

Research Paper

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A new species of *Parapharyngodon* Chatterji, 1933 (Oxyuroidea: Pharyngodonidae), parasitic in *Osteocephalus taurinus* (Anura: Hylidae) from Brazil

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Abstract

Parapharyngodon politoedi n. sp. is described here, based on specimens found in the large intestines of *Osteocephalus taurinus* from the Caxiuanã National Forest, State of Pará, Brazil. The new species is assigned to *Parapharyngodon* based on the presence of non-embryonated eggs with sub-terminal opercula, when in the ovjector. *Parapharyngodon politoedi* belongs to a group of species with three pairs of cloacal papillae and differs from its congeners by morphometric aspects, such as the length of the spicule, and the combination of the following morphological characters: ovaries never encircling the oesophagus, tail shape in females, cloacal lips, sharply pointed spicule and presence of genital cone. This is the second species of nematode reported to parasitize *O. taurinus* and the eleventh species of *Parapharyngodon* from hylids in the Neotropical region.

Introduction

The anuran genus *Osteocephalus* Steindachner, 1862, comprises approximately 24 species and belongs to the family Hylidae Rafinesque, 1815 (Frost, 2017). Among the species of this genus, *Osteocephalus taurinus* Steindachner, 1862, is widely distributed throughout the Amazon, Guyana and Suriname basins. This species is characterized as having a smooth dorsum in females and a grainy one in males, having arboreal and nocturnal habits and commonly being found in primary or secondary forests (Lima *et al.*, 2012; Frost, 2017).

The nematode genus *Parapharyngodon* Chatterji, 1933, has 53 valid species, nine of which are distributed in the Neotropical region; currently, only three species of this genus have been reported from hylid frogs (Araújo-Filho *et al.*, 2015; Bursey & Goldberg, 2015; Velarde-Aguilar *et al.*, 2015; Ramallo *et al.*, 2016; Pereira *et al.*, 2017).

According to Campião *et al.* (2014), almost 92% of anurans in Brazil have not been examined for helminth parasites. Currently, the only nematodes reported to parasitize *O. taurinus* are *Physalopteroides venancioi* Lent, Freitas, & Proença 1946, *Ochoterenella vellardi* Travassos, 1929, and *Kentropyxia hylae* Feitosa, Furtado, Santos & Melo 2015 (Camião *et al.*, 2014; Feitosa *et al.*, 2015). The present work reports and describes a new species of *Parapharyngodon* parasite from the large intestines of *O. taurinus* from the Caxiuanã National Forest, Brazil.

Materials and methods

Eight specimens of *O. taurinus* were collected in the Caxiuanã National Forest, Melgaço Municipality, State of Pará, Brazil (1°47′32.3″S, 51°26′02.5″W) during expeditions to collect helminth parasites of amphibians and reptiles in March 2014 and 2015. The hosts were euthanized by cardiac injection of 2% lidocaine hydrochloride, and their internal organs were examined under a dissecting microscope. The helminths were rinsed in saline and fixed in heated 70% alcohol. For morphological and morphometric analysis, the nematodes were cleared in Aman’s lactophenol and observed using an Olympus BX41 microscope (Olympus, Tokyo, Japan) with an attached drawing tube. All measurements are in micrometres unless otherwise indicated and are presented as the range followed by the mean in parentheses.

For scanning electron microscopy (SEM), the nematodes were dehydrated through a graded ethanol series, critical-point dried with carbon dioxide and coated with gold–palladium. Some

nematode samples were examined using a JEOL JSM-5800LV (Jeol Inc., Peabody, Massachusetts, USA) at the Universidad Nacional del Nordeste (UNNE), Argentina.

Results

Systematics

Family: Pharyngodonidae Travassos, 1920.

Genus: *Parapharyngodon* Chatterji, 1933.

Parapharyngodon politoedi

Taxonomic summary

Type host. *Osteocephalus taurinus* Steindachner, 1862.

Site in host. Large intestine.

Infection parameters. Prevalence 100%.

Type locality. Caxiuanã National Forest, Pará, Brazil (1°47' 32.3"S, 51°26'02.5"W).

