Taxonomical considerations and molecular phylogeny of the closely related genera *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus* (Nematoda: Telotylenchinae), with one new and four known species from Iran

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Abstract

During several nematological surveys in cultivated and natural habitats in Khuzestan and Zanjan provinces of Iran, a new species, *Bitylenchus parvulus* n. sp., two new records for Iran namely *Tylenchorhynchus agri* and *T. graciliformis*, and a population of *Bitylenchus parvus* and one of *Sauertylenchus maximus* were recovered and characterised based upon morphological and molecular approaches. The new species is characterized by lip region with five to seven annuli, stylet 17.7 (17.0-18.5) μm long, sub-cylindrical tail narrowing abruptly near terminus, cuticle near anterior part of vulva wrinkled and post-rectal sac occupied whole of tail cavity. The phylogenetic analyses were carried out using molecular data from D2-D3 expansion segments of 28S rDNA for all studied species and the partial 18S rDNA for the new species. The representatives of *Bitylenchus* and *Sauertylenchus* formed distinct clades from *Tylenchorhynchus* members supporting the hypothesis in which *Bitylenchus* and *Sauertylenchus* could be considered as valid genera but rejecting the 'large-genus' concept for *Tylenchorhynchus*. Also, *S. ibericus* was proposed as a junior synonym of *S. maximus* based on the results from morphological and phylogenetic analysis. Furthermore, an identification key for all known species included in three genera *Bitylenchus*, *Tylenchorhynchus* and *Sauertylenchus* is presented herein. The number of transverse annuli on the lip region and presence/absence of post-rectal sac were considered as main diagnostic characters for classifying of the species into seven groups, and other morphological and morphometric characters were subsequently used for distinguishing species in each group.

Introduction

Nematode members of the genus *Tylenchorhynchus* Cobb, 1913, known under the common name "stunt nematodes", are one of the largest groups of plant-parasitic nematodes which are migratory ectoparasites of the roots of many plants, including various agricultural crops and native plants (Siddiqi, 2000; Handoo *et al.*, 2014). *Bitylenchus* Filipjev, 1934 was proposed as a subgenus of the genus *Tylenchus* Bastian, 1865 by Filipjev (1934) with *Tylenchus* (*Bitylenchus*) *dubius* (Bütschli, 1873) Filipjev, 1934 as its type species; however, it was synonymized with *Tylenchorhynchus* by Filipjev (1936). Some nematologists (Fortuner & Luc, 1987; Handoo, 2000; Geraert, 2011) considered *Bitylenchus* as a synonym of *Tylenchorhynchus*, but others recognized both as valid genera (Gomez-Barcina *et al.*, 1992; Siddiqi, 2000; Andrássy, 2007; Handoo *et al.*, 2014; Ghaderi *et al.*, 2014). The genus *Bitylenchus* was differentiated from *Tylenchorhynchus* in having areolated outer bands of lateral fields, a large post-anal intestinal sac containing intestinal granules and fasciculi, relatively more thickened cuticle at the female tail tip, and gubernaculum lacking a crest (Gomez-Barcina *et al.*, 1992). The genus *Sauertylenchus* Sher, 1974 is also similar to *Bitylenchus* except for the difference in the structure of the lip region which has an offset labial disc and the first labial annulus divided into six sectors and a relatively longer stylet (Sher, 1974; Siddiqi, 1986, 2000; Gomez-Barcina *et al.*, 1992).

Siddiqi (2000) listed 105 valid species under *Tylenchorhynchus*, 29 valid species under *Bitylenchus* and further five species under *Sauertylenchus*. Geraert (2011) considered 133 species under *Tylenchorhynchus* (the genus *Bitylenchus* was considered as its synonym) and only one species under the genus *Sauertylenchus* (*S. labiodiscus* Sher, 1974). Subsequently, *Tylenchorhynchus bambusi* Singh, Lal, Rathour & Ganguly, 2010; *B. hispaniensis* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014; *B. capsicumi* Zarina & Akhtar, 2014; *T. mediterraneus* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014 and *T. iranensis* Azimi, Mahdikhani-Moghadam, Rouhani & Rajabi Memari, 2016 were described from India, Spain, Pakistan, Spain, and Iran, respectively. Sturhan (2014) transferred *Paratrophurus striatus* Castillo, Siddiqi & Gomez-Barcina, 1989 to the genus *Tylenchorhynchus* and named it *T. casigo* (Castillo, Siddiqi & Gomez-Barcina, 1989) Sturhan, 2014.

Recently, Handoo *et al.* (2014) studied relationships between the genera *Bitylenchus* and *Tylenchorhynchus* using integrative taxonomy. In this sense, the use of integrative taxonomy combined with the phylogenetically test of alternative hypotheses confirmed the separation of these two genera, rejecting the Fortuner & Luc's conceptual view of *Tylenchorhynchus* as a large genus (Fortuner & Luc, 1987). Because of the few and difficult characters to study in this group of nematodes, the use of molecular markers for species identification in the genus is of high importance. The use of nuclear ribosomal RNA genes [D2–D3 expansion segments of the large ribosomal subunit (28S), internal transcribed spacer (ITS), and partial small ribosomal subunit (18S)] have been proven useful in the latest phylogenetical studies of this group of nematodes (Handoo *et al.*, 2014; Azizi *et al.*, 2016; Hosseinvand *et al.*, 2019a; Gharakhani *et al.*, 2019; Hosseinvand *et al.*, 2020).

Taxonomic position of certain genera incorporated into the large genus *Tylenchorhynchus*, particularly *Bitylenchus* and *Sauertylenchus* is still controversial despite several phylogenetic and morphological studies. Sufficient morphological information is not available on several known species, which make their identification a challenging task. So far, several identification keys have been given for species included in these genera, mostly under *Tylenchorhynchus sensu lato* (Allen, 1955; Brzeski, 1998; Brzeski & Dolinski, 1998; Handoo, 2000; Geraert, 2011). In the present study, we provided a revised and new updated key for species included in the genera *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus*. Moreover, we described a new species from *Bitylenchus* and provided additional morphological and molecular information on four other known species namely *B. parvus* (Allen, 1955) Jairajpuri, 1982; *Sauertylenchus maximus* (Allen, 1955) Siddiqi, 2000; *Tylenchorhynchus agri* Ferris, 1963 and *T. graciliformis* Siddiqi & Siddiqui, 1983.

Materials and methods

Taxonomic review of Bitylenchus, Sauertylenchus and Tylenchorhynchus

The original and several relevant published descriptions of the species included in the genera *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus* were studied in detail. Based on obtained information from these descriptions, as well as those given in the monograph by Geraert (2011), we provided an updated and revised identification key for all known species in these genera. For practical reasons, we prefer to include all species in a single key due to close morphological affinity of the genera and the problems arise when assigning a correct taxon for a given population. Besides the characters implemented in the key by Geraert (2011), we used new additional characters such as the number of tail annuli, areolation of the lateral field, and the shape of gubernaculum for obtaining a better resolution to separate closely related species.

The taxonomic position of the valid species and their synonyms are mainly based on Siddiqi (2000).

Sampling and morphological identification

Soil samples were collected from the rhizosphere of different wild plants in Khuzestan and Zanjan provinces of 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. Drawings were made through a drawing tube attached to a light microscope. Specimens were identified at species level using available identification keys (Geraert, 2011).

Molecular characterization

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) to ensure confirming specimens with the unidentified populations. All necessary morphological and morphometric data (pictures and measurements) of the studies specimens in temporary slides were recorded using the abovementioned camera-equipped microscope. This was followed by DNA extraction from single individuals as described by Subbotin *et al.* (2000). The D2-D3 region was amplified using the D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′-TCGGAAGGAACCAGCTACTA-3′) primers (Nunn, 1992). Finally, the 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 of 28S rRNA and 18S rRNA sequences of different genera of Telotylenchinae Siddiqi, 1960 were obtained from GenBank and used for phylogenetic reconstruction. Outgroup taxa for each dataset were chosen following previous published studies (Handoo *et al.*, 2014; Azizi *et al.*, 2016; Hosseinvand *et al.*, 2019a). The D2-D3 region alignment consisted of 54 sequences as ingroups and two sequences of *Psilenchus hilarulus* de Man, 1921 (KP313831) and *Coslenchus paramaritus* Hosseinvand, Eskandari, & Ghaderi, 2019b (MK542004) as outgroups. The 18S rRNA alignment consisted of 30 sequences as in groups and *Geocenamus quadrifer* (Andrássy, 1954) Brzeski, 1991 (AY993997) and *P. hilarulus* (KX798728) as outgroups. The newly obtained sequences were edited and aligned with other relevant sequences available in GenBank using Muscle alignment tool implemented in MEGA7 program (Kumar *et al.*, 2016). The ambiguously aligned parts and divergent regions were removed from the alignments using the online version of Gblocks 0.91b (Castresana, 2000) (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The best-fit model of nucleotide substitution used for the phylogenetic analysis was statistically selected using jModelTest 2.1.10 (Darriba *et al.*, 2012) with the Akaike Information Criterion (AIC). Phylogenetic trees were generated with Bayesian inference method using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012). 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 genes, was run with four chains for 2×10^6 generations. A combined analysis of the two genes was not undertaken due to some sequences not being available for all species. 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 visualized and saved using FigTree software V.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) (Rambaut, 2014) and edited with Adobe® Acrobat® XI Pro 11.0.1.

Results

Key to the species of Bitylenchus, Sauertylenchus and Tylenchorhynchus

All known species of these three genera are treated in a single identification key here (see discussion for justification). For practical reasons, we separate this complex nematode group in several morphotype groups, based on the main diagnostic characters (mainly the number of lip region annuli and presence/absence of post-rectal sac) used for species delineation in these genera.

