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Paurodontella parapitica n. sp. (Nematoda: Hexatylina, Sphaerularioidea) from Kermanshah Province, Western Iran

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Abstract: Paurodontella parapitica n. sp., collected from the rhizosphere of an apple tree in Kermanshah province, western Iran, is described. The new species is characterized by a body length of 505 to 723 μm (females) and 480 to 600 μm (males), lip region continuous by depression; 7 to 8 μm broad, 3 to 4 μm high, stylet length 7 to 9 μm or 1 to 1.3 times the lip region diameter, short postuterine sac of 4 to 6 μm long, lateral fields with five to six incisures; outer incisures crenated and inner incisures weakly crenated, excretory pore situated 90 to 100 μm from anterior end; functional males common in the population, with spicules 24 to 26 μm long. Tail of both sexes similar, almost straight and elongate-conoid. The new species resembles in morphology and morphometrics to four known species of the genus, namely *P. apitica*, *P. minuta*, *P. myceliophaga*, and *P. sohailai*. The results of phylogenetic analyses based on sequences of D2/D3 expansion region of 28S rRNA gene revealed this genus is polyphyletic in four different clades in Tylenchid.

Key words: 28S D2/D3, new species, molecular phylogeny, morphology, taxonomy.

The genus *Paurodontella* was proposed to accommodate its type species *P. minuta* Husain & Khan (1968) based on the following features: short and robust body, convex-conoid ditylenchoid tail, and stem-like extension of the basal pharyngeal bulb projecting into the intestine, giving the appearance of another isthmus, uterus often with an offset diverticulum, bursa adanal, and spicules ditylenchoid. According to the classification by Siddiqi (2000), *Paurodontella* belongs to the subfamily Paurodontinae Thorne, 1941, family Paurodontidae Thorne, 1941, superfamily Sphaerularioidea Lub-bock, 1861, suborder Hexatylina Siddiqi, 1980 in the order of Tylenchida Thorne, 1949. Currently, the genus *Paurodontella* has 10 valid nominal species (Handoo et al., 2010).

Siddiqi (2000) pointed out that the family Paurodontidae is a junior synonym of Sphaerulariidae since the genera included in these families are morphologically similar and have similar life cycles. Biologically, in Paurodontidae, a fungus-feeding generation is well known and nothing is known about entomoparasitic forms, while in Sphaerulariidae an entomoparasitic form is present (Siddiqi, 2000). Some other nematologists (Chizhov, 2004; Andrássy, 2007; Handoo et al., 2010) followed Siddiqi's opinion considering Paurodontidae as a synonym of Sphaerulariidae. In this study, Siddiqi's (2000) scheme was followed since such synonymy will make it easier to study members of this diverse group of nematodes.

In recent years, the nematode fauna of the superfamily Sphaerularioidea in Iran received considerable attention. Several known species of this diverse group of nematodes have been reported from Iran: *Hexatylus mulveyi* Das, 1964, *Stictylus mucronatus* (Thorne & Malek, 1968) Siddiqi, 1986 (Kheiri, 1972, Gharakhani et al., 2009), *Deladenus durus* (Cobb, 1922) Thorne, 1941 and *Prothallonema obtusum* (Thorne, 1941) Siddiqi, 1986 (Jahanshahi Afshar et al., 2014). Recently, two new genera of the family, *Abursanema iranicum* Yaghoubi, Pourjam, Pedram, Siddiqi & Atighi, 2014 and *Veleshkinema iranicum* Miraeiz, Heydari, Álvarez-Ortega, Pedram & Atighi, 2015, and a new species *Paurodontella iranica* Golhasan, Heydari & Miraeiz, 2016 were described from Iran with molecular data of ribosomal RNA gene.

A survey was conducted on Sphaurolaroid nematodes in Iran during 2013–15. A population belonging to *Paurodontella* was collected from western Iran that morphologically did not match with any known species of the genus. A new species of *Paurodontella parapitica* is described from Iran and its partial sequence of LSU rRNA gene was provided.

