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Parasitism by Amblyomma rotundatum Koch, 1844 (Acarina: Ixodidae) on Rhinella marina Linnaeus, 1758 (Anura Bufonidae) in the Amazon Forest: geographic expansion across Madeira River

Host-parasite interactions between ticks and wild species are important for examining the ecology and distribution of ticks, as well as the consequences of these interactions for hosts and diseases. Ticks have medical importance because they are vectors of microorganisms and pathogens, such as viruses, bacteria, and protozoa, that can be transmitted to humans and other animals, causing serious disease. Like other anuran species, Rhinella marina also harbors a wide variety of parasites. This occurs mainly because of its large body size and because it occupies terrestrial environments, but also because it has direct contact with aquatic environments (larval and reproductive stages), thus presenting several opportunities for parasitic infections. This work has as main objective to describe the occurrences of host-parasite interactions between the species Rhinella marina (Linnaeus, 1758) and Amblyomma rotundatum (Koch, 1844) geographically expanding their occurrence in the Amazon rainforest through the Madeira River. During a herpetofauna survey, the first individual of R. marina was observed at 08:37 PM on September 20, 2020. The location is near a transect within a primary forest. This individual was parasitized by a single tick identified as Amblyomma rotundatum. On a second expedition, a second individual of R. marina was observed at 11:38 PM on January 5, 2021. The location is rural road C-01 in the municipality of Porto Velho-RO, which passes within a primary forest and deforested private properties. This individual was parasitized by nine ticks identified as Amblyomma rotundatum. An important observation to be made is that the occurrences of A. rotundatum parasitizing R. marina are 57 km apart and cross an important geographical barrier, the Madeira River of the Amazon basin. This work geographically expands the occurrence of A. rotundatum parasitism on R. marina and describes the natural histories of this host-parasite interaction of these species.

Keywords: Cane Toad; Tick; Amazon; Parasitism; Madeira River.

Parasitismo por Amblyomma rotundatum Koch, 1844 (Acarina: Ixodidae) na Rhinella marina Linnaeus, 1758 (Anura: Bufonidae) na Floresta Amazônica: expansão geográfica através do Rio Madeira

As interações hospedeiro-parasita entre carrapatos e espécies silvestres são importantes para examinar a ecologia e distribuição de carrapatos, bem como as consequências dessas interações para os hospedeiros e doenças. Os carrapatos possuem importância médica pois são vetores de microrganismos e patógenos, tais como vírus, bactérias e protozoários, que podem ser transmitidos a humanos e outros animais, causando doenças graves. Como outras espécies de anuros, Rhinella marina também abriga uma grande variedade de parasitos. Isso ocorre principalmente por seu grande tamanho corporal e por ocupar ambientes terrestres, porém também por possuir contato direto com ambientes aquáticos (fase larval e reprodutiva), dessa forma apresenta diversas oportunidades para infecções parasitárias. Este trabalho tem como principal objetivo descrever as ocorrências das interações hospedeiro-parasita entre as espécies Rhinella marina (Linnaeus, 1758) e Amblyomma rotundatum (Koch, 1844) expandindo geograficamente sua ocorrência na Floresta Amazônica através do rio Madeira. Durante um levantamento de herpetofauna, o primeiro indivíduo de R. marina foi observado às 08:37 PM do dia 20 de setembro de 2020. O local é próximo a um transecto dentro de uma floresta primaria. Este indivíduo estava parasitado por um único carrapato identificado como Amblyomma rotundatum. Em uma segunda expedição, um segundo indivíduo de R. marina foi observado às 23:38 PM do dia 5 de janeiro de 2021. O local é a estrada rural C-01 no município de Porto Velho-RO, que passa dentro de uma floresta primaria e de propriedades privadas desmatadas. Este indivíduo estava parasitado por nove carrapatos identificados como Amblyomma rotundatum. Uma observação importante a ser feita é a de que as ocorrências de A. rotundatum parasitando R. marina estão distante 57 km e ultrapassam uma importante barreira geográfica, o rio Madeira da bacia amazônica. Esse trabalho expande geograficamente a ocorrência do parasitismo de A. rotundatum em R. marina e descreve as histórias naturais dessa interação hospedeiro-parasita destas espécies.

