

***Butlerius butleri* Goodey, 1929 (Rhabditida) from Iran with the Phylogenetic Position of the Species**

EBRAHIM SHOKOOHI,^{1,2} HADI PANAHI,¹ HENDRIKA FOURIE,² AND JOAQUÍN ABOLAFIA³

Abstract: A population of *Butlerius butleri* Goodey, 1929 was isolated from vermicompost in Kerman in the Kerman Province of Iran during a nematode survey that was conducted during 2014. This population of *B. butleri* is characterized by the presence of a dorsal thorn-like tooth (4 to 5 µm long), long spicules (44 to 47 µm long), gubernaculum (33 to 37 µm or more than half of the spicule length), three pairs of precloacal papillae, five pairs of postcloacal papillae (papillae V3 and V5 comprising three small papillae), and a long filiform tail (304 to 409 µm in females, 312 to 380 µm in males). Molecular and phylogenetic analysis of *B. butleri* individuals from this Iranian population based on 18S ribosomal deoxyribonucleic acid (rDNA) sequence placed this species close to *Pseudodiplogasteroides compositus* (AB597237) and an unidentified *Pseudodiplogasteroides* species (AB597238). Measurements, illustrations, and the phylogenetic tree, including the position of *B. butleri* are provided.

Key words: *Butlerius*, description, Iran, phylogeny, taxonomy, 18S rDNA.

Biocontrol of nematode pests is becoming more important as highly effective, but toxic nematicides are progressively being withdrawn from world markets (Zuckerman and Esnard, 1994; Nico et al., 2004). *Butlerius* constitute a genus that can play an important role in reducing population levels of plant parasitic nematodes (Khan and Kim, 2007). Moreover they also contribute toward stimulating the cycling of plant nutrients, which may enable plants to better withstand any nematode burden on their roots (Yeates and Wardle, 1996). Although members of the family Diplogasteridae occupy many different ecological niches, they are not rich in terms of species being identified (Abolafia, 2006). This group of nematodes represent predators (Khan and Kim, 2007), with *Mononchoide* being the most important genus (Khan and Kim, 2007). Another predator species, *Butlerius degrissei* however, also feeds on bacteria present in the vicinity of its niche if plant parasitic nematodes are not present (Grootaert et al., 1977). The taxonomic position of the genus *Butlerius* was established by Goodey in 1929. Individuals belonging to this genus are characterized by the presence of long labial setae, stomas that are divided in two parts: thorn-like teeth and filiform tails. Individuals of this species inhabit rotting materials and compost, but rarely aquatic niches (Andrássy 2005). Andrássy (1984) proposed the genus *Monobutlerius* to represent the monodelphic species, whereas later Ebsary (1986) proposed the genus to be *Parabutlerius*. Sudhaus and Fürst von Lieven (2003), however, considered both these genera as *Butlerius*. In addition, Andrássy (2005) suggested separation of *Butlerius* and *Monobutlerius* mainly based on their female genital systems (monodelphic and amphidelphic)

and presence/absence of the postvulval sac. Finally, Ahmad et al. (2009) redescribed *B. butleri* Goodey, 1929 and synonymized this species with *B. filicaudatus* Adam, 1930 and *B. singularis* Lordello and Zamith, 1959.

Thus far, the genus *Butlerius* has not been studied using rDNA. Therefore, the goals of this investigation were to (i) amplify the 18S rDNA marker using deoxyribonucleic acid (DNA) from the Iranian *Butlerius* specimens, (ii) describe the juvenile stage of *B. butleri* (not described previously) from Iran, and (iii) elucidate the phylogenetic position of this *B. butleri* species within members of the family Diplogasteridae.

The manuscript thus presents a new taxonomic report of *B. butleri* collected from vermicompost in Iran along with its molecular characterization and phylogenetic position based on use of the 18S rDNA marker.

MATERIAL AND METHODS

Nematode extraction: Nematodes were extracted from vermicompost samples by means of the Baermann (1917) funnel technique. Extracted individuals were then fixed with a hot 4% formaldehyde solution and transferred to anhydrous glycerin using the method of De Grisse (1969). Measurements of the nematode individuals and their corresponding organs or structures were done with an Olympus CH-2 light microscope (Japan) that is furnished with an ocular micrometer and/or a curvimeter and drawing tube. The terminology used to describe the morphology of the stoma and spicules of specimens represent those proposed by De Ley et al. (1995) and Abolafia and Peña-Santiago (2006), respectively.

Phylogenetic analysis: DNA was extracted from nematode individuals using the methods of Subbotin et al. (2006) and Mehdizadeh et al. (2013). Five individuals were picked using a fine-tip needle and transferred to a 1.5 ml capacity Eppendorf tube containing 25 µl of double distilled water. The presence of the specimens in the tubes was verified using an Olympus CH-2 light microscope. The tube, containing the nematode

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¹Nematology Laboratory, Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

²Unit for Environmental Sciences and Management, Potchefstroom, North-West University, South Africa.

³Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Campus "Las Lagunillas" s/n, 23071-Jaén, Spain.