Group 1. Lip region without annuli, smooth; post-rectal sac absent or very slightly overlapping rectum (presented in the original description of *T. amgi* but not drawn)

Group 2. The number of transverse annuli on lip region (excluding labial disc) usually 2 or 3, rarely 4; post-rectal sac absent or very slightly overlapping rectum

Group 3. The number of transverse annuli on lip region (excluding labial disc) usually 2 or 3, rarely 4; post-rectal sac present

Group 4. The number of transverse annuli on lip region (excluding labial disc) usually 4 or 5, rarely 6; post-rectal sac absent or very slightly overlapping rectum

Group 5. The number of transverse annuli on lip region (excluding labial disc) usually 4 or 5, rarely 6; post-rectal sac present

Group 6. The number of transverse annuli on lip region (excluding labial disc) mostly 6-10, fine; post-rectal sac absent or very slightly overlapping rectum

Group 7. The number of transverse annuli on lip region (excluding labial disc) mostly 6-10, fine; post-rectal sac present

Dichotomous keys for species identification in proposed morphospecies groups:

Group 1.

1. Average of stylet length below 15 µm ….. *T. cynodoni* Kumar, 1981

- Average of stylet length 17-22 µm ….. 2

- Average of stylet length 23 µm ….. *T. robustus* Thorne & Malek, 1968 (lip region described as having no visible transverse annuli; however, this species may have 6-10 mostly fine annuli, and belongs to the group 6)

2. Average value of c' ratio 2 to 3.5 ….. 3

- Average value of c' ratio nearly 4 or more ….. 6

3. Average of cuticular annuli on the ventral side of tail below 30 ….. 4

- Average of cuticular annuli on the ventral side of tail more than 40 ….. *T. sudanensis* (Decker, Yassin & El-Amin, 1975) Castillo, Siddiqi & Gomez-Barcina, 1989

4. Lip region distinctly offset; tail sub-cylindrical ….. *T. oryzae* Kaul & Waliullah, 1995

- Lip region continuous to slightly offset; tail cylindrical ….. 5

5. Lip region hemispherical, basal bulb saccate; stylet knobs posteriorly or laterally directed ….. *T. parasudanensis* (Elbadri, Moon, Lee & Choo, 2010) Geraert, 2011

- Lip region conoid truncate, basal bulb pyriform; stylet knobs anteriorly directed ….. *T. tuberosus* Zarina & Maqbool, 1994

6. Cuticular annuli on the ventral side of tail below 25 in average ….. 7

- Cuticular annuli on the ventral side of tail more than 35 in average ….. 8

7. Stylet with anteriorly directed basal knobs; cuticular annuli 1.0-1.2 µm apart ….. *T. amgi* Kumar, 1981

- Stylet with posteriorly directed basal knobs; cuticular annuli 1.5-2.4 µm apart ….. *T. leviterminalis* Siddiqi, Mukherjee & Dasgupta, 1982

8. Lip region hemispherical, slightly offset, tail cylindrical, very slightly arcuate ventrally ..... *T. ooti* Siddiqi, 2008

- Lip region conoid-truncate, continuous, tail cylindrical to sub-clavate with characteristic shallow depression on its dorsal side…….. *T. microcephalus* Siddiqi & Patel, 1990

Group 2.

1. Average of stylet length below 18 µm ….. 2

- Average of stylet length 18-22 µm ….. 17

- Average of stylet length more than 23 µm ….. 28

2. Cuticular annuli on the ventral side of tail more than 25 in average ….. 3

- Cuticular annuli on the ventral side of tail below 25 in average ….. 5

3. Average of body length more than 800 µm ….. *T. handooi* Khan, 2004

- Average of body length less than 700 µm ….. 4

4. c' = 2.7-3.2; stylet 16-17 µm ….. *T. karnalensis* Saha, Singh, Lal & Kaushal, 2002

- c' = 4; stylet 14-16 µm ….. *T. delhiensis* Chawla, Bhamburkar, Khan & Prasad, 1968

5. Lip region distinctly offset ….. 6

- Lip region continuous or slightly offset …. 7

6. Basal bulb saccate and offset from intestine; cuticular annuli 1.6-1.8 µm wide at mid-body; tail slightly ventrally curved; c = 17-24 ….. *T. microconus* Siddiqi, Mukherjee & Dasgupta, 1982

- Basal bulb pyriform with slight overlapping, cuticular annuli 2.7 µm wide at mid-body; tail straight; c = 14-17 ….. *T. aspericutis* Knobloch, 1975

7. Tail cylindrical ….. 8

- Tail conical to sub-cylindrical …… 9

8. Lateral field marked by peculiar oblique striae ….. *T. gossypii* Nasira & Maqbool, 1996

- Lateral field not marked by such striae ….. *T. lucknowensis* Singh & Jain, 1983

9. Average of stylet length below 14 µm ….. *T. tritici* Golden, Maqbool & Handoo, 1987

- Average of stylet length more than 14 µm ….. 10

10. c' = 3.9 ….. *T. elamini* Elbadri, Moon, Lee & Choo, 2010

- c' = 2.0-3.5 ….. 11 (including 8 similar species)

11. Average of body length less than 600 µm ….. 12

- Average of body length more than 600 µm ….. 16

12. Stylet knobs anteriorly directed ….. 13

- Stylet knobs posteriorly directed ….. 14

13. Lip region with 2 transverse annuli; c' = 2.3-2.8; average of stylet length more than 17 µm ….. *T. varicaudatus* Singh, 1971

- Lip region with 3-4 transverse annuli; c' = 3.0-3.4; average of stylet length less than 17 µm ….. *T. qasimii* Ramzan, Handoo & Fayyaz, 2008

14. Tail annuli 20-24 ….. *T. iarius* Saha, Gaur & Lal, 1998

- Tail annuli 11-20 ….. 15

15. c' = 2.3-2.9 ….. *T. rudis* Siddiqi, 2008

- c' = 3.2 ….. *T. haki* Fotedar & Mahajan, 1971

16. Tail annuli 13-16; cuticular annuli about 3 µm wide at mid-body; spicules with distinct terminal notch ….. *T. mashhoodi* Siddiqi & Basir, 1959

- Tail annuli 17-21; cuticular annuli 2.6 µm wide at mid-body; spicules without terminal notch ….. *T. bohrrensis* Gupta & Uma, 1980

- Tail annuli 23; cuticular annuli about 2.2 µm wide at mid-body; spicules without terminal notch ….. *T. elegans* Siddiqi, 1961

17. Tail annuli more than 25 in average ….. 18

- Tail annuli less than 25 in average ….. 21

18. Tail clavate; c' = 4; tail with distinct hyaline near 10 µm thick ….. *T. clavicaudatus* Seinhorst, 1963

- Tail conical to sub-cylindrical; c' below 3.5; tail without distinct hyaline ….. 19

19. Lip region distinctly offset ….. *T. sanwali* Kumar, 1982

- Labial region continuous to slightly offset ….. 20

20. Tail conical to almost funnel-shaped with narrow terminus ….. *T. paratriversus* Brzeski, 1992

- Tail regularly conical with hemispherical terminus ….. *T. thermophiles* Golden, Baldwin & Mundo-Ocampo, 1995

21. Tail clavate ….. 22

- Tail conical to sub-cylindrical ….. 23 (two similar species)

22. Pharynx 115-160 µm; spicules 19-23 µm; tail hyaline 7-13 µm thick ….. *T. crassicaudatus* Williams, 1960

- Pharynx 105-120 µm; spicules 21-26 µm; tail hyaline 5-8 µm thick ….. *T. kegasawai* Minagawa, 1995

23. Tail terminus striated; longitudinal striae present on anterior end ….. *T. claytoni* Steiner, 1937

- Tail terminus smooth; longitudinal striae absent ….. 24

24. Male not found, even in populations with very high number of females ….. *T. annulatus* (Cassidy, 1930) Golden, 1971

- Males usually present ….. 25

25. Lip framework refractive; stylet knobs concave, 5 µm across ….. *T. ancorastyletus* Ivanova, 1983

- Lip framework non-refractive; stylet knobs usually not concave, 4 µm or less ….. 26

26. c' = 2.0; stylet 19-23 µm; stylet knobs posteriorly directed ….. *T. mexicanus* Knobloch & Laughlin, 1973

- c' = 2.7-3.0; stylet 17-20 µm; stylet knobs laterally or anteriorly directed ….. 27 (three similar species)

27. Lip region annuli three; body length 620-710 µm; spicules 20-22 µm ….. *T. badliensis* Saha & Khan, 1982

- Lip region annuli two; body length 570-630 µm; spicules 23-24 µm ….. *T. coffeae* Siddiqi & Basir, 1959

- Lip region annuli 2; body length 580-660 µm; spicules 24-26 µm ….. *T. musae* Kumar, 1981

28. Body length 700-800 µm; tail hyaline very thick (12-16 µm); spicules 21-25 µm ….. *T. clavus* Khan, 1990

- Body length 800-1000 µm; tail hyaline normal (below 8 µm thick); spicules 28-33 µm ….. *T. silvaticus* Ferris, 1963

Group 3.

1. Average of stylet length less than 18 µm; spicules 24-28 µm ….. *T. microcephaloides* (Zarina, Siddiqi & Shahina, 2004) Geraert, 2011

- Average of stylet length between 18 and 22 µm; spicules shorter than 25 µm ….. 2

- Average of stylet length more than 24 µm; spicules shorter than 25 µm ….. *T. botrys* Siddiqi, 1985

2. Cuticular annuli on the ventral side of tail below 25 in average; tail terminus smooth (except in *T. kashmirensis*) ….. 3

- Cuticular annuli on the ventral side of tail 25-35 in average; tail terminus striated ….. *B. equatorialis* Talavera & Siddiqi, 1995

- Cuticular annuli on the ventral side of tail more than 35 in average; tail terminus smooth ….. *T. neoclavicaudatus* Mathur, Sanwal & Lal, 1979

3. Lip region distinctly offset; tail terminus striated ….. *T. kashmirensis* Mahajan, 1974

- Lip region continuous or slightly offset; tail terminus smooth ….. 4

4. Tail cylindrical to clavate ….. 5 (two similar species)

- Tail conical to sub-cylindrical ….. 6

5. Tail cylindrical to slightly clavate ….. *T. nudus* Allen, 1955

- Tail distinctly clavate….. *T. bambusi* Singh, Lal, Rathour & Ganguly, 2010

6. c' = 2.8-4.1; post-rectal sac filling less than 20% of tail length ….. *T. alami* Shaw & Khan, 1996

- c' = 2.1-2.6; post-rectal sac filling about 45-70% of tail length ….. 7

7. Spicules 21-25 µm; stylet knobs with concave anterior surfaces ….. *T. agri* Ferris, 1963

- Spicules 18 µm; stylet knobs rounded ….. *T. ewingi* Hopper, 1959

Group 4.

