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Description of *Paurodontella longicaudata* n. sp. (Tylenchomorpha, Paurodontidae), the cryptic species of *Paurodontella gilanica*, recovered from Golestan province, northern Iran

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Abstract

Two populations of *Paurodontella longicaudata* n. sp. were recovered from natural forests of Golestan province, northern Iran. The type population of the new species was characterized by 405-579 µm long females having 7.5-8.4 µm long stylet with asymmetrical knobs, four lines in the lateral fields, basal pharyngeal bulb with long stem-like extension projecting into the lumen of intestine, small post-vulval uterine sac (PUS) 4.0-4.8 µm long, elongate conoid tail with 84-96 µm length and finely rounded tip. Males were not recovered, but spheroid sperm cells were observed inside female genital tract. Morphological differences between the new species and seven known species of the genus, mainly having conical tail, namely *P. asymmetrica*, *P. balochistanica*, *P. gilanica*, *P. minuta*, *P. myceliophaga*, *P. parapitica* and *P. persica*, were discussed. The new species is the morphologically closest species to *P. gilanica*, forms a tentative cryptic species showing remarkable nucleotide differences in two small and large subunit ribosomal RNA gene (SSU and LSU rDNA D2-D3) markers. In Bayesian phylogenetic analyses using the SSU and LSU, it formed a clade with *Paurodontella gilanica*. This is another new evidence on cryptic speciation in nematodes, indicating "apparently minor" morphological differences which were corroborated with the remarkable molecular differences.

Keywords: Free living, morphology, mycetophagous, new species, phylogeny, SSU and LSU

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توصیف گونه *Paurodontella longicaudata* n. sp. (Tylenchomorpha, Paurodontidae)، گونه کریپتیک *Paurodontella gilanica*، جمع‌آوری شده از استان گلستان، شمال ایران

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چکیده:

دو جمعیت از *Paurodontella longicaudata* n. sp. از جنگل‌های استان گلستان، شمال ایران، جداسازی شدند. جمعیت تیپ جدید دارای ماده-هایی به طول 405 تا 579 میکرومتر، استایلت به طول7/5 تا 8/4 میکرومتر با گره‌های نامتقارن، سطوح جانبی دارای چهار شیار طولی، حباب انتهای مری با امتداد ساقه مانند بلند که به روده نفوذ کرده، کیسه‌ی عقبی رحم (PUS) به طول 4/0 تا 4/8 میکرومتر، دم مخروطی کشیده به طول 84-96 میکرومتر با انتهای گرد است. نرها برای هیچ یک از جمعیت‌ها پیدا نشد، اما اسپرم‌های کروی درون سیستم تناسلی ماده‌ها مشاهده شد. تفاوت‌های ریخت‌شناختی بین گونه‌ی جدید و هفت گونه‌ی شناخته شده از جنس که همگی دارای دم مخروطی هستند، به نام‌های *P. asymmetrica*، *P. balochistanica*، *P. gilanica*، *P. minuta*، *P. myceliophaga*، *P. parapitica* و *P. persica* مورد بحث قرار گرفته است. گونه‌ی *P. gilanica* شبیه‌ترین گونه به گونه‌ی جدید بود، گونه کریپتیک احتمالی آن را تشکیل می‌دهد و دارای تفاوت‌های قابل توجه در توالی دو مارکر ژنومی یعنی ژن رمزگردان RNA زیرواحد کوچک و بخشی از زیرواحد بزرگ ریبوزومی (SSU و LSU rDNA D2-D3) بود. در تجزیه و تحلیل فیلوژنی بیس با استفاده از توالی‌های دو ناحیه ژنومی نام برده در بالا، گونه جدید با گونه *Paurodontella gilanica* یک کلاد تشکیل داد. این مطالعه، مثال جدیدی از گونه زایی کریپتیک در نماتدهاست و نشان می‌دهد تفاوت‌های جزئی ریخت شناسی با تفاوت‌های چشمگیر در توالی-های مولکولی حمایت می‌شود.

