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First record of *Eustrongylides* sp. (Nematoda: Dioctophymatidae) larvae parasitizing snakes in the Neotropical region

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ABSTRACT

Eustrongylides uses piscivorous birds as definitive hosts, oligoquets as intermediate hosts and fish as second intermediate hosts. However, amphibians and reptiles can act as secondary or paratenic hosts. The following work presents the first record of *Eustrongylides* larvae in snakes in the Neotropics. The coelomic cavity of 101 snakes belonging to *Helicops infrataeniatus*, *Philodryas aestiva, Philodryas olfersii, Philodryas patagoniensis, Erythrolamprus poecilogyrus*, *Erythrolamprus jaegeri, Atractus reticulatus, Dipsas ventrimaculatus, Thamnodynastes strigatus, Phalotris lemniscatus* and *Bothrops alternatus* from southern Brazil were examined. Six *H. infrataeniatus* were parasitized with larvae of *Eustrongylides* sp. The encounter of these larvae only in this specie can be related to the aquatic habit of the snake, which allows ecological interactions with the definitive and intermediate hosts of the *Eustrongylides* cycle. The occurrence of these parasites in a Neotropical snake is registered for the first time, contributing to the knowledge of the helminth diversity associated to Brazilian snakes.

Key Words: *Helicops infrataeniatus*; Helminths; Parasitism; Reptiles; Serpentes.

Eustrongylides (Jägerskiöld, 1909) (Nematoda: Dioctophymatidae) was proposed for Dioctophymatidae species that cause tumors on the wall of the proventriculus, mainly from piscivorous birds (Measures, 1988). Some species were reported in Gaviiformes, Podicipediformes, Pelecaniformes, Anseriformes, Charadriiformes, Ciconiiformes and Passeriformes (Measures, 1988). *Eustrongylides* consists of three species: *Eustrongylides tubifex* Jägerskiöld, 1909, *Eustrongylides ignotus* (Jägerskiöld, 1909) and *Eustrongylides excisus* (Jägerskiöld, 1909) (Melo *et al.*, 2016).

Regarding the geographical distribution of these parasites, *Estrongylides ignotus* has the widest distribution, occurring in the Nearctic, Neotropical and Australian regions. *Eustrongylides excisus* occurs in Europe and *E. tubifex* is present only in the Holartic and Neotropical regions (Measures, 1988).

The life cycle of these parasites is not well known (Xiong *et al.*, 2009), however, some studies indicate that these helminths use piscivorous birds as definitive hosts, oligoquets as intermediate hosts and usually fish as intermediate second hosts, with amphibians and reptiles as intermediate or paratenic hosts (Measure, 1988; Friend and Franson, 1999; Melo *et al.*, 2016). *Eustrongylides* species may occasionally parasitize humans, thus demonstrating zoonotic potential (Xiong *et al.*, 2009; Eiras *et al.*, 2016).

Little is known about the parasitism of *Eustrongylides* species in wild snake populations, but larvae of these parasites have been reported in some Colubridae from North America, Europe and Asia,

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such as: *Nerodia sipedon* (Linnaeus, 1758), *Natrix natrix* (Linnaeus, 1751) and *N. tessellata* (Laurenti, 1768) (Bursey, 1986; Biserkov, 1995; Mihalca, 2007; Yildirimhan *et al.*, 2007). In acrochordid snakes from Australia there are also reports of the occurrence of these parasites (Jones, 1978). In captivity, larvae of *Eustrongylides* have been observed in some colubrid snakes such as *Coluber constrictor* (Linnaeus, 1758), *Masticophis flagellum* (Shaw, 1802), *Nerodia sipedon*, *Pituophis melanoleucus* (Daudin, 1803), *Thamnophis sirtalis* (Linnaeus, 1758), as well as in the viperid snakes *Bothrops atrox* (Linnaeus, 1758) and *Agkistrodon contortrix* (Linnaeus, 1766) in North America (Winsor, 1948 *in* Bursey, 1986). In Brazil, while these nematodes have been reported in some hosts, such as birds (Vicente *et al.*, 1995; Bernardon *et al.*, 2017), amphibians (Melo *et al.*, 2016) and fish (Isaac *et al.*, 2004; Meneguetti *et al.*, 2013; Carvalho *et al.*, 2017), we know of no reports of their occurrence in snakes. The following work presents the first record of *Eustrongylides* larvae in wild snakes from the Neotropical region.

