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On the synonymy of *Trophotylenchulus asoensis* and *T. okamotoi* with T. *arenarius*, and intra-generic structure of *Paratylenchus* (Nematoda: Tylenchulidae)

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Abstract

Two populations of the genus *Trophotylenchulus* and 10 species of the genus *Paratylenchus* from Iran were characterized based on morphometric, morphological and molecular characters. Our observations on the two populations of *Trophotylenchulus* from Iran revealed that *T. asoensis* and *T. okamotoi* have been distinguished from *T. arenarius*, on the basis of the features which cannot be longer considered as stable diagnostic characters. One of the populations shows a mixed combination of the characters of *T. arenarius* and *T. asoensis*; it has morphometrics more similar to *T. arenarius* but shows affinities with *T. asoensis* in the tail terminus shape of females and second-stage juveniles (J2) and in having a reduced stylet in males. The other population fit well with *T. okamotoi*; it has females with generally bluntly rounded tails typical for *T. okamotoi*, but sometimes with finely rounded tail termini, like those of *T. arenarius* or *T. asoensis*. The sequences of D2–D3 expansion segments of 28S rRNA gene for the two populations are identical with each other, but only 4 bp (0.67%) difference with *T. arenarius* sequence deposited in the GenBank. Considering no stable difference allow separating species, synonymy of *T. asoensis* and *T. okamotoi* with *T. arenarius*, which has already been proposed, is supported and confirmed here. All studied *Paratylenchus* species with stylets longer than 40 µm, except *P. straeleni*, formed a basal cluster to *Cacopaurus pestis* and species of *Paratylenchus* bearing stylets shorter than 40 µm; thus, validity of *Gracilacus* cannot be rejected using our data sets. However, the synonymy of *Paratylenchoides* was supported by the positioning of *P. sheri* within representatives of *Paratylenchus* in the inferred phylogenetic tree.

Keywords

Gracilacus, Identification, Phylogeny, *Paratylenchus,* Taxonomy, *Trophotylenchulus,* Tylenchulidae, 28S rRNA.

Raski (1956) described a nematode species as *Sphaeronema arenarium* with females being most commonly individual ectoparasites who do not form colonies, as can be seen in the endoparasitic species, *S. californicum* (Raski and Sher, 1952). After observations of new specimens, Raski (1957) concluded that *S. arenarium* should be placed in a different genus than *S. californicum*. He established Tylenchulidae as a new family, and further proposed two new genera: *Trophonema* with *T. arenarium* (Raski, 1956, 1957) and *Trophotylenchulus* with *T. floridensis* (Raski, 1957) as type species. Posteriorly, the same author explained that *Trophotylenchulus* differs from *Trophonema* in the modification of lip region and the more posterior position of the excretory pore. These two genera were distinguished by two characters: a circumoral elevation is present in female heads of *Trophotylenchulus* but absent in *Trophonema*; and all stages of *Trophotylenchulus*

are encapsulated in round, brittle structure but various stages of *Trophonema* are covered with gelatinous matrix (Raski, 1991). However, Siddiqi (1999) considered these characters to be variable and, therefore, synonymized *Trophonema* with *Trophotylenchulus*.

The genus *Trophotylenchulus sensu lato* has currently 14 valid species (Ghaderi et al., 2016). From these, 11 species belong to *Trophotylenchulus sensu stricto* and 3 species (*T. arenarium*, *T. asoense* and *T. okamotoi*) to the genus *Trophonema*. Minagawa (1983) described *T. okamotoi* based on bluntly rounded tail end in females and differentiated *T. asoensis* by differences in the length of female tail, male gubernaculum and juvenile body size. Gomez-Barcina and Castillo (1990) synonymized *Trophonema asoensis* and *T. okamotoi* with *Trophonema arenarium* because they believed that most diagnostic features used for separating these species are minor quantitative differences, and tail terminus shape of females and juveniles is also a variable intraspecific character. However, these nematodes were considered as separate valid species in the subsequent taxonomic works (Raski, 1991; Inserra et al., 1993; Brzeski, 1998; Siddiqi, 2000; Andrássy, 2007; Ghaderi et al., 2016).

Current taxonomic views on the systematics of the genera included in the family Tylenchulidae have been reviewed recently (Mokaram Hesar et al., 2019); the authors concluded that the genera *Cacopaurus* (Thorne, 1943), *Paratylenchus sensu stricto* and *Gracilacus* (Raski, 1962) in the subfamily Paratylenchinae (Thorne, 1949) are clustered in one clade in the inferred trees of 28S rRNA and ITS gene sequences.

The present study provides morphological and molecular support on the synonymy of *T. asoensis* (Minagawa, 1983; Siddiqi, 1999) and *T. okamotoi* (Minagawa, 1983; Siddiqi, 1999) with *T. arenarius* (Raski, 1956; Siddiqi, 1999) based on the observations made on two populations from Iran. Moreover, morphological and molecular characterization of 10 species of *Paratylenchus* (Micoletzky, 1922) was used to obtain a better insight into the intra-generic structure of this genus.

Materials and methods

Nematode populations and morphological characterization

The populations of *Trophotylenchulus* and *Paratylenchus* were recovered from the rhizosphere of different plants in different localities of the three Iranian provinces: Khuzestan, Kohgiluyeh and Boyer-Ahmad, and Chaharmahal and Bakhtiari (Table 1). The nematodes were extracted from the soil and plant samples, using the rapid centrifugal-flotation method (Jenkins, 1964), killed and fixed by hot FPG (4:1:1, formaldehyde: propionic acid: glycerol), and then processed to anhydrous glycerol (De Grisse, 1969), and finally mounted in glycerol on permanent slides using paraffin wax. Morphometric and morphological characters of the nematode populations were studied by a light microscope, equipped with a Dino-eye microscope eyepiece camera in conjunction with its Dino Capture version 2.0 software. The specimens were identified using available identification keys (Ghaderi et al., 2016).

DNA extraction

For molecular analysis, a single female nematode of each of the populations was transferred into a drop of distilled water on a microscopic slide and examined under the light microscope. Each specimen was washed three times in deionized water and then transferred into an Eppendorf tube with 25µl distilled water. Posteriorly, 25µl lysis buffer (23.75µl NaCl 0.2M and Tris-HCL 0.2M, 1.0µl β-mercaptoethanol and 0.25µl proteinase K) was added to each Eppendorf tube. Nematode specimen was crushed with a microhomogeniser for 2min. The tubes were incubated at 65°C (1hr) and then at 95°C (15min) (Tanha Maafi et al., 2003).

PCR amplification

DNA amplification was carried out based on the protocols described by Tanha Maafi et al. (2003). The D2–D3 expansion regions of the 28S rRNA gene were amplified with the forward D2A (5′–ACAAGTACCGTGAGGGAAAGTTG–3′) and the reverse D3B (5′–TCGGAAGGAACCAGCTACTA–3′) primers (Nunn, 1992). The PCR was performed in a final volume of 30µl containing: 3µl DNA template, 1µl of each PCR primer, 15µl PCR mastermix (Amplicon, Denmark) and 10µl deionized water. The following PCR program was used: denaturation 94°C for 5min; 35 cycles × (denaturation 94°C, 30sec; anneling 54°C, 30sec; extension 72°C, 70sec) and a final extension 72°C for 5min.

The PCR products were purified using the QIAquick Gel Extraction Kit (Takapozist, Iran) according to the manufacturer's instruction and used for direct sequencing. The PCR products were sequenced in both directions at BioNeer Inc. (Korea).

Phylogenetic analysis

The obtained sequences (Table 1) were compared with sequences of other taxa in GenBank with a BlastN homology search and then the closest

Table 1. Species and populations of *Paratylenchus* and *Trophotylenchulus*, used in the present study.

Species	Locality	Associated plant	GenBank accession No.
Paratylenchus bukowinensis (Micoletzky, 1922)	Boldaji, Chaharmahal and Bakhtiari	Alfalfa (*Medicago sativa* L.)	MN088372
P. coronatus (Colbran, 1965)	Dehdasht, Kohgiloyeh and Boyer-Ahmad	Alfalfa (*Medicago sativa* L.)	–
P. goodeyi (Oostenbrink, 1953)	Sureshjan, Chahrmahal and Bakhtiari	Rough bluegrass (*Poa trivialis* L.)	–
P. nawadus (Khan et al., 1967)	Behbahan, Khuzestan	Gundelia (*Gundelia tournefortii* L.)	MN088373
P. paraperaticus (Kashi et al., 2009)	Andimeshk, Khuzestan	Howthorn (*Crataegus aronia* L.)	–
P. projectus Jenkins, 1956	Dezful, Khuzestan	Jujube (*Ziziphus spina-christi* L.)	–
P. sheri (Raski, 1973; Siddiqi, 1986)	Dezful, Khuzestan	Ziziphus (*Ziziphus nummularia* (Burm. F.) Wight et Arn.)	MN088374
P. similis (Khan et al., 1967)	Andimeshk, Khuzestan	Howthorn (*Crataegus aronia* L.)	MN088375
P. teres (Raski, 1976; Siddiqi, 1986)	Dehdez, Khuzestan	Mount Atlas mastic or Baneh (*Pistacia atlantica* Desf.)	MN088376
P. variabilis (Raski, 1975a, b)	Basht, Kohgiloyeh and Boyer-Ahmad	Unknown citrus	–
Trophotylenchulus arenarius (Raski, 1956; Siddiqi, 1999)	Baghmalek, Khuzestan	Weeping willow (*Salix babylonica* L.)	MK733978
T. arenarius	Dehdasht, Kohgiloyeh and Boyer-Ahmad	Unknown grasses in a canebrake	MK733979

sequences were selected for phylogenetic analyses. The sequences of D2–D3 segments of 28S rRNA gene were aligned with ClustalX 1.83 (Thompson et al., 1997), using default parameter values and were manually edited if necessary. Phylogenetic analyses of the sequence datasets were based on Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and 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 +G) for the D2-D3 segments of 28S rRNA gene were run with four chains for 2×10^6 generations, respectively. The Markov chains were sampled at intervals of 100 generations. Two runs were conducted for 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) were given for appropriate clades. Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance valuesbased on whole alignment, with adjustment for missing data with PAUP* 4.0b 10 (Swofford, 2003). Trees were visualized using TreeView (Page, 1996).

Results

Systematics

An amended description of *Trophotylenchus arenarius sensu lato* is given based on the two populations from southwestern Iran. The population with bluntly rounded tail was recovered from the rhizosphere and roots of weeping willow (*Salix babylonica* L.) in Baghmalek, Khuzestan province, and the other

population with narrow tail was collected from soil and plant material of a canebrake in Dehdasht, Kohgiluyeh and Boyer-Ahmad province. All morphological and morphometric characters of the first population fit well with those of the original description of *T. okamotoi* (Minagawa, 1983). The second population comes closest to *T. asoensis* particularly in the tail terminus shape of females and J2s and in having a reduced stylet in males, but it has morphometric data more similar to *T. arenarius*. In fact, attributing this population to each of the mentioned species cannot be done with certainty. Considering morphological and molecular results, we consider both populations as *T. arenarius* (see Discussion).

Moreover, four new records of *Paratylenchus* for Iranian nematofauna are described and illustrated here. Morphological and morphometric characters of these four species (Figs. 5–10; Tables 3 and 4) along with morphometric data of six other *Paratylenchus* species (Tables 4 and 5) from Iran are also given. Phylogenetic relationships of *Trophotylenchulus* populations and five *Paratylenchus* species within other representatives of Tylenchulidae are inferred (Fig. 11).

Trophotylenchulus arenarius (Raski, 1956; Siddiqi, 1999).

(Figs. 1–4; Table 2).

Description

Female

The body is obese particularly in the region between pharynx and vulva, regularly tapering towards both ends, strongly ventrally curved. Cuticular annuli are 0.9 to 1.4 µm wide at mid-body, lateral field is indistinct. The cephalic region is hemispherical, lacking perioral disc and transverse annuli, continuous with the body contour; cephalic framework is slightly sclerotized.

