Spatio-temporal variation and the use of host body surface by ectoparasites of the chelonians *Phrynops geoffroanus* and *Mesoclemmys tuberculata* in areas of the Caatinga and Atlantic Forest in northeast Brazil

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Abstract

Ectoparasites such as hematophagous leeches and monogeneans are common in chelonians, occupying different parts of the body. Thus, the present study aimed to identify and describe the fauna of ectoparasites that infest *Phrynops geoffroanus* and *Mesoclemmys tuberculata* to evaluate the effect of host conditions and seasonality (dry and rainy season) on the abundance and composition of ectoparasites. We verified the presence of ectoparasites in 73.2% of the examined turtles, with four species of leeches belonging to Glossiphoniidae, *Haementeria brasiliensis* sensu Cordero, 1937, *Helobdella cf. adiastola*, *Haementeria sp1.*, and *Haementeria sp2.*, and one monogenean Polystomatidae, *Polystomoides brasiliensis*. For both chelonians, we observed a significant difference in the abundance of ectoparasites in relation to sex, biome, and season, which was unrelated to length and mass. Leeches were more frequent in the cavities of the hind limbs in *P. geoffroanus*, and the anterior limbs of *M. tuberculata*. The general spatial niche overlap of ectoparasites was high, except for that of the monogenean *P. brasiliensis*, which did not overlap with those of other leech species. The present study is the first report of the presence of *H. brasiliensis* and *P. brasiliensis* parasitizing *M. tuberculata*, and *Helobdella cf. adiastola* in a phoretic relationship with *P. geoffroanus* and *M. tuberculata*. Finally, the differences in infestation levels may reflect ecological factors, differences in behavioral patterns of the hosts, and different anthropic alterations suffered in the Caatinga and Atlantic Forest biomes.

Keywords Hirudinea · Leeches · Monogenae · Niche breadth · Overlap · Host

Introduction

Parasites potentially affect the life history of their hosts (Møller et al. 1990; Møller et al. 2003). The number of conservation studies concerning issues relating to population health, including parasitism, is increasing (Møller 1999; Deem et al. 2001). Parasitism and with predation and competition act as great selective forces in the evolution of the species and are

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responsible for population and community structure (Gibbons et al. 2000; Menezes 2000; Silva and Araújo 2008). However, little is known about the dynamics of parasites in natural populations of reptiles, especially freshwater turtles (Ryan and Lambert 2005; Readel et al. 2008). Moreover, ectoparasites like hematophagous leeches are common in aquatic turtles (Ringuelet 1944b; Sawyer 1986; Watermolen 1996; Light and Siddall 1999; Siddall and Borda 2004; Cubas et al. 2006), usually occupying the pleural cavity of the arms, legs, neck, tail, and even the mouth (Jacobson et al. 1989; Herbst 1994).

Leeches are known environmental bioindicators (Sawyer 1986; Christoffersen 2007). The high degree of endemism of aquatic leeches makes them potentially useful as indicator species of water quality and undisturbed environments, since their habitat specificity makes them very sensitive to any form of anthropic influence (Christoffersen 2007). Koperski (2005) also emphasizes that the Hirudinea present a low level of mobility, high degree of oxygen absorption, and low diversity in comparison with other invertebrates, attributes that strengthen their position as bioindicators of environmental quality. However, some studies have reported a relationship between the occurrence of leeches and levels of organic pollutants, physical-chemical factors of water and food availability (Sawyer 1986; Miserendino and Gullo 2014; Brites and Rantin 2004), considering that in some cases, these factors could promote even more damage to hosts, as they can favor attack by parasites, leading to large infestations (Brites and Rantin 2004).

The Hirudinea, in addition to being parasites, are important components of the benthic macroinvertebrates of rivers and streams and is a taxon with a remarkable degree of endemism in the Neotropical region (Ringuelet 1944a; Christoffersen 2009). With the exception of some cosmopolitan species, the vast majority have a relatively low dispersion capacity (Ringuelet 1944b; Gullo 2014), which provides a great diversity of endemic species (Siddall and Borda 2004; Christoffersen 2007, Christoffersen 2008).

Seven families of aquatic leeches are present in the Neotropical region: Glossiphoniidae (Vaillant, 1890), Psicolidae (Johnson, 1965), Ozobranchiidae (Pinto, 1921), Cilicobellidae (Ringuelet, 1972), Semiscolecidae (Scriban and Autrum, 1934), Praobdellidae (Sawyer, 1986), and Macrobdellidae (Richardson, 1969) (Soós 1970; Christoffersen 2008; César et al. 2009; Christoffersen 2009; Phillips et al. 2010). Glossiphoniidae comprises the largest family in number of freshwater species, including predatory species of macroinvertebrates and the temporal ectoparasites of fish, turtles, amphibians, mammals, and even aquatic birds (Ringuelet 1985a; Sawyer 1986; Oceguera-Figuerola 2012). In the Neotropical region, studies of the biodiversity of Hirudinea are scarce (Ringuelet 1944b, 1981, 1985a; Gullo 1998; Christoffersen 2007; Gullo 2007; César et al. 2009; Christoffersen 2009), and no natural history information is available for freshwater environments (Gullo 2014). Iwama and Arruda (2016) also point out that in Brazil, little is known about the diversity of leeches and that no major studies on Brazilian leech fauna have been conducted. Despite their ecological potential as bioindicator organisms, basic studies on the taxonomy, biology, and ecology of leeches are practically neglected in tropical regions (Christoffersen 2007; Iwama and Arruda 2016).