One hundred and one specimens belonging to 11 species of snakes were examined: *Helicops infrataeniatus* Jan, 1865 (n=65), *Philodryas aestiva* (Duméril, Bibron & Duméril, 1854) (n=1), *P. olfersii* (Lichtenstein, 1823) (n=4), *P. patagoniensis* (Girard, 1858) (n=6), *Erythrolamprus poecilogyrus* (Wied-Neuwied, 1825) (n=10), *E. jaegeri* (Günther, 1858) (n=2), *Atractus reticulatus* (Boulenger, 1885) (n=1), *Dipsas ventrimaculatus* (Boulenger, 1885) (n=3), *Thamnodynastes strigatus* (Günther, 1858) (n=2), *Phalotris lemniscatus* (Duméril, Bibron & Duméril, 1854) (n=1) (Dipsadidae) and *Bothrops alternatus* (Duméril, Bibron & Duméril, 1854) (n=6) (Viperidae).

The snakes came from the municipalities of Capão do Leão (31° 45' 48" S - 52° 29' 02" W), Pelotas (31° 46' 19 "S - 52° 20' 33 "W), Rio Grande (32° 02' 06 "S - 52° 05' 55 "W), Encruzilhada do Sul (30° 32' 38 "S - 52° 31' 19 "W) and Dom Pedrito (30° 58' 58 "S - 54° 40' 23 "W), Rio Grande do Sul. Fifty of them were collected dead on roads from March 2017 to June 2019. The collection of specimens were licensed by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio No. 38913). Four snakes were donated by the Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres of the Federal University of Pelotas (NURFS-CETAS/UFPel), where they died after a rehabilitation attempt. The other hosts were donated by the Vertebrate Laboratory of the Federal University of Rio Grande (n= 36) and Vertebrate Zoology Laboratory of the Federal University of Pelotas (n= 11), where the specimens were fixed in formalin and conserved in 70°GL alcohol.

The hosts were necropsied for analysis of the coelomic cavity. The larvae were fixed in AFA, conserved in 70°GL alcohol and clarified with Amann lactophenol for morphological identification according to Measures (1988). The parasitological indices were calculated according to Bush *et al.* (1997). The specimens were deposited in the helminth collection of the Laboratory of Wild Animal Parasitology (CHLAPASIL n° 831-836). The photomicrographs were done in an Olympus® BX 41 microscope with attached camera system and the images were prepared in Adobe Photoshop® CS5.

Among the total hosts examined, six (5.9%) were parasitized by fourth stage larvae of *Eustrongylides* sp. (Fig. 1), which were found in the coelomic cavity of *Helicops infrataeniatus* (Fig. 2), with a prevalence of 9.2% and mean intensity of infection of 1.83 helminths/host. Female larvae were larger than male larvae, which measured 75-115 mm and 65-80 mm length, respectively.

Helicops infrataeniatus is a non-venomous aquatic snake that can reach about one meter in length. It is distributed in southern Brazil, Paraguay, Uruguay and Argentina (Quintela and Loebmann, 2009; Abegg and Entiauspe-Neto, 2012). The diet of the species includes mostly fish and amphibians (Aguiar and Di-Bernardo, 2004), which were possibly the source of infestation of the snakes in this study.

The transmission of *Eustrongylides* species occurs through the trophic chain involving aquatic organisms such as oligochaetes (primary intermediate hosts), fish, anurans, snakes (secondary and/or paratenic intermediate hosts) and birds (definitive hosts). Bird infection occurs by ingestion of the infective fourth-stage larva present in the secondary and/or paratenic intermediate hosts (Measure, 1988; Moravec, 1994; Friend and Franson, 1999).

