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Leech Infestation Patterns between Native and Invasive Freshwater Turtles: Implications for Invasion Success

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The increasing global introduction of alien species in recent decades underscores the need to understand the factors driving their establishment and spread in order to mitigate their ecological impacts. As alien species often thrive due to the absence of natural enemies (*e.g.*, parasites), we investigated leech parasitism in freshwater turtles, focusing on an assemblage with over 10 years of co-occurrence between native species (*Phrynops geoffroanus*, *Hydromedusa tectifera*) and invasive sliders (*Trachemys dorbigni*, *Trachemys scripta elegans*). We used traps to capture 62 turtles to assess host characteristics (species, sex, body size, and body region) that influence leech prevalence and infestation intensity in southern Brazil. Our findings revealed that native turtles exhibited an 18-fold higher prevalence of hematophagous leech than invasive species, with *T. dorbigni* being the only species in which no leech infestation was observed. Infestation intensity also varied among species, with native turtles harboring more leeches. robability of leech infestation increased with

body size, and the hind limbs were significantly more infested. The diminished presence of ectoparasites on invasive *Trachemys* throughout this coexistence period supports the enemy release hypothesis, suggesting that the lack of natural enemies may be a potential driver facilitating their invasion success. These results provide baseline data for future studies exploring key factors in the success of the slider invasions.

Keywords: Aquatic biodiversity, Host-parasite interaction, Invasive species, Parasitism, Testudines

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BACKGROUND

In the context of climate change and the ongoing loss of biological diversity, Brazil remains a globally recognized biodiversity hotspot (IUCN 2024). The introduction of invasive alien species, the second leading cause of biodiversity loss (Pyšek and Richardson 2010), poses a significant threat to these ecosystems. With over 500 alien species with invasive potential across Brazil and significant gaps in knowledge regarding their population dynamics and impacts on native species (Zenni et al. 2024), understanding the mechanisms driving the success of these species is increasingly crucial for mitigating their impacts (Ricciardi et al. 2017).

The absence of natural enemies, such as competitors, predators, pathogens, and parasites, influences species' success when introduced into new areas (Enders et al. 2018). For example, the enemy release hypothesis predicts that an alien species introduced to a new area leaves the old enemies as pathogens and predators, which evolved in their natural range. The center of this hypothesis is that natural enemies play an essential role in regulating populations (Roy et al. 2011), a proposition supported for some alien species (*e.g.*, Hartshorn et al. 2022; Wolfe 2002), and uncorroborated or partially accepted for others (*e.g.*, Colautti et al. 2004; Paula et al. 2021).

In aquatic and semiaquatic reptiles, hematophagous leeches (Hirudínea) are among the most common ectoparasites (Brites and Rantin 2004; Readel et al. 2008). This infestation can trigger their hosts' anemia and favor fungal and bacterial infections in lesions caused by the parasite's buccal structure (Brites 2002; Trivalairat et al. 2023). In chelonians, leeches are also vectors of hemoparasites, such as *Haemogregarina* sp. and *Trypanosoma* sp., and may transmit these parasites both intra and interspecifically (*e.g.*, Brites and Rantin 2004; Soares et al. 2014; Goes et al. 2018). Preliminary observations in the study area confirmed that hematophagous leeches parasitize

freshwater turtles, and the infestation seems to differ between native and alien chelonians (Lima 2021).

Here we aim to examine the potential role of the enemy release hypothesis as a driving mechanism behind the invasion of *Trachemys dorbigni* and *Trachemuys scripta elegans*. To attempt this aim, we took a quantitative and comparative assessment of hematophagous leeches parasitizing native and invasive freshwater turtles in southern Brazil. Specifically, we conducted analysis to determine leeches' prevalence and infestation intensity in native and invasive hosts, as well as verified if there are more invasive sliders than native turtles in this assemblage. We predict invasive sliders are more abundant and present lower ectoparasite infestation since they are alien species and suffer less impact from natural enemies (Enders et al. 2018). Additionally, we explore additional factors potentially influencing leech infestation in all parasitized turtles, including the relationship with host sex, body region (*i.e.*, anterior and posterior), and body size. We expect that sex and host size (carapace length) influence leech infestation, with more females parasitized (larger than males), considering they have a greater area available for colonization by ectoparasites (Readel et al. 2008). Finally, the posterior region of turtles is expected to exhibit a higher leech infestation because this site offers more protection from desiccation and predators (McCoy et al. 2007).

