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**Systematic position of the genus *Atetylenchus* (Nematoda: Tylenchidae)
with description of two new species**

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Summary – Two new and one known species of the genus *Atetylenchus* recovered from Dezful, Khuzestan province, southwestern Iran are illustrated and described using morphological, morphometric and molecular data. *Atetylenchus dezfuliensis* n. sp. is characterized by medium-sized body 700-998 μm , stylet 10.0-11.5 μm long, characteristic tail, first tapering regularly but then cylindrical to slightly clavate, 90-136 μm long, phasmids located at one-third anterior part of tail and short spicules 18.7 μm long. *Atetylenchus longilabiatius* n. sp. is characterized by small-sized body 593-720 μm , prominent lip region, stylet 9.5-12.0 μm long, conical tail with hook-shaped terminus, 42-63 μm long, phasmids located near mid-tail and short spicules 17.5 μm long. *Atetylenchus* cf. *graminus* characterized by small-sized body 643-787 μm , stylet 10.7-11.7 μm , vulva at 51.7-55.1 % of body and conical tail with finely rounded end, 63-69 μm . Molecular data are provided for the characterization of this genus from these two new and one known species using ribosomal genes (18S and 28S rRNA). This genus is molecularly separated from *Psilenchus*, but closely related in both genes. These molecular markers showed different phylogenies for both genera, partial 18S showed their relationship with Tylenchidae, while 28S rRNA showed their relationship with Merliniidae. A key to the genera is provided for species identification.

Keywords – 18S rRNA, D2-D3 of 28S rRNA, molecular, morphology, phylogeny, rRNA, taxonomy.

Introduction

Khan (1973) proposed the genus *Atetylenchus* for *Tylenchus abulbosus* Thorne, 1949, a species of *Tylenchus* without stylet knobs. Sher (1974) differentiated it from *Psilenchus* de Man, 1921 by the absence of slit-like amphidial aperture on the lip region and the low, flat lip region, as well as difference in the position of the median bulb: in *A. abulbosus* (Thorne, 1949) Khan, 1973, the median bulb is slightly anterior to middle of pharynx while in *Psilenchus* species, it is distinctly behind the middle. A second species was added by Siddiqi (1986) after studying a paratype of *Psilenchus graminus* Bajaj, Kaushik & Bhatti, 1982. Then, Geraert & Raski (1987) transferred *Leipotylenchus amiri* Maqbool & Shahina, 1984 to the genus *Atetylenchus*. In this paper, the authors restudied by SEM the material used by Sher (1974) and found that large slit-like amphidial apertures are present together with six papillae around the oral opening. Fourth and fifth members of *Atetylenchus* were described as *A. metaporus* Erum & Shahina, 2008 and *A. minor* Yaghoubi, Pourjam, Atighi & Pedram, 2015 from Pakistan and Iran, respectively (Erum & Shahina, 2008; Yaghoubi *et al.*, 2015).

Siddiqi (1986; 2000) observing the didelphic nature of the female reproductive system and the presence of phasmids, separated *Atetylenchus* from Tylenchidae Örley, 1880, and placed it in family Psilenchidae Paramonov, 1967 under superfamily Dolichodorioidea Chitwood in Chitwood & Chitwood, 1950 (Siddiqi, 1986). Geraert & Raski (1987) did not consider the paired female genital system a character of sufficient value to sustain a separate subfamily or family and then synonymized Psilenchinae Paramonov, 1967 with Boleodorinae Khan, 1964. Holterman *et al.* (2006, 2009) and Bert *et al.* (2008), studying the molecular phylogeny of Tylenchida, Thorne, 1949; found unresolved support for the position of *Psilenchus*, which is treated as a sister-group to the complex Tylenchidae-Anguinae or as a sister-group to all derived plant parasites, depending on the method used. Geraert (2008) considered *Atetylenchus* and *Psilenchus* under Boleodorinae in Tylenchidae. However, close relationships of *Psilenchus* (Subbotin *et al.*, 2006; Ghaderi *et al.*, 2014) and *Atetylenchus* (Yaghoubi *et al.*, 2015) species with the members of Merliniidae Siddiqi, 1971 (Ryss, 1993) in the inferred phylogenetic trees support treating Psilenchidae as a separate family from Tylenchidae. However, more sequences from *Atetylenchus* are needed from both molecular markers (18S and 28S rRNA) in order to validate this hypothesis.

