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Two new species of *Parapharyngodon* (Oxyuroidea: Pharyngodonidae) from the enigmatic *Bipes canaliculatus* and *Bipes tridactylus* (Squamata: Bipedidae)

Dos especies nuevas de *Parapharyngodon* (Oxyuroidea: Pharyngodonidae) de los enigmáticos *Bipes canaliculatus* and *Bipes tridactylus* (Squamata: Bipedidae)

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Abstract. Two species of *Parapharyngodon* collected from *Bipes canaliculatus* and *Bipes tridactylus* are herein described. *Parapharyngodon lamothei* n. sp. was collected from the 4-toed worm lizard *Bipes canaliculatus* from southern Mexico. It is unique in possessing 6 lips, a gubernaculum, a fringed cloacal lip, and a single medial papilla in addition to 3 paired papillae. *Parapharyngodon maestro* n. sp., was collected from the 3-toed worm lizard *Bipes tridactylus,* and it is characterized by having 3 bilobed lips, a simple cloaca, a relatively long spicule, and by possessing 3 pairs of caudal papillae. These are the third and fourth species of the genus recorded in Mexico.

Key words: Nematoda, Oxyuridae, Bipedidae, *Parapharyngodon*, *Bipes*, Mexico.

Resumen. En este trabajo se describen 2 especies del género *Parapharyngodon*: *Parapharyngodon lamothei* n. sp. y *Parapharyngodon maestro* n. sp., recolectadas del intestino de los bipédidos *Bipes canaliculatus* y *Bipes tridactylus*, respectivamente. La primera se caracteriza por poseer 6 labios, gubernáculo, labio cloacal ornamentado y las papilas caudales distribuidas en 3 pares y 1 papila sencilla media ventral inmediatamente postcloacal. *Parapharyngodon maestro* n. sp. se diferencia por poseer 3 labios lobulados, por su estructura cloacal lisa y por presentar 3 pares de papilas caudales en el caso de los machos.

Palabras clave: Nematoda, Oxyuridae, Bipedidae, *Parapharyngodon*, *Bipes*, México.

Introduction

Bipedidae (Squamata: Amphisbaenia: Bipedidae) includes 3 species of worm lizards endemic to Mexico. The distribution of the species is restricted to the Baja California Peninsula and to the Balsas River basin near the Pacific coast of southern Mexico. Their habits are fossorial and they probably feed on arthropods and soft-bodied invertebrates associated with the soil (Kearney, 2003). There are 2 species in mainland Mexico and 1 in the Baja California Peninsula.

Corresponding to the former group, *Bipes tridactylus* (Dugès, 1894) and *Bipes canaliculatus* Latreille in Sonnini and Latreille, 1801 occur in southern Mexico. Both species are separated by the Sierra Madre del Sur. *Bipes canaliculatus* occurs in the valley of the Balsas River. There is no record of its parasite fauna and little information is known about its habits in general. It occurs in a variety of soils, including sand and rocky substrates (Hodges and Pérez-Ramos, 2001). *Bipes tridactylus* occurs in southern Mexico in a handful of localities near the Pacific coast. There has been no detailed study about their habits and habitat.

Herein we present the nematode fauna for both species including the description of 2 new species of *Parapharyngodon* Chatterji, 1933. These are the fifteenth and sixteenth species of the genus in the New World and the third and fourth recorded in Mexico. According to Bursey and Goldberg (2005) and Bursey et al. (2007) the species may be differentiated using traits of the tail of females, size of spicule, number of caudal papillae and ornamentation on cloacal lip.

Materials and methods

Amphisbaenians were captured in the field as described

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in Hodges and Pérez-Ramos (2001). Those collected in 1998 were brought back alive to the laboratory and others were euthanized with an overdose of sodium pentobarbital in the field. The digestive tract was examined using a dissecting miscroscope. Worms found were fixed using Berland fluid, stored in 70% ethanol, and cleared in lactophenol.

