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TELORCHIS SPP. (DIGENEA: TELORCHIIDAE) IN *TRACHEMYS DORBIGNI* (DUMÉRIL & BIBRON, 1835) (TESTUDINES: EMYDIDAE) IN SOUTHERN BRAZIL

TELORCHIS SPP. (DIGENEA: TELORCHIIDAE) EN *TRACHEMYS DORBIGNI* (DUMÉRIL & BIBRON, 1835) (TESTUDINES: EMYDIDAE) EN EL SUR DE BRASIL

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Abstract

Trachemys dorbignii, the Brazilian slider turtle, is native to Uruguay, Argentina and Brazil. In Brazil the species is abundant in the southern region. This research investigated the helminth fauna of this species. We examined sixty chelonians from aquatic environments of urban and rural areas in two municipalities in the State of Rio Grande do Sul, Brazil. During the research we found *Telorchis achavali* and *Telorchis corti* (Digenea). This is the first record of *T. achavali* in Brazil.

Keywords: Brazilian slider turtle - freshwater turtle - *Telorchis corti* - *Telorchis achavali*.

Resumen

Trachemys dorbignii es nativo de Uruguay, Argentina y Brasil, en el territorio brasileño es abundante en el extremo sur del país. Para la investigación de la fauna de helmintos de *T. dorbignii* se examinaron sesenta tortugas de ambientes acuáticos de zonas urbanas y rurales de dos municipios de Rio Grande do Sul, Brasil. Durante la investigación se encontraron *Telorchis achavali* y *Telorchis corti* (Digenea). Este es el primer registro de *T. achavali* en Brasil.

Palabras clave: tortuga pintada - *Telorchis corti* - *Telorchis achavali*.

INTRODUCTION

Trachemys dorbigni (Duméril and Bibron, 1835), the Brazilian slider turtle, is restricted in its distribution to the southern countries of South America, occurring in Brazil, Uruguay and Argentina (Fritz & Havaš, 2007), and are found inhabiting fluvial ecosystems, such as dams, rivers, ponds, and wetlands (Quintela & Loebmann, 2009). In the Brazilian territory, the species is native to the State of Rio Grande do Sul; however its distribution has been expanded to other Brazilian states, probably due to the illegal trafficking of wild animals (Bujes & Verrastro, 2007; Quintela & Loebmann, 2009).

Telorchiidae Looss, 1899 (Digenea) is compound with five subfamilies, among them the Telorchiinae Looss, 1899 which is represented by five genera, and *Telorchis* Lühe, 1899 is the type genus. The species of *Telorchis* usually parasitize the intestine of amphibians and reptiles, especially turtles from North America, South America, Europe, and Asia (Font & Lotz, 2009).

In freshwater turtles from the Neotropical Region 25 species of *Telorchis* (Lenis, 2009) have been reported. In *T. dorbigni* there have been records of *Telorchis dubius* Mañé-Garzon & Hollman-Spector, 1968 and *Telorchis achavali* Mañé-Garzon & Hollman-Spector, 1973 (Mañé-Garzon & Hollman-Spector, 1968; Mañé-Garzon & Hollman-Spector, 1973) in Uruguay and *Telorchis corti* Stunkard, 1915 was reported in Brazil (Pesenti *et al.*, 2009). In this context, the current study aims report the presence of *Telorchis* spp. in *Trachemys dorbigni* in Brazil.

MATERIALS AND METHODS

From July 2010 to December 2012, 60 turtles were collected under license (n°23196) of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). Twenty-eight specimens of *T. dorbigni* were captured in four lakes in Centro Agropecuário da Palma

belonging to the Universidade Federal de Pelotas (UFPel), which is located in the rural area of the Municipality of Capão do Leão; the other 32 chelonians were collected in channels in urban areas of the Municipality of Pelotas, and both municipalities are found in the State of Rio Grande do Sul, Brazil.

The animals were manually captured with the aid of netting and transported in plastic boxes (56 L) to the laboratory, where the chelonians were weighed and measured. After morphometry the animals were anesthetized with a combination of ketamine 10% and xylazine 2%, and after analgesia they were given an intrathecal injection of lidocaine hydrochloride 2% (Amado *et al.*, 1994), as recommended by Resolution N° 1000/2012 of Brazilian Federal Board of Veterinary Medicine (CFV, 2012). Fifty-six of these animals were frozen for later necropsy, and the others were necropsied after death. Their organs were individualized and their content and mucous membrane were examined. Some specimens of *Telorchis* were fixed under light compression in AFA (70°GL ethanol, formalin 37% and glacial acetic acid) and preserved in 70°GL alcohol. Others specimens were stained in Delafield's hematoxylin or Carmine's Langeron, and mounted as permanent slides in Canada balsam and identified according Mañé-Garzon & Hollman-Spector (1973) and MacDonald & Brooks (1989).

