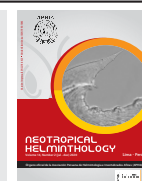




## Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

### INTESTINAL HELMINTHS OF *HYLODES HEYERI* HADDAD, POMBAL & BASTOS, 1996 (ANURA: HYLODIDAE) IN THE ATLANTIC FOREST, BRAZIL

### HELMINTOS INTESTINALES DE *HYLODES HEYERI* HADDAD, POMBAL & BASTOS, 1996 (ANURA: HYLODIDAE) EN EL BOSQUE ATLÁNTICO, BRASIL

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## ABSTRACT

Cosmocercid nematodes are common parasites of amphibians and reptiles. Although it is a well-known family, knowledge about its host diversity is lacking. Herein, we expand the knowledge of the anuran parasitic fauna of the family Hylodidae and the geographic distribution of three species of cosmocercid nematodes (*Cosmocerca brasiliensis* Travassos, 1925, *Cosmocercoides sauria* Ávila, Strüssmann & Silva, 2010, and an unidentified cosmocercid species) found in two male torrent frogs *Hylodes heyeri* Haddad Pombal & Bastos, 1996. Our report includes new data on the geographic distribution and the morphology of cosmocercid parasites and further enlightens the parasitic interactions of Neotropical amphibians.

**Keywords:** amphibians – Cosmocercidae – endoparasites – Nematoda – new host

## RESUMEN

Los nematodos cosmocercidos son parásitos comunes de anfibios y reptiles. Aunque es una familia bien conocida, falta conocimiento sobre la diversidad de sus hospederos. En este documento, ampliamos el conocimiento de la fauna parasitaria de anuros de la familia Hylodidae y la distribución geográfica de tres especies de nematodos cosmocercidos (*Cosmocerca brasiliensis* Travassos, 1925, *Cosmocercoides sauria* Ávila, Strüssmann & Silva, 2010 y cosmocercid) encontrados en dos ranas torrentes macho *Hylodes heyeri* Haddad Pombal & Bastos, 1996. Nuestro reporte incluye nuevos datos sobre la distribución geográfica y la morfología de los parásitos cosmocercidos y aclara aún más las interacciones parasitarias de los anfibios neotropicales.

**Palabras clave:** anfibios – Cosmocercidae – endoparasitos – nematoda – nuevos hospedadores

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## INTRODUCTION

Nematodes are typical macroparasites of amphibians (Koprivnikar *et al.*, 2012). The interaction between these two animal groups is an outcome of their coevolutionary history and the biological constraints of host species (Campião *et al.*, 2016). Unfortunately, we have a long way to go to a comprehensive perspective on the complexity of the interactive network between frogs and helminths. One important step to reduce this gap is by searching for descriptive notes about the occurrence of hosts, which also generates data on the geographical ranges of the parasites (Toledo *et al.*, 2018).

While Cosmocercidae nematodes are a common parasite of amphibians (Campião *et al.*, 2014), the list of hosts they interact with is not fully known. Therefore, focusing on poorly studied groups of frogs in parasite research is a promising approach to reveal new interactions (Campião *et al.*, 2015).

Here we study helminths in a torrent frog species of the family Hylodidae, which is composed of 47 species (Segalla *et al.*, 2019). *Hylodes* (Fitzinger, 1826) is the most diverse genus of Hylodidae and includes 26 species, all endemic to Brazil (Frost *et al.*, 2020; Segalla *et al.*, 2019). *Hylodes heyeri* Haddad Pombal & Bastos, 1996 was described from the municipality of Eldorado, São Paulo State, but occurs in other localities in Paraná and Santa Catarina State (Lingnau, 2004; Araújo *et al.*, 2010; Monteiro *et al.*, 2014). It is a diurnal species exhibiting vocal activity and visual signals on rocks of typical Atlantic forest streams (Haddad *et al.*, 1996; Lingnau & Bastos, 2007; de Sá *et al.*, 2016).

Despite the high number of Hylodidae species, their parasites are rarely studied, with only one report of a filarial parasite infecting *H. heyeri* (D'Bastiani *et al.*, 2018).

Here we describe the infection of *H. heyeri* with cosmocerciid nematode species, expanding our knowledge of the anuran parasitic fauna of the family Hylodidae and the geographic distribution of these helminths. Besides, we provide a brief morphological description of cosmocerciid parasites.