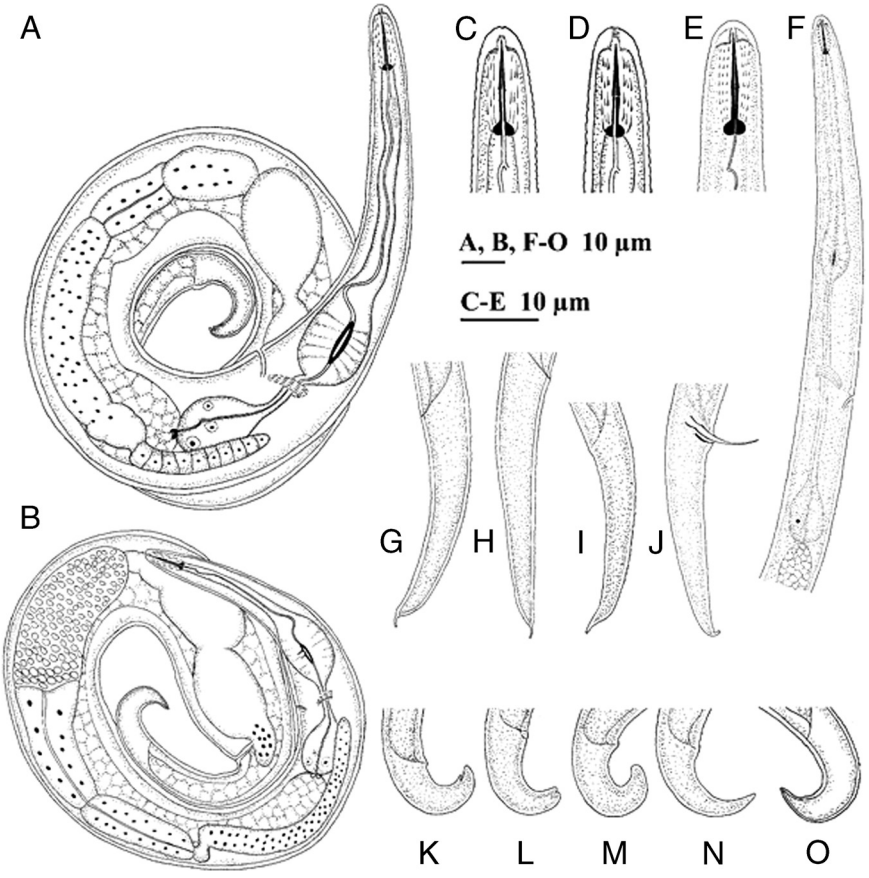

Figure 1: *Trophotylenchulus arenarius* from Iran: Dehdasht population with narrow tail terminus in females (B, E–H, J, O) and Baghmalek population with blunt tail terminus in females (A, C, D, I, K–N). Female (A, B, K–O), male (F, J) and second-stage juvenile (C–E, G–I). A, B: entire body; C–E: head and stylet; F: anterior end and pharyngeal region; G–O: posterior end.

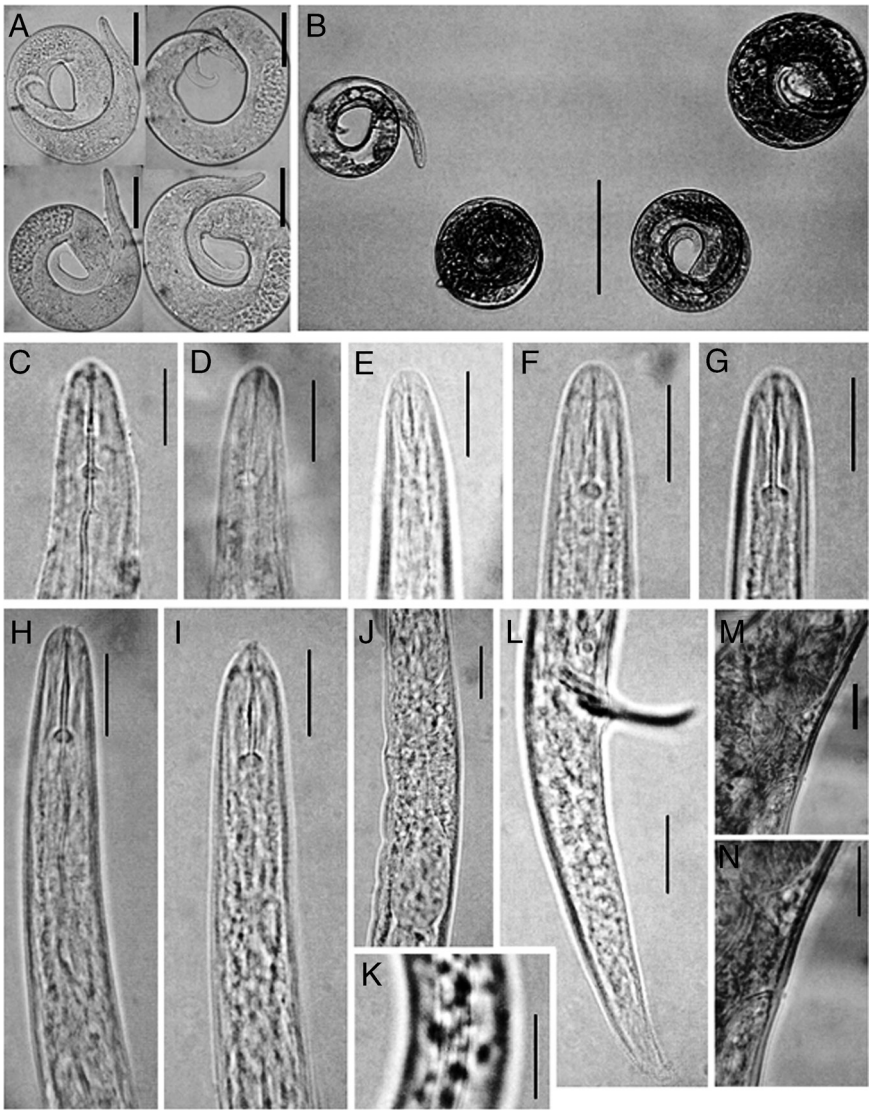

Figure 2: *Trophotylenchulus arenarius* from Iran: Dehdasht population (A, C, E, F, H, J–L) and Baghmalek population (B, D, G, I, M, N). Female (A–D, M, N), male (E, L) and second-stage juvenile (F–K). A, B: entire body; C–G: head and stylet; H–J: anterior end and pharyngeal region; K: lateral field at mid-body; L: posterior end; M, N: pharyngeal median bulb and excretory pore (scale bars: A = 50 µm; B = 100 µm; C–N = 10 µm).

Stylet is strong, conus is nearly as long as shaft, but sometimes slightly shorter or longer; basal knobs are round, slightly sloping backward, about 3 µm across. DGO is situated at 3.5 to 5.0 µm posterior the stylet knobs. Procorpus amalgamated with median bulb which has elongate-oval distinct valvular apparatus; median bulb is 14 to 23 µm in width; basal bulb is pyriform. Isthmus is short and narrow; nerve ring is located at anterior end of isthmus. Excretory pore is located at 72 to 104 µm from the anterior end (18–25% of body length) at the end of isthmus; duct with wide lumen leads backward to a large renette cell. The reproductive system is monodelphic-prodelphic. Ovary is well developed, outstretched without flexures reaching the basal pharyngeal posterior bulb. Spermatheca is round, globular to slightly oval sperm cells; short post-vulval is present. Vulva is a transverse slit flush with body, without cuticular flaps, at 89 to 114 µm anterior to tail terminus. Vagina is perpendicular with body or directed anteriorly. Anus is distinct with raised lips. Tail is arch-shaped, curved ventrally with a finely rounded terminus (Dehdasht population) and a bluntly rounded, indented or finely rounded terminus (Baghmalek population).

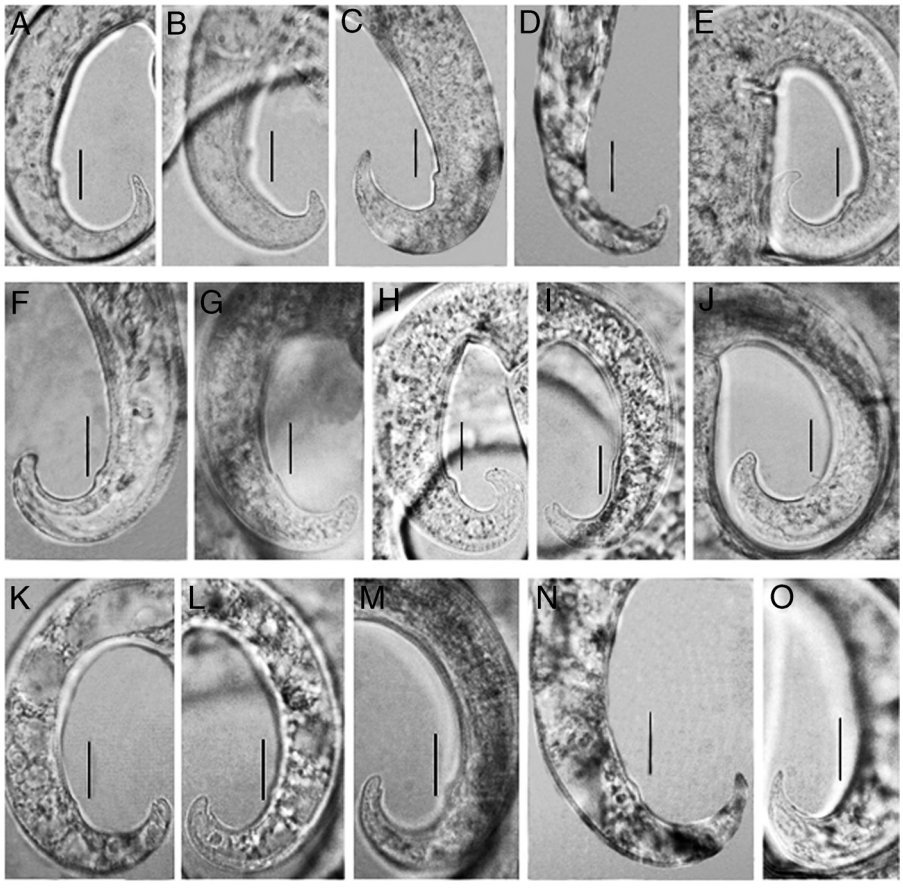

Figure 3: Posterior end of females of *Trophotylenchulus arenarius* from Iran: Dehdasht population (A–E) and Baghmalek population (F–O) (scale bars = 10 µm).

Male

Only one male was found in Dehdasht population. Body is vermiform, open C-shaped habitus after fixation. Lateral field is indistinct, when viewed under light microscope. Cuticular annuli are 1.4 µm apart at mid-body. The cephalic region is round, lacking perioral disc and transverse annuli. Stylet and pharynx are reduced; median and basal pharyngeal bulbs are not distinct, having single testis, anteriorly outstretched. Spicules are slightly arcuate ventrally, protruded. Gubernaculum is short and simple, trough-shaped in lateral view. Tail is elongated conical, with a terminal projection.

Second-stage juvenile (J2)

Body is slender, ventrally curved to C-shaped habitus after fixation. Lateral field is usually indistinct, with four longitudinal lines. The cephalic region is conical, smooth, and continuous with the body contour. Stylet is 2.1 to 2.6 head widths long; conus is slightly longer than shaft; basal knobs are round. Dorsal pharyngeal orifice is 3.7 to 4.2 µm behind the stylet knobs. Pharynx is normally developed, median bulb is oval, posterior bulb is saccate to pyriform. Excretory pore is located at 75 to 82 µm from anterior end. Tail is ventrally arcuate, regularly tapering, terminus mostly with a slender acute projection in both populations, the projection is slightly shorter in Baghmalek specimens.

Paratylenchus goodeyi (Oostenbrink, 1953).
(Figs. 5A–E and 6; Table 3).

Description

Female

Mature females are slightly stout, body tapers posterior to vulva, body ventrally curved or open to close C-shaped after fixation. Cuticle is fine but distinct transverse annules, 1.1 to 1.5 µm wide in mid-body. Lateral field is with four lines, with equal intervals.