MATERIALS AND METHODS

Study area and target species

The study site included a complex of open-water lakes in an urban remnant of Atlantic Forest hotspot in Paraná state, southern Brazil (23°25'S, 51°55'W). This fragmented forest is part of a designed Conservation Unit and functions as a public park. Hydrologically, the waterbodies are connected by a canal that flows downstream; however, this channel serves as a barrier to aquatic fauna movement, restricting their dispersal beyond the park boundaries (for more details, see Rocha et al. 2025).

We focused on four turtle species (Fig. 1), two native *Hydromedusa tectifera* Cope, 1970 and *Phrynops geoffroanus* (Schweigger 1812), and two invasive species, *Trachemys dorbigni* (Duméril and Bibron 1835) and *T. scripta elegans* (Wied 1838). Although *T. dorbigni* is indigenous to Brazil, their presence in Paraná lies beyond their natural distribution range (Guedes et al. 2023). Both alien taxa are commonly traded as pets and are currently among the most popular freshwater turtle kept in captivity in Brazil (Alves et al. 2019). Their introduction is likely the result of owner releases, as recorded in many Brazilian states (*e.g.*, Ciccheto et al. 2018; Santos et al. 2020; Fonseca et al. 2021)

and other regions worldwide (*e.g.*, Thomson et al. 2010; Alcalde et al. 2012; Taniguchi et al. 2017). Owing to their successful establishment and spread, the two congeneric sliders are classified as invasive exotic species in Brazil (Zenni et al. 2024). While *T. dorbigni* has demonstrated invasive potential throughout the Americas (Fonseca et al. 2021), *T. scripta* is recognized as one of the world's top 100 worst invasive species (GISD 2024).

Fig. 1. Freshwater turtles sampled: A, *Hydromedusa tectifera*; B, *Phrynops geoffroanus*; C, *Trachemys scripta elegans*; D *Trachemys dorbigni*. C and D are invasive alien species.

Sampling

The fieldwork was carried out in two sessions, both in summer, during nine consecutive days between 08:00 and 17:00 h. The first session was between February 14 to 22, 2022, and the second was between February 01 and 09, 2023. We used two trapping methods simultaneously: I) eight funnel traps baited with chicken gizzard and sardine (1 m long × 0.50 m external diameter × 0.25 m entrance diameter), maintained in operation for eight hours per day and checked every four hours, set near and parallel to the margins, and distant 20 meters from each other; II) four devices of hookless fishing with clip (Rocha et al. 2024), baited with chicken gizzard, in operation during four hours per day, and distant 10 m apart from each other. The sampling effort for funnel trap amounted to 576 hours per session, totaling 1.152 hours of sampling, while for hookless fishing, it was 144 hours per session, with a total of 288 hours.

After being captured, we transported the chelonians to the Ichthyoparasitology laboratory at the Universidade Estadual de Maringá, separated into different wet bags. Then, we marked the turtles using unique numbers glued to the carapace and through a combination of notches in the marginal scutes (Cagle 1939). Once the mark was established, we counted and removed all leeches attached to the turtles. All ectoparasites were fixed in 70% alcohol and identified to the lowest taxonomic level possible (Sawyer 1972). Finally, we measured the curvilinear carapace and plastron (length and width) and determined sex based on secondary sexual traits (*e.g.*, tail length, cloaca position; Rueda-Almonacid et al. 2007). After these procedures, turtles were released at the same site where they were captured.

Data analysis

We employed Chi-square analysis in the R statistical computing environment (R Core Team 2024) to compare i) the total number of individuals captured of invasive and native species, ii) the total number of leeches (infestation intensity, Bush et al. 1997) recorded between sexes (male and female) and turtle body region (anterior and posterior). Due to the small number of parasitized individuals for some turtle species (*i.e.*, *H. tectifera* and *T. scripta elegans*), we compared leech infestation among species using a Kruskal-Wallis test. Finally, we performed a logistic regression analysis in R to evaluate the relationship between the prevalence of leech infestation (presence/absence) and turtle body size. Four individuals were recaptured during the sampling period, and all were parasitized at each capture. However, each capture event was treated as an independent observation in the analyses, as all leeches were removed from the turtles during their captures.