The objectives of this study were: *i*) to describe two new species and one known species of *Atetylenchus* by morphological and molecular approaches using the D2-D3 segments, and partial 18S rRNA gene sequences; *ii*) provide a key for species identification in this genus; and *iii*) to determine the phylogenetic relationships of *Atetylenchus* with the representatives of Psilenchinae, Boleodorinae, and Merliniidae.

Material and methods

NEMATODE SAMPLING AND MORPHOLOGICAL IDENTIFICATION

Soil samples were collected from the rhizosphere of different wild plants in Dezful, Khuzestan province, southwestern Iran. Nematodes were extracted by the tray method (Whitehead & Hemming 1965), then killed and fixed by hot FPG (4: 1: 1, formaldehyde: propionic acid: glycerol), and processed to anhydrous glycerol (de Grisse 1969). The nematodes were transferred to a drop of glycerol and a surrounding ring of paraffin wax on permanent slides and studied using a light microscope, equipped with a Dino-eye microscope eye-piece camera in conjunction with its Dino Capture version 2.0 software. Specimens were identified at species level using available identification keys (Geraert, 2008).

NEMATODE MOLECULAR IDENTIFICATION

For molecular analyses, and in order to avoid mistakes in case of mixed populations in the same sample, two specimens from each sample were temporarily mounted in a drop of 1M NaCl containing glass beads (to avoid nematode crushing/damaging specimens) to ensure specimens conformed with the unidentified populations. All necessary morphological and morphometric data by taking pictures and measurements using the above camera-equipped microscope were recorded. This was followed by DNA extraction from single individuals as described by Subbotin *et al.* (2000). The D2-D3 segments were amplified using the D2A (5'-ACAAGTACCGTGAGGGAAAGTTG-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3') primers (De Ley *et al.*, 1999). Finally, the portion of 18S rRNA was amplified using primers 988F (5'-

CTCAAAGATTAAGCCATGC-3'), 1912R (5'-TTTACGGTCAGAACTAGGG-3'),
1813F (5'-CTGCGTGAGAGGTGAAAT-3') and 2646R (5'-
GCTACCTTGTTACGACTTTT-3') (Holterman *et al.*, 2006).

All PCR assays were carried out according to the conditions described by Archidona-
Yuste *et al.* (2016). Then, the amplified PCR products were purified using ExoSAP-IT
(Affimetrix, USB products) and used for direct sequencing on a DNA multicapillary
sequencer (Model 3130XL genetic analyser; Applied Biosystems, Foster City, CA,
USA), using the BigDye Terminator Sequencing Kit V.3.1 (Applied Biosystems, Foster
City, CA, USA), at the Stab Vida sequencing facilities (Caparica, Portugal). The newly
obtained sequences were submitted to the GenBank database under the accession
numbers indicated on the phylogenetic trees.

PHYLOGENETIC ANALYSES

D2-D3 segments and partial 18S rRNA sequences of different *Atetylenchus* and
Psilenchinae, Boleodorinae, and Merliniidae species were obtained from GenBank and
used for phylogenetic reconstruction. Outgroup taxa for each dataset were chosen
following previous published studies (Yaghoubi *et al.*, 2015; Qiao *et al.*, 2019).
Multiple sequence alignments of both genes were made using MAFFT V.7.205 (Katoh
& Standley, 2013). Sequence alignments were manually visualised using BioEdit (Hall,
1999) and edited by Gblocks ver. 0.91b (Castresana, 2000) in Castresana Laboratory
server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) using options for
a less stringent selection (minimum number of sequences for a conserved or a flanking
position: 50% of the number of sequences +1; maximum number of contiguous non-
conserved positions: 8; minimum length of a block: 5; allowed gap positions: with half).
Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI)
using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). The best-fit model of DNA
evolution was obtained using JModelTest V.2.1.7 (Darriba *et al.*, 2012) with the Akaike
Information Criterion (AIC). The best-fit model, the base frequency, the proportion of
invariable sites, and the gamma distribution shape parameters and substitution rates in
the AIC were then used in MrBayes for the phylogenetic analyses. The general time-
reversible model with invariable sites and a gamma-shaped distribution (GTR + I +
G) for the D2-D3 segments of 28S rRNA and 18S rRNA gene were run with four

chains for 2×10^6 generations, respectively. A combined analysis of the two genes was not undertaken due to some sequences not being available for all species. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for each analysis. After discarding burn-in samples and evaluating convergence the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority-rule consensus tree. Posterior probabilities (PP) are given on appropriate clades. Trees from all analyses were visualised using FigTree software V.1.42 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

*Atetylenchus dezfuliensis*¹ n. sp.