## MATERIAL AND METHODS

On September 16<sup>th</sup>, 2017, we collected two specimens of *H. heyeri* by hand during visual encounter surveys in two localities from São Paulo State: one adult male specimen (snout-vent length [SVL] 38.5 mm) from Parque Estadual da Caverna do Diabo (-24.636329°S, -48.403442°W) in the municipality of Eldorado and on September 13<sup>th</sup>, 2017, and another adult male specimen (SVL 42 mm) from Parque Estadual do Turístico do Alto Ribeira (-24.533361S°, -48.699261°W) in the municipality of Iporanga, São Paulo State, Southeast Brazil.

We searched all internal organs for helminths using a stereoscope, soon after the frogs were euthanized. After this, the hosts were fixed in 10% formalin, preserved in 70% alcohol, and deposited at Museu de Zoologia “Prof. Adão José Cardoso” at the Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, Brazil (under the codes ZUEC-AMP 24061 and ZUEC-AMP 24023). The nematodes found were cleared with lactophenol and the taxonomic determination was conducted by comparing morphological traits with published descriptions (Vicente *et al.*, 1991, Anderson *et al.*, 2009, Gibbons, 2010).

Voucher helminth specimens were deposited in the Helminthological Collection of the Institute of Biosciences, São Paulo State University – UNESP (CHIBB), in the municipality of Botucatu, São Paulo, Brazil.

**Ethics aspects:** The collections were authorized by Instituto Chico Mendes de Conservação da Biodiversidade – ICMBIO for collecting permission (ICMBio permit 57611-2). The procedures were approved by the Ethics Committee on the Use of Animals (CEUA#126) of the Institute of Biosciences, São Paulo State University – UNESP.

## RESULTS

Both collected specimens of *H. heyeri* were infected with at least one helminth parasite. In the

small intestine of the individual from Iporanga, we found one specimen of *Cosmocerca brasiliensis* Travassos, 1925 (CHIBB 8852). The nematode had a small and slender body, spicules of 210.9 mm, gubernaculum measuring 195.3 mm, and three precloacal and four postcloacal pairs of caudal papillae, and 11 pairs of rosettes (Figure 1; Table 1). More detailed morphometry was not performed, because the anterior part of the body was cut and separated for later molecular analysis, which unfortunately was not successful. However, the measurements performed on the posterior part of the body were consistent with previous *C. brasiliensis* description (Table 1).

In the large intestine of the host specimen from Eldorado, we found one adult male *Cosmocercoides sauria* Ávila, Strüssmann &

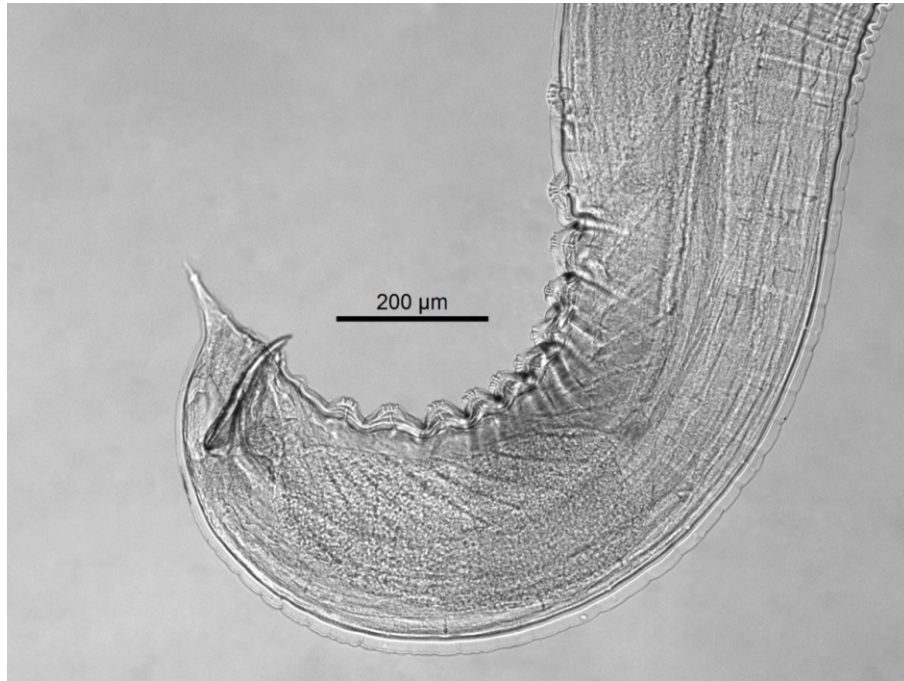
Silva, 2010 (CHIBB 8853) and seven adult cosmocercioid females (CHIBB 8854). The *C. sauria* male was 2.3 mm long and 205 mm wide at midbody, with pharyngeal portion 20.4 mm long, corpus 257.7 mm long, bulb length 55.3 mm, bulb width 63.5 mm, nerve ring 103.6 from the anterior extremity, excretory pore 174.8 mm from anterior end. Its tapering tail was 59.5 mm long. Lateral alae were present, gubernaculum 105.5 mm, equal spicules 103.7 mm, and four pairs of rosette papillae (Figure 2).