Monogeneans are parasites of mainly semi-aquatic and aquatic vertebrates, principally fish (Reichenbach-Klinke 1966), but are also found in amphibians (Yamaguti 1963), mammals (Stunkard 1924), and freshwater and marine turtles (Platt 2000; Ávila et al. 2010; Domènech et al. 2016).

Polystomatidae (Monogenea) are found exclusively parasitizing chelonians, often infecting the conjunctival sac, urinary bladder, and oral cavity (Du Preez and Lim 2000; Platt 2000; Ávila et al. 2010; Du Preez and Van Rooyen 2015; Domènech et al. 2016). We know little about the life cycle of these parasites, and most of the scarcely available information summarizes records of hosts and sites of infestation. For example, only recently was a monogenean, Polystomoides brasiliensis Vieira et al., 2008, described in chelonians in Brazil (Vieira et al. 2008).

Parasitism by leeches has been reported in Phrynops geoffroanus (Schweigger, 1812) (Ringuelet 1981; Brites and Rantin 2004; Ferronato et al. 2009), and the presence of monogenoids P. brasiliensis and Polystomoides sp. has been recorded (Vieira et al. 2008). Information related to ectoparasites of Mesoclemmys tuberculata (Lüderwaldt, 1926) is unavailable at this time.

The objective of this study was to identify and describe the fauna of ectoparasites that infest P. geoffroanus and M. tuberculata, to evaluate the effect of host conditions (maximum carapace length (CL) and body mass), sex, and seasonality (dry and rainy season) on the abundance and composition of ectoparasites. We also describe patterns in the use of the microhabitat (body surfaces of the chelonians) and the differences found in fixation site preference between ectoparasites and compare infestation rates in each host between the different biomes occurring in environments of the Caatinga and Atlantic forest in an area of northeastern Brazil.

Materials and methods

Study sites

Samples of P. geoffroanus and M. tuberculata were obtained from six localities, three in the Caatinga, and three in the Atlantic Forest in the State of Sergipe, Brazil (Fig. 1). The selected locations in the Caatinga area were the Monumento Natural do Rio São Francisco (09°41.202′ S, 037°42.963′ W—municipality of Poço Redondo), Monumento Natural Grota do Angico (09°40.852′ S, 037°41.408′ W—municipality of Poço...
Redondo), and the Bacia do Rio Real (11°10.730′ S, 038°02.300′ W—municipality of Tobias Barreto). The selected Atlantic Forest areas were the Parque Nacional Serra de Itabaiana (10°45.251′ S, 037°20.511′ W—municipality of Areia Branca), Reserva Biológica de Santa Isabel (10°35.766′ S, 036°40.366′ W—municipality of Pirambú), and Refúgio de Vida Silvestre Mata do Junco (10°31.895′ S, 037°03.142′ W—municipality of Capela).

**Turtle collection**

The samples were obtained in semiannual campaigns (dry and rainy season), over a year, lasting 10 consecutive days at each of the six collection sites, three in the Caatinga area, and three in the Atlantic Forest, totaling 120 days of sampling. The study was developed in 2014, and we defined the rainy and dry seasons based on information on the distribution of precipitation in the areas (historical averages) (Hijmans et al. 2005). The months from April to August were considered as the rainy season, and the remaining months were assigned to the dry season.

To capture individuals, we used at each collection point 20 hoop traps placed in rivers, streams, and lagoons. To attract tortoises, the traps were baited with beef and checked every 3 h and remained in the water between 06:00 and 18:00, with occasional nocturnal collections. In environments with less turbid waters, the active search was performed between 07:00 and 17:00, using free diving with the aid of a mask, snorkel, and fins.

The analyses were performed with adult specimens (male and female) and juveniles. Each captured adult animal was sexed according to external morphological characteristics and secondary sexual characters, such as plastral concavity and tail length (Berry and Shine 1980; Ernst and Barbour 1989; Molina 1990; McCord et al. 2001; Corazza and Molina 2004; Goulart 2004; Rueda-Almonacid et al. 2007; Martins and Souza 2008; Brito et al. 2009; Molina et al. 2012; Forero-Medina et al. 2013; Marques et al. 2013; Moura et al. 2015; Santana et al. 2016). Individual marking of chelonian specimens was performed by perforations in the marginal shells, following the methodology described by Cagle (1939), to avoid repeat data from the same specimen in the same expedition and to allow individual registration in the different periods of the study.

