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Diet and helminth parasites of freshwater turtles *Mesoclemmys tuberculata*, *Phrynops geoffroanus* (Pleurodira: Chelidae) and *Kinosternon scorpioides* (Criptodyra: Kinosternidae) in a semiarid region, Northeast of Brazil

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Abstract. In this study, the *Kinosternon scorpioides*, *Mesoclemmys tuberculata* and *Phrynops geoffroanus* freshwater turtles collected in the Cariús River, State of Ceará, were analysed as to their diet composition and presence of helminths. Among the 63 examined turtles 55 (87.3%) were parasitized. We found three Nematoda species (*Physaloptera retusa*, *Serpinema monospiculatus* and *Spiroxys figueiredoi*) and one Trematoda species (*Gorgoderina* sp.). *Phrynops geoffroanus* had the highest indexes of prevalence (97.56%) and mean intensity of infection (33.5), followed by *M. tuberculata* (70% and 12.64, respectively) and *K. scorpioides* (50% and three, respectively). Host body size was positively related to helminths abundance in both male and female Chelidae species. A significant difference in helminths abundance between the sexes was found only in *P. geoffroanus*, where females had more parasites than males. Regarding diet, the main food items ingested by *M. tuberculata* were Odonata nymphs (Aeshnidae and Libellulidae), whilst *P. geoffroanus* feeds mainly on Diptera larvae (Chironomidae), Odonata nymph (Aeshnidae) and Notonectidae, and only seeds were found in the stomach contents of *K. scorpioides*. Here, we present the first record of *S. monospiculatus* parasitizing *K. scorpioides*, *Gorgoderina* sp. and *P. retusa* were reported for the first time in *P. geoffroanus*, and *M. tuberculata* represents a new host to *P. retusa* and *S. figueiredoi*.

Keywords. Testudines, feeding habits, helminths, Nematoda, Trematoda, host gender, host body size.

INTRODUCTION

Freshwater turtles are important components in the food webs of aquatic ecosystems, act in the processing of organic matter, nutrient cycling, dispersion of riparian vegetation, as well as in the maintenance of water quality (Moll and Moll, 2004). Despite that, studies regarding feeding ecology and helminths associated with freshwater turtles are still limited (Souza, 2004; Anjos, 2011; Moura et al., 2014).

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In South America, researches about the helminth diversity associated with continental turtles are mostly driven in Argentina, Brazil and Uruguay (Mascarenhas et al., 2013). In Brazil, Testudines is represented by 36 species and 18 genera included in eight families (Costa and Bérnils, 2015). Nevertheless, data about helminth fauna composition of freshwater turtles are poorly known (Anjos, 2011) as most studies only report species descriptions and records of new hosts.

In Brazil, the helminth fauna associated with *Kinosternon scorpioides* (Linnaeus, 1766) (the scorpion mud turtle), includes the nematodes *Serpinema magathi* (Sprehn, 1932) and *Spiroxys figueiredoi* Freitas and Dobbin Jr., 1962 (Freitas and Dobbin Jr., 1971; Viana et al., 2016), and the digenean trematodes *Nematophila grandis* (Diesing, 1839), *Telorchis rapidulus* Dobbin, 1957 and *Telorchis diaphanus* Freitas and Dobbin Jr., 1959 (Fernandes and Kohn, 2014). The occurrence of *K. scorpioides*, reaches from the east side of Panama to northern South America and from the Amazon basin to northern Argentina (Berry and Iverson, 2011). This species has a wide habitat ecological tolerance as it can be found in weirs, lakes, streams, rivers, marshlands and other aquatic environments modified by anthropogenic activities (Vanzolini et al., 1980; Rueda-Almonacid et al., 2007). It has an omnivore habit and a diverse diet, which includes insects and their larvae, spiders, gastropods, worms, crustaceans, fishes, frog eggs, tadpoles, adult frogs, snake scales, bird eggshells, parts of mammals, algae, fruits, nuts, seeds, flowers, and aquatic plants (Vanzolini et al., 1980; Rueda-Almonacid et al., 2007; Berry and Iverson, 2011).

Currently, little is known about the natural history of the tuberculate toad-headed turtle, *Mesoclemmys tuberculata* (Lüederwaldt, 1926), including its diet and helminth fauna. The nematode *Serpinema monospiculatus* Freitas and Dobbin Jr., 1962 is the unique parasite recorded for this turtle species (Freitas and Dobbin Jr., 1971). This freshwater turtle is endemic to Brazil (Moura et al., 2014; Turtle Taxonomy Working Group, 2014) with a wide distribution in the Northeast region of the country (Iverson, 1992; Bour and Zaher, 2005; Moura et al., 2014). It has been recorded mainly in the Caatinga biome and in some Atlantic Forest and Cerrado areas (Silveira and Valinhas, 2010; Moura et al., 2015; Santana et al., 2015). The species is associated with coastal ecosystems and semiarid regions, where inhabits a variety of aquatic environments, such as rivers, ponds, permanent lakes, and temporary streams (Vanzolini et al. 1980; Bour and Zaher, 2005; Santana et al., 2016).