Materials and Methods

Sampling, Extraction, Mounting, and Drawing

More than 50 soil samples were collected from the rhizosphere of several orchards in Kermanshah province, western Iran in 2014. Nematodes were extracted using a modified combined sieving and centrifugation flotation (Jenkins, 1964) and tray method (Whitehead and Hemming, 1965). Specimens examined under light microscopy (LM) were heat-killed by adding 4% hot formaldehyde solution and processed to pure glycerin using De Grisse's method (1969) and mounted on permanent slides. Measurements and drawings were made using a microscope (Nikon E200) equipped with a drawing tube and a digital camera.

DNA extraction, PCR, and sequencing

Nematode DNA was extracted from single individuals using worm lysis buffer (2 mM KCl, 10 mM Tris–Cl, 2 μl proteinase K (600 μg/ml), 5 mM $MgCl_2$, 12 μl ddH_2O; pH 8.2) (Williams et al., 1992). For the molecular study, single nematode specimens were examined by LM, and then transferred to a small drop of AE buffer (10 mM Tris–Cl, 0.5 mM EDTA; pH 9.0, Qiagen) on a clean slide and squashed using a clean cover slip. The suspension was collected by adding 20 μl AE buffer. DNA sample was stored at −20°C until used as polymerase chain reaction

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(PCR) templates. The D2/D3 expansion segments of 28S rRNA gene were amplified using forward primer D2A (5′-ACA AGT ACC GTG AGG GAA AGT TG-3′) and reverse primer D3B (5′-TCG GAA GGA ACC AGC TAC TA-3′) (Nunn, 1992). The 25-µl PCR mixture contained 12.5-µl 2X GoTaq DNA polymerase mix (Promega Corporation, Madison, WI), each of a 1.2-µl forward and reverse primers solution (5 pM), 6 µl distilled water, and 4 µl of DNA template. The thermal cycling program was as follows: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. PCR products were purified after amplification using ExoSAP-IT (Affymetrix, USB Products), quantified using a Nanodrop spectrophotometer (Nanodrop Technologies) and sequenced directly for both strands using the same primers referred to above with an ABI 3730XL sequencer (Macrogen Corporation, South Korea). The newly obtained sequence was submitted to GenBank database under accession number KU522237. PCR on the ITS1 and 18S sequences was not successful despite many attempts.

Phylogenetic analyses

DNA sequences were edited with ChromasPro1.5 2003-2009 (Technelysium Pty Ltd, Helensvale, Australia) and aligned using ClustalW (http://workbench.sdsc.edu; Bioinformatics and Computational Biology group, Department of Bioengineering, UC San Diego, CA). All available species of *Paurodontella* and other species from GenBank were also selected for phylogenetic analysis (see Table 1 for selected sequences of LSU D2/D3). The model of base substitution in the 28S D2/D3 sequence data was evaluated using MODELTEST version 3.06 (Posada and Criandall, 1998) based on the Akaike-supported model (Arnold, 2010). Bayesian analysis was performed to confirm the tree topology for each gene separately using MrBayes 3.1.0 (Huelsenbeck and Ronquist, 2001) running the chain for 1,000,000 generations and setting the 'burnin' at 1,000. Markov Chain Monte Carlo methods were used within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. The λ^2 test for homogeneity of base frequencies and phylogenetic trees were performed using PAUP* version 4.0 (Sinauer Associates, Inc. Publishers, Sunderland, MA).

Table 1. Species used for analysis of phylogenetic relationships and the accession numbers for LSU D2/D3 sequences deposited in GenBank.

Species	Accession number	Authors
Paurodontella parapitica n. sp.	KU522237	Present paper
Abursanema iranicum	KF885742	Yaghoubi et al., 2014
Contortylenchus	DQ328731	Subbotin et al., 2006
Deladenus siricidicola	AY633444	Ye et al., 2007
Deladenus sp.	JX104313	Unpublished
Ditylenchus dipsaci	HQ219218	Vovlas et al., 2011
Fergusobia sp.	AY589356	Ye et al., 2007
Fergusobia sp.	AY633446	Ye et al., 2007
Fergusobia sp.	FJ386990	Davies et al., 2010
Howardula phyllotretae	DQ328728	Subbotin et al., 2006
Howardula sp.	JX291131	Chizhov et al., 2012
Parasitylenchus sp.	DQ328729	Subbotin et al., 2006
Paurodontella auriculata	KR920361	Unpublished
Paurodontella iranica	KP642168	Golhasan et al., 2016
Paurodontella sp.	KP00034	Unpublished
Psyllotylenchus sp.	KF373738	Koshel et al., 2014
Rubzovinema sp.	KF155283	Koshel et al., 2014
Rubzovinema sp.	KF373736	Koshel et al., 2014
Sphaerularia cf. *bombi*	AB733665	Unpublished
Sphaerularia cf. *bombi*	AB733664	Unpublished
Spilotylenchus sp.	KF373740	Koshel et al., 2012
Subanguina sp.	KT205568	Unpublished
Veleshkinema iranicum	KM401545	Miraeiz et al., 2015

