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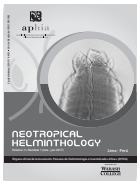

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

LARVAE OF *DIOCTOPHYME RENALE* (GOEZE, 1782) (NEMATODA: ENOPLIDA) AND *CONTRACAECUM* SP. (NEMATODA: ANISAKIDAE) IN FRESHWATER TURTLES (TESTUDINES: CHELIDAE) FROM SOUTHERN BRAZIL

LARVAS DE *DIOCTOPHYME RENALE* (GOEZE, 1782) (NEMATODA: ENOPLIDA) Y *CONTRACAECUM* SP. (NEMATODA: ANISAKIDAE) EN TORTUGAS DE AGUA DULCE (TESTUDINES: CHELIDAE) DEL SUR DE BRASIL

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ABSTRACT

Dioctophyme renale (Goeze, 1782) has been reported in several species of wild carnivores and in domestic dogs. Aquatic oligochaetes act as intermediate hosts, frogs and fish act as paratenic hosts. *Contracaecum* spp. parasites fish-eating birds and mammals associated with aquatic environments act as intermediate hosts, and fish act as intermediate and paratenic hosts. This work aims to report the occurrence larvae of *D. renale* and *Contracaecum* sp. in freshwater turtles. Nine specimens of *Phrynops hilarii* (of Duméril & Bibron, 1835) and 21 specimens of *Acanthochelys spixii* (Duméril & Bibron, 1835) were collected from Rio Grande do Sul, Brazil. Four specimens of *P. hilarii* were infected by third-stage larvae of *D. renale* and one specimen of *A. spixii* was infected by third-stage *Contracaecum* sp. larvae. This is the first record of larvae of *D. renale* and *Contracaecum* sp. in *P. hilarii* and *A. spixii*, respectively.

Keywords: *Acanthochelys spixii* – giant kidney worm – nematodes – *Phrynops hilarii* – third-stage larvae

RESUMEN

Dioctophyme renale (Goeze, 1782) se ha reportado en varias especies de carnívoros silvestres y en perros domésticos. Los oligoquetos acuáticos son hospedadores intermedios, ranas y los peces actúan como hospedadores paraténicos. *Contracaecum* spp. es parásito de aves y mamíferos que alimentan de peces, invertebrados actúan como hospedadores intermedios, y los peces actúan como hospedadores intermediarios y paraténicos. Este trabajo tiene como objetivo registrar larvas de *D. renale* y *Contracaecum* sp. en tortugas de agua dulce. Se examinaron nueve *Phrynops hilarii* (Duméril & Bibron, 1835) y 21 *Acanthochelys spixii* (Duméril & Bibron, 1835), colectados in Rio Grande do Sul, Brasil. Cuatro ejemplares de *P. hilarii* estuvieron infectados por larvas de tercer estadio de *D. renale* y una *A. spixii* estuvo infectada por larvas de tercer estadio de *Contracaecum* sp. Este es el primer registro de larvas de *D. renale* y *Contracaecum* sp. en *P. hilarii* y *A. spixii*, respectivamente.

Palabras clave: *Acanthochelys spixii* – gusano gigante del riñón – larvas de tercer estadio – nematodos – *Phrynops hilarii*

INTRODUCTION

Dioctophyme renale (Goeze, 1782) (Nematoda: Enoplida) has been registered in wild carnivores and domestic dogs (definitive hosts), parasitizing the right kidney and, occasionally, both kidneys and the peritoneal cavity (Anderson, 2000). The life history of *D. renale* was studied in North America, where fishes and amphibians act as paratenic hosts when they become infected by ingesting aquatic oligochaetes (intermediate hosts) with third-stage larvae (Mace & Anderson, 1975; Measures & Anderson, 1985). Third-stage *D. renale* larvae were reported in frogs, fish, and freshwater turtles in Brazil (Pedrassani *et al.*, 2009; Abdallah *et al.*, 2012; Mascarenhas & Müller, 2015a; Mascarenhas *et al.*, 2016). However, there is a large gap in the knowledge about the life cycle of *D. renale*, despite several reports of the parasitic helminth in domestic dogs and wild animals (Mascarenhas & Müller, 2015a).