**Procedures**

Immediately after capture, turtles were examined for the presence of ectoparasites, before ectoparasites could migrate to other regions of the host body due to desiccation caused by animal handling. The ectoparasites found were narcotized...
according to the technique of Klemm (1995), which consists of soaking them in 10% ethanol to facilitate their removal. The ectoparasites were removed with forceps, fixed in 4% formaldehyde, and then washed with distilled water and preserved in 70% ethanol.

In the laboratory, these parasites were identified according to Ringuelet (1985b) and Sawyer (1986) and the occurrences were quantified. Infestation levels were defined based on the frequency and abundance of parasites found in each animal. After identification, the parasitological indices evaluated were the prevalence (percentage of hosts infected with at least one parasite), abundance (total number of parasites), and mean parasitism intensity (mean number of parasites per infected host) calculated according to Bush et al. (1997). In order to identify microhabitats (regions in the hosts’ body), after removal of the ectoparasites, they were placed in specific flasks for each different region of the body, allowing the verification regarding the positioning of these parasites, where they were found (pleural cavities of the anterior limbs and lower, neck, tail, carapace, and plastron). The specimens were deposited in the Collection of Hirudinea of the Federal Rural University of Pernambuco (UFRPE).

Statistical analyses

The spatial niche breadths (body regions) were calculated using the inverse diversity index of the Simpson (1949):

$$B = \frac{1}{\sum_{i=1}^{n} p_i^2}$$

where \( p \) is the proportion of the resource category (spatial) used \( i \) and \( n \) is the number of resource categories adopted. \( B \) ranges from 1 (exclusive use of a resource type) to \( n \) homogeneous use of all resource types). Similarities in the use of the resources by the different species were investigated through the symmetrical overlapping formula (Pianka 1973):

$$\varphi_{jk} = \frac{\sum_{i=1}^{n} p_{ij} p_{ik}}{\sqrt{\sum_{i=1}^{n} p_{ij}^2 \sum_{i=1}^{n} p_{ik}^2}}$$

where the symbols are the same as those mentioned above, but \( j \) and \( k \) represent different species. Values close to zero indicate that there is no similarity in resource use; values close to one indicate a similar use of resources.

In order to verify the influence of sex/life stage (males, females, and juveniles) of hosts, as well as differences between biomes and stations on the abundance of ectoparasites, a generalized linear model (GLM) was used, assuming the Poisson distribution (Hudson et al. 2002). A simple linear regression (Zar 1999) was used to analyze the relationship of maximum carapace length (CL) and body mass of turtles to the abundance of ectoparasites. Infection rates (calculated infection prevalence and mean intensity as well as ecoparasitological terminology) followed those defined by Bush et al. (1997). The normality of the data was analyzed by the Shapiro-Wilk test. In order to investigate the overlap of spatial niches, the Ecosim niche overlay module (Gotelli and Ellison 2013) was employed. Other statistical analyses were performed using the program R (R Development Core Team, 2011), and the significance level for hypothesis testing was 5%.

Results

In total, 154 turtles were captured, 99 \( P . \) geoffroanus (11 males, 24 females, and 64 juveniles), and 55 \( M . \) tuberculata (24 males, 26 females, and 5 juveniles). The animals were more abundant in the Caatinga with 132 captured individuals, 93 \( P . \) geoffroanus (8 males, 22 females, and 63 juveniles), and 40 \( M . \) tuberculata (18 males, 21 females, and 1 juvenile). In the Atlantic Forest, only 21 individuals were captured, 6 \( P . \) geoffroanus (three males, two females, and one juvenile), and 15 \( M . \) tuberculata (six males, five females, and four juveniles).

We verified the presence of ectoparasites in 113 turtles (prevalence 73.2%). A total of 2609 ectoparasites were collected (Table 1), including 2346 Glossiphoniidae leeches represented by four species, \( Haementeria \) brasiiliensis sensu Cordero, 1937, \( Helobdella \) cf. adiastola, \( Haementeria \) sp1., and \( Haementeria \) sp2. (Fig. 2), the latter being the only one that occurred exclusively in \( M . \) tuberculata. We also collected 263 monogeneans, all the specimens were identified as \( P . \) brasiiliensis (Fig. 3).

For both chelonians, we observed a significant difference in the abundance of ectoparasites in relation to sex (Table 2), with \( P . \) geoffroanus females showing the highest infestations rates, whereas in \( M . \) tuberculata, males had the highest indices (Table 3). The juvenile hosts presented lower values of prevalence and mean intensity of infestation than those found in adults (Table 3).

As for the biomes, we observed significant differences in infestation levels (Table 2): In \( P . \) geoffroanus, the Atlantic Forest specimens showed higher infestation rates when compared with Caatinga, whereas for \( M . \) tuberculata, the Caatinga showed higher infestation rates (Table 4). The influence of season on the abundance of ectoparasites was also significant in \( P . \) geoffroanus but was not significant in \( M . \) tuberculata (Table 2), with the highest rates occurring in the dry period. We also verified in both species a significant difference in the total abundance of ectoparasites considering the interaction between the biome and the season (Table 2).

