



Gastrointestinal helminths associated with three species of freshwater turtles in the Pampa biome, southern Brazil

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Abstract

Maintenance of the life cycle of a wide diversity of helminths depends on the aquatic environment. Freshwater turtles are considered one of the main groups in these ecosystems due to their high density and biomass. However, there is little information on the infection parameters of helminths associated with the Chelidae species *Hydromedusa tectifera*, *Phrynops hilarii*, and *Acanthochelys spixii* that occur in the Pampa biome. Therefore, this study aimed at analyzing the gastrointestinal helminth assemblage of the three species. Analyses showed that *A. spixii* was associated with 11 helminth *taxa* while *P. hilarii* and *H. tectifera* were parasitized by eight *taxa* each. The gastrointestinal helminth assemblage of *A. spixii* and *H. tectifera* showed high similarity by the Morisita index. Infections suggest that the helminth fauna is directly related to the diet of the hosts, since *A. spixii*, *P. hilarii*, and *H. tectifera* are generalist and/or opportunistic with diets composed of various invertebrates, which, in turn, are part of the life cycles as intermediate hosts of most of the identified helminths. Thus, this study highlights not only the importance of helminthological research for the understanding of the biology of host organisms, but also the need for conservation of flooded areas characteristic of the Pampa biome, which is essential to maintain the diversity of the ecosystem.

Keywords *Phrynops* · *Hydromedusa* · *Acanthochelys* · Similarity · Parasitological indices · Roadkill fauna

Introduction

Freshwater ecosystems are fundamental to the development of helminths because the chain of transmission of many species depends on the aquatic environment, where various organisms (mollusks, crustaceans, insects, fishes, anurans, turtles, and others) cohabit and are essential to maintain the life cycle of several helminths. Freshwater systems have complex trophic relations and parasites are part of food webs

which reflect prey-predator interactions (Brooks and Hoberg 2000; Marcogliese 2004; Dobson et al. 2008). The diversity of helminths associated with freshwater turtles and parasite-host relations is little known and explored while studies that provide information to elucidate their life history in South America are scarce (Mascarenhas and Müller 2021).

Freshwater turtles are considered one of the main groups in aquatic ecosystems, due to their high density and biomass (Iverson 1982; Congdon et al. 1986; Souza and Abe 2000). Chelidae (Testudines: Pleurodira) is composed of 58 species with representatives in South America, Australia, Indonesia, Papua New Guinea, and Timor-Leste (Rhodin et al. 2017). In Brazil, Chelidae is represented by 20 species (Costa and Bérnils 2018); 11 out of them have been recorded as helminth hosts in the country (Mascarenhas and Müller 2021).

The Pampa biome stretches over three South American countries: Uruguay, Argentina, and Brazil. In the Brazilian territory, the biome is restricted to Rio Grande do Sul (RS) state, which corresponds to 63% of the whole area (Bencke et al. 2016). *Acanthochelys spixii* (Duméril & Bibron, 1835), *Hydromedusa tectifera* Cope, 1870, and *Phrynops hilarii*

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(Duméril & Bibron, 1835) (Chelidae) are common species in this biome. These three turtles have had their distribution registered in Argentina, Brazil, and Uruguay (Rhodin et al. 2017; Sanchez et al. 2019) while *H. tectifera* and *P. hilarii* have also been recorded in Paraguay (Rhodin et al. 2017).

Acanthochelys spixii, *H. tectifera*, and *P. hilarii* were recorded mainly as hosts of Digenea and Nematoda in Brazil (Bernardon et al. 2013; Mascarenhas et al. 2013; Novelli et al. 2014; Mascarenhas et al. 2016; Mascarenhas et al. 2017; Bullard et al. 2019; Chaviel et al. 2020), in Uruguay (Mañé-Garzón and Gil 1961a, 1961b, 1961c; Brooks and Holcman 1993), and Argentina (Lombardero and Moriena 1977; Palumbo et al. 2016, 2020; Palumbo and Diaz 2018). Monogenea have been reported in Brazil (Chaviel et al. 2020) and Uruguay (Mañé-Garzón and Gil 1961d, 1962a, 1962b) whereas Cestoda were reported in Uruguay (Cordero 1946), and Brazil (Mascarenhas and Müller 2021). Most studies had a taxonomic focus and were carried out with a small number of hosts. There is a gap in the knowledge about the infection parameters of the gastrointestinal helminths that parasitize these species. Parasitological indices are tools that help to understand parasite-host relations (Von Zuben 1997; Rózsa et al. 2000; Reiczigel et al. 2005). Therefore, this study aimed to analyze the gastrointestinal helminth assemblage of three species of Chelidae—*A. spixii*, *H. tectifera*, and *P. hilarii*—which occur in the Pampa biome in the extreme south of Brazil where there are extensive wetlands with swamps and lagoons mainly on the Coastal Plain of RS.