Type material. Holotype (MPEG 154); allotype (MPEG 155), paratypes (MPEG 156–175), where MPEG is Museu Paraense Emilio Goeldi, Belém, Pará Brazil.

Etymology. The species epithet is derived from the fictional character named 'Politoed' a frog-type Pokémon from the Pokémon Universe.

Description

General. Small, robust, fusiform nematodes (fig. 1a, e). Cuticle thin with marked transverse annulations along body, excepting tail end. Sexual dimorphism evident. Oral opening triangular, surrounded by three bilobed lips (figs 1b, c and 2b, c), bearing six labial papillae in males: two on each lip and a pair of small amphidial pores in ventro-lateral lips (figs 1b, 2c); females with four labial papillae: two on dorsal lip, one papilla and one amphidial pore on each ventro-lateral lip (figs 1c and 2b). Oxyuroid oesophagus divided in corpus, short isthmus and large bulb. Nerve ring at anterior third of oesophagus, excretory pore post-bulbar (fig. 1a, e). Males with well-developed lateral alae, absent in females (fig. 2a).

Male. Based on holotype and ten paratypes. Total length 1.07–1.73 (1.41) mm, width at oesophagus–intestinal junction 91–209 (118). Lateral alae beginning close to isthmus, ending close to cloacal aperture, 0.73–1.31 (1) mm long. Oesophagus 337–468 (369) long, representing 26% of total body length. Oesophageal corpus 275–325 (299), isthmus 1–2.4 (1) and bulb 61–77 (69) long, bulb width 60–83 (74). Nerve ring and excretory pore at 99–145 (126) and 371–992 (536), respectively, from anterior end. Caudal alae absent. Anterior cloacal lip with discreet fringes (fig. 2e, f). Genital cone present, mammilliform-shaped with terminal ornamentations, fused with posterior cloacal lip. Posterior cloacal lip triangular, smooth edged (fig. 2e, f). Three pairs of sessile caudal papillae: one on anterior cloacal lip, one ad-cloacal, one fused pair situated ventrally on caudal appendage, and a pair of lateral phasmids on caudal appendage (fig. 2e). Spicule poorly sclerotized, sharply pointed 53–75 (64) long (fig. 1g). Cuticular projections beginning almost at mid-length of body (fig. 2a). Tail region ending with cuticular, sharp-pointed, caudal appendage 52–92 (78) long.

Female. Based on allotype and ten paratypes. Total length 2.46–3.9 (3) mm, width at vulvar region 237–542 (391), at oesophagus–intestinal junction 208–405 (325). Lateral alae absent. Oesophagus 580–709 (649) long, representing 22% of total body length. Oesophageal corpus 471–584 (527), isthmus 1–3.15 (2) and bulb 100–132 (120) long, bulb width 108–142 (127). Nerve ring and excretory pore at 126–211 (167) and 711–995 (893), respectively, from anterior end. Vulva, no prominent lips situated at mid-region of the body 711–995 (893) from anterior end (fig. 1a). Ovary post-bulbar, never encircling oesophagus (fig. 1a). Uterus didelphic, amphidelphic, coiled, filled with eggs. Eggs thick-shelled, non-embryonated, with sub-terminal single operculum 93–125 (118) × 27–64 (49) (fig. 1h). Tail conical, narrow, 379–489 (422) long (figs 1d and 2d).

Discussion

This new species is assigned to *Parapharyngodon* Chatterji, 1933, based on the following morphological characters, as proposed by Bursey *et al.* (2013): females with non-embryonated eggs and eggs with a sub-terminal operculum. According to Bursey *et al.* (2013), the absence of a genital cone in *Parapharyngodon* is another important character to differentiate the genus; however, genital cones are observed in *P. politoedi* n. sp., as well as in *P. thulini* Rahimian, Pazoki and Habashi, 2014, *P. almoriensis* Karve, 1949, and *P. echinatus* Rudolphi, 1819.