Palavras-chave: Sapo-cururu; Carrapato; Amazônia; Parasitismo; Rio Madeira.

Topic: **Parasitologia**

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INTRODUCTION

Host-parasite interactions between ticks and wild species are important for examining the ecology and distribution of ticks, as well as the consequences of these interactions for hosts and diseases (HORTA et al., 2011). Ticks have medical importance because they are vectors of microorganisms and pathogens, such as viruses, bacteria, and protozoa, which can be transmitted to humans and other animals, causing serious diseases (MASSARD et al., 2004). This work has as main objective to describe the occurrences of host-parasite interactions between the species *Rhinella marina* (Linnaeus, 1758) and *Amblyomma rotundatum* (Koch, 1844) geographically expanding their occurrence in the Amazon rainforest through the Madeira River.

THEORETICAL REVIEW

Anurans of the genus *Rhinella* have a worldwide distribution, with greater frequency in regions with tropical and humid climates, being considered synanthropic, found in Central America, South America, and South Texas (North America). There are more than 200 species of this genus in the world, with *Rhinella marina* being the most studied (ROBERTS et al., 2000; MONTI et al., 1994; JARED et al., 2009; GADELHA et al., 2012).

Rhinella marina is a species with nocturnal habits and an active life in open environments, feeding predominantly on slugs, land snails, chilopods, grasshoppers, cockroaches, beetles, ants, and small rodents. Studies of the stomach contents of the species reveal that terrestrial gastropods constitute more than 40% of the diet of these individuals (HINCKLEY, 1963; BAILEY, 1976; GRANT, 1996). They constitute the class of vertebrate animals common in Brazil, ranging in size from 2 to 20 cm in length; protuberance above the eyes to the snout; a large paratid gland behind each eye; absence of teeth and interdigital membrane on the front legs; dry, warty skin; horizontal pupils and a golden iris. The species has no lower eyelid, but a nictitating membrane (FROST et al., 2006).

The introduction of this species outside its place of origin is a much-debated topic until today. The increasing number of *R. marina* in Australia, Fiji and Hawaii has had a significant impact on the number of native species in these regions, that is, according to experts, the invasion of this species has caused a reduction in the population of several resident predators such as crocodiles, snakes, marsupials, lizards and other anurans that are poisoned when trying to feed on these frogs (BURNETT, 1997; DOODY et al., 2006; GRIFFITHS et al., 2007; CROSSLAND et al., 2008; LETNIC et al., 2008).

Forest and Amazon Basin

The Amazon Forest is an extensive territory with more than 4.1 million square kilometers (km²), being present in nine countries in South America, and about 64% of this territory belongs to Brazil (VERÍSSIMO et al., 2014; CARVALHO et al., 2016). Possessing a vast biodiversity, relevant biomass, hot and humid climate with an expressive rainfall index of 2,000 ml/year, being the Amazon Forest a significant

contributor to the conservation of watersheds in the country (BRAGA, 1979; VERÍSSIMO et al., 2014).

As the largest hydrographic basin in Brazil and in the world, the Amazon basin extends over approximately 6 million km² and is present in six South American countries: Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, and Venezuela (ANA, 2015). The Amazon Hydrographic Region, according to the National Water Resources Council (BRASIL, 2003) is inserted in the Amazon River hydrographic region, however it is the Brazilian territorial part covering about 3,870 million km², present in seven states: Acre, Amazonas, Rondônia, Roraima, Amapá, Pará and northwest of Mato Grosso, standing out as the main rivers, which feed this network, the rivers Negro, Xingu, Madeira, Tapajós and Purus (ANTAQ, 2013).