Results and Discussion

Systematics

Paurodontella parapitica n. sp.
(Table 2; Figs. 1,2)

Description

Females: Body cylindrical, robust, almost straight, or sometimes slightly ventrally curved upon heat relaxation. Cuticle thick, mostly under 2 µm thick, finely annulated. Lateral field 8 to 9 µm wide at mid-body, occupying about one-fourth (22% to 26%) of the corresponding body diameter, marked by five to six incisures, outer incisures crenated and wider and inner incisures weakly crenated. Lip region high, truncate, annulated; continuous with body contour; labial framework slightly sclerotized. Stylet distinct with well-developed, rounded basal knobs, conus occupying ca. 37.5% to 44.4% of its total length. Amphidial aperture on the tip of labial lips. Dorsal gland orifice 1.5 to 2.5 µm posterior to stylet knobs. Neck region, from anterior to base of pharynx, 110 to 132 µm long or comprising ca. 16.5% to 22.8% of total body length. Pharyngeal corpus, a cylindrical tube, with long and fusiform median bulb, without valvular apparatus. Isthmus cylindrical, encircled by nerve ring at the base. Excretory duct prominent with excretory pore situated at the basal pharyngeal bulb region. Basal pharyngeal bulb 32 to 40.5 µm long with long posterior extension projecting into intestine, ca. 18 to 23 µm long and forming another isthmus-like structure ending in a shape similar to stylet knobs. Hemizonoids distinct, 1.5 to 2 µm long or 1 to 2 body annuli just anterior to excretory pore. Ovary prodelphic, outstretched with oocytes arranged in two files, spermatheca filled with numerous spherical sperms. Prominent, small, anterior projection attached to oviduct forming a uterine diverticulum of variable size which may function as spermatheca. Quadricolumella of ca. 9 to 10 cells. Vulva a transverse slit, vagina somewhat oblique to body axis, reaching more than halfway across body. Postuterine

Table 2. Morphometrics of *Paurodontella parapitica* n. sp. All measurements in μm and in the form: mean ± SD (range).

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	‘	10	5
L	625	610.7 ± 57.8 (505–723)	521.6 ± 46.4 (480–600)
a	26	25.7 ± 3.0 (20.6–30.1)	25 ± 3.8 (20.0–29.1)
b	5.2	5.1 ± 0.6 (4.4–6.1)	4.9 ± 0.6 (4.3–5.7)
c	8.3	8.6 ± 0.6 (7.7–9.5)	7.0 ± 0.4 (6.4–7.5)
c′	6.8	5.2 ± 0.9 (3.9–6.8)	5.7 ± 0.7 (5.0–6.8)
V or T	78.4	81.4 ± 1.4 (78.4–83.2)	42.2 ± 9.2 (31.2–52.5)
Lip region height	3	3.3 ± 0.3 (3.0–4.0)	3.3 ± 0.4 (3.0–4.0)
Lip region width	7.5	7.2 ± 0.3 (7.0–8.0)	7.1 ± 0.7 (6.0–8.0)
Stylet length	9	8.0 ± 0.7 (7.0–9.0)	7.8 ± 0.8 (7.0–9.0)
Nerve ring from anterior end	75	67.3 ± 5.5 (60–75)	55.2 ± 6.8 (47–62)
E. pore from anterior end	100	94.4 ± 3.9 (90–100)	79.4 ± 6.4 (74–90)
Pharynx length	120	119.5 ± 6.7 (110–132)	106.6 ± 5.5 (100–115)
Postuterine sac length	4	4.4 ± 0.7 (4.0–6.0)	-
Ovary length or testis	320	374.5 ± 49.5 (320–491)	218.6 ± 43 (163–265)
Body diameter at vulva	20	20 ± 1.9 (18–24)	-
Distance vulva—posterior end	135	112.8 ± 10.2 (100–135)	-
Anal (cloacal) body diameter	11	14 ± 1.9 (11–17)	13.4 ±1.8 (11–15)
Tail length	75	70.9 ± 6.6 (60–83)	75 ± 3.5 (70–80)
Spicules length (arc line)	-	-	25.2 ± 0.8 (24–26)
Gubernaculum length	-	-	7.6 ± 0.5 (7.0–8.0)
Bursa (% of tail)	-	-	24.4 ± 2.3 (22–28)