The measurements are expressed in micrometers (μm), unless otherwise specified. Means and standard deviations appear in parentheses. The ecological terms were used according to Bush *et al.* (1997). The photomicrographs were taken using an Olympus BX 41 Microscope, with a camera adapter system. Representative specimens of trematodes were deposited in two collections: Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro State, Brazil, and in the Coleção de Helminhos do Laboratório de Parasitologia de Animais Silvestres from the Universidade Federal de Pelotas (CHLPAS-UFPel), Rio Grande do Sul State, Brazil. Representative specimens of the hosts were deposited in the

collection at the Coleção Herpetológica do Museu Carlos Ritter (CHMR-UFPel), Rio Grande do Sul State, Brazil (Numbers 78 and 79).

RESULTS

Of the 60 hosts examined 48.3% were parasitized by *Telorchis* spp. In the urban area only *T. corti* were found; in the rural area *T. corti*, *T. achavali*, and immature specimens of *Telorchis* were found (Table 1). There was no association between species in the same host in the rural area. *Telorchis corti* was the species with higher mean intensity and mean abundance

in the rural area; however, it occurred in only one host.

Telorchis corti and *T. achavali*, described below, are Digenea from the small intestine of *T. dorbigni* in southern Brazil. Besides these two species, specimens of *Telorchis* were found in six hosts in the rural area, however, their specific determination was not possible, due to the stage of maturation of some specimens, they were considered to be juveniles due to their immature reproductive structures, and the low mean intensity of the infection (2.16 helminths/host) (Table 1).

Table 1. Prevalence, infection mean intensity and mean abundance of *Telorchis corti*, *Telorchis achavali* and immature specimens of *Telorchis* spp. (Digenea: Telorchidae) from *Trachemys dorbigni* (Testudines: Emydidae) in rural and urban environment in southern Brazil.

	Urban (N = 32)			Rural (N = 28)		
	Prevalence (%)	Mean intensity	Mean abundance	Prevalence (%)	Mean intensity	Mean abundance
<i>Telorchis corti</i>	65.62	20.38	13.37	3.5	130	4.64
<i>Telorchis achavali</i>	-	-	0	3.5	3	0.1
Immature specimens of <i>Telorchis</i> spp.	-	-	0	21.42	2.16	0.46

Telorchis corti Stunkard, 1915 (Figs. 1 - 6)

Description. Based on 24 helminths. Body elongate 4.8 – 11.25mm (7.43mm ± 1.89) long by 250 - 930 (581.66 ± 219.33) wide (the level of the ovary). Oral sucker subterminal, 75 – 202.5 (123.33 ± 31.31) long by 95 - 210 (136.56 ± 29.98) wide, prepharynx short, pharynx muscular with 60 – 110 (74.1 ± 11.7) long by 60 - 120 (83.47 ± 17.46) wide. Oesophagus 210 – 460 (299.16 ± 77.56) long. Double caeca, unbranched, extending back to posterior body at a distance 75 – 200 (122.91 ± 31.37) from posterior extremity; distance from the bifurcation of the caeca to the anterior extremity 350 – 1000 (608.08 ± 176.91). Acetabulum intercaecal, 85 – 225 (159.47 ± 42.2) long by 77.5 – 200 (136.84 ± 39.11) wide (19 specimens measured). Testes oval to spherical, vertical and intercaecal and situated at a distance 200 – 650

