at the tail end. The new species was provisionally assigned to the group 2 of intraspecies grouping of *Aphelenchoides*. However, this is an artificial framework (Mobasseri et al., 2018), and in some cases, the species could not easily assigned to either of those groups. In their extensive study, van Megen et al. (2009) investigating a broad range of taxa using SSU rDNA sequences, concluded that the tail tip characters could support some cladogenesis events, and also correlated the tail tip characters with the resolved relations between two genera, *Laimaphelenchus* Fuchs, 1937 and *Aphelenchoides*. In our LSU tree (Fig. 9), although the new species and *A. heidelbergi* (two species with similar tail end structure) are in close phylogenetic affinity, however three other species having warty mucro at the tail tip (*A. huntensis, A. paraxui, A. xui*) are in distant positions. The presently described new species has five lines in the lateral field and further future sequencings of isolates having similar tail end structure or five lines at the lateral fields are needed for further elucidation of their tentative phylogenetic affinities.

Just recently, Kanzaki et al. (2018) revised the taxonomic status of *Aphelenchoides arachidis* and *A. subtenuis*, transferred them to the genus *Robustodorus* and redefined the latter genus based on new observations. The morphological and phylogenetic analyses however supported such a new placement for these two species. *Robustodorus* is currently monophyletic in both SSU and LSU analyses. According to Kanzaki et al. (2018), both species are related with higher plant species and together with some other aphelenchoidids, an obligate plant parasitic lifestyle is assumed for *Robustodorus* spp. (Kanzaki et al., 2018). An unknown, or a plant feeding habit is already reported for *R. megadorus* (Allen, 1941) Andrássy, 2007 (Hunt, 1993) too. The fungus feeding habits of *A. subtenuis*, is, as far as we know, missing. On the other hand, present population of *Aphelenchoides helicus* was successfully reared on fungus plates of *Botrytis cinerea,* and its potential plant feeding ability needs further future experiments/studies. The close morphological studies of fresh females of Iranian population of *Aphelenchoides helicus* revealed the stylet knobs are well developed, teardrop-shaped and the other morphological characters fit well with the newly defined characters for *Robustodorus*. Surprisingly, the species clustered into the clade of *Robustodorus* spp. in both SSU and LSU analyses, and in conclusion, both morphological and molecular phylogenetic data well support the placement of the species under *Robustodorus* as *R. helicus* n. comb.

In our 28S phylogeny, the species *Aphelenchoides rotundicaudatus* Fang et al., 2014 formed a clade with three *Martininema* spp. This relation was already shown in the study of Kanzaki et al. (2018). This is a surprising observation, especially with regarding the similarity in position of excretory pore of the species and *Martininema* spp. However, the position of the species in18S tree is well distant from *Martininema* spp., and the alternative hypotheses tests on monophyly of *Martininema* spp. + *Aphelenchoides rotundicaudatus* using two Shimodaira-Hasegawa (SH) test (Shimodaira-Hasegawa, 1999) and comparison of marginal likelihood estimates between the first tree and the topologically constrained tree using Bayes factors calculated using the harmonic means of both trees likelihood values (Nylander et al., 2004; Kass and Raftery, 1995), was rejected (data not shown).

## Remarks

While present study, some wide morphometric data ranges or poor interpretations/illustrations were detected for some species that are compared with *A. giblindavisi* n. sp.

A wide range of PUS length (33–106 µm) is reported for *A. ensete* in its original description (Swart et al., 2000). A 10–47 µm long PUS range is given for *A. heidelbergi* in its original description (Zhao et al., 2007) and the given range for this trait for *A. xui* is 68–132 (Wang et al., 2013). As recently emphasized (Pedram et al., 2018a, 2018b), such a wide ranges for PUS length should be used with caution in delimiting of species and separating the close species based solely on this trait should be avoided. Measuring of this trait in fresh females in temporary slides could be an alternative approach, much helpful in correct measuring of this organ.

In the case of *A. ensete*, it is noted that the warty appearance of mucro at tail tip of the species could be due to bacterial accumulation, however, original SEM data show the mucro is warty in its surface.

The wide and heavily muscular procorpus, and weak and small valvular plates of metacorpus as well as the offset head, separated from the body by a deep constriction as illustrated for *A. huntensis* in line drawings (Esmaeili et al., 2016b), seem to be in conflict with the light microphotographs of the species and need further confirmation.

The accession number KX977428 that is assigned to the species *A. obtusus* Thorne and Malek, 1968 in GenBank database, is assigned to *A. salixae* Esmaeili et al., 2017 too (Esmaeili et al., 2017b), and for this reason, this accession number is shown in our LSU tree as "*A. obtusus/salixae*?".

The species *A. paraxui* is illustrated by centrally located valve at median bulb and a conus equal to shaft in line drawings (Esmaeili et al., 2017a), while light microphotograps show posteriorly located well-developed valve in metacorpus, and a conus, shorter than the shaft.

And finally, the stylet knobs in the form of two small swellings, as drawn for an Iranian population of *Robustodorus helicus* n. comb. by Adeldoost et al. (2017) is not confirmed and the species has three well-developed teardrop-shaped knobs.

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