(Figs. 1 & 2; Table 1)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body slightly curved to C-shaped after fixation. Cuticle thick and finely annulated *ca* 1 μm at mid-body. Lateral field with three equally distant bands and four incisures, 6.3 (6.0-7.3) μm wide at mid-body. Lip region truncated, with four or five transverse annuli, 3.4 (3.2-3.7) μm high and 6.8 (6.4-7.3) μm wide. Amphidial apertures short with longitudinal grooves at the front. Stylet weak, 1.5 (1.3-1.7) times of the lip region diameter; conus 41 (38-45)% of the total stylet length, without distinct basal knobs but with swollen arms at base of shaft. Orifice of dorsal pharyngeal gland slightly less than of stylet length. Pharyngeal procropus cylindrical, median bulb well developed, 8.3 (8.0-

¹ The species epithet refers to Dezful, the Iranian city where the new species was collected.

9.2) μm wide and 17.2 (17-18) μm length, occupies 48 (41-55)% of the corresponding body diameter. Basal pharyngeal bulb nearly cylindroid, 9.8 (9.0-11.0) μm wide and 22.5 (22.0-24.0) μm long. Excretory pore at level of the beginning of basal bulb, 97 (88-114) μm from anterior end. Deirids at level of excretory pore, hemizonid one to two annuli anterior to the pore. Nerve ring surrounding middle part of isthmus, located 71 (71-87) μm from anterior end. Reproductive system with two well-developed genital tracts, nearly equal lengths, anterior gonad 194 (121-249) μm and the posterior one 200 (160-250) μm long, ovaries outstretched without any flexure, oocytes arranged in a single row. Spermatheca rectangular, with globular to slightly oval sperm, slightly less than 1 μm in diameter. Vulva a transverse slit, vagina perpendicular to the body axis, extending towards into body less than half of the vulval body diameter. Vulva to anus distance 2.2-2.9 times of the tail. Tail elongated clavate, tapering regularly until near terminus but becoming slightly broader near terminus and appearing as cylindrical to slightly clavate, and ending to a finely rounded terminus. Phasmids located at 38 (28-43) μm from anus level or ca. 32 (27-38)% of the tail length.

Male.

Similar to female in general characteristics. Body slightly curved. Lateral field with four incisures. Pharynx slightly shorter than that of female. Basal pharyngeal bulb pyriform to slightly cylindroid. Testis outstretched, sperm cells observed in a single row. Spicules slender, ventrally arcuate, bursa short and at cloacal region, 30-31 μm in length; gubernaculum short with a slight proximal curvature.

TYPE MATERIAL

Three paratype females and one paratype male were deposited in the collection of the Department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran. Two paratype females were deposited in the collection of the Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran. Two paratype females and one paratype male were deposited in the nematode collection of the Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Spain.

TYPE HOST AND LOCALITY

The present population was recovered from the rhizosphere of *Astragalus* sp. and mosses in Dezful, Khuzestan province, southwestern Iran (GPS coordinates 32°36'22" N, 48°33'48" E).

DIAGNOSIS AND RELATIONSHIPS

Atetylenchus dezfuliensis n. sp. is characterized by medium-sized body (700-998 µm), lip region truncated without pronounced lobes, median pharyngeal bulb located at 43-47% of total pharynx, tail elongated conical at anterior half and cylindrical to slightly clavate at posterior half, 90-136 µm long, phasmids located at one-third anterior part of tail, and short spicules 18.5 µm long.