1. Average of stylet length below 18 µm ….. 2 (some species have a stylet near to 18 µm; comparison with those bearing 18-22 µm stylet is recommended)

- Average of stylet length 18-22 µm ….. 15

- Average of stylet length more than 22 µm ….. 28

2. Cuticular annuli on the ventral side of tail below 30 in average ….. 3

- Cuticular annuli on the ventral side of tail more than 30 in average ….. 11

3. Lip region distinctly offset ….. 4

- Lip region continuous or slightly offset ….. 5

4. Tail terminus smooth; spicules 24-26 µm ….. *T. variannulatus* Siddiqi, 2008

- Tail terminus striated; spicules 14 µm ….. *T. leucaenus* Azmi, 1991

- Tail terminus striated; male unknown ….. *T. crotoni* Pathak & Siddiqui, 1997

5. Body ventrally contracted behind vulva ….. *T. contractus* Loof, 1964

- Body not contracted behind vulva ….. 6

6. Tail with broadly rounded terminus; lateral field strongly areolated; gubernaculum hammer-shaped ….. *T. aerolatus* Tobar Jiménez, 1970

- Tail with narrower terminus; lateral field not strongly areolated; gubernaculum simple ….. 7

7. Spermatheca non-functional; males absent or very rare ….. *T. clarus* Allen, 1955 (*T. variannus* Mavlyanov, 1978 is a possible synonym)

- Spermatheca functional; males usually present ….. 8

8. Stylet length 11-13 µm ….. *T. nordiensis* Khan & Nanjappa, 1974

- Stylet length 15-19 µm ….. 9 (three similar species; also they should be compared with *T. zeae* and *T. chirchikensis* which have only slightly longer stylet)

9. Tail terminus flattened ….. *T. shivanandi* Shaw & Khan, 1992

- Tail terminus hemispherical ….. 10

10. Spicules 18-21 µm ….. *T. brassicae* Siddiqi, 1961

- Spicules 21-26 µm …… *T. spinaceai* Singh, 1976

11. Tail terminus striated ….. *T. mangiferae* Luqman & Khan, 1986

- Tail terminus smooth ….. 12

12. Cuticle on either side of vulva irregular ….. *B. usmanensis* (Khurma & Mahajan, 1988) Siddiqi, 2000

- Cuticle near vulva normal ….. 13

13. Lip region hemispherical; vulva with lateral membrane ….. *T. fatimae* Khan, Saeed & Akhter, 2004

- Lip region sunken and dome-shaped; vulva without lateral membrane …... 14

14. Lip region with 5-6 transverse annuli; lateral field areolated ….. *B. quaidi* (Golden, Maqbool & Handoo, 1987) Siddiqi, 2000

- Lip region with 4 transverse annuli; lateral field apparently not areolated ….. *T. minutus* Karapetjan, 1979

15. Cuticular annuli on the ventral side of tail below 25 in average ….. 16

- Cuticular annuli on the ventral side of tail between 25 and 35 in average ….. 25

- Cuticular annuli on the ventral side of tail more than 35 in average ….. 27

16. Tail cylindrical to clavate ….. 17

- Tail conical to sub-cylindrical ….. 19

17. Body length 730-830 µm; c' = 3.0-3.8 …… *T. idahoensis* Siddiqi, 2008

- Body length 570-710 µm; c' = 2.5-3.0 …… 18

18. Tail clavate; tail annuli about 15 ….. *T. dactylurus* Das, 1960

- Tail cylindrical; tail annuli 8-10 ….. *T. georgiensis* Eliashvili, 1971

19. Lip region distinctly offset ….. 20

- Lip region continuous or slightly offset ….. 22

20. Tail with very narrow terminus ….. *T. aduncus* de Guiran, 1967

- Tail terminus not so narrow ….. 21

21. Body length 460-660 µm; tail terminus striated …… *T. ismaili* Azmi & Ahmad, 1991

- Body length 670-830 µm; tail terminus smooth ….. *T. graciliformis* Siddiqi & Siddiqui, 1983

22. Tail with very narrow terminus ….. *T. paulettae* Bloemers & Wanless, 1998

- Tail terminus not so narrow ….. 23

23. Tail terminus striated; spicules 26-29 µm ….. *T. irregularis* Wu, 1969

- Tail terminus smooth; spicules less than 25 µm ….. 24 (three similar species)

24. Body length 530-640 µm ….. *T. zeae* Sethi & Swarup, 1968

- Body length 620-700 µm ….. *T. chirchikensis* Mavlyanov, 1978

- Body length 700-780 µm ….. *T. projectus* Khan, 1990

25. Body length 520-590 µm; lip region with 5 annuli; stylet knobs rounded ….. *T. ebriensis* Seinhorst, 1963

- Body length 600-760 µm; lip region with 4 annuli; stylet knobs with concave anterior end ….. 26

26. Tail terminus narrow, striated ….. *T. eremicolus* Allen, 1955

- Tail terminus hemispherical, smooth ….. *T. digitatus* Das, 1960

27. Tail with finger-shaped outgrowth; c' = 2.2 ….. *T. erevanicus* Karapetjan, 1979

- Tail with distinct hyaline (9-12 µm); c' = 5 ….. *T. areoterminalis* Siddiqi, 2008

- Tail normal; c' = 2.8 …… *T. hordei* Khan, 1972

28. Tail with elongated-conical with narrow terminus; tail annuli more than 30 ….. *T. antarctictus* Wouts & Sher, 1981

- Tail bifurcated; tail annuli 26-27 ….. *T. bicaudatus* Khakimov, 1973

- Tail suddenly constricted ventrally before middle, distal part hook-shaped with a bursa-like structure formed by lateral fields; tail annuli less than 25 ….. *T. bursifer* Loof, 1960

- Tail conical; tail annuli 15-22 ….. *T. cylindricus* Cobb, 1913

- Tail almost cylindrical; tail annuli near 25 ….. *S. velatus* (Sauer & Annells, 1981) Siddiqi, 2000

- Tail almost cylindrical; tail annuli 14-15 ….. *T. tarjani* Andrássy, 1969

Group 5.

1. Average of stylet length below 18 µm ….. 2

- Average of stylet length more than 18 µm ….. 7

2. Tail annuli more than 40 ….. *T. paracanalis* Khan, 1991

- Tail annuli less than 40 ….. 3

3. Average of stylet length below 15 µm; vulva without distinct epiptygma ….. 4

- Average of stylet length more than 15 µm; vulva without epiptygma ….. *B. capsicumi* Zarina & Akhtar, 2014

- Average of stylet length more than 15 µm; vulva with distinct epiptygma ….. *B. queirozi* (Monteiro & Lordello, 1976) Jairajpuri, 1982

4. Cuticle posterior to vulva with irregular ondulations …… *B. ventrosignatus* (Tobar Jiménez, 1969) Jairajpuri, 1982

- Cuticle posterior to vulva normal ….. 5

5. Tail terminus smooth ….. *T. subcylindricus* Singh & Jain, 1982

- Tail terminus striated ….. 6

6. Tail conical with narrow terminus; spicules 20-23 µm ….. *B. swarupi* (Singh & Khera, 1978) Jairajpuri, 1982

- Tail almost cylindrical with hemispherical terminus; spicules 23-26 µm ….. *B. cuticaudatus* (Ray & Das, 1983) Siddiqi, 1986

7. Tail annuli less than 25 ….. *T. kamlae* Shaw & Khan, 1996

- Tail annuli 25-35 ….. 8

- Tail annuli 35-45 ….. *B. bryobius* (Sturhan, 1966) Jairajpuri, 1982

8. Body length 480-800 µm; spicules 18-26 µm ….. 9

- Body length 830-1300 µm; spicules 33-35 µm ….. *S. pratensis* (Gomez-Barcina, Siddiqi & Castillo, 1992) Siddiqi, 2000

9. Lip region usually with 4 transverse annuli ….. *B. colombianus* Siddiqi, 1985

- Lip region with 5 or 6 transverse annuli ….. 10 (two similar species)

10. Stylet length 16-20 µm ….. *B. iphilus* Minagawa, 1995

- Stylet length 19-21 µm ….. *B. malinus* (Lin, 1992) n. comb.

Group 6.

1. Average of stylet length below 17 µm ….. 2 (two similar species)

- Average of stylet length 18-22 µm ….. 3

- Average of stylet length more than 22 µm ….. 6

2. Body length 580-700 µm; stylet 16-17 µm ….. *T. latus* Allen, 1955

- Body length 710-730 µm; stylet 14-16 µm ….. *T. persicus* Sultan, Singh & Sakhuja, 1991

3. Tail annuli less than 20 ….. *T. shimizui* Talavera, Watanabe & Mizukubo, 2002

- Tail annuli 20-35 ….. 4

- Tail annuli more than 35 ….. 5

4. Lip region continuous; c' = 1.7-2.3; spicules 29-34 µm ….. *T. casigo* (Castillo, Siddiqi & Gomez-Barcina, 1989) Sturhan, 2014

- Lip region offset; c' = 3.4-5.0; spicules 20-22 µm ….. *T. teres* (Khan & Darekar, 1979) Siddiqi, 1986

5. Lip region distinctly offset; body length 800-1200 µm; tail regularly sub-cylindrical ….. *T. mediterraneus* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014

- Lip region continuous; body length 750 µm; tail contracted behind anus ….. *T. manubriatus* Litvinova, 1946

6. Tail annuli 16-30; vulva with protruding double epiptygma ….. *T. siccus* Nobbs, 1990

- Tail annuli more than 30; epiptygma absent ….. 7

7. Tail conical ending to a very narrow terminus; stylet 23-26 µm ….. *T. tenuicauda* Wouts & Sher, 1981

- Tail cylindrical ending to a hemispherical terminus; stylet 26-31 µm …... *T. kegenicus* Litvinova, 1946

Group 7.