The helminths recorded in the Geoffroy's side-necked turtle, *Phrynops geoffroanus* (Schweigger, 1812) in Brazil, are the nematodes *S. monospiculatus*, *S. figueiredoi*, *Brevimulticaecum* sp. (larvae) and *Physaloptera* sp. (larvae) (Freitas and Dobbin Jr., 1971; Silva, 2014), the digenean trematodes *N. grandis*, *Telorchis birabeni* Mañé-Garzón and Gil, 1961, *Prionosomoides scalaris* Freitas and Dobbin Jr., 1967, *Cheloniodiplostomum testudinis* (Dubois, 1936) and *Cheloniodiplostomum* sp. (Freitas and Dobbin Jr., 1967; Novelli et al., 2013; Silva, 2014), and the monogenean trematodes *Polystomoides brasiliensis* Vieira, Novelli, Sousa and Souza-Lima, 2008 and *Polystomoides* sp. (Vieira et al., 2008; Silva, 2014). *Phrynops geoffroanus* is a neotropical freshwater turtle that occurs in South America, in the east side of the Andes, from the Colombian Amazon to the south of Brazil, Uruguay and north of Argentina (Pritchard and Trebbau, 1984; Ernst and Barbour, 1989). This species inhabits ponds, streams and large rivers (Medem, 1960; Pritchard and Trebbau, 1984), being also common in rivers and streams of urban areas (Souza and Abe, 2000; Souza, 2004; Martins et al., 2010). Although this is an omnivore turtle species it could exhibit a prevailing carnivorous habit, feeding on insects, crustaceans, fishes, molluscs, annelids and carrion in addition to sewage organic matter in environments impacted by human activity (Fachín-Terán et al., 1995; Souza and Abe, 2000; Souza, 2004; Martins et al., 2010).

Thus, the current research aimed to analyse the diet and the helminth infection parameters in freshwater turtle populations in a river section in the semiarid region of Northeastern Brazil. This study will enrich the knowledge about helminth fauna associated with freshwater turtles and the diet of the studied species.

MATERIALS AND METHODS

Study site

Fieldwork was carried out in a stretch of the Cariús River (7º05'20.0"S, 39º40'37.0"W) in the Nova Olinda municipality (7º05'30.0"S, 39º40'50.0"W, 445 m a.s.l.), Ceará State, Northeast of Brazil. Local climate is semiarid hot tropical with an average annual temperature varying from 24 to 26 °C. The rainy season occurs between January and May with an average annual rainfall of 682.7 mm (IPECE, 2015). The study site is placed in an urban area rounded by buildings where water quality is frequently affected by household waste and sewage. Besides pollution, removal of riparian forest for the development of agricultural crops and pastures probably has contributed to the river silting process.

Collecting and laboratory procedures

The turtles were collected between October 2014 and April 2015, using fish hooks baited with pieces of chicken.

A total of 63 freshwater turtles were collected: 41 individuals of *P. geoffroanus* (17 males and 24 females), 20 *M. tuberculata* (five males, nine females and six juveniles), and two specimens of *K. scorpioides* (both females).

The captured individuals were transported in plastic boxes (60 liters) to the Laboratório de Zoologia da Universidade Regional do Cariri (LZ-URCA), where they were anesthetized by administration of xylazine 2% and ketamine hydrochloride 1%, and euthanized with a lethal injection of lidocaine chlorohydrate 2%. These specimens were weighed in a digital scale (to the nearest 0.5 g) and measured with a digital caliper (precision 0.05mm) as to their straight-line carapace length (CL). The specimens' gender was determined through a gonads analysis during the dissection process. Turtles with no testicles developed or devoid of vitellogenic follicles in the ovaries and/or eggs in the oviducts, were considered juveniles. Next, gastrointestinal tract, heart, liver, lungs, urinary bladder and body cavity were examined for helminths using a stereomicroscope.

All helminths found were counted and preserved in 70% ethanol. Nematodes were cleared in Hoyer's solution (Everhart, 1957) and the trematodes were stained with hydrochloric carmine and assembled on slides temporary with Hoyer's solution. Then, the helminths were analysed under an optical microscope and identified according to Freitas and Dobbin Jr. (1971), Vicente et al. (1993) and Bray et al. (2008). Afterwards, we calculated the prevalence, mean intensity of infection and abundance according to Bush et al. (1997). Helminths were deposited in the Coleção Parasitológica da Universidade Regional do Cariri (URCA-P 1075-1202).

Diet analyses

The food items present in the stomach were collected during the turtles' dissection and examined under a stereomicroscope. The specimens found as diet components were fixed in 10% formaldehyde and preserved in 70% ethanol. Then, we used a specialized literature (Pérez, 1988; Fernandez and Dominguez, 2001; Mugnai et al., 2010) to identify the least possible taxonomic level (usually order or family) of each specimen.

