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Tadpoles of *Boana geographica* (Spix, 1824) (Anura: Hylidae) parasitised by *Argulus* sp. (Branchiura: Argulidae) in a Central Amazonia forest stream

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Boana geographica (Spix, 1824) is an arboreal and nocturnal anuran that reproduces throughout the year in the Amazon. Females use ponds and stream backwaters to deposit about 2000 black eggs, from which conspicuous black and unpalatable larvae hatch (Caldwell, 1989). The tadpoles form large (up to 2000–3000 individuals) schools that occupy the upper layer of the water column, where they stay close to each other (Caldwell, 1989; Lima et al., 2008). However, gregarious behavior can bring costs to organisms, such as increased parasite infestation risk (by environmental contagious or contact-transmitted) (Alexander, 1974; Moller et al., 1993). Besides, physical injuries caused by parasites like skin lesions can further increase the risk of secondary infection by other parasites or pathogens, negatively affecting the fitness or even the survival of the host (Kabata, 1970; Lehmann, 1993; Loehle, 1995; Varga et al., 2019).

Argulid crustaceans (Branchiura) present several apomorphic characters in its appendages that are supposed to be very efficient to a parasitic life (Malta, 1983; Moravec et al. 1999). The genus *Argulus* Müller, 1785 contains 151 described species, of which 132 are currently considered as valid species (Walter and Boxshall, 2020). *Argulus* species are ectoparasites that are commonly found in freshwater and marine fishes, but that also occasionally parasitise amphibians (Stuhlmann, 1981, Poly, 2003; Piasecki and Avenant-Oldewage, 2008; Tanzola and Villegas-Ojeda, 2017), reptiles (Ringuelet, 1943), and aquatic invertebrates (Jackson and Marcogliese, 1995). Among the amphibians, species of *Argulus* have been found parasitising Caudata (salamanders) and Anura (frogs) but with few records of anuran larvae as hosts (Table 1).

In this work we report the occurrence of an undescribed species of *Argulus* (in process of description) parasitising tadpoles of the hylid anuran *Boana geographica* (Fig. 1). The parasites were identified by one of us (JCOM, an experienced taxonomist in ectoparasitic crustaceans) based on morphological characteristics (morphology of

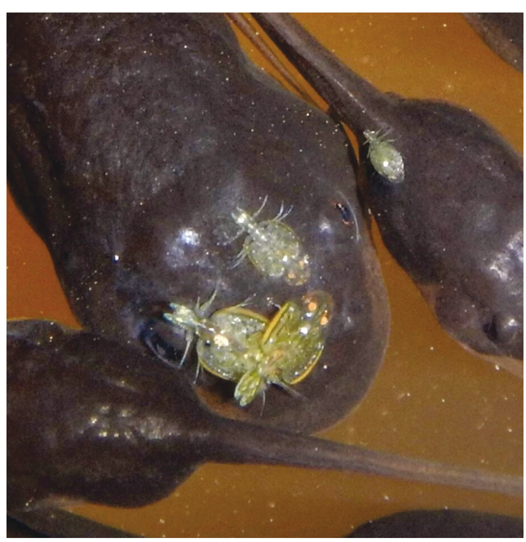

Figure 1. Tadpoles of *Boana geographica* parasitised by *Argulus* sp. in a Central Amazon forest stream.

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Table 1. Records of *Argulus* species (Branchiura) as parasites of Amphibia (adults and larvae). * Detected in the host only in 1969; ** provided for description in 1996, receiving in 2003 the name *A. ambystoma*; *** information unknown.