1. Average of stylet length 10-11 µm ….. *B. depressus* Jairajpuri, 1982

- Average of stylet length 12-15 µm ….. 2

- Average of stylet length 15-19 µm ….. 6

- Average of stylet length 19-22 µm ….. 10

- Average of stylet length 22-30 µm ….. 13

- Average of stylet length 35-40 µm ….. *S. labiodiscus* Sher, 1974

2. Cuticle near vulva irregular ….. 3

- Cuticle near vulva normal …… 4

3. Longitudinal striae present on anterior end (pharyngeal region); spicules 23-27 µm ….. *B. brevilineatus* (Williams, 1960) Jairajpuri, 1982

- Longitudinal striae absent; spicules 21-23 µm ….. *B. goffarti* (Sturhan, 1966) Jairajpuri, 1982

4. Lip region continuous …… *B. mediocris* Talavera & Siddiqi, 1995

- Lip region offset ….. 5

5. Lateral field not areolated; lip region with 6-7 transverse annuli; spermatheca with spheroid sperm ….. *B. vulgaris* (Upadhyay, Swamp & Sethi, 1972) Jairajpuri, 1982

- Lateral field areolated; lip region with 7-9 transverse annuli; spermatheca lacking sperm ….. *B. singularis* Siddiqi & Sharma, 1994

6. Lip region offset ….. 7

- Lip region continuous ….. 9

7. Lip region with 5-7 transverse annuli; epiptygma present; intestinal fasciculi absent; tail sub-cylindrical, abruptly narrowing near terminus ….. ***B. parvulus* n. sp.**

- Lip region with 6-10 transverse annuli; epiptygma absent; intestinal fasciculi present; tail sub-cylindrical to cylindrical, with normal hemispherical terminus ….. 8

8. c' = 2.2-3.7; spermatheca filled with sperm, males common…… *B. dubius* (Bütschli, 1873) Filipjev, 1934

- c' = 3.1-4.4; spermatheca empty, males absent ….. *B. tobari* (Sauer & Annells, 1981) Siddiqi, 1986

9. Gubernaculum with crest, distinctly curved proximally ….. *T. iranensis* Azimi, Mahdikhani-Moghadam, Rouhani & Rajabi Memari, 2016

- Gubernaculum lacking crest, simple ….. *B. parvus* (Allen, 1955) Jairajpuri, 1982 (three other species, *B. teeni* (Hashim, 1984) Siddiqi, 1986, *B. huesingi* (Paetzold, 1958) Jairajpuri, 1982 and *B. hispaniensis* Handoo, Palomares-Rius, Cantalapiedra-Navarrete, Liébanas, Subbotin & Castillo, 2014 are very similar)

10. Tail annuli less than 30; c' below 2 ….. *B. kidwaii* (Rashid & Heyns, 1990) Siddiqi, 2000

- Tail annuli more than 30; c' more than 2 ….. 11

11. Post-rectal sac covers one-third of tail length at maximum ….. *B. serranus* Gomez-Barcina, Siddiqi & Castillo, 1992

- Post-rectal sac covers almost the total tail length ….. 12

12. Stylet knobs anteriorly directed; c' = 2.7; Stylet knobs anteriorly directed ….. *B. canalis* (Thorne & Malek, 1968) Jairajpuri, 1982

- Stylet knobs posteriorly directed; c' = 3-4; Stylet knobs posteriorly directed ….. *B. natalensis* (Kleynhans, 1984) Siddiqi, 1986

13. Labial region distinctly offset; average of stylet length 25 µm; vulva with epiptygma ….. *S. pamiricus* (Ivanova, 1989) n. comb.

- Labial region slightly offset; average of stylet length below 25 µm; vulva without epiptygma ….. 14

14. Tail terminus annuli finer than other tail annuli ….. *S. maximus* (Allen, 1955) Siddiqi, 2000

- Tail terminus annuli as wide as other tail annuli ….. *S. ibericus* (Mahajan & Nombela, 1987) Siddiqi, 2000

Descriptions and observations on Iranian populations

A total of 81 individuals including 62 female and 19 male specimens belonging to seven species were used for morphological analyses. Description of the new species and some species are given and observations are made on other species.

Bitylenchus parvulus **n. sp.**

ZooBank (zoobank.org) identifier: urn:lsid:zoobank.org:pub: 9089459F-7E88-4039-80EB-F0B65B600CFC.

(Figs 1, 2 & 3)

Measurements

The morphometrics of *B. parvulus* n. sp. are present in Table 1.

Description

Female. Medium sized, ventrally arcuate to J-shaped after heat fixation. Lateral field with four incisures, outer bands areolated, 29 (21-37)% of the corresponding body diameter. Cuticle annuli delicate, 1.0 (0.5-1.5) µm wide at mid-body. Cuticle just anterior to vulva with irregular undulations (wrinkling) at its ventral side. Labial framework slightly sclerotized; lip region slightly offset from body contour by a distinct depression, with five to seven annuli, 3.6 (3.0-4.0) µm high and 7.4 (6.5-8.5) µm wide. Amphidial apertures indistinct. Stylet well-developed, conus 9.1 (8.5-9.5) µm, about one half of stylet length, stylet knobs rounded, slightly sloping backward, 3.7 (3.0-4.5) µm in width. Dorsal pharyngeal gland orifice (DGO) 2.8 (2.0-3.5) µm from stylet knobs. Procorpus cylindrical, as long as isthmus, median bulb well developed, ovate, with distinct valve, 17.5 (15.0-19.5) µm long and 11.6 (10.0-13.5) µm wide, occupying 48 (57-66)% of the corresponding body diameter. Nerve ring at anterior half of isthmus, 91 (80-101) µm from anterior end. Excretory pore position varies from posterior end of isthmus to anterior end of basal bulb. Hemizonid 5-6 annuli anterior to excretory pore, 109 (96-119) µm from anterior end. Basal bulb pocket-shaped, offset from intestine, 27 (22-34) µm in length and 13.5 (10.0-15.5) µm in width. Cardia small. Intestine generally without distinct fasciculi. Post-rectal sac occupies almost entire tail cavity. Reproductive system didelphic-amphidelphic, gonads straight, oocytes arranged in single row, spermatheca non-offset, usually without sperm, uterus long, vagina perpendicular to the body axis, 8.7 (7.5-10.5) µm long, occupying 24 (22-26) % of the vulval body width. Vulva near mid-body, as a transverse slit, with small epiptygma. Phasmids at 42 (31-50)% of tail length. Tail sub-cylindrical, often dorsally convex, usually narrowing abruptly near terminus giving a bluntly digitate appearance to its striated terminus.

Male. Body ventrally curved to J-shaped. Cuticle annuli 0.8 (0.5-1.0) µm at mid-body. Lateral field occupying 29 (26-37)% of body width, not areolated. Labial framework not sclerotised. Labial region slightly offset from body with five to six annuli, 6.8 (6.5-7.5) µm wide and 3.5 (3.0-4.5) µm high. Stylet knobs well developed, slightly sloping backward, 3.4 (3.0-3.5) µm in width. Median bulb oval, 10.0 (9.0-11.0) µm × 14.5 (13.5-16.5) µm. Basal bulb pyriform, 11.4 (10.5-12.0) µm × 21.5 (18.0-26.5) µm. Testis single, 430 (417-460) µm long, occupying 65.5 (63.0-68.0)% of total body length. Spicules curved ventrally, with velum and pointed tip. Gubernaculum simple, crescent-shaped, about one-third of spicules length. Bursa elongated, 58 (51-62) µm long. Tail conical, ending to a pointed terminus.

Type locality

The new species was recovered from the rhizosphere of milk vetch (*Astragalus* sp.) in Sendan mountain, Zanjan province, northwestern Iran (GPS coordinates: 49º07'13"N, 36º42'41"E).

Type material

Holotype, 16 paratype females and 6 paratype males were deposited in the nematode collection of the Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran; and two paratype females and one paratype male at the USDA Nematode Collection (*Bitylenchus parvulus* T-7480p).

Etymology

The species epithet refers to the proximity of the new species with the other known species, *B. parvus*.

Diagnosis and relationships

The new species is characterized by lip region with five to seven annuli, stylet 17.7 (17.0-18.5) µm long, sub-cylindrical tail with digitate terminus, cuticle just anterior to vulva wrinkled, and post-rectal sac occupied whole of tail cavity.

Regarding general characters, lip region annuli, tail shape and post-rectal sac, *Bitylenchus parvulus* n. sp. is similar to a group of some closely related species namely: *B. dubius*; *B. tobari* (Sauer & Annells, 1981) Siddiqi, 1986; *B. hispaniensis*; *B. huesingi* (Paetzold, 1958) Jairajpuri, 1982; *B. parvus*; *B. serranus* Gomez-Barcina, Siddiqi & Castillo, 1992 and *B. teeni* (Hashim, 1984) Siddiqi, 1986. The new species differs from *B. dubius* and *B. tobari* in the number of lip region annuli (5-7 *vs* 6-9 and 8-10, respectively), epiptygma (present *vs* absent), fasciculi (absent *vs* present), unique shape of tail terminus (suddenly narrowing near terminus giving a bluntly digitate shape to tail terminus *vs* normal hemispherical tail terminus), and cuticle anterior to the vulva wrinkled (*vs* usually normal). It can be further distinguished from *B. tobari* by difference in c' ratio (2.8 (2.3-3.3) *vs* 3.8 (3.1-4.4)) and reproduction behavior (spermatheca filled with sperm and males are common *vs* spermatheca empty and males absent).