There was no significant relationship between maximum carapace length (CL) and abundance of
ectoparasites in \textit{P. geoffroanus} ($F_{1,62} = 1.436; R^2 = 0.054; P = 0.156$) and \textit{M. tuberculata} ($F_{1,46} = 0.019; R^2 = 0.0001; P = 0.985$), nor between the mass and the abundance of ectoparasites in \textit{P. geoffroanus} ($F_{1,62} = -0.800; R^2 = $)
0.054; \( P = 0.427 \) and in \( M. \ tuberculata \) \((F_{1,46} = -0.049; \ R^2 = 0.0001; \ P = 0.961)\).

Regarding the positioning of ectoparasites, leeches were more frequent in the cavities of the hind limbs in \( P. \ geoffroanus \), with a smaller spatial niche breadth, presenting a more specialized behavior regarding the use of the micro-habitat. In contrast, in \( M. \ tuberculata \), the same species of ectoparasites were distributed more homogeneously over the different parts of the body, with a greater abundance in the anterior limb cavities, resulting in a broader special niche that was more general with respect to microhabitat (i.e., parts of the host body) use (Table 1; Fig. 4). The monogenean \( P. \ brasiliensis \) was found using only the oral and pharyngeal cavity of the hosts (Fig. 4), where they were fixed through multiple suckers. This monogenetic species frequently showed leakage behavior, migrating towards the host esophagus when the turtles’ mouths were open.

The spatial niche overlap of leeches was high, ranging from 0.74 to 0.98. We found the lowest value of niche overlap between \( Haementeria \) sp2. and \( Helobdella \ cf. \ adiastola \) and the largest between \( Haementeria \) sp1. and \( H. \ brasiliensis \), both parasitizing \( M. \ tuberculata \) (Table 5). However, the monogenean \( P. \ brasiliensis \) did not present spatial niche overlap with the other ectoparasites (Table 5).

We have observed the presence of areas of redness and small rashes on the skin in some \( P. \ geoffroanus \) parasitized by \( H. \ brasiliensis \). In these cases, it was not possible to determine if these pathologies were caused by bruises generated by leeches, antihistamine reaction, or blood vessel dilation. Larger blood vessels were also observed, visible through the skin in areas of fixation and feeding of this leech; however, no greater fixation lesions were observed from these ectoparasites.

**Discussion**

The diversity of species found in the present study resulted in new identifications of the leeches \( H. \ brasiliensis \), \( Helobdella \ cf. \ adiastola \) in the Brazilian northeast. This is the first report of the presence of these ectoparasites in \( M. \ tuberculata \) and the first record of the presence of \( Helobdella \ cf. \ adiastola \) in \( P. \ geoffroanus \). In addition, the leeches \( Haementeria \) sp1. and \( Haementeria \) sp2. are potentially non-described species, although further investigation is still needed. This result is not surprising because studies with Hirudinea in the Neotropical region are still scarce (Gullo 1998, 2007; César et al. 2009; Christoffersen 2007, 2009), with long periods without collection of any new information (Iwama and Arruda 2016).

\( Phrynops \) \( geoffroanus \) and \( M. \ tuberculata \) shared three species of leeches and the Monogenea; only the leech \( Haementeria \) sp2. was not common among them, present only in \( M. \ tuberculata \). Even with the phylogenetic distance between \( M. \ tuberculata \) and \( P. \ geoffroanus \) (Pleurodira: Chelidae), the similarity in the composition of ectoparasites found indicates a certain degree of generalism of registered parasites, although most of the parasitological studies in reptiles and amphibians show high overlap in the composition of parasites between phylogenetically related host species (Brooks et al. 2006; Brito et al. 2014).

In \( P. \ geoffroanus \), the Hirudinea \( H. \ brasiliensis \) was the most prevalent ectoparasite, whereas \( M. \ tuberculata \) presented the highest prevalence for \( Haementeria \) sp1. These differences...
in prevalence rates may be related to differences in host uses of microhabitats (ponds and rivers) (Sawyer 1986; Gullo 2014), as well as specific behaviors presented, such as displacement near the substrate where leeches reside, favoring infestations (Roca et al. 2005; Ryan and Lambert 2005; McCoy et al. 2007).

Glossiphoniidae leeches often parasitize amphibians and reptiles, such as aquatic chelonians and crocodilians (Ringuelet 1944b; Sawyer 1972; Siddall and Gaffney 2004; Roca and García 2008). Haementeria brasiliensis sensu Cordero, 1937, occurs in Argentina and Brazil (Pinto 1920; Ringuelet 1981; Brandão and Garda 2000; Christoffersen 2009). In chelonians, H. brasiliensis has already been recorded parasitizing P. geoffroanus in Pernambuco State (Ringuelet 1981).