Contracaecum spp. (Nematoda: Anisakidae) occurs in the stomach and small intestine of birds and mammals piscivorous (definitive hosts). The eggs of *Contracaecum* species are eliminated in the definitive hosts feces, and the development of the second-stage larvae free occurs in the water. Later on, it is ingested by invertebrates or fishes (intermediated hosts), in which occurs the development of the third-stage larvae infective, which are found, generally, free or encapsulated in the cavity (Anderson, 2000).

Dioctophyme renale and some species of *Contracaecum* have recognized zoonotic potential, since several cases have been reported in humans, mainly due to fish consumption (Eiras *et al*., 2015). *Dioctophyme renale* was related to skin and kidneys infection in humans from Asia (Hanjani *et al*., 1968; Urano *et al*., 2001; Sardjono *et al*., 2008; Katafigiotis *et al*., 2013; Tokiwa *et al*., 2014). Anisakiasis in humans may be caused by *Contracaecum* spp. and other species of Anisakidae and there are several cases in the world (Eiras *et al*., 2015). Even though here are not cases in Brazil, there are records of *Contracaecum* larvae in several species of fish allowed for human consumption, such as *Hoplias malabaricus* (Bloch, 1794), *Hoplerythrinus unitaeniatus* (Spix & Agassiz, 1829), *Salminus brasiliensis* (Cuvier, 1816) and *Rhamdia quelen* (Quoy & Gaimard, 1824) (Martins *et al.,* 2005; Barros *et al*., 2007; Madi & Da Silva, 2009; Mesquita *et al.,* 2014).

Phrynops hilarii (Duméril & Bibron, 1835) (Chelidae) occurs in Uruguay, Paraguay, Argentina and Brazil (Santa Catarina and Rio Grande do Sul) (Djik *et al*., 2014). This specie is considered one of the most abundant in the Rio Grande do Sul, being commonly observed in urban environments, as well as in the countryside (Bujes & Verrastro, 2008). *Acanthochelys spixii* (Duméril & Bibron,

1835) (Chelidae) can be found in Uruguay, Argentina and Brazil (Djik *et al*., 2014). In Brazil, it occurs in the states of Bahia, Goiás, Minas Gerais, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul, and in the Federal District (Brasil *et al*., 2011; Djik *et al*., 2014). In the state of Rio Grande do Sul, this species is associated with sandy lagoon environments, wetlands and seasonal or semi-permanent ponds (Bujes & Verrastro, 2008).

In this paper, we report for the first time the occurrence of third-stage *D. renale* larvae in freshwater turtle *P. hilarii* and third-stage *Contracaecum* sp. larvae in *A. spixii*.

MATERIALS AND METHODS

Thirty freshwater turtles, nine specimens of *P. hilarii* and 21 of *A. spixii*, were examined, which were dead run over victims on highways in the southern of the State of Rio Grande do Sul, Brazil. The cities were Pelotas (31°46'19"S - 52°20'33"W), Capão do Leão (31°46'30"S - 52°26'55"W), Rio Grande (32°10'60"S - 52°50'55"W) and Santa Vitória do Palmar (33°32'20"S - 53°20'59"W). The collections were made under license from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/n° 47397). From the total of examined turtles, seven were donated by Núcleo de Reabilitação da Fauna Silvestre (NURFS-UFPel) after their death during the rehabilitation process. Turtles were necropsied, examined and nematodes were fixed in AFA (70°GL ethanol, formalin 37% and glacial acetic acid), preserved in 70°GL glycerinated alcohol, cleared and mounted in Amann's lactophenol (phenol, lactic acid, glycerin and water). Specimens were identified according to Mace & Anderson (1975), Measures & Anderson (1985), Martins *et al*. (2005) and Pedrassani *et al.* (2009). Vouchers were deposited in the "Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres (CHLAPASIL/UFPel)" (Numbers 680 and 681), Rio Grande do Sul State, Brazil. Parasitological indexes followed Bush *et al.* (1997). Photomicrographs were prepared in the microscope Olympus BX 41 with a camera system and the plates were made in the Adobe PhotoshopCS5.