Larvae are often found parasitizing several fish species in Brazil, such as *Hoplias malabaricus* (Bloch, 1794) (Characiformes), *Cichla piquiti* Kullander & Ferreira, 2006 and *Plagioscion squamosissimus* (Heckel, 1840) (Perciformes) (Isaac *et al.*, 2004; Martins *et al.*, 2009; Meneguetti *et al.*, 2013; Carvalho *et al.*, 2017). Amphibians were also reported as hosts of *Eustrongylides* larvae in northern Brazil (Melo *et*

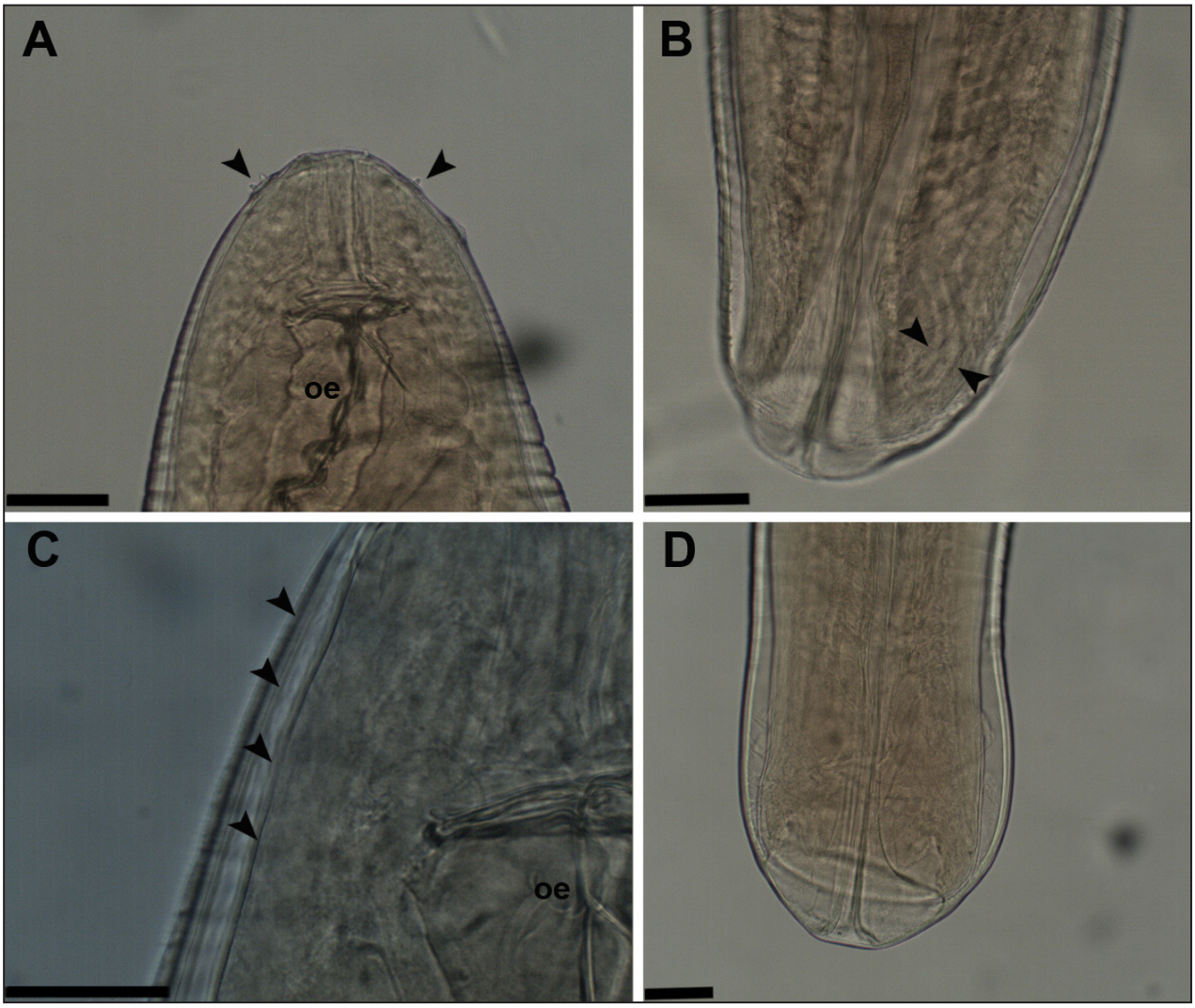

Figure 1. Fourth stage larvae from *Eustrongylides* sp. (Enoplida: Dioctophymatidae) found in the coelomic cavity of *Helicops infrataeniatus* Jan, 1865 (Serpentes: Dipsadidae) in the extreme south of Brazil. A - Anterior extremity of female larva (Arrows indicate the cephalic papillae; *oe*: esophagus; bar – 100 µm). B - Posterior extremity of the female larva (Arrows indicate the primordial vulvar; bar - 100µm). C - Arrows indicate cuticle layers (*oe*: esophagus; bar - 12,5 µm). D - Posterior end of the male larva (bar – 60 µm).

al., 2016). There are no previous records of larvae of this nematode associated with snakes in Brazil; however, there are records in North America (Bursey, 1986), Europe (Mihalca *et al.*, 2007; Biserkov, 1995; Yildirimhan *et al.*, 2007) and Australia (Lichtenfels and Lavies, 1976; Jones, 1978).