The genus Helobdella consists of the largest and most diverse group, containing almost half of the diversity of species of Neotropical Hirudinea fauna (Christoffersen 2007, Christoffersen 2009). Helobdella adiastola has a wide distribution in South America, occurring in Argentina, Uruguay, Paraguay, Peru, and Brazil (César et al. 2009; Christoffersen 2009; Gullo 2014; Iwama and Arruda 2016). Its redescription occurred only recently (Iwama and Arruda 2016); the species is associated with floating vegetation (César et al. 2009) in areas with stones in the substrate of the river bed (Gullo 2014). Thus, it is quite possible that individuals of this species are only in a phoretic relationship, since the host species are invertebrates, mainly mollusks (Ringuelet 1944b; Darrigran et al. 1998; Vega et al. 2006), which may explain the low rates of prevalence and intensity recorded in the chelonians.

Species of the genus Haementeria occur throughout the Neotropical region (Christoffersen 2007; Siddall and Borda 2004), and terrestrial species normally parasitize mammals, while the aquatic species are parasites of crocodilians and freshwater turtles (Sawyer and Kinard 1980; Sawyer 1986). This genus features giant species such as the giant Amazon leech, Haementeria ghiliani, which to approximately 50 cm (Siddall et al. 2001); however, the Haementeria sp. registered in our study parasitizing M. tuberculata corresponds to a small species.

| Table 3 | Values by sex/life stage (males, females, and juveniles) of the prevalence P (%), infestation intensity (IF), and abundance (A) for each species of ectoparasitic associated with freshwater turtles Phrynops geoffroanus (Schweigger, 1812) and Mesoclemmys tuberculata (Lüderwaldt, 1926) in areas of the Caatinga and Atlantic Forest in northeast Brazil |
|---------|--------------------------------------------------------------------------------------------------|-----------|-----------|-----------|
| Parasite | Phrynops geoffroanus Males (n = 11) | Females (n = 24) | Juveniles (n = 64) |
| Hirudinea | | | |
| Glossiphoniidae | | | |
| Haementeria brasiliensis sensu Cordero, 1937 | 81.81 15.55 ± 19.02 140 | 83.33 26.9 ± 29.22 538 | 50.76 14.48 ± 19.65 478 |
| Helobdella cf. adiastola | 9.09 51* 51 | 4.16 1* 1 | 4.61 2 ± 0.63 6 |
| Haementeria sp1. | 18.18 7.5 ± 4.4 15 | 8.33 28.5 ± 10.69 57 | 0 0 0 |
| Monogenea | | | |
| Polystomatidae | | | |
| Polystomoides brasiliensis | 72 1.87 ± 1.17 15 | 58.33 2.07 ± 2.03 29 | 12.3 2 ± 1,20 16 |
| Total | 90.9 22.1 ± 28.85 221 | 91.66 28.40 ± 29.71 625 | 50.76 15.15 ± 20.06 500 |
| Parasite | Mesoclemmys tuberculata Males (n = 24) | Females (n = 26) | Juveniles (n = 5) |
| Hirudinea | | | |
| Glossiphoniidae | | | |
| Haementeria brasiliensis sensu Cordero, 1937 | 25 8 ± 7.2 48 | 15.38 13.5 ± 7.7 54 | 0 0 0 |
| Helobdella cf. adiastola | 0 0 0 | 15.38 5.5 ± 3.86 22 | 20 10 ± 5.77 10 |
| Haementeria sp1. | 70.83 26.58 ± 25.15 452 | 88.46 15.73 ± 17.27 362 | 40 8 ± 8.38 16 |
| Haementeria sp2. | 12.5 25.33 ± 10.63 76 | 7.69 10 ± 3.87 20 | 0 |
| Monogenea | | | |
| Polystomatidae | | | |
| Polystomoides brasiliensis | 66.66 5.43 ± 5.25 87 | 61.53 7.12 ± 7.04 114 | 40 1* 2 |
| Total | 87.5 31.57 ± 28.07 663 | 92.3 23.83 ± 19.68 572 | 60 9.33 ± 7.02 28 |

*Values referring to the intensity of total infestation
The monogenea *P. brasiliensis* presented high prevalence in both hosts. *Polystomoides brasiliensis* was described from specimens that were parasitizing *Hydromedusa maximiliani* and *P. geoffroanus* in the municipality of Juiz de Fora, Minas Gerais, Brazil (Vieira et al. 2008). In the present study, we describe the first record of *P. brasiliensis* parasitizing *M. tuberculata* and considerably increasing the distribution of this parasite. Information on the life cycle of this group of parasites is scarce in the literature, especially for *P. brasiliensis*, which was the first monogenetic described in chelonians. The monogeneans Polystomatidae are found exclusively parasitizing chelonians, infecting various parts of the body such as the eyes, urinary bladder, and oral cavity (Du Preez and Lim 2000; Platt 2000; Ávila et al. 2010; Du Preez and Van Rooyen 2015; Domènech et al. 2016).