Both length (L) and width (W) of the intact items were measured using a digital caliper (precision 0.01 mm) as well as their volume (V) was estimated by the ellipsoid's formula (Dunham, 1983):

$$V = \frac{4}{3}\pi\left(\frac{W}{2}\right)^2\left(\frac{L}{2}\right)$$

We examined the frequency (F, number of samples with a specific food item category), abundance (N, number of specimens of each category found in stomach samples) and volume (V, value calculated to each category with the ellipsoid's formula above) of each turtle species' diet items. We calculated the relative importance index (I) to identify the importance of each category for diet composition by the following formula (Powell et al., 1990):

$$I = \frac{F\% + N\% + V\%}{3}$$

where F%, N% and V% are, respectively, the percent values of frequency, number and volume of each food item category.

Statistical analyses

We used a simple linear regression (Zar, 2010) to assess the relationship between the turtle body size (CL) and helminths abundance in both male and female individuals from representative species only (from which there were at least five adults of each gender). Helminth abundance is defined as the total number of parasites at the individual host level regardless of whether or not the host is infected (Bush et al., 1997). Juvenile hosts were removed from this analysis to avoid possible ontogenetic effects bias. The General Linear Model (GLM) with Poisson distribution was used to verify whether hosts' gender affects parasites abundance (Wilson et al., 2002). Since the sexual dimorphism in body size can strongly influence the abundance of helminths, we used a Student's t-test (Zar, 2010) to determine whether there were significant differences in CL and body mass between the sexual genders. The normality of data was confirmed through the Kolmogorov-Smirnov test (Zar, 2010). Statistics were performed in the Statistica software, version 8.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

Among the 63 examined turtles 55 were parasitized (87.3% overall prevalence). In general, we collected 1,520 helminths, which correspond to an overall intensity of infection of 27.63.

The Nematoda found were *Physaloptera retusa* Rudolphi, 1819 (Physalopteridae), *Serpinema monospiculatus* Freitas and Dobbin Jr., 1962 (Camallanidae) and *Spiroxys figueiredoi* Freitas and Dobbin Jr., 1962 (Gnathostomatidae). *Gorgoderina* sp. (Gorgoderidae) was the single Trematoda species recorded in this study (Table 1). All of the recorded helminth species here have a heteroxenic life cycle.

Regarding the body size, *M. tuberculata* carapace length was positively related to helminth abundance in males (F = 59.47, R^2 = 0.93, P = 0.003) and females (F = 6.28, R^2 = 0.39, P = 0.03) (Fig. 1, A and B). The same pattern was observed to *P. geoffroanus* males (F = 11.46, R^2 = 0.39, P = 0.004) and females (F = 64.12, R^2 = 0.73, P < 0.0001) (Fig. 1, C and D).

Regarding the helminths abundance, we found a significant difference in the parasite load between the sexes in *P. geoffroanus* being females more parasitized than males (GLM, Wald = 148.2, df = 1, P ≤ 0.001). We found

Table 1. Prevalence (P) (%), mean intensity of infection (MII), range (R), and sites of infection (S)* of helminths associated with freshwater turtles from Cariús River, Ceará State, Northeast of Brazil. *BC = body cavity, LI = large intestine, L = lungs, SI = small intestine, S = stomach.

Taxon / family / species	*Mesoclemmys tuberculata* Overall (n= 20)			*Phrynops geoffroanus* Overall (n= 41)			*Kinosternon scorpioides* Overall (n= 2)		
	P (%)	MII (R)	S	P (%)	MII (R)	S	P (%)	MII (R)	S
Nematoda									
Camallanidae / *Serpinema monospiculatus*	45	13.77 (1-32)	LI, SI	95.12	29.12 (2-131)	BC, LI, L, SI, S	50	3 (–)	LI
Gnathostomatidae / *Spiroxys figueiredoi*	50	5.1 (2-9)	SI, S	70.73	6.41 (1-18)	S	–	–	–
Physalopteridae / *Physaloptera retusa*	5	2 (-)	LI	7.3	1.33 (1-2)	SI, S	–	–	–
Trematoda									
Gorgoderidae / *Gorgoderina* sp.	–	–	–	9.75	3.5 (1-6)	SI	–	–	–
Total	70	12.64 (1-37)		97.56	33.5 (3-135)		50	3 (–)	