Specimens were measured using Sigmascan Pro™ Image Analyzer (Albinger et al., 1995) attached to a Zeiss™ ultraphot microscope. Measurements are given in micrometers (µm). The measurements of the 2 males studied are offered. For each character studied in females, the range is given first, followed by sample mean, and coefficient of variation in parentheses. The coefficient of variation (Sokal and Rohlf, 1995), allows to compare the relative amounts of variation in populations with different means. The measurements of the holotypes and allotypes are summarized on Table 1. Drawings were made with a Wild microscope equipped with a drawing tube.

The worm lizards were deposited in the Colección Herpetológica del Museo de Zoología Alfonso L. Herrera (MZFC), whereas nematodes were deposited in the Colección Nacional de Helmintos (CNHE), México D.F., Mexico and in the collection of The Harold W. Manter Laboratory of Parasitology (HWML) of the University of Nebraska (Lincoln, Nebraska).

Description

Parapharyngodon lamothei n. sp. (Figs. 1 – 5, 11)

General. Pharyngodonydae: Robust specimens with blunt ends; conspicuous cuticular annulation along the entire body; each ring consists of 2 parts that meet at lateral lines of body (Fig 1). Anterior end with 6 conspicuously separated lips; 2 lateral amphids and 2 pairs of cephalic papillae (Fig. 2). Sexual dimorphism in size and cuticular ornamentation consisting of lateral alae present in males.

Males (*based on 2 specimens; measurements of holotype on Table 1*). Body length 2 104 - 2 253, maximum width 185 - 214; lateral alae start slightly anterior to esophageal bulb, ends near tail, length 1 479 - 1 781 (Fig. 3), maximum width of 28 - 38. Stoma funnel shaped 9 - 13; total length of esophagus 422 - 428; corpus 345 - 361 in length, 29 - 33 width; short isthmus; esophageal bulb 77 long by 85 - 86 wide. Nerve ring and excretory pore located at 132 - 154 and 727 - 752 from anterior end respectively (Fig. 3). Spicule 64 - 66 long, 8 - 9 width at manubrium; gubernaculum or accessory piece in form of a folded cuticular flange surrounding the lamina of the spicule, V-shaped in ventral view, 30 - 32 long, 8 wide (Fig. 4). Precloacal lip fringed. Tail projected dorsally, divided into a stalk and a whip-shaped spinneret, total length 76 - 84. Cloaca opens terminally with 1 pair of subventral precloacal papillae, single medial postcloacal papilale, 1 pair sublateral and postcloacal, and 1 pair at the end of stalk of spinneret, sharing a mammilla (Fig. 4). Phasmids postcloacal, present in caudal stalk. Precloacal lip with short blunt projections. Cuticular rings at the caudal level with grooves associated to glands.

Females (*based on 10 specimens; measurements of allotype on Table 1*). Body length 3 488 - 5 302, 4 457 (14%); maximum width 334 - 581, 503 (15%). Stoma straight 11 - 20, 15 (23%, n=9); total length of esophagus 818 - 1 209, 1 085 (12%); corpus length 700 - 1 077, 946 (13%), width 35 - 46, 40 (11%, n=9); esophageal bulb 118 - 143, 130 (5%) in length, 120 - 156, 146 (7%) width. Nerve ring and excretory pore located 150 - 288, 208 (24%, n=7) and 995 - 1 380, 1 217 (10.4%) from anterior end respectively. Vulva near equator of body located 1 694 - 2 472, 2 077 (13%) from anterior end (Fig. 1). Ovejector 422 - 607, 493 (13%, n=7) in length, maximum width 71 - 110, 92 (16%, n=7). Muscular sphincter 86 - 127, 110 (15%, n=7) long and 70 - 94, 83 (10%, n=7) wide. Didelphic, prodelphic. Eggs oval, symmetrical, containing non differentiated embryo, textured thick shell (with punctations), 92 - 119, 106 (5%, n=120) long, 34 - 44, 38, (6%, n=120) wide (Fig. 5). Distance of anus to tip of caudal appendage 230 - 294, 272 (7%), conical caudal appendage tapers to a point, with the tip directed posteriad (Fig. 1).