sac short, 0.2 to 0.3 times vulval-body diameter or 6.7% to 13.3% of vulva to anus distance. Vulva-anus distance 35 to 60 μm (42.6 ± 7.8 μm). Tail almost straight, elongate-conoid, within five anal body diameter or 1.3 to 2.1 (1.7 ± 0.3) times vulva-anus distance long and ending in an acute terminus with a short mucro at terminus. Phasmids not observed.

Males: Similar to female in general morphology except for sexual characters. Testis single, outstretched, with spermatogonia arranged in a single row and spermatocytes in double rows, sperms spherical, 1.6 to 2.4 μm in diameter. Spicules paired, arcuate, cephalated, and gubernaculum simple. Bursa adanal or ditylenchoid, enveloping 20% to 35% of tail. Bursal margins strongly annulated. Tail exactly as in females. Phasmids not observed.

Entomoparasitic generation: not found.

Diagnosis and Relationships

Paurodontella parapitica n. sp. is an amphimictic species and is characterized by body length of 505 to 723 μm (females) and 480 to 600 μm (males). Lip region high, annulated with 3 to 4 μm high, 7 to 8 μm wide in females, stylet 7 to 9 μm long with distinct basal knobs. Lateral fields with five to six incisures. Basal bulb with long stem-like extension projecting into the intestine. Excretory pore located at the base of the pharynx, 56 to 86 μm from anterior end, and postuterine sac short (4–6 μm). Tails of both sexes similar, almost straight, elongate-conoid and terminus ending a short mucro. Spicule 24 to 26 μm long.

The new species belongs to the genus *Paurodontella* on the basis of commonly shared characters, i.e., an elongate fusiform, nonmuscular, nonvalvate median pharyngeal bulb, basal bulb with long stem-like extension projecting into the intestine, a prominent small anterior projection attached to oviduct branching to form a uterine diverticulum, and having an adanal bursa.

Due to presence of a relatively long body, shape of posterior body and an oviduct branching to form an uterine diverticulum, the new species *Paurodontella parapitica* n. sp. comes close to four known species in the genus, namely *P. apitica* (Thorne, 1941) Husain & Khan, 1968, *P. minuta* Husain & Khan, 1968, *P. myceliophaga* Handoo, Iqbal, Kazi & Fayyaz, 2010 and *P. sohailai* (Maqbool, 1982) Fortuner & Raski, 1987 in morphology and morphometric characters.

From *P. myceliophaga*, it differs by the longer body (L = 505–723 vs. 432–512 μm in females), greater a ratio (a = 20.6–30.1 vs. 18.7–21.8), more posteriorly located excretory pore and nerve ring (90–100 vs. 56–86 μm and 60–75 vs. 40–56 μm, respectively), lateral field with five to six vs. four incisures and longer spicule (24–26 vs. 20.8–21.5 μm long). *Paurodontella parapitica* n. sp. is different from *P. minuta* in having longer body (body length 505–723 vs. 290–400 μm in females), greater a ratio (20.6–30.1 vs. 17–23), presence vs. absence of post-uterine sac, excretory pore situated opposite to the base of pharyngeal bulb vs. opposite to the nerve ring level, longer spicule (24–26 vs. 15–20) and lateral field with five to six vs. four incisures. The new species differs from *P. apitica* by having greater a ratio (20.6–30.1 vs. 16–22), postuterine sac present vs. absent, lateral lines five to six vs. six to seven, stem-like extension of the basal pharyngeal bulb projecting into intestine vs.