(419.79 ± 126.27) from posterior extremity. Anterior testes 130 – 470 (280.83 ± 93.15) long by 90 – 470 (261.25 ± 99.75) wide and posterior testes 140 – 480 (300.41 ± 103.1) long by 90 – 450 (251.66 ± 94.57) wide. Cirrus sac extending from the anterior region of the acetabulum at the anterior margin of ovary. Seminal vesicle bipartite. Genital pore ventral, immediately pre-acetabular, intercaecal, mediano or slightly sinistral. Ovary spherical 100 – 250 (167.91 ± 46.71) long by 80 – 300 (175.83 ± 64.05) wide, intercaecal, median. Distance between ovary and acetabulum 1100 – 2430 (1590.83 ± 382.51) and distance between ovary and anterior testes 1700 – 4500 (2851.04 ± 859.85). Seminal receptacle, Laurer's canal, and Mehl's gland presents. Uterus between ovary and anterior testes. Vitelline follicles in continuous longitudinal lateral rows, extending from

posterior extremity cirrus sac to near zone pretestes, occupying the middle third of the body. Eggs 27.5 – 38 (31.87 ± 2.29) long by 15 – 20 (18.27 ± 1.54) wide. Excretory vesicle Y-shape; pore terminal.

Host: *Trachemys dorbigni* (Duméril & Bibron, 1835), Brazilian slider turtle

Infection site: small intestine

Collection site: Pelotas, Rio Grande do Sul, Brazil ($31^{\circ}46'16.9''\text{S}$ - $52^{\circ}18'45.9''\text{W}$; $31^{\circ}46'24.3''\text{S}$ - $52^{\circ}19'13.8''\text{W}$; $31^{\circ}46'22.0''\text{S}$ - $52^{\circ}19'04.2''\text{W}$; $31^{\circ}45'44.3''\text{S}$ - $52^{\circ}19'46.7''\text{W}$; $31^{\circ}45'48.6''\text{S}$ - $52^{\circ}19'47.0''\text{W}$); Centro Agropecuário da Palma, Capão do Leão, Rio Grande do Sul, Brazil ($31^{\circ}47'50.0''\text{S}$ - $52^{\circ}29'43.0''\text{W}$).

Deposited specimens: 481-504 (CHLPAS/UFPel), 37846–37850 (CHIOC).

Telorchis achavali Mañé-Garzon & Hollman-Spector, 1973 (Figs. 7 - 12)

Description. Based on 3 helminths. Body elongate 13.12 – 17.55mm ($14.8\text{mm} \pm 2.39$) long by 575 – 1125 (783.33 ± 298.25) wide (the level of the ovary). Oral sucker subterminal 125 - 195 (167.5 ± 37.33) long by 155 – 220 (194.16 ± 34.49) wide, prepharynx short, pharynx muscular 77.5 – 125 (105.83 ± 25.04) long by 10.5 – 152.5 (128.33 ± 22.68) wide. Oesophagus absent. Double caeca, unbranched, extending back to posterior body at a distance 25 – 100 (66.66 ± 38.18) long from posterior extremity. Acetabulum intercaecal 120 – 200 (166.66 ± 41.63) diameter. Testes spherical and vertical, intercaecal situated in posterior extremity, anterior testes 230 – 380 (306.66 ± 75.05) long by 230 – 370 ($310 \pm 72,11$) wide and posterior testes 280 – 360 ($316.66 \pm 40,41$) long by 280 – 360 (316.66 ± 40.4) wide situated 250 – 325 (300 ± 43.3) from posterior extremity. Cirrus sac muscular and well developed. Genital pore ventral, median, intercaecal, pre-acetabular. Ovary rounded, intercaecal with 340 – 500 (403.33 ± 85.04) long by 290 - 430 (383.33 ± 80.82). Distance between ovary and acetabulum 3750 – 5500 (4333.33 ± 1010.36) and distance between ovary and anterior testes 5650 – 7600 (6458.33 ± 1016.83). Seminal receptacle and

Laurer's canal not observed. Mehli's gland present. Uterus between ovary and anterior testes. Vitelline follicles extending from the middle portion of the cirrus sac to near zone pretestes, occupying the middle third of the body. Eggs 30 – 32.5 (31.66 ± 1.44) long by 15 – 17.5 (16.66 ± 1.44) wide. Excretory pore situated in posterior extremity.

Host: *Trachemys dorbigni* (Duméril & Bibron, 1835), Brazilian slider turtle

Infection site: small intestine

Collection site: Centro Agropecuário da Palma, Capão do Leão, Rio Grande do Sul, Brazil ($31^{\circ}48'01.1''\text{S}$ - $52^{\circ}30'48.6''\text{W}$).

Deposited specimens: 505-507 (CHLPAS/UFPel).