Taxonomic summary

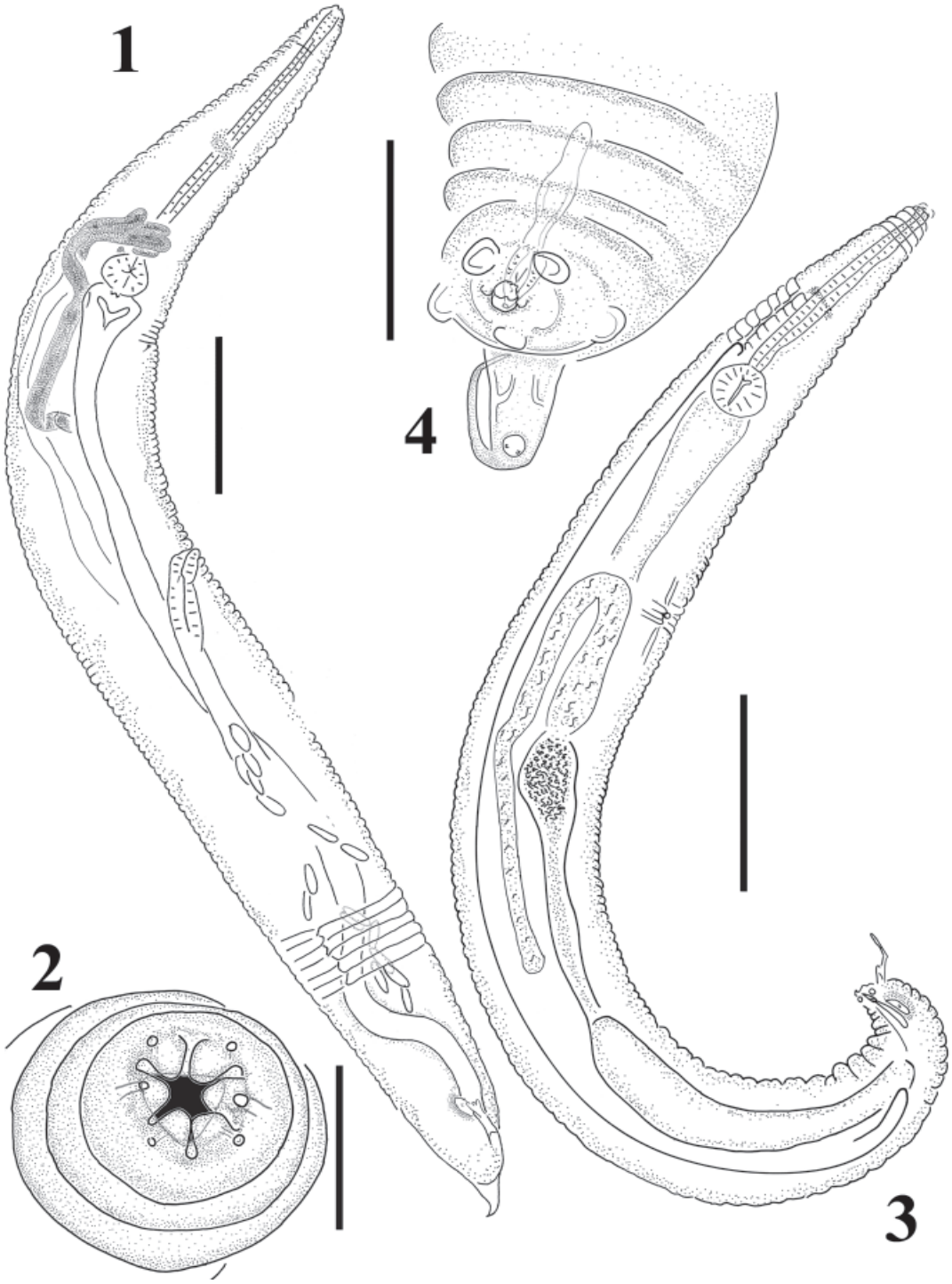
Type-host: *Bipes canaliculatus* Latreille in Sonnini and Latreille, 1801.
Symbiotype: MZFC11534.
Site of infection: posterior end of intestine.
Type-locality: MEXICO: Guerrero, Municipio Cocula, 2.5 km N Atzcala, 17º 59’ 10” N, 99º 39’ 59” W, 720 m, 21 November 1998.
Prevalence: 100% (3/3).
Other localities: MEXICO: Guerrero, Municipio Copalillo, Hueyatl. 17° 53’ 38’’ N; 99° 07’ 28’’ W; 690 m, 17 November 1998, (MZFC 11523); Municipio Cocula, 1 km N Atzcala. 17° 58’ 52’’ N; 99° 39’48’’; 423 m; 21 November 1998, (MZFC 11533).
Type-specimens: holotype CNHE 5909; allotype CNHE 5911; paratypes CNHE 5910, CNHE 5912, and CNHE 5913; HWML 48582 through HWML 48584.
Etymology: the species is named after Dr. Rafael Lamothe-Argumedo, head of the CNHE and professor of several generations of biologists at Universidad Nacional Autónoma de México (UNAM) for the last 50 years.