Material and methods

Collection of hosts

Seventy-five specimens of freshwater turtles—*A. spixii* (13 males: 11 females: 1 undetermined), *H. tectifera* (22 males: 9 females), and *P. hilarii* (14 females: 5 males)—were collected in Pelotas (31° 44' 45.6" S to 52° 21' 43.3" W), Capão do Leão (31° 45' 15.4" S to 52° 27' 05.2" W), Rio Grande (32° 20' 58.5" S to 52° 32' 43.7" W), and Santa Vitória do Palmar (33° 32' 20" S to 53° 20' 59" W), RS, Brazil, between May 2007 and May 2020. Fifty-nine dead turtles were collected on roads and highways and sixteen turtles were donated by Núcleo de Reabilitação da Fauna Silvestre e Centro de Triagem de Animais Silvestres (NURFS/CETAS/UFPel), where they had died during the rehabilitation process. Twenty-seven hosts (13 *A. spixii*, 9 *H. tectifera*, and 5 *P. hilarii*) were processed immediately after death while 48 hosts (22 *H. tectifera*, 14 *P. hilarii*, and 12 *A. spixii*) were individually frozen and subsequently necropsied. Sexing was done during necropsy with the examination of their

reproductive system. The study was licensed by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio no. 38913).

Collection, preparation, and identification of helminths

In the necropsy process, firstly, the plastron was 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 a 150- μ m mesh sieve. The celomatic cavity was also examined. Nematoda, Digenea, Cestoda, Acanthocephala, and Monogenea were fixed in cold AFA (ethanol 70°GL, formalin 37%, and glacial acetic acid) for 48 h, and then preserved in ethanol 70°GL. Some specimens of Digenea, Cestoda, Acanthocephala, and Monogenea were stained in Delafield's hematoxylin or Langeron's carmine, and mounted as permanent slides in Canada balsam while Nematoda were mounted on semi-permanent slides with Amann's lactophenol (Amato et al. 1991). Identification of helminths was performed according to Hedrick (1935), Measures and Anderson (1985), Moravec et al. (1995), González and Hamann (2008), Petter and Quentin (2009), Janwan et al. (2011), and Mascarenhas and Müller (2017) in the case of Nematoda. Mañé-Garzón and Gil (1961a), Mañé-Garzón and Gil (1961b), Mañé-Garzón and Gil (1961c), Lombardero and Moriena (1977), Dubois (1979), Brooks and Holcman (1993), and Kostadinova (2005) were used to identify Digenea. Petrochenko (1971) was used for Acanthocephala, Cordero (1946) and Khalil et al. (1994) for Cestoda, and Pichelin (1995) for Monogenea.

Vouchers were deposited in the helminth collection at the Laboratório de Parasitologia de Animais Silvestres, Universidade Federal de Pelotas (CHLAPASIL-UFPel) (304–317, 322–326, 329, 345–350, 518, 569–583, 587–604, 680–681, 779–791, 881–896), the helminthological collection at the Instituto Oswaldo Cruz (CHIOC) (36,733, 36,734, 38044a-38044c, 35,999, 36,734, 38045a-38045e), Brazil, and the invertebrate collection at the Museo de La Plata, Argentina (MLP-He) (6836–6841).

Data analysis

The assemblage of gastrointestinal helminths of the three species of freshwater turtles was analyzed by indices of prevalence (P%), mean intensity of infection (MII), and mean abundance (MA) (Bush et al. 1997).

Infections among host species were compared by using P% and MII values which were submitted to the Fisher's exact test ($p \leq 0.05$) and the bootstrap test ($p \leq 0.05$), respectively, for helminths that occurred concomitantly in the three host species with a prevalence greater than or equal to 10%

(Bush et al. 1990). Analyses were performed by the Quantitative Parasitology 3.0 software (Reiczigel et al. 2019).