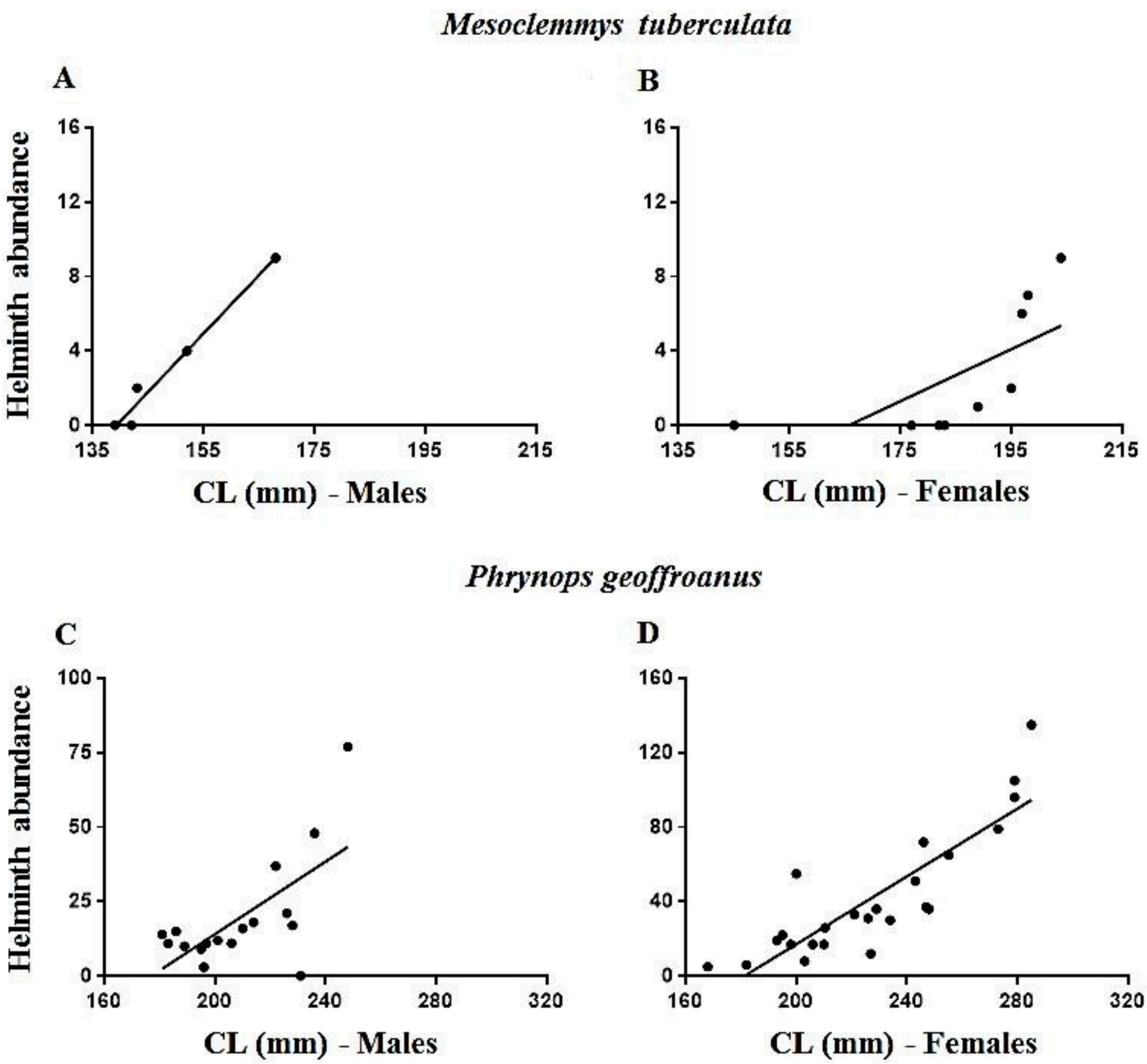

Fig. 1. Relationship between carapace length (CL) and helminths abundance in *M. tuberculata* (A and B) and *P. geoffroanus* (C and D) adult individuals.

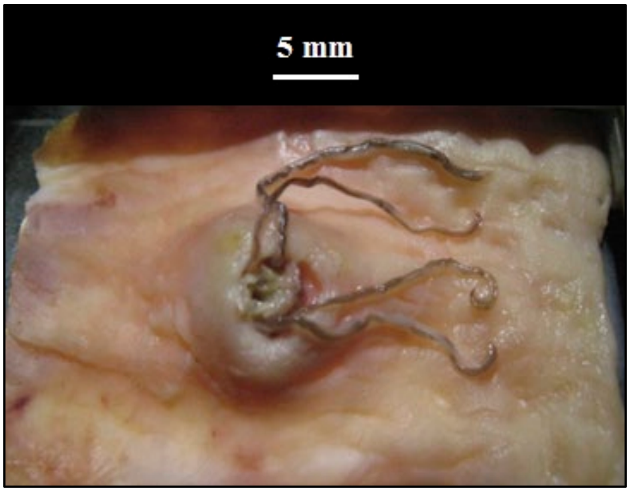

Fig. 2. *Spiroxys figueiredoi* nematodes attached to gastric mucosa of *Phrynops geoffroanus* forming an ulcer.

no significant difference in helminths abundance between *M. tuberculata* males and females (GLM, Wald = 2.45, df = 1, P = 0.11).

