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Cryptaphelenchus varicaudatus n. sp. (Rhabditida: Ektaphelenchinae) from Tehran Province, Iran

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Abstract: Cryptaphelenchus varicaudatus n. sp. is described and illustrated. It was isolated from bark samples of dead or dying pine (*Pinus* spp.) trees with bark beetle frass and galleries, in Tehran province. The new species has 275- to 367-μm-long females (a medium-sized species) with distinctly annulated cuticle having three bands in lateral fields, lip region continuous with body contour, delicate knobbed stylet, monodelphic–prodelphic reproductive system with distinct spermatheca, short postvulval uterine sac (PUS), transverse vulval slit with raised posterior lip and body narrowing behind it, sclerotized vagina, simple intestine ending in a blind sac, having no rectum but vestigial anus in some specimens, and distal body end tip (tail tip) with variation in morphology (shape), from sharply or slightly pointed to bluntly rounded. Males of the new species are common, but less frequent than females, characterized by shorter body (235–278 μm long) compared to females, their posterior body end more ventrally bent, arcuate separate spicules with well-developed wide condyles, distinct rostrum having sharp, attenuated tip. The precloacal single supplement (P1) and the distally located pair of caudal papillae close to tail tip were only observed. The new species is morphologically compared with the species of the genus having short PUS and similar body end morphology. In molecular phylogenetic analyses using 1520- and 698-nt-long sequences of small subunit (SSU) and large subunit (LSU) rDNA D2/D3 fragments, the new species formed a clade with two currently available GenBank-derived, unspecified isolates/sequences in SSU and three other isolates/sequences in LSU trees, respectively.

Key words: Aphelenchoidinae, description, LSU, phylogeny, rDNA, SSU, taxonomy.

In the checklist of Aphelenchoidea Fuchs, 1937, Hunt (2008) listed 25 valid species under the genus *Cryptaphelenchus* Fuchs, 1937. These include uncertainties on the taxonomic status and identity of some species that arose from their poor descriptions and unavailability of type specimens. There were controversies in assigning the type species (*C. macrogaster* [Fuchs, 1915] Rühm, 1956 in Massey [1974] and *C. minutus* [Fuchs, 1930] Fuchs, 1937 in Hunt [2008]) and besides no molecular data for most species in databases; some other problems with some key features of the genus such as small body size and difficulties in studying of internal body organ details and overlapping of morphometric data ranges between species further deteriorate taxonomic studies of *Cryptaphelenchus* spp. However, certain problems have made the genus as a difficult one to study. After Hunt's checklist on Aphelenchoidea including the chronologically newest species of *Cryptaphelenchus*, namely *C. diversispicularis* being described around 30 years ago, only two other species namely *C. dominicus* Poinar, 2011 and *C. iranicus* Esmaeili, Heydari, Majd Taheri, Fang, and Li, 2016 are described to date. On the other hand, there are currently only a few DNA sequences of *Cryptaphelenchus* spp. in GenBank, with most of their descriptions still not available. In the present study, an improved solution for taxonomic study of the species of the genus is proposed. A recently recovered species from Tehran province, representing an unknown species of *Cryptaphelenchus*, is described and illustrated and its phylogenetic affinities with the few currently available sequences are discussed.

Some previous studies on ektaphelenchid genera described species of the genera *Ektaphelenchus* Fuchs, 1937, *Ektaphelenchoides* Baujard, 1984, and *Devibursaphelenchus* Kakuliya, 1967 from Iran (Atighi et al., 2012, 2013a, 2013b; Pedram et al., 2012; Aliramaji et al., 2014a, 2014b, 2015; Yaghoubi et al., 2014; Alvani et al., 2016). In the present study, the recently recovered population of *Cryptaphelenchus* is described as *C. varicaudatus* n. sp.

Materials and Methods

Sampling, nematode extraction, and morphological observation: Several wood and bark samples were collected from parks in the city of Tehran. The tray method (Whitehead and Hemming, 1965) was used to extract nematodes from the barks. Nematodes of interest were handpicked under a Nikon SMZ1000 stereomicroscope, heat killed by adding boiling 4% formalin solution, transferred to anhydrous glycerine according to De Grisse (1969), mounted on permanent slides, and examined using a Nikon Eclipse E600 light microscope. Photographs were taken using an Olympus DP72 digital camera attached to an Olympus BX51 microscope powered with differential interference contrast. Drawings were made using a drawing tube attached to the microscope and were redrawn using CorelDRAW® software version 16.