E-mail: ebrahim.shokoohi@nwu.ac.za; eshokoohi@uk.ac.ir

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individuals, was submerged in liquid nitrogen to allow freezing of the specimens. The frozen specimens were then crushed in the tube using the tip of a needle and the content vortexed and mixed. Following this procedure, 2 μl of tissue lysis buffer and 2 μl proteinase K (20 $\text{mg}\cdot\text{ml}^{-1}$) were added to the nematode substrate and the homogenate was incubated at 56°C for 1 hr and then at 95°C for 10 min. The supernatant was then extracted from the tube and stored at -20°C. Following this step, the forward primer SSU_F_04 (5'-GCTTGTC TCAAAGATTAAGCC-3') and reverse primer SSU_R_26 (5'-CATTCTTGCAAATGCTTTCG-3') (Blaxter et al., 1998) were used in the polymerase chain reaction (PCR) reactions for amplification of the partial sequence of the 18S rDNA. Subsequently, PCR was conducted with 8 μl of the PCR product of the nematode specimens to which 2.5 μl of PCR buffer, 0.5 μl of deoxynucleotide, 1 μl of MgCl_2 , 0.3 μl of Taq polymerase (Sinagen, Iran), 1 μl of each primer listed above (10 $\text{pmol}\cdot\mu\text{l}^{-1}$), and finally double distilled water added to comprise a final volume of 25 μl . The amplification was done using an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany), with the following program: initial denaturation for 3 min at 94°C, 37 cycles of denaturation for 45 sec at 94°C, extension for 45 sec at 56°C, and annealing for 1 min at 72°C, and finally an extension cycle of 6 min at 72°C followed by a holding temperature of 4°C. After DNA amplification, 5 μl of product was loaded on a 1% agarose gel in TBE buffer (40 mM Tris, 40 mM boric acid, and 1 mM ethylenediaminetetraacetic acid) for evaluation of the DNA bands. The bands were stained with 50 mM ethidium bromide and visualized and photographed on an ultraviolet transilluminator. The DNA product was next stored at -20°C prior to sequencing, and then sequenced with the primers used for amplification as explained above. The PCR product purified for sequencing by the Macrogen Corporation (Republic of Korea). Publicly available sequences for other Diplogasteroidea were obtained from NCBI GenBank for this study. Also an outgroup, *Bunonema reticulatum* Richters, 1905 (AY593925) for 18S rDNA phylogenetic analysis based on a study by Mehdizadeh et al. (2013), was obtained for comparison. The ribosomal small subunit (SSU) sequences were analyzed and aligned using BioEdit (Hall, 1999). The length of alignment is 1,863 bps, and the base substitution model was evaluated using jModeltest 0.1.1 (Posada, 2008). Phylogenetic trees were generated using the Bayesian inference method as implemented in the program Mr Bayes 3.1.2 (Ronquist and Huelsenbeck, 2003). The analysis HKY+G model was selected using jModeltest 0.1.1 (Posada, 2008). The selected model was initiated with a random starting tree and run with the Markov chain Monte Carlo for 10^6 generations. The Bayesian tree was ultimately visualized using the TreeView program (Page, 1996).

RESULTS SYSTEMATICS

Butlerius butleri Goodey, 1929 (Figs. 1,2, Table 1)

Description

Adult females: Body slightly arcuate ventrally when heat-killed. Cuticle 1.2 to 1.8 μm thick, lacking punctuation, with 14 to 16 prominent longitudinal ridges at midbody, all ridges equidistant and bearing fine transverse striations, and 0.9 to 1.1 μm wide at midbody. Lip region continuous with body contour, consisting of six fused lips, edge of lip region with six additional cephalic setae (5.9 to 6.6 μm long). Amphids with aperture oval shaped, located at level of the dorsal tooth, about 4.8 to 5.2 μm wide. Stoma about 1.5 to 2.3 times longer than wide. Cheilostom with 8 to 10 narrow rib-like plates (cheilorhabdia), bifurcate at the apex, and extend beyond the labial contour when posterior stomatal elements are retracted; inner wall of cheilostom cuticularized. Second part of stoma consisting of gymnostom and stegostom, both unisotropic with subventral walls slightly longer than dorsal side. Gymnostom 10- μm wide, forming a cuticular isomorphic cylinder with its anterior edge not serrated; its dorsal side cuticularized, thickened at the middle that remains more uniformly in its shape. Promesostegostom wider in dorsal side and relatively thin in subventral side. Metastegostom bearing dorsally a prominent thorn-like tooth, 3.8 to 4.8 μm long and 4.8 to 5.2 μm wide, pointed toward anterior part, having the duct of the dorsal gland, subventrally a smaller sickle-shaped right tooth. Posterior part of stegostom (= meta- and telostegostom) forming a cylindrical tube, 5.6 to 7.5 μm wide and 6.6 to 7.5 μm long; a minute denticle observed in the subventral wall of the stegostom. Pharynx diplogasteroid; pharyngeal procorpus cylindrical, 2.1 to 2.3 times metacarpus length; metacarpus swollen; postcorpus (isthmus + basal bulb) robust, shorter in males, lacking glottoid apparatus; isthmus-basal bulb junction not distinguishable. Cardia conoid, surrounded by intestinal tissue. Neck region comprising 16% to 19% of total body length. Nerve ring at 55% to 61% of neck length, at isthmus level. Excretory pore situated at level of the anterior part of isthmus, at 147 to 181 μm from the anterior end or at 61% to 71% of neck length. Hemizonid in front of the excretory pore. Deirid not visible. Reproductive system didelphic-amphidelphic, with both branches equally developed and reflexed terminus often reaching near the vulva level; ovaries totally reflexed (= antidromous flexion) with oocytes arranged in one, then in two rows in the germinative zone; germinative and growth zones slightly broad, distinctly separated from each other; oviducts narrow and short, with the spermatheca not set off from the uterus; uteri 1.7 to 2.8 times as long as the corresponding body diameter; a pair of