واژه‌های کلیدی: آزادزی، ریخت‌شناسی، فیلوژنی، قارچ‌خوار، گونه جدید، مولکولی

Introduction

The genus *Paurodontella* was established in 1968 with *P. minuta* Husain & Khan, 1968 as its type species. Within the family Paurodontidae Thorne 1941 *sensu* Siddiqi (2000), *Paurodontella* is mainly characterized by presence of stylet knobs, location of excretory pore near the nerve ring, basal bulb with long (rarely short) stem-like pharyngeal extension into the intestine, short or sometimes no post-vulval uterine sac (PUS), tail of both sexes similar, conoid, and cloacal bursa (Siddiqi 2000; Yaghoubi *et al*. 2018). Currently, *Paurodontella* contains 15 nominal species (Handoo *et al.* 2010; Yaghoubi *et al.* 2018; Esmaeili *et al.* 2019). Among them, five species including *P. iranica* Golhasan, Heydari & Miraeiz 2016; *P. parapitica* Esmaeili, Heydari & Ye 2016a; *P. persica* Esmaeili, Heydari & Ye 2016b; *P. gilanica* Yaghoubi, Pourjam & Pedram 2018 and *P. composticola* Esmaeili, Heydari, Kheiri & Ye 2019 have originally been described from Iran. A compendium including useful morphological and morphometric data for delimiting two similar genera *Paurodontella* and *Paurodontus* Thorne 1941 and their species, has already been published by Yaghoubi *et al.* (2018).

In this study, two populations of the genus *Paurodontella* were recovered from the bark and rotten wood samples of dead forest trees collected in Golestan province, northern Iran. These specimens are described herein as *Paurodontella longicaudata* n. sp. using morphological and molecular criteria.

Materials and methods

Sampling, nematode extraction, mounting and drawing

Specimens of *P. longicaudata* n. sp. were obtained from wood and bark samples collected from natural forests of Golestan province using the tray method (Whitehead & Hemming 1965). Live specimens were handpicked under a Nikon SMZ1000 stereomicroscope, heat killed by adding hot 4% formalin solution, transferred to anhydrous glycerin and mounted on permanent slides according to De Grisse (1969). The permanent slides were examined using a Nikon Eclipse E600 light microscope. Photomicrographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope equipped with differential interference contrast. Drawings were made using a drawing tube attached to the microscope and were digitally drawn using the CorelDRAW software version 17.

DNA extraction, PCR, and sequencing

An individual live nematode specimen of each population was picked out, examined on a temporary slide, washed and transferred to a small drop of TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0; Qiagen) on a clean slide and squashed with the aid of a cover slip and a plastic pipette tip. The suspension was collected by adding 15 µl TE buffer. Two DNA samples were prepared in total, one for the type, and one for the second population. The DNA samples were stored at −20°C until used as PCR template. Primers for LSU rDNA D2-D3 expansion segments amplification were forward KK28S-1 (5'-AAGGATTCCCTTAGTAACGGCGAGTG-3') (Kiontke *et al.* 2004) and reverse1006R (5'-GTTCGATTAGTCTTTCGCCCCT-3') (Holterman *et al.* 2008). Primers for partial amplification of SSU rDNA were forward primer 22F (5'-TCCAAGGAAGGCAGCAGGC-3') (Dorris *et al.* 2002) and reverse primer 18S 1573R (5'-TACAAAGGGCAGGGACGTAAT-3') (Mullin *et al.* 2005), and forward primer 988F (5'-TTTACGGTCAGAACTAGGG-3') and reverse primer 1912R (5'-CTCAAAGAGATTAAGCCATGC-3') (Holterman *et al.* 2006). The thermocycling program for amplification of both loci was as follows: denaturation at 95°C for 4 min, followed by 32 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 40 sec, and extension at 72°C for 80 sec. A final extension was performed at 72°C for 10 min. The PCR products were purified and sequenced directly for both strands using the same primers used in PCR with an ABI 3730 XL sequencer (Bioneer, South Korae). Sequences were deposited into the GenBank database under the accession numbers MZ015006 and MZ015007 for SSU, and MZ015005 and MZ015008 for LSU rDNA.

Phylogenetic analyses

The newly obtained SSU and LSU sequences were compared with those of other sphaerularioids available in GenBank using the BLAST homology search program. The retrieved sequences from the database were updated according to the previous studies

(Kanzaki *et al.* 2016; Yaghoubi *et al.* 2018; Esmaeili *et al*. 2019). The abovementioned datasets with including the newly generated sequences for the new species were aligned using the Q-INS-i algorithm of the online version of MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013). The Gblocks program (version 0.91b) with all three less stringent parameters (http://phylogeny.lirmm.fr/ phylo-cgi/one-task.cgi?task-type=gblocks), was used for post-editing of both alignments, i.e., to eliminate poorly aligned regions or divergent positions (Castresana 2000). The model of base substitution was selected using MrModeltest 2 (Nylander 2004). The Akaike-supported model, a general time reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I) was used in both SSU and LSU analyses. Bayesian analyses were performed using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) and a random starting tree, running the chains for 10×10^6 generations for both datasets. The burn-in phase was set at 25% of the converged runs. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic tree (Larget & Simon 1999) using the 50% majority rule. To visualize the results of each run-in order to check the effective sample size of each parameter, Tracer v1.6 (Rambaut & Drummond 2009) was used. The classic rhabditid taxa were selected as outgroups (for species names and accession numbers see trees). The output files of MrBayes were visualized using Dendroscope v3.2.8 (Huson & Scornavacca 2012) and drawn in CorelDRAW version 17.