DNA extraction, PCR, and sequencing: Considering small body size of the studied nematode species, DNA was extracted from 5 to 6 individuals (each DNA sample was extracted from 5–6 females) by direct squashing of nematodes in pure water or TE buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0, Qiagen) on a clean slide.

PCR was carried out in a total volume of 30 μl (19.2 μl distilled water, 3 μl 10× PCR buffer, 0.6 μl 10 mM dNTP mixture, 1.2 μl 50 mM $MgCl_2$, 1.2 μl of each primer [10 pmol/μl], 0.6 μl *Taq* DNA polymerase [5 unit/μl,

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CinnaGen, Tehran, Iran] and 3 µl DNA template) (Soleymanzadeh et al., 2016). The thermal cycling program for amplifying two fragments was as follows: denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 40 s, and extension at 72°C for 80 s. A final extension was performed at 72°C for 10 min. Primers for 28S rDNA D2/D3 amplification were forward primer D2A (5′-ACAAGTACCGTGAGGGAAAGT-3′) and reverse primer D3B (5′-TGCGAAGGAACCAGCTACTA-3′) (Nunn, 1992). Primers for amplification of 18S rDNA were forward primer 1096F (5′-GGTAATTCTGGAGCTAATAC-3′) and reverse primer 2646R (5′-GCTACCTTGTTACGACTTTT-3′) (Holterman et al., 2006) or reverse primer SSU13R (5′-GGGCATCACAGACCTGTTA-3′) (Dorris et al., 2002). The PCR products were sequenced in both directions using the same primers with an ABI 3730XL sequencer. Newly obtained sequences of the studied species were deposited in GenBank (accession number KY828212 for partial 18S rDNA and accession number KY828211 for partial 28S rDNA D2/D3).

Phylogenetic analysis: The newly obtained 18S and 28S rDNA sequences were compared with those of other aphelenchid species available in GenBank using the BLAST homology search program. For reconstruction of phylogenetic relationships, two independent 18S and 28S datasets were prepared. The selected DNA sequences (representatives of almost all Ektaphelenchinae Paramonov, 1964 available in GenBank and several other aphelenchid species/genera were selected for both 18S and 28S datasets) were aligned using the Q-INS-I algorithm of online version of MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/) (Katoh and Standley, 2013). The Gblocks program (version 0.91b) with all the three less stringent parameters, a server tool at the Castresana Lab (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) was used for postediting of the alignments, i.e., to eliminate poorly aligned regions or divergent positions.

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 18S and 28S analyses. Bayesian analysis was performed using MrBayes v 3.2 (Ronquist et al., 2012) running the chains for 10 million generations for both datasets. After discarding burn-in samples, the remaining samples were retained for further analyses. The Markov chain Monte Carlo method within a Bayesian framework was used to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule. Convergence of model parameters and topology were assessed based on average standard deviation of split frequencies and potential scale reduction factor values. Adequacy of the posterior sample size was evaluated using autocorrelation statistics as implemented in Tracer v.1.6 (Rambaut and Drummond, 2009). A maximum likelihood (ML) tree was reconstructed by using RaxmlGUI 1.1 (Silvestro and Michalak, 2011) software using the same nucleotide substitution model as in the BI in 1,000 bootstrap (BS) replicates for both datasets. Two panagrolaimid species were used as outgroup taxa in the SSU tree. The species *Panagrellus redivivus* (Linnaeus, 1767) Goodey, 1945 (accession number in LSU tree) was used as outgroup taxon in the LSU tree. The output files of the phylogenetic programs were visualized using Dendroscope V.3.2.8 (Huson and Scornavacca, 2012) and redrawn in CorelDRAW software version16. The Bayesian posterior probability (BPP) and ML BS values exceeding 0.50% and 50%, respectively, are given on appropriate clades in the shape BPP/ML BS.

Results

Cryptaphelenchus varicaudatus n. sp.[*] (Figs. 1,2)

Measurements: See Table 1.