According to recent publications (Velarde-Aguilar *et al.*, 2015; Pereira *et al.*, 2017; Rizvi *et al.*, 2017) the morphological characteristics frequently used to differentiate the species of this genus are the number of caudal papillae pairs, the morphology of the anterior cloacal lip and the ovary position.

The presence of three pairs of caudal papillae in *P. politoedi* n. sp. is a morphological character shared by 17 other species of the genus: *P. alvarengai* Freitas, 1957; *P. calotis* Johnson, 1966; *P. grismeri* Bursey & Goldberg, 2007; *P. guerreroensis* Bursey & Goldberg, 2015; *P. hugoi* Pereira, Campiã, Luque & Tavares, 2017; *P. hylidae* Velarde-Aguilar, Mata-López, Guillén-Hernández & León-Règagnon, 2015; *P. iguanae* Telford, 1965; *P. japonicus* Bursey & Goldberg, 1999; *P. kasauli* Chatterji, 1935; *P. kenyaensis* Bursey & Goldberg, 2005; *P. maestro* Jiménez, León-Règagnon & Pérez-Ramos, 2008; *P. ocalaensis* Bursey & Telford, 2002; *P. pavlovskiyi* Markov, Ataev & Bogdanov, 1968 (Sharpilo, 1976); *P. thulini*; *P. tuberculata* Rizvi, Maity & Bursey, 2017; *P. tyche* Sulahian & Schacher, 1968; and *P. verrucosus* Freitas & Dobbin, 1959.

Parapharyngodon politoedi n. sp. is similar to *P. thulini*, *P. japonicus*, *P. tyche* and *P. tuberculata* in that it presents ovaries that do not encircle the oesophagus; however, *P. politoedi* n. sp. can be easily distinguished from *P. japonicus* and *P. tyche*, since in both the latter species, the anterior lip of the cloaca is smooth, whereas in *P. politoedi* it is echinate (Sulahian & Schacher, 1968; Bursey & Goldberg, 1999; Rahimian *et al.*, 2014; Rizvi *et al.*, 2017).

The new species also shares the morphology of the spicule tip and cloacal lip and the presence of the genital cone with *P. thulini*; however, these species can be differentiated by the shape of the lateral alae, i.e. well-developed lateral alae beginning close to the isthmus and ending close to the cloacal aperture in *P. politoedi* n. sp., while in *P. thulini*, the lateral alae are rounded at the level of the genital cone and enlarged to form broad vanes in the cloacal region. Additionally, these species can be differentiated by the morphology of the anterior end in males and females; in *P. thulini*, males have a cephalic extremity with three membranes, and females have a subtriangular oral opening surrounded by six