Results

***Paurodontella longicaudata* n. sp.**

(Figs 1 & 2)

Measurements

See Table 1.

Female (Free-living)

Small-sized nematodes. Body slightly ventrally arcuate after fixation. Lateral fields with four lines. Cephalic region rounded in corners, continuous with body contour, cephalic framework weak. Stylet fine, the conus about 30-40% of the total length, with asymmetrical basal knobs. Procorpus cylindroid, slightly swollen posteriorly, forming a fusiform metacorpus-like differentiation without valve, isthmus narrow, basal bulb with long stem-like extension projecting into the lumen of intestine. Excretory pore posterior to nerve ring. Hemizonid just anterior to excretory pore. Nerve ring enveloping isthmus. Intestine simple, rectum and anus functional. Reproductive system monodelphic-prodelphic, ovary with single and double row of oocytes in anterior and posterior part of ovary, oviduct tubular, spermatheca axial, elongate-ellipsoid, with spheroid sperm cells in most examined specimens, crustaformeria quadricolumellate, apparently with four to five cells at each column, uterus short, vagina with moderately sclerotized wall, short post-vulval uterine sac, less than corresponding body diameter long. Tail elongate conoid, gradually narrowing toward distal end, never filiform, with finely rounded tip.

Male

Not found.

Type habitat and locality

The type population was recovered from the wood and bark samples of a dead broadleaf forest tree, collected in Golestan province, northern Iran, during October 2019. GPS coordinate is 36°40.33.8'N, 54°07.00.4'E

Another locality

The second population of the new species was recovered from the wood and bark samples of a dead broadleaf forest tree, collected in Golestan province, northern Iran, during October 2019. GPS coordinate is 36°45.54.8'N, 54°28.23.0'E

Type specimens

Holotype female, 10 paratype females deposited in Nematology Collection of Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. The voucher specimens of the second population were deposited in the WaNaCo collection, Wageningen, The Netherlands (http://www.waneco.eu/). The LSID code of this publication is: urn:lsid:zoobank.org:pub:2D654893-D771-4626-9479-9A30590DFB58.

Etymology

The specific epithet refers to the elongate conoid tail of the new species.