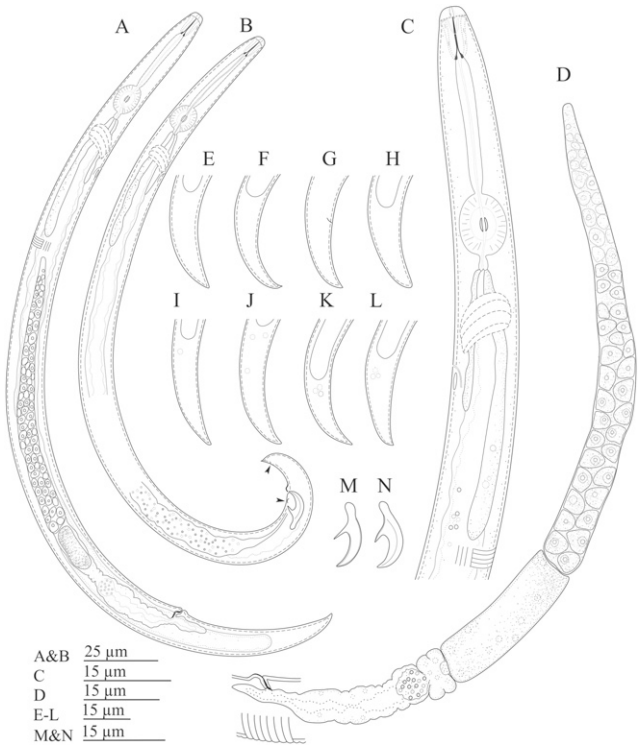

Fig. 1. Line drawings of *Cryptaphelenchus varicaudatus* n. sp. A, B. Female and male entire body. C. Pharynx details. D. Female reproductive system. E–L. Variation in morphology of posterior body end. M, N. Spicules.

[*] The specific epithet refers to observed variation in body end (tail tip) morphology.

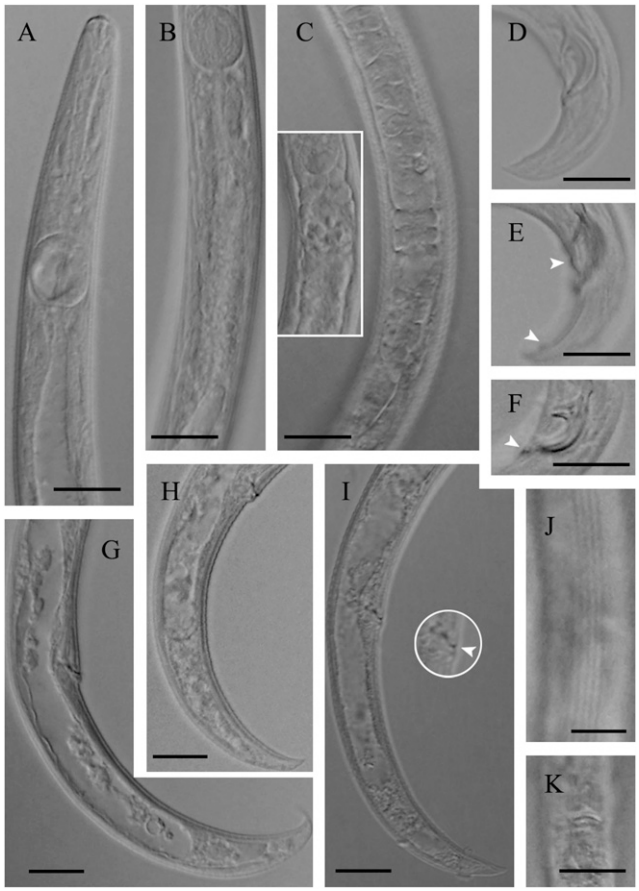

FIG. 2. Light microphotographs of *Cryptaphelenchus varicaudatus* n. sp. A. Anterior pharynx. B. Posterior pharynx, and proximal end of ovary. C. Partial female reproductive system showing empty and filled spermatheca. D–F. Male posterior body end and spicules details. G–I. Details of female posterior body region (I, arrow showing sclerotized vagina). J. Three bands in lateral line. K. Vulval slit in ventral view. All scale bars = 10 μm; J = 5 μm.

TABLE 1. Morphometrics of *Cryptaphelenchus varicaudatus* n. sp.

	Female		Male
	Holotype	Paratypes	Paratypes
n	-	19	9
L	317	315.0 ± 25.3 (275–367)	256.0 ± 15.4 (235–278)
a	25.5	24.0 ± 1.4 (21.7–26.5)	23.5 ± 1.7 (21–26)
b	6.7	6.8 ± 0.6 (5.9–8.0)	6.2 ± 0.3 (5.9–6.7)
c	-	-	15.1 ± 1.0 (14.0–16.4)
c'	-	-	1.9 ± 0.1 (1.8–2.0)
V	78.2	79.5 ± 1.6 (75.1–81.8)	-
Stylet total length	8	7.6 ± 0.4 (7.0–8.5)	7.0 ± 0.7 (6–8)
m	43.8	41.0 ± 2.8 (37.5–43.8)	-
MB%	89.5	86.2 ± 3.4 (76.6–89.6)	-
Pharynx	47	46.8 ± 3.0 (41–53)	41.5 ± 2.3 (38–44)
E. pore	59	59.0 ± 3.8 (52–65)	52.2 ± 1.8 (50–55)
Body width at median bulb	11	11.5 ± 0.7 (10–12)	-
at midbody	12.5	13 ± 1 (12–15)	10.9 ± 1.0 (9–12)
at cloaca	-		9.1 ± 0.7 (8–10)
PUS	8.5	8.4 ± 0.6 (8–9)	-
Tail	-	-	16.8 ± 1.2 (16–17)