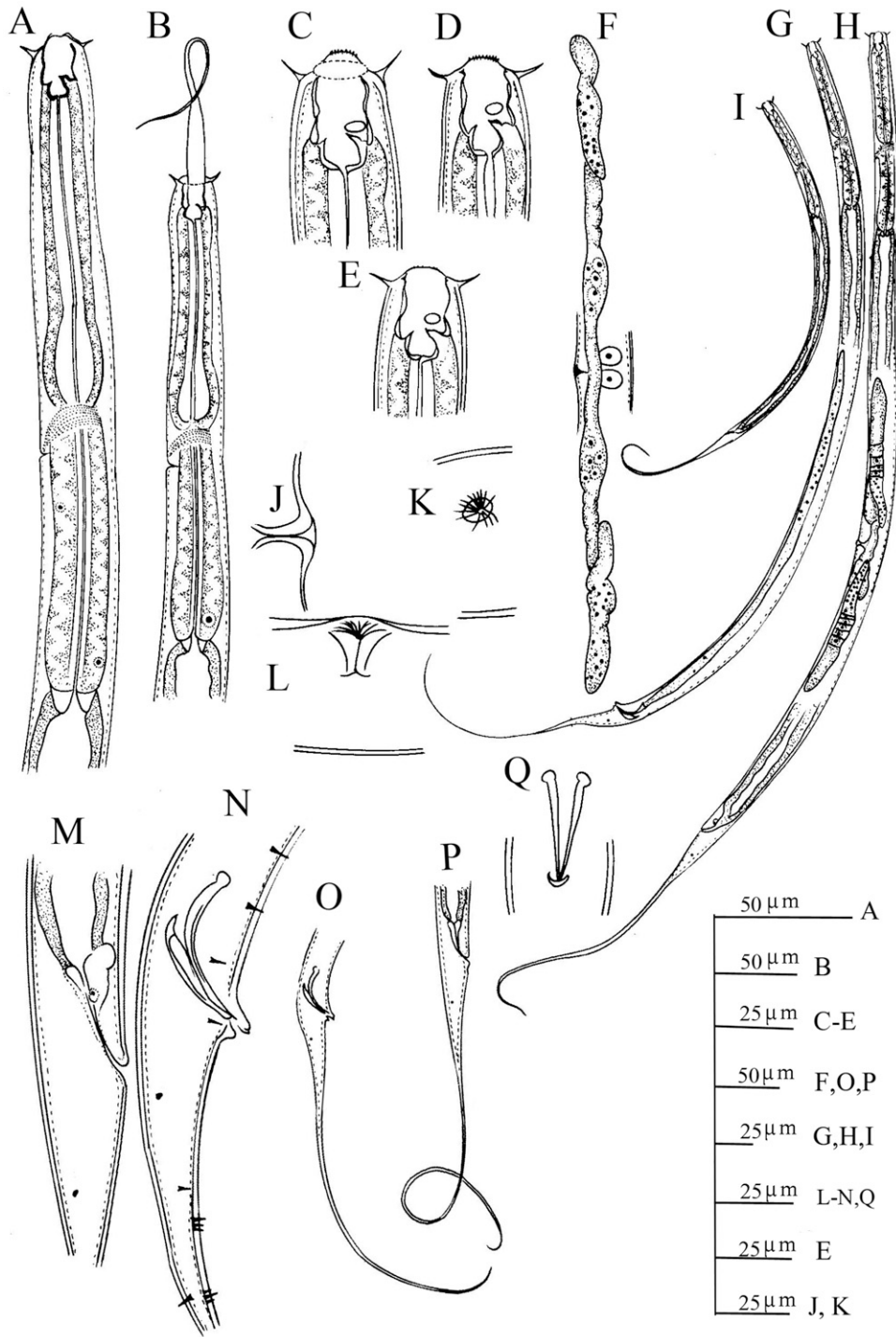


FIG. 1. *Butlerius butleri* Goodey, 1969. A. Anterior region. B. Anterior end with prey. C-E. Stoma. F. Reproductive system. G. Entire male. H. Entire female. I. Entire juvenile. J-L. Vulval position (K: superficial view). M, P. Female posterior end. N, O. Male posterior end. Q. Spicules.

dumb-bell-shaped pouches present at proximal part of uterus (ovijector), 21 to 25 μm long, connecting both uteri; vagina with narrow lumen and extending inwards less than half of the corresponding body diameter; vulva pre-equatorial, lips weakly cuticularized, protruding, pore-like aperture. Rectum 1.0 to 1.3 times anal body diameter long. Phasmids (Ph) prominent, situated at 6% to 10% of tail length. Tail

first conical and then filiform, 8.3 to 8.9 times the vulva-anus distance long.

Male: Similar to female body with longer cephalic setae (6.6 to 9.4 μm). Reproductive system monorchid. Spicules stout, paired, separate in ventral view, smoothly ventrally arcuate in lateral view; rounded manubrium; calamus very short, lamina thin, ventrad curved end. In lateral view, the gubernaculum is