The leeches found in chelonians are normally observed occupying soft body parts favorable to fixation, such as the pleural cavity of the arms and legs, neck, tail, and even the mouth (Jacobson et al. 1989; Herbst 1994; Rodrigues and Silva 2015). According to Brites and Rantin (2004), the attachment of leeches mainly to the inguinal cavity is related to the presence of thinner skin in these areas of the body, probably favoring the attachment and feeding of leeches. Another advantage of attachment to the locomotor limb cavity may be related to the protection against desiccation and the friction with water flow (Ernst 1971; Hulse and Routman 1982; Brites and Rantin 2004).

Table 4  Values of prevalence *P* (%), infection intensity (IF), abundance (A), and spatial niche breadth (*B*) for each ectoparasite associated with freshwater turtles *Phrynops geoffroanus* (Schweigger, 1812) and *Mesoclemmys tuberculata* (Lüderwaldt, 1926) in areas of the Caatinga and the Atlantic Forest in northeast Brazil. The values next to the biome correspond to the total number of specimens with at least one ectoparasite. The absence of a parasite species is indicated by a hyphen

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<th><em>Phrynops geoffroanus</em> (Atlantic forest, <em>n</em> = 5)</th>
<th>Caatinga (Atlantic forest) (n = 60)</th>
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<td>Parasite</td>
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<td><em>Haementeria brasiliensis</em> sensu Cordero, 1937</td>
<td>50 56 ± 39.31 168 63.44 16.74 ± 21.59 988</td>
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<td><em>Helobdella cf. adiastola</em></td>
<td>16.66 51* 51 4.3 1.75 ± 0.49 7</td>
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<td><em>Haementeria sp1.</em></td>
<td>66.66 18 ± 20.67 72 – – – –</td>
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<td><em>Haementeria sp2.</em></td>
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<td>Monogenea</td>
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<td><em>Polystomatidae</em></td>
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<tr>
<td><em>Polystomoides brasiliensis</em></td>
<td>50 2 ± 1.3 6 29.03 2 ± 1.60 54</td>
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<td>Total</td>
<td>83.33 59.4 ± 36.12 297 64.52 17.48 ± 21.75 1049</td>
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<th><em>Mesoclemmys tuberculata</em> (Atlantic forest, <em>n</em> = 13)</th>
<th>Caatinga (Atlantic forest) (n = 35)</th>
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<td><em>Haementeria brasiliensis</em> sensu Cordero, 1937</td>
<td>6.66 1* 1 22.5 11.22 ± 8.29 101</td>
<td></td>
</tr>
<tr>
<td><em>Helobdella cf. adiastola</em></td>
<td>6.66 10 ± 2.7 10 10 5.5 ± 3.2 22</td>
<td></td>
</tr>
<tr>
<td><em>Haementeria sp1.</em></td>
<td>53.33 10.12 ± 9.67 81 85 22.02 ± 22.54 749</td>
<td></td>
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<tr>
<td><em>Haementeria sp2.</em></td>
<td>– – – –</td>
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<tr>
<td>Monogenea</td>
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<tr>
<td><em>Polystomatidae</em></td>
<td></td>
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<tr>
<td><em>Polystomoides brasiliensis</em></td>
<td>66.66 9.3 ± 8.94 93 60 4.58 ± 4.29 110</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>86.66 14.23 ± 15 185 87.5 30.8 ± 24.89 1078</td>
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</table>
curves its neck in the form of an “S” inside the carapace, forming fine folds in the skin of the neck providing favorable places for fixation of leeches (Ernst 1971; Brites and Rantin 2004). In a different way, P. geoffroanus and M. tuberculata are chelonians of the Pleurodira Suborder, horizontally curving the neck and presenting thicker skin in this region, which according to Brites and Rantin (2004) is what makes it difficult for leeches to affix themselves to this region of the body.

The most prevalent theories used to explain the variation of leech infestations among chelonians are related to the use of the microclimate by host chelonians, such as basking, which favors the desiccation and predation of leeches (Ernst 1971; McAluliffe 1977; Dodd Jr 1988), whereas the increased use of the substrate where leeches normally reside increases parasitism rates (Ryan and Lambert 2005; McCoy et al. 2007).

However, the use of the microclimate does not seem to explain all the variation of the infestations, and we need to consider other characteristics of the host that can also explain the variation in parasitism by leeches: the amount of soft tissue exposed for fixation and feeding of leeches (Brooks et al. 1990; Graham et al. 1997; McCoy et al. 2007), sex (Brooks et al. 1990; Graham et al. 1997; Tucker et al. 2005), reproductive phase (MacCulloch 1981; Dodd Jr 1988; Brooks et al. 1990), body size (Brooks et al. 1990; Graham et al. 1997; McCoy et al. 2007), and environmental variation (Sawyer 1986; Readel et al. 2008). Host body size is often considered a determinant of the abundance and richness of parasites (Poulin 2007; Poulin and Leung 2011; Kamiya et al. 2014). Normally, host body size has a positive correlation with parasite abundance, thus suggesting that larger hosts provide more space and can host a larger number of parasites (Brooks et al. 1990; Graham et al. 1997; McCoy et al. 2007; Poulin 2007; Bielecki et al. 2012). However, linear regressions between the size and abundance of ectoparasites in both host species were not significant, although the lowest rates were
recorded in juveniles regardless of the species, which corroborates the hypothesis that body size may influence rates of infestation. In the absence of a correlation between the size and abundance of ectoparasites, it is possible that in adults, other factors may be more influential on the variation in the abundance of ectoparasites, such as sex (Tucker et al. 2005; Brooks et al. 1990; Graham et al. 1997) and reproductive phase (MacCulloch 1981; Dodd Jr 1988; Brooks et al. 1990).

