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New records of helminths in Chelidae freshwater turtles (Testudines) in South America

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RESUMO

Novos registros de helmintos em tartarugas de água doce Chelidae (Testudines) na América do Sul. Estudos helmintológicos com tartarugas de água doce Chelidae na América do Sul foram conduzidos na Argentina, Uruguai e Brasil, onde foram relatados nematoides, digenéticos e monogenéticos. Nesse contexto, o objetivo do estudo é registrar novas ocorrências de helmintos gastrintestinais associados a *Acanthochelys spixii*, *Hydromedusa tectifera* e *Phrynops hilarii*. Foram examinados 65 hospedeiros, provenientes da região sul do Rio Grande do Sul, Brasil. Sete *taxa* pertencentes a Nematoda, Digenea e Monogenoidea são reportados em novos hospedeiros na América do Sul bem como, um Nematoda e dois Digenea tem novo registro de hospedeiro para o Brasil. Assim, o estudo amplia o conhecimento sobre a diversidade de helmintos e a distribuição geográfica dos *taxa* encontrados parasitando tartarugas Chelidae.

Palavras-chave: *Acanthochelys spixii*, Digenea, *Hydromedusa tectifera*, Nematoda, *Phrynops hilarii*

ABSTRACT

Helminthological studies with Chelidae freshwater turtles in South America were conducted in Argentina, Uruguay and Brazil, where nematodes, digeneans and monogeneans were reported. In this context, the study aims to report new cases of gastrointestinal helminth parasites of *Acanthochelys spixii*, *Hydromedusa tectifera,* and *Phrynops hilarii*. A total of 65 hosts from the southern region of Rio Grande do Sul, Brazil, were examined. Seven *taxa* belonging to Nematoda, Digenea, and Monogenoidea have new cases on hosts Chelidae in South America, as well as a Nematoda and two Digenea have new host cases for Brazil. Thus, the study expands the knowledge about the diversity of helminths and the geographic distribution of *taxa* associated with Chelidae turtles.

Keywords: *Acanthochelys spixii*, Digenea, *Hydromedusa tectifera*, Nematoda, *Phrynops hilarii*

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The parasites are important in the conservation of biological resources and ecosystems, as well as acting as bioindicators, are important pieces in studies of environmental impact or even conservation of host species, due to the close host-parasite interaction (Marcogliese, 2004). However, parasitological studies are often neglected, being regarded when somehow the parasites cause diseases in their hosts or degrade biological products, causing economic damages (Marcogliese, 2004; Amato & Amato 2010).

Studies on helminths that parasitize freshwater turtles of family Chelidae in South America were conducted in Brazil (Freitas & Dobbin Jr., 1971; Vieira et al., 2008; Ávila et al., 2010; Bernardon et al., 2013; Novelli et al., 2013; Mascarenhas et al., 2013; Silva, 2014; Novelli et al., 2014; Vieira et al., 2016; Mascarenhas et al., 2016; Mascarenhas et al., 2017), Argentina (Lombardero & Moriena, 1977; Palumbo et al., 2016), and Uruguay (Cordero, 1946; Mañé-Garzón & Gil, 1961a, b, c, d; Mañé-Garzón & Gil, 1962a, b; Brooks & Holcman, 1993). In Brazil, digenetics (Bernardon et al., 2013; Mascarenhas et al., 2016) and nematodes (Bernardon et al., 2013; Mascarenhas et al., 2013, 2017) were reported parasitizing the *Acanthochelys spixii* (Duméril & Bibron, 1835), *Hydromedusa tectifera* Cope, 1870 and *Phrynops hilarii* (Duméril & Bibron, 1835). The aim of this study was to report new cases of gastrointestinal helminths of Chelidae freshwater turtles in South America.