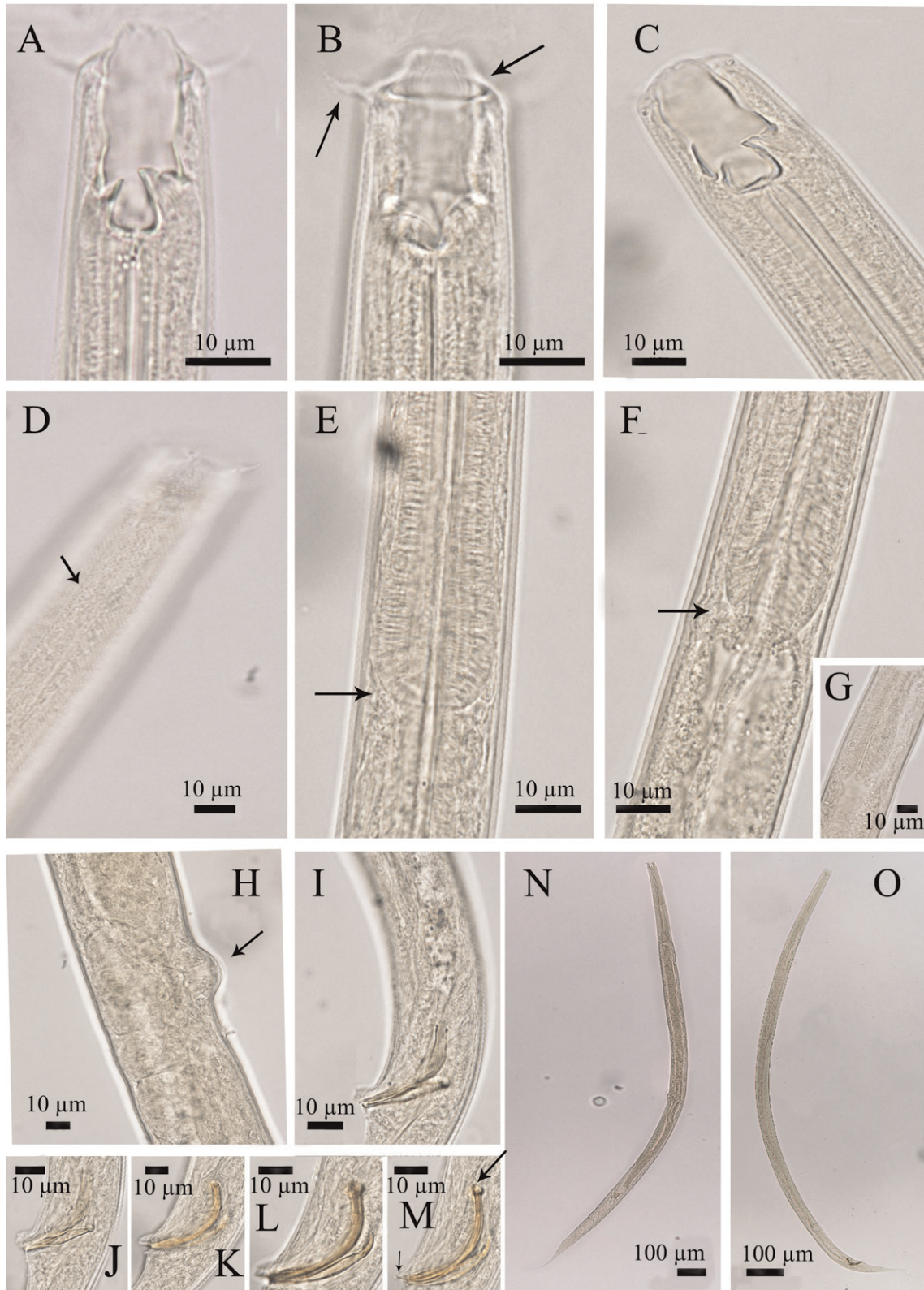


FIG. 2. *Butlerius butleri* Goodey, 1969 (LM). A–C. Female stoma (in B: arrow indicates oral opening). D. Cuticle surface. E. Median bulb. F, G. Terminal bulb. H. Vulva. I–M. Spicules and gubernaculum. N. Entire female. O. Entire male.

expanded proximally and rounded distally. Eight pairs of genital papillae (P1 to P3 precloacal + P4 to P8 postcloacal) and a pair of Ph present, which P6 and P8

comprising three papillae, according to the nomenclature by Sudhaus and Fürst von Lieven (2003). All papillae are seta like, and P6 and P8 are rather smaller

TABLE 1. Measurements (micrometer) of *Butlerius butleri* Goodey, 1929 individuals sampled from the Kerman Province of Iran followed by the standard deviations (\pm) and the upper and lower values in parenthesis.

	Females ($n = 7$)	Males ($n = 5$)	Juveniles ($n = 3$)
L	1,500 \pm 144.1 (1,285–1,695)	1,404 \pm 118 (1,266–1,533)	918 \pm 224 (685–1,133)
a	37.3 \pm 3.2 (34.0–41.2)	41.6 \pm 1.8 (39.4–43.7)	33.7 \pm 6.2 (29.1–40.8)
b	5.6 \pm 0.3 (5.1–6.3)	6 \pm 0.3 (5.6–6.3)	4.4 \pm 0.5 (4.1–5.0)
c	4.1 \pm 0.3 (3.7–4.8)	3.9 \pm 0.3 (3.5–4.1)	3.7 \pm 0.2 (3.4–3.9)
c'	24.3 \pm 3.6 (21.1–31.7)	12.2 \pm 1.3 (11.1–14.4)	12.4 \pm 1.4 (11.2–13.9)
V	46.3 \pm 0.08 (45–51)	-	-
Lip region wide	20.9 \pm 1 (19–22)	19.5 \pm 1 (19–21)	17.9 \pm 1.1 (17–19)
Amphid width	5	2.0, 2.8	2.0–2.5
Stoma length	24.1 \pm 2.8 (21–29)	23.3 \pm 3.5 (21–29)	20.8 \pm 0.6 (20–21)
Cheilostom width	10.3 \pm 1.5 (9–13)	9.2 \pm 0.9 (8–10)	6.8 \pm 1.4 (5–8)
Stegostom length	4.9 \pm 0.8 (4–7)	5.1 \pm 0.5 (4–6)	3.9 \pm 0.3 (3.8–4.2)
Stegostom width	11.3 \pm 1.5 (10–13)	10.2 \pm 0.5 (10–11)	8 \pm 1.4 (7–10)
Teeth length	4.3 \pm 0.5 (4–5)	4.8 \pm 0 (4.8)	2.4 \pm 0.2 (2–3)
Teeth width	4.9 \pm 0.2 (4.8–5.2)	4.5 \pm 0.6 (4–5)	3.4 \pm 0 (3.4)
Corpus length	36.8 \pm 2.9 (33–40)	31.8 \pm 1.4 (30–34)	25.3 \pm 5.7 (19–31)
Procorpus length	82.9 \pm 9.2 (67–93)	79.7 \pm 4.1 (74–85)	79.1 \pm 22.5 (57–102)
Metacarpus length	37.1 \pm 5.4 (31–43)	32.9 \pm 1.8 (30–35)	26.7 \pm 0.3 (26–27)
Metacarpus width	24.8 \pm 1.7 (23–28)	20.76 \pm 0.92 (20–22)	18.6 \pm 2.8 (15–20)
Isthmus length	82.8 \pm 7.0 (70–91)	67.3 \pm 4.8 (63–74)	60.1 \pm 15.8 (42–70)
Bulb length	119.8 \pm 11.2 (103–134)	100.2 \pm 6.3 (93–109)	77.8 \pm 16.7 (68–98)
Excretory pore position	168.3 \pm 11.7 (152–182)	161.3 \pm 10.4 (147–173)	134.5 \pm 22.8 (109–152)
Nerve ring position	150.9 \pm 12.4 (132–167)	139 \pm 5.5 (132–145)	123 \pm 19.3 (101–137)
Neck length	265.8 \pm 21.5 (232–295)	234.46 \pm 7.99 (227–244)	205.6 \pm 38.7 (161–229)
Neck base diameter	31.3 \pm 3.1 (29–36)	29.4 \pm 2.4 (27–32)	26.1 \pm 2.7 (23–26)
Midbody diameter	40.3 \pm 5.1 (35–49)	33.8 \pm 3.3 (29.3–38.4)	27.1 \pm 3.2 (24–30)
Anal body diameter	24.3 \pm 3.6 (21–32)	29.4 \pm 2.9 (26–34)	19.9 \pm 4.9 (14–24)
Vagina	16.7 \pm 2.25 (15–21)	-	-
Vulva to anus distance	462.1 \pm 44.7 (395–525)	-	-
Anterior gonad	244.5 \pm 32.6 (185–284)	-	-
Posterior gonad	227 \pm 28.2 (190–261)	-	-
Anterior ovary	61.7 \pm 11.9 (47–76)	-	-
Posterior ovary	62.2 \pm 10.6 (53–83)	-	-
Anterior oviduct	41.9 \pm 9.4 (28–53)	-	-
Posterior oviduct	36.8 \pm 7.5 (26–47)	-	-
Anterior uterus	84.7 \pm 13.3 (63–100)	-	-
Posterior uterus	74.3 \pm 9.7 (63–92)	-	-
Spicule length	-	46 \pm 1.2 (44–47)	-
Gubernaculum length	-	34.2 \pm 1.7 (33–37)	-
Tail length	365.7 \pm 32 (304–410)	357 \pm 28 (312–380)	243 \pm 46.6 (200–296)