RESULTS AND DISCUSSION

Four specimens of *P. hilarii* were infected by third-stage larvae of *D. renale* (Fig. 1) with one larva per cyst occurring in the muscles, body cavity (surface of esophagus, stomach, lung, liver) and serous of stomach. The mean intensity (MI) was 3.75 helminths/host (1-5 larvae). Third-stage larvae of *Contracaecum* sp. (Fig. 2) occurred in one specimen of *A. spixii* with six helminths.

The first reported of third-stage *D. renale* larvae in freshwater turtle was in *Trachemys dorbigni* (Duméril & Bibron, 1835) (Testudines: Emidydae) (n=32) with prevalence of 87.5% and mean intensity of infection of 13.9 (Mascarenhas & Müller, 2015a,b). This report was in urban area from Pelotas, where also was reported third-stage *D. renale* larvae in fish, *Hoplosternum littorale* (Hancock, 1828) (Siluriformes: Callichthyidae), with 4.5 helminths/host (1-5 larvae), four hosts parasited (Mascarenhas *et al*., 2016). Mascarenhas & Müller (2015a) suggests that the significant prevalence of larvae in turtles in the city of Pelotas might be related to characteristics of the urban zone, where to the presence of parasitized domestic dogs, which spread eggs through their urine; hence, contaminating urban water bodies that turtles and oligochaetes cohabit. Additionally, the process of eutrophication caused by the demand for organic matter and other household, wasted in these aquatic urban environments, may have favored an increased oligochaeta population, contributing to maintenance of the *D. renale* cycle (Mascarenhas & Müller, 2015a). High densities of oligochaetes are usually found in altered aquatic environments due to an increase in food resources and a decrease in oxygen supply, and the ensuing decrease of predators and/or competitors (Martins *et al*., 2008). Dogs population in Pelotas is approximately 66,723 (46,706 semi-domestic, 6,672 street dogs and 13,345 domestic dogs) (Municipal Administration, 2012). Therefore, the report of third-stage *D. renale* larvae in freshwater turtle and fish in urban areas of Pelotas is an alert for canine dioctophymatosis.

About larvae of *Contracaecum*, Foster *et al.* (1998) examined 41 specimens of *Apalone ferox* (Schneider, 1783) (Testudines: Trionychidae) in Southeastern Florida (USA) and registered larvae