Among the different infestation rates verified in relation to sex, the highest infestations recorded in *P. geoffroanus* females may be slightly related to body size (Brooks et al. 1990; Graham et al. 1997; McCoy et al. 2007), considering that females are larger than males (Molina 1990; Moura et al. 2015). However, in the Uberabinha River in Minas Gerais State, Brazil, Brites and Rantin (2004) even registering larger females found that the males of *P. geoffroanus* presented a slightly higher number of leeches. However, the environmental conditions in the area of this study presented a strong degree of anthropization by contamination of the river both in urban and rural areas, which potentially influenced the parasitism rates obtained. In *M. tuberculata*, males showed the highest parasite indices; the behavior may be a stronger determinant of the infestation rates of males in this species, since males usually cover larger territories, thus increasing the chances of infestation (Brooks et al. 1990; Graham et al. 1997; Tucker et al. 2005).

Seasonal variations in leech parasitism rates are usually associated with behavioral responses of host turtle species to seasonal changes in the environment (Ernst 1971; Koffler et al. 1978; Graham et al. 1997). In a study in North America, Koffler et al. (1978) found that in *Clemmys insculpta*, leeches vary seasonally, with the lowest rates of infestation in the dry season, attributing the large decline of infestations during the summer to the reproductive behavior of the species, where *C. insculpta*, which is a semiaquatic species, remains for most of the dry period in the terrestrial environment basking and looking for food and places to nest.

In our study, we obtained an antagonistic result, where the dry period had the highest rates of infestation, both for *P. geoffroanus* and *M. tuberculata*. However, *P. geoffroanus* and *M. tuberculata* present exclusively aquatic behavior (Bonin et al. 2006), and during the dry season, many water bodies are reduced to small portions, thus concentrating the ectoparasites in a smaller area, potentiating encounters with hosts and raising the rates of infestation for the period. Another hypothesis is that the higher water temperatures are indicated as facilitators in the reproduction of leeches (Brites and Rantin 2004). In a study with the Hirudinea *Erpobdella octoculata*, Sawyer (1986) showed that water temperature plays an important role in the rate of incubation, growth, and sexual maturation, and during the reproduction period in leeches, the fecundity of *E. octoculata* increases with increasing water temperature, so the same could have occurred for the species of leeches recorded in our study.

Prevalence rates, infestation intensities, and ectoparasite abundances were lower in *P. geoffroanus* than in *M. tuberculata*. It is known that the freshwater chelonians that use the substrate at the bottom of water bodies usually have higher ectoparasite loads than other chelonians that use the water column more, since they end up emerging from the water more often (Ernst 1986; Brooks et al. 1990; Ryan and Lambert 2005), forcing detachment of host leeches to avoid desiccation and consequently reducing ectoparasite loads (Ernst 1971; MacCulloch 1981). This is probably the best hypothesis to explain the differences found in infestations of *P. geoffroanus* and *M. tuberculata*, despite the absence of behavioral information in natural environments of these two species. It is important to emphasize that presence of *M. tuberculata* has been recorded in small bodies of water, especially in the dry season, which makes it more likely to be in contact with the substrate, so we suggest that the use of microhabitats by these chelonians may influence the ectoparasite rates.

The largest spatial niche breadths found in leeches in *M. tuberculata* are probably related to the high infestation rates presented by this host, which suggests that it was the result of a possible competition for spatial resources between leech species. Theoretically, if two or more species use the same limiting resource, this may induce species to share this resource through the use of different categories, thus ensuring the coexistence and maintenance of populations (Vitt 1981; Giacomini 2007). The processes that act to reduce niche overlap are known as stabilizers (Tilman 1990). Thus, we verified that this mechanism of widening of the niche breadth in the leeches occurs as an ecological adjustment minimizing the competition to assert the coexistence of these ectoparasites.

Regarding the biomes, we observed differences in infestation levels, and in *P. geoffroanus*, the Atlantic Forest specimens showed higher rates of infestation when compared to the Caatinga, whereas for *M. tuberculata*, the Caatinga presented higher infestation rates. Considering the interaction between the biome and the season, this significant difference in the total abundance of ectoparasites was also verified. Variations in the characteristics of the habitats where the chelonians were collected can directly influence the patterns of abundance and diversity of ectoparasites (Sawyer 1986; César et al. 2009; Gullo 2014). Yet, many Hirudinea are sensitive to different forms to anthropic influences (Christoffersen 2007), and some can be considered tolerant to the disturbances derived from anthropization (Miserendino and Brand 2009). Thus, differences in the levels of infestation between the biomes may be a reflection of the distinct ecological factors in the biomes.
differences in the behavioral patterns of the hosts, and the different anthropic changes occurring in the biomes.