than others. Phasmid is clearly visible, located at 1.4 to 1.7 anal body diameter, at 10% to 14% of tail length. Tail conical and then filiform.

Juveniles (fourth stage): Body slightly arcuate ventrally when heat-killed. Cuticle 0.8- μ m thick, lacking punctation, with 13 to 16 prominent longitudinal ridges at midbody, all ridges equidistant and bearing fine transverse striations, 0.9- μ m wide at midbody. Lip region continuous with body contour, consisting of six fused lips, edge of lip region with six additional cephalic setae, 4.7 to 6.1 μ m long. Amphids with aperture oval shaped, located at level of the dorsal tooth, about 4 to 5 μ m wide. Buccal cavity about two times longer than wide. Cheilostom with eight narrow rib-like plates (cheilorhabdia) that are bifurcate at the apex and extend beyond the labial contour when posterior stomatal elements are retracted; inner wall of cheilostom cuticularized. Second part of stoma consisting of gymnostom and stegostom, both unisotopic with subventral walls

slightly longer than dorsal side. The gymnostom, about 6.6- to 9.4- μ m wide, forms a cuticular isomorphic cylinder with its anterior edge not serrated; its dorsal side cuticularized, thickened at the middle that remains more uniformly in shape. Promesostegostom wide in dorsal side and relatively thin in subventral side. Metastegostom bearing dorsally, a prominent claw-like tooth, 2.3 to 2.8 μ m long and 3.4 μ m wide, pointed toward anterior part, with the duct of the dorsal gland and subventrally, a smaller sickle-shaped right tooth. Posterior part of stegostom (= metastegostom and telostegostom) forming a cylindrical tube, 5.6 to 6.6 μ m wide and 4.7 to 6.12 μ m long; a minute denticle observed in the subventral wall of the stegostom. Neck region comprising 20 to 24 times of total body length. Pharynx diplogasteroid; pharyngeal procorpus cylindrical, 2.1 to 3.8 times metacarpus length; metacarpus swollen; isthmus robust, longer in males. Cardia conoid, surrounded by intestinal tissue. Nerve ring at 57%

to 62% of neck length at isthmus level. Excretory pore situated at level of the anterior part of isthmus, at 108 to 150 μm from the anterior end, and at 62% to 67% of neck length. Hemizonid in front of excretory pore. Deirid not visible.

Locality and habitat: Specimens of the species were collected in Kerman in the Kerman Province of Iran (GPS coordinates: N 30°15'27.10; E 57°06'13.59) during 2014 from vermicompost.

Diagnosis: Specimens from the Iranian population collected are morphologically similar to those that were originally described as of *B. butleri* by Goodey (1929) and represent the first report of this species in Iran. However, its morphometrics differ with regard to the "a" (34 to 41 vs. 18 to 23) and "V" (45 to 51 vs. 58 to 64) values of the *B. butleri* specimens described by Goodey (1929). These differences, compared to the type specimens, have also been reported for a Korean population (Ahmad et al., 2009). Compared to this Korean species, the Iranian specimens have a shorter tail (304 to 410 μm vs. 361 to 570 μm) in female specimens and a longer gubernaculum (33 to 37 μm vs. 25 to 33 μm) in male specimens. Andrassy (2005) reported *B. butleri* specimens with a shorter body length (1.3 to 1.7 mm in females and 1.3 to 1.5 mm in males vs. 1.1 to 1.55 mm in females and 0.95 to 1.15 mm in males) and different "V" value (45 to 51 vs. 40 to 43). The 18S sequence of the 914 bps for the *Butlerius butleri* Goodey, 1929 from Iran described in this study was deposited in GenBank under accession number KP453998.