Snakes can include in their diet of large variety of vertebrates (Voris and Murphy, 2002; Abegg *et al.*, 2005; Costa *et al.*, 2009), enabling the transmission of parasites through complex trophic networks (Marcogliese, 2004). Herons, such as *Syrigma sibilatrix* (Temminck, 1824) (Ardeidae) feed on *Helicops infrataeniatus* as observed by Franz *et al.* (2007) in Campo Belo do Sul in the state of Santa Catarina, Brazil. In the region of our present study, *Eustrongylides ignotus* was reported in herons *Ardea cocoi* Linnaeus, 1766 and *Nycticorax nycticorax* (Linnaeus, 1758) (Ardeidae) (Bernardon *et al.*, 2017), and larvae of this nematode were found in siluriform fish, *Callichthys callichthys* (Linnaeus, 1758), as well as in *Hoplias malabaricus* (Carolina Silveira Mascarenhas, com. pess.). The record of fourth stage larvae of *Eustrongylides* sp. in *H. infrataeniatus* suggests that the species may act as a trophic bridge in the parasite cycle, since the snake has aquatic habits, feeds on fish and serves as a food resource for birds such as herons. These records highlight the importance of the region for maintenance of the *Eustrongylides* species life cycle and warns about the presence of a nematode with zoonotic potential (Eiras *et al.* 2016).