Parasite	Host	Locality (country)	Year	Reference
Argulus foliaceus (Linnaeus, 1758)	*Lissotriton vulgaris* (Linnaeus, 1758)	UK	1938	Bower-Shore, 1940
Argulus americanus Wilson, 1902	*Pseudobranchus s. axanthus* Netting and Goin, 1942	USA	1939	Goin & Ogren, 1956
Argulus sp.	*Ambystoma dumerilii* (Dugès, 1870)	MX	1968*	Poly, 2003
Argulus ambystoma Poly, 2003	*Ambystoma dumerilii*	MX	1981**	Poly, 2003
Argulus sp.	*Pseudobranchus striatus* (LeConte, 1824)	USA	1995	Wolfe et al. 2001
Argulus foliaceus / (Monoculus gyrini) (Cuvier, 1798)	Unknown species	***	1798	Wilson, 1902
Argulus americanus Wilson, 1902	*Lithobates heckscheri* Wright, 1924	USA	1955	Goin & Ogren, 1956
Argulus sp.	Unknown species	BRA	1980	Malta & Varella, 2009
Argulus hylae- nomen nudum Lemos de Castro & Gomes-Correa, 1985	*Boana geographica* Spix, 1824	BRA	1985	Lemos de Castro & Gomes-Correa, 1985
Argulus sp.	*Lithobates heckscheri*	USA	1995	Wolfe et al. 2001
Argulus americanus	*Lithobates heckscheri*	USA	1999	Clark, 2001
Argulus diversus Wilson, 1944	*Lithobates heckscheri*	USA	1999	Clark, 2001
Argulus ventanensis Tanzola & Villegas-Ojeda, 2017	*Boana pulchellus* (Duméril & Bibron, 1841)	ARG	2017	Tanzola & Villegas-Ojeda, 2017
Argulus foliaceus	Unknown species	***	***	Sauer, 1977

the carapace, buccal apparatus, antennae, antennules, legs, and other anatomical details). The occurrence was observed in a partially dammed stretch of a *terra firme* (non-floodable) forest stream inserted in an old-growth secondary forest area of Dimona Farm, one of the study sites of the Biological Dynamics of Forest Fragments Project (BDFFP) of the Instituto Nacional de Pesquisas da Amazônia (INPA), Amazonas State, Brazil (2.3904°S, 60.1708°W). *Argulus* was not found parasitising any other vertebrate host at the study site.

Part of the tadpoles schools (n = 100) were randomly collected with a hand-net, taken to the laboratory at INPA and separated according to size (following the stages proposed by Gosner, 1960), individually measured (Total Length, in millimeters) and inspected for the presence of ectoparasites The parasitological indices: prevalence (P%), mean abundance (MA) and mean intensity (MI) were calculated according to Margolis et al. (1982). After these initial observations, the parasitised tadpoles (n = 41) were separated in individual containers with water for the measurement of both tadpoles and its parasites, and parasites counting.

We used simple linear regressions to verify if the size of parasites was related to the size of the host tadpole, and if the number of ectoparasites was related to the size of tadpoles. We were confident in using the host's total length as the independent variable because all analysed tadpole hosts had intact tails. Specimens samplings and experimental research were carried out in accordance with animal welfare standards certified by INPA's Committee for Ethics in Research with Animals (permit CEUA # 054/2016) and authorised by SISBIO licenses # 40224-1; 40224-2; 40224-3.

The ectoparasite *Argulus* sp. (n = 61) was present in less than half of the tadpoles examined (P = 41%; n = 100), with a mean abundance of 0.61 (range: 1-6 parasites per tadpole) and mean intensity of 1.49 (range: 0–6 parasites per tadpole). Information on parasitic infestations are important functional aspects of anurans biodiversity and may potentially help revealing how each species interacts with its environment (Leivas et al., 2018). In the case of branchiuran ectoparasites, this information is especially important because of the scarcity of such records in the literature for amphibians. The current record represents a new occurrence of a species of *Argulus* parasitising tadpoles, and a new record of this branchiuran genus for streams of Central Amazonia, increasing the range of aquatic habitats occupied by these ectoparasites.