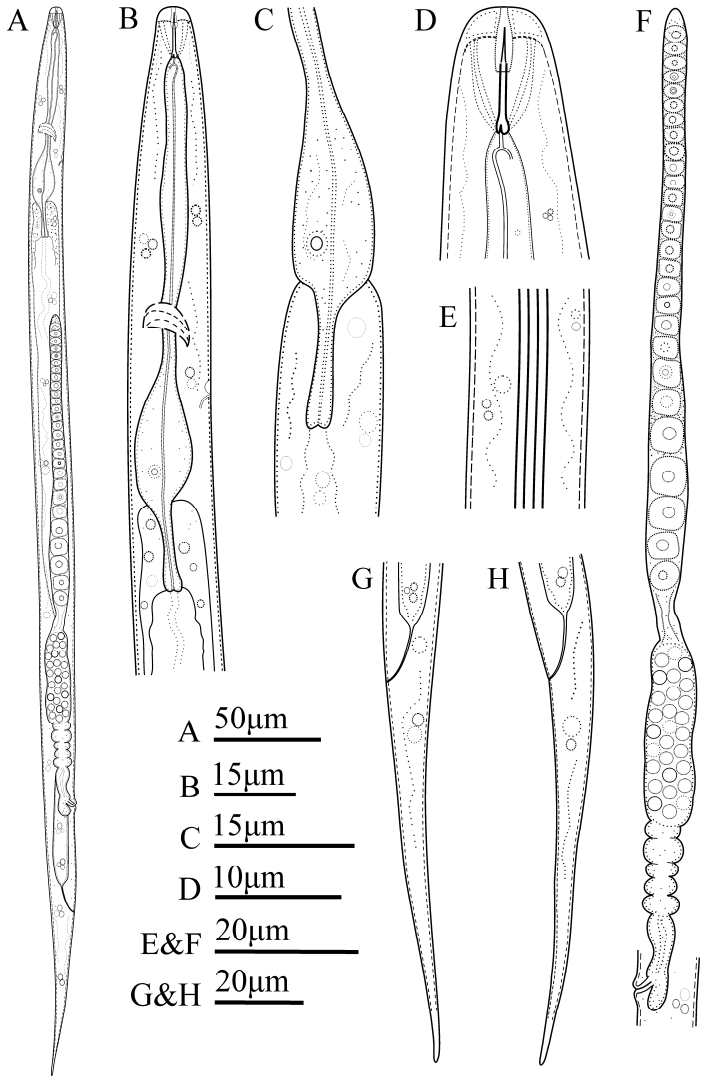

Fig. 1. Line drawings of *Paurodontella longicaudata* n. sp. Female. A: Entire body; B: Pharynx; C: Pharyngo-intestinal junction; D: Anterior body region; E: Lateral field; F: Reproductive system; G & H: Tail.

Differential diagnosis

The type population of *P. longicaudata* n. sp. is mainly characterized by four lines in the lateral fields, long stem-like pharyngeal extension projecting into the lumen of intestine, short PUS and elongate conoid tail, 84-96 µm long with finely rounded tip. It is further characterized by 7.5-8.4 µm long stylet with asymmetrical basal knobs and 405-579 µm long females usually having sperm inside their genital tract. The new species was compared with morphologically similar species of the genus mainly having conical tail and/or comparable number of lateral lines, PUS and pharyngeal extension as follow.

The new species differs from *P. asymmetrica* (Tikyani & Khera 1968) Sumenkova, 1975 by having a longer body (509 (405-579) *vs* 330-380 µm), shorter stylet (8.3 (7.5-8.4) *vs* 13-14 µm), asymmetrical stylet knobs (*vs* posteriorly directed, symmetrical), higher *a* ratio (33.6 (28.5-36.5) *vs* 16.5-19.0), lower *c* ratio (5.7 (4.8-6.1) *vs* 9.7-10.8), more anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 81-84) and longer (88 (84-96) *vs* 34 µm) conoid tail with finely rounded tip (*vs* cylindrical with very acute terminus).

Paurodontella longicaudata n. sp. is differentiated from *P. balochistanica* Handoo, Iqbal, Kazi & Fayyaz 2010 by having shorter body (509 (405-579) *vs* 735 (720-750) µm), shorter stylet (8.3 (7.5-8.4) *vs* 10.5 (10-

11) µm), lower *b* ratio (4.7 (3.8-5.2) *vs* 5.9 (5.8-6.0)), lower *c* ratio (5.7 (4.8-6.1) *vs* 17.5 (17.1-17.8)), greater *c'* ratio (7.6 (6.7-8.4) *vs* 3.1 (3.0-3.2)), anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 88.3 (88.0-88.6)) and longer tail (88 (84-96) *vs* 42 µm) without a terminal mucron (*vs* present).