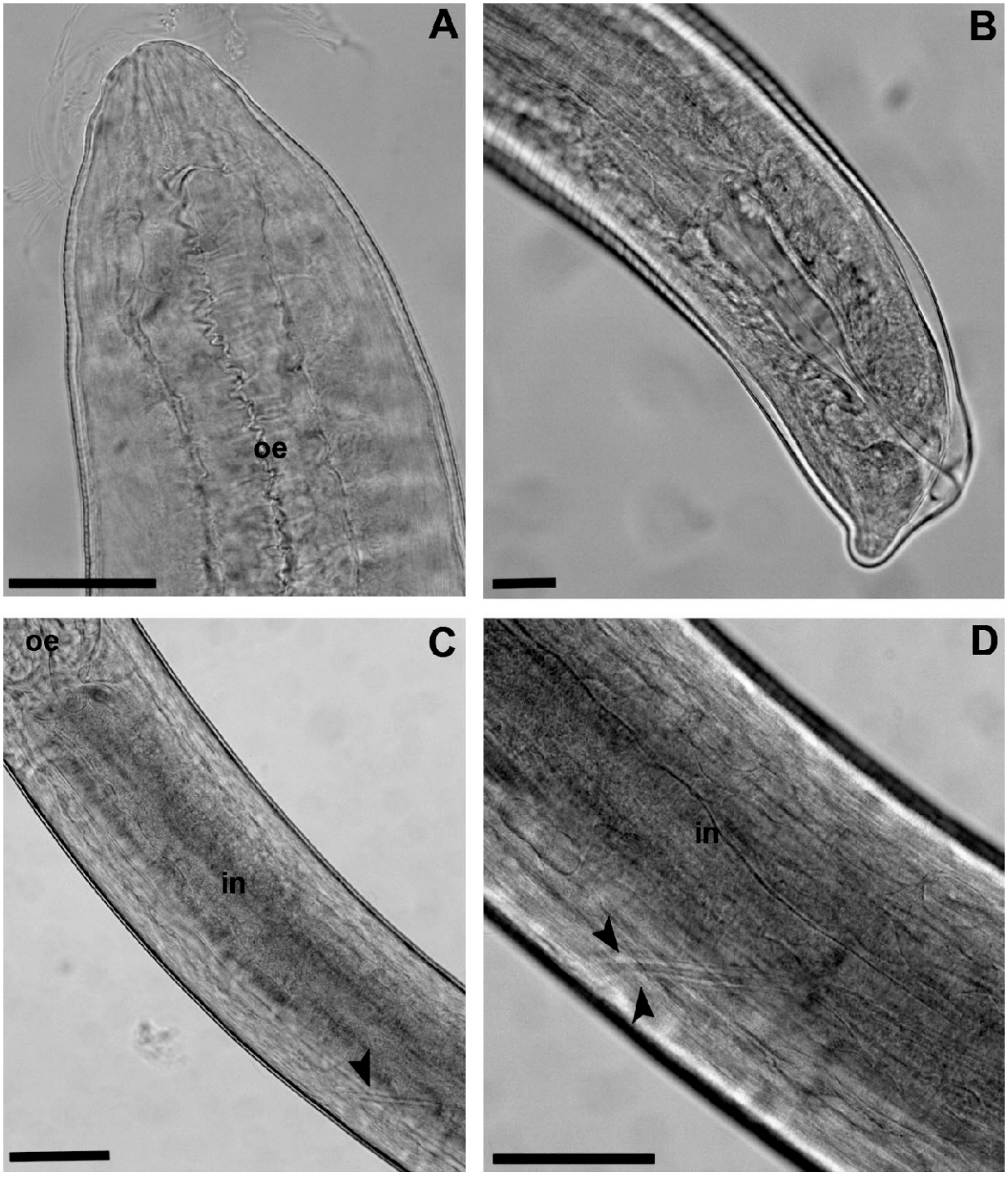

Figure 1. Third-stage larva of *Dioctophyme renale* parasite of *Phrynops hilarii* (Duméril & Bibron, 1835) (Testudines: Chelidae) from southern Brazil. **A –** Anterior extremity of female larva (oe – oesophagus) (Bar = 62 µm). **B –** Posterior extremity of male larva (Bar = 37 µm)**. C –** Genital primordium (arrow) of female larva near to junction oesophagus-intestine (oe – oesophagus, in – intestine) (Bar = 290 µm)**. D –** Detail of genital primordium (arrows) and intestine (in) of female larva (Bar = 290 µm).

of *Contracaecum* in the stomach of 29% hosts with mean intensity of 13 (1-66 larvae), also related to one adult male of *Contracaecum multipapillatum* (Drasch, 1882) in the small intestine of one host. Foster *et al.* (1998) suggest that infection of turtles was by ingestion of freshwater fish, which are part of the species diet. Similarly, the infection of *A. spixii* can occurs by ingestion of fish, since this species also uses fish as a food source (Huckembeck *et al*., 2007). This is the first record of *Contracaecum* larvae in freshwater turtle, *A. spixii*.