Table 1. Measurements of the type specimens of *Parapharyngodon lamothei* n. sp. and *Parapharyngodon maestro* n. sp. collected from *Bipes canaliculatus* and *Bipes tridactylus*, respectively

	Parapharyngodon lamothei n. sp.	*Parapharyngodon maestro* n. sp.
HOLOTYPE	CNHE 5909	CNHE5904
Body length	2104	1955
Maximum width	185	127
Length of lateral ala	1479	1380
Width of lateral ala	38	N/A
Stoma	13	115
Anterior end to:		
Nerve ring	154	112
Excretory pore	727	671
Length of esophagus	422	345
Length of corpus	345	273
Width of corpus	29	27
Bulb length	77	72
Bulb width	85	80
Spicule	64 x 8	71 x10
Stalk of spinneret	17	21
Total length of tail	76	81
ALLOTYPE	CNHE5911	CNHE5905
Body length	5302	4231
Maximum width	487	388
Stoma	20	24
Anterior end to:		
Nerve ring	288	164
Excretory pore	1380	1300
Vulva ant end	2432	2077
Length of esophagus	1209	942
Length of corpus	1067	752
Width of corpus	--	42
Bulb length	143	133
Bulb width	154	158
Ovejector	422	373
Tail	283	218
Eggs	106 x 36	104 x 33

Remarks

Parapharyngodon lamothei is unique in featuring a gubernaculum and blunt projections on the cloacal lip. It also shows 3 paired papillae, with a single medial postcloacal. It most resembles *Parapharyngodon verrucosus* Freitas and Dobbin, 1959 in most of the measurements, arrangement of the caudal papillae and surface of cloacal lip. However, *P. verrucosus* has 3 lips, smooth eggs, and no gubernaculum. *Parapharyngodon lamothei* also resembles *Parapharyngodon osteopili* Adamson, 1981 in the size of spicule and in the shape of the tail of females. Nevertheless, 3 differences must be noted. First, there are no lateral alae in males of *P. osteolepi*,



Figures 1-4. *Parapharyngodon lamothei* n. sp. 1, lateral view of female, showing annulation, shape of tail and relative position of vulva, nerve ring and excretory pore. Scale bar = 500 µ. 2, *en face* view of female showing 6 lips. Scale bar = 30µ. 3, lateral view of male, showing general internal morphology and details of cuticle. Scale bar = 300 µ. 4, ventral view of the tail of a male showing spicule, gubernaculum, and caudal papillae. Scale bar = 50µ.