M. tuberculata females were significantly bigger than males regarding their carapace length (Student's t-test, t = 4.15, P = 0.001) and body mass (Student's t-test, t = 4.48, P = 0.0007). Females of *P. geoffroanus* were also significantly bigger than males in relation to their carapace length (Student's t-test, t = 2.09, P = 0.04) and body mass (Student's t-test, t = 3.75, P = 0.0007).

In the current research, all turtles with at least two individuals of *S. figueiredoi* presented stomach ulcers (58.73%). All individuals of *S. figueiredoi* were attached to the same ulcer in these turtles, but there was only one ulcer per host (Fig. 2). We observed this pathology in nine specimens of *M. tuberculata* (45%) and 28 *P. geoffroanus* (68.29%). The other collected helminths were randomly distributed in the sites of infection.

Diet composition

Among the 63 analysed stomachs, only 26 presented food items (41.27%). We collected 17 samples of *P. geoffroanus* (15 females and two males), eight of *M. tuberculata* (six juveniles and two females) and one of *K. scorpioides* (female). This samples distribution pattern unable comparative analysis between both genders and between adults and juveniles (in the case of *M. tuberculata*). Overall, we identified 28 categories of food items in the diet of turtles' assemblage (Table 2).

We recognized 18 categories of food items in *M. tuberculata* diet (Table 2). Libellulidae (25%), Aeshnidae (20%) and Chironomidae (15%) were the most frequent categories, whilst Libellulidae (48.53%), Notonectidae (13.23%) and Aeshnidae (10.29%) were numerically more important. Aeshnidae (63.23%) and Libellulidae (16.27%) had the greatest volumes and also the greatest relative importance index of all categories (Aeshnidae, 70.30%, and Libellulidae, 22.62%).

We identified 17 categories of food items in the diet of *P. geoffroanus* (Table 2). Meat (23.8%), Chironomidae (14.28%), leaves (11.9%) and Formicidae (9.52%) were the most frequent items, whereas Chironomidae (71.15%), Formicidae (8.65%) and Notonectidae (5.8%) were the numerically most important and Aeshnidae (45.45%), Chironomidae (24.7%) and Notonectidae (14.15%) had the greatest volumes. Regarding the relative importance of each category, Chironomidae (60.5%), Aeshnidae (18.56%) and Notonectidae (11.55%) were the main consumed items.

Only seeds were found in the stomach contents of *K. scorpioides* (100%) (Table 2).

DISCUSSION

Both *M. tuberculata* and *P. geoffroanus* share the same three helminth species. *Gorgoderina* sp. was found only in *P. geoffroanus*. Also, the unique parasite species found in *K. scorpioides* (*S. monospiculatus*) was infecting all three host species. Parasitological studies involving other herpetofauna groups have been noticing parasites composition overlaps between phylogenetically close host species (see Brooks et al., 2006; Brito et al., 2014). The phylogenetic closeness between *M. tuberculata* and *P. geoffroanus* (Pleurodira: Chelidae) may contribute to the occurrence of a similar helminths composition among these species.

For *K. scorpioides*, the less diverse helminth fauna and lower diversity of food items (seeds only) here recorded, probably reflects the small sample for this species (n = 2). *Kinosternon scorpioides* is the most widely distributed turtle species in the New World and has comparatively a vastly diverse diet along their home range (see Berry and Iverson, 2011). In addition, host species with large geographical ranges usually harbour a richer helminth fauna (Poulin, 2004).

Serpinema monospiculatus and *S. figueiredoi* were the most prevalent parasites among the Chelidae species. Differences in prevalence indexes could be related to the way how helminth species are transmitted to their definitive hosts (Pereira et al. 2012b) and the host's food habits could influence this transmission as well. Although there is no available information about *S. monospiculatus* life cycle, studies about other camallanid nematodes (espe-

Table 2. Diet composition of *Mesoclemmys tuberculata*, *Phrynops geoffroanus* and *Kinosternon scorpioides* collected in Cariús River, Ceará State, Brazil. F = Frequency of occurrence, N = Number of preys, V = Volume (mm^3) and I = Relative importance index. Percentages are shown in parentheses.