The richness of gastrointestinal helminths in relation to the number of examined hosts of each species was analyzed using a species accumulation curve. Similarity among assemblages of gastrointestinal helminths of host species was verified by a cluster analysis with the use of the Morisita index for quantitative data. The analysis was tested considering helminths grouped in families. Analyses were performed by the Paleontological Statistics—PAST 2.17 (Hammer et al. 2012).

Results

Sixty hosts (80%) were parasitized by at least one gastrointestinal helminth species, which were represented by Nematoda, Digenea, Cestoda, Acanthocephala, and Monogenea; 2,035 helminths were found (483 in *A. spixii*, 345 in *H. tectifera*, and 1,207 in *P. hilarii*). Five *A. spixii*, eight *H. tectifera*, and two *P. hilarii* composed the group of 15 negative specimens. The species accumulation curve indicated that the richness of the gastrointestinal helminth assemblages of *P. hilarii* was representative with the 19 specimens examined and that after the sixth host the number of helminths found did not increase (Fig. 1C). In contrast, the helminth species accumulation curve for *A. spixii* (Fig. 1A) and *H. tectifera* (Fig. 1B) indicated that the number of hosts examined was not representative.

Acanthochelys spixii was associated with 11 helminth taxa while *P. hilarii* and *H. tectifera* were parasitized by eight taxa each. Infection sites and parasitological indices are shown in Tables 1, 2, and 3. In the cluster analysis, the gastrointestinal helminth assemblages of *A. spixii* and *H. tectifera* had the highest similarity (Fig. 2).

Nematodes were the most representative helminths, since that found species belonged to Gnathostomatidae, Camallanidae, Anisakidae, Physalopteridae, Dioctophymatidae, and Pharyngodonidae. *Spiroxys contortus* (Rudolphi, 1819) (Gnathostomatidae) occurred in the three host species, with indices of prevalence of 56.00% (*A. spixii*), 52.63% (*P. hilarii*), and 45.16% (*H. tectifera*) while mean intensity of infection ranged from 5.07 to 10.71 helminths/host (Tables 1, 2 and 3). There was no significant difference in prevalence ($p > 0.7$) and mean intensity of infection ($p > 0.2$) of *S. contortus* among the three freshwater turtle species.

Camallanus emydidius Mascarenhas & Müller, 2017 (Camallanidae) was found in 32.25% of *H. tectifera* specimens, with mean intensity of infection of 6.3 helminths/host (Table 2). In *A. spixii*, *Camallanus* sp. showed lower indices than those of *C. emydidius* (Table 1). Pharyngodonidae specimens were found in *A. spixii* and *H. tectifera* with low infection indices (Tables 1 and 2).