RESULTS

We captured 62 turtles, comprising 31 *P. geoffroanus*, one *H. tectifera,* 15 *T. dorbigni*, and 15 *T. scripta elegans.* There was no significant difference in the number of turtles captured between native and invasive species ($\chi^2 = 0.06$, *d.f.* = 1, $p = 0.79$). Among all turtles, 19 were found to have hematophagous leeches (*Placobdella* sp.) attached to their bodies, representing a prevalence of 30% on the assemblage. Both native and invasive species were infested by leeches, with different prevalence.

Native turtles exhibited a higher prevalence of leeches (56%) in contrast to invasive taxa (3%) (Fig. 2). Additionally, leech infestation varied significantly among turtle species (H = 71.047, *d.f.* =

2, $p < 0.001$) (Table 1). Of the parasitized individuals, 89% were *P. geoffroanus* ($n = 17$, ♀ = 4, ♂ = 12, juvenile = 1), representing over half of all individuals of this species captured, with infestation intensities ranging from 1 to 96 leeches per turtle. The single individual captured by *H. tectifera* (male) displayed the most severe leech infestation, with over 300 ectoparasites on its body and apparent weakness. Concerning the invasive species, only *T. s. elegans* showed parasitism, with one individual (female) parasitized by a single leech.

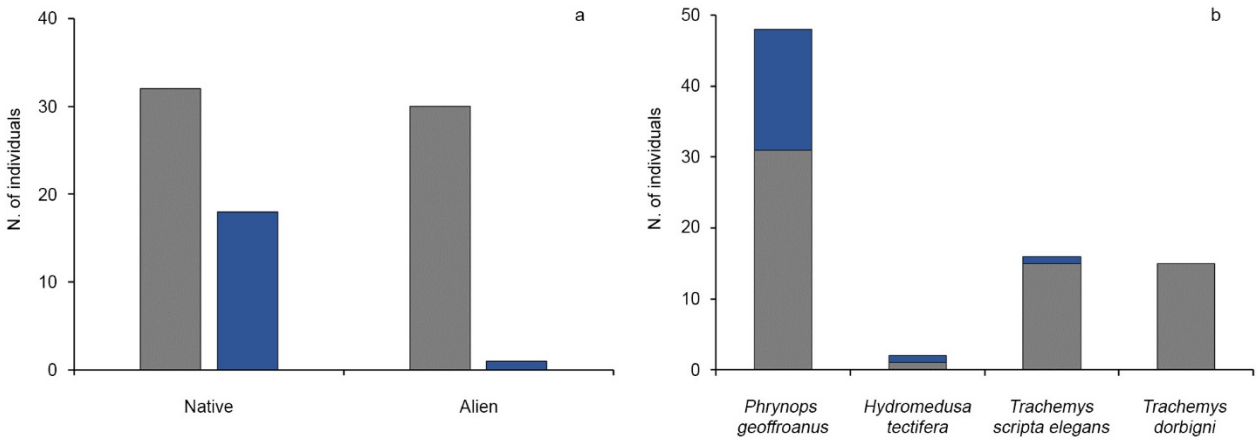

Fig. 2. Leech parasitism in a freshwater turtle assemblage after over 10 years of co-occurrence between native and invasive species. Gray bars represent the total number of turtles sampled, while orange bars indicate those parasitized by leeches (*Placobdella* sp). (a) Comparison between native and invasive alien species groups; (b) Distribution of parasitism among each turtle species. The invasive species include *Trachemys scripta elegans* and *Trachemys dorbigni*, whereas native species are *Phrynops geoffroanus* and *Hydromedusa tectifera*.

Table 1. Freshwater turtles parasitized by the Leech *Placobdella* sp. in Atlantic Forest in southern Brazil. The alien species are in gray. Number of turtles captured (N), Parasitized (PA), values of Prevalence (P) and Infestation intensity (IF ± SE) are presented for each turtle species. A hyphen indicates the absence of parasites

Turtle species	N	PA	P (%)	IF
Hydromedusa tectifera	1	1	100	378
Phrynops geoffroanus	31	17	54	9 (± 3.6)
Trachemys dorbigni	15	-	-	-
Trachemys scripta elegans	15	1	6	1

Among all species sampled, we recorded a similar number of males ($n = 25$) and females ($n = 23$), with fewer juveniles ($n = 14$). The prevalence of leeches in males was 52% ($n = 13$), in females was 22% ($n = 5$) and in juveniles was 7% ($n = 1$) (Table 2). However, no significant difference in leech infestation intensity was found between males and females ($\chi^2 = 3.55$, *d.f.* = 1, p = 0.05; Fig. 2a). Concerning the distribution of leech infestation on turtle bodies, leeches were found in both the anterior (29%; $n = 181$) and posterior (71%; $n = 443$) regions, with significantly higher infestation intensity in the cavities of the hind limb of turtles ($\chi^2 = 110.01$, *d.f.* = 1, $p < 0.01$; Fig. 2b).