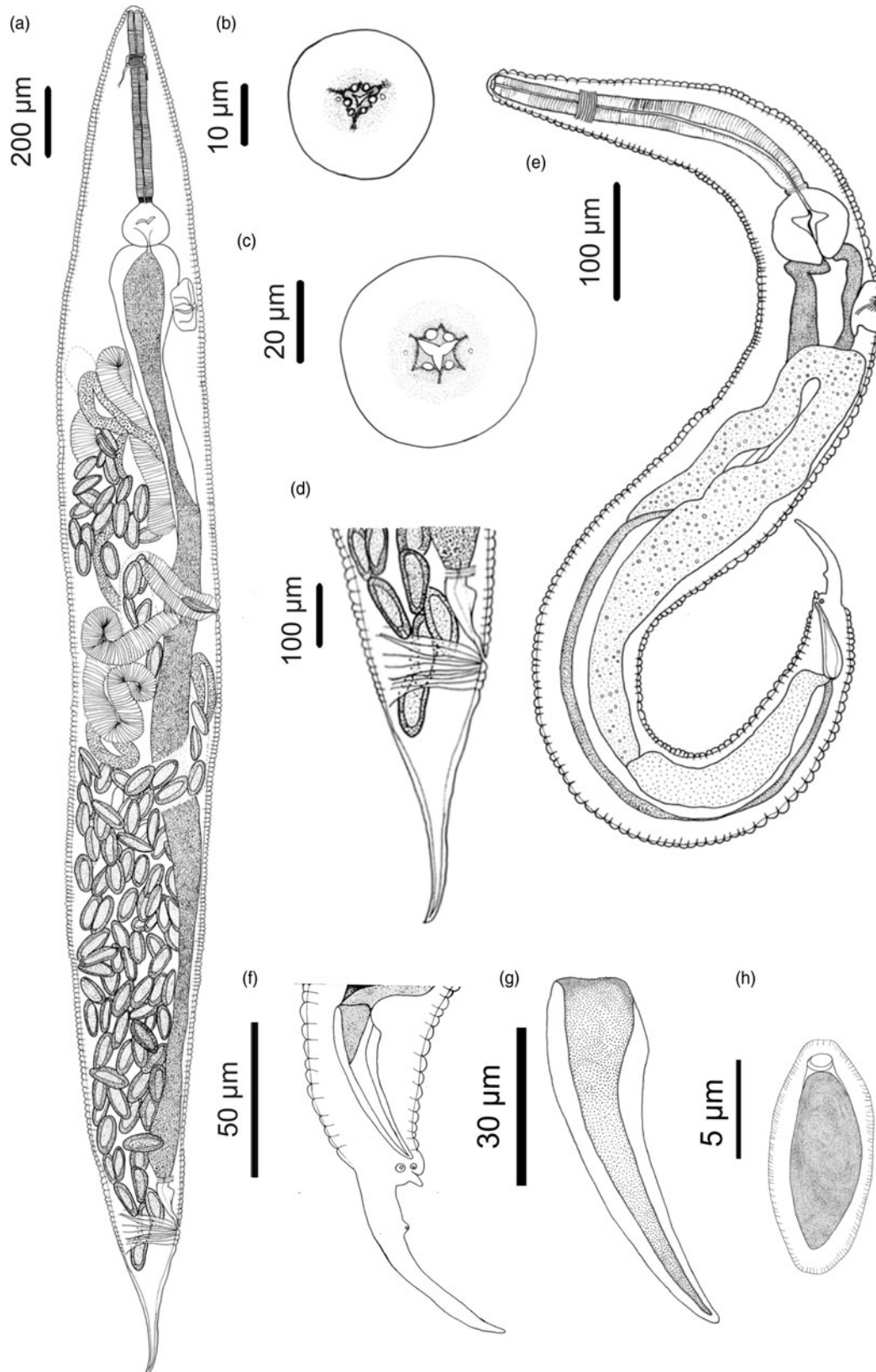


Fig. 1. *Parapharyngodon politoedi* n. sp. line drawings: (a) entire body of female; (b) male apical view; (c) female apical view; (d) female posterior end, lateral view; (e) entire male; (f) male posterior end, lateral view; (g) spicule; (h) egg.

prominent spherical processes, while in *P. politoedi* n. sp., the oral opening in both sexes is triangular, surrounded by three bilobed lips.

In addition, *P. politoedi* n. sp. can be distinguished easily from *P. tuberculata* by the presence of a genital cone (absent in the latter species), morphology of the spicule tip (sharp vs. blunt,