Category	*Mesoclemmys tuberculata* (n = 8)				*Phrynops geoffroanus* (n = 17)				*Kinosternon scorpioides* (n = 1)			
	F (%)	N (%)	V (%)	I	F (%)	N (%)	V (%)	I	F (%)	N (%)	V (%)	I
Crustacea												
Ostracoda	-	-	-	-	1 (2.38)	2 (1.92)	1.01 (0.02)	0.00	-	-	-	-
Insecta												
Coleoptera												
Elmidae (adult)	-	-	-	-	1 (2.38)	1 (0.96)	21.19 (0.50)	0.20	-	-	-	-
Elmidae (larvae)	-	-	-	-	1 (2.38)	1 (0.96)	0.62 (0.01)	0.00	-	-	-	-
Gyrinidae	-	-	-	-	1 (2.38)	2 (1.92)	18.84 (0.45)	0.18	-	-	-	-
Scirtidae	1 (5)	2 (2.94)	3139.73 (2.90)	0.80	-	-	-	-	-	-	-	-
Diptera												
Diptera (pupae)	1 (5)	1 (1.47)	23.55 (0.02)	0.00	1 (2.38)	1 (0.96)	13.08 (0.31)	0.13	-	-	-	-
Chironomidae	3 (15)	4 (5.90)	202.16 (0.18)	0.15	6 (14.28)	74 (71.15)	1036.43 (24.70)	60.50	-	-	-	-
Sciomyzidae	-	-	-	-	1 (2.38)	1 (0.96)	150.72 (3.60)	1.47	-	-	-	-
Ephemeroptera												
Baetidae	-	-	-	-	1 (2.38)	1 (0.96)	35.05 (0.83)	0.34	-	-	-	-
Heteroptera												
Belostomatidae	2 (10)	2 (2.94)	4211.05 (3.85)	2.14	-	-	-	-	-	-	-	-
Corixidae	2 (10)	2 (2.94)	256.69 (0.23)	0.13	-	-	-	-	-	-	-	-
Naucoridae	1 (5)	1 (1.47)	-	-	-	-	-	-	-	-	-	-
Nepidae	1 (5)	1 (1.47)	1714.85 (1.57)	0.43	-	-	-	-	-	-	-	-
Notonectidae	2 (10)	9 (13.23)	544.52 (0.50)	0.30	2 (4.76)	6 (5.80)	593.77 (14.15)	11.55	-	-	-	-
Hymenoptera (wasp)	-	-	-	-	1 (2.38)	1 (0.96)	100 (2.38)	0.97	-	-	-	-
Hymenoptera												
Formicidae	-	-	-	-	4 (9.52)	9 (8.65)	66.56 (1.60)	2.60	-	-	-	-
Megaloptera												
Corydalidae	1 (5)	2 (2.94)	4841.35 (4.43)	1.23	-	-	-	-	-	-	-	-
Odonata (adult)	-	-	-	-	1 (2.38)	1 (0.96)	200 (4.76)	1.95	-	-	-	-
Odonata (nymph)												
Aeshnidae	4 (20)	7 (10.29)	69139.13 (63.23)	70.30	1 (2.38)	1 (0.96)	1907.55 (45.45)	18.56	-	-	-	-
Coenagrionidae	1 (5)	1 (1.47)	427.43 (0.39)	0.10	-	-	-	-	-	-	-	-
Libellulidae	5 (25)	33 (48.53)	17795.32 (16.27)	22.62	-	-	-	-	-	-	-	-
Orthoptera (terrestrial)	1 (5)	1 (1.47)	-	-	-	-	-	-	-	-	-	-
Reptilia												
Squamata												
Snakes												
Epictia sp.	1 (5)	1 (1.47)	7037.78 (6.43)	1.80	-	-	-	-	-	-	-	-
Bone fragmente	1 (5)	-	-	-	-	-	-	-	-	-	-	-
Plant material												
Leaves	-	-	-	-	5 (11.90)	-	-	-	-	-	-	-
Seeds	1 (5)	1 (1.47)	4.71 (0.00)	0.00	3 (7.14)	3 (2.88)	52.16 (1.24)	1.52	1 (100)	28 (100)	1501.96 (100)	100
Unidentified material	1 (5)	-	-	-	2 (4.76)	-	-	-	-	-	-	-
Meat	1 (5)	-	-	-	10 (23.80)	-	-	-	-	-	-	-
Total numbers	-	68	109338.3	-	-	104	4197.01	-	-	28	1501.96	-

cially *S. trispinosum* [Leidy, 1852]) showed that freshwater copepods act as intermediate hosts, and aquatic snails, fishes, amphibians and odonates as paratenic hosts until the definitive host's infection (Moravec and Vargas-Vázquez, 1998; González and Hamann, 2007; Wiles and Bolek, 2015). *Spiroxys figueiredoi* life cycle is also unknown. However, *Spiroxys* larvae have been reported from freshwater copepods and Odonata nymphs (as intermediate hosts), and fishes and amphibians, which are used as paratenic hosts (Hedrick, 1935; Anderson, 2000).

According to other studies, *M. tuberculata* feeds mainly on fish (Vanzolini et al., 1980) and *P. geoffroanus* mainly on aquatic insects, crustaceans and fish (Medem, 1960; Souza, 2004; Martins et al., 2010). In the current research, the *M. tuberculata* most consumed items were Odonata nymphs (Aeshnidae and Libellulidae), whilst *P. geoffroanus* feed mainly on Diptera larvae (Chironomidae), Odonata nymph (Aeshnidae) and Notonectidae. Thus, the high consumption of odonates (in this study) and fish could influence the *Serpinema* and *Spiroxys* recruitment, consequently increasing their infection indexes.