Moreover, our results indicate that the probability of leech infestation increased significantly with body size for both, males (β = 0.67, p = 0.03, odds ratio = 1.97, 95% CI = 1.27–4.59) and females (β = 0.31, p = 0.03, odds ratio = 1.37, 95% CI = 1.07–2.01; Fig. 3).

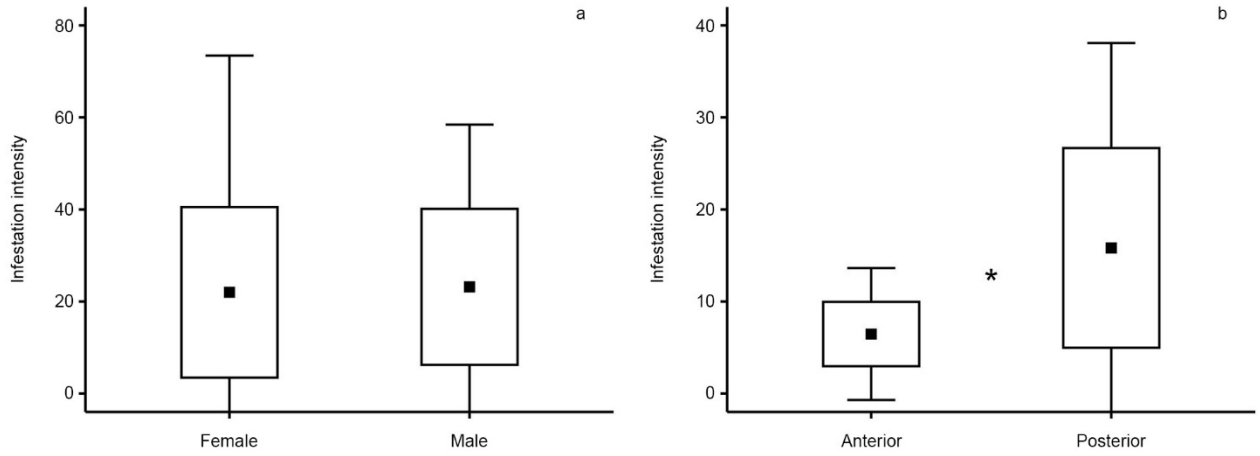

Fig. 3. Boxplots showing the intensity of leech infestation in freshwater turtles by (a) body region (anterior and posterior) and (b) sex (females and males). Black squares indicate mean leech counts, boxes represent standard errors, and whiskers denote 95% confidence intervals. An asterisk between boxes indicates a statistically significant difference between groups (p < 0.05). Lower bounds of 95% confidence intervals were truncated at zero when calculations yielded negative values. One outlier (n = 378 leeches in the posterior region) was excluded from the plot but was included in all statistical analyses.

Table 2. Summary of statistical analyses examining leech parasitism in freshwater turtles. Values are presented as mean ± standard error (SE) and 95% confidence intervals (CI)

Response variable	Predictor	Group	n	Mean ± SE	95% CI	Test	p-value
Prevalence	Body size (mm)	Females	23	0.22 ± 0.08	1.07–2.01	Logistic regression	*
		Males	25	0.52± 0.09	1.27–4.59		*
Infestation intensity	Sex	Females	5	22 ± 18.52	-29.43–73.43	Chi-square	0.05
		Males	13	23.18 ± 16.94	-12.06–58.42		
	Body region	Anterior	56	6.46 ± 3.49	-0.70 –13.63	Chi-square	***
		Posterior		15.8 ± 10.84	-6.43 – 38.07		

n, indicates sample size. (*) for $p \leq 0.05$, (***) for $p \leq 0.001$.