The Madeira basin corresponds to about 23% of the total Amazon basin, with its main tributaries: Beni and Mamoré, with their Andean origin, are in Bolivia (SOUSA et al., 2001). The Madeira River with its approximately 1,420,000 km² of total area is twice as large as any other Amazon River basin (ANEEL, 2002). The Brazilian portion of this river is 580,000 km², having an average width of 1,000 meters (m), whose average depth oscillates, according to the dry and rainy seasons, between 2.80 m during the dry season and 8.20 m during the flood season, and can reach an admirable 13 m of depth in the seasons with higher rainfall (GOULDING, 1979; DNIT, 2018).

METHODOLOGY

We conducted field observations at the Military Police Jungle Base in the municipality of Porto Velho, state of Rondônia, Brazil, in the southwestern Brazilian Amazon. The site is close to a transect within a primary forest. This base is used as a training site for the local police. Figure 1 shows the total area of the base (outlined in red lines) and the exact location of the first individual of *R. marina* found.

Figure 1: Military Police Jungle Base/RO area.

Later, another expedition was conducted where a second individual of *R. marina* was observed. The site is a rural road called C-01. This road cuts through an area of primary Amazonian rainforest with some deforested private properties. Figure 2 shows the area around the road and the exact location of the second individual of *R. marina* found.

Figure 2: Road C-01 area.

The data were collected using the herpetology methodology of active search and occasional encounters in the years 2020 and 2021. The record was made with a Nikon model P900 camera. From the morphological analyses it was possible to identify the species using current taxonomic keys (ARAGÃO et al., 1961; ONOFRIO et al., 2006; MARTIN et al., 2010).

RESULTS AND DISCUSSION

During a herpetofauna survey, the first individual of *R. marina* (*R. marina 1* in Figure 4) was observed at coordinates 9.1054°S 64.0191°W at 08:37 PM on September 20, 2020 (Figure 3A). The location is near a transect within a primary forest. This individual was parasitized by a single tick identified as *Amblyomma rotundatum* (Figure 3B).

On a second expedition, a second individual of *R. marina* (*R. marina 2* in Figure 4) was observed at coordinates 8.6011°S, -63.8824°W, at 11:38 PM on January 5, 2021 (Figure 3C). The location is rural road C-01 which passes within a primary forest and deforested private property. This individual was parasitized by nine ticks (Figure 3C) identified as *Amblyomma rotundatum* (Figure 3D).

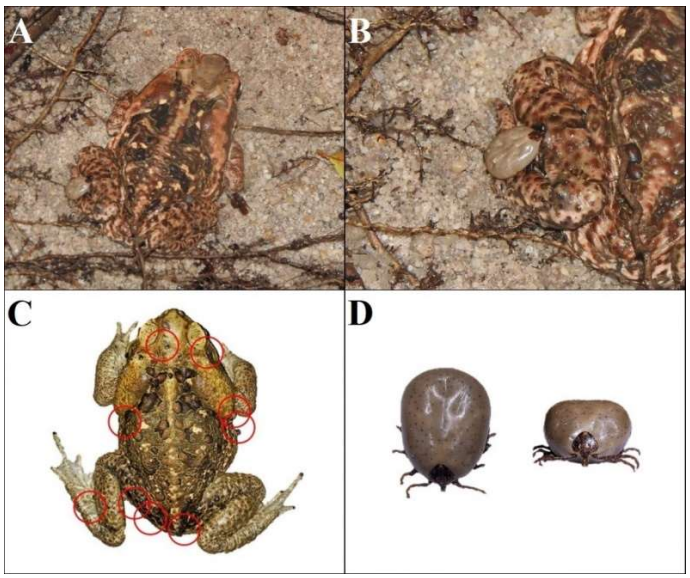

Figure 3: A) *R. marina* first individual; B) *A. rotundatum* parasitizing *R. marina*; C) *R. marina* second individual parasitized by nine *A. rotundatum*; D) Macroscopic record of *A. rotundatum*.

An important observation to be made is that the occurrences of *A. rotundatum* parasitizing *R. marina* are 57 km distant and cross a geographical barrier, the Madeira River of the Amazon basin. The map demonstrating the range of occurrences as well as geographic expansion across the Madeira River can be seen in Figure 4.