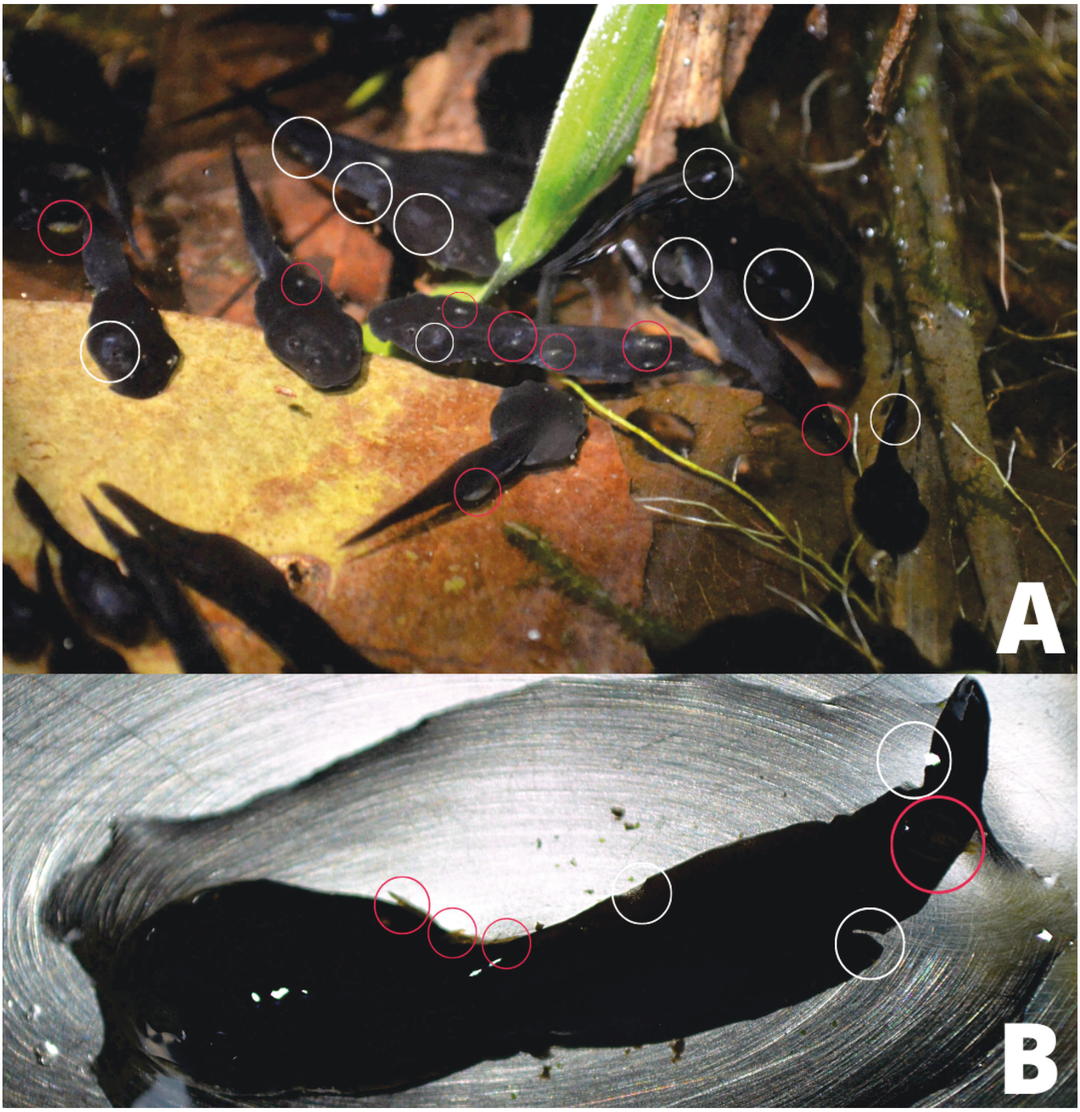

Figure 2. Parasitism by *Argulus* sp. A- The parasite *Argulus* sp. (red circles) and skin lesions caused by fungi (not identified) of the tadpoles of *Boana geographica* (white circles). B- Mechanical injuries caused by the exfoliation of the parasite *Argulus* sp. under the epithelium of the tadpoles of *Boana geographica.*