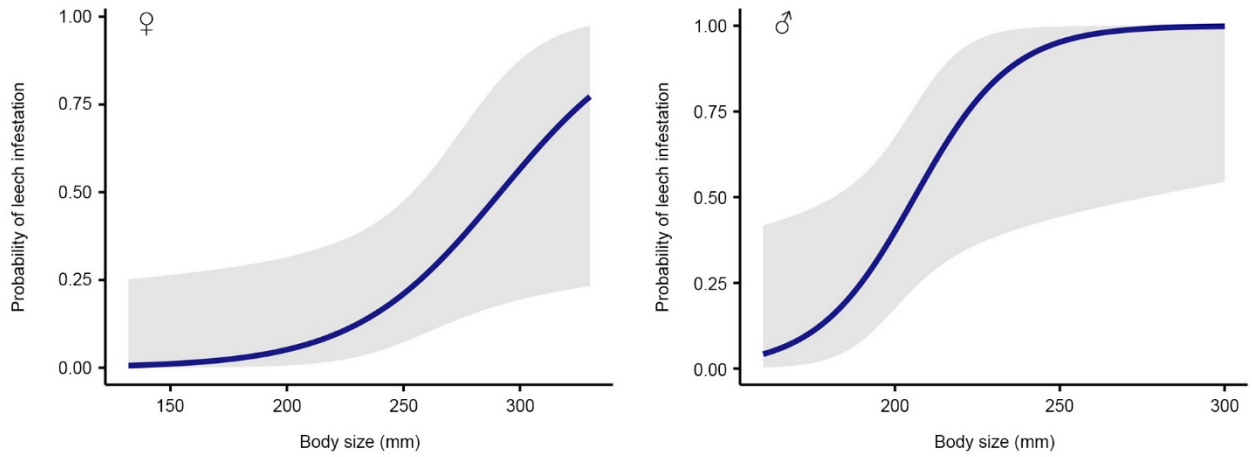

Fig. 4. Logistic regression models showing the relationship between body size (mm) and the probability of leech infestation in freshwater turtles: (♀) females and (♂) males. Shaded grey areas represent the 95% confidence intervals around the predicted probabilities (blue lines).

DISCUSSION

Native turtles exhibited a higher level of leech parasitism than exotic taxa, likely attributable to parasite-host coevolution and specificity among hosts. In Brazil, this leech is frequently found parasitizing *P. geoffroanus* from urban areas (*e.g.*, *Placobdella bistriata,* see Brites and Rantim 2004). Previous study conducted at the same area (Lima 2021) recorded *Placobdella* sp. parasitizing *P. geoffroanus*, along with another species of leech (*Helobdella adiastola*). Conversely, the few preceding studies on ectoparasites of *H. tectifera* did not record *Placobdella* parasitizing this turtle (Lima 2021; Teixeira 2015); only *Helobdella* was found attached on their plastron and carapace (*H. adiastola* and *H. triserialis*). One record in Rio Grande do Sul state, Brazil, registered leeches from Rhynchobdellida order on *H. tectifera* without specification of the ectoparasite genus (Alcalde et al. 2021; Huckembeck and Quintela 2013). Both *Placobdella* and *Helobdella* belong to Rhynchobdellida; however, while *Placobdella* establishes a trophic relationship with turtles, *Helobdella* leeches do not (Marrone et al. 2016). Instead, they are commonly found in a symbiotic association with turtles for dispersal (Davy et al. 2009) and to access their prey, including other leeches (Perera et al. 2019; Richardson et al. 2017a b). Even though *Hellobdella* was recorded on some turtles, we did not include this data in our analysis under the assumption that they do not feed on turtle blood and, therefore, cause no deleterious effects to the turtle species. Although *P. geoffroanus* accounted for most parasitized individuals, the observed association between body size and leech prevalence, as well as the higher infestation intensity in the posterior body region, highlights the influence of host traits on parasite–host interactions. These factors, along with

anthropogenic pressures and ecological disturbances, are expected to shape the dynamics of this association (Readel et al. 2008; Brooks and Hoberg 2007; McCoy et al. 2007).