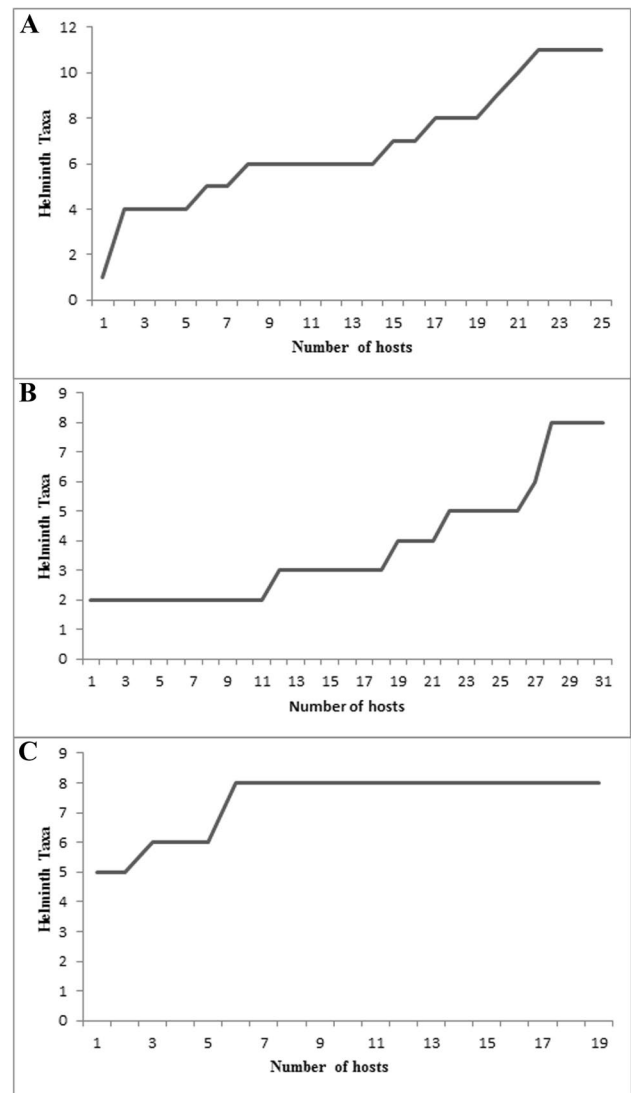


Fig. 1 Accumulation curve of gastrointestinal helminth species associated with freshwater turtles, *Acanthochelys spixii* (A), *Hydromedusa tectifera* (B), and *Phrynops hilarii* (C) in the Pampa biome, southern Brazil

Larval forms of nematodes occurred in the three host species. *Dioctophyme renale* (Goeze, 1782) (Dioctophymatidae) larvae were found with a high prevalence value in *P. hilarii* 26.32% (Tables 1, 2, and 3). *Contracaecum* sp. (Anisakidae) and *Physaloptera* sp. (Physalopteridae) larvae were found in *A. spixii* (Table 1) while a larva of *Gnathostoma* sp. (Gnathostomatidae) was found in *H. tectifera* (Table 2).

Digeneans *Cheloniodiplostomum* sp. (Proterodiplostomidae) and *Telorchis* spp. (Telorchidae) were found in all three Chelidae species (Tables 1, 2, and 3). *Cheloniodiplostomum* sp. and *Telorchis platensis* Mañé-Grazón & Gil, 1962, showed the highest mean intensity of infection, 35.2 helminths/host, and 42.25 helminths/host, respectively, in *A. spixii* (Table 1). *Hydromedusa tectifera* was parasitized

Table 1 Helminth parasites of *Acanthochelys spixii* (Duméril & Bibron, 1835) (Testudines: Chelidae) ($n=25$) and their respective site of infection (SI), prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range (R) in southern Brazil. *SE* standard error

Helminths	SI	P %	MII (\pm SE)	MA (\pm SE)	R
NEMATODA					
Gnathostomatidae	Stomach, small and large intestine	56.00	5.07 (\pm 2.59)	2.84 (\pm 1.52)	1–38
<i>Spiroxys contortus</i>					
Camallanidae	Small and large intestine	20.00	4.6 (\pm 1.72)	0.92 (\pm 0.49)	1–11
<i>Camallanus</i> sp.					
Anisakidae	Celomatic cavity	8.00	3.5 (\pm 2.5)	0.28 (\pm 0.24)	1–6
<i>Contracaecum</i> sp. (larvae)					
Physalopteridae	Stomach	4.00	1	0.04 (\pm 0.04)	1
<i>Physaloptera</i> sp. (larvae)					
Diectophymatidae	Cysts in the stomach wall and large intestine	8.00	1	0.08 (\pm 0.06)	1
<i>Diectophyme renale</i> (larvae)					
Pharyngodonidae gen. sp.	Large intestine	4.00	3	0.12 (\pm 0.12)	3
DIGENEA	Small and large intestine	20.00	35.2 (\pm 23.49)	7.04 (\pm 5.16)	6–128
Proterodiplostomidae					
<i>Chelonioidiplostomum</i> sp.					
Telorchidae	Small and large intestine	16.00	45.25 (\pm 40.60)	7.24 (\pm 6.67)	1–167
<i>Telorchis platensis</i>					
Cryptogonimidae	Small intestine	4.00	10	0.4 (\pm 0.04)	10
<i>Caimanicola brauni</i>					
MONOGENEA	Oral cavity	12.00	2.66 (\pm 0.88)	0.32 (\pm 0.20)	1–4
Polystomatidae					
<i>Polystomoides</i> sp.					
ACANTHOCEPHALA	Small intestine	4.00	1	0.04 (\pm 0.04)	1
Polymorphidae gen. sp. (immature)					

Table 2 Helminth parasites of *Hydromedusa tectifera* Cope, 1870 (Testudines: Chelidae) ($n=31$) and their respective site of infection (SI), prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range (R) in southern Brazil. *SE* standard error