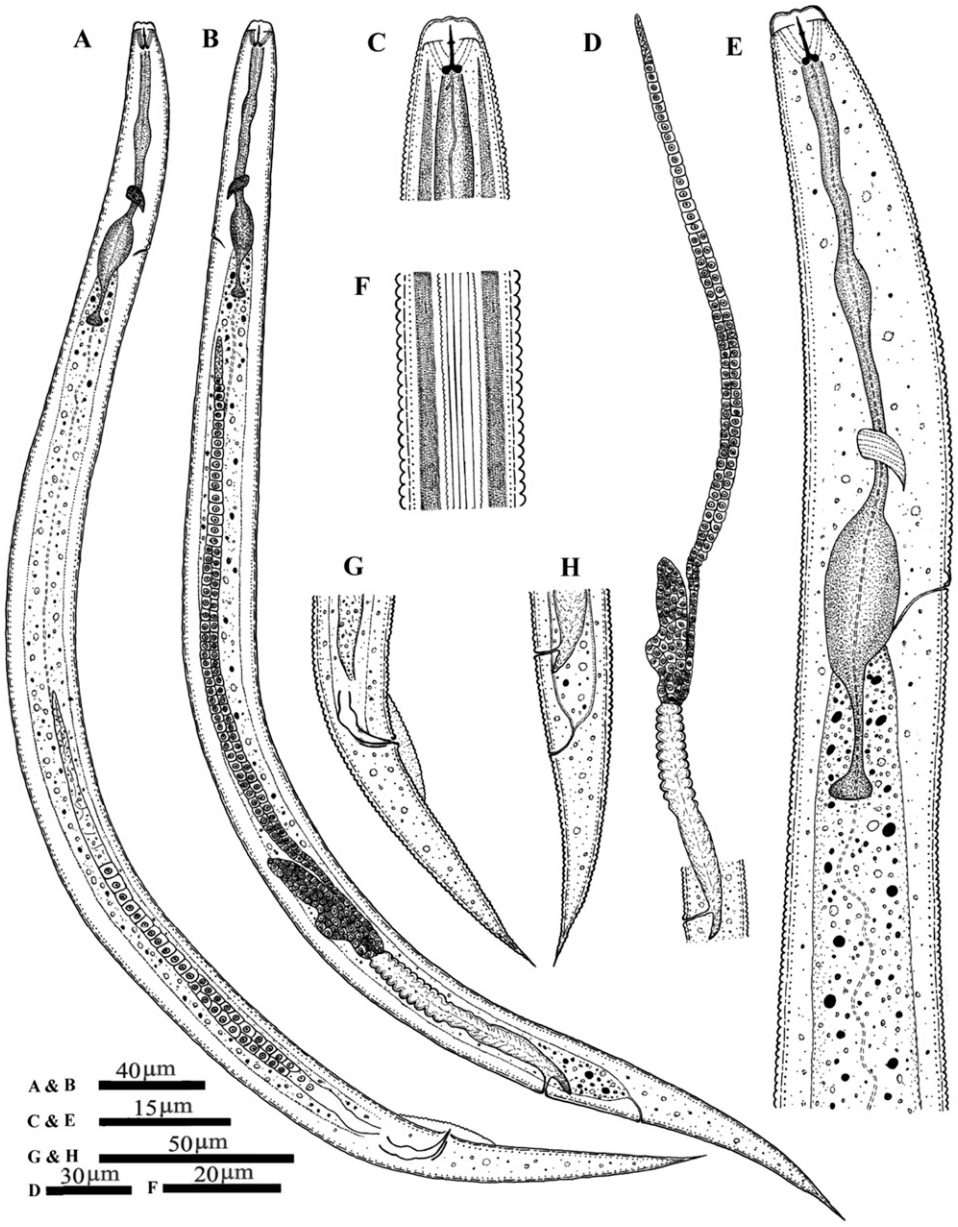

Fig. 1. Line drawings of *Paurodontella parapitica* n. sp. A. Male entire body. B. Female entire body. C. Female anterior end. D. Female oviduct and uterine diverticulum. E. Pharyngeal region of female. F. Lateral field of female. G. Male posterior body. H. Female posterior body.