Atetylenchus dezfuliensis n. sp. can be differentiated from all species in the genus by its characteristic tail which is first conical but then not tapering towards terminus and has a cylindrical to slightly clavate in its posterior half (typically conical or filiform in other species). It further can be distinguished from *A. abulbosus* by slightly shorter body length (700-998 vs 1000-1460 µm), smaller stylet (10.0-11.5 vs 13-16 µm), and considerably shorter spicules (18.5 vs 33-37 µm). From *A. graminus*, it differs by longer tail (90-136 vs 63-75 µm), and slightly shorter stylet (10.0-11.5 vs 13 µm). From *A. amiri*, it can be separated by stylet (without vs with weak knobs), slightly longer tail (90-136 vs 80-85 µm), the number of lateral incisures (4 vs 5) and slightly shorter stylet (10.0-11.5 vs 12-13 µm). The new species differs from *A. metaporus* by slightly shorter body (700-998 vs 970-1240 µm), smaller stylet (10.0-11.5 vs 12-19 µm) and much shorter tail (90-136 vs 165-230 µm). It may be distinguished from *A. minor* by distinctly longer tail (90-136 vs 51-62 µm), (well-developed ovaries vs less-developed), and slightly shorter stylet (10.0-11.5 vs 11.5-12.5 µm). *A. dezfuliensis* n. sp. can be considered as a separate species from *A. longilabiatu*s n. sp. by differences in tail shape (clavate vs conical), lip region (truncated without raised lobes vs with pronounced lobes at anterior end), tail length (90-136 vs 42-63 µm), and slightly longer body size (700-998 vs 593-720 µm).

Previously, a population of *A. graminus* has been reported from Dezful, Iran (Husseinvand *et al.*, 2016). It should be noted that morphological and morphometric characters of a female specimen from that population fit well with those of *A. dezfuliensis* n. sp. (see Fig. 4J in that study). Indeed, the upper limit of the recorded range of tail length (125 µm) and V (50) for *A. graminus* belongs to that specimen, which now it can be considered as a representative of *A. dezfuliensis* n. sp.

*Atetylenchus longilabiatu*² n. sp.

(Figs. 3 & 4; Table 1)

MEASUREMENTS

See Table 1.

DESCRIPTION

Female

Body almost slightly curved in posterior part of body after fixation. Cuticle thick and finely annulated *ca* 1 µm at mid-body. Lateral field with four incisures that inner band narrower than the outer bands, 6.1 (5.0-7.0) µm wide at mid-body. Cephalic region truncated, with four or five transverse annuli, 3 (2.5-3.5) µm high and 7.1 (7.0-7.5) µm wide; four distinct raised lobes at anterior end, giving a disc-like structure on lip region. Amphidial apertures longitudinal slit, slightly sinuate. Stylet weak, 1.6 (1.3-1.8) times longer than labial region diameter; conus 43 (38-45)% of the total length, without distinct basal knobs but with swollen arms at base of shaft. Orifice of dorsal pharyngeal gland about equal of stylet length. Procorpus cylindrical, median bulb well developed, 9.6 (9.0-10.5) µm wide and 18.2 (15.5-20.0) µm long that occupies 65.5 (57-75)% of the corresponding body diameter. Basal bulb nearly cylindroid, 11.3 (9.5-13.0) µm in width and 24.0 (20.0-29.0) µm in length. Excretory pore at level of anterior part of basal bulb, 83 (73-90) µm from anterior end. Deirids at the same level with the pore. Hemizonid one to two annuli anterior to the pore. Nerve ring at middle of isthmus, located 71 (60-80) µm from anterior end. Reproductive system didelphic-amphidelphic, with two well-developed genital branches but unequal lengths, anterior branch usually with one or two flexures. Ovaries with a single row of oocytes; spermatheca in-line with gonad, bearing globular to slightly oval sperm cells, about 1 µm in diameter. Vulva a

² The species epithet refers to the expanded and pronounced lip region in females and males of the species.

transvers slit, vagina perpendicular to the body axis, extending less than half of the corresponding body diameter. Vulva-anus distance 3.3-5.6 times of the tail length. Tail conical, relatively short, strongly bent ventrally ending to a hook-shaped (beak-lick appearance) terminus. Phasmids around middle of tail.

Male

Similar to female in general characteristics. Body straight and slightly shorter than female. Lateral field with four incisures. Raised lobes on lip region more expanded and pronounced. Pharynx slightly shorter than that of female, basal bulb pyriform. Testis outstretched, sperm cells arranged in single row. Spicules slender, ventrally arcuate, bursa adcloacal and short, 28 μm in length; gubernaculum simple and short.

TYPE MATERIAL

Seven paratype females and a paratype male were deposited in the collection of the Department of Plant Protection, College of Agriculture, University of Zanjan, Zanjan, Iran. Two paratype females were deposited in the collection of the Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, Iran. Three paratype females were deposited in the nematode collection of the Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Spain.

TYPE HOST AND LOCALITY

Recovered from the rhizosphere of *Cynodon dactylon* (L.) Pers. and mosses in Dezful, Khuzestan province, southwestern Iran (GPS coordinates 32°29'27" N, 48°45'16"E).