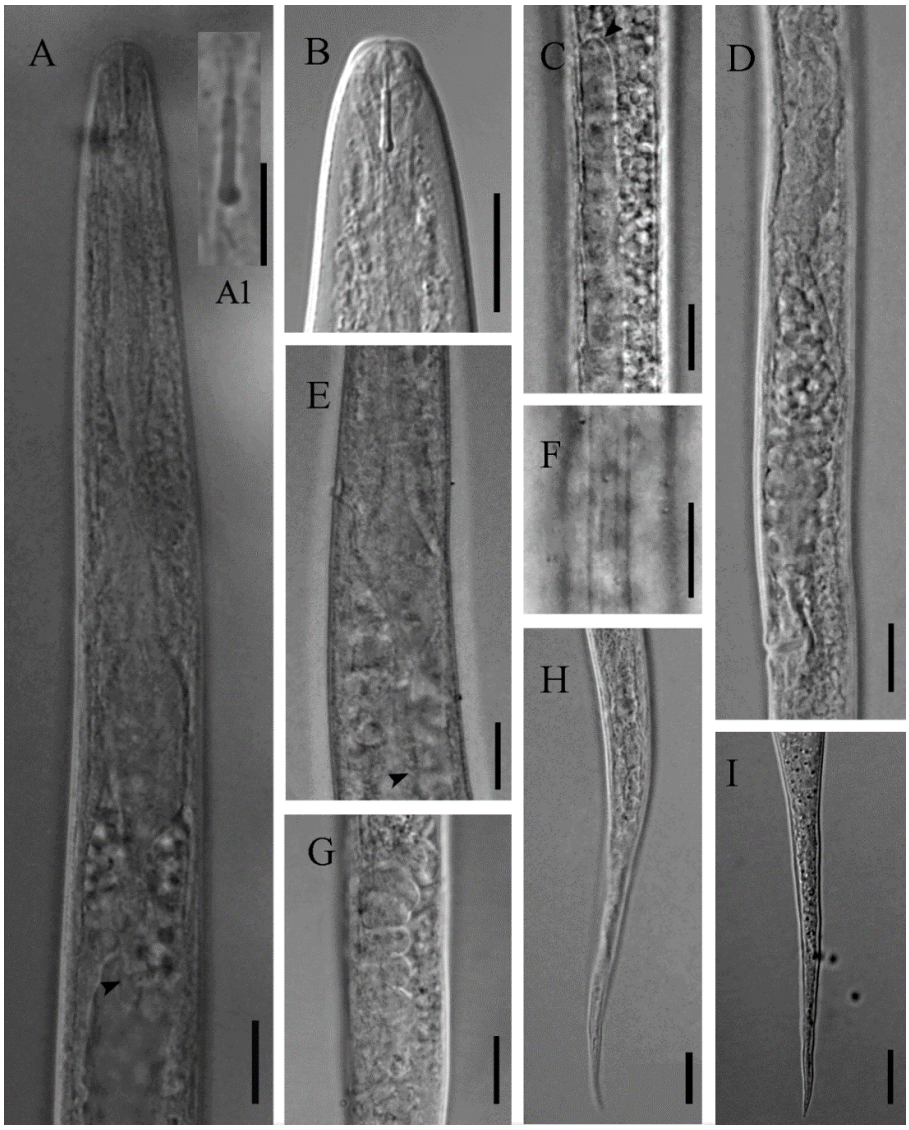

Fig. 2. Light microphotographs of *Paurodontella longicaudata* n. sp. Female. A: Pharynx; A1: Stylet; B: Cephalic region and stylet (fresh specimen); C: Distal part of ovary; D: Part of female reproductive system; E: Variation in pharyngeal extension into intestine; F: Lateral field; G: Vulva and crustaformeria; H & I: Tails. (A1= 5 µm; A-I = 10 µm). (Arrowheads showing the end of the pharyngeal extension).

The new species differs from *P. gilanica* by having longer pharynx (109 (106-118) *vs* 98 (87-106) µm), more anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 77.9 (76.7-79.6)) and longer tail (88 (84-96) *vs* 75 (69-89) µm) elongate conoid, gradually narrowing toward distal end, never filiform (*vs* the filiform distal end is common).

The new species separates from *P. minuta* Husain & Khan 1968 by having longer body (510 (405-579) *vs* 290-400 µm), shorter stylet (8.3 (7.5-8.4) *vs* 8-10 µm), asymmetrical knobs (*vs* symmetrical), greater *a* ratio (33.6 (28.5-36.2) *vs* 17.0-23.0), lower *c* ratio (5.7 (4.8-6.1) *vs* 8-13), anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 78-90) and tail shape (elongate conoid *vs* almost straight, ending in an acute terminus).

It differs from *P. myceliophaga* Handoo, Iqbal, Kazi &Fayyaz 2010 by having greater *a* ratio (33.6 (28.5-36.2) *vs* 19.8 (18.7-21.8)), lower *c* ratio (5.7 (4.8-6.1) *vs* 8.4 (7.6-9.3)), anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 81.0 (77.9-85.9)), longer pharynx (109 (106-118) *vs* 77 (64-92) µm) with elongate stem-like extension (*vs* gradually narrowing to form the extended region), longer (88 (84-96) *vs* 56 (48-64) µm) conoid tail narrowing distally and ending to a finely rounded tip (*vs* conical, ending to a mucro).

The two species *P. longicaudata* n. sp. and *P. parapitica* can be distinguished from each other by having lower *c* ratio (5.7 (4.8-6.1) *vs* 8.6 (7.7-9.5)), greater *c'* ratio (7.6 (6.7-8.4) *vs* 5.2 (3.9-6.8)), lateral fields with four *vs* five or six lines, shorter pharynx (109 (106-118) *vs* 119.5 (110-132) µm), anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 81.4 (78.4-83.2) and longer tail (88 (84-96) *vs* 70.9 (60-83) µm) ending to a finely rounded tip (*vs* to a short mucro).

And finally, the new species has the following discernible characters from *P. persica*: longer stylet (8.3 (7.5-8.4) *vs* 6.2 (5.5-7.0) µm), lower *b* ratio (4.7 (3.8-5.2) *vs* 6.1 (5.4-7.8)), lower *c* ratio (5.7 (4.8-6.1) *vs* 9.1 (7.2-10.8)), greater *c'* ratio (7.6 (6.7-8.4) *vs* 4.1 (3.3-4.6)), longer pharynx (109 (106-118) *vs* 84 (62-95) µm), anteriorly located vulva (*V* = 73.7 (68.4-76.9) *vs* 81.5 (77.4-83.1)) and longer tail (88 (84-96) *vs* 55 (50-65) µm) ending to a finely rounded tip (*vs* mucronated).