reaching to, but not into intestine, and presence of males vs. absence. It differs from *P. sohailai* by a longer body (L = 505–723 vs. 400–500 µm in females), lateral lines five to six vs. seven, longer spicule (24–26 vs. 18–20) and tail shape (straight, ending in an acute terminus with a mucro at terminus vs. slightly curved ventrally with some swelling at the tail terminus).

Nonvalvate median pharyngeal bulb and a stem-like basal bulb with an extension projecting into the intestine can be also observed in some other members of the family Paurodontidae including species of *Abursanema*, *Misticius* Massey, 1967; *Paurodontus* Thorne, 1941 and *Paurodontoides* Jairajpuri & Siddiqi, 1969. The new species differs from members of these genera by the presence of a prominent small anterior projection attached to oviduct branching to form a uterine diverticulum.

Paurodontella parapitica n. sp. can be distinguished from *Abursanema* by having a stylet with basal knobs (vs. stylet without knobs), five to six incisures in lateral field (vs. two), and the presence of bursa (vs. absence). The new species differs from *Misticius* by having five incisures in lateral field (vs. absence of lateral field), excretory pore opening near base of isthmus to opposite basal bulb (vs. near the stylet base), and having an adanal (vs. subterminal) bursa. It can be distinguished from *Paurodontus* by having basal pharyngeal bulb with long stem-like extension projecting into the intestine (vs. short), and by the presence of a rudimentary post-uterine sac (vs. prominent). It can also be distinguished