Between May 2007 and May 2018, 65 specimens of freshwater turtles: *A. spixii* (13 males: 10 females); *H. tectifera* (17 males: 8 females) and *P. hilarii* (5 males: 12 females) were collected in Pelotas (31° 45'02.8" S - 52° 20'38.7" W; 31° 43'49.1" S - 52° 22'43.3" W; 31° 44'45.6" S - 52° 21'43.3" W; 31° 44'47.9" S - 52° 24'28.7" W; 31° 45'34.6" S - 52° 24'09.4" W; 31° 45'54.2" S - 52° 23'03.3" W; 31° 46'11.6" S - 52° 21'57.9" W), Capão do Leão (31° 47'36.7" S - 52° 24'28.8" W; 31° 45'15.4" S - 52° 27'05.2" W), Rio Grande (32° 20'58.5" S - 52° 32'43.77" W) and Santa Vitória do Palmar (33° 15'16.22" S - 53° 05'00.1" W), Rio Grande do Sul, Brazil. Fifty-one turtles were collected dead on roads and highways of these municipalities and fourteen hosts were donated by Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres (NURFS/CETAS/UFPel), where the animals died during the rehabilitation process. The study was licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio n° 38913).

The identification of the hosts was performed according to Quintela & Loebmann (2009) and the sexing was done during necropsy with the examination of the reproductive system. Forty-four hosts were frozen (20 *H. tectifera*, 12 *A. spixii,* and 12 *P. hilarii*), and 21 were processed immediately after death (11 *A. spixii*, 5 *H. tectifera*, and 5 *P. hilarii*). In the necropsy process, the plastron was first removed by osteotomy of its lateral processes and section of the circumferential integument. The digestive system was removed, individualized (oral cavity, esophagus, stomach, small and large intestines), and washed in

150 µm mesh sieve. The cavity was also washed and its content examined. Nematoda, Digenea, and Monogenea were fixed in cold AFA (ethanol 70°, formalin 37%, and glacial acetic acid) for 48 hours, and then preserved in ethanol 70°. Some specimens of Digenea and Monogenea were stained in Delafield's hematoxylin or Carmine's Langeron, and mounted as permanent slides in Canada balsam, and Nematoda were mounted on semi-permanent slides with Amann's lactophenol (Amato et al., 1991). The identification of helminths was performed according to Hedrick (1935), Measures & Anderson (1985), Anderson et al. (2009), Mascarenhas & Müller (2017) to Nematoda; Mañé-Garzón & Gil (1961d), Travassos et al. (1969), Brooks & Holcman (1993) to Digenea; and Pichelin (1995) to Monogenoidea. Vouchers were deposited in the "Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres" (CHLAPASIL/UFPel), at Laboratório de Parasitologia de Animais Silvestres, Departamento de Microbiologia e Parasitologia of Universidade Federal de Pelotas, Rio Grande do Sul State, Brazil. The parasitological index, prevalence, mean intensity, and mean abundance of infection were estimated according to Bush et al. (1997).

New cases of gastrointestinal helminths in *A. spixii*, *H. tectifera* and *P. hilarii* were reported, as follow:

Camallanus emydidius Mascarenhas & Müller, 2017 (Nematoda: Camallanidae)

Host: *Hydromedusa tectifera*.

Prevalence, mean abundance, mean intensity of infection, and intensity: 36.00%, 2.44, 6.77 helminths/host, and 1-30.

Stage: Adult.

Site of infection: Small intestine.

Specimens deposited: CHLAPASIL (306, 313, 314, 316, 779).

Notes: *Camallanus emydidius* was described parasitizing *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae), in the same region of the present study, with prevalence and mean intensity of infection of 100% and 30.31 helminths/host, respectively (Mascarenhas & Müller, 2017). It is the second case of *C. emydidius* in South America and the first in *H. tectifera*.

Spiroxys contortus (Rudolphi, 1819) (Nematoda: Gnathostomatidae)

Host: *Phrynops hilarii.*

Prevalence, mean abundance, mean intensity of infection, and intensity: 58.82%, 4.52, 7.70 helminths/host, and 1-42.

Stage: Adult.

Site of infection: Stomach, small and large intestines.

Specimens deposited: CHLAPASIL (780, 781).

Notes: *Spiroxys contortus* was recorded in Argentina parasitizing *P. hilarii* with prevalence (P) of 70% and mean intensity of infection (MII) of 3 helminths/host and *H. tectifera* with P = 70% and MII = 1.7 helminths/host (Palumbo et al., 2016). In Brazil, the species was registered in *H. tectifera* with MII of 14.2 helminths/host (Mascarenhas et al., 2013) and *T. dorbigni* with MII of 70.17 helminths/host (Mascarenhas & Müller, 2015a). It is the first case of *Spiroxys contortus* in *P. hilarii* in Brazil.