From *B. parvus*, *B. serranus* and *B. teeni*, It differs by having a distinctly offset lip region (*vs* continuous or slightly offset) and female tail shape (digitate *vs* not digitate). It can be further distinguished from *B. hispaniensis* and *B. parvus* by presence a distinct epiptygma (*vs* absence), and absence of intestinal fasciculi (*vs* presence), from *B. huesingi* by stylet knobs shape (sloping backward *vs* rounded), from *B. serranus* by slightly shorter stylet (17.7 (17.0-18.5) *vs* 19.0-22.0 μm), slightly posterior position of vulva (V = 56.0 (54.5-60.0) *vs* 49-54 %) and slightly shorter spicules (23.2 (21.5-25.0) *vs* 26-31 μm), and from *B. teeni* by absence of intestinal fasciculi (*vs* presence).

Iranian population of *Bitylenchus parvus* (Allen, 1955) Jairajpuri, 1982

(Fig. 6)

Measurements

The morphometrics of *B. parv*us are presented in Table 2.

This population was found around the rhizosphere of rice (*Oryza sativa* L.) in Dezful, Khuzestan province, southwestern Iran (GPS coordinates: 32º27'87"N, 48º46'11"E). The morphological and morphometric characters of the Iranian population of *B. parvus* well fit with other populations recorded in literature (Allen, 1955; Geraert, 2011; Ghaderi *et al.*, 2014), except that tail terminus is usually smooth.

Iranian population of *Sauertylenchus maximus* (Allen, 1955) Siddiqi, 2000

(Figs. 4 & 5)

Measurements

The morphometrics of *Sauertylenchus maximus* are presented in Table 3.

Description

Female. Body ventrally curved to C-shaped, about 1 mm in length. Lateral field with four incisures, about one-fourth to one-third or 30.0 (25.5-35.5)% of body diameter, with areolation at entire body. Body annuli deep, 2.2 (2.0-2.5) μm wide at pharynx, 1.7 (1.5-2.0) μm wide at mid-body. Lip region high, non-offset or offset by a slight depression, with 6-8 distinct annuli, 8.4 (8.0-9.0) µm wide and 4.3 (4.0-5.0) high. Stylet slender, conus 11.5 (11.0-12.0) µm, occupying 53 (51-56)% of total stylet length, knobs sloping backward, 2.9 (2.5-3.0) µm wide. DGO 2.2 (1.5-3.0) µm posterior to stylet knobs. Procorpus cylindrical, about as long as isthmus. Median bulb masculine with distinct refractive valve, 14 (13-15) µm wide and 21.0 (19.0-23.5) µm long, occupying 66 (60-70)% of body diameter. Nerve ring at middle of isthmus, 118 (111-125) µm from anterior end. Excretory pore at level with anterior end of basal bulb. Hemizonid 3-8 annuli anterior to excretory pore, or 133 (127-143) µm from anterior end. Basal bulb pyriform and offset, 14.7 (13.5-16.0) µm wide and 30 (28-36) µm long. Cardia small. Post-rectal sac occupies entire tail cavity. Ovaries paired, vulva a transverse slit, without epiptygma, spermatheca indistinct, uterus short, vagina perpendicular to the body axis, 12 (9-16) µm or 47 (35-57)% of vulval body diameter. Phasmids at 43 (37-52) % of tail length. Tail cylindrical, curved ventrally ending to a rounded striated terminus.

Male. Not found.

Locality

This population was recovered from the rhizosphere of milk vetch (*Astragalus* sp.) in Sendan mountain, Zanjan Province, northwestern Iran (GPS coordinates: 36º42'31"N, 49º07'23"E).

Remarks

Sauertylenchus maximus is very similar to *Bitylenchus canalis* (Thorne & Malek, 1968) Jairajpuri, 1982; *B. serranus* and *S. ibericus* (Mahajan & Nombela, 1987) Siddiqi, 2000. It differs from *B. canalis* by lip region morphology (rounded *vs* truncated), stylet knobs shape (sloping posteriorly *vs* anteriorly) and the number of tail annuli (25-52 *vs* 66). It differs from *B. serranus* by annuli characters in tail tip (as wide as other tail annuli *vs* wider), and post-rectal sac length (occupied almost whole of tail cavity *vs* short post-rectal sac, 10-19 µm long). The present population fits well with *S. maximus* but differs by annuli on tail terminus as same as other tail annuli (*vs* finer than other tail annuli). Mahajan & Nombela (1987) differentiated *S. ibericus* from *S. maximus* in having an offset lip region and lacking areolation in the caudal region. However, lip region is illustrated as slightly offset similar to that observed for *S. maximus*. Therefore, areolation of the lateral field on tail region and the width of tail terminus annuli remain as the only diagnostic characters between *S. maximus* and *S. ibericus*. The present population shows areolation of the lateral field on tail region (similar to *S. maximus*) but tail terminus annuli are generally as wide as other tail annuli (similar to *S. ibericus*). As the present population of *S. maximus* has an offset labial disc distinctly separable from lip region annuli, and the first cephalic annuli divided into six sectors, we prefer to follow Siddiqi (2000) for maintaining this species in *Sauertylenchus*, not in *Bitylenchus*.

Iranian population of *Tylenchorhynchus agri* Ferris, 1963

(Figs. 7 & 8)

Measurements

The morphometrics of *Tylenchorhynchus agri* are presented in Table 4.

Description

Female. Body slightly ventrally curved to C-shaped after heat fixation. Lateral field occupies 30 (28-33)% of the corresponding body diameter. Body annuli delicate, 2.2 (2.0-2.5) µm wide at pharynx, and 1.7 (1.5-2.0) µm at mid-body. Labial region slightly offset, bearing four to five annuli, 7.5 (7.4-7.7) µm wide and 3.4 (3.5-4.0) µm high. Stylet conus 8.8 (8.5-9.0) µm, 53 (50-56)% of total stylet length, knobs rounded, 3.9 (3.5-4.5) µm wide. DGO 2.4 (2.0-2.7) µm posterior to stylet knobs. Procorpus cylindrical, about as along as isthmus. Median bulb ovate with distinct valve, 11 (10-12) µm wide, 16.5 (16-17) µm long, occupying 61 (55-70) % of the corresponding body diameter. Isthmus slender. Basal bulb pyriform to slightly elongate, offset from intestine, 12 (11-14) µm wide and 32 (29-36) µm long. Cardia hemispherical. Nerve ring at anterior half of isthmus, at 85 (83-89) µm from anterior end. Excretory pore at anterior part of basal bulb. Hemizonid one to two annuli anterior to excretory pore, at 99 (96-106) µm from anterior end. A small post-rectal sac occupying 30 (26-36)% of tail cavity. Ovaries paired and straight, spermatheca rounded and non-offset, without sperm. Uterus short, vagina perpendicular to the body axis, 7.5 (7.0-8.0) µm, occupying 38 (34-43)% of the vulval body width, vulva with small epiptygma. Phasmids located at 37 (23-30) % of tail length. Tail sub-cylindrical ending to a smooth terminus.

Male. Not found.

Locality

This population was recovered from the rhizosphere of desert poplar (*Populus euphratica* Oliv.) in Dezful region, Khuzestan province, southwestern Iran (GPS coordinates: 32º11'36"N, 48º42'67"E).

Remarks

Tylenchorhynchus agri is similar to *T. alami* Shaw & Khan, 1996; *T. claytoni* Steiner, 1937; *T. crassicaudatus* Williams, 1960; *T. ewingi* Hopper, 1959; *T. haki* Fotedar & Mahajan, 1971 and *T. mexicanus* Knobloch & Laughlin, 1973. It is different from *T. alami* by lip region morphology (slightly offset *vs* continuous), tail shape and length (sub-cylindrical, 32-39 µm long *vs* conoid, 48 µm long), spicules length (21-25 *vs* 17-18 µm), and post-rectal sac (filling 30% *vs* less than 20% of tail length). From *T. claytoni*, it can be distinguished by longitudinal striae (absent *vs* present) and post-rectal sac (present *vs* absent). It differs from *T. crassicaudatus* by tail length (32-39 *vs* 38-63 µm) and post-rectal sac (present *vs* absent), from *T. ewingi* by annuli of lip region (4-5 *vs* 3 annuli), spicules (21-25 *vs* 18 µm) and stylet knobs shape (with concave anterior surfaces *vs* rounded), from *T. haki* by slightly longer stylet (17-23 *vs* 16-18 µm), tail characters (sub-cylindrical, 32-39 µm long *vs* conoid, 27 µm long), and from *T. mexicanus* by lip region characters (slightly offset *vs* distinctly offset), tail shape (sub-cylindrical *vs* conoid) and post-rectal sac (present *vs* absent).

Iranian population of *Tylenchorhynchus graciliformis* Siddiqi & Siddiqui, 1983

(Figs. 9 & 10)

Measurements

The morphometrics of *Tylenchorhynchus graciliformis* are presented in Table 4.