DISCUSSION

Phylogenetic position of B. butleri based on 18S rDNA: The base substitution model evaluated in this study was the HKY+G model ($-\ln L = 15028.8913$; AIC = 1258.246; K = 2.5854; freqA = 0.2474; freqC = 0.2118; freqG = 0.2270; freqT = 0.3139; R(a)[AC] = 0.04; R(b)[AG] = 0.14; R(c)[AT] = 0.06; R(d)[CG] = 0.05; R(e)[CT] = 0.16; R(f)[GT] = 0.06; Gamma shape = 0.3350). The phylogenetic tree, including the 18S rDNA sequence for *B. butleri* specimens from Iran, which is a first report, contains three main clades (Fig. 3). Hence, the genus *Butlerius* is placed close to the genera *Diplogastrellus* and *Pseudodiplogasteroides* with a 1.00 posterior probability. Mayer et al. (2009) studied the phylogenetic relationship of 12 diplogasterid taxa using SSU rDNA and suggested that *Diplogastrellus* and *Pseudodiplogasteroides* placed close to each other. Results from other studies also placed *Diplogastrellus* as a sister group with *Pseudodiplogasteroides* (Kanzaki et al., 2012, 2014, 2015). According to Sudhaus and Fürst von Lieven (2003), *Butlerius* and *Diplogastrellus* present similarities, namely the presence of their labial sensilla (longer vs. shorter, respectively); structure of their stoma with the presence of a well-developed dorsal tooth in the stegostom (thorn-like in

Butlerius and triangular in *Diplogastrellus*); female reproductive systems for the prodelpic species and male specimens lacking bursas. Additionally, *Butlerius* showed visible dissimilarities with *Pseudodiplogasteroides*, namely (i) the stomas of specimens belonging to *Pseudodiplogasteroides* are tube-shaped and narrower vs. the barrel shaped and wider stomas in *Butlerius* specimens (Sudhaus and Fürst von Lieven, 2003) and (ii) the terminal terminal/pharyngeal bulb bears remnants of the haustulum for *Pseudodiplogasteroides* but is absent in *Diplogastrellus* specimens. However, specimens of *Diplogastrellus* and *Pseudodiplogasteroides* have similar female reproductive systems (prodelpic and didelphic amphidelphic genital branches). Also the males of these two genera have no bursas. Although individuals from these two genera differ in terms of their gymnostom and terminal bulb morphology as discussed above, both are considered as paraphyletic groups (Sudhaus and Fürst von Lieven, 2003).

Genus Butlerius: Sudhaus and Fürst von Lieven (2003) listed 15 valid species under the genus *Butlerius* compared to Andrassy (2005) who only proposed 10 of which the female specimens all have amphidelphic reproductive systems. The other five species, which are prodelpic, are categorized by Andrassy (2005) under the genus *Monobutlerius* Andrassy, 1984. According to the morphology of their stomas, *Butlerius* and *Monobutlerius* (representing both mono- and didelphic females) are similar and proposed as being synonyms. Later Ahmad et al. (2009) redescribed *B. butleri* from South Korea and proposed *B. filicaudatus* Adam, 1930 and *B. singularis* Lordello and Zamith, 1959 as its junior synonyms. Thus, at present 13 valid species are placed under the genus *Butlerius* (Tables 2 and 3).

List of species:

- Butlerius* Goodey, 1929
- syn. *Butlerioides* Lordello and Zamith, 1959
- syn. *Butleriellus* Meyl, 1960
- syn. *Mesodiplogasteroides* Khera, 1969
- syn. *Monobutlerius* Andrassy, 1984
- syn. *Parabutlerius* Ebsary, 1986

Type species:

- B. butleri* Goodey, 1929
- syn. *B. singularis* Lordello and Zamith, 1959
- B. filicaudatus* Adam, 1930
- Butleriellus filicaudatus* (Adam, 1930) Meyl, 1960

Other species:

- B. canadensis* Ebsary, 1986
- B. degrissei* Grootaert and Jaques, 1979
- syn. *Monobutlerius degrissei* (Grootaert and Jaques, 1979) Andrassy, 1984
- B. demani* (Schneider, 1923) Andrassy, 1984
- syn. *Diplogaster demani* Schneider, 1923
- B. gerlachi* Meyl, 1957
- syn. *Monobutlerius gerlachi* (Meyl, 1957) Andrassy, 1984