Dictophyme renale Goeze, 1782 (Nematoda: Dioctophymatidae)

Host: *Acanthochelys spixii*

Prevalence, mean abundance, mean intensity of infection, and intensity: 4.34%, 0.04, 1 helminth/host, and 1.

Stage: third-stage larvae.

Site of infection: Large intestine.

Specimens deposited: CHLAPASIL (783).

Notes: In the same region of the current study of larvae of *D. renale* were reported in *P. hilarii* (Mascarenhas et al., 2017) with a mean intensity of infection of 3.75 helminths/host and in *T. dorbigni* with 13.9 helminths/host (Mascarenhas & Müller, 2015b). According to Mascarenhas et al. (2018) the presence of larvae of *D. renale* in freshwater turtles indicates that these vertebrates may be sentinel species for the occurrence of dioctophymatosis in the region. It is the first case of *Dioctophyme renale* larvae in *A. spixii* in South America.

Physaloptera sp. (Nematoda: Physalopteridae)

Host: *Acanthochelys spixii.*

Prevalence, mean abundance, mean intensity of infection, and intensity: 4.34%, 0.04, 1 helminth/host and 1.

Stage: third-stage larvae.

Site of infection: Stomach.

Specimens deposited: CHLAPASIL (782).

Notes: According Anderson (2000), *Physaloptera* species occur mainly in the stomach of reptiles, birds, mammals, and, rarely, amphibians and fish. The nematodes are generally found firmly adhered to the gastric mucosa of definitive hosts. However, it is believed that helminths normally do not feed on the mucosa, but rather on the contents present in the host's stomach, which influences the development of

helminths (third-stage to adult). In the non-fed host, the nematode remains in third instar larvae attached to the gastric mucosa. *Physaloptera* larvae were reported in the stomach of *Phrynops geoffroanus* (Schweigger, 1812), in São Paulo, with a mean intensity of infection of 10 helminths/host (Silva, 2014). In Northeast Brazil, *Physaloptera retusa* Rudolphi, 1819 was reported in *P. geoffroanus* and *Mesoclemmys tuberculata* (Lüederwaldt, 1926) with prevalence and mean intensity of infection of 7.3%, 1.33 helminths/host and 5%, 2 helminths/host, respectively (Pereira et al., 2018). It is the first case of helminths belonging to *Physaloptera* parasitizing *A. spixii* in South America.

Caimanicola brauni Mañé-Garzón & Gil, 1961 (Digenea: Cryptogonimidae)

Hosts: *Acanthochelys spixii* and *Phrynops hilarii.*

Prevalence, mean abundance, mean intensity of infection, and intensity: *A. spixii* (4.34%, 0.43, 10.00 helminths/host and 10), *P. hilarii* (35.29%, 37.47, 106.16 helminths/host and 1-347).

Stage: Adult.

Site of infection: Small intestine.

Specimens deposited: CHLAPASIL (322 – 326, 329, 579 - 582).

Timoniella ostrowski Brooks & Holcman, 1993 (Digenea: Cryptogonimidae).

Host: *Phrynops hilarii.*

Prevalence, mean abundance, mean intensity of infection, and intensity: 11.76%, 1.11, 9.50 helminths/host, and 4-15.

Stage: Adult.

Site of infection: Small intestine.

Specimens deposited: CHLAPASIL (786 - 791).

Notes: Digeneans Cryptogonimidae inhabit a variety of piscivorous ectothermic amniote vertebrates throughout the tropical and subtropical regions of the world (Brooks; Holcman, 1993). *Caimanicola brauni* (= *Acanthostomum brauni*) and *Timoniella ostrowski* were reported parasitizing *P. hilarii* in Uruguay (Mañé-Garzón & Gil, 1961d; Brooks & Holcman, 1993). It is the first case of *C. brauni* parasitizing *A. spixii* and the second case of *T. ostrowski* in South America. In addition, this is the first case of *C. brauni* and *T. ostrowski* parasitizing *P. hilarii* in Brazil.