The seven cosmocercioid females presented 4.5–5.4 mm long and 409.3–506.2 mm wide, pharyngeal portion 41.2–58.5 mm, esophagus total 693.6–720.1 mm, with corpus 484.7–498.8 mm, isthmus 38.1–51.3 mm and bulb 99.7–124.8 x 135.7–151.9 mm, excretory pore 533.6–559.8

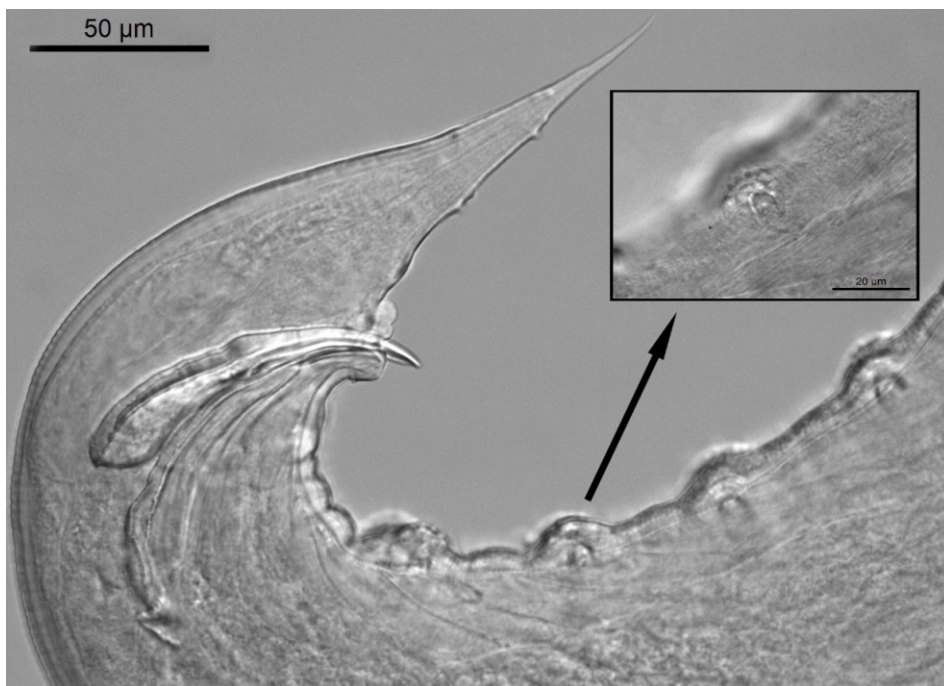
**Table 1.** Morphometric comparison of *Cosmocerca brasiliensis*. All measurements are in micrometers, except where stated.

Parameter	Present study	Data from Travassos (1931)		Data from Dyer & Altig (1976)	
		Male	Female	Male	Female
Sex (N = individuals)	Male (N=1)	Male	Female	Male (N=5)	Female (N=8)
Length (mm)	-	5.5	9	2.24–3.99	9.67–12.67
Width	-	52	96	180–270	320–490
Pharyngeal portion	-	48–72	48–72	39–47	68–81
Esophagus	-	60–92	060–92	400–509	780–960
Corpus	-	-	-	-	-
Isthmus	-	-	-	-	-
Bulb	-	100–170	100–170	78–96	140–169
Excretory pore	-	-	-	258–390	450–660
Nerve ring	-	-	-	122–231	260–327
Spicules	210.9	150–200	-	158–185	-
Gubernaculum	195.3	-	-	140–156	-
Rosette	11 pares	9–11 pairs	-	8 pairs	-
Caudal papillae	-	0:3:4	-	-	-
Anus	-	28–32	56–70	-	-
Vulva from anterior end (mm)	-	-	-	-	3.8–5.1
Tail	228.8	-	-	-	530–740
Eggs	-	-	0.90 x 54	-	62–93 x 31 55

from anterior end, nerve ring 232.1–242 mm from anterior end, vulva 2.2–3 mm from the anterior end, tail 374.5–401.8 mm long. Eggs were 63.6–73.6 x 33.7–47.4 mm (Figure 3).