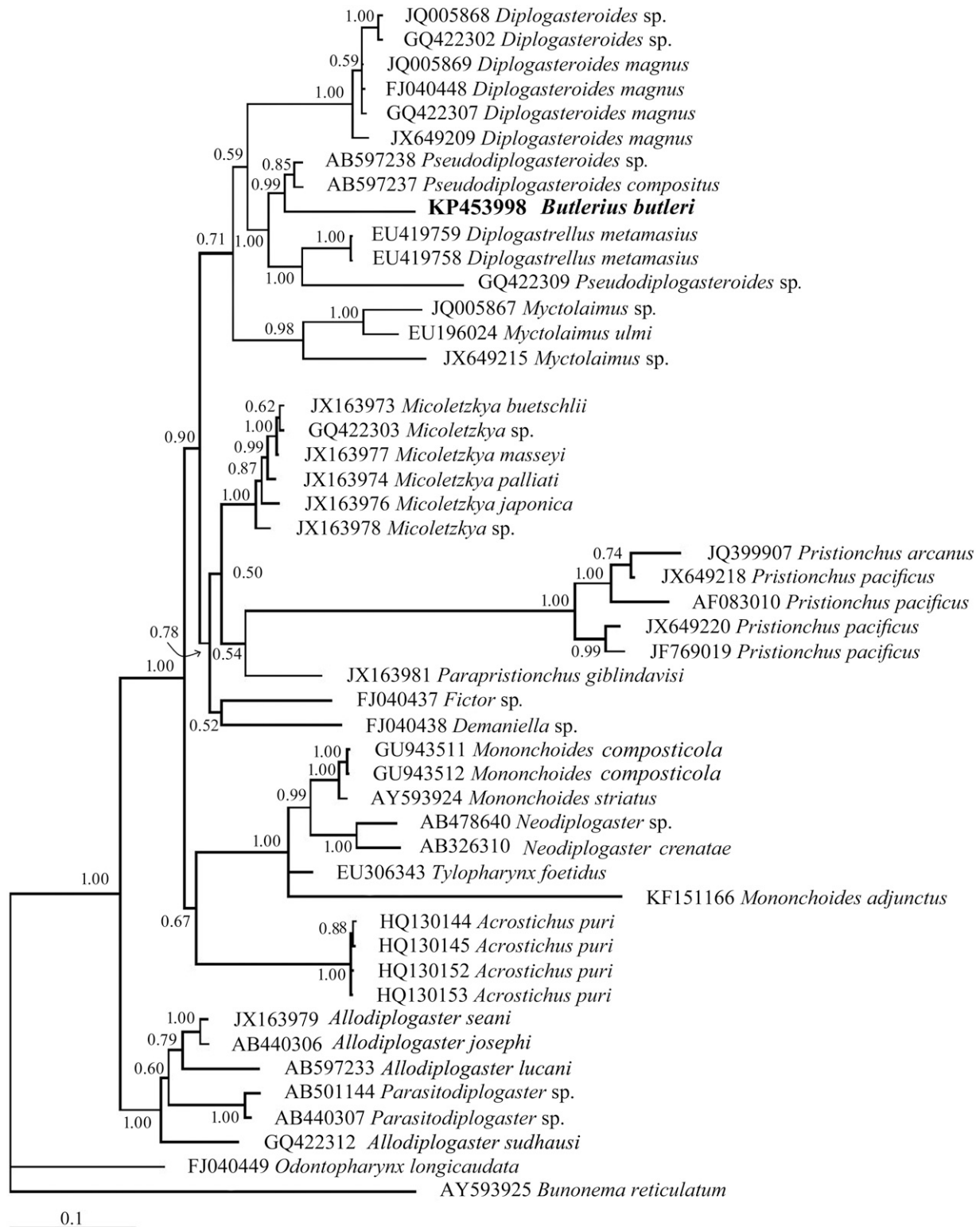


FIG. 3. The Bayesian inference tree of *Butlerius butleri* from Iran and other related taxa based on Bayesian 50% majority rule consensus tree as inferred from a 1,863 bps alignment of 18S ribosomal deoxyribonucleic acid. The branches contain posterior probability values.

B. kaplini (Ryss, 1989) Sudhaus and Fürst von Lieven, 2003

syn. *Monobutlerius kaplini* Ryss, 1989

B. longipyge (Khera, 1969) Sudhaus and Fürst von Lieven, 2003

syn. *Mesodiplogasteroides longipyge* Khera, 1969

B. macrogubernaculum (Chitambar, 1990) Sudhaus and Fürst von Lieven, 2003

syn. *Monobutlerius macrogubernaculum* Chitambar, 1990

TABLE 2. Morphometrical data of didelphic species of *Butlerius* Goodley, 1929.

Species	Sex	L	a	b	c	c'	V	Longitudinal		Tail	Spicules	Precloacal papillae	Postvulvar		Country	Reference
								Striae	Striae				sac/gubernaculum	sac/gubernaculum		
<i>B. butleri</i>	♀	1.11-1.55	18.3-23.3	4.2-6.2	3.3-5.9	?	40.5	Absent	?	263-336 ^a	-	-	-	United Kingdom	Goodey (1929)	
	♂	0.97-1.15	19.1-19.6	5.0-6.6	4.0-4.6	?	-	Absent	?	243-250 ^a	39	3	28	The Netherlands	Adam (1930)	
	♀	1.15-1.90	33.0-43.0	4.8-6.0	3.3-4.7	?	48	Absent	?	348-404 ^a	-	-	-	Hungary	Andrássy (2005)	
	♂	2.35	38.0	5.0	4.2	?	-	absent	?	560 ^a	?	3	?	Korea	Ahmad et al. (2009)	
	♀	1.11-1.55	18.0-23.0	4.2-6.2	3.3-5.8	15.0-20.0	40-43	40-45	Absent	?	263-333 ^a	-	-	26-28	Iran	Present paper
<i>B. ficicaudatus</i>	♂	0.95-1.15	19.0-22.0	5.0-6.6	4.0-4.6	?	-	Absent	?	243-250 ^a	-	-	-	Canada	Ebsary (1986)	
	♀	1.33-1.85	33.9-46.5	5.4-6.3	3.4-4.2	14.1-19.0	40-45	Absent	?	361-570	-	-	-	Germany	Schneider (1923)	
	♂	1.19-1.64	43.2-48.1	4.9-5.8	3.2-3.7	13.8-16.4	-	30	?	336-503	39-49	-	25-33	Brasil	Meyl (1957)	
	♀	1.28-1.69	34.0-41.2	5.1-6.3	3.7-4.8	21.1-31.7	45-51	Absent	?	304-410	44-47	-	-	India	Khera (1969)	
	♂	1.26-1.53	39.4-43.7	5.6-6.3	3.5-4.1	5.6-6.3	-	42-48	?	312-380	-	-	-	United States of America	Pillai and Taylor (1968)	
<i>B. canadensis</i>	♀	1.00-1.21	35.0-42.0	5.3-5.7	3.0-3.4	17.0-20.0	41-43	Absent	?	324-370	-	-	-	China	Rahm (1938)	
	♂	1.00-1.86	32.0-58.0	5.6-7.3	3.2-3.8	10.0-13.5	-	?	?	234-324	21-27	2	13-14	Lithuania	Skwarra (1921)	
	♀	1.18-1.21	40.0-40.6	4.7	3.7-5.0	15.3 ^a	42-48	Absent	?	?	-	-	-			
	♂	1.00-1.14	42.0-47.7	4.4-4.9	5.9-7.6	?	51-54	10	?	?	-	-	-			
	♀	0.89-1.00	43.0-48.1	4.1-4.4	9.6-9.7	8	-	40-42.5	?	125 ^a	40 ^a	4	17 ^a			
<i>B. demani</i>	♀	1.00-1.20	25.0-28.0	6.5-6.8	3.1-3.5	?	-	Absent	?	318	-	-	-			
	♂	0.75-0.85	26.0-28.0	6.5-6.8	3.0-3.3	-	-	35-45	?	274	30-32	3	12-13			
	♀	0.98-1.25	26.0-30.0	6.7-8.1	3.1-5.0	7.8 ^a	39-47	Absent	?	453 ^a	-	-	-			
<i>B. micans</i>	♂	0.79-1.10	28.0-45.0	6.3-8.7	2.9-3.7	-	-	Absent	?	?	25-29	2	13-14			
	♀	0.95-1.23	30.0-36.0	4.2-5.3	4.1-4.9	48.0-51.0	?	?	?	-	-	-	-			
	♂	1.12-1.59	35.0-40.0	4.8-5.2	3.0-4.7	-	-	?	?	-	-	-	-			
<i>B. spirifer</i>	♂	1.00	24.8	6.1	3.2	41.6	-	?	?	?	-	-	-			
	♀	0.89	36.5	5.6	3.4	?	-	?	?	?	-	-	-			
	♂	0.89	36.5	5.6	3.4	?	-	?	?	?	-	-	-			