Helminths	SI	P%	MII (\pm SE)	MA (\pm SE)	R
NEMATODA					
Gnathostomatidae	Esophagus, stomach, small and large intestines	45.16	10.71 (\pm 4.10)	4.84 (\pm 2.06)	1–57
<i>Spiroxys contortus</i>					
<i>Gnathostoma</i> sp. (larva)	Small intestine	3.22	1	0.03 (\pm 0.03)	1
Camallanidae	Small intestine	32.25	6.3 (\pm 2.96)	2.03 (\pm 1.07)	1–30
<i>Camallanus emydidius</i>					
Diectophymatidae	Celomatic cavity (surface of stomach, and muscles) and serous of stomach	3.22	9	0.29 (\pm 0.29)	9
<i>Diectophyme renale</i> (larvae)					
Pharyngodonidae gen. sp.	Large intestine	3.22	1	0.03 (\pm 0.03)	1
DIGENEA	Small intestine	16.13	22.6 (\pm 17.14)	3.65 (\pm 2.94)	1–91
Telorchidae					
<i>Telorchis</i> sp.					
Proterodiplostomidae	Small intestine	3.22	2	0.06 (\pm 0.06)	2
<i>Chelonioidiplostomum</i> sp.					
CESTODA					
Proteocephalidae	Small intestine	9.68	2.67 (\pm 1.67)	0.26 (\pm 0.20)	1–6
<i>Ophiotaenia</i> sp.					

by two species of Digenea, mainly *Telorchis* sp., with the highest mean intensity of infection, 22.6 helminths/host. It was the highest value among all gastrointestinal helminths found in this host species (Table 2). *Phrynosoma hilarii* was parasitized by five Digenea taxa (Table 3). Cryptogonimidae

was represented by two species, *Caimanicola brauni* (Mañé-Garzón & Gil, 1961) and *Timoniella ostrowski* Brooks, 1980. The former occurred with prevalence of 31.58% and mean infection intensity of 106.17 helminths/host while *T. ostrowski* occurred with lower prevalence (10.53%) and

Table 3 Helminth parasites of *Phrynops hilarii* (Duméril & Bibron, 1835) (Testudines: Chelidae) ($n=19$) and their respective site of infection (SI), prevalence (P%), mean intensity of infection (MII), mean abundance (MA), and range (R) in southern Brazil. SE standard error

HELMINTHS	SI	P%	MII (\pm SE)	MA (\pm SE)	R
NEMATODA					
Gnathostomatidae	Esophagus, stomach, small and large intestines	52.63	7.7 (\pm 3.92)	4.05 (\pm 2.21)	1–42
<i>Spiroxys contortus</i>					
Diactophymatidae	Celomatic cavity (surface of esophagus, stomach, lung, liver, and muscles) and serous of stomach	26.32	4.8 (\pm 1.50)	1.26 (\pm 0.62)	1–10
<i>Diactophyme renale</i> (larvae)					
DIGENEA					
Proterodiplostomidae	Small intestine	52.63	42.9 (\pm 12.16)	22.58 (\pm 8.03)	1–132
<i>Cheloniodiplostomum testudinis</i>					
Telorchidae	Small intestine	21.05	3 (\pm 1.41)	0.63 (\pm 0.39)	1–7
<i>Telorchis birabeni</i>					
Cryptogonimidae	Small intestine	31.58	106.17 (\pm 55.35)	33.53 (\pm 20.10)	1–347
<i>Caimanicola brauni</i>					
<i>Timoniella ostrowski</i>	Small intestine	10.53	9.5 (\pm 5.5)	1 (\pm 0.81)	4–15
Echinostomatidae	Small intestine	10.53	2	0.21 (\pm 0.14)	2
<i>Prionosomoides</i> sp.					
MONOGENEA					
Polystomatidae	Oral cavity	21.05	1.25 (\pm 0.25)	0.26 (\pm 0.13)	1–2
<i>Polystomoides</i> sp.					

mean infection intensity was 9.5 helminths/host. *Cheloniodiplostomum testudinis* (Dubois, 1936) was found in 52.63% with mean intensity of infection of 42.9 helminths/host (Table 3). *Prionosomoides* sp. (Echinostomatidae) was found in *P. hilarii* with prevalence of 10.53% and mean intensity of 2.00 helminths/host (Table 3).