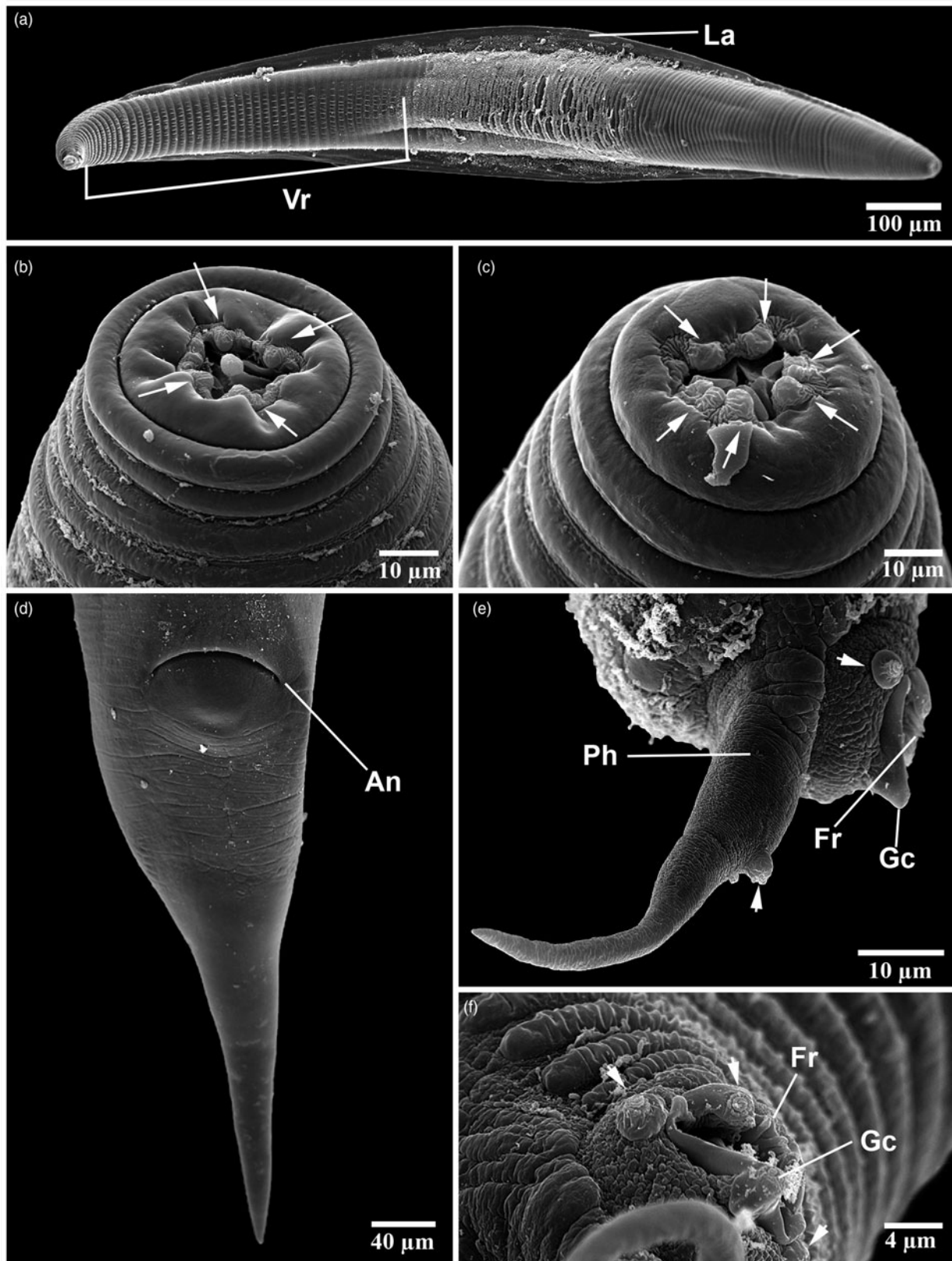


Fig. 2. *Parapharyngodon politoedi* n. sp. scanning electron micrographs: (a) entire male, ventral view (La, lateral alae; Vr, ventral ridges); (b) female, apical view, labial papillae (arrows); (c) male, apical view (arrows, labial papillae); (d) female posterior end (An, anus); (e) male posterior end, lateral view (Ph, phasid; Fr, cloacal internal fringes; Gc, genital cone; arrowheads, caudal papillae); (f) male posterior end, ventral view (Fr, cloacal internal fringes; Gc, genital cone; arrowheads, caudal papillae).

respectively) and the shape of the tail; according to Rahimian et al. (2014) *P. tuberculata* has a tail with a stout spike shape, while *P. politoedi* n. sp. has a conical tail. Regarding morphometric characters, the new species also has a smaller spicule (53–75 in *P. politoedi* n. sp. vs. 112–115 in *P. tuberculata*).