The role of *P. hilarii* and *A. spixii* in cycle of *D. renale* and *Contracaecum* sp. is questionable, because the freshwater turtles are not easily predator by definitive hosts of these helminths. In relation to *D. renale*, it is emphasized that *P. hilarii* examined, in this study, were found dead on highways in the urban area, which can facilitate a possible predation by stray dogs, which have poor selective eating habits. Therefore, further studies are needed to understand the life cycle of *D. renale* in the region, for the importance of this parasite in public health.

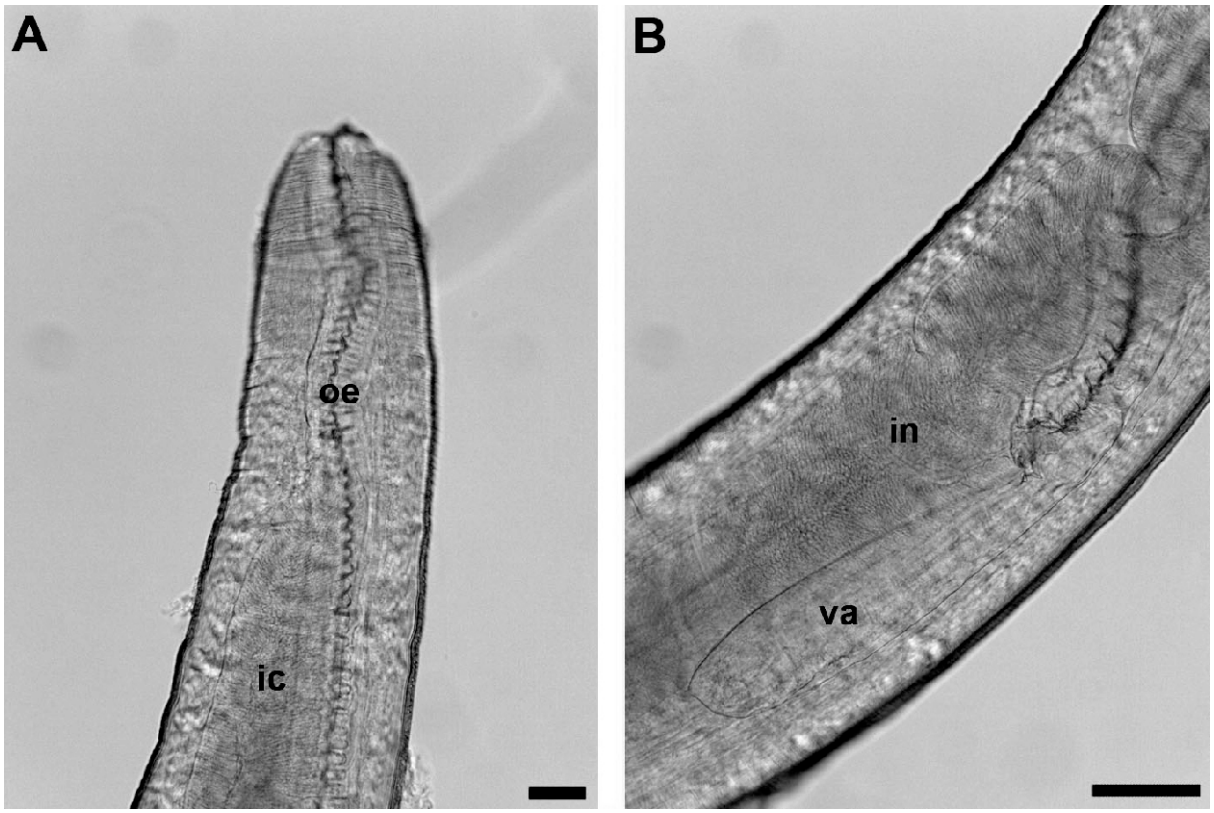

Figure 2. Third-stage larva of *Contracaecum* sp. parasite of *Acanthochelys spixii* (Duméril & Bibron, 1835) from southern Brazil. **A –** Anterior extremity of larva (oe – oesophagus, ic – intestinal caecum) (Bar = 87 µm)**. B –** Detail of ventricular appendix (va) and intestine (in) (Bar = 85 µm).

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