Monogenea was represented by Polystomatidae found in the oral cavity of *A. spixii* and *P. hilarii* with indices of prevalence of 12.00% and 21.05%, respectively (Tables 1 and 3). Cestoda occurred only in *H. tectifera* with low infection indices (Table 2). An immature form of Polymorphidae (Acanthocephala) was found in the small intestine of *A. spixii* with low infection indices (Table 1).

Discussion

Trophic interactions are considered the main routes of infection for gastrointestinal helminths, since infective forms of various species are transmitted through prey-predator interactions (Dobson et al. 2008; Marcogliese 2004). According to Dobson et al. (2008), 75% of linkages in trophic chains involve a parasite species.

Ferguson and Smales (2006) identified 11 species of helminths (Nematoda, Digenea, Aspidogastrea, Monogenea, and Amphilinidae) associated with *Emydura macquarii* (Gray, 1830) (Chelidae) and suggested that helminth infections were influenced by the diet of the hosts, since most parasites are transmitted through trophic interactions involving intermediate hosts. Similarly, Pereira et al. (2018) and Palumbo et al. (2021) highlighted the importance of diet in helminth infections in Chelidae species in the Neotropical

region. Although there is little knowledge about the life cycle of helminths which parasitize freshwater turtles of Pampa biome, this study may state, based on studies of congener species or species of the same family, that most nematodes, as well as digenetic, acanthocephalid, and cestode species, have heterogenic life cycles that involve the trophic chain and that they are transmitted through the ingestion of infective forms found in intermediate or paratenic hosts.

Copepod crustaceans act as intermediate hosts of *S. contortus* as well as species of *Camallanus* Railliet & Henry, 1915, *Contraecaecum* Railliet & Henry, 1912, and *Gnathostoma* Owen, 1836 (Hedrick 1935; Anderson 2000; Janwan et al. 2011), while *D. renale* develops its infective form in freshwater oligochaetes (Mace and Anderson 1975) and some species of *Physaloptera* Rudolphi, 1819, use beetles, cockroaches, and crickets as intermediate hosts (Anderson 2000). Fishes can also act as intermediate and/or paratenic hosts for some species of *Gnathostoma*, *Contraecaecum*, *Camallanus*, and *D. renale* (Measures and Anderson 1985; Moravec et al. 1995; Anderson 2000). Additionally, *Camallanus trispinosus* (Leidy, 1852) larvae were found in four species of Zygoptera (Odonata) (Wiles and Bolek 2015), while *Spiroxys* spp. larvae were found in mollusks, odonates, fishes, and anurans, suggesting that they may act as potential paratenic hosts (Hedrick 1935; Bartlett and Anderson 1985; Moravec et al. 1995; Anderson 2000; Santos et al. 2009; González and Hamann 2010). Digenetic helminths belonging to Cryptogonimidae, Echinostomatidae, Telorchidae, and Proterodiplostomidae have mollusks, fishes, and anurans as intermediate hosts (Cribb et al. 2003; Miller and Cribb 2008; Font and Lotz 2008; Esteban and Muñoz-Antoli 2009). Acanthocephalans, belonging to Polymorphidae, and