PUS = postvulval uterine sac.
All measurements are in μm and in the form: mean ± SD (range).

DESCRIPTION

Females (type population)

Slender nematodes: Body ventrally curved after heat relaxation, open to moderately close *C*, slightly tapering toward both ends, more toward posterior end. Cuticle with distinct annuli (annulus 1.1–1.5 μm wide) and four equally distant lines, forming three bands. Lip region continuous with body contour, 4.0 to 5.5 μm wide, 1.5 to 2.5 μm high, with moderately sclerotized framework. Stylet delicate, lacking a distinct lumen (the lumen might probably present, but not seen due to small size and low width), conus ca. 3 μm, knobs small, ca. 1 μm distant from each other, apparently drop shaped, sloping backward. Procorpus slender, its lumen and border lines hardly visible in mounted individuals in glycerin, metacorpus rounded to oval (in few individuals, probably due to preparation pressures), its valve distinct, platelets well sclerotized, centrally to slightly anteriorly located, pharyngeal glands forming a relatively long dorsal overlap. Intestine simple, ending in a blind sac, rectum always invisible, anus usually not developed, sometimes vestigial. Nerve ring at about less than one metacorpus length posterior to it. Hemizonid at 58 to 63 μm distance from anterior end. Reproductive system a monodelphic–prodelphic single tube, composed of an outstretched ovary, its oocytes mostly in two rows, except in germinal zone at proximal end, sometimes with a mature oocyte in distal region, oviduct short with four cells in lateral view, rounded spermatheca with or without fine spheroid sperm cells, in latter case with empty chamber, crustaformeria and uterus not discernible from each other, uterus thick walled, vagina sclerotized, slightly anteriorly directed, PUS 0.6 to 0.7 vulval body width (VBW) long, vulva a transverse slit, its posterior lip raised, and body remarkably narrowing posterior to it. No specific structure at the junction of uterus and PUS. Vulva-body end distance 50 to 83 μm or 4.2 to 5.9 times VBW long. Posterior body end conical, slightly ventrally bent, distal tip sharply or slightly pointed, to bluntly rounded.

Males (type population)

Slender nematodes: General morphology, anterior end characters and pharynx similar to that of females, smaller. Reproductive system monorchic, testis not

TABLE 2. Species of the genus *Cryptaphelenchus* having reliable/accessible morphological and morphometric data, some useful morphological characters for species delimitation, and used references.