Table 1. Morphometrics of two populations of *Paurodontella longicaudata* n. sp. All measurements are in µm and in the form: mean ± s.d. (range).

Character	Type population (females)		Second population
	Holotype	Paratypes	Females
n	-	7	4
L	519	510 ± 61 (405-579)	456.5 ± 12.0 (441-470)
a	32.4	33.6 ± 2.7 (28.5-36.2)	29.9 ± 0.7 (29.4-30.7)
b	4.4	4.7 ± 0.6 (3.8-5.2)	4.2 ± 0.1 (4.1-4.3)
c	5.9	5.7 ± 0.5 (4.8-6.1)	5.1 ± 0.3 (4.6-5.3)
c'	6.7	7.6 ± 0.6 (6.7-8.4)	8.9 ± 1.0 (8.0-9.9)
V	75.1	73.7 ± 2.7 (68.4-76.9)	71.7 ± 1.6 (69.6-73.4)
m	36.8	33.8 ± 3.6 (28.6-38.7)	38.6 ± 4.4 (32.6-42.9)
Cephalic region height	3	3.1 ± 0.4 (3-4)	3.0 ± 0.0 (3-3)
Cephalic region width	6	6.3 ± 0.5 (6-7)	6.0 ± 0.8 (5-7)
Stylet conus	3	2.8 ± 0.3 (2.3-3.0)	2.9 ± 0.1 (2.8-3.0)
Stylet length	8.2	8.3 ± 0.4 (7.5-8.4)	7.6 ± 0.7 (7.0-8.6)
Pharynx length	118	109.0 ± 4.2 (106-118)	107.6 ± 1.7 (106-110)
Excretory pore from anterior end	73	74 ± 10 (57-80)	70 ± 7 (65-75)
Hemizonid from anterior end	70	75.7 ± 5.5 (70-81)	72 ± 5 (65-79)
Post-vulval uterine sac (PUS)	4.3	4.5 ± 0.3 (4.0-4.8)	3.9 ± 0.4 (3.5-4.2)
Tail	88	88.0 ± 3.8 (84-96)	90.5 ± 3.7 (88-96)