Gorgoderina sp. and *P. retusa* had the lowest registered prevalence. *Gorgoderina* species have a complex life cycle, in which bivalve molluscs commonly act as first intermediate hosts and aquatic insect larvae and tadpoles as second intermediate hosts (Coil, 1954). Data on *P. retusa* life cycle are scarce. Nevertheless, Anderson (2000) reports that infection caused by *Physaloptera* spp. on definitive hosts begins with the ingestion of insects containing infective larvae (in third stage). Arthropods such as cockroaches, crickets, grasshoppers and beetles act as intermediate hosts to many *Physalopteras* pecies larvae (e.g., *P. hispida, P. maxillaris, P. preaeputilialis* and *P. rara*) (Schell, 1952; Lincoln and Anderson, 1975).

Although terrestrial insects can occasionally be consumed by freshwater turtles (Souza and Abe, 2000; Santana, 2012), aquatic insects and its larvae are included in the most important food resources for these reptiles (Souza and Abe, 2000; Souza, 2004; Martins et al., 2010). In this study, the diet of Chelidae populations was composed mostly by aquatic invertebrates, with a predominance of insect larval stages, and few terrestrial specimens were found in *M. tuberculata* (Orthoptera and Snake) and *P. geoffroanus* (Hymenoptera and adult Odonata) diet. This low frequency of terrestrial invertebrates and the absence of molluscs and tadpoles in the diet could reduce the probability of infection by *Physaloptera* and *Gorgoderina* species, respectively, which reflects the low prevalence and infection intensity indexes.

The host body size is an important feature that frequently determines parasites species richness and abundance (Poulin, 2007; Poulin and Leung, 2011; Kamiya et al., 2014). Correlations between host body size and abundance and richness of helminths are common in studies with lizards (Martin et al., 2005), an approach still poorly explored for turtle parasites. Several studies report a positive relationship between the host body size and abundance and diversity of parasite species (e.g., Poulin, 2007; Anjos et al., 2012; Kamiya et al., 2014), which indicates that larger host species can provide greater space and, consequently, support a higher number of parasites (Poulin, 2007). Hence, our linear regression results to both Chelidae species are in accordance with previous statements, since the largest turtles (both males and females) had greater helminth abundance than smaller turtles.

Females of *P. geoffroanus* were more parasitized than males. Our data showed that *P. geoffroanus* females were significantly bigger and heavier than males. This sexual dimorphism related to body also recorded in other researches (e.g., Medem, 1960; Brites, 2004) must have contributed to a higher parasite load in female hosts. Although *M. tuberculata* females were significantly bigger than males, there was no significant difference in helminth abundance between the sexual genders. Further studies with larger samples of adult turtles are needed for a better understanding of the infection patterns in this Chelidae species.

In addition, physiological and behavioural differences related to sexual gender of the host (such as differences in habitat use, diet, reproductive behaviour, hormone levels, time of activity) may result in parasite abundance differences between males and females (Aho, 1990; Pereira et al., 2012b). Nevertheless, such ecological aspects about most of Brazilian turtle species have not been investigated in great depth (Souza, 2004).

Regarding the available data on the helminth fauna associated with *P. geoffroanus* in Brazil, Silva (2014) developed the research with the best known turtle sampling (n = 11), in which a prevalence of 90.90% was recorded. Our research recorded the major infection prevalence (97.56%). However, we did observe a relatively low helminth richness (four species). In turn, Silva (2014) recorded the major helminth species richness of *P. geoffroanus* (10 species) in the Reserva Particular do Patrimônio Natural Foz do Rio Aguapeí protected area, located in a transition area (ecotone) between Cerrado and Atlantic Forest biomes, Southeastern Brazil. Nevertheless, the population analysed by Silva (2014) had lower mean intensity of infection (11.7) when compared to our study (33.5). Variability in habitat characteristics (such as variations in climate conditions and watershed or landscape alterations) in which turtles were collected could influence the parasites abundance and diversity patterns due to less human-impacted areas usually have high helminth diversity and the hosts are parasitized by a lower number of individual parasites (McKenzie, 2007; Anjos et al., 2012). Thus, anthropogenic pressures on Cariús River and its vicinity could lead to lower helminth diversity and higher prevalence and infection intensity indexes as recorded here.

Regarding the helminths we recorded, *Gorgoderina* species are commonly found in the urinary bladder of anurans and caudates (Amphibia) around the world (Coil, 1954; Mata-López et al., 2005). Now considering the freshwater turtles of Brazil, among 29 species 13 have been reported to harbour trematode species (Fernandes and Kohn, 2014). Despite that, *Gorgoderina* spp. has not been recorded in association with these reptiles. It is probably that *Gorgoderina* adults accidentally occurred in the small intestine of *P. geoffroanus* because *Gorgoderina* usually become mature inside the bladder (Coil, 1954).