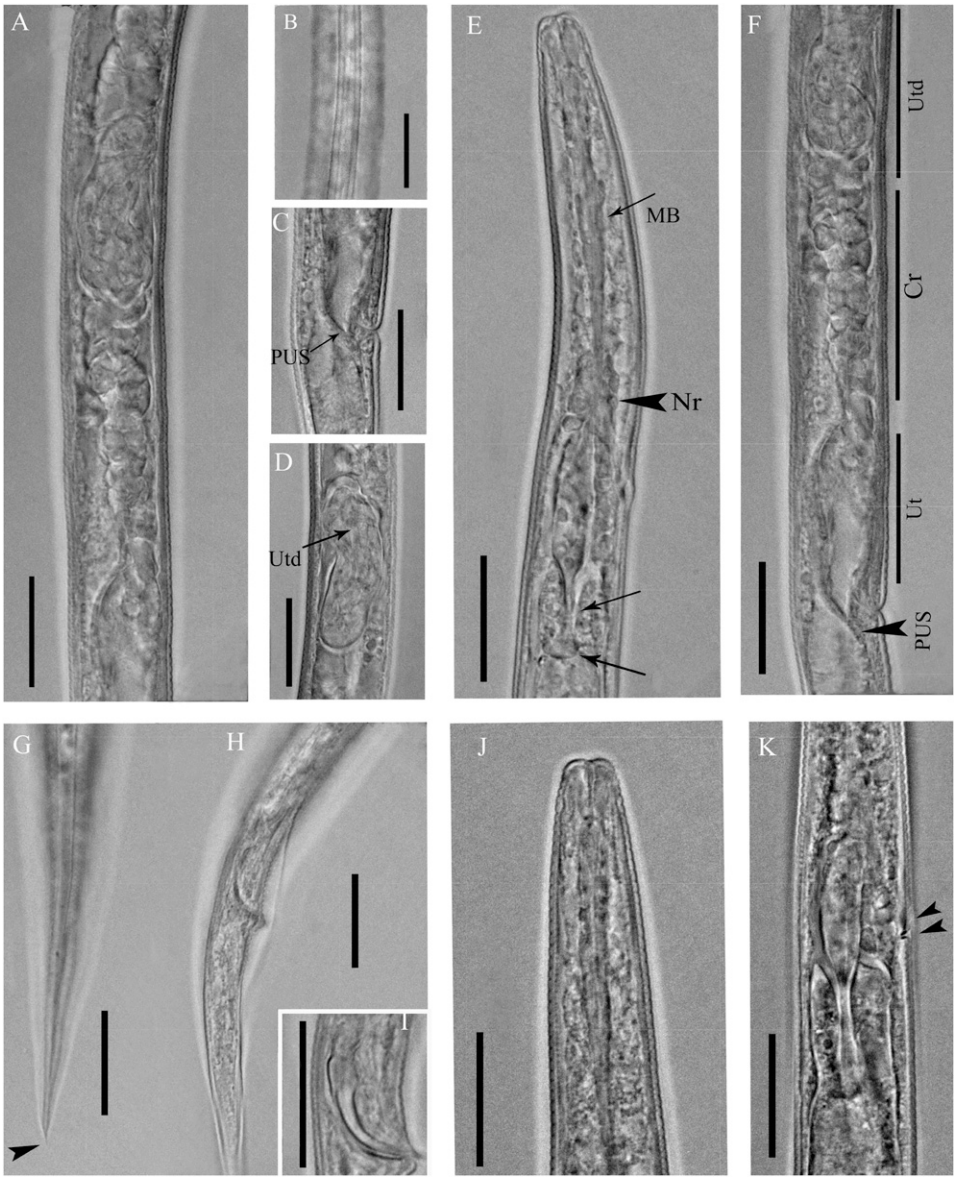

Fig. 2. Photomicrographs of *Paurodontella parapitica* n. sp. A. Part of female reproductive system. B. Lateral field. C. Vulva region showing postuterine sac. D. Vulva region showing uterine diverticulum. E. Female pharyngeal region. F. Part of female reproductive system showing details. G. Female posterior body (tail). H. Male posterior body. I. Spicules in details. J. Female anterior end. K. Basal pharyngeal bulb region. Cr = crustaformeria; MB = median bulb; Nr = nerve ring; Pus = post-uterine sac; Ut = uterus; Utd = uterine diverticulum. (All scale bars = 20 μm).

from *Paurodontoides* by having rudimentary postuterine sac (vs. prominent) and having an adanal (vs. terminal) bursa.

Type habitat and locality

The new species recovered from a soil sample associated with the rhizosphere of an apple tree in Cheshmeh-e-Nezami district of Gilan-e Gharb City, Kermanshah Province, western Iran during August 2013 (GPS coordinates: N 33°59′, E 46°12′, 1,248 m above sea level).

Type material

Holotype female, two paratype females, and two paratype males (Slides SPP001 and SPP002) deposited in nematode collection of the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Iran. Two female paratypes deposited at each of the following collections: CABI Europe-UK, Egham, Surrey, UK and Department of Nematology, Agricultural University, Wageningen, the Netherlands.

Etymology

The specific epithet was chosen because the new species is morphologically similar to *P. apitica*.

Molecular phylogeny

The 433-bp 28S D2/D3 sequence data was less than 94% homologous from any available DNA sequences from GenBank. The BlastN search revealed the highest

match with *Rubzovinema* sp. (KF373736, KF155281, KF155282, and KF155283) with 93% to 94% identity, *Psyllotylenchus* sp. (KF373738) with 94% identity and *Spilotylenchus* sp. (KF373740) with 91% identity. The new species is very different from three available sequences in the genus of *Paurodontella*. It differs from *Paurodontella auriculata* (KR920361) with 79.4% identity, *Paurodontella* sp. (KP000034) with 75.4% identity, and *P. iranica* (KP642168) with 74.6% identity. Phylogenetic analysis (Fig. 3) using Bayesian method rooted with *Subanguina* sp. (TK205568) and *Ditylenchus dipsaci* (HQ219218) revealed *Paurodontella parapitica* n. sp. is in a clade with *Rubzovinema* sp. (Tylenchida; Hexatylina; Sphaerularioidea; Neotylenchidae), *Psyllotylenchus* sp. (Tylenchida; Hexatylina; Iotonchioidea; Parasitylenchidae), and *Spilotylenchus* sp. (Tylenchida; Hexatylina; Iotonchioidea; Parasitylenchidae) with 100% support. This clade is close to *Parasitylenchus*, *Howardula*, *Paurodontella*, *Fergusobia*, and *Deladenus* with 99% support. Four species in the genus *Paurodontella* are not in a monophyletic clade which revealed their morphological similarity is a convergence in evolution and they are not a natural monophyletic group derived from a common ancestor based on the limited sequence data. It is worthy to investigate the taxonomy of the genus *Paurodontella* when more species are described and sequenced in the future.