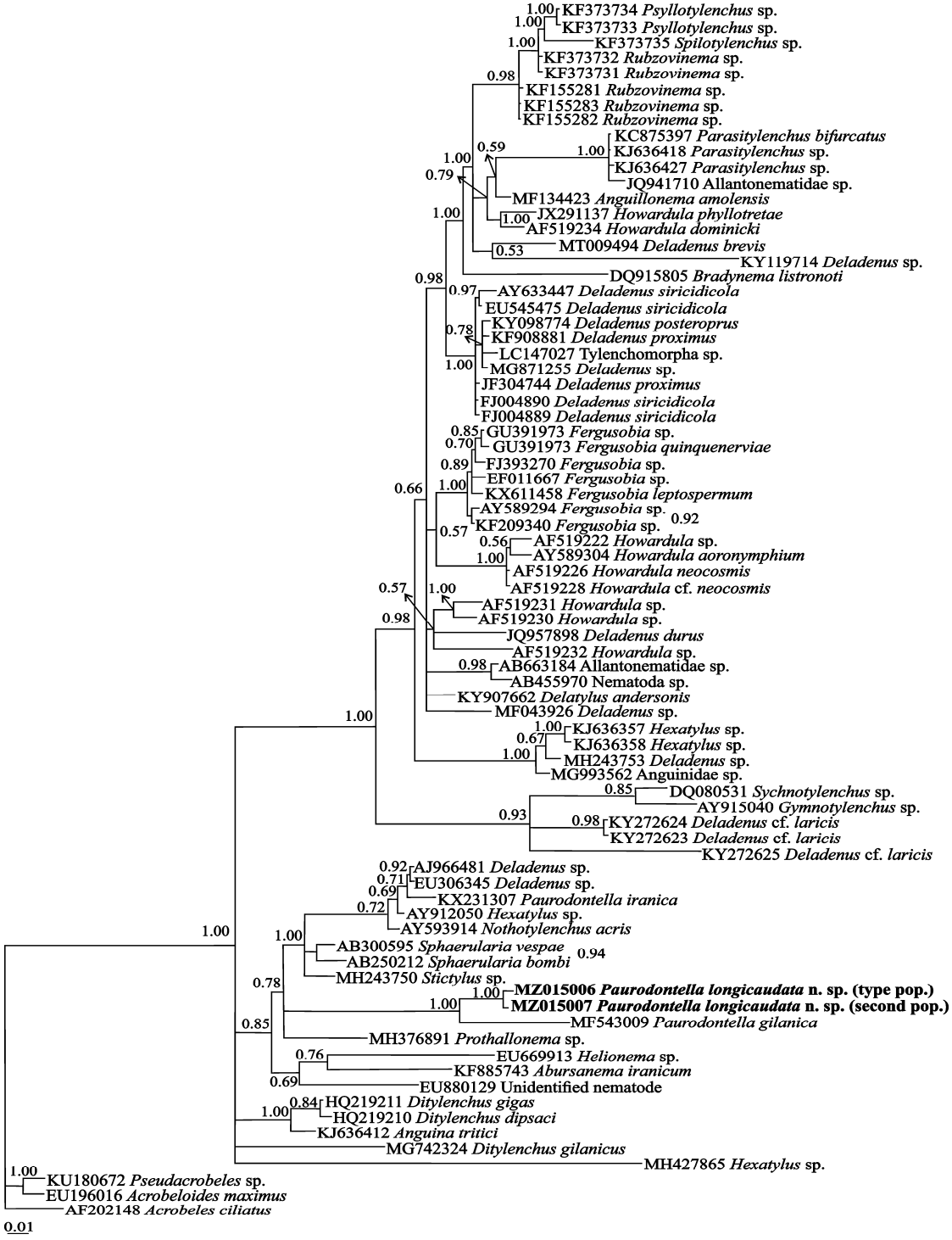

Fig. 3. Bayesian 50% majority rule consensus tree inferred using partial SSU rDNA of *Paurodontella longicaudata* n. sp. under the GTR + G + I model. Bayesian posterior probabilities (BPP) more than 50% are given for appropriate clades. New sequences are in bold font.

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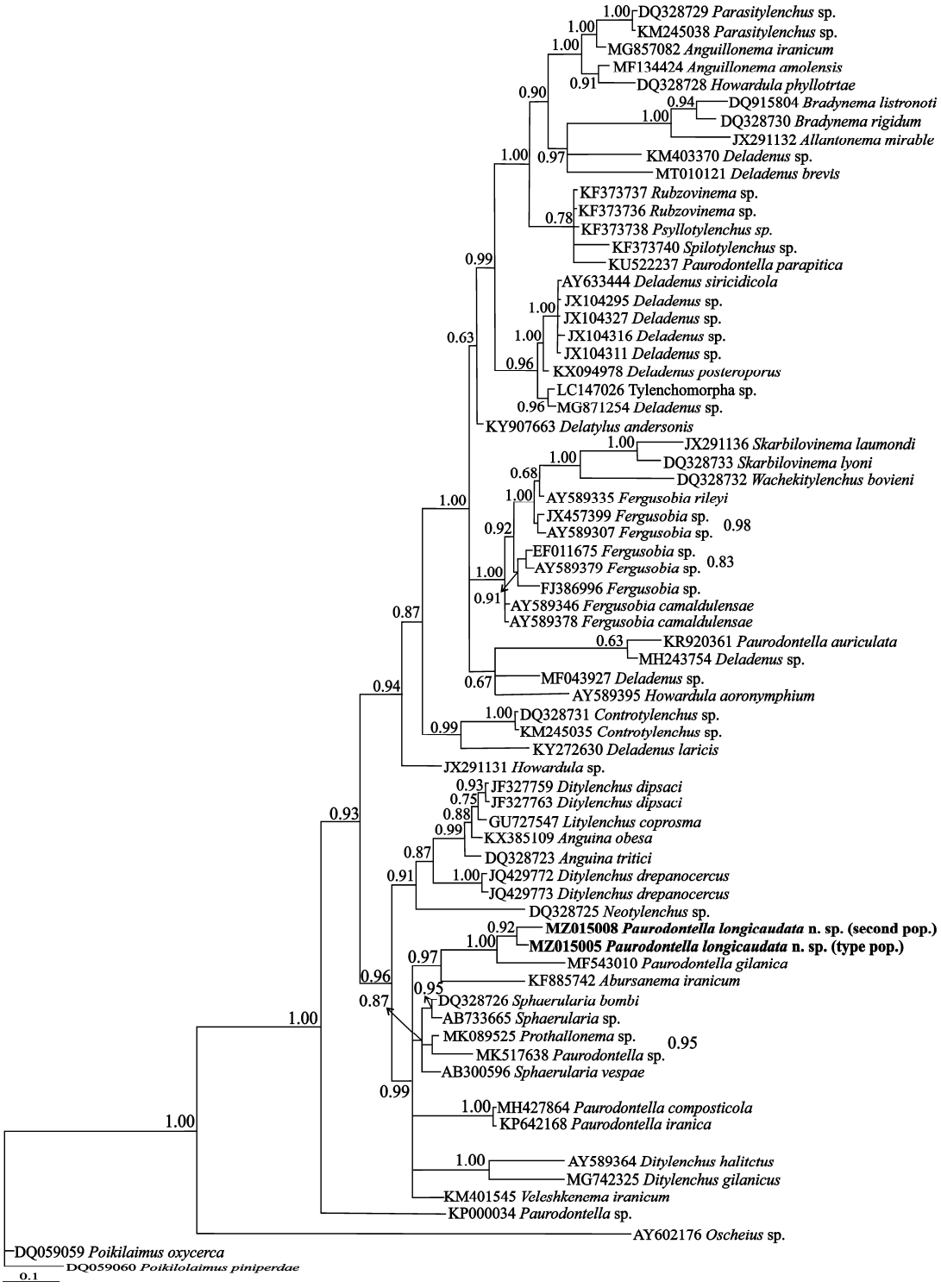