DISCUSSION

According to MacDonald & Brooks (1989), *T. corti* is the most problematic species of the genus, and 13 synonyms have been suggested. The species occurs in several hosts, shows a wide geographic distribution exhibiting a high degree of intra-specific variation.

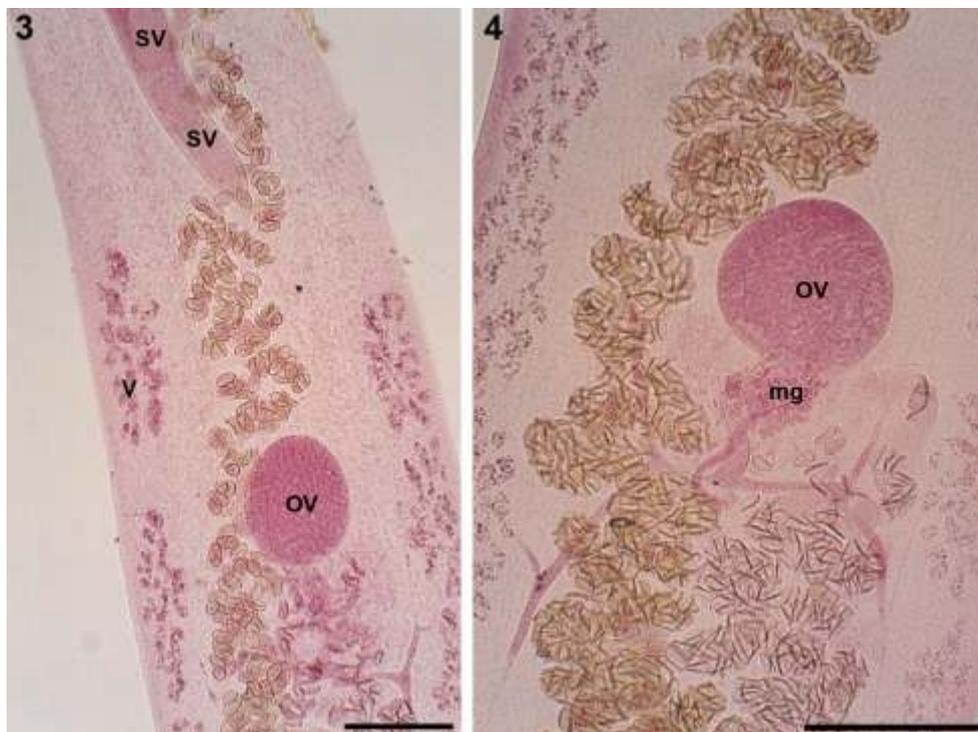
Moravec & Vargas-Vázquez (1998) considered that *T. corti* is conspecific with *Telorchis attenuata* Goldberger, 1911 reported in *Trachemys scripta* (Schoepff, 1792) in Mexico (Moravec & Vargas-Vázquez, 1998). The identification of the species found in *T. dorbigni* in southern Brazil was done according to the description and the key proposed by MacDonald & Brooks (1989).

In South America, *T. corti* was reported parasitizing the small intestine of *Trachemys callirostris* (Gray, 1856) in Colombia, with lower indexes to those found for this helminth in *T. dorbigni* in Brazil (Table 1). In the Colombian chelonian *T. corti* occurred in 0.76% of the hosts with intensity of 18.5 and abundance of 0.14 (Lenis & Vélez, 2011).

In Brazil, *T. corti* was reported in a specimen of *T. dorbigni* that was run over and killed on a highway in the Municipality of Capão do Leão,



Figures 1 – 2. *Telorchis corti* stained with Langeron's carmine. **1** – Anterior region, oral sucker (os); pharynx (ph); Oesophagus (oe); caeca (c) (bar = 70 μ m). **2** – Median region, genital pore (gp); acetabulum (ac); cirrus sac (sc) (bar = 82.5 μ m)



Figures 3 – 4. *Telorchis corti* stained with Langeron's carmine. **3** – Median region, seminal vesicle (sv); vitellaria (v); ovary (ov) (bar = 150 μ m). **4** - Median region, ovary (ov); Mehli's gland (mg) (bar = 260 μ m).