Species of the *Physaloptera* Rudolphi, 1819 are usually reported as amphibians, birds, mammals and reptiles parasites (Pereira et al., 2012a). Among the *Physaloptera* spp. recorded in Brazilian reptiles (*P. bonnie*, *P. liophis*, *P. lutzi*, *P. monodens*, *P. obtusíssima*, *P. retusa* and *P. tupinambae*), *P. retusa* have been found frequently in the stomach of lizards, parasitizing 36 species (Ávila and Silva, 2010; Araujo-Filho et al., 2014). In Brazil, only *Physaloptera* larvae were found in *P. geoffroanus* in São Paulo State (Silva, 2014). Accounting that *Physaloptera* infection goes through intermediate hosts (terrestrial insects which are occasionally consumed by freshwater turtles), *P. retusa* must has occasionally occurred in *M. tuberculata* (large intestine) and *P. geoffroanus* (stomach and small intestine).

Serpinema Yeh, 1960 frequently parasitizes freshwater turtles (Anderson, 2000). In Brazil, *S. monospiculatus* was found in association with *M. tuberculata* (type host), *M. nasuta* (Schweigger, 1812) and *P. geoffroanus* in the State of Pernambuco (Freitas and Dobbin Jr., 1971; Vicente et al., 1993), and *S. magathi* was found in *K. scorpioides* in the States of Pernambuco, Pará (Freitas and Dobbin Jr., 1971) and Maranhão (Viana et al., 2016). *Serpinema magathi* was originally described as *Camallanus magathi* by Sprehn (1932) from *Kinosternon integrum* Le Conte, 1854 collected in Bolivia. Then, Caballero (1939) recorded *S. magathi* (=*Camallanus parvus*) parasitizing *Kinosternon hirtipes* Wagler, 1830, in Mexico (Freitas and Dobbin Jr., 1971). In Costa Rica, Bursey and Brooks (2011) reported *S. magathi* as a *Kinosternon leucostomum* (Duméril and Bibron, 1851) parasite.

Nematodes from *Spiroxys* Schneider, 1866 are usually found in the gastrointestinal tract of freshwater turtles (Hedrick, 1935; Berry, 1985) and parasite amphibians and reptiles (Roca and García, 2008) just like definitive hosts such as frogs, salamanders and snakes (Berry, 1985). In Brazil, *S. figueiredoi* was found in *K. scorpioides* (type host) in the States of Pernambuco, Pará (Freitas and Dobbin Jr., 1971) and Maranhão (Viana et al., 2016). In São Paulo, it was also found parasitizing *P. geoffroanus* and *Pseudoboa nigra* (Duméril, Bibron and Duméril, 1854) (Dipsadidae) (Silva, 2014). Some undescribed *Spiroxys* specimens were recorded as *Mesoclemmys vanderhaegei* (Bour, 1973) (Chelidae) parasites in the State of Mato Grosso do Sul (Ávila et al., 2010). In the State of Rio Grande do Sul, *Spiroxys* sp. was also found in *Phrynops hilarii* (Duméril and Bibron, 1835) (Chelidae) and in *Trachemys dorbigni* (Duméril and Bibron, 1835) (Emydidae) (Bernardon et al., 2013, 2014). In the same State, *S. contortus* (Rudolphi, 1819) was recorded in association with the *Acanthochelys spixii* (Duméril and Bibron, 1835), *Hydromedusa tectifera* Cope, 1870 (Chelidae) and *T. dorbigni* freshwater turtles (Mascarenhas et al., 2013; Mascarenhas and Müller, 2015). In Costa Rica, *S. figueiredoi* was found in *K. leucostomum* and in *K. scorpioides* (Bursey and Brooks, 2011). Our study reports the second occurrence of *S. figueiredoi* parasitizing *P. geoffroanus* in Brazil.

Researches that draw forth the effects of parasitism on freshwater turtles are still scarce. In the current study, we recorded for the first time, the occurrence of erosive lesions in the gastric mucosa in *M. tuberculata* and *P. geoffroanus* individuals caused by *S. figueiredoi* adults. Likewise, in other countries specimens of *Spiroxys* were found causing pathological changes in their hosts. In the United States, McAllister et al. (1993) reported the involvement of *S. contortus* larvae in forming gastric granulomas in *Apalone spinifera pallida* (Webb, 1962)(Trionychidae). In Europe, Miclăuş et al. (2008) found *S. contortus* adults causing pylorus irreversible lesions in the stomach of *Emys orbicularis* (Linnaeus, 1758) (Emidydae). In addition, Burke and Rodgers (1982) suggest that the occurrence of gastric ulcers caused by nematode scan reduce the hosts' appetite and stunt their growth. The high frequency of gastric ulcers in the sampled Chelidae species is atypical and demands for future research efforts.

Among the collected helminths, *S. monospiculatus* is a new record to *K. scorpioides*. *Gorgoderina* sp. and *P. retusa* were recorded for the first time in *P. geoffroanus*. *Mesoclemmys tuberculata* represents a new host record for *P. retusa* and *S. figueiredoi*.

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