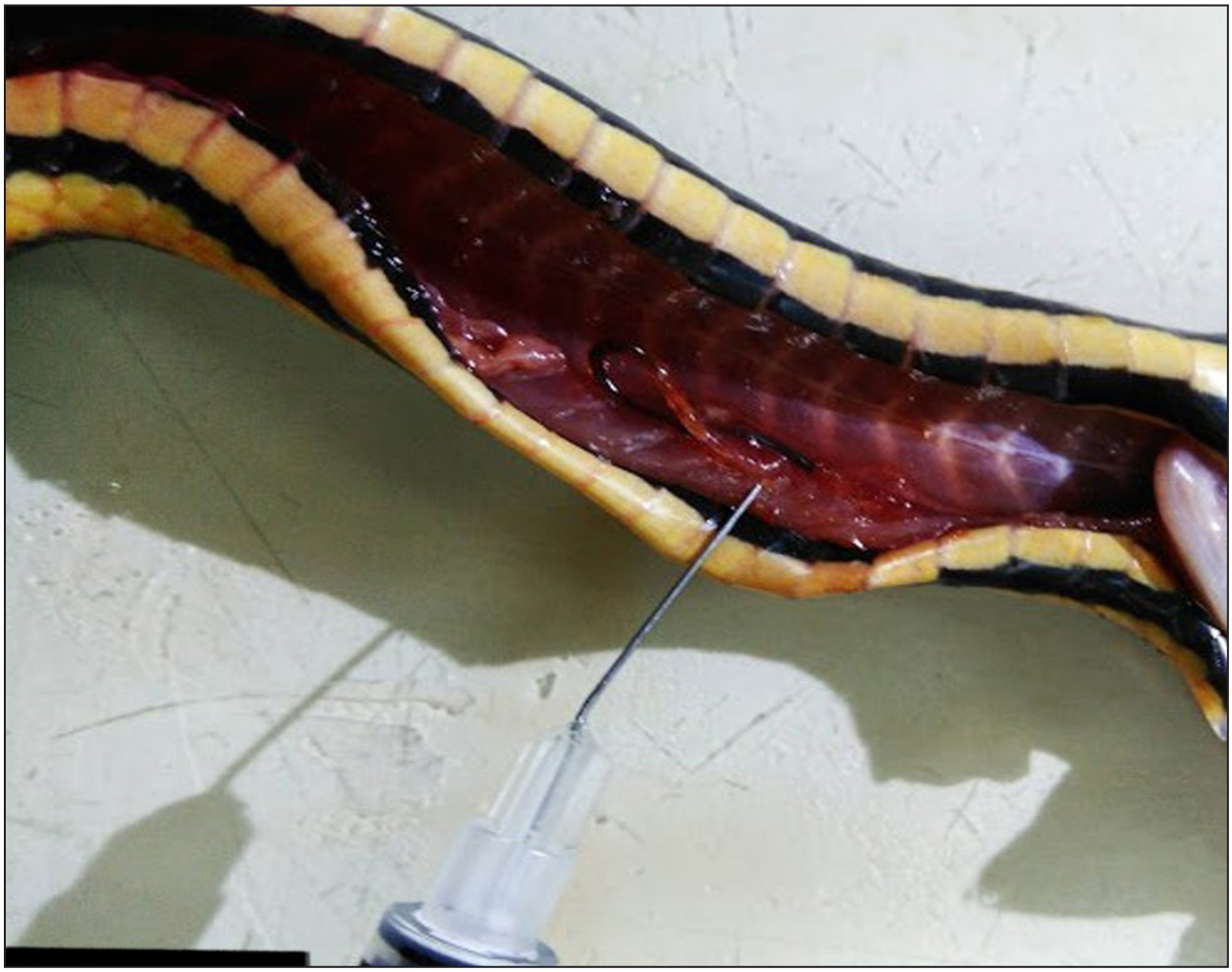

Figure 2. *Eustrongylides* sp. fourth stage larva (Enoplida: Dioctophymatidae) found in the coelomic cavity of *Helicops infrataeniatus* Jan, 1865 (Serpentes: Dipsadidae) in the extreme south of Brazil (Bar – 12 mm).

It is important to point out that the other snakes used in this study present predominantly terrestrial habits, such as *Bothrops alternatus, Thamnodynastes strigatus, Philodryas patagoniensis*, or semi-aquatic habit as *Erythrolamprus jaegeri* and *E. poecilogyrus* (Achaval and Olmos, 2003), which may hinder or even make completely impossible the transmission of this nematode. However, future studies should be conducted to complement the results presented herein to help in the understanding of parasitic relationships involving *Eustrongylides* species and snakes in the Neotropical region.

The occurrence of *Eustrongylides* larvae in *Helicops infrataeniatus* registered for the first time, is the second finding of helminths parasitizing this species in Brazil, since there is a previous record of parasitism by *Opisthogonimus serpentis* Artigas, Ruiz & Leão 1943 (Digenea: Opsitogonimidae) (Fernandes and Kohn, 2014).

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