The results of this study show the importance of the use of freshwater turtle populations to investigate the general trends in host-parasite dynamics. The relationship of environmental characteristics and host conditions that influenced ectoparasitism in *P. geoffroanus* and *M. tuberculata* provides baseline data for further analysis of parasitism, thus fostering future studies of how these parasites may influence the health, physiology, and behavior of the chelonians.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

**Appendix 1. Voucher list**

The reference specimens can be found at Collection of Hirudinea of the Federal Rural University of Pernambuco (CH-UFRPE) under numbers *Haementeria brasiliensis* sensu Cordero, 1937: (CH-UFRPE29; CH-UFRPE31; CH-UFRPE39; CH-UFRPE41; CH-UFRPE47; CH-UFRPE48; CH-UFRPE49; CH-UFRPE50), *Helobdella cf. adiastola*: (CH-UFRPE32; CH-UFRPE40), *Haementeria sp1.:* (CH-UFRPE35; CH-UFRPE36; CH-UFRPE37; CH-UFRPE38; CH-UFRPE46), *Haementeria sp2.:* (CH-UFRPE44; CH-UFRPE45).

**Appendix 2. Diagnosis of leech species**

*Haementeria brasiliensis* sensu Cordero, 1937

**Diagnose:** One pair of circular eyespots; paramarginal dark spots absent on ventral and dorsal surfaces; 7–9 longitudinal rows of inconspicuous tubercules on dorsal surface; Lang’s organs absent; gonopores separated by one annulus; mid-body somite trianulate, {[(a1) + (a2)] + (a3)}; diffuse salivary glands; 2 pairs of compact salivary glands; 2 pairs of mycetomes, connected to esophagus by independent ducts; 7 pairs of gastric caeca; 6 pairs of testisacs.

**Remarks:** *Haementeria brasiliensis* sensu Cordero, 1937, was originally described in *Oligobdella* and transferred to *Hamenteria* by Oceguera-Figueroa (2012). However, the author did not account for *H. brasiliensis* sensu Weber, 1915, creating a synonym problem.

*Haementeria sp1.*

**Diagnose:** One pair of eyespots; semi-circular pigmented area on the nuchal region; one pair of paramarginal dark spots on dorsum and venter of each a2; 9 longitudinal lines of large tubercles; 1 pair of Lang’s organs on a2; gonopores separated by 1 primary annulus; mid-body somites primarily trianulate, {[(a1) + (a2) + (a3)]}; diffuse salivary glands; 2 pairs of spherical bacteriomes; 7 pairs of gastric caeca, first pair with an anterior ramification, last pair forming a ramified post-caeca; pre-atrial loop present; 6 pairs of testisacs.

**Remarks:** specimens found on this study are morphology similar to *Placobdella* species in Ringuette (1985a) and Cordero (1937). However, many studies consider *Placobdella* to be restricted to North America, except for *P. costata*, in Europe (Oceguera-Figueroa 2012; Siddall et al. 2005). Specimens were identified using the proposed characters in Oceguera-Figueroa (2012).

*Haementeria sp2.*

**Diagnose:** One pair of circular eyespots; no metameric pattern of pigmentation; 4 longitudinal rows of tubercules on the dorsal surface; gonopores separated by two primary annuli; mid-body somite trianulate, {[(b1 + b2) + (b3 + b4) + (b5 + b6)]} on dorsal surface, {[(b1 + b2) + (a2) + (b5 + b6)]}; 2 pairs of compact salivary glands; 2 pairs of mycetomes, connected to esophagus by independent ducts; 7 pairs of gastric caeca; 6 pairs of testisacs.

**Remarks:** this species resemble *Haementeria lutzi* and *Haementeria depressa*, but the position of tubercules and details on the internal morphology do not allow a precise identification of this species. Further studies are needed to understand the taxonomic status of this species.

*Helobdella cf. adiastola* Ringuette, 1972

**Diagnose:** One pair of eyespots, close to each other; no metameric pattern of pigmentation; no tubercles or papillae on the dorsal and ventral surface; nuchal scute on the dorsal surface; gonopores separated by 1 annulus; mid-body somite trianulates, {[(a1) + (a2) + (a3)]}; diffuse salivary glands; bacteriomes absent.

**Remarks:** *H. adiastola* is morphologically similar to scute-bearing species from North America and Europe (Saglam et al. 2018). *Helobdella stagnalis* was also reported in South America, but recent studies have suggested that this species is restricted to North America. Therefore, Iwama and Arruda (2016) suggested that the name *H. adiastola* should be used for South American scute-bearing specimens.

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Gullo BS (2014) Biodiversidade de Hirudinae em ambientes dulceacuícolas serranos (Provincia de Buenos Aires), Argentina. Rev Mus La Plata (Zoo) 23:11–18


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