**Figure 1.** Posterior region of male *Cosmocerca brasiliensis*.



**Figure 2.** Posterior region of male *Cosmocercoides sauria*. Highlighted rossete.

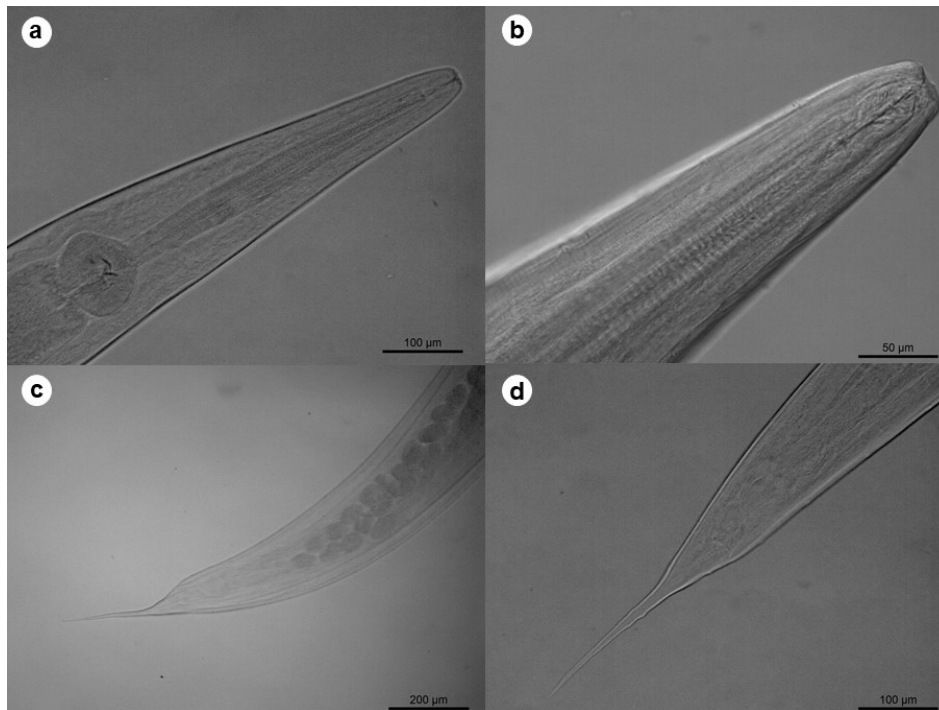


Figure 3. Cosmocerciid female. (a-b) Anterior end, and (c-d) posterior end.

## DISCUSSION

Our results contribute to the knowledge on the parasitic interactions of Neotropical amphibians. The three nematode taxa found in *H. heyeri* is an important contribution to the poorly known parasites of this frog family, as previously registers of parasites in this species were limited to one report of a filarial nematode (D'Bastiani *et al.*, 2018), while other helminth species have been reported in other species of Hylodidae (Campião *et al.*, 2014).

*Cosmocerca brasiliensis* is an anuran intestinal parasite and has been reported from Brazil, Ecuador, Guayana, and Peru (Campião *et al.*, 2014). In Brazil, this nematode species was found parasitizing the intestine of *Rhinella icterica* (Spix, 1824) in Santa Catarina State (Santos *et al.*, 2013), *Dendropsophus cachimbo* (Napoli & Caramaschi, 1999) in Pará State, and *Boana faber* (Wied, 1821), *Ischnocnema guentheri* (Steindachner, 1864), *Proceratophrys appendiculata* (Günther, 1873), *Thoropa miliaris* (Spix, 1824), and *Rhinella crucifer* (Wied, 1821) in Rio de Janeiro State (Campião *et al.*, 2014). This is the first record of *C.*

*brasiliensis* parasitizing an anuran in São Paulo State, expanding the geographical distribution of this parasite and adding *H. heyeri* as a newly reported host.

*Cosmocercoides sauria* has been described in the small and large intestines of the gymnophthalmid lizard *Iphisa elegans elegans* Gray, 1851 from the municipality of Vale de São Domingos, Mato Grosso State, Brazil (Ávila *et al.*, 2010) and no other report on the occurrence of this helminth has been published. Therefore, here we report for the first time the interaction between this parasite and its amphibian host. Also, our record expands its geographical distribution to the southeastern region of Brazil.

In the same host infected with a male of *C. sauria*, seven cosmocerciid adult female specimens were also found. However, these females were not identified as *C. sauria* since their morphometrical data were inconsistent with the data reported by this nematode species (Ávila *et al.*, 2010) and so characterizing a mixed infection.

Our results reinforce the necessity of more studies on the parasitic fauna of Neotropical amphibians,

especially of hylodid frogs, a promising family to find unknown host-parasites interactions.

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