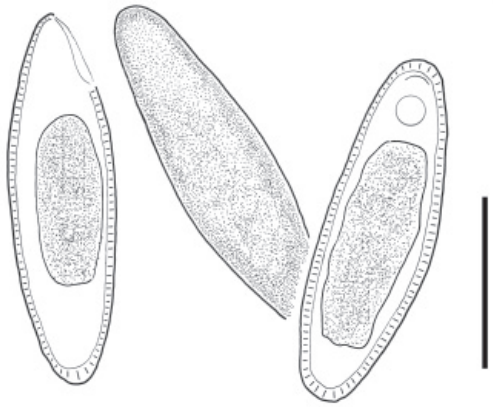


Figure 5. Eggs of *Parapharyngodon lamothei* n. sp. Scale bar = 50 µ.

second, *P. lamothei* has a conspicuous gubernaculum, and finally, there are 6 conspicuously separated lips around the stoma of *P. lamothei*.

Other species showing similarities to *P. lamothei* include *Parapharyngodon scleratus* Travassos, 1923, *Parapharyngodon alvarengai* Freitas, 1957, *Parapharyngodon senisfasciecaudus* Freitas, 1957, and *Parapharyngodon colonensis* Bursey, Goldberg, and Telford, 2007. The similarities between *P. colonensis* and *P. lamothei* include the number of lips, size of the spicule and shape of the tail of females. However, the differences consist in that the lateral alae of *P. lamothei* is shorter, the eggs are symmetrical as opposed to assymetrical in *P. colonensis,* and *P. lamothei* has single postcloacal papillae; postcloacal papillae are paired in *P. colonensis*.

Parapharyngodon scleratus has a longer spicule than *P. lamothei* and an additional pair of sublateral papillae. Additionally, females of *P. scleratus* have spike-shaped tails. Both *P. alvarengai* and *P. senisfasciecaudus* have 3 bilobed lips, longer spicules, spines on the cloacal lip, asymmetric eggs with smooth shells, and the lateral alae start at the level of the mid section of the esophagus. In *P. lamothei,* lateral alae begin slightly anterior to the esophageal bulb, the cloacal lip is fringed as opposed to echinate, eggs are textured, and there are 6 lips.

Parapharyngodon lamothei is the only species in the genus featuring a conspicuous gubernaculum or accessory piece. This structure is typical of species in *Thelandros* Wedl, 1862; however, we have included *P. lamothei* in *Parapharyngodon* because it does not have a genital cone and the eggs are released containing embryos in an early stage of the development. *Parapharyngodon lamothei* is the third member of this genus recorded in Mexico.

Parapharyngodon maestro n. sp. (Figs. 6 – 10, 12)