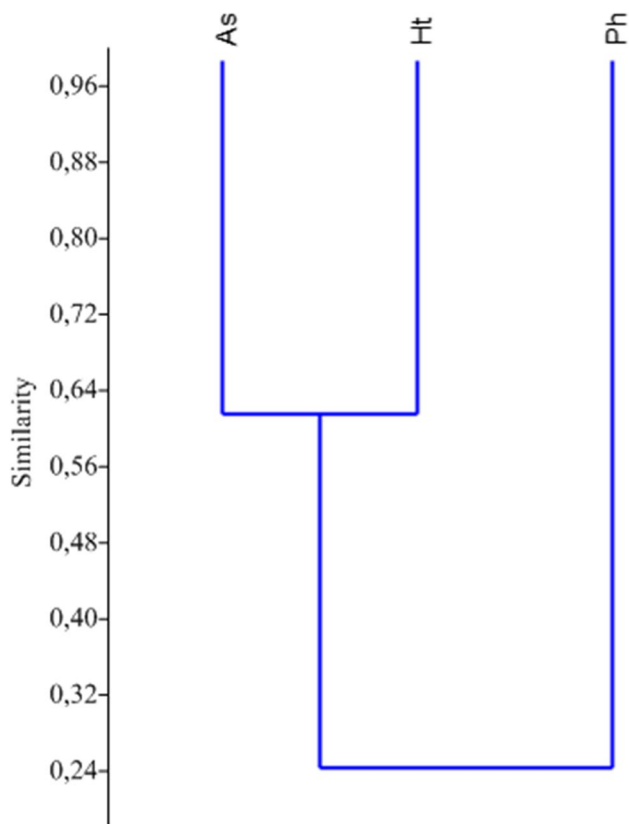


Fig. 2 Cluster analysis by the Morisita index considering the groups Gnathostomatidae, Camallanidae, Anisakidae, Physalopteridae, Diocotophymatidae, Pharyngodonidae (Nematoda), Proterodiplostomidae, Telorchhiidae, Cryptogonimidae, Echinostomatidae (Digenea), Proteocephalidae (Cestoda), Polystomatidae (Monogenea), and Polymorphidae (Acanthocephala) that compose the gastrointestinal helminth assemblage of freshwater turtles *Acanthochelys spixii* (As), *Hydromedusa tectifera* (Ht), and *Phrynops hilarii* (Ph) in the Pampa biome, southern Brazil

Proteocephalidae cestodes develop their infective forms in aquatic crustaceans, such as copepods and amphipods (Petrochenko 1971; Dezfuli and Giari 1999; Scholz and Chamberrier 2003).

From this perspective, helminth infections observed in the three Chelidae species of the Pampa biome were found to be related to the diet of the hosts. Studies of trophic ecology of *P. hilarii*, *A. spixii*, and *H. tectifera* carried out in Argentina and Brazil considered them generalist and/or opportunistic species, once their diet was composed of several invertebrates (insects, gastropods, crustaceans, hirudines, arachnids, and oligochaetes), anurans, fishes, and vegetal fragments (Bonino et al. 2009; Alcalde et al. 2010; Brasil et al. 2011; Assmann et al. 2013; Chaviel et al. 2021). Stomach and intestinal contents of 41 specimens (21 *A. spixii* and 20 *H. tectifera*) used by this study were examined by Chaviel et al. (2021), who recorded gastropods, malacostracks, amphipods, hirudineans,

insects (hemiptera, odonates, coleoptera), fish (*Phalliceros caudimaculatus* [Hensel, 1868]), and tadpoles. In the diet of *A. spixii*, insects were the main food resources, while for *H. tectifera*, the most important food item was gastropods (Chaviel et al. 2021). However, there was no significant difference in the volume of items consumed by the two species and, in addition, the authors pointed out that the trophic niche range showed similar values for *A. spixii* and *H. tectifera* suggesting uniformity in food consumption (Chaviel et al. 2021). Thus, the similarity in the helminth assemblage of *A. spixii* and *H. tectifera* may be a reflection of the similar diet of the species. Therefore, the generalist and/or opportunistic diet of the host species may favor parasite transmission. Infection indices of helminths reinforce the importance of diet in the transmission of parasites to freshwater turtles, since some species (e.g., *S. contortus*, *C. emydidius*, *D. renale*, *C. testudinis*, *T. platensis*, and *C. brauni*) transmitted through the ingestion of intermediate and/or paratenic hosts showed high prevalence and/or mean intensity of infection. Mascarenhas et al. (2021), in the same region of this study, reported helminth fauna of *Trachemys dorbigni* (Duméril & Bibron, 1835) (Emydidae) and highlighted the importance of diet in helminth infections for this freshwater turtle species.

Regarding monoxenic helminths, Monogenea, belonging to Polystomatidae, and nematodes Pharyngodonidae (Oxyuroidea) were found. *Polystomoides* Ward, 1917, species were recorded in the oral cavity and esophagus of Chelidae and Emydidae turtles in Uruguay (Mañé-Garzón and Gil 1961d, 1962a; Mañé-Garzón and Holcman-Spector, 1968), Brazil (Vieira et al. 2008; Mascarenhas et al. 2021), and Colombia (Lenis and García-Prieto, 2009). In Uruguay, 11 specimens of *P. hilarii* were examined and two species of *Polystomoides* were reported with infection intensity of 1–17 helminths and prevalence of 54.5% (Mañé-Garzón and Gil 1961d, 1962a). In the same region of this study, Mascarenhas et al. (2021) recorded *Polystomoides rohdei* Mañé-Garzon and Holcman-Spector, 1968, in *T. dorbigni* with prevalence of 81.66% ($n = 52/60$) and mean intensity of 16.81 helminths/host (1–132). Low infection indices found in *A. spixii* and *P. hilarii* may be related to the aquatic ecosystems, which may not offer adequate conditions to maintain free-living forms. Moreover, it should be highlighted that *Polystomoides* species exhibit peculiar reproductive characteristics regarding egg production (Price 1939). Paul (1938) reported that *Polystomoides oris* Paul, 1938, produced from two to three eggs/day and Pichelin (1995) reported that *Polystomoides australiensis* Rohde and Pearson, 1980, produced about six eggs/day. Pharyngodonidae is a group of oxyuroids that parasitizes all vertebrate classes, except birds (Petter and Quentin 2009). Low infection rates of this group in *A. spixii* and *H. tectifera* suggest that these infections are infrequent or accidental.