^a Data extracted from the illustration.
 ? = the measure could not be obtained.

TABLE 3. Morphometrical data of monodelphic species of *Butlerius* Goodey, 1929.

Sex	L	a	b	c	c'	V	Longitudinal striae	Tail	Spicules	Precloacal papillae	Postvulvar sac/gubernaculum	Country	Reference
<i>B. degrassi</i>													
♀	1.08-1.31	32.0-40.0	5.0-5.8	2.7-3.6	?	50-55	Finely striation	290-400	-	-	57 ^a	Belgium	Grootaert and Jaques (1979)
♂	1.0-1.10	39.0-42.0	4.9-5.7	3.1-3.7	-	-	Finely striation	276-345	32-34	3	-	Hungary	Andrássy (2005)
♀	1.08-1.30	32.0-40.0	5.0-5.8	2.7-3.8	10-15	50-55	Finely striation	300-400	-	-	?		
♂	0.97-1.10	39.0-42.0	4.9-5.7	3.1-3.7	-	-	striation	270-350	?	3	?		
♀	0.76-0.60	33.0-34.0	4.5-5.0	13.1-14.4	3.8-4.1	56-58	Absent	43-53	-	-	24	Turkmenistan	Ryss (1989)
♂	0.68-0.86	27.7-34.3	4.0-6.8	3.2-5.0	8.1-14.2	52-59	Striated	143-249	-	-	36-60	United States	Chitambar (1990)
♀	0.59-0.79	28.3-41.3	4.1-5.0	3.2-4.7	6.0-12.7	-	Striated	126-230	32-35	2	18-23		
♂	1.32-1.53	28.0-40.0	4.2-4.8	2.7-3.4	16-22	49-56	Absent	410-554	-	-	31	Saint Lucia	Hunt (1980)
♂	1.03-1.27	31.9-39.1	4.1-4.6	2.9-3.4	12.4-16.0	-	Absent	353-401	73-86	3	23		
♀	1.00-1.22	26.5-33.3	4.6-5.4	2.6-2.8	-	45-50	Absent	385-436	-	-	49	United States	Taylor (1964)
♂	0.77-1.07	28.2-40.0	4.8-6.3	2.6-2.7	-	-	Absent	296-373	41	3	16		

^a Data extracted from the illustration. ? = the measure could not be obtained.

B. macrospiculum Hunt, 1980
 syn. *Monobutlerius macrospiculum* (Hunt, 1980)
 Andrassy, 1984
B. micans Pillai and Taylor, 1968
B. monhystera Taylor, 1964
 syn. *Monobutlerius monhystera* (Taylor, 1964)
 Andrassy, 1984
B. okai Rahm, 1938
 syn. *Butlerioides okai* (Rahm, 1938) Lordello and Zamith, 1959
B. spirifer (Skwarra, 1921) Zullini and Loof, 1980
 syn. *Diplogaster spirifer* Skwarra, 1921

Key to species

- 1) Female genital system monodelphic 2
- Female genital system didelphic..... 6
- 2) Spicules long, 73 to 86 µm..... *macrospiculum*
- Spicules short, less than 45 µm 3
- 3) Female tail conical elongated, 43 to 53 µm long *kaplini*
- Female tail filiform, less than 140-µm long 4
- 4) Gubernaculum long, 18 to 23 µm, 1/2 to 1/3 spicules length; two precloacal papillae present *macrogubernaculum*
- Gubernaculum short, more than 18 µm, about 1/3 spicules length; three precloacal papillae present..... 5
- 5) Spicules 41 µm, gubernaculum keel like..... *monhystera*
- Spicules 32 to 34 µm, gubernaculum triangular *degrassi*
- 6) Cuticle with longitudinal striation present 7
- Cuticle with longitudinal striation absent..... 11
- 7) Pharyngeal lumen prominent, zip-like..... 8
- Pharyngeal lumen not prominent, normal 9
- 8) Spicules with manubrium offset by constriction, not strongly narrowed; ventral part of gubernaculum paralld..... *spirifer*
- Spicules with manubrium not offset by constriction, strongly narrowed; ventral part of gubernaculum emarginated *micans*
- 9) Female tail shorter (c = 5.9 to 7.6); vulva post-equatorial (V = 51 to 54); four precloacal papillae present..... *gerlachi*
- Female tail longer (c ≤ 5.0); vulva pre-equatorial (V ≤ 48); two precloacal papillae present or absent male 10
- 10) Female tail longer (c = 3.0 to 3.4; c' = 17.20)..... *canadensis*
- Female tail shorter (c = 3.7 to 5.0; c' = 15.3) *demani*
- 11) Gubernaculum absent *okai*
- Gubernaculum present 12
- 12) Spicules 30 to 32 µm long; gubernaculum 12 to 13 µm long *longipyge*

Spicules 39 to 49 μm long; gubernaculum 25 to 33 μm long..... *butleri*

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