Description

Female. Body ventrally curved to C-shape. Lateral field occupied one fourth to one third of body width, outer bands crenate, 32 (23-39)% of body width in diameter. Body annuli delicate, 1.9 (1.0-2.3) µm at pharynx, and 1.7 (1.5-1.9) µm at mid-body. Lip region slightly offset bearing usually five, rarely six annuli, 6.8 (6.5-7.5) µm wide, and 3.2 (3.0-4.0) µm high. Stylet delicate, conus 8.7 (8.0-9.5) µm, 52 (50-54)% of total stylet length, knobs laterally directed, 4.1 (3.5-4.5) µm wide. DGO 2.2 (1.5-3.0) µm posterior to stylet knobs. Procorpus cylindrical, about as along as isthmus. Median bulb ovate with distinct valve, 10.7 (10.0-11.0) µm wide, 16.8 (15.5-18.0) long, occupying 64 (59-69)% of body width in diameter. Isthmus slender. Basal bulb elongate-saccate, offset from intestine to slightly overlapping anterior end of intestine, 12.3 (11.0-14.0) µm wide, 31 (27-38) µm long. Cardia hemispherical. Nerve ring at middle of isthmus, 86 (76-93) µm from anterior end. Excretory pore at anterior part of basal bulb. Hemizonid one to three annuli anterior to excretory pore, 104 (97-109) µm from anterior end. Deirid indistinct. Post-rectal sac absent. Reproductive system didelphic-amphidelphic, gonads straight, spermatheca rounded and non-offset, filled with spheroid sperm, 2-3 µm in diameter. Uterus short, vagina perpendicular to body axis, 9.0 (7.5-12.0) µm, occupying 44 (35-53)% of vulval body diameter. Vulva prominent equipped with small epiptygma. Phasmids located at 32 (23-40)% of tail. Tail sub-cylindrical bearing 22 (18-26) cuticular annuli on its ventral side, ending to a smooth terminus.

Male. General characters similar to those of female. Body ventrally curved to J-shaped. Body annuli 1.6 (1.5-2.0) µm wide at mid-body. Lateral field with four incisures, 29 (23-36)% of body width. Lip region bearing four to five annuli, 6.1 (6.0-6.5) µm wide and 2.9 (2.5-3.0) µm high. Stylet comparable with that of female, conus 51 (50-52)% of total stylet length; knobs sloping backward. DGO 1.9 (1.5-2.0) µm posterior to stylet knobs. Median bulb 10.0 (9.5-11.0) µm wide and 13.5 (12.0-15.0) µm long. Basal bulb elongate-saccate, slightly shorter than female, 11.4 (10.0-13.0) µm wide and 28 (25-30) µm in length. Nerve ring located at 87 (80-93) µm from anterior end. Deirids indistinct. Spicules tylenchoid. Gubernaculum with crest. Bursa extending to end of tail, 61 (53-67) µm in length.

Locality

The present population of T. graciliformis was recovered from the rhizosphere of tamarisc (*Tamarix* sp.) in Dezful region, Khuzestan province, southwestern Iran (GPS coordinates: 32º16'66"N, 48º47'56"E).

Remarks

All morphological and morphometric characteristics of the present population fit well with those of the original population of *Tylenchorhynchus graciliformis* differing only in the lip region demarcation; in the present population, lip region is generally slightly offset from the rest body (drawn and illustrated as distinctly offset in the original description). *T. graciliformis* is similar to *T. aduncus* de Guiran, 1967; *T. brassicae* Siddiqi, 1961; *T. clarus* Allen, 1955; *T. contractus* Loof, 1964; *T. aerolatus* Tobar Jiménez, 1970, *T. striatus* Allen, 1955 and *T. ismaili* Azmi & Ahmad, 1991. It differs from *T. aduncus* by tail shape (sub-cylindrical *vs* conical), shape of stylet knobs (laterally directed *vs* posteriorly directed), spermatheca shape (spherical *vs* ovoid), spicules length (19-21 *vs* 22-25 µm) and shape of gubernaculum (with a simple crest *vs* characteristic hammer-shaped). It can be distinguished from *T. aerolatus* by absence of areolation in lateral field (*vs* having regular areolation), the shape of gubernaculum (with simple crest *vs* hammer-shaped), and body length (678-780 *vs* 540-700 µm). It differs from *T. brassicae* by annuli of lip region (5-6 *vs* 4 annuli), stylet length (17-19 *vs* 16-17 µm) and tail characters (sub-cylindrical, 36-51 µm *vs* conical, 23-40 µm in length). The present population can be differentiated from *T. clarus* by lip region morphology (offset *vs* non offset), number of tail annuli (16-20 *vs* 7-20 annuli), presence of males and spermatheca filled with sperm (*vs* absence of males and empty spermatheca), from *T. contractus* and *T. striatus* by lip region characters (offset *vs* non offset), and from *T. ismaili* by body size (678-780 *vs* 460-660 µm) and tail terminus striation (smooth *vs* striated).

Molecular characterization of Tylenchorhynchus, Bitylenchus and Sauertylenchus species from Iran

The amplification of D2-D3 expansion segments of 28S, and partial 18S rRNA genes yielded single fragments of 669 and 1695 bp, respectively after discarding primer sequences. D2-D3 for *B. parvulus* n. sp. (MK473884) differed from the closest related species, *B. parvus* (KJ585431) by 14 different nucleotides and 0 indels (98% similarity), from *B. hispaniensis* (KJ461548; KJ461544; KJ461547 and MG770479) by 13-15 different nucleotides and 1 indels (98% similarity); from *B. huesingi* (KX789756) by18 different nucleotides and 1 indel (97% similarity); and from *B. dubius* (DQ328707 and EU368590) by 20 different nucleotides and 0 indel (97% similarity). Two sequences for *B. parvulus* n. sp. (MT232322-MT232323) differing in 3 nucleotides (99,8% similar) were obtained for the 18S rRNA marker. These sequences closely matched with several species, such as *B. parvus* (KX789742) and *B. teeni* (KJ636394) by 19, 21 different nucleotides and 1, 3 indels (99% similarity), respectively. D2-D3 from Iranian population of *B. parvus* (MT193835) was identical to *B. parvus* (KJ585431) from Fars, Iran. D2-D3 from the Iranian population of *S. maximus* (MK473883) closely matched with several populations of *S. maximus* (KX789749, KX789755 and KX789748) differing by 1-2 bp and 0 indel (99% similarity). Finally, D2-D3 from Iranian populations of *T. agri* and *T. graciliformis* (MT193837 and MT193836) showed that these isolates had the highest similarity (100% and 97.2%) with *T. agri* and *T. zeae*, respectively.

Phylogenetic relationships of studied species

Phylogenetic relationships within *Bitylenchus*, *Sauertylenchus*, *Tylenchorhynchus* and representatives of other genera of the subfamily Telotylenchinae from the analysis of the partial 28S rRNA and 18S rRNA gene sequences are given in Figures 11 and 12, respectively. The phylogenetic analysis of the 28S rRNA separated the genus *Bitylenchus* and *Sauertylenchus* in different clades, at exception of *B. brevilineatus* (Williams, 1960) Jairajpuri, 1982 (KJ461533). The D2-D3 sequences of *B. parvulus* n. sp. clustered well (PP = 99) with two sequences of *B. dubius* (DQ328707 and EU368590). *B. parvulus* n. sp. formed a well-supported clade (PP = 100) with *B. parvus* (KJ585431 and MT193835), *B. huesingi* (KX789756) and four sequences of *B. hispaniensis* (KJ461548; KJ461544; KJ461547 and MG770479). Our isolate of *S. maximus* (MK473883) formed a well-supported clade (PP = 99) with populations of *S. maximus* (KX789749, KX789755 and KX789748) deposited in GenBank. Iranian isolate of *B. parvus* (MT193835) clustered well (PP = 100) with *B. parvus* (KJ585431). The Iranian isolate of *T. agri* (MT193837) formed a clade (PP = 99) with three isolates of *T. agri* (KX622690, MG491667 and KJ475549). The Iranian isolate of *T. graciliformis* (MT193837) formed a clade (PP = 100) with four isolates of *T. zeae* Sethi & Swarup, 1968 (MN757911, KJ461563, KJ461565and KM068058).

The 18S rRNA phylogeny showed that *B. parvulus* n. sp. (MT226922 and MT226923) formed a moderately supported clade (PP = 84) with *B. parvus* (KX789742) and a high supported clade (PP = 100) with other species of the genus *Bitylenchus,* as *B. dubius* (EU306352, AY284601 and EU306352), *B. teeni* (KJ636395 and KJ636394), *B. huesingi* (KX789739) and *B. serranus* (KX789743), .

Discussion

In the present study, we provided a key for identification of the all known species treated in the three closely related genera *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus*. A new species, *B. parvulus* n. sp., was described and illustrated during this study, morphological and additional molecular data were obtained for some previously described species (*T. agri*, *B. parvus* and *S. maximus*), and new molecular and morphological data were presented for *T. graciliformis*.

Some morphological (Gomez-Barcina *et al.*, 1992; Siddiqi, 2000) and molecular data (Handoo *et al.*, 2014; Ghaderi *et al.*, 2014; present study) support that *Bitylenchus* and *Sauertylenchus* are separable genera from *Tylenchorhynchus*. However, the validity of the genus *Sauertylenchus* still needs to be tested with more studies, particularly by inclusion of the type species of this genus (Handoo *et al.*, 2014).

Although *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus* are treated as the three separated valid genera in literature, there is no adequate information on the important diagnostic characters such as lateral field areolation, lip region structure, post-rectal sac presence, or the shape of gubernaculum on many of described species in literature. Moreover, there is no agreement on the taxonomic position of certain species; for instance, *S. maximus* in Siddiqi (2000) has been considered as *B. maximus* in Andrássy (2007) or *T. maximus* in Geraert (2011). Which is why, the identification of species in *Tylenchorhynchus*, *Bitylenchus* and *Sauertylenchus* remains as a challenging task and certain species from one genus may come close to certain species from the other. Considering these problems in identification of species, all known species of these three genera are treated in a single identification key here.

The following studies have tried to provide identification keys or diagnostic compendiums for identification of species in the genus *Tylenchorhynchus*: Ganguly *et al.*, 2013 (compendium to 158 species), Geraert, 2011 (key for 133 species including *Bitylenchus* species), Andrássy, 2007 (key for 16 European species), Handoo, 2000 (key and compendium for 111 species), Brzeski & Dolinski, 1998 (compendium for 177 species of *Tylenchorhynchus sensu lato*), Brzeski, 1998 (key for 9 European species and compendium for 160 species of *Tylenchorhynchus sensu lato*), Hooper, 1978 (key and compendium for 55 species), Tarjan, 1973 (key and compendium for 46 species), Tarjan, 1964 (key and diagnostic compendium for 88 species of *Tylenchorhynchus sensu lato*), Thorne & Malek, 1968 (key for 9 species), Allen, 1955 (key for 34 species of *Tylenchorhynchus sensu lato*). For the genus *Bitylenchus*, only two keys are provided separately: Jairajpuri (1982) and Andrássy (2007).