DIAGNOSIS AND RELATIONSHIPS

Atetylenchus longilabiatatus n. sp. is characterized by short body length (593-720 μm), lip region with four distinct pronounced lobes giving a disc-like structure, median pharyngeal bulb located at 42-48% of pharynx, tail short conical, 42-63 μm , strongly bent ventrally assuming hook-shaped, phasmids located near the middle of tail, and short spicules 17.5 μm long.

Atetylenchus longilabiatus n. sp. can be distinguished from all known species in the genus by expanded and pronounced lobes at anterior end of females and males giving a disc-like structure on lip region. The population of the present study also differs from *A. abulbosus* by shorter body length (593-720 vs 1000-1460 μm), shorter tail (42-63 vs 120 μm), smaller stylet (10.0-12.0 vs 13-16 μm), and shorter spicules (17.5 vs 33-37 μm), as well as in the position of phasmids (41-55 % of tail length vs below centre of tail). From *A. graminus*, the new species differs by shorter body length (593-720 vs 810-1007 μm), slightly shorter stylet (10.0-12.0 vs 13 μm), and shorter tail (42-63 vs 63-75 μm). It can be differentiated from *A. amiri* by shorter body length (593-720 vs 800-1000 μm), stylet (without vs with weak knobs), shorter tail (42-63 vs 80-85 μm), V ratio (52-56 vs 49-51), tail shape (strongly curved ventrally vs straight) and tail terminus shape (regularly tapering vs becoming broader just near the terminus and ending to a pointed projection). Compared to *A. metaporus*, it has a shorter body length (593-720 vs 970-1240 μm), smaller stylet (10.0-12.0 vs 12-19 μm) and different tail shape (conical vs filiform) and tail length (42-63 vs 165-230 μm). The new species comes close to *A. minor* in morphometric characters and tail shape but differs by lip region (with prominent lobes vs simple), ovary length (very long vs short) and lateral field (simple vs crenate outer incisures).

***Atetylenchus* cf. *graminus* (Bajaj, Kaushik and Bhatti, 1982) Siddiqi, 1986**

Body straight to slightly curved after fixation. Cuticle thick and finely annulated *ca* 1 μm at mid-body. Lateral field with three equally distant bands and four incisures, 5.3 (4.5-6.0) μm wide at mid-body. Lip region truncated, with four transverse annuli, 3.5 (3.0-3.5) μm high and 6.6 (6.5-7.0) μm wide. Cephalic framework weakly sclerotized. Amphidial apertures inconspicuous. Stylet weak, 1.7 (1.6-1.8) times of the lip region diameter; conus 39 (38-40) % of the total stylet length, without distinct basal knobs but with swollen arms at base of shaft. Orifice of dorsal pharyngeal gland slightly less than of stylet length. Pharyngeal procorpus cylindrical, median bulb well developed, 9.4 (8.5-10.5) μm wide and 17.7 (16-19) μm length, occupies 61 (57-66) % of the corresponding body diameter. Basal pharyngeal bulb nearly cylindroid, 11.0 (10.0-14.0) μm wide and 29.0 (24.0-35.0) μm long. Excretory pore at level of the beginning of basal bulb, 81 (70-102) μm from anterior end. Deirids at level of excretory pore, hemizonid one to two annuli

anterior to the excretory pore. Nerve ring surrounding middle part of isthmus, located (66-87) μm from anterior end. Reproductive system with two well-developed genital tracts, nearly equal lengths, anterior gonad 178 (164-176) μm and the posterior one (149-161) μm long, ovaries outstretched without any flexure, oocytes arranged in a single row. Spermatheca rectangular, without sperm. Vulva a transverse slit, vagina perpendicular to the body axis, extending towards into body less than half of the vulval body diameter. Tail conical and ending to a finely rounded terminus. Phasmids located at 24 (22-26) μm from anus level or ca. 36 (34-40) % of the tail length.