Species	*L* (μm) (female)	PUS status	Vulva-body end/VBW	Reference
C. macrogaster (Fuchs, 1915) Rühm, 1956	?	No visible PUS	ca. 6	Fuchs, (1915), Figs. 40–44. Type species in Massey (1974, page 208)
C. aedili Lazarevskaya, 1961	324–416	No PUS	ca. 4.9	Lazarevskaya (1961), original description
C. bidentati (Rühm, 1954) Paramonov, 1964	365–422	No PUS	ca. 4.6	Drawing in Rühm (1954), page 234
C. cirrus Massey, 1974	300	No PUS	ca. 5.3	Massey (1974), original description
C. diversispicularis Korentchenko, 1987	370–538	No PUS	?	Korentchenko (1987), original description
C. ipinius Massey, 1974	430	No PUS	ca. 4.8	Massey (1974), original description
C. ligniperdae Kurashvili, Kakulia, and Devdariani, 1980	300	No PUS	ca. 4	Kurashvili et al. (1980), original description
C. quadridens Kakulia, 1963	210–255	No PUS	ca. 4.7	Data and drawing in Kakulia (1989), page 56
C. ryjikovi Kurashvili, Kakulia, and Devdariani, 1980	275	No PUS	ca. 3.3	Kurashvili et al. (1980), original description
C. vorontzovi Kurashvili, Kakulia, and Devdariani, 1980	275–330	No PUS	ca. 4.6	Kurashvili et al. (1980), original description
C. weiseri Devdariani, 1975	200–230	No PUS	ca. 4.3	Data and drawing in Kurashvili et al. (1980), page 71
C. bicoloris Devdariani, 1971	?	ca.2.2 times VBW	ca. 4.2	Data and drawing in Kurashvili et al. (1980), pages 68–69
C. borlossi Lazarevskaya, 1963	275–390	ca. 1.5–1.6 times VBW	4.8–5.7	Lazarevskaya (1963), original description
C. hectographi Rühm, 1957 in Rühm and Chararas, 1957	285–308	ca. 2.3 times VBW	ca. 5.3	Rühm (1957) in Rühm and Chararas (1957), original description
C. iranicus Esmaeili, Heydari, Majd Taheri, Fang, and Li, 2016	250–330	ca. 0.6–1.0 times VBW	6.4–7.6	Esmaeili et al. (2016), original description
C. koerneri Rühm, 1956	270–372	ca. 2.7 times VBW	ca. 4.8	Rühm (1956), original description
C. latus (Thorne, 1935) Rühm, 1956,	400	ca. 1.8 times VBW	ca. 3.8	Data and drawing in Thorne (1935), page 140
C. leptocaudus Rühm, 1956,	325–382	ca. 2.9 times VBW	ca. 6.1	Rühm (1956), original description
C. malpighius (Fuchs, 1937) Rühm, 1956	207–263	ca. 2.4 times VBW	ca. 6.9	Data and drawing in Fuchs (1937), pages 362–363
C. varicaudatus n. sp.	275–367	ca. 0.6–0.7 times VBW	4.2–5.9	Present study

PUS = postvulval uterine sac; VBW = vulval body width.
The ratios are calculated from drawings or morphometric data.

clearly seen in examined individuals, sperm in vas deferens fine, spheroid, similar to those inside females' spermatheca. Posterior body end much ventrally bent. Spicules arcuate, separate, with well-developed wide condylus and distinct rostrum, and attenuated tip with no differentiation. The precloacal supplement (P1, at 3–6 μm distance anterior to cloacal opening) and the distally located pair of caudal papillae at 3 to 4 μm to tail tip were only observed. Posterior cloacal lip sclerotized, body remarkably narrowing posterior to it. Tail conical, dorsally convex, ventrally slightly concave with sharp tip.

Type habitat and locality: The new species was recovered from bark samples of dead or dying *Pinus* spp. having frass and galleries of bark beetles collected in Pounak region, city of Tehran, Tehran province, Iran.

Type material: Holotype female and paratype females deposited in the Nematode Collection at the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. Several voucher individuals were deposited in each of the following collections: UGent Nematode Collection of the Nematology Research Unit, Department of Biology, Ghent University, Ghent; Belgium USDA Nematode Collection, Beltsville, MD; and WANECO collection, Wageningen, the Netherlands (http://www.waneco.eu/).

Diagnosis and relationships: Cryptaphelenchus varicaudatus n. sp. has medium-sized females compared to body length of the species in Table 2 and belongs to the group of species having a PUS. It is further characterized by having distinctly annulated cuticle and three bands in lateral fields, lip region continuous with body contour and moderately sclerotized cephalic framework, weak but knobbed stylet, mondelphic–prodelphic reproductive system with short PUS, sclerotized vagina, simple intestine ending in a blind sac, vestigial anus in some specimens, and distal body end tip (tail tip) with variation in morphology, from sharply or slightly pointed to bluntly rounded tip, less frequent smaller males with typical form of arcuate separated spicules with well-developed wide condyles, distinct rostrum with sharp tip and pointed end, a precloacal single supplement (P1), and one pair of caudal papillae close to tail tip. Compared to all species of the genus having a PUS, except *C. iranicus* having a short PUS but with different posterior body end (tail) shape, the new species has a remarkably smaller PUS that is 0.6 to 0.7 times VBW long. The detailed comparison with some morphologically close species is as follows.