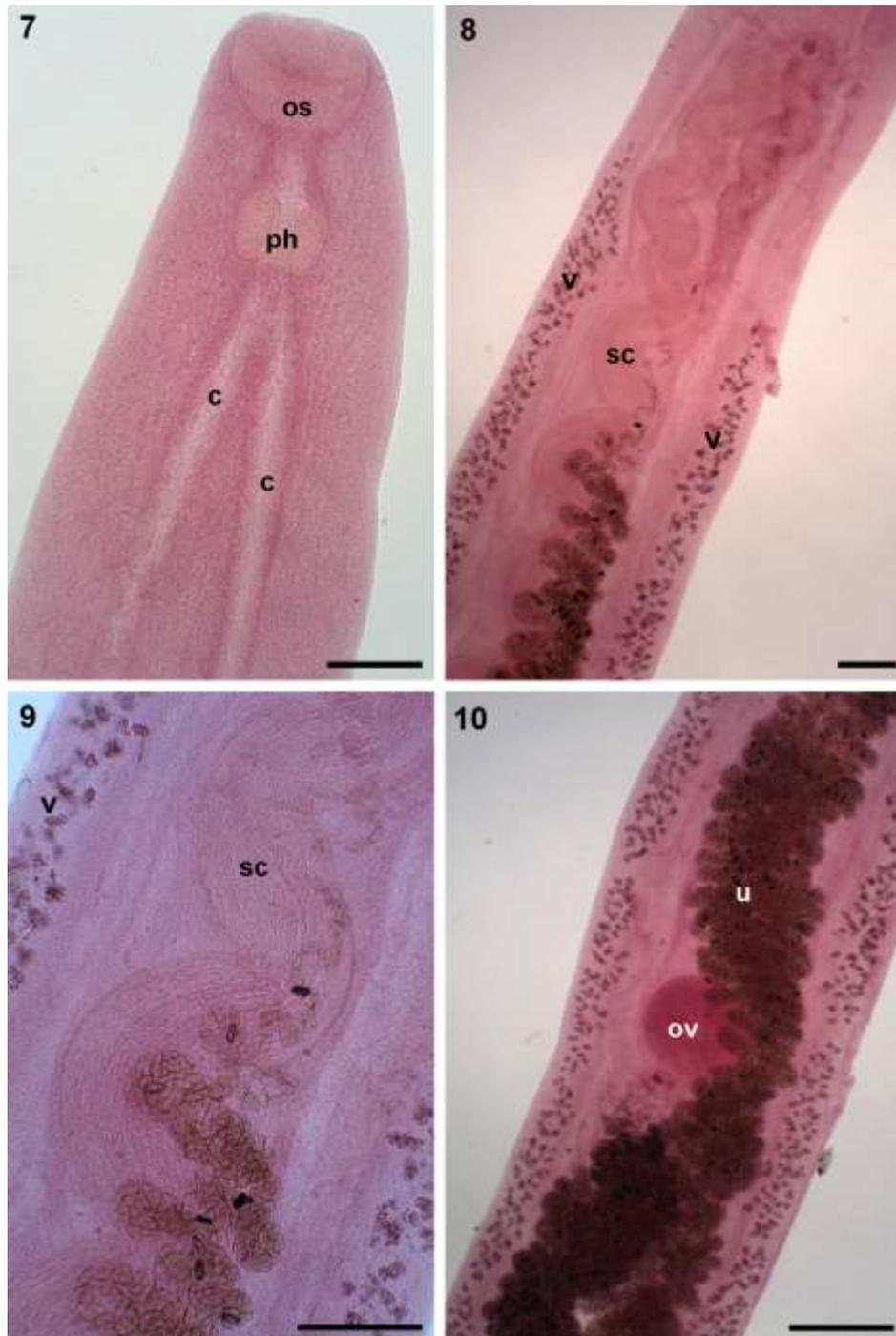
Figures 5 – 6. *Telorchis corti* stained with Delafield's hematoxylin. **5** – Posterior region, uterus (u); anterior teste (at); posterior teste (pt); caeca (c); vitellaria (v) (bar = 290µm). **6** - Posterior region, posterior teste (pt); caeca (c) (bar = 100µm).

Rio Grande do Sul State. Sixty-eight helminths were found parasitizing the small intestine of that host (Pesenti *et al.*, 2009).