Polystomoides sp. (Monogenoidea: Polystomatidae)

Host: *Acanthochelys spixii.*

Prevalence, mean abundance, mean intensity of infection, and intensity: 13.04%, 0.34,

2.66 helminths/host, and 1-4.

Stage: Adult.

Site of infection: Oral cavity.

Specimens deposited: CHLAPASIL (784, 785).

Notes: Two species of *Polystomoides* (*P. uruguayensis* Mañé-Garzón & Gil, 1961 and *P. fuguesi* Mañé-Garzón & Gil, 1962) were reported in *P. hilarii* from Uruguay, with intensity of infection of 1 – 17 helminths (Mañé-Garzón & Gil, 1961a, 1962a). In Brazil, *P. brasiliensis* Vieira, Novelli, Sousa & Sousa-Lima, 2008 was reported in *P. geoffroanus* and *H. maximiliani* (Mikan, 1820), from Minas Gerais, with intensity of infection of 6 – 30 helminths (Vieira et al., 2008). In the same region of the present study, Mascarenhas (2014) reported *P. rohdei* Mañé-Garzon & Hollman-Spector, 1968 in *T. dorbigni* with prevalence of 81.66% and intensity of infection of 1 – 132 helminths. It is the first case of helminths belonging to *Polystomoides* in *A. spixii* in South America.

Most gastrointestinal helminths can be acquired through the ingestion of intermediate and/or paratenic hosts, which transmit the infective forms through the trophic chain (Dobson et al., 2005). Although there is little knowledge about the life cycle of helminths found parasitizing the freshwater turtles studied in this research, it is possible to affirm that most present heteroxenic life cycle, based on studies of similar species or species of the same family. *Spiroxys contortus* and representatives of Camallanidae use crustaceans as intermediate hosts (Hedrick, 1935; Anderson, 2000). *Dioctophyme renale*, a helminth recognized by its zoonotic potential, commonly parasitizes the kidney of domestic and wild mammals, with oligochaetes as intermediate hosts and fish and amphibians as paratenic hosts (Anderson, 2000). Some species of *Physaloptera* use beetles, cockroaches, and crickets as intermediate hosts (Anderson, 2000). Molluscs, fish, and anurans act as intermediate hosts of digeneans belonging to Cryptogonimidae (Cribb et al., 2003; Miller & Cribb, 2008). The host species of the present study were considered generalist and/or opportunistic, with the diet composed of invertebrates (Insecta, Gastropoda, Crustacea, Hirudinea, Arachnida, Malacostraca, and Oligochaeta), anurans, fish and plant fragments (Huckembeck et al., 2007; Bonino et al., 2009; Alcade et al., 2010; Brasil et al., 2011; Assman et al., 2013). In this context, helminth infections may be related to the diet of the freshwater turtles, since these infections may be influenced by the habitat and eating habits of their hosts (Ferguson & Smales, 2006).

According to Mascarenhas et al. (2016) road-killed animals can offer interesting results for helminthological research as observed for freshwater turtles, providing important information about the diversity of their parasites, and about the biology of the hosts, that may contribute to the conservation

of the involved species and their habitats. Most of the helminthological studies related to Chelidae freshwater turtles in the Southern area of Brazil were performed with animals collected on roads and highways, demonstrating the importance of the use of this type of material in helminthological research. The diversity of helminth parasites of *A. spixii*, for example, was unknown until 2013 when the first studies using animals found dead on roads in the south of Brazil began to reveal digenetic and nematodes helminths parasitizing the species (Mascarenhas et al., 2013, 2016, 2017).

The present study reports four new cases of helminths [*Dioctophyme renale* (larvae), *Physaloptera* sp. (larvae), *Caimanicola brauni* and *Polystomoides* sp.] associated with *Acanthochelys spixii* in South America. Additionally, it reports *Camallanus emydidius* in *Hydromedusa tectifera* for the first time. The Nematoda *Spiroxys contortus* and the Digenea *Caimanicola brauni* and *Timoniella ostrowski* are reported for the first time in *Phrynops hilarii* in Brazil.

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