The low number of *H. tectifera* individuals captured was consistent with previous reports, given the declining population trend in the area (Grou et al. 2024, $n = 5$; Rocha et al. 2025, $n = 2$). Nevertheless, data obtained from a single individual still informative considering the scarcity of information on ectoparasitism in this species. The high ectoparasite infestation intensity in *H. tectifera* may be attributed to their bottom-dwelling behavior and the absence of aerial basking activity (Readel et al. 2008; Ryan and Lambert 2005; Santana et al. 2019). Leeches of this genus is well known for parasitizing freshwater turtles (Neely et al. 2020), but they often select hosts less prone to basking, reducing their risk of desiccation (Ryan and Lambert 2005). As *H. tectifera* exhibits crepuscular-nocturnal habits and does not bask aerially (Molina and Leynaud 2017), this turtle's high infestation of leeches is expected despite never being recorded. Importantly, the combination of intense parasitism and the potential competitive disadvantage faced by this native species against invasive sliders likely contributes to its ongoing population decline, particularly within this closed system, where no dispersal routes are available.

In its original distribution range, *T. scripta* is commonly parasitized by the same leech genus, *Placobdella* (*P. parasitica*; Neely et al. 2020; Unger et al. 2019). However, in a previous study conducted in the same area (Lima 2021), there were no records of *Placobdella* on *T. scripta elegans*; only *Helobdella diastola* was recorded on both invasive species, *T. scripta elegans* and *T. dorbigni*. The lack of ectoparasite studies on turtles species in Brazil (*i.e.*, leeches) contributes to the limited knowledge about this association. In Eastern Europe, for instance, where *T. scripta* was introduced and coexisted with indigenous turtles, there are records of both native leech infestation and infection by local haemogregarine parasites in *T. scripta* (Maričić et al. 2023). There are also records of host-switching endoparasites from *T. scripta* to native species in the Mediterranean region (Meyer et al. 2015). Therefore, additional studies are needed to evaluate the parasite-host association in both native and non-native species within a tropical context, which is essential to address the impact of aliens on native turtles.

In addition to the extremely low parasitism observed in alien species, the difference in infestation between the two congeneric invaders may be linked to differences in their duration of exposure to local parasites. *Trachemys scripta elegans* appears to have coexisted with these parasites for a longer period than *T. dorbigni*. *Trachemys scripta* became the most popular turtle in the pet trade between 1989 and 1997 (GISD 2024) and was subsequently banned in Brazil in 1998 (IBAMA Ordinance n. 93, July 7, 1998), despite ongoing illegal trade within the country. In contrast, records of *T. dorbigni* in the Brazilian pet trade date back to the 1990s, while records beyond their original distribution range have only emerged in recent decades (since 2000), as well

as occurrences of hybridization between these two congeneric species (Tortato et al. 2014; Figueiredo 2014; Santos et al. 2020). This temporal difference may have facilitated a greater integration of local parasites by *T. scripta elegans*, whereas *T. dorbigni* still in early stages of spread, may not yet have established interactions with native ectoparasites.

The long-term persistence and increasing predominance of both *Trachemys* species in this urban freshwater system, particularly over the past decade (20% increase over an eight-year monitoring period; Grou et al. 2024), may be facilitated by their reduced parasite load. Nevertheless, few studies have examined parasitic interactions involving these assemblages. The combination of behavioural advantages previously reported for *T. scripta* over native species (Cadi and Joly 2003; Polo-Cavia et al. 2010, 2011; Pearson et al. 2015; Taniguchi et al. 2017; Lambert et al. 2019), its recognized threat to native turtles such as *P. geoffroanus* and *H. tectifera* (Martins et al. 2014), and the apparent release from parasite pressure may enhance their ecological performance and contribute to their competitive success. This highlights the potential role of parasitism, or its absence, as an overlooked but ecologically relevant mechanism in the invasion process of freshwater turtles.

CONCLUSIONS

Our findings revel the absence or scarcity of ectoparasites in invasive *Trachemys* sliders, despite decades of their introduction and prolonged exposure to new environmental conditions, supporting the enemy release hypothesis as a potential driver behind their invasion success. In contrast, the native *P. geoffroanus* exhibited high levels of leech infestation, suggesting a possible parasite-mediated disadvantage in environments increasingly dominated by alien species. Although *H. tectifera* was represented by a single individual, its intense parasitism, combined with competitive pressure from invasive sliders, underscore the need for further investigation into the vulnerability of more specialized native taxa. Overall, our results emphasise the importance of parasite-host dynamics in understanding invasion processes, particularly in urban environments where biological invasions are increasingly common.

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