In Uruguay, two species were described, *T. dubios* and *T. achavali*, parasitizing the small intestine of *T. dorbigni* (Mañé-Garzon & Hollman-Spector, 1968; Mañé-Garzon & Hollman-Spector, 1973). The main characteristic that distinguished *T. corti* (the present study) from *T. dubios* (Mañé-Garzon & Hollman-Spector, 1968) was that the latter have a transversely elongate ovary. Also, *T. dubios* (Mañé-Garzon & Hollman-Spector, 1968) differed from *T. achavali* (the present study), since the latter did not have an esophagus, the ovary was spherical, and the cirrus sac was muscular and well developed. In the present study, *T. achavali* differed from *T. corti*, mainly because it was greater than *T. corti*, did not have an esophagus, and its cirrus sac was muscular and well-developed. This is the first record of *T. achavali* in Brazil.

Telorchis corti and *T. achavali*, as well as the other species of *Telorchis*, have a tegument with spines (Stunkard, 1915; Mañé-Garzon & Hollman-Spector, 1973; Font & Lotz, 2009). We did not observe spines in the specimens found in *T. dorbigni*, which probably have been lost in the freezing process of the hosts. We examined the specimens that were prepared by Pesenti *et al.* (2009), in which we could clearly observe the spines, which are abundant in the anterior region of the body. It is important to highlight that in this case the Digenea were prepared shortly after the death of the host.

Regarding the life cycle of the species of *Telorchis*, Font & Lotz (2009) reported the participation of pulmonate mollusks as the first intermediate hosts in which the cercariae develop; tadpoles and mollusks are the second intermediate hosts where cercariae develop to metacercariae. The parasites reach the sexual maturity in amphibians and reptiles, which become infected by eating tadpoles and mollusks with metacercariae.



Figures 7 – 10. *Telorchis achavali* stained with Langeron's carmine. **7** - Anterior region, oral sucker (os); pharynx (ph); caeca (c) (bar = 152.5 μ m). **8** - Median region, cirrus sac (sc); vitellaria (v) (bar = 210 μ m). **9** - Median region, vitellaria (v); cirrus sac (sc) (bar = 210 μ m). **10** - Median region, uterus (u); ovary (ov) (bar = 430 μ m).



Figures 11 – 12. *Telorchis achavali* stained with Langeron's carmine. **11** - Posterior region, uterus (u); anterior testes (at); posterior testes (pt); caeca (c) (bar = 330 μ m). **12** - Posterior region, anterior testes (at); posterior testes (pt); caeca (c) (bar = 330 μ m).

Studies on the diet may help investigations of helminth fauna and vice versa. Lenis (2009) studied digenetic helminths of *T. callirostris* and *Podocnemis lewyana* Duméril, 1852, as well as the diet of these hosts, through analysis of stomach and intestinal contents of some individuals, and collected limnic mollusks to identify the possible intermediate hosts, concluding that the omnivorous diet of *T. callirostris* could favor the transmission of digenetic helminths, for example, *T. corti* that uses mollusks and tadpoles as intermediate hosts.

Regarding *T. dorbigni*'s diet, there are studies carried out in the State of Rio Grande do Sul, Brazil, with individuals in captivity (Lema & Ferreira, 1990) and in the wild environment (Pereira, 1998; Hahn, 2005; Bujes *et al.*, 2007). Lema & Ferreira (1990) reported that chelonians fed on snakes, frogs, teleosts, saurians, caecilians, Oligochaeta, bovine meat, and lettuce. Moreover, they observed the consumption of floating plants (*Eichornia* spp.

and *Elodea* spp.). Pereira (1998), by analyzing feces from nine individuals, recorded as food items insects, grass, seeds, algae, fragment of bones, and scales. The ingestion of mollusks was reported by Hahn (2005) who studied the stomach content of 75 turtles and reported that 85.27% of the diet was composed of plant matter and 13.23%, and the total volume was represented by animal matter (mollusks, crustaceans, arthropods, Hirudinea, and vertebrates). With regard to vertebrate as a component of *T. dorbigni*'s diet, Hahn (2005) identified Anura, in two turtles, and Characiformes in another. Bujes *et al.* (2007) recorded *T. dorbigni* preying on the golden mussel *Limnoperna fortunei* (Dunker, 1857), a bivalve mollusk originating from Southeast Asia and introduced into Rio Grande do Sul State in the 1970s (Mansur *et al.*, 2003). They also analyzed the fecal content from eight specimens and recorded the presence of gastropods, crustaceans, plant matter, sand, and synthetic material.

During the collection of helminths we noticed that some turtles ingested gastropod mollusks, however, future studies should be conducted to identify the food items that were part of the diet of the turtles examined.

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