Fig. 4. Bayesian 50% majority rule consensus tree inferred using partial LSU rDNA D2-D3 of *Paurodontella longicaudata* n. sp. under the GTR + G + I model. Bayesian posterior probabilities (BPP) more than 50% are given for appropriate clades. New sequences are in bold font.

Molecular profiles and phylogenetic status

Partial SSU rDNA phylogeny

The two newly obtained SSU rDNA sequences for the new species were 868 (the type population, accession number MZ015006) and 897 nt long (the second population, accession number MZ015007). A restricted BLAST search against Paurodontidae members using the SSU sequence of the type population revealed it has the highest identity with *Paurodontella gilanica* (MF543009, 95.49% identity yielded from 35 mismatches,) and *Sphaerularia* sp. (MT002878, 91.85% identity). The SSU dataset to infer this phylogeny, included 75 sequences of the ingroup taxa and three sequences of classic rhabditid outgroups. Figure 3 represents the Bayesian phylogenetic tree inferred using this dataset. In this tree, the new species has formed a maximally supported clade with *P. gilanica* (MF543009). Currently the GenBank database is poor with the SSU sequences for the genus, but, based upon available data, *Paurodontella* seems non-monophyletic using this marker.

D2-D3 fragments of LSU rDNA phylogeny

The two newly obtained LSU D2-D3 sequences of the new species were 979 (the type population, accession number MZ015005) and 686 nt long (the second population, accession number MZ015008). The BLAST search using the LSU sequence of the type population revealed it has the highest identity with an unidentified isolate of *Prothallonema* sp. (accession number MK089525, 80.12% identity) and *Sphaerularia* sp. (accession number MT002910, 70.9% identity). Its identity with *P. gilanica* (MF543010) was 79% (more than 70 mismatches and 50 gaps). A number of 66 sequences of the ingroup taxa and three sequences of classic rhabditids as outgroup taxa were used for inferring the LSU phylogeny. Figure 4 represents the Bayesian phylogenetic tree inferred using this dataset. In this tree, the genus has been appeared as polyphyletic and the species of the genus didn't form a clade and the new species has formed a maximally supported clade with *P. gilanica*.

Discussion

According to recent studies, most sphaerularioid genera are not monophyletic using ribosomal markers (Mobasseri *et al.* 2017; Heydari *et al.* 2020). The similar observation was also made here, in both SSU and LSU trees.

The two genera *Paurodontella* and *Paurodontus* represent two similar forms under the family Paurodontidae. In the recent study, Yaghoubi *et al.* (2018) listed the useful morphological characters for species delimitation under these two close genera. In the present study, two populations of *Paurodontella* were recovered from two distant points in northern Iran. The comparisons with valid species under the genus revealed they belong to an unknown species, being described herein as *P. longicaudata* n. sp. The species *P. gilanica* has the closest morphology to it and is regarded as its cryptic species. Both species have similar morphology and close morphometric data ranges, but the number of nucleotide differences in two ribosomal markers were remarkable, as already presented. Formerly, several examples of cryptic forms were reported for soil inhabiting nematodes (e.g. Cantalapiedra-Navarrete *et al*. 2013; Soleymanzadeh *et al*. 2016; Archidona-Yuste *et al*. 2016; Naghavi *et al*. 2022).

Conflict of interest

All the authors certify that they do not have any conflict of interest.

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