Anderson (1985) emended the genus *Paurodontella* and briefly gave several generic diagnostic characters to distinguish *Paurodontus* and *Paurodontella*. Constant differences were the length of the post-uterine sac (at least one body diameter in length vs. shorter in *Paurodontella*), slender body (a >30 vs. <30 *Paurodontella*), and vulva-anus distance (>50 μm vs. <50 μm). All other characters in the emended diagnosis for *Paurodontella* were found in one or more species in either genus. In addition, Sumenkova (1975) transferred *Paurodontus aberrans* Nandakumar & Khera, 1969 and *Neopaurodontus asymmetricus* Tikyani & Khera, 1968 to *Paurodontella*. The differences noted between the species in *Paurodontus* and *Paurodontella* were not accepted as sufficient diagnostic characters at generic level by Fortuner and Raski (1987); they considered *Paurodontella* as a junior synonym of *Paurodontus*. Siddiqi (2000) gave a detailed diagnosis and again validated both genera *Paurodontella* and *Paurodontus*, as two valid genera in subfamily Paurodontinae, belonging to family Paurodontidae (Massey, 1967).

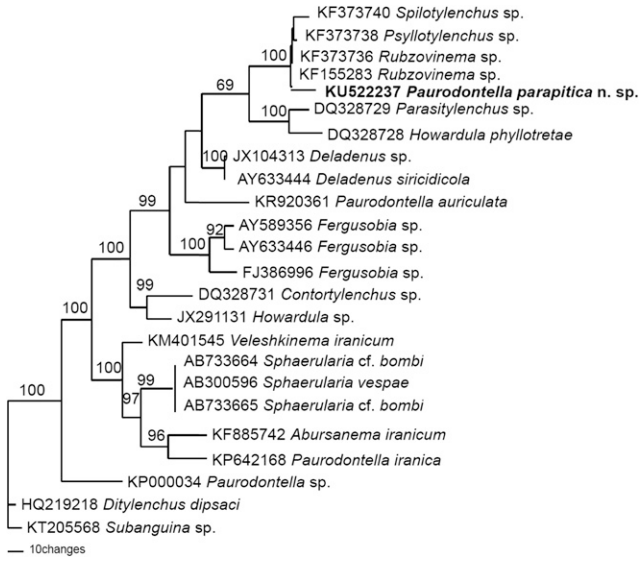

Fig. 3. The 10001st Bayesian tree inferred from Revised 28S D2/D3 under TVM+I+G model (-lnL = 3258.8145; AIC = 6535.6289; freqA = 0.1843; freqC = 0.2082; freqG = 0.3284; freqT = 0.2791; R(a) = 1.3629; R(b) = 4.8955; R(c) = 2.5959; R(d) = 0.7212; R(e) = 4.8955; R(f) = 1; Pinva = 0.2327; Shape = 0.4722). Posterior probability values exceeding 50% are given on appropriate clades.

Anderson (1985) studied morphology of the head for *P. auriculata* by light and scanning electron microscopy and showed that the lateral lips are absent and the amphid apertures are deep, vertical slits extending from the oral disc to the base of the head and dividing the submedian lips. However, the importance of these features as diagnostic characters must be determined with more detailed morphological and\or molecular studies on more species for an integrated generic revision of these genera, namely *Paurodontus* and *Paurodontella*.

This study described a new species of *Paurodontella* with its molecular characterization on D2/D3 expansion segment of 28S rDNA. It is the first molecular phylogenetic study for this genus. *Paurodontella* contains a dozen of species with diverse morphological characters. Our molecular results revealed the polyphyly of this genus, based on only four sequences available in GenBank. Clearly, more species, additional molecular markers and other morphological data are needed to help understanding the taxonomy and molecular phylogeny of the Paurodontidae in the future.

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