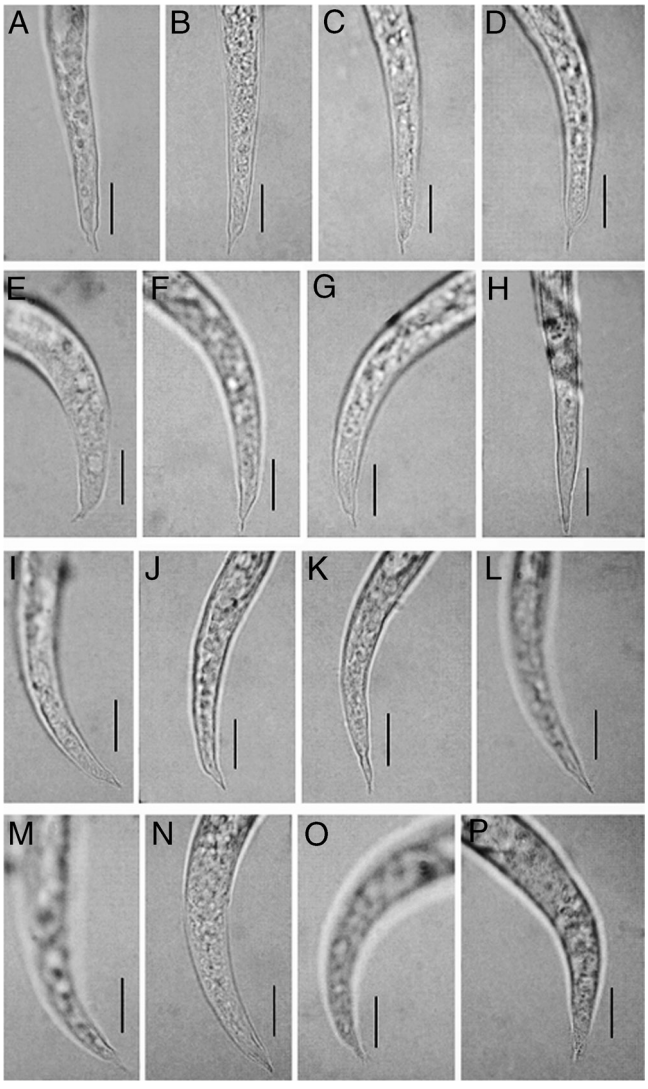

Figure 4: Posterior end of second-stage juveniles of *Trophotylenchulus arenarius* from Iran. Dehdasht population (A–H) and Baghmalek population (I–P) (scale bars = 10 µm).

Cephalic region is continuous to the body contour, almost rounded with delicate transverse striations, and submedian lobes on head are not visible in lateral view. Stylet is long and slender, slightly curved, and basal knobs are rounded and 2.2 to 2.9 µm in width. Deirids are distinct, opposite to excretory pore in some individuals; the pore is located at the level of pharyngeal posterior bulb. Vulva flush with body, with small cuticular flaps usually lower than lips. Reproductive system is monodelphic-prodelphic and ovary outstretched. Spermatheca is oval, filled with globular sperm. Post-vulval uterine sac is not present. Tail is convex-conoid, often ventrally curved, with acute to finely rounded terminus. Phasmids are not observed.

Male

Not found.

Remarks: morphometric and morphological characteristics of the Iranian population fit well with those of the original description (Oostenbrink, 1953) and other populations of *P. goodeyi* reported worldwide (Szczygiel, 1974; Castillo et al., 1989; Brzeski, 1995, 1998). This species may be compared with *P. robustus* (Wu, 1974), *P. crenatus* (Corbett, 1966), *P. ivorensis* (Luc and de Guiran, 1962) and *P. straeleni* (De Coninck, 1931; Oostenbrink, 1960). *Paratylenchus robustus* has aspermagonium instead of spermatheca with sperm, and a longer stylet (58–73 vs 47–51 µm). In *P. crenatus*,

Table 2. Morphometric characters of the two populations of *Trophotylenchulus arenarius* from Iran.

Character\ population	Dehdasht population			Baghmalek population		Total	
n	10 females	5 J2	1 male	10 females	5 J2	20 females	10 J2
L	416 ± 36.6 (356–476)	313 ± 9.8 (301–326)	500	478 ± 42.5 (382–544)	346 ± 21.8 (324–380)	447 ± 50.1 (356–544)	330 ± 23.7 (301–380)
a	11.6 ± 0.7 (10.2–12.6)	26.9 ± 1.9 (24.5–29.6)	37.3	11.1 ± 0.8 (9.8–12.4)	26.5 ± 1.5 (24.5–28.5)	11.3 ± 0.8 (9.8–12.6)	26.7 ± 1.6 (24.5–29.6)
b	3.6 ± 0.3 (3.1–4.2)	2.9 ± 0.1 (2.7–3.2)	5.1	3.7 ± 0.1 (3.3–4)	3.1 ± 0.1 (2.9–3.3)	3.6 ± 0.2 (3.1–4.2)	3 ± 0.2 (2.7–3.3)
c	12.7 ± 1.1 (11.1–15.1)	8.7 ± 0.4 (8.2–9.3)	9.4	12.7 ± 1.3 (11.1–15.1)	9.9 ± 0.8 (8.5–10.6)	12.7 ± 1.2 (11.1–15.1)	9.3 ± 0.9 (8.2–10.6)
c'	2.7 ± 0.2 (2.4–3.1)	4.8 ± 0.2 (4.5–5)	4.1	2.9 ± 0.3 (2.5–3.6)	4.2 ± 0.3 (3.6–4.7)	2.8 ± 0.3 (2.4–3.6)	4.5 ± 0.4 (3.6–5)
V	75.9 ± 2.2 (73.2–78.5)	–	–	77.3 ± 1.5 (74.5–79.9)	–	76.6 ± 1.9 (73.2–79.9)	–
Stylet	13.5 ± 0.5 (12.3–14.5)	12.5 ± 0.3 (12–13)	9.6	13.5 ± 0.7 (12.4–14.3)	13.2 ± 0.6 (12.5–13.8)	13.5 ± 0.6 (12.3–14.5)	12.9 ± 0.5 (12–13.8)
Conus	6.7 ± 0.4 (6–7.5)	6.2 ± 0.2 (6–6.6)	–	7.1 ± 0.8 (6–8.3)	6.9 ± 0.3 (6.5–7.3)	6.9 ± 0.6 (6–8.3)	6.5 ± 0.4 (6–7.3)
m (conus/stylet %)	49.8 ± 2.2 (46.6–52.9)	49.4 ± 0.9 (48.4–50.7)	–	52.8 ± 5.6 (44.4–62.9)	52.5 ± 1.3 (51–54.4)	51.3 ± 4.4 (44.4–62.9)	50.9 ± 1.9 (48.4–54.4)
Pharynx	114 ± 12 (99–129)	108 ± 8.7 (92–113)	97	129 ± 9.2 (106–140)	109 ± 5.7 (102–117)	122 ± 12.8 (99–140)	108 ± 7 (92–117)
Median bulb	68.3 ± 5.6 (60–78)	60.4 ± 5.1 (56–69)	45	70.9 ± 3.7 (62.7–76)	55.6 ± 2.9 (52–60)	69.6 ± 4.8 (60–78)	58 ± 4.7 (52–69)
MB	59.9 ± 3.9 (55.2–66.6)	56.2 ± 4.6 (51.8–61.6)	46	55.1 ± 2.6 (51–59)	50.9 ± 0.5 (50–51.3)	57.5 ± 4.1 (51–66.6)	53.6 ± 4.2 (50–61.6)
Excretory pore	88.9 ± 9.8 (77–104)	77.2 ± 4.4 (71–83)	70	96.3 ± 9 (72.4–103)	78.2 ± 3.1 (75–82)	92.6 ± 9.9 (72.4–104)	77.7 ± 3.6 (71–83)
Head-vulva	316 ± 35.2 (261–373)	–	–	370 ± 39.4 (284–435)	–	343 ± 45.6 (261–435)	–
Head-anus	383 ± 34.9 (327–441)	277.4 ± 10 (265–289)	447	440 ± 41.7 (349–506)	312 ± 21.7 (291–344)	412 ± 47.6 (327–506)	294 ± 24 (265–344)
Tail length	32.9 ± 3.3 (28–37)	35.8 ± 1.3 (34–37)	53	37.6 ± 3.4 (32.9–43)	34.8 ± 2.7 (32–39)	35.2 ± 4.1 (28–43)	35.3 ± 2.1 (32–39)
Body width	35.8 ± 3.7 (30–41.5)	11.6 ± 0.5 (11–12.5)	13.4	43.1 ± 5.4 (35.2–55)	13 ± 1.3 (12–15.5)	39.4 ± 5.9 (30–55)	12.3 ± 1.2 (11–15.5)
Anal body width	11.8 ± 0.8 (10.5–13.5)	7.4 ± 0 (7.3–7.5)	12.8	12.9 ± 0.9 (11.5–14.5)	8.3 ± 0.5 (7.5–9)	12.3 ± 1 (10.5–14.5)	7.8 ± 0.5 (7.3–9)
Annulus width	1.2 ± 0.1 (1.0–1.4)	–	1.4	1.0 ± 0.1 (0.9–1.3)	–	1.1 ± 0.1 (0.9–1.4)	–
Spicules	–	–	21	–	–	–	–
Gubernaculum	–	–	4.5	–	–	–	–

Measurements are in µm and in the form of average ± SD (range).

the posterior edge of female annules is crenate, and stylet is longer (61–73 vs 47–51 µm). Our population differs from *P. ivorensis* by more posterior position of vulva (79–87 vs 73–77) and the shape of spermatheca (oval vs rounded) and differs from *P. straeleni* by different shapes of lip region (rounded vs truncate) and spermatheca (oval vs rounded). The present population was recovered from the rhizosphere of rough bluegrass (*Poa trivialis* L.) in Sureshjan county, Chaharmahal and Bakhtiari province, southwestern Iran.

Paratylenchus nawadus (Khan, Prasad and Mathur, 1967).

(Figs. 5F–J and 7; Table 3).

Description

Female

The body tapers suddenly posterior to vulva; the body is ventrally curved to C-shaped after fixation. Cuticle is with fine transverse annules, 1.1 to 1.5 µm wide in mid-body. Lateral field is with four lines. Cephalic region is continuous to the body contour, truncated to slightly raised at anterior end; submedian lobes are on head, appeared very small in lateral view. Stylet is medium-sized, and basal knobs are rounded to slightly directed backward, being 2.2 to 3.2 µm in width. Excretory pore is located at the level of pharyngeal posterior bulb. The posterior lip of vulva is lower than the anterior one, with distinct raised semi-circular cuticular flaps. The reproductive system is monodelphic-prodelphic, spermatheca oval, and filled with globular sperm. Post-vulval uterine sac is short. Tail is convex-conoid, often slightly ventrally curved, with finely to bluntly rounded terminus. Phasmids are not observed. Fourth-stage juvenile (J4) is similar to female in general characteriztics, but lacking stylet.

Male

Similar to female in general characteriztics, the body is almost straight to slightly ventrally curved after fixing. Cuticular annules are 1.2 to 1.4 µm apart in mid-body.

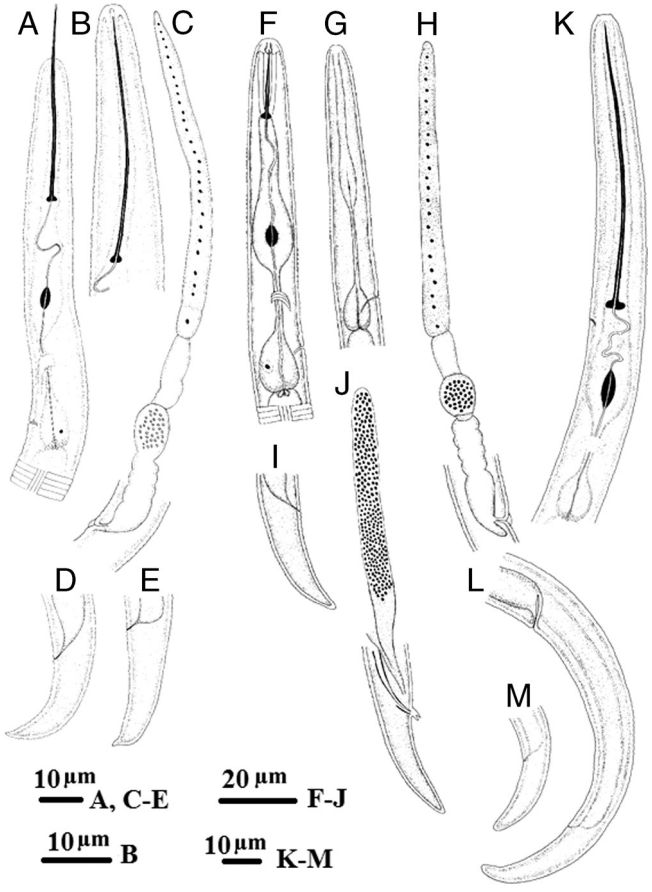

Figure 5: Diagnostic characters of *Paratylenchus goodeyi* (A–E), *P. nawadus* (F–J) and *P. teres* (K–M) from Iran. Female (A–F, H, I, K–M) and male (G, J). A, B, F, G, K: anterior end and pharyngeal region; C, H, J: reproductive system; D, E, I, L, M: posterior end.

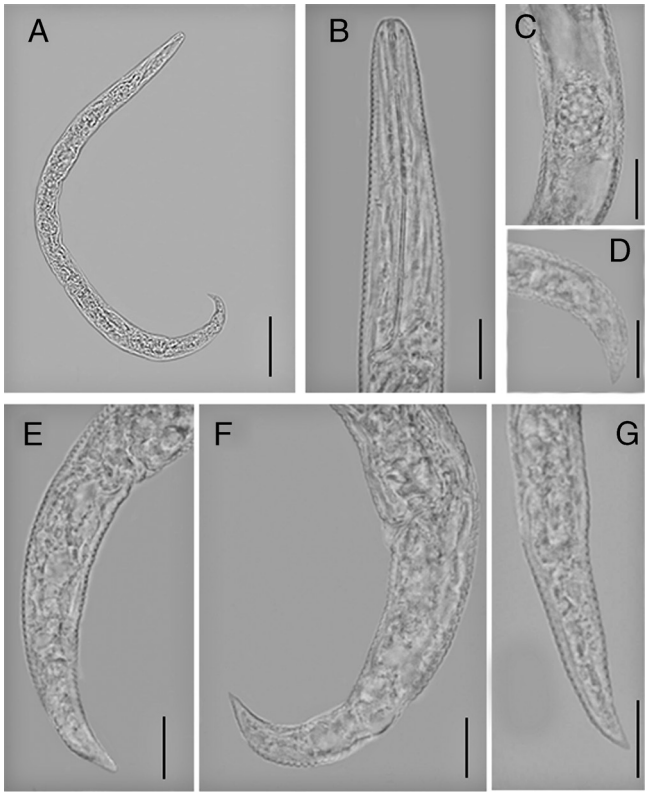

Figure 6: *Paratylenchus goodeyi* from Iran. Female (A–G). A: entire body; B: anterior end; C: part of reproductive system; D–G: posterior end (scale bars: A = 20 µm; B–G = 5 µm).

Lateral field is with four lines. Stylet is absent and pharynx degenerate. Spicules are slightly curved ventrally, protruding from the body in some individuals; gubernaculum is simple. Tail is conoid, slightly curved ventrally or dorsally, with finely rounded terminus.

Remarks: comparing with the original description of *P. nawadus* (Khan et al., 1967), the present population has a lower value for b in females (3.1–3.8 vs 4.2–5.3) and slightly larger spicules (22–25 vs 19–22 µm). *Paratylenchus nawadus* mostly comes close to *P. arculatus* (Luc and de Guiran, 1962) (= *P. nainianus*; Edward and Misra, 1963); however, our population differs from the latter by having a shorter stylet (20 (19–22) vs 24 (22–27)) vs 26 (21–32) µm in Edward and Misra (1963) and Brzeski et al. (1999), respectively), shorter distance between stylet knobs and base of median bulb valve (typically more than vs less than stylet length) and in marked reduction of body width posterior to the vulva. Our population was recovered from the rhizosphere of gundelia (*Gundelia tournefortii* L.) in Behbahan county, Khuzestan province, southwestern Iran.

Paratylenchus sheri (Raski, 1973; Siddiqi, 1986). (Figs. 8 and 9; Table 3).

Description

Female

The body tapers gradually at both ends; the body is ventrally curved to C-shaped after fixation. Cuticle is with distinct transverse annules, 1.2 to 1.5 µm wide in mid-body. Lateral field is with four equally-distant lines; in cross sections, each three bands protrude markedly. The cephalic region is truncated conical; submedian lobes protrude on head surrounding the oral aperture when observed from lateral view. Cephalic framework is with relatively strong sclerotization. Stylet is robust with well-developed basal knobs sloping backward, 4.1 to 4.6 µm in width. Excretory pore is located at level of pharyngeal posterior bulb. Vulva is a deep transverse slit, with large raised semi-circular cuticular flaps. The reproductive system is monodelphic-prodelphic, spermatheca on lateral side of the gonad, oval to elongated shape, and filled with globular sperm. Post-vulval uterine sac is absent. Tail tapers gradually and is slightly ventrally curved, with subacute, finely rounded, or bluntly rounded terminus. Phasmids are not observed.

Table 3. Morphometric characters of *Paratylenchus goodeyi*, *P. nawadus* and *P. sheri* from Iran.

Characters\ species	*P. goodeyi*	*P. nawadus*		*P. sheri*	
n	8 females	10 females	5 males	12 females	6 males
L	335 ± 38.8 (263–368)	358 ± 24.1 (330–400)	353 ± 10.1 (340–367)	424 ± 12.5 (406–446)	449 ± 24.7 (412–488)
a	20.7 ± 2.2 (15.4–22.5)	25.2 ± 2.5 (21.1–29.3)	28 ± 1.6 (26.1–30.3)	25.1 ± 1.6 (22–27)	33.2 ± 2 (30.8–36.1)
b	4.1 ± 0.7 (2.8–5.3)	3.5 ± 0.2 (3.1–3.8)	3.5 ± 0.2 (3.3–3.9)	4 ± 0.1 (3.8–4.2)	4.5 ± 0.2 (4.1–4.8)
c	12 ± 1.3 (9.3–13.2)	12.7 ± 1.6 (10.1–15.6)	11.1 ± 1 (10–12.5)	11.4 ± 0.5 (10.7–12.5)	10.5 ± 1.4 (8.5–12.8)
c'	2.9 ± 0 (2.8–3)	3 ± 0.3 (2.5–3.6)	3.1 ± 0.3 (2.5–3.6)	3.6 ± 0.1 (3.3–3.9)	3.9 ± 0.5 (3.2–4.6)
V	81.8 ± 2.4 (79.4–87.4)	83.1 ± 0.7 (81.8–84.2)	–	80.2 ± 1 (78.5–82.3)	–
Stylet	48.8 ± 1.4 (46.9–51.2)	20.5 ± 1 (18.7–22)	–	24.1 ± 0.5 (23–25)	–
m (conus/stylet %)	76.7 ± 1.2 (74.8–78.1)	65 ± 2.4 (61.1–69.9)	–	64.5 ± 1.3 (62.5–66.5)	–
Pharynx	82.5 ± 9.9 (67–93.5)	99.8 ± 6.9 (91.7–112)	100 ± 4.8 (93–105.6)	104.6 ± 4.6 (98–112)	99.1 ± 8.3 (85–109)
MB	73 ± 6.6 (65.8–80.5)	56.4 ± 1 (55.1–58)	–	56.3 ± 1 (54.7–58)	–
Excretory pore	77 ± 9 (65–89)	82.5 ± 5.1 (77–89)	74.6 ± 8.4 (67–88.7)	91.6 ± 3.2 (87–96)	86 ± 10.3 (66–95)
Head-vulva	274 ± 31.2 (209–300)	298 ± 20.3 (274.6–332)	–	340 ± 12.4 (319–360)	–
Head-anus	307 ± 38.8 (235–340)	329 ± 23 (305–369)	321 ± 9.5 (313–336)	389 ± 11.4 (372–409)	406 ± 20.7 (376–441)
Tail length	27.8 ± 0.5 (27–28.5)	28.4 ± 3.9 (22.1–34.4)	31.8 ± 3 (27–34.9)	37 ± 1.9 (33–40)	43.1 ± 7 (34–53)
Body width	16.2 ± 1.3 (13.9–17.4)	14.2 ± 1.2 (12.5–16.3)	12.5 ± 0.5 (11.8–13)	16.9 ± 0.9 (15.5–18.5)	2.8 ± 0.2 (2.5–3.2)
Annulus width	1.3 ± 0.1 (1.2–1.5)	1.3 ± 0.1 (1.1–1.5)	1.3 ± 0.1 (1.2–1.4)	1.3 ± 0.1 (1.1–1.5)	1.3 ± 0.1 (1.2–1.5)
st/L ×100	14.7 ± 1.7 (13.1–18.2)	5.7 ± 0.4 (5.1–6.4)	–	5.7 ± 0.1 (5.5–5.8)	–
Spicules	–	–	23.4 ± 1.4 (22–25.2)	–	24.8 ± 0.6 (24–25.5)
Gubernaculum	–	–	4.5 ± 0.4 (4.1–5.2)	–	4.8 ± 0.2 (4.5–5)

Measurements are in µm and in the form of average ± SD (range).

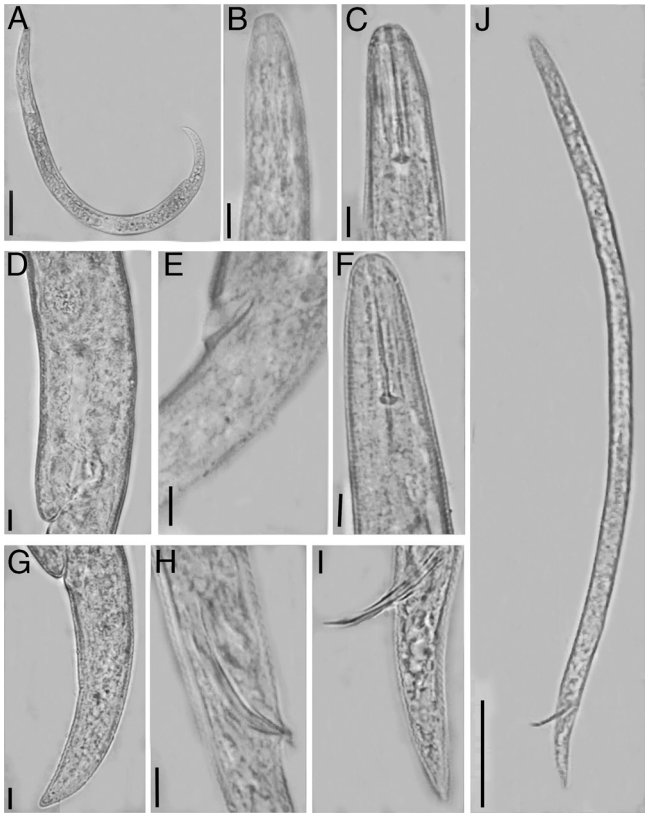

Figure 7: *Paratylenchus nawadus* from Iran. Female (A, C–G) and male (B, H–J). A, J: entire body; B, C, F: anterior end; D, E: vulval region; G, I: posterior end; H: spicules (scale bars: A = 20 µm; B–I = 5 µm, J = 50 µm).

Male

Similar to female in general characteristics, the body is almost straight to ventrally or dorsally curved after fixing. Cuticular annules are 1.2 to 1.5 µm apart in mid-body. Lateral field is with four lines. Stylet is lacking; pharynx degenerates and is less developed, only a simple outline can be distinguished. Spicules are slightly curved ventrally, gubernaculum is simple, and penial tube bears a distinct appendage on its posterior part. Tail is elongated conoid, slightly curved ventrally, with finely rounded terminus.

Remarks: Raski (1973) proposed *Paratylenchoides* for his two new species and distinguished it from *Paratylenchus* based on stronger cephalic sclerotization, cephalic region dorso-ventrally narrower, and having a small, narrow, rounded protrusion on the anterior surface of the conoid lip region. However, this genus was not considered as a valid genus in subsequent taxonomic studies (Raski and Luc, 1987; Raski, 1991; Brzeski, 1998; Siddiqi, 2000; Andrássy, 2007; Decraemer and Hunt, 2013; Ghaderi et al., 2014, 2016). Raski (1973) expressed that *P. sheri* differs from *P. israelensis* (Raski, 1973) in the shorter, less robust stylet, lesser sclerotization of the head, and in the uniform outline of the lateral field as seen in cross section. Regarding these characters, our population is more similar to *P. sheri*; however, the length of stylet of our population, 24 (23–25) µm, overlaps with *P. israelensis*, 26 (24–28) µm, from Israel (Raski, 1973), and populations of *P. sheri*, 22 (20–23) µm, 23 (21–25) µm, and 21 (18–23) µm from France (Raski, 1973), Spain (Gomez-Barcina et al., 1990) and Italy (Brzeski, 1995). The Iranian population was recovered from the rhizosphere of a desert tree, ziziphus (*Ziziphus nummularia* (Burm. F.) Wight et Arn.) from Dezful, Khuzestan province, southwestern Iran.

Paratylenchus teres (Raski, 1976; Siddiqi, 1986). (Figs. 5K–M and 10; Table 4).

Description

Female

The body is ventrally curved to open C-shaped after fixation, more strongly curved posterior to vulva. Cuticle is with fine transverse annules, 0.9 to 1.3 µm

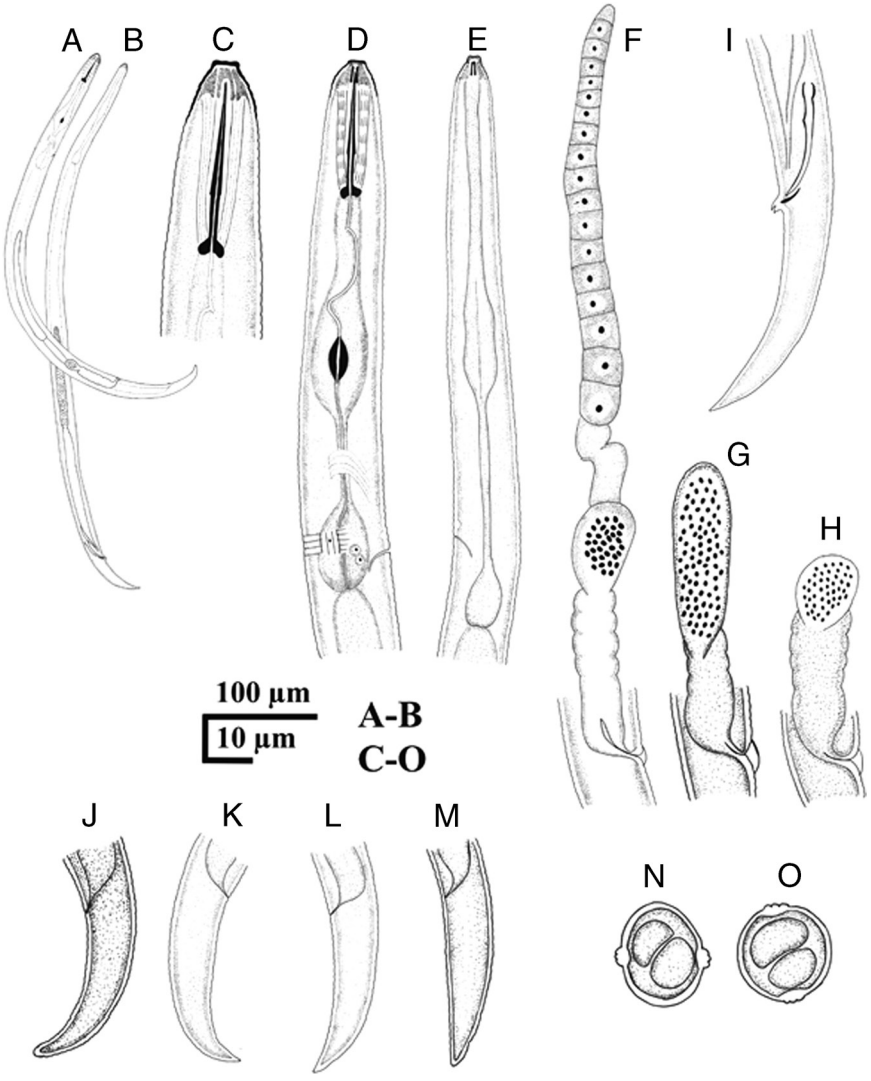

Figure 8: Diagnostic drawings of *Paratylenchus sheri* from Iran. Female (A, C, D, F–H, J–O) and male (B, E, I). A, B: entire body; C: head and stylet; D, E: anterior end and pharyngeal region; F–H: reproductive system; I–M: posterior end; N, O: cross sections at mid-body.

wide in mid-body. Lateral field is with four lines. The cephalic region is truncated to slightly raised at anterior end; submedian lobes on head are distinct in lateral view. Stylet is long and flexible, basal knobs are large and rounded, being 3.6 to 5.5 µm in width. Excretory pore is located at the level of pharyngeal median bulb and anterior to its valve. Vulva flush with body, lacking cuticular flaps. The reproductive system is monodelphic-prodelphic, spermatheca small, without sperm, and attached to the gonad from lateral side. Post-vulval uterine sac is absent. Tail is convex-conoid, often ventrally curved, with finely rounded terminus. Phasmids are not observed. Fourth-stage juvenile (J4) is similar to female in general characteriztics, but with reduced stylet (14–19 µm) bearing small basal knobs and having a tail with bluntly rounded terminus (Table 5).

Male

Not found.

Remarks: the present population is similar to the previously recovered populations of *P. teres* from Brazil (Raski, 1976) and Spain (Peña Santiago and Geraert, 1991), but it extends lower limit for range of stylet length. *P. teres* can be distinguished from *P. peraticus* (Raski, 1962) and *P. enatus* (Raski, 1976) by having a shorter stylet (61–83 vs 81–95 vs 82–104 µm, respectively); furthermore, *P. peraticus* has cuticular flaps around vulva. *Paratylenchus teres*

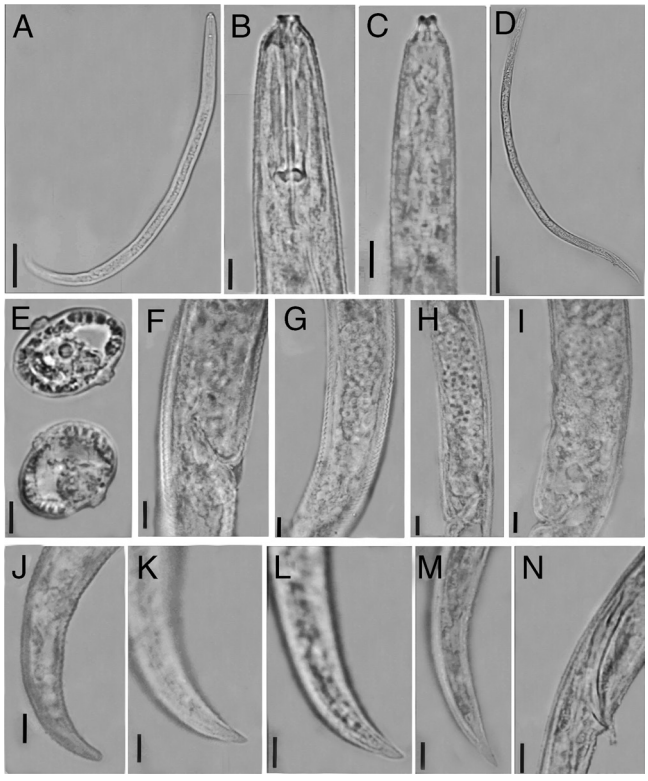

Figure 9: *Paratylenchus sheri* from Iran. Female (A, B, E–M) and male (C, D, N). A, D: entire body; B, C: anterior end; E: cross sections from near mid-body; F–I: vulval region and part of reproductive system; J–M: posterior end; N: cloaca and genital organs (scale bars: A, D = 50 µm; B, C, E–I = 5 µm).

also differs from *P. oostenbrinki* (Misra and Edward, 1971), *P. macrodorus* (Brzeski, 1963) and *P. steineri* (Golden, 1961) by having set off head bearing distinct submedian lobes protruding on lip region (vs in line head lacking distinct submedian lobes). Van den Berg (1989) and Brzeski (1995) discussed on the possibility of *P. teres* to be a junior synonym of *P. steineri*, but Brzeski and Háněl (1999) clarified that the lack of a stylet in the J4 of *P. steineri* clearly differentiate this species from *P. teres*. The length of J4 stylet is comparable in the Iranian and Brazilian populations of *P. teres*. Our population was recovered from the rhizosphere of Mount Atlas mastic (*Pistacia atlantica* Desf.), which is called Baneh in Iran, near the Shivand waterfall in Dehdez county, Khuzestan province, southwestern Iran.

Phylogenetic analysis

The D2–D3 alignment was 596 bp long and consisted of 88 sequences as ingroups and three sequences including *Aglenchus agricola* (De Man, 1884; Andrássy, 1954), *Basiria gracilis* (Thorne, 1949) and *Coslenchus costatus* (De Man, 1921; Siddiqi, 1978) as outgroups. Two new sequences for *Trophotylenchulus* species of the D2–D3 expansion fragments of 28 S rRNA gene were obtained in the present study. Phylogenetic relationships within *Trophotylenchulus* species and other representatives of the family Tylenchulidae inferred from the analysis of this partial 28 S rRNA gene sequences with collapsed branches, with PP less than 50%, are given in Figure 11.

The BI tree revealed good separation between the four subfamilies in trees inferred from 28 S rRNA gene sequences. Four distinct clades were formed, congruent with the morphological classification of the subfamilies in Ghaderi et al. (2016). Representatives of Paratylenchinae (Thorne, 1949) included several species of *Paratylenchus sensu lato* and *Cacopaurus pestis* (Thorne, 1943) formed clade I, with two distinct subclades 1A and 1B. Three populations of *Trophotylenchulus arenarius*, two ones of *T. floridensis* (Raski, 1957) and five *Tylenchulus* species (subfamily Tylenchulinae Skarbilovich, 1947) formed a well-supported clade II, *T. floridensis* having

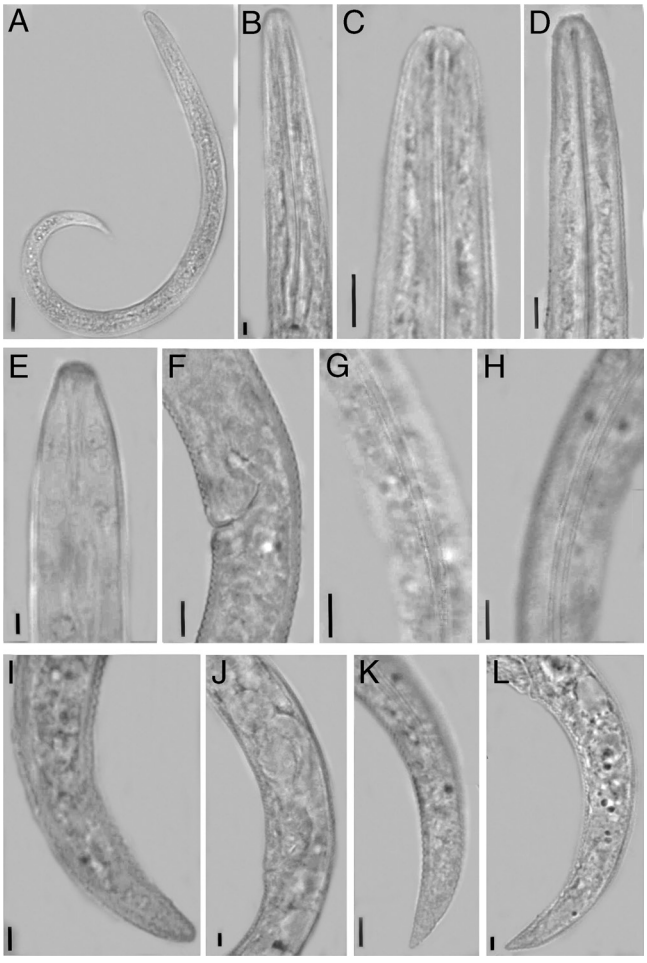

Figure 10: *Paratylenchus teres* from Iran. Female (A–D, F–H, J–L) and fourth-stage juvenile (E, I). A: entire body; B–E: head and stylet; F, J: vulval region; G, H: lateral field; I–L: posterior end (scale bars: A = 20 µm; B–I = 5 µm).

a basal position in this subfamily. The representatives of Sphaeronematinae (Raski and Sher, 1952) and Meloidoderitinae (Kirjanova and Poghossian, 1973) formed basal clades III and IV, respectively.

Together with another sequence of *T. arenarius* (AY780971), the two sequences of this species from Iran (MK733978 and MK733979) formed a high-supported clade (PP = 100%) to other representatives of Tylenchulinae including *Trophotylenchulus floridensis*, *Tylenchulus semipenetrans* (Cobb, 1913), *T. musicola* (Tanha Maafi et al., 2012), *T. graminis* (Inserra et al., 1988), *T. furcus* (Van den Berg and Spaull, 1982), and *T. palustris* (Inserra et al., 1988).

The sequences of the four *Paratylenchus* species from Iran, *P. bukowinensis*, *P. nawadus*, *P. sheri* and *P. similis* (having a stylet under 40 µm, four lateral lines, and with cuticular vulval flaps) clustered among other *Paratylenchus* species bearing stylet shorter than 40 µm in subclade IA, but *P. teres* (having a stylet longer than 40 µm, four lateral lines, and without cuticular vulval flaps) formed a sister cluster with *P. wuae* in a basal group to *C. pestis* and other *Paratylenchus* species in subclade IB; this basal group containing only long-stylet bearing species (Fig. 11).

Discussion

Different populations of the three *Trophotylenchulus* closely related species, *T. arenarius*, *T. asoensis* and *T. okamotoi*, have been recovered from America, Asia and Europe so far. *Trophotylenchulus arenarius* has been described from USA and later has been recorded from Spain (Gomez-Barcina and Castillo, 1990) and Germany (Sturhan, 2014). *Trophotylenchulus asoensis* has been recovered from Japan (Minagawa, 1983) and Iran (Gharakhani et al., 2007), and *T. okamotoi* has been recovered from Japan (Minagawa, 1983), Venezuela,

Table 4. Morphometric characters of *Paratylenchus teres*, *P. bukowinensis* and *P. coronatus* from Iran.

Characters\ species	*P. teres*	*P. bukowinensis*		*P. coronatus*
n	8 females	7 females	5 males	6 females
L	337 ± 26.7 (285–365)	382 ± 41 (304–425)	367 ± 25.3 (325–390)	308 ± 24.1 (273–347)
a	22.5 ± 1.4 (19.5–23.9)	24.6 ± 2.1 (22.1–28.6)	30.1 ± 2.5 (27–32.8)	23.7 ± 2.2 (20.5–26.5)
b	2.4 ± 0.1 (2.2–2.6)	3.9 ± 0.3 (3.3–4.5)	4.1 ± 0.4 (3.4–4.6)	3.7 ± 0.3 (3.4–4.2)
c	15.5 ± 2.5 (12.3–19.1)	15.5 ± 1.7 (12.1–17.3)	11.2 ± 0.4 (10.7–11.8)	15.2 ± 1.5 (13–17.7)
c′	2.8 ± 0.2 (2.5–3.3)	2.7 ± 0.1 (2.6–2.8)	3 ± 0.2 (2.8–3.3)	2.4 ± 0 (2.3–2.5)
V	75.7 ± 1.2 (74.6–78)	85.6 ± 4.7 (83.4–96.4)	–	80.5 ± 0.9 (79.1–82.1)
Stylet	76.7 ± 6.4 (61.3–82)	25.4 ± 1.4 (22.7–26.7)	11.7 ± 1 (10–13)	30.2 ± 1 (28.9–32)
m (conus/stylet %)	89.5 ± 0.7 (88.2–90.7)	63.3 ± 1.2 (61.2–64.9)	–	68.9 ± 1.8 (66.7–71.1)
Pharynx	139 ± 10.7 (117–149)	97.6 ± 6 (90.3–105.7)	90 ± 12.2 (79–110)	82.5 ± 5.1 (79–92.8)
MB	70.5 ± 1.4 (68–72.3)	58.2 ± 1.4 (56.7–60.6)	–	57.7 ± 3.2 (51.5–60.5)
Excretory pore	81.5 ± 5.5 (68–85)	97.6 ± 6 (90.3–105.7)	91 ± 7.3 (79–98)	82.5 ± 5.1 (79–92.8)
Head-vulva	255.6 ± 20.8 (219.7–285)	327 ± 39.4 (255–369)	–	248 ± 19.5 (220–280)
Head-anus	315 ± 27.4 (264–345)	357 ± 41 (279–401)	335 ± 23 (297–357)	287 ± 24.5 (252–327)
Tail length	22.1 ± 2.9 (19–27)	24.5 ± 0.6 (23.5–25.1)	32.6 ± 2.6 (28–34)	20.2 ± 0.4 (19.5–21)
Body width	14.9 ± 0.5 (14.2–15.8)	15.5 ± 1.9 (12.9–19.2)	12.2 ± 0.5 (11.5–13)	12.9 ± 1 (11.7–14.5)
Annulus width	1.2 ± 0.1 (0.9–1.3)	1.4 ± 0.2 (1.2–1.7)	1.3 ± 0.1 (1.2–1.4)	1.2 ± 0.1 (1.1–1.3)
st/L × 100	22.8 ± 1.2 (21.1–24.6)	6.7 ± 0.5 (6.3–7.5)	3.2 ± 0.3 (2.7–3.6)	9.9 ± 0.9 (8.5–11.1)
Spicules	–	–	20.1 ± 0.8 (19.5–21.5)	–
Gubernaculum	–	–	4.5 ± 0.4 (4.2–5)	–

Measurements are in µm and in the form of average ± SD (range).

Costa Rica and USA (Inserra et al., 1993) and Poland (Brzeski, 1998).

Gomez-Barcina and Castillo (1990) synonymized *T. asoensis* and *T. okamotoi* with *T. arenarius* because they believed that most diagnostic features used for separating these species are minor quantitative differences. They further noted that the shape of female tail terminus is a variable intraspecific character and is not sufficient for separating these species. However, these nematodes were treated as separate valid species in the subsequent taxonomic works (Raski, 1991; Inserra et al., 1993; Brzeski, 1998; Siddiqi, 2000; Andrássy, 2007; Ghaderi et al., 2016).

Raski (1991) distinguished the three species based on the same characters mentioned by Minagawa (1983); they used morphometric differences of female tail, male gubernaculum and J2 body size for separating of *T. asoensis* from *T. arenarius*, and the shape of female tail end for differentiation of *T. okamotoi* from both species.

Inserra et al. (1993) found populations of *T. okamotoi* in Florida, Costa Rica and Venezuela which showed intraspecific morphometric variability. However, swollen females of their populations had tapering tails with bluntly rounded or small rounded termini like those of *T. okamotoi* paratypes from Japan and unlike the pointed or minutely digitate tail termini of *T. arenarius* paratypes from California. They accepted the strong arguments of Gomez-Barcina and Castillo (1990) to support synonymy of the three species, but finally preferred to maintain the validity of the described species until more information is available on the morphological variability of *T. arenarius* topotypes. Brzeski (1998) classified

Table 5. Morphometric characters of *Paratylenchus paraperaticus*, *P. projectus*, *P. similis* and *P. variabilis* from Iran.

Characters\ species	*P. paraperaticus*	*P. projectus*	*P. similis*	*P. variabilis*
n	5 females	5 females	22 females	17 females
L	303 ± 17.2 (283–325)	405 ± 28.1 (363–437)	358 ± 44.4 (281–451)	325 ± 18.1 (291–355)
a	25.6 ± 0.6 (25–26.4)	20.9 ± 1.6 (18.7–23.4)	22.7 ± 1.9 (17.2–26.5)	23.2 ± 1.5 (20.7–27.1)
b	2.4 ± 0.1 (2.2–2.5)	4.3 ± 0.3 (4–4.7)	3.8 ± 0.3 (3–4.6)	3.9 ± 0.2 (3.5–4.3)
c	8.6 ± 0.8 (7.4–9.8)	13.2 ± 1 (11.5–14.5)	10.9 ± 0.6 (10–12.6)	16.1 ± 1 (14.2–17.7)
c'	3.2 ± 0.1 (3–3.4)	3 ± 0.1 (2.7–3.2)	3.4 ± 0.2 (2.9–4.1)	2.4 ± 0.1 (2.2–2.5)
V	75.4 ± 1.9 (72.2–77.3)	81.8 ± 0.8 (80.4–83)	81.2 ± 2 (75.3–84.3)	85.2 ± 1 (83.4–87.4)
Stylet	80.1 ± 2.2 (76.7–82.6)	25.4 ± 0.5 (24.3–25.9)	16.9 ± 0.8 (15.5–18.9)	14.1 ± 0.6 (12.8–14.9)
m (conus/stylet %)	90.5 ± 5.3 (87.6–100)	62.2 ± 2.8 (58.4–66.5)	61.8 ± 2 (57.6–65.8)	62.5 ± 2.6 (57.3–69.9)
Pharynx	124.4 ± 6.5 (113.2–128.9)	92.6 ± 5.1 (88.1–102.1)	92.5 ± 6.2 (79.6–114.6)	82.1 ± 3.1 (76.4–87.8)
MB	76.6 ± 3.3 (74.1–82.3)	58.6 ± 2.6 (56.5–63.6)	54.1 ± 2.1 (45.6–56.4)	50.5 ± 1.9 (45.5–53.9)
Excretory pore	83.4 ± 2.3 (81.7–87.4)	92.6 ± 5.1 (88.1–102.1)	86.2 ± 4.8 (76–102)	72.7 ± 2.6 (69–78)
Head-vulva	229 ± 16.5 (208–247)	331 ± 25.1 (292–358)	291 ± 32.5 (220–346)	277 ± 17.2 (246–304)
Head-anus	268 ± 18.6 (245–292)	374 ± 28 (332–406)	325 ± 41.8 (254–413)	305 ± 18.1 (270–335)
Tail length	35 ± 1.8 (33–38)	30.6 ± 1.5 (29.1–33.1)	32.5 ± 2.8 (27–38)	20.1 ± 0.5 (19–21.5)
Body width	11.8 ± 0.6 (10.9–12.5)	19.3 ± 2.1 (16.8–22.6)	15.7 ± 1.7 (13.5–21.2)	14 ± 0.9 (11.4–15.4)
Annulus width	0.9 ± 0.1 (0.8–1.1)	1.5 ± 0.1 (1.4–1.6)	1.1 ± 0.1 (0.9–1.4)	1.3 ± 0.1 (1.1–1.5)
st/L × 100	26.5 ± 0.8 (25.4–27.4)	6.3 ± 0.5 (5.6–7.0)	4.8 ± 0.5 (3.9–5.8)	4.4 ± 0.3 (3.8–4.7)

Measurements are in µm and in the form of average ± SD (range).

populations of Poland as *T. okamotoi*, but he admitted that controversy exists about the identification of these three species.

Two populations of *Trophotylenchulus* were recovered during the present study which one of them (Baghmalek population) had characteriztics of *T. okamotoi* (females with usually bluntly rounded tail end) and the other (Dehdasht population) had morphometric characters more similar to *T. arenarius* (*c* ratio in females = 11–15, male gubernaculum 4.5 µm and second-stage juveniles longer than 320 µm) than to *T. asoensis*, but the shape of tail terminus in females or juveniles was more similar to the latter species. Furthermore, a reduced stylet was observed in a single male recovered during present study that this is in agreement with the original description of *T. asoensis*; males of the type population *T. arenarius* should be restudied for this character because they were described and illustrated without stylet. Swollen females of the population similar to *T. okamotoi* had a tail bearing usually bluntly rounded terminus; however, indented or finely rounded termini were also common in the population. Swollen females of the other population had a conoid tail with finely rounded terminus, and the only recovered male also had a conoid tail with a short terminal projection. Juveniles in both populations had similar tails with a usually slender acute projection at end; however, the projection was slightly shorter in the population similar to *T. okamotoi*. Finally, it may be concluded that all morphological (particularly the shape of female/juvenile tail terminus) or morphometric (*c* ratio of females, the length of gubernaculum in males, and the body size of second-stage juveniles) characters do not show any distinct border delimitating the three species. On the contrary, the two partial 28S rRNA sequences of the populations from Iran had complete identity with each other and show only 4 bp (0.67%) difference with another sequence of *T. arenarius* in the

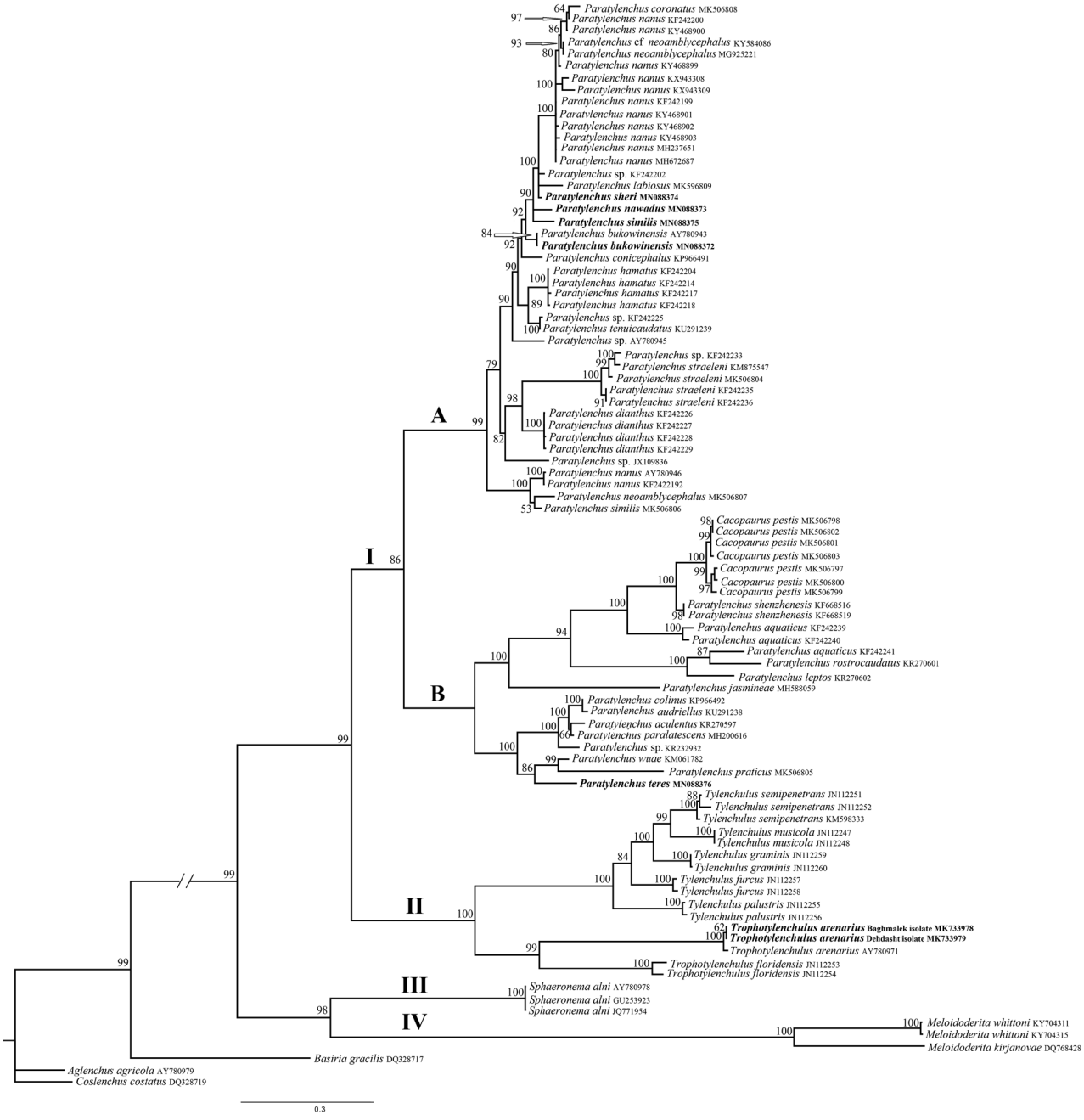

Figure 11: The 50% majority rule consensus tree from Bayesian analysis generated from the D2–D3 expansion segments of 28 S rRNA gene dataset under the GTR + I + G model. Posterior probabilities for BI analysis more than 50% are given for appropriate clades. The new sequencesare indicated in bold.

GenBank (AY780971). They formed a clade with high probability support (PP = 100%) with *T. floridensis* and *Tylenchulus* spp. Considering current knowledge from morphological and molecular studies, we support here *T. asoensis* and *T. okamotoi* as junior synonyms of *T. arenarius*, as it has already been proposed based on morphological observations by Gomez-Barcina and Castillo (1990).

The other interesting result inferred from the phylogenetic analysis of the present study, distant position of the three sequences of *T. arenarius* (as representatives of *Trophonema sensu*; Raski, 1957)

from the two sequences of *T. floridensis* (as a representative of *Trophotylenchulus sensu*; Raski, 1957). The two isolates of *T. floridensis* (JN112253 and JN112254) had 19 bp (3.18%) difference with each other, but their differences with the three isolates of *T. arenarius* were 130 to 138 bp (22.2–23.6%). Considering such a relatively high divergence, the possibility of *Trophonema* as a valid genus cannot be excluded with the present data. However, future taxonomic studies coupled with DNA sequencing on additional representatives of *Trophonema* and *Trophotylenchulus* will help to clarify relationships within and between these two genera.

From the five sequenced *Paratylenchus sensu lato* during the present study, three species (*P. bukowinensis*, *P. nawadus* and *P. similis*) belong to the genus *Paratylenchus sensu stricto*, but *P. teres* belongs to the *Gracilacus sensu* (Raski, 1962), and *P. sheri* belongs to the *Paratylenchoides sensu* (Raski, 1973). Although all species of *Gracilacus* formed a basal cluster in subclade IB to the species with stylet shorter than 40 µm (*Paratylenchus sensu stricto*) in the subclades IA and IB, *P. straeleni* (the other species with a stylet longer than 40 µm) occupied an unresolved position among short-stylet bearing species of the subclade IA.

Raski (1973) distinguished *Paratylenchoides* (Raski, 1973) from *Paratylenchus* based on stronger cephalic sclerotization, head dorso-ventrally narrower, and in having a small, narrow, rounded protrusion on the anterior surface of the conoid lip region. The only representative of this genus, *P. sheri*, occupied an unresolved position among other *Paratylenchus* species (subclade IA) including *P. nanus*, *P. similis*, *P. bukowinensis*, *P. conicephalus* and *P. hamatus*. Therefore, the validity of *Paratylenchoides* cannot be supported with the present phylogenetic analysis. It should be considered as a junior synonym of *Paratylenchus*, as already been stated by several studies (Raski and Luc, 1987; Raski, 1991; Brzeski, 1998; Siddiqi, 2000; Andrássy, 2007; Decraemer and Hunt, 2013; Ghaderi et al., 2014, 2016) based on information obtained from morphological observations.

Ghaderi et al. (2014, 2016) provided a grouping for all *Paratylenchus sensu lato* species, falling them into 11 groups based on stylet length, the number of lateral lines and the presence of advulval flaps. Groups 1 to 5 included short-stylet (<40 µm) species (*Pratylenchus* spp.), and groups 6 to 11 contain representatives of long-stylet (>40 µm) species (*Gracilacus* spp.). These groupings are sometimes in congruence with our results. In the tree (Fig. 11), clade IA mainly contains representatives of Group 3 (stylet < 40 µm, lateral lines = 4, advulval flaps present) but also has four isolates of *P. straeleni* from Group 10 (stylet > 40 µm, lateral lines = 4, advulval flaps present) and several unknown species (without any available morphological characters). With the present information, we cannot explain the clustering of *P. straeleni* with short-bearing stylet species. A more detailed morphological and molecular study on different populations of this species is recommended to clarify the unusual position of *P. straeleni* in the 28 S rRNA and ITS (present study; Van den Berg et al., 2014; Mokaram Hesar et al., 2019) phylogenetic trees.

The first cluster of subclade IB contains *C. pestis*, *P. shenzhenesis* (Wang et al., 2013) from Group 3, three species of Group 2 (stylet < 40 µm, lateral lines = 3, advulval flaps present), and *P. jasminae* Phani et al., 2019 from Group 4 (stylet < 40 µm, lateral lines = 4, advulval flaps absent); the other cluster of the subclade IB can be identified as *Gracilacus* species (with stylet > 40 µm),with three (Groups 8 and 9) or four (Groups 10 and 11) lateral lines and either with (Groups 8 and 10) or without (Groups 9 and 11) advulval flaps.

As a starting point for a detailed analysis and molecular characterization of the genus *Paratylenchus*, Van den Berg et al. (2014) found some species complexes containing several species in *P. aquaticus* (Merny, 1966), *P. hamatus* (Thorne and Allen, 1950) and *P. nanus* (Cobb, 1923). They further noticed that the validity of *Gracilacus* cannot be rejected with their data sets. Our phylogenetic analysis revealed that such species complexes may be present also for *P. similis* because two isolates of the species and also an isolate of the very closely related species, *P. labiosus* Anderson and Kimpinski, 1967, occupied different positions in the tree. Regardless to the position of *P. straeleni*, the validity of *Gracilacus* is supported here as seven representatives of *Gracilacus* formed a distinct basal cluster from other *Paratylenchus* species. Nevertheless, it seems that the perfect outline of the *Paratylenchus sensu lato* still needs the inclusion of more sequences of known or probably undescribed species.

References

Anderson, R. V. and Kimpinski, J. 1967. *Paratylenchus labiosus n.* sp. (Nematoda: Paratylenchidae) from Canada. Canadian Journal of Zoology 55:1992–6.

Andrássy, I. 1954. Revision der Gattung *Tylenchus* Bastian, 1865 (Tylenchidae: Nematoda). Acta Zoologica Academiae. Scientiarum Hungaricae 1:5–42.

Andrássy, I. 2007. Free-Living Nematodes of Hungary (Nematoda, Errantia), II Hungarian Natural History Museum, Budapest.

Brzeski, M. W. 1963. *Paratylenchus macrodorus* n. sp. (Nematoda: Paratylenchidae), a new plant parasitic nematode from Poland. Bulletin de l'Académie Polonaise des Sciences, Classe II 11:277–80.

Brzeski, M. W. 1995. Paratylenchinae: morphology of some known species and descriptions of *Gracilacus bilineata* sp. n. and *G. vera* sp. n. (Nematoda: Tylenchulidae). Nematologica 41:535–65.

Brzeski, M. W. 1998. Nematodes of Tylenchina in Poland and Temperate Europe Muzeum I Instytut Zoologii PAN, Warszawa.

Brzeski, M. W. and Háněl, L. 1999. Paratylenchinae: postembryonic developmental stages of *Paratylenchus straeleni* (De Coninck, 1931) and *P. steineri* Golden, 1961 (Nematoda: Tylenchulidae). Nematology 1:673–80.

Brzeski, M. W., Háněl, L., Nico, A. I. and Castillo, P. 1999. Paratylenchinae: redescription of *Paratylenchus arculatus* Luc and de Guiran, 1962, a new senior synonym of *P. nainianus* Edward and Misra, 1963 (Nematoda: Tylenchulidae). Nematology 1:375–80.

Castillo, P., Gonzàlez-Pais, M. A. and Gomez-Barcina, A. 1989. El género *Gracilacus* Raski, 1962 en Espana (Paratylenchinae: Tylenchida). Revista Ibérica de Parasitologia 49:321–8.

Cobb, N. A. 1913. Notes on *Mononchus* and *Tylenchulus*. Journal of the Washington Academy and Sciences 3:287–8.

Cobb, N. A. 1923. Notes on *Paratylenchus*, a genus of nemas. Journal of the Washington Academy and Sciences 13:254–7.

Colbran, R. C. 1965. Studies of plant and soil nematodes. 10. *Paratylenchus coronatus* n. sp. (Nematoda: Criconematidae), a pin nematode associated with citrus. Queensland Journal of Agricultural and Animal Sciences 22:277–9.

Corbett, D. C. M. 1966. Central African nematodes. II. *Paratylenchus crenatus* n. sp. (Nematoda: Criconematidae) from Malawi. Nematologica 12:101–4.

Darriba, D., Taboada, G. L., Doallo, R. and Posada, D. 2012. jModel Test 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

De Coninck, L. A. P. 1931. Sur trois espèces nouvelles de nématodes libres trouvés en Belgique. Bulletin du Musée royal d'histoire naturelle de Belgique 7:1–5.

De Grisse, A. T. 1969. Redescription ou modifications de quelques techniques utilisées dans l'étude des nématodes phytoparasitaires. Mededlingen van den Rijksfaculteit Landbouwwetenschappen Gent 34:351–69.

De Man, J. G. 1884. Die, Frei in der Reinen Erde und im Süssen Wasser Lebenden Nematoden der Niederländischen Fauna: Eine Systematisch-faunistische Monographie EJ Brill, Leiden, 206pp.

De Man, J. G. 1921. Nouvelles recherches sur les nematodes terricoles de la Hollande. Capita Zoologica 1:3–62.

Decraemer, W. and Hunt, D. J. 2013. Structure and classification, in Perry, R. N. and Moens, M. (Eds), Plant Nematology CABI Publishing, Wallingford, pp. 3–39.

Edward, J. C. and Misra, S. L. 1963. *Paratylenchus nainianus* n. sp. (Nematoda: Criconematidae) from Uttar Pradesh, India. Nematologica 9:215–7.

Ghaderi, R., Geraert, E. and Karegar, A. 2016. The Tylenchulidae of the World: Identification of the Family Tylenchulidae (Nematoda: Tylenchida) Academia Press, Ghent.

Ghaderi, R., Kashi, L. and Karegar, A. 2014. Contribution to the study of the genus *Paratylenchus* Micoletzky, 1922 sensu lato (Nematoda: Tylenchulidae). Zootaxa 3841:151–87.

Gharakhani, A., Pourjam, E. and Karegar, A. 2007. Some plant parasitic nematodes (Criconematoidea and Longidoridae) in Kerman province orchards. Iranian Journal of Plant Pathology 43:372–97.

Golden, A. M. 1961. *Paratylenchus steineri* (Criconematidae) a new species of plant nematode. Proceedings of the Helminthological Society of Washington 28:9–11.

Gomez-Barcina, A. and Castillo, P. 1990. *Trophonema arenarium* (Nematoda: Tylenchulidae) and its junior synonyms. Nematologica 36:404–7.

Gomez-Barcina, A., Castillo, P. and Gonzalez Pais, M. A. 1990. Four species of the genus *Paratylenchus* Micoletzky from southeastern Spain. Nematologia Mediterranea 18:169–77.

Inserra, R. N., Vovlas, N. and Crozzoli, R. 1993. Geographical distribution, hosts and biological characteriztics of *Trophonema okamotoi* (Nematoda: Tylenchulidae). Nematologica 39:328–45.

Inserra, R. N., Vovlas, N. and O'Bannon, J. H. 1988. Morphological and biological characters of diagnostic significance in *Tylenchulus* and *Trophotylenchulus* species. Nematologica 34:412–21.

Jenkins, W. R. 1956. *Paratylenchus projectus*, new species (Nematoda: Criconematidae), with a key to the species of *Paratylenchus*. Journal of the Washington Academy of Science 46:296–8.

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

Kashi, L., Karegar, A. and Kheiri, A. 2009. *Paratylenchus paraperaticus* sp. n. (Tylenchida: Tylenchulidae) found in the rhizosphere of walnut trees in Hamadan province, Iran. Nematology 11:641–7.

Khan, E., Prasad, S. K. and Mathur, V. K. 1967. Two new species of the genus *Paratylenchus* Micoletzky, 1922 (Nematoda: Criconematidae) from India. Nematologica 13:79–84.

Kirjanova, E. S. and Poghossian, E. E. 1973. [Redescription of *Meloidoderita kirjanovae* Poghossian, 1966 (Nematoda: Meloidoderitidae) n. fam.]. Parazitologiya 7:280–5.

Luc, M. and de Guiran, G. 1962. Deux nouveaux *Paratylenchus* (Nematoda-Criconematidae) de Côte d'ivoire. Nematologica 7:133–8.

Merny, G. 1966. Nématodes d'Afrique tropicale: un nouveau *Paratylenchus* (Criconematidae), deux nouveaux *Longidorus* et observations sur *Longidorus laevicapitatus* Williams, 1959 (Dorylaimidae). Nematologica 12:385–95.

Micoletzky, H. 1922. Die Freilebenden Erd-Nematoden. Archiv für Naturgeschichte, Berlin A 87:1–650.

Minagawa, N. 1983. Descriptions of two new species of nematode genus *Trophonema* Raski, 1957 (Tylenchida: Tylenchulidae). Applied Entomology and Zoology 18:90–7.

Misra, S. L. and Edward, J. C. 1971. Two new species of the genus *Paratylenchus* with description of their larval stages and a note on *P. nawadus* a synonym of *P. nainianus*. The Allahabad Farmer 45:345–50.

Mokaram Hesar, A., Karegar, A. and Ghaderi, R. 2019. Phylogenetic relationships of *Cacopaurus pestis* Thorne, 1943 within representatives of the Tylenchulidae Skarbilovich, 1947 as inferred from ITS and D2–D3 expansion segments of 28S-rRNA sequences. Nematology 21:971–94.

Nunn, G. 1992. Nematode Molecular Evolution. Ph.D. thesis, University of Nottingham, Nottingham, 228pp.

Oostenbrink, I. M. 1953. A note on *Paratylenchus* in the Netherlands with the description of *P. goodeyi* n. sp. (Nematoda, Criconematidae). Tijdschrift Over Pantenziekten 59:207–16.

Oostenbrink, M. 1960. The family criconematidae, in Sasser, J. N. and Jenkins, W. R. (Eds), Nematology University of North Carolina Press, Chapel Hill, NC, pp. 196–205.

Page, R. D. 1996. TreeView: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12:357–8.

Peña Santiago, R. and Geraert, E. 1991. New data on *Aorolaimus perscitus* (Doucet, 1980) and *Gracilacus teres* Raski, 1976 (Nematoda: Tylenchida) associated with olive (Olea europez L.) in the province of Jaén, Spain. Nematologica 36:408–16.

Phani, V., Somvanshi, V. S., Rao, U. and Khan, M. R. 2019. *Paratylenchus jasmineae* sp. n. (Nematoda: Paratylenchinae) from rhizosphere of *Jasminum sambac* in India. Nematology 21:469–78.

Raski, D. J. 1956. *Sphaeronema arenarium*, n. sp. (Nematoda: Criconematidae), a nematode parasite of Salt Rush, *Juncus leseurii* Boland. Proceedings of the Helminthological Society of Washington 23:75–7.

Raski, D. J. 1957. *Trophotylenchulus* and *Trophonema*, two new genera of Tylenchulidae n. fam. (Nematoda). Nematologica 2:85–90.

Raski, D. J. 1962. Paratylenchidae n. fam. with descriptions of five new species of *Gracilacus* n. g. and an emendation of *Cacopaurus* Thorne, 1943, *Paratylenchus* Micoletzky, 1922 and Criconematidae Thorne, 1943. Proceedings of the Helminthological Society of Washington 29:189–207.

Raski, D. J. 1973. *Paratylenchoides* gen. n. and two new species (Nematoda: Paratylenchidae). Proceedings of the Helminthological Society of Washington 40:230–3.

Raski, D. J. 1975a. Revision of the genus *Paratylenchus* Micoletzky, 1922 and descriptions of new species. Part I of three parts. Journal of Nematology 7:15–34.

Raski, D. J. 1975b. Revision of the genus *Paratylenchus* Micoletzky, 1922 and descriptions of new species. Part II of three parts. Journal of Nematology 7:274–95.

Raski, D. J. 1976. Revision of the genus *Paratylenchus* Micoletzky, 1922 and descriptions of new species. Part III of three parts. Journal of Nematology 8:97–115.

Raski, D. J. 1991. Tylenchulidae in agricultural soils, in Nickle, W. R. (Ed.), Manual of Agricultural Nematology Marcel Dekker, New York, NY, pp. 761–94.

Raski, D. J. and Luc, M. 1987. A reappraisal of Tylenchina (Nemata). 10. The superfamily Criconematoidea Taylor, 1936. Revue de Nématologie 10:409–44.

Raski, D. J. and Sher, S. A. 1952. *Sphaeronema californicum*, nov. gen nov. spec., (Criconematidae'. Sphaeronematinae, nov. subfam.) an endoparasite of the roots of certain plants. Proceedings of the Helminthological Society of Washington 19:77–80.

Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–4.

Siddiqi, M. R. 1978. The unusual position of the phasmids in *Coslenchus costatus* (de Man, 1921) gen. n., comb. n., and other Tylenchidae (Nematoda: Tylenchida). Nematologica 24:449–55.

Siddiqi, M. R. 1986. Tylenchida: Parasites of Plants and Insects Commonwealth Institute of Parasitology, Slough, 645pp.

Siddiqi, M. R. 1999. *Trophotylenchulus colbrani* sp. n. and *T. cunctus* sp. n. (Criconematina: Tylenchulidae) from Mbalmayo forest in Cameroon. International Journal of Nematology 8:179–84.

Siddiqi, M. R. 2000. Tylenchida: Parasites of Plants and Insects 2nd ed., CABI Publishing, Wallingford.

Skarbilovich, T. S. 1947. On the taxonomic reorganization of nematodes of family Anguillulinidae Baylis and Daubney, 1926. in Russian, Doklady Annual SSSR 57:307–8.

Sturhan, D. 2014. Plant-parasitic nematodes in Germany-an annotated checklist. Soil Organisms 86:177–98.

Swofford, D. L. 2003. PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4, Sinauer, Sunderland, MA, 128pp.

Szczygiel, A. 1974. Plant parasitic nematodes associated with strawberry plants in Poland. Zeszyty Problemowe Postepów Nauk Rolniczch 154:1–132.

Tanha Maafi, Z., Amani, M., Stanley, J. D., Inserra, R. N., Van den Berg, E. and Subbotin, S. A. 2012. Description of *Tylenchulus musicola* sp. n. (Nematoda: Tylenchulidae) from banana in Iran with molecular phylogeny and characterization of species of *Tylenchulus* Cobb, 1913. Nematology 14:353–69.

Tanha Maafi, Z., Subbotin, S. A. and Moens, M. 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. Nematology 5: 99–111.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–82.

Thorne, G. 1943. *Cacopaurus pestis* n. g., n. spec. (Nematoda: Criconematidae), a destructive parasite of the walnut, *Juglans regia*. Linn. Proceedings of the Helminthological Society of Washington 10:78–83.

Thorne, G. 1949. On the classification of the Tylenchida new order (Nematoda: Phasmidia), Proceeding of Helminthological Society of Washington 16:37–73.

Thorne, G. and Allen, M. W. 1950. *Paratylenchus hamatus* n. sp. and *Xiphinema* index n. sp., two nematodes associated with fig roots, with a note on *Paratylenchus anceps* Cobb, Proceeding of Helminthological Society of Washington 17:27–35.

Van den Berg, E. 1989. More species of pin nematodes from Southern Africa (Paratylenchinae: Nemata). Phytophylactica 21:221–6.

Van den Berg, E. and Spaull, V. W. 1982. Two new species of Tylenchuloidea (Nematoda) on sugar cane. Phytophylactica 14:131–44.

Van den Berg, E., Tiedt, L. R. and Subbotin, S. A. 2014. Morphological and molecular characterization of several *Paratylenchus* Micoletzky, 1922 (Tylenchida: Paratylenchidae) species from South Africa and USA, together with some taxonomic notes. Nematology 16:323–58.

Wang, K., Xie, H., Li, Y., Xu, C. L., Yu, L. and Wang, D. W. 2013. *Paratylenchus shenzhenensis* n. sp. (Nematoda: Paratylenchinae) from the rhizosphere soil of *Anthurium andraeanum* in China. Zootaxa 3750:167–5.

Wu, L. Y. 1974. *Paratylenchus robustus* n. sp. (Paratylenchinae: Nematoda) from forest soil in Ontario. Canadian Journal of Zoology 52:1423–5.