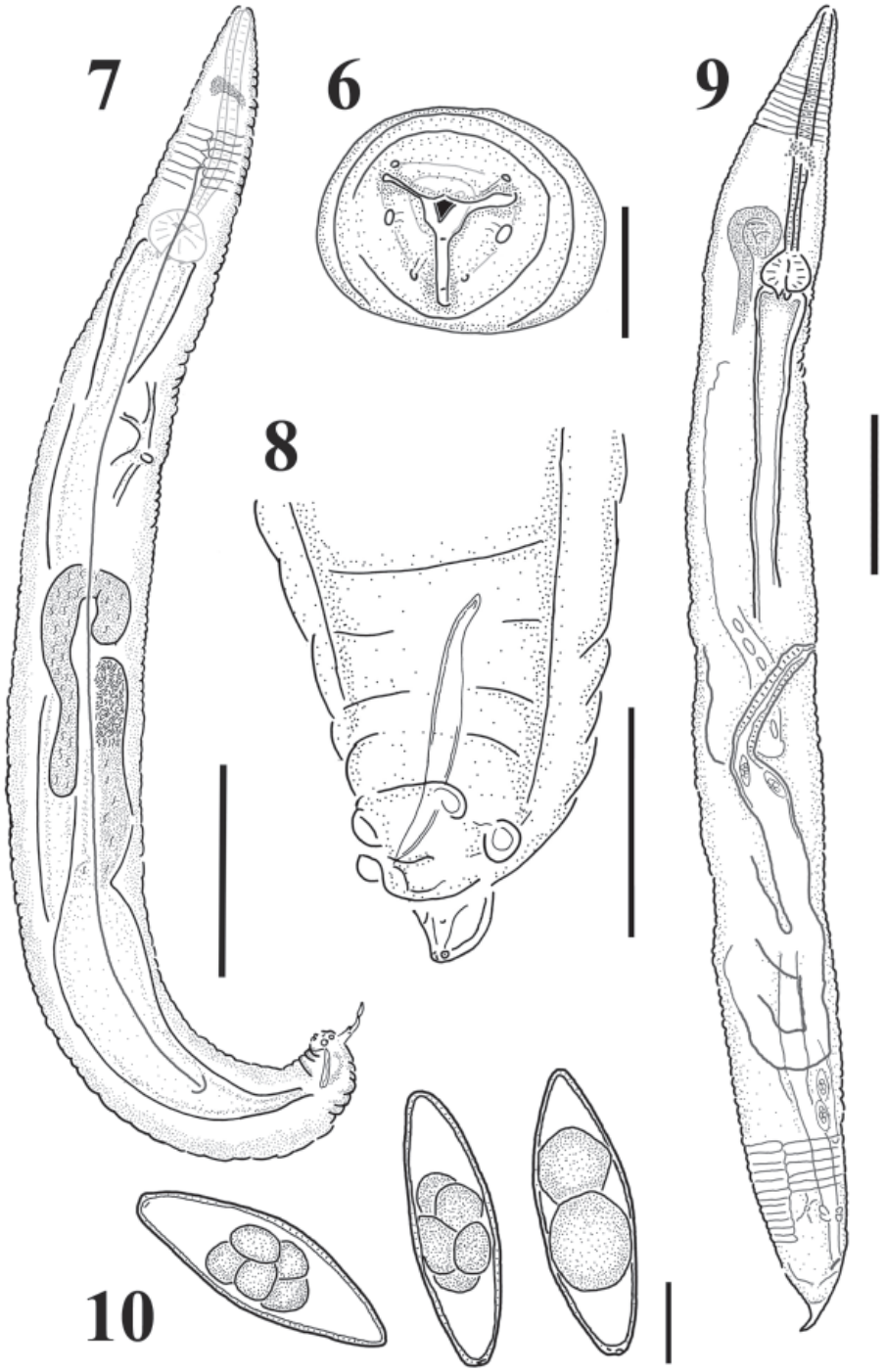
General. Pharyngodonidae. Robust specimens with blunt ends; conspicuous cuticular annulation along the entire body; each ring consists of 2 parts that meet at lateral lines of body. Anterior end with 3 lips, slightly bilobed (Fig. 6). Two lateral amphids and 2 pairs of cephalic papillae. Stoma conical in shape. Sexual dimorphism in size and cuticular ornamentation, consisting in lateral alae present in males.

Males (*based on 2 specimens*; *measurements of holotype in Table 1*). Body length 1 955 - 1 979, maximum width 127 - 152; lateral alae starts slightly anterior to esophageal bulb, ends near tail, length 1 380 - 1 323 (Fig. 7). Stoma wider than long 9 long by 13 wide; total length of esophagus 345; corpus 273 - 287 in length, 23 - 27 in width; short isthmus; esophageal bulb 58 - 72 long by 59 - 80 wide. Nerve ring and excretory pore located at 112 and 617 - 728 from anterior end respectively (Fig 7). Spicule 62 - 70 long, 8 wide at manubrium (Fig. 8). Cloacal lip with no ornamentations. Cloaca opens terminally with 1 pair of subventral precloacal papillae, 1 pair sublateral postcloacal papillae, and 1 pair at the end of stalk of caudal spinneret (Fig. 8); phasmids open in stalk of caudal spinneret. Tail spinneret projected dorsally, total length including stalk 63 - 81.