To help identifying *Atetylenchus* species, a key is proposed below:

KEY TO THE SPECIES OF *ATETYLENCHUS*

1. Tail very long (165-230 μm), filiform *A. metaporus* Erum & Shahina, 2008
 - Tail shorter (under 140 μm), conical or clavate 2
2. Body length 1-1.5 mm; stylet = 13-16 μm *A. abulbosus* (Thorne, 1949) Khan, 1973
 - Body length under 1 mm; stylet 13 μm or below 3
3. Stylet with weak knobs; tail becoming broader near the terminus and ending to a pointed projection *A. amiri* (Maqbool & Shahina, 1984) Geraert & Raski, 1987
 - Stylet without knobs; tail regularly tapering or clavate 4
4. Lip region with four pronounced lobes giving a disc-like structure; phasmids at mid-tail *A. longilabiatatus* n. sp.
 - Lip region simple, without such prominent lobes; phasmids at anterior half of tail 5
5. Tail first conical, but then cylindrical to slightly clavate, 90-136 μm *A. dezfuliensis* n. sp.
 - Tail conical, regularly tapering towards terminus, 51-75 μm 6
6. Body length under 0.7 mm; tail 51-62 μm ; V = 54-59 *A. minor* Yaghoubi, Pourjam, Atighi & Pedram, 2015
 - Body length more than 0.7 mm; tail 63-75 μm ; V = 45-51 *A. graminus* (Bajaj, Kaushik & Bhatti, 1982) Siddiqi, 2000

MOLECULAR CHARACTERISATION AND PHYLOGENETIC RELATIONSHIPS OF *ATETYLENCHUS* WITH OTHER PSILENCHINAE, BOLEODORINAE AND MERLINIIDAE GENERA

The amplification of D2-D3 expansion segments of 28S, and partial 18S rRNA, genes yielded single fragments of approximately 900, and 1800 bp, respectively, based on estimation using gel electrophoresis. Six new D2-D3 of 28S rRNA gene sequences were obtained in the present study (MN807619-MN807624), these sequences showed a 84-86% similarity values to other far related genera as *Pratylenchoides*, *Scutylenchus* or *Amplimerlinius* and 82% similarity with the close related genus *Psilenchus* (MK639384) using Blastn search in NCBI web. The partial 18S rRNA from *Atetylenchus* spp. (MN807625-MN807629) matched well with the close related genus *Psilenchus* ranging the similarities from 97.5% to 97.2% followed by other genera as *Coslenchus* (AY284583) or *Aglenchus* (MK639387) with 97% and 96.8% similarities, respectively. The only partial 18S rRNA sequence from *Atetylenchus minor* (KP730045) showed important problems in some sections of the sequence in the alignment used for the phylogenetic analysis. For this reason this sequences showed no similarity with our sequences and other deposited in GenBank with related genera.

There is no intraspecific variability for D2-D3 segments of 28S rRNA markers for two specimens in *A. longilabiatus* n. sp. and for *A. cf. graminus*. While, for *A. dezfuliensis* n. sp. there are four nucleotides differences (99.4% similarity) in D2-D3 segments of 28S rRNA and 2 nucleotide difference (99.8% similarity) in the partial 18S rRNA between the two specimens studied.

The genus *Atetylenchus* is well-supported as a genus in both markers (PP = 1.00) (Figs. 5, 6). It is related phylogenetically to *Psilenchus* in both markers studied, but a moderate and good support in the partial 18S rRNA (Fig. 5) and D2-D3 region of 28S rRNA (Fig. 6) phylogenetic trees, respectively (PP = 0.91 and PP= 0.98: clade III and II). Other members of the family Tylenchidae related with the subfamily Boleodorinae with data available in GenBank are included in other clades as *Basiria* and *Boleodorus*, clade I in partial 18S rRNA and clade III in D2-D3 region of 28S rRNA. The phylogenetic relationship among the species described is similar for both markers. *Atetylenchus longilabiatus* n. sp. is closely related to *A. cf. graminus* (PP = 1.00) and both species are related in the clade with *A. dezfuliensis* n. sp. (PP = 1.00).