Considering the geographical distribution, nine *Parapharyngodon* species have been described from the Neotropical region: *P. alvarengai*; *P. binae* Pereira, Sousa & Lima, 2011; *P. hugoi*; *P. largitor* Alho & Rodrigues, 1963; *P. riojensis* Ramallo, Bursey & Goldberg, 2002; *P. sanjuanensis* Ramallo, Bursey, Castillo & Acosta, 2016; *P. scleratus* Travassos, 1923; *P. silvo* Araújo Filho, Brito, Almeida, Morais & Ávila, 2015; and *P. verrucosus* (Araújo-Filho et al., 2015; Bursey & Goldberg, 2015; Ramallo et al., 2016; Pereira et al., 2017).

Of these, only *P. alvarengai* and *P. hugoi* have three pairs of caudal papillae; however, in the new species the ovary never encircles the oesophagus, being easily distinguished from *P. alvarengai*, which has a smooth anterior cloacal lip and a prebulbar ovary (encircling the oesophagus), and from *P. hugoi*, which shares the ovary position and cloacal lip morphology with the new species but can be differentiated by close examination of the cloacal lips. Although the cloacal lips are echinate in both species, in *P. hugoi*, the lip flanges are well developed, while in the new species they are delicate; furthermore, the genital cone of *P. hugoi* has a papilla-like structure in its ventral side, which is absent in *P. politoedi* n. sp. Other differences include the shape of the cephalic end. In males of *P. hugoi* this has a triangular aperture internally outlined by cuticular projections and surrounded by six well-developed papillose lips; and, in females, it has an oval oral aperture surrounded by six flattened lips. In *P. politoedi* n. sp., both males and females have triangular oral openings and different shapes and distributions of papillae and lips.

According to Pereira et al. (2017), *Parapharyngodon* apparently exhibits low host specificity, and one species can be found infecting different host families. However, we will compare the new species with other *Parapharyngodon* spp. described from hylids. Thus, three other congeners have been described from hylid frogs: *P. hugoi*, a parasite of *Trachycephalus typhonius* (Linnaeus, 1758) from Brazil; *P. duniae* Bursey & Brooks, 2004, from *T. venulosus* (Laurenti, 1768) from Costa Rica; and *P. hylidae*, a parasite of *Tripurion petasatus* (Cope, 1865) from Mexico (Bursey & Brooks, 2004; Velarde-Aguilar et al., 2015; Pereira et al., 2017).

Parapharyngodon politoedi n. sp. can be differentiated from *P. duniae* by the number of cloacal papillae, 3 pairs vs. 3 pairs + 1, respectively; by the position of the ovary (not encircling the oesophagus vs. encircling it); and by having a larger spicule (53–75 µm vs. 40–49 µm).

The new species is distinguished easily from *P. hylidae* by the presence of a gubernaculum in the latter species (absent in *P. politoedi* n. sp.). Other differences include the morphology of the oral opening and the distribution of papillae in the oral opening, lateral alae (in *P. hylidae*, these end far anterior to the cloaca vs. close to the cloaca in *P. politoedi* n. sp.), morphology of the posterior cloacal lip (in *P. hylidae*, this is divided into three lobes vs. triangular and smooth edged in *P. politoedi* n. sp.) and cuticular transversal and lateral thick striations on the lateral flanks of females in *P. hylidae*, which are absent in the new species. The differences between the new species and *P. hugoi* have been shown above.

In the present work, SEM analysis showed details not usually easily observed by light microscopy, especially of the morphology of the oral opening. According to Pereira et al. (2017), few species

of *Parapharyngodon* have been observed using SEM, and the study of Velarde-Aguilar et al. (2015) confirms that the use of this technique allows a detailed description of the anterior end, which usually contains characters important for interspecific differentiation and sexual dimorphisms in *Parapharyngodon*.

Thus, the present work describes a new species of the genus *Parapharyngodon*, which includes ten species from the Neotropical region. This species is the fourth species of this genus described parasitizing hylid frogs, and the second species of nematodes described in this host species, *O. taurinus*, from Brazil and the eastern Brazilian Amazon. Thus, this report adds new knowledge of the helminth fauna of our region.

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Conflict of interest. None.

Ethical standards. All applicable institutional, national and international guidelines for the care and use of animals were followed. The present study was approved by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Brazil, through licence SISBIO 30772-4.

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