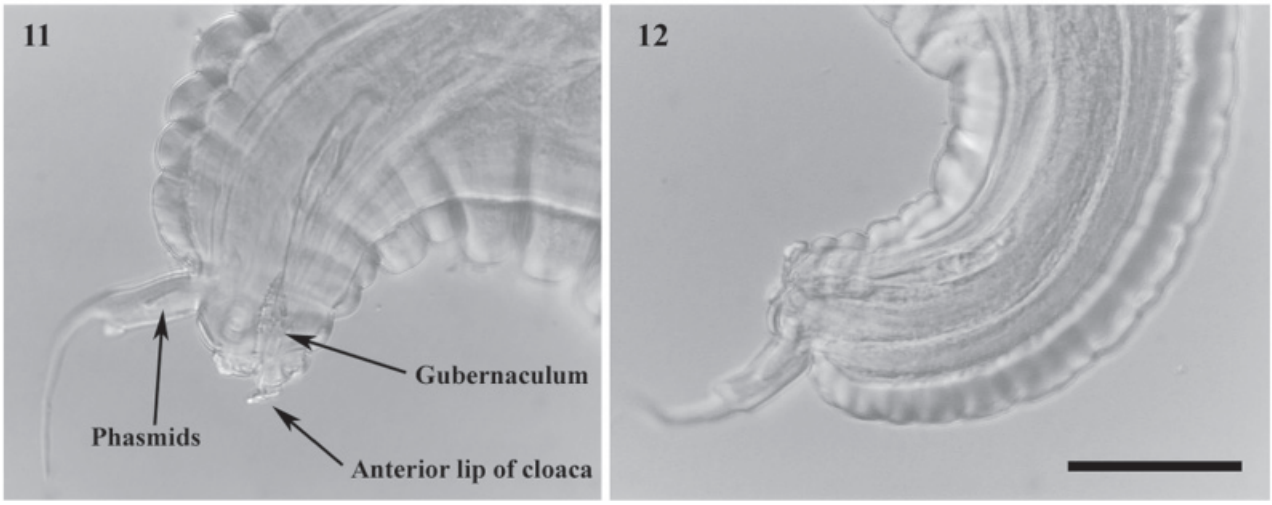
Females (*based on 5 specimens; measurements of allotype in Table 1*). Body length 4 038 - 6 231, 5 169 (20%); maximum width 188 - 406, 315 (30%) (Fig. 9). Stoma short 24 - 48, 35 (31%, n=4). Total length of esophagus 700 - 995, 867 (17%); corpus length 585 - 852, 724 (17%), width 39 - 72, 47 (31%). Esophageal bulb 112 - 145, 133 (10%) in length, 130 - 158, 139 (8%) in width. Nerve ring and excretory pore located 152 - 164, 157 (4%, n=3) and 820 - 1 300, 1 196 (18%) respectively from anterior end. Vulva near mid-body located 1 577 - 2 307, 2 054 (15%) from anterior end. Ovejector 373 in length, maximum width 53. Didelphic, prodelphic; eggs oval, symmetrical, containing non differentiated embryo, flexible shell, 88 - 109, 100 (5%, n=57) long, 27 - 36, 33, (6%, n=57) wide (Fig. 10). Distance of anus to tip of caudal appendage 218 - 251, 232 (6%), conical caudal appendage tapers to a point, with the tip directed posteriad.

Taxonomic summary

Type-host: Bipes tridactylus (Dugès, 1894)
Site of infection: posterior end of intestine.
Symbiotype: MZFC 19278.
Type-locality: MEXICO: Guerrero: Municipio Tecpan de Galeana, San Luis de la Loma, 17° 16’ 19” N, 100° 53’ 28” W, 20 m, 29 June 2004.



Figures 6-10. *Parapharyngodon maestro* n. sp. 6, *en face* view of female showing 3 bilobed lips. Scale bar = 30µ. 7, lateral view of male showing general internal morphology and details of cuticle. Scale bar = 300 µ. 8, ventral view of tail of a male, showing the position and arrangement of the caudal papillae. Scale bar = 50 µ. 9, lateral view of female, showing annulation, shape of tail and relative position of vulva, nerve ring and excretory pore. Scale bar = 500 µ. 10, eggs. Scale bar = 30 µ.