We have observed that the specialised structures for fixation of this parasite cause severe abrasion to the epithelium of tadpole hosts (observed in 56% of 41 parasitised tadpoles). The thin, permeable skin of anurans, which is particularly vulnerable to cutaneous lesions (Varga et al., 2019), may cause parasitised tadpoles to die either directly as a result of the mechanical injuries or from secondary infections caused by bacteria and fungi, besides other complications caused by *Argulus* on its hosts (Fig. 2A, B) (e.g., Kabata, 1970; Lehmann, 1993; Loehle, 1995; Moravec et al., 1999; Varga et al., 2019).

The argued unpalatability of the tadpoles of *B. geographica* (Caldwell, 1989) seems to be insufficient to prevent infestation by *Argulus* sp. However, it is possible that the association with these toxic tadpoles present some additional benefits to the ectoparasite. For instance, it is possible that associating to *B. geographica* tadpoles results in a reduction in the risk of parasite death by accidental predation by fish and other

Table 2. Fish species co-habiting the study site with *Boana geographica* parasitised tadpoles and examined for the presence of *Argulus* sp.

Fish species (Family)	Number of examined specimens
Apistogramma hyppolitae (Cichlidae)	83
Apistogramma agassizii (Cichlidae)	4
Crenuchus spilurus (Crenuchidae)	360
Copella nattereri (Lebiasinidae)	37
Nannostomus marginatus (Lebiasinidae)	55
Hoplias malabaricus (Erythrinidae)	3
Hemigrammus pretoensis (Characidae)	6
Hyphessobrycon aff. *melazonatus* (Characidae)	23
Helogenes marmoratus (Cetopsidae)	1

piscivorous animals, a hypothesis that could be tested experimentally.

There was no relation between the size of the tadpoles of *B. geographica* and of the ectoparasite *Argulus* sp. ($r^2 = 0.012$, $p = 0.396$, $n = 61$). This may be considered surprising, given that several studies have detected positive correlations between parasite and host body sizes (see Poulin, 1999, and included citations) because a larger host may offer a greater area of attachment to the parasites (Poulin, 1995). However, our results showed a weak positive relationship between the size of the host tadpole and the number of attached ectoparasites ($r^2 = 0.107$, $p = 0.018$, $n = 39$). This may represent a simple relationship to the host's body area, but it is also possible that larger tadpoles survive longer after infestation and resist better to the ill effects of parasitic energetic spoliation (Lehman, 1993 and included citations; Mikheev et al., 2015). Experiments on the relationship between the attachment time of *Argulus* sp. ectoparasites and the eventual death of their *B. geographica* tadpole hosts are under way and may help clarifying the effects of these parasitic infestations on the host's fitness.

The interaction recorded here between the branchiuran ectoparasite *Argulus* sp. and tadpoles of the anuran *B. geographica* is surprising, since the tadpole's lifetime up to its metamorphosis is relatively short (approximately 3 months under natural conditions; our pers. obs.). This indicates that the ectoparasite should have some survival strategy to deal with the loss of the tadpoles hosts after exiting from the aquatic environment. In order to check for possible alternative hosts in the study site we checked dozens of fishes of several species co-habiting the stream, but none was found parasitised by *Argulus* sp. (Table 2). So, if *Argulus* sp. is in fact a specific parasite of tadpoles of this anuran species it would be necessary the presence of host tadpoles in the study site throughout the year in order to allow the maintenance of the ectoparasite´s population. In this sense, the occurrence of reproductive activity of *B. geographica* across the seasons in the study area (our personal *in situ* observations across five years; 2015–2019) may have allowed the persistence of the association of the ectoparasite with its host species. Alternatively, the ectoparasite may be able to perceive that the host is about to metamorphose nearly leaving the water, then moving temporarily to another unknown aquatic host organism and returning to a tadpole host later. Studies in progress may help understanding the dynamics of this unusual parasite-host relationship in streams of the Brazilian Central Amazonia.

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