Compared to poorly known species, *C. bicoloris*, the new species has basic difference in PUS length (0.6–0.7

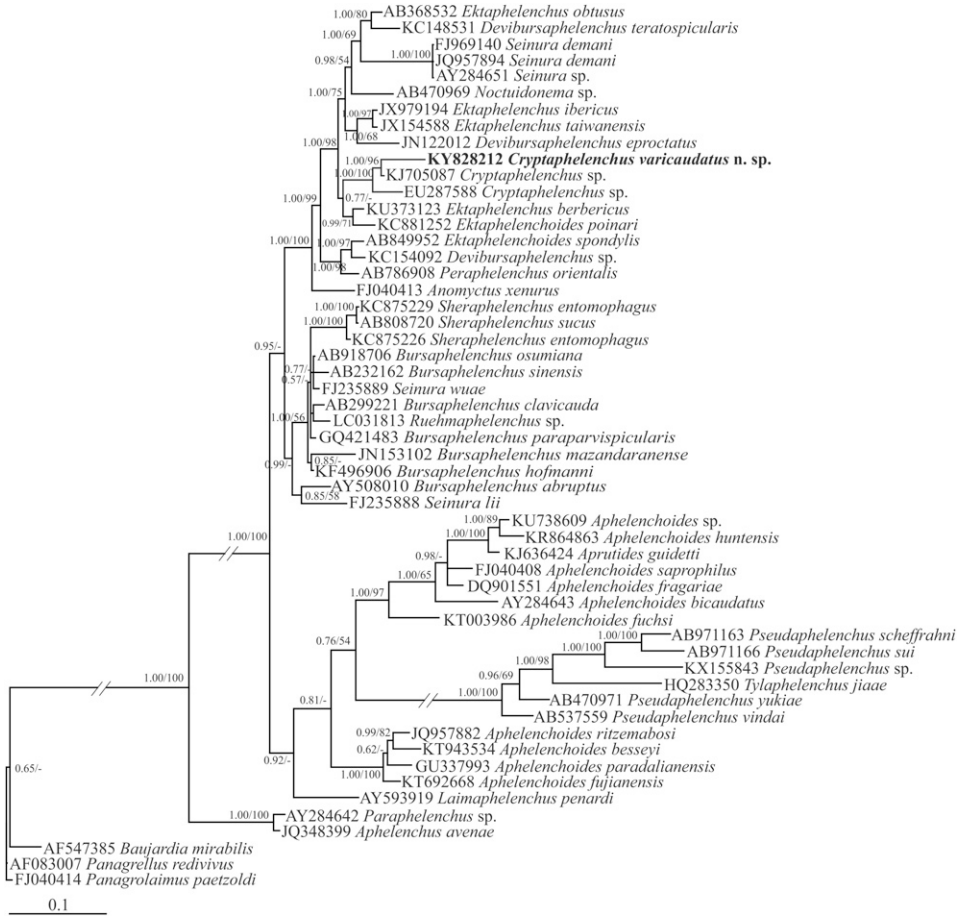

FIG. 3. Bayesian 50% majority rule consensus tree inferred from SSU rDNA sequence of *Cryptaphelenchus varicaudatus* n. sp. under the GTR + G + I model. Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap (ML BS) values greater than 50% are given for appropriate clades in the form: BPP/ML BS. New sequence is in bold font.

times VBW *vs* ca. 2.2), body end not narrowing distally (*vs* narrowing) and differences in male tail characters, e.g., shape (dorsally convex, ventrally concave with sharp tip *vs* conical, uniformly narrowing distally), *c* (14.0–16.4 *vs* 11) and *c′* value (1.8–2.0 *vs* ca. 2.3). There are other differences in number and arrangement of male supplements between the two species.

Compared to *C. borlossi*, beside basic difference in PUS length (0.6–0.7 times VBW *vs* 1.5–1.6), the new species has shorter stylet (7–8 *vs* 10–11 μm) with distinct knobs separate from each other (*vs* knobs much smaller, and apparently not separate) and well-developed rostrum with sharp tip (*vs* apparently short and blunt).

Compared to *C. latus*, beside difference in PUS length (0.6–0.7 times VBW *vs* ca. 1.8 times), the new species has very small drop-shaped knobs separated from each other (*vs* well developed, and rounded), moderately annulated cuticle (*vs* apparently coarsely annulated), and much shorter males (235–278 *vs* 400 μm long).

Compared to *C. malpighius*, beside difference in PUS length (0.6–0.7 times VBW *vs* ca. 2.4), the new species has longer females (275–367 *vs* 207–263 μm) and males (235–278 *vs* 183–228 μm) and female distal body end not narrowing toward tip (*vs* narrowing).