According to D2-D3 of 28S, and partial 18S rRNA genes phylogeny, we accept the hypothesis of *Bitylenchus, Sauertylenchus* and *Tylenchorhynchus* as valid genera and reject the 'large-genus idea' advocated by Fortuner & Luc (1987). Similar results were obtained in some recently published molecular phylogenies (Handoo *et al.*, 2014, Ghaderi *et al.*, 2014; Azimi *et* *al.*, 2016). Diagnosis and identification of *Bitylenchus, Sauertylenchus* and *Tylenchorhynchus* species based solely on morphometric features is quite problematic because there is a continuous range in values of morphological-morphometric data within populations of the same species, as well as amongst species (Handoo *et al.*, 2014). As a result, it is important to include molecular markers in the description of topotypes from already described and new species, as it has been suggested by other authors (Gutiérrez-Gutiérrez *et al.*, 2010, Zamora Araya *et al.*, 2016) and the probability of cryptic speciation in plant-parasitic nematodes (Palomares-Rius *et al.*, 2014; Gharakhani *et al.*, 2019).

Bitylenchus parvulus n. sp. is molecularly closely related to *B. dubius* and *B. parvus*, and in both phylogenetic trees (D2-D3 expansion segments of 28S rRNA and partial 18S rRNA) formed a monophyletic position as valid species inside the genus *Bitylenchus*. This species moreover could be separated from these closely related species in regard to morphological and morphometric characteristics. The validity of *Bitylenchus* as a separated genus from *Tylenchorhynchus* has been already discussed in Handoo *et al.* (2014) and the addition of more representatives of this genus, as *B. parvulus* n. sp., reinforce this idea..

Morphological characters of the Iranian isolate of *T. agri* (MT193837) were similar to type species, and in D2-D3 tree formed a clade with three isolates of *T. agri* (KX622690, MG491667 and KJ475549). *Tylenchorhynchus agri* formed a sister clade with *T. annulatus* in the 28S rRNA tree. Additonally, these species could be separated morphologically because *T. annulatus* (MH142612) differs by the number of lip region annuli and absence of post-rectal sac from *T. agri*. Our isolate of *T. graciliformis* formed a clade with *T. zeae*, which could be also separated morphologically by slightly shorter stylet length and the number of tail annuli. .

Tylenchorhynchus iranensis (KU248449 and KU248450) was transferred to the genus *Bitylenchus* by Ghaderi & Karegar (2018). The authors noticed that this species is not different from *B. parvus*, *B. teeni* and *B. huesingi*, but according to the shape of gubernaculum, having a distinct crest in the relevant description (Azimi *et al.,* 2016) and its phylogenetic position within *Tylenchorhynchus* species in the 28S rRNA tree, it could be considered as a valid species in the genus *Tylenchorhynchus* and not in *Bitylenchus*.

Sauertylenchus ibericus is very close to *S. maximus* and differs only by tail terminus annuli (as wide as *vs* smaller than other tail annuli). *Sauertylenchus maximus* (MK473883) isolate from Iran has the annuli of tail terminus as wide as the other tail annuli (seldom smaller than tail annuli). This sequence is identical to and formed a clade with *S. maximus*. As no stable diagnostic character can be observed and sequences of *S. maximus* and *S. ibericus* are identical, synonymy of these two species is proposed here, being *S. maximus* the valid species and *S. ibericus* a junior synonym. However, the study of topotypes of both species may clarify the exact taxonomic status of them.

Conclusions

1. In this paper, we provided an identification key to all known species treated in closely related genera, *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus*.
2. Confirmed the Siddiqi's classification for transferring *S. maximus* to the genus *Sauertylenchus* as representatives of this species formed a separate clade from *Bitylenchus* species.
3. Described a new species namely *B. parvulus* n. sp. and characterized four known species (*B. parvus, S. maximus, T. agri* and *T. graciliformis*) by morphological and molecular characters, two of them (*T. agri* and *T. graciliformis*) were new records from Iran.
4. Proposed the synonymization of *S. ibericus* with *S. maximus*.

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Conflict of interest. The authors declare no conflicts of interest.

Ethical standards. No specific permits were required for the indicated fieldwork studies. The soil samples were obtained in public areas, forests, and other natural areas and do not involve any species endangered or protected in Iran, nor are the sites protected in any way.

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Figure legends

Fig. 1. *Bitylenchus parvulus* n. sp. Female (A, C, E, F, H-K) and male (B, D, G, L). A, B: Entire body; C: Anterior end; D, E: Anterior end and pharyngeal region; F: reproductive system; G: Spicules and Gubernaculum; H-L: Posterior end.

Fig. 2. *Bitylenchus parvulus* n. sp. Female (A, B, D, E, F, I-K) and male (C, G-H). A: Anterior body and pharyngeal region; B-C: Entire body; D: Anterior end; E: Lateral field; F: Pharyngeal basal bulb; G-H, K: Posterior end; I-J: Vulva region. (Scale-bars: B, C = 100 μm, A, D-K = 10 μm).

Fig. 3. *Bitylenchus parvulus* n. sp. Female (A-N). A-E: Anterior end; F-H: Vulva region; I-N: Posterior end. (Scale-bars: A-N = 10 μm).

Fig. 4. *Sauertylenchus maximus*. Female (A-I). A: Entire body; B-C: Anterior end; D: Reproductive system; E: Anterior end and pharyngeal region; F-H: Posterior end; I: Lateral field.

Fig. 5. *Sauertylenchus maximus*. Female (A-L) and male (B-C). A: Anterior body and pharyngeal region; B-C: Anterior end; D: Entire body; E: Vulva region; F: Lateral field; G-L: Posterior end. (Scale-bars: A-G = 10 μm).

Fig. 6. *Bitylenchus parvus*. Female (A, D-G) and male (B-C). A-B: Anterior end; C-G: Posterior end. (Scale-bars: A-G = 10 μm).

Fig. 7. *Tylenchorhynchus agri*. Female (A-F). A: Entire body; B: Anterior end and pharyngeal region; C: Anterior end; D-E: Posterior end; F: Reproductive system.

Fig. 8. *Tylenchorhynchus agri*. Female (A-G). A: Entire body; B: Anterior end and pharyngeal region; C: Anterior end; D: Pharyngeal basal bulb; E-G: Posterior end. (Scale-bars: A = 100 μm, B-G = 10 μm).

Fig. 9. *Tylenchorhynchus graciliformis*. Female (A, C-E, F-H) and male (B, D, I-J). A-B: Entire body; C-D: Anterior end and pharyngeal region; E: Reproductive system; F-I: Posterior end; J: Spicules and gubernaculum.

Fig. 10. *Tylenchorhynchus graciliformis*. Female (A, D, E-J) and male (B-C, H). A-B: Entire body; C-D: Anterior end; E: Vulva region; F-H: Posterior end. (Scale-bars: A-B = 100 μm, C-H = 10 μm).

Fig. 11. Phylogenetic relationships among *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus* species with representatives of other Telotylenchinae 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). Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).

Fig. 12. Phylogenetic relationships among *Bitylenchus*, *Sauertylenchus* and *Tylenchorhynchus* species with representatives of other Telotylenchinae genera. Bayesian 50 % majority rule consensus tree as inferred from the 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). Newly obtained sequences in this study are shown in bold. (Scale bar = expected changes per site).

Table 1. Morphometric characters of *Bitylenchus parvulus* n. sp. recovered from Iran. All measurements are in μm, and in the form: mean ± s.d. (range) and coefficient of variation.

	Holotype	Paratypes			
		Females		Males	
n	-	18	CV	7	CV
L	816	697 ± 77.6 (542-834)	11.1	633 ± 32.9 (582-672)	5.2
H-V	448	390 ± 40.0 (323-455)	10.2	-	-
H-a	761	649 ± 73.9 (498-775)	11.3	593 ± 29.6 (545-627)	4.9
Stylet	18.5	17.7 ± 0.4 (17.0-18.5)	2.7	16.7 ± 0.5 (16-18)	3.3
m	52.0	51.4 ± 1.7 (49-56)	3.4	53 ± 1.1 (52-55)	2.1
b	5.3	5.0 ± 0.5 (4.2-5.8)	9.9	4.7 ± 0.1 (4.4-4.9)	3.1
a	32.5	30.7 ± 3.0 (26-38)	9.9	34 ± 1.8 (32-38)	5.4
c	14.8	14.6 ± 1.0 (12-17)	6.8	15.7 ± 1.5 (13.1-17.7)	9.7
c'	2.9	2.8 ± 0.2 (2.3-3.3)	8.8	2.2 ± 0.2 (1.9-2.5)	9.0
V	54.9	56.0 ± 1.3 (54.5-60.0)	2.4	-	
Median bulb	76.0	66.9 ± 5.3 (58-77)	7.9	63.2 ± 2.8 (60-69)	4.4
MB	49.7	48.8 ± 2.5 (45-57)	5.1	48.3 ± 2.0 (46-51)	4.2
Excretory pore	121	107 ± 8.7 (93-126)	8.2	102 ± 3.0 (97-107)	3.0
Pharynx	153	137 ± 9.3 (123-155)	6.8	131 ± 5.9 (123-140)	4.5
Body width	25.0	22.6 ± 1.5 (20-25)	6.9	18.5 ± 0.6 (17.7-19.5)	3.5
Lateral field	7.8	6.6 ± 1.0 (5-8)	16.4	5.4 ± 0.8 (4.5-7.0)	15.8
Vulva body width	25.0	23.1 ± 1.8 (19-26)	7.9	-	
Anal body width	19.0	16.5 ± 2.1 (13-20)	12.8	17.6 ± 0.9 (16.5-19.5)	5.5
Tail length	55.0	47.5 ± 4.5 (42-59)	9.5	40.5 ± 5.4 (34.8-50.0)	13.4
Phasmid	22.0	19.8 ± 3.3 (13.5-26)	16.9	17.9 ± 3.6 (13-23)	20.3
Tail annuli	48.0	46 ± 2.9 (42-52)	6.3	-	-
Spicules	-	-	-	23.2 ± 1.4 (21.5-25.0)	6.2
Gubernaculum	-	-	-	10.2 ± 1.1 (9-12)	10.8
Bursa length	-	-	-	58 ± 7.1 (52-73)	12.3

Table 2. Morphometric characters of *Bitylenchus parvus* recovered from Iran. All measurements are in μm, and in the form: mean ± s.d. (range).