Figures 11-12. 11, *Parapharyngodon lamothei* n. sp., showing relative size of gubernaculum, spicule, caudal papillae, and cloacal lip. 12, *Parapharyngodon maestro* n. sp., showing spicule, caudal papillae, and simple cloacal lips. Scale bar = 50 µ.

Prevalence: 80%
Other localities: MEXICO: Guerrero: Municipio Tecpan de Galeana, Río Tecpan, 17°14' 08" N, 100° 37' 36" W, 65 m, 28 June 2004.
Type-specimens: holotype CNHE 5904; allotype CNHE 5905; paratypes CNHE 5906, CNHE 5907 and CNHE 5908; HWML 48580 and HWML 48581.
Etymology: the Spanish word *maestro* is used as a noun in apposition to the genus name. We use it because among his students, Dr. Rafael Lamothe-Argumedo is known as *Maestro*, meaning professor, moral compass, and mentor.

Remarks

Parapharyngodon maestro is diagnosed by the possession of 3 slightly bilobed lips, a funnel shaped buccal capsule, smooth cloacal lip, eggs with flexible shells, and the presence of 3 pairs of caudal papillae. *Parapharyngodon maestro* is similar to *P. colonensis, P. osteopili*, *Parapharyngodon duniae* Bursey and Brooks, 2004, and *Parapharyngodon ocalaensis* Bursey and Telford, 2002. *Parapharyngodon maestro* is different from *P. ocalaensis* in that *P. maestro* has a longer spicule and the lateral alae start at the level of the nerve ring in *P. ocalaensis*. The eggs of this species are thick shelled, punctated, and the tail of females is spike shaped. The differences with *P. osteopili* and *P. colonensis* include the starting point of the lateral alae (absent in *P. osteopili*), the 4 pairs of caudal papillae, and the textured thick shelled eggs present in both species. Finally, *P. maestro* is different from *P. duniae* in that the latter has a smaller spicule, 7 caudal papillae, pectinated cloacal lip, and textured thick shelled eggs.

Parapharyngodon maestro resembles *P. lamothei* in most characters of the anatomy of females. There are, however, 3 conspicuous differences between them. First, *P. maestro* has 3 slightly bilobed lips, whereas *P. lamothei* presents 6 (Figs. 2, 6); second, *P. maestro* has no gubernaculum or accessory piece (Figs. 11, 12); third, cloacal lips are smooth in *P. maestro*, and finally, *P. lamothei* shows a single medial papillae (immediately postcloacal), not present in *P. maestro*.

Discussion

In recent publications some features have been redescribed and modified from their original description. That includes the nature of the cloacal lip of *Parapharyngodon senisfaciecaudus* Freitas, 1957, which was described as smooth in the original description ["*Formações pectinadas na borda anterior da abertura cloacal ausentes"* p. 452 (Freitas, 1957)]. However, the same structure is described as echinate in the table included in Bursey and Goldberg (2005). This may have been a minor typological error in the construction of the table or an error in the interpretation of the original description. Therefore, the cloacal lip of *P. senisfasciecaudus* must be considered smooth. We have found this character to be extremely useful in the taxonomy of the species.

It is our perception that the tail of females may be of little help in identifying species in this genus. The reason

is that the perception of a "stout spike" or "conical" tail may be caused by the relative width of the body, which depends on the number of eggs in the uteri. The shape of eggs as asymmetrical or symmetrical may be a better indicator of similarity among species. For instance the eggs in *P. lamothei* are symmetrical (even in lateral view) and punctated or textured, whereas the eggs in *P. alvarengai* and *P. senisfaciecaudus* are described as smooth. The surface of the eggs in *P. lamothei* is similar to the surface of those of both *Parapharyngodon cubensis* Barus and Coy Otero, 1969 and *Parapharyngodon riojensis* Ramallo, Bursey and Goldberg, 2002.

Both species of *Parapharyngodon* herein described are the first representatives of the genus to be discovered in members of Bipedidae in the New World. To our knowledge this represents the first report on any species of nematode occurring in species of *Bipes*.

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