Molecular phylogenetic studies: Partial sequencings of SSU and LSU rDNA D2/D3 fragments yielded single sequences of 1,520 and 698 nt long, respectively. BLAST search using these sequences revealed the SSU sequence has the highest coverage and identity with an unidentified species of *Cryptaphelenchus* (*Cryptaphelenchus* sp., accession number EU287588, 95% coverage, 96% identity) and the partial LSU sequence has the highest coverage and identity (96% and 94%, respectively) with *C. iranicus* (accession number: KT895255). Almost all available SSU and LSU rDNA sequences deposited in GenBank database for Ektaphelenchinae members and representatives of several other aphelenchid genera were selected for reconstructing of the 18S and 28S phylogenetic trees. Several classic rhabditid genera/species (see Figs. 3 and 4 for the species and accession numbers) were selected as outgroup taxa for both phylogenetic trees according to previous phylogenetic studies on Tylenchomorpha De Ley and Blaxter, 2002. Two separate 18S and 28S datasets were prepared for reconstruction of phylogenetic trees. The 18S dataset included 54 sequences, composed of 1,470 total characters

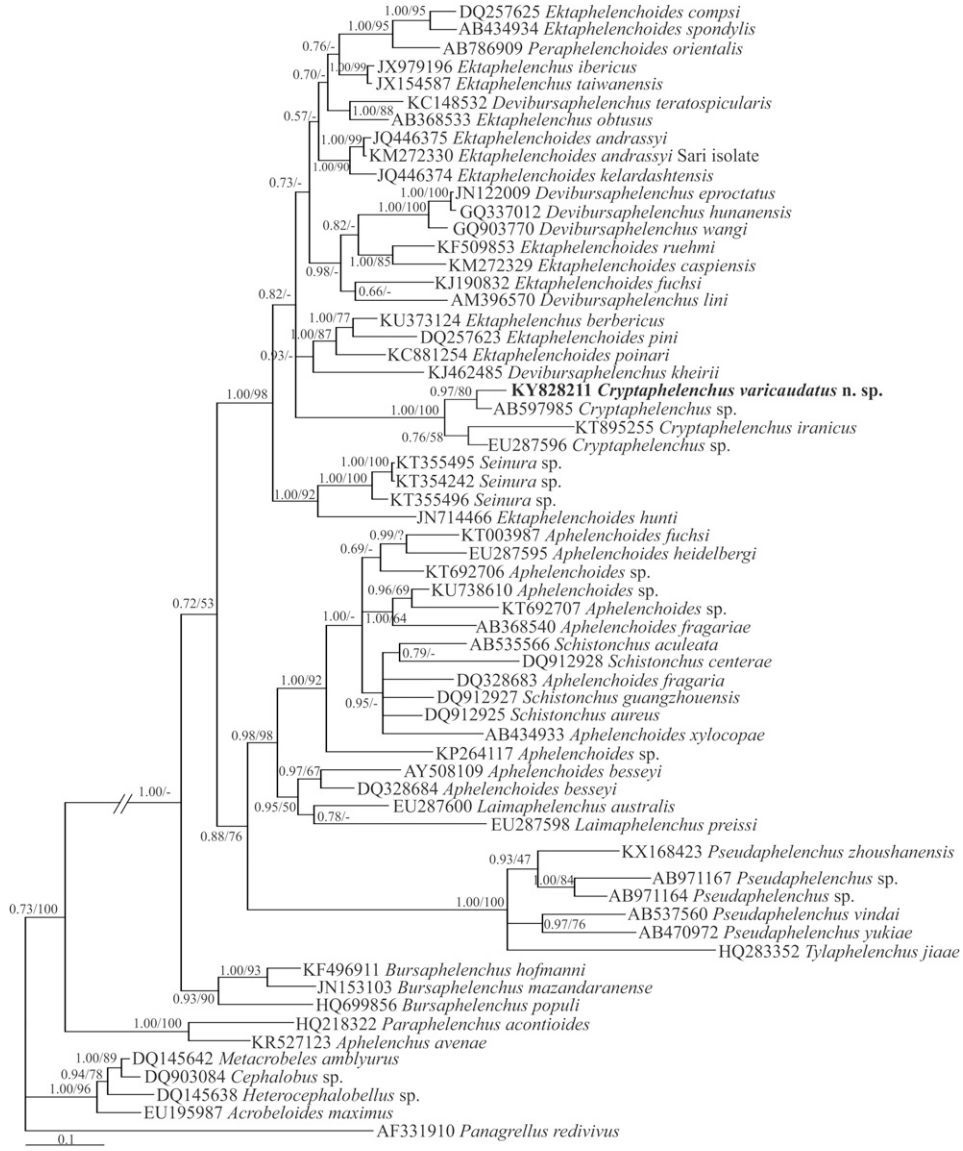

FIG. 4. Bayesian 50% majority rule consensus tree inferred from LSU rDNA D2-D3 sequence of *Cryptaphelenchus varicaudatus* n. sp. under the GTR + G + I model. Bayesian posterior probabilities (BPP) and maximum likelihood bootstrap (ML BS) values greater than 50% are given for appropriate clades in the form: BPP/ML BS. New sequence is in bold font.