Discussion

This research increased the number of species and the molecular diversity in the genus *Atetylenchus*. Our data from the partial 18S rRNA include this genus within Tylenchidae, which is in agreement with their close morphology with the genus *Psilenchus* and the position under Boleodorinae in Tylenchidae suggested by Geraert (2008). In this point, it is in disagreement with the molecular position of *A. minor* (Yaghoubi *et al.*, 2015), in which this marker included this genus with Merliniidae. We did not include this sequence in our analysis because of showed a very low similarity and many mismatches in one side of this sequence when compared to our sequences from three species of the genus *Atetylenchus* and other sequences in our dataset. In our 28S rRNA phylogeny, the position of *Atetylenchus* is highly supported with Merliniidae and other families. A similar result is showed for the genus *Psilenchus* with a broader phylogeny in Qing *et al.* (2017) for 28S rRNA. Even with the recent use of a combined dataset of both ribosomal markers, the genus *Psilenchus* and *Atetylenchus* are placed in a position of the tree with *Pratylenchoides magnicauda* (Thorne, 1935) (two of the three sequences used in this dataset are now transferred to *Psilenchus* genus from *Atetylenchus* in GenBank, KX789734 and KX789727) (Qing & Bert, 2019). We agree more with the position inside Tylenchidae because 18S rRNA marker is more suited for the understanding of genus evolution in context of families because of it is a high conserved gene and shows lower mutation rate in comparison to 28S rRNA. Even for some specific genera (*Malenchus* and *Filenchus*) the 28S rRNA showed high substitutions rates using substitution saturation test (Qing *et al.*, 2017). Further research is required to elucidate systematic position of the genus *Atetylenchus* and the broad phylogeny of Tylenchidae using other markers or combination of them as suggested by Qing and Bert (2019).

In summary, the present study describes the biodiversity of the genus *Atetylenchus* with two new species, includes molecular markers and a key for their species identification. The position of this genus is valid and well-supported using ribosomal molecular markers.

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Table 1. Morphometric characters of *Atetylenchus dezfuliensis* n. sp., *A. longilabiatius* and *A. cf. graminus* recovered from Iran. All measurements are in μm , and in the form: mean $\pm$ s.d. (range) and coefficient of variation.

Character	<i>A. dezfuliensis</i> n. sp.				<i>A. longilabiatius</i> n. sp.				<i>A. cf. graminus</i>	
	Holotype	Paratype females		Paratype males	Holotype	Paratype females		Paratype male	Females	
n	-	8	CV	2	-	12	CV	1	6	CV
L	818	808 ± 107 (700-998)	13.	815, 820	616	640 ± 44 (593-720)	6.8	541	711 ± 52.2 (643-787)	7.3
L'	682	690 ± 95 (610-867)	13.	713, 719	7.5	584 ± 40 (543-657)	6.9	483	646 ± 50.5 (579-718)	7.8
Stylet	11.5	10.7 ± 0.5 (10.0-11.5)	4.7	10.5	11.4	11.0 ± 0.7 (9.5-12.5)	6.6	10.0	11.2 ± 0.3 (10.5-11.5)	3.3
m	40.0	41.0 ± 2.4 (38.0-45.4)	5.9	38.5, 40.8	43.0	43.0 ± 2.1 (38.4-45.7)	4.9	42.9	39 ± 0.8 (38.4-40.1)	2.2
a	37.2	36.5 ± 4.1 (32.8-44.1)	11.	45.8, 51.3	30.8	31.0 ± 3.6 (25.4-40.1)	11.5	38.6	33 ± 2.6 (30.1-37.5)	7.9
b	6.4	6.4 ± (0.6 (5.8-7.7)	10.	5.3	6.0	5.0 ± 0.3 (4.9-6.3)	6.6	5.3	5.8 ± 0.5 (5.3-6.8)	9.9
c	6.0	6.8 ± 0.6 (6-7.7)	8.8	8.0, 8.1	10.6	11.0 ± 1.3 (9.6-14.6)	11.6	9.3	64 ± 2.1 (63-69)	3.2
c'	8.8	7.7 ± 0.7 (6.6-8.7)	9.6	6.4, 6.7	4.9	4.0 ± 0.6 (3.7-5.6)	12.7	4.5	5.4 ± 0.2 (5.1-5.9)	5.1
V	47.3	49.4 ± 1.7 (47.3-51.6)	3.4	-	54.2	54.0 ± 1.1 (52.1-56.1)	2.0	-	53 ± 1.3 (51.7-55.1)	2.5
DGO	7.0	7 ± 0.3 (6.5-7.5)	4.7	7.0, 7.5	19.5	5.0 ± 0.6 (4.5-7.0)	12.3	5.0	6.7 ± 0.4 (6.0-7.5)	6.4
Median bulb	60.0	56.8 ± 4.5 (49.0-65.0)	7.9	65, 67	75.0	51.0 ± 2 (49.0-55.0)	3.9	48.0	49 ± 3.9 (45.0-55.0)	7.9
MB	47.2	45.7 ± 1.4 (43.5-47.0)	3.2	50.0, 51.9	47.6	45.0 ± 1.6 (42.0-47.5)	3.6	46.5	40 ± 1.6 (38.5-43.5)	3.9