	Present study		Geraert, 2011	Ghaderi *et al.*, 2014
	Females	Males	Female/Male	Female/Male
n	7	5	?	26
L	758 ± 60.3 (644-834)	622 ± 32.8 (582-672)	650-910	745 (630-863)
H-V	419 ± 31.2 (358-455)	-	-	-
H-a	707 ± 55.7 (600-775)	583 ± 29.2 (545-626)	-	-
Stylet	18.2 ± 0.2 (18.0-18.7)	16.6 ± 0.4 (16-17)	16.5-19.5	17.6 (16.5-19)
b	5.2 ± 0.3 (4.7-5.8)	4.7 ± 0.1 (4.4-4.9)		6.0 (5.1-7.3)
a	32.6 ± 3.7 (26-38)	34 ± 2.1 (32-38)	25-31	31.3 (28-34.9)
c	14.9 ± 0.6 (14-16)	16 ± 1.1 (14.6-17.7)	12-17	14.6 (12-17)
c'	2.9 ± 0.1 (2.5-3.0)	2.2 ± 0.2 (1.9-2.5)	2.3-3.3	2.9 (2.4-3.4)
V	55.3 ± 0.8 (54.5-57.0)	-	50-57	53 (50-56)
Median bulb	72 ± 2.6 (68-77)	62.1 ± 1.6 (60-64)	-	-
MB	50.4 ± 3.2 (46-57)	47.4 ± 1.6 (46-49)	-	-
Excretory pore	115 ± 7.8 (101-126)	101 ± 2.7 (97-103)	-	-
Pharynx	144 ± 6.4 (135-155)	131 ± 5.1 (128-140)	125-148	-
Body width	23 ± 1.8 (20-25)	18.5 ± 0.5 (18-19)	23-33	-
Lateral field	7.7 ± 0.2 (7.5-8.0)	5.0 ± 0.3 (4.5-5.5)	-	-
Anal body width	17.5 ± 1.9 (15-20)	17 ± 0.6 (16-18)	-	-
Tail length	51 ± 5.0 (44-59)	39 ± 4.2 (35-46)	43-63	33-48
Phasmid	21.8 ± 2.3 (19-26)	17 ± 3.4 (13-21)	-	-
Tail annuli	48 ± 2.5 (45-52)	-	42-58	45-50
Spicules	-	23.5 ± 0.8 (23-25)	28-31	23.9 (22-26)
Gubernaculum	-	9.5 ± 0.6 (9.0-10.5)	13-13.5	-
Bursa length	-	56 ± 3.0 (52-60)	-	-

Table 3. Morphometric characters of *Sauertylenchus ibericus* recovered from Iran and its comparison with original description, as well as with populations of *S. maximus*. All measurements are in *µ*m, and in the form: mean ± s.d. (range) and coefficient of variation.

	S. ibericus			*S. maximus*	
	Present study Females		Mahajan & Nombela, 1987 Females	Geraert, 2011 Females	Panahandeh & Pourjam, 2014 Females
n	13	CV	10	?	10
L	1108 ± 55.5 (1012-1191)	5.	1130 (990-1270)	940-1620	1329 (1120-1348)
H-V	582 ± 34.8 (496-620)	5.9	-	-	-
H-a	1056 ± 53.2 (966-1137)	5.	-	-	-
Stylet	21.8 ± 0.8 (20-23)	3.6	24.1 (21-26)	20-24.5	-
a	42 ± 2.1 (39-46)	5.1	43 (40-48)	28-58	41.6 (36.2-46.5)
b	6.1 ± 0.3 (5.5-6.6)	5.2	6.4 (5.6-7.1)	-	6.7 (6.0-7.3)
c	21.3 ± 1.3 (19.0-23.1)	6.2	21.8 (18.4-25.1)	16-26	20.4 (18.7-23.5)
c'	2.7 ± 0.1 (2.5-3.0)	5.6	2.7 (2.3-3.3)	1.9-4.1	2.9 (2.3-4.0)
V	52.5 ± 2.3 (46-58)	4.5	53.1 (51-56)	47-58	52 (50-54)
Median bulb	96± 3.3 (90-100)	3.5	-	-	-
MB	53.5 ± 1.6 (51-57)	2.9	57.6 (53-61)	51-58	-
Excretory pore	137.1 ± 5.1 (130-148)	3.7	143 (111-176)	-	-
Pharynx	179 ± 5.1 (170-189)	2.8	-	153-192	-
Body width	26.5 ± 1.5 (24-29)	5.7	-	23-32	-
Lateral field	8.0 ± 0.8 (6.8-10.0)	10.7	-	8-11	-
Vulva body width	25.7 ± 1.5 (24-28)	6.	-	-	-
Anal body width	18.8 ± 1.3 (17-21)	7.1	-	-	-
Tail length	52 ± 4.0 (46-59)	7.7	-	42-83	-
Phasmid	22.5 ± 3.3 (19-28)	14.7	-	19-30	-
Tail annuli	32± 1.8 (28-35)	5.9	32-36	25-52	39-47

Table 4. Morphometric characters of *Tylenchorhynchus graciliformis* and *T. agri* recovered from Iran. All measurements are in μm, and in the form: mean ± s.d. (range) and coefficient of variation.

	T. graciliformis				*T. agri*	
	females	CV	males	CV	females	CV
n	16		7		8	
L	722 ± 33.5 (678-779)	4.6	703 ± 17.4 (682-729)	2.4	659 ± 31.3 (615-709)	4.7
H-V	405 ± 24.2 (374-471)	5.9	-		358 ± 14.0 (338-381)	3.9
H-a	680 ± 31.0 (636-728)	4.5	660 ± 17.9 (636-686)	2.7	608 ± 29.1 (567-654)	4.7
Stylet	16.8 ± 0.8 (15.5-18.5)	4.9	16.9 ± 0.3 (16.5-17.5)	2.3	17.6 ± 0.4 (17-18)	2.6
m	52 ± 1.2 (50-54)	2.4	51.6 ± 0.9 (50-53)	1.7	50.0 ± 0.2 (50.0-50.5)	0.5
a	37 ± 2.9 (32-45)	8.0	36 ± 1.3 (34-39)	3.7	31 ± 1.6 (29-34)	5.3
b	5.3 ± 0.4 (4.6-6.1)	7.7	5.2 ± 0.1 (5.0-5.5)	2.7	4.6 ± 0.2 (4.2-4.9)	4.8
c	17.3 ± 1.1 (15.2-19.1)	6.4	16.3 ± 0.7 (14.8-17.1)	4.8	12.9 ± 0.2 (12.5-13.3)	2.1
c'	3.1 ± 0.3 (2.6-3.8)	11.2	2.6 ± 0.1 (2.5-2.8)	4.6	3.6 ± 0.1 (3.4-3.8)	3.6
V	56 ± 2.4 (53-64)	4.2	-		54 ± 1.0 (53-56)	1.9
Median bulb	60 ± 2.8 (56-65)	4.7	61 ± 2.7 (57-64)	4.5	63 ± 2.1 (61-67)	3.3
MB	44 ± 1.6 (41-48)	3.6	46 ± 1.5 (44-48)	3.3	44 ± 1.2 (42-46)	2.7
Excretory pore	104 ± 3.9 (97-109)	3.8	106 ± 6.1 (102-120)	5.8	103 ± 3.3 (99-109)	3.2
Pharynx	136 ± 6.1 (125-145)	4.5	133 ± 3.7 (127-139)	2.8	143 ± 5.8 (134-150)	4.0
Body width	19.5 ± 1.4 (16-22)	7.3	19 ± 0.7 (18-20)	3.8	21 ± 0.9 (19.5-22.0)	4.7
Lateral field	6.0 ± 0.9 (5.0-7.5)	14.9	5.6 ± 0.7 (4.5-7.0)	12.7	6.4 ± 0.3 (6-7)	5.7
Vulva body width	20.0 ± 1.4 (17.5-22.5)	7.3	-		19.5 ± 1.2 (17.5-21.0)	6.2
Anal body width	13.5 ± 1.4 (11-16)	10.6	16 ± 0.4 (15-16.5)	2.8	14 ± 0.8 (13-15)	5.9
Tail length	42 ± 3.7 (36-51)	8.8	43 ± 1.5 (41-46)	3.7	51 ± 2.4 (48-55)	4.8
Phasmid	13.5 ± 2.5 (9-18)	19.1	15 ± 1.6 (13.5-18.0)	10.6	19 ± 1.7 (17-22)	9.1
Tail annuli	22.5 ± 2.2 (19-26)	10.1	-		26.4 ± 2.5 (23-30)	9.4
Spicules	-		19.7 ± 0.6 (19-21)	3.5	-	
Gubernaculum	-		10.3 ± 0.6 (9.5-11.0)	6.6	-	
Bursa length	-		61.4 ± 4.7 (53-67)	7.7	-	
Post-rectal sac	-		-		15.3 ± 2.3 (13-20)	15.5
PAS/Tail	-		-		30.1 ± 3.3 (26.5-36.0)	11.2