having 758 variable characters. Figure 3 represents the phylogenetic tree inferred using this dataset. The three currently available SSU sequences of *Cryptaphelenchus* formed a clade in this tree with maximal BPP and ML BS values (1.00/100%) inside the major clade, containing several ektaphelenchid genera. This clade again formed a clade with two species of the genera *Ektaphelenchus* and *Ektaphelenchoides* (accession numbers KU373123 and KC881252) with moderate BPP but with no support with the ML method. More SSU sequences will be helpful to further elucidate the phylogenetic relations of *Cryptaphelenchus* spp. using this genomic fragment. The 28S dataset included 62 sequences, composed of 502 total characters with 313 characters being variable. Figure 4 represents the phylogenetic tree inferred using this dataset. The new species and three other species have formed a fully supported clade in both BI and ML methods in this tree. In the clade of *Cryptaphelenchus* spp., the new species is in close phylogenetic affinity with an unidentified species (*Cryptaphelenchus* sp., accession number: AB597985). As in the case for SSU sequences, there are currently only few LSU sequences for this interesting rare nematode genus. Further tentative sequences will help to assess its monophyletic nature and unravel the phylogenetic relation of its species with other ektaphelenchid species and genera.

DISCUSSION

Some species of *Cryptaphelenchus* are poorly described and there are no updated data or new reports for many of them. From the valid species listed by Hunt (2008), reliable morphological or morphometric data are

accessible only for 19 species. These are listed in Table 2 in the present study with some useful morphological characters for species delimitation being included. The new species was also morphologically compared with these species. Massey (1974) regarded the species *C. macrogaster* as the type species, a framework followed in the present study too. Species of the genus could morphologically be divided in two groups, those with and those without a PUS (an update to Hunt [1993], reporting the genus lacks PUS). The recently described species, *C. iranicus*, (disregarding the species *C. dominicus* described in 2011), is also included in Table 2. Overlaps in the range of some morphometric data highlight the usefulness of some other morphological features such as PUS and shape of posterior body end in distinguishing *Cryptaphelenchus* spp. Some other characters such as shape of spicules are less variable, and have conserved morphology (i.e., the spicules of *Cryptaphelenchus* spp. are arcuate, their tips pointed, the condyles well developed, wide/round, the rostrum distinctly developed usually with sharp tip). The total body length range of the genus is 207 to 430 μm for 17 species (all species, except the range 370–538 μm for *C. diversispicularis* Korentchenko, 1987 and two species body length showed by "?" in Table 2). Thus, the remarkable small body size, a rounded median bulb (sensu Kanzaki and Giblin-Davis, 2012), and probably the shape of spicules could be regarded as key morphological characters demarcating the genus *Cryptaphelenchus*.

The biology and feeding habits are other issues, still needing further studies. Hunt (1993) made a discussion on bionomics of the genus. According to him, and subsequent confirmation by Kanzaki and Giblin-Davis (2012), most species are in association with frass and galleries of bark beetles, and are apparently mycetophagous. A predatory feeding behavior, however, is not presumed for the genus (compared to predatory behavior common in the subfamily). Although the new species was not reared on fungus plates, an unidentified species of the genus was successfully cultured on *Botrytis cinerea* in this lab. The recently described species, *C. iranicus*, was also successfully cultured on *Botryotinia fuckeliana*.

Including molecular data for tentative future new species or newly recovered populations of known species will further reveal usefulness of these markers in taxonomy of *Cryptaphelenchus*. In the present study, partial SSU and LSU data of the new species, in addition to previously available sequences, again confirmed placement of *Cryptaphelenchus* in Ektaphelenchinae. However, the monophyletic nature of the genus could not be tested using only the few available sequences. The basal placement of the genus in Ektaphelenchinae as hypothesized by Kanzaki and Giblin-Davis (2012) needs further sequences and analyses, however, this placement could be deduced in the SSU tree with currently available data. The present study did not aim to discuss phylogenetic relations of Ektaphelenchinae, but emphasizes inclusion of molecular data from at least two genomic regions of the species in the future descriptions, so, the phylogenetic affinities of this rare genus could be assessed. In the present paper, a solution to classic taxonomic studies of rare genus *Cryptaphelenchus* spp. is proposed and the list of the species accessible morphological data was presented and a proposal for comparing of the recovered populations in the future was given. Two new molecular sequences were also provided.

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