Similarly, low indices recorded for larvae of *Gnathostoma* sp., *Physaloptera* sp., and *Contracaecum* sp., as well as helminths belonging to Echinostomatidae and Polymorphidae, may also show that these parasites are occasional or incidental to the freshwater turtles in the region. On the other hand, parasitological parameters observed for *S. contortus*, *Camallanus* spp., *Cheloniidiplostomum* spp., *Telorchis* spp., *C. brauni*, *T. ostrowski*, *D. renale*, and Cestoda, together with records of helminths belonging to these groups in Brazil (Bernardon et al. 2013), Uruguay (Mañé-Garzón and Gil 1961a, 1961b, 1961c; Brooks and Holcman 1993), and Argentina (Lombardero and Moriena 1977; Palumbo et al. 2016; Palumbo and Diaz 2018) suggest that these parasites are common in assemblages of gastrointestinal helminths of Chelidae in the South American continent. However, few studies have reported infection indices. Bernardon et al. (2013) analyzed seven *P. hylarii*, in the same region of this study, and recorded *Spiroxys* sp., *Camallanus* sp., and *Cheloniidiplostomum* sp. with prevalence of 28.57%, 28.5%, and 100%, respectively; *Cheloniidiplostomum* sp. occurred with the highest mean intensity, 25.29 helminths/host. In Argentina, Palumbo et al. (2016) analyzed stomach contents of 47 *P. hylarii* and 25 *H. tectifera* and reported *S. contortus* with prevalence of 70% in both host species. However, the mean infection intensity was lower than the one recorded by this study, 3 helminths/host in *P. hylarii* and 1.7 helminths/host in *H. tectifera*. Palumbo et al. (2018) reported *C. testudinis* ($n = 19$ specimens) in *H. tectifera* ($n = 1$ examined host) and *Cheloniidiplostomum argentinensis* Palumbo & Díaz, 2018 ($n = 191$ specimens) and *C. testudinis* ($n = 7$ specimens) in *P. hylarii* ($n = 1$ examined host). In the same region of the present study, *S. contortus*, *C. emydidius*, and *D. renale* larvae, as well as *Telorchis* Lühe, 1899 species, were also recorded in *T. dorbigni* with significant infection indices (Mascarenhas et al. 2021), a fact that shows that freshwater turtle species Chelidae and Emydidae share some helminth groups in southern Brazil. However, it is interesting to note that the accumulation curve of helminth species found in *A. spixii* and *H. tectifera* demonstrated that sampling was not representative in the region of the present study, suggesting that increasing the number of hosts examined may reveal new records of helminths associated with the gastrointestinal tract of both host species.

Helminthological studies focusing on freshwater turtles point to the importance of the conservation of aquatic environments, which support complex life cycles that depend on trophic interactions and conditions favorable to the development of free-living forms of the parasites and their hosts. The area of this study is on the Coastal Plain, where an important lagoon complex comprises the Patos, Mirim, and Mangueira Lagoons. Thus, the region has ecosystems that enable the development and maintenance of rich biological diversity. Therefore, the importance of helminthological

research should be highlighted, since it can provide information about host organisms and their habitats, contributing to knowledge of host biology, environmental stress, trophic chains, and biodiversity. Thus, the use of roadkill fauna by helminthological studies proves to be a viable alternative for certain groups of hosts, such as freshwater turtles, and generates information that can assist conservation programs for aquatic ecosystems.

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Declarations

Conflict of interest The authors declare no competing interests.

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