Excretory pore	95	97 ± 10.2 (90-120)	10.	98, 103	103	83 ± 4.9 (74-90)	6.0	87	81 ± 11.8 (70-102)	14.4
Pharynx	127	124 ± 9.7 (104-139)	7.8	129, 130	25.0	114 ± 5.4 (103-121)	4.8	103	122 ± 7.8 (110-133)	6.4
Lip region to vulva	387	399 ± 44.6 (350-485)	11.	-	558	351 ± 23 (322-399)	6.6	-	380 ± 27.6 (350-415)	7.2
Vulva-anus	295	291 ± 51 (249-382)	17.	-	1.5	232 ± 20 (208-279)	8.5	-	266 ± 26.2 (229-303)	9.8
Annulus width	1.3	1.1 ± 0.1 (1-1.5)	11.	1.3	1.2	1.1 ± 0 (1.0-1.5)	6.9	1.0	1.2 ± 0 (1.0-1.5)	6.3
Body width	22.0	22 ± 0.6 (20.5-22.5)	3.1	16, 17.8	20.0	20.0 ± 3 (15.0-28.0)	14.7	14.0	21 ± 1.4 (19.0-22.5)	6.7
Tail / Vulva-Anus	0.5	0.4 ± 0.0 (0.3-0.5)	11.	-	0.3	0.2 ± 0.0 (0.2-0.3)	13.4	-	0.2 ± 0.0 (0.2-0.3)	8.2
Vulval body width	21.5	21.1 ± 0.9 (19.5-22.0)	4.4	-	18.0	18.0 ± 2.4 (15.5-24.0)	13.0	-	20 ± 1.6 (18.5-21.5)	8.2
Anal body width	15.5	15.1 ± 1.1 (12.5-16.5)	7.6	15, 16	11.8	11.0 ± 0.8 (10.5-14.0)	7.6	13.0	11.9 ± 0.7 (11-13)	6.0
Tail length	136	118 ± 16.8 (90-136)	14.	101, 102	58.0	56 ± 7.1 (42-63)	12.5	58	64 ± 2.1 (63-69)	3.2
Spicules	-	-	-	18.7	-	-	-	17.5	-	-
Gubernaculum	-	-	-	7.5, 8.3	-	-	-	6.5	-	-

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Figure legends

Fig. 1. *Atetylenchus dezfuliensis* n. sp. Female (A, C, E, G, H, G) and male (B, D, F, I). A, B: Entire body; C, D: Anterior end and pharyngeal region; E, F: Stylet and lip region; G: reproductive system; H, I: Posterior end.

Fig. 2. *Atetylenchus dezfuliensis* n. sp. Female (A, C-E, G, I-L) and male (B, F, H). A, B: Entire body. C, D: Anterior body and pharyngeal region; E, F: Stylet and lip region; G: Position of phasmid on tail; H: Spicules and gubernaculum. I-K: Posterior end; L: Lateral field. (Scale-bars: A, B= 100 μ m, C, I= 20 μ m, D-H, J-L = 10 μ m).

Fig. 3. *Atetylenchus longilabiatus* n. sp. Female (A, C, E-H, J-L) and male (B, D, I, M). A, B: Entire body; C, D: Anterior end and pharyngeal region; E-G: Lip region; H: Reproductive system; I-K: Posterior end; L, M: Stylet and lip region.

Fig. 4. *Atetylenchus longilabiatus* n. sp. Female (A, C-E, G-K) and male (B, F, L). A, B: Entire body; C, D, F: Anterior end; E, H: pharyngeal region; G, I, J, L: Posterior end; K: Cross section of mid-body. (Scale-bars: A, B= 100 μ m, C-L = 10 μ m).

Fig. 5. Phylogenetic relationships among *Atetylenchus* species with Psilenchinae, Boleodorinae and Merliniidae genera. Bayesian 50% majority rule consensus tree as inferred from partial 18S rRNA gene sequence alignment under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).

Fig. 6. Phylogenetic relationships among *Atetylenchus* species with Psilenchinae, Boleodorinae and Merliniidae genera. Bayesian 50% majority rule consensus tree as inferred from D2-D3 expansion segments of 28S rRNA sequence alignment under the general time-reversible model of sequence evolution with correction for invariable sites and a gamma-shaped distribution (GTR + I + G). Posterior probabilities greater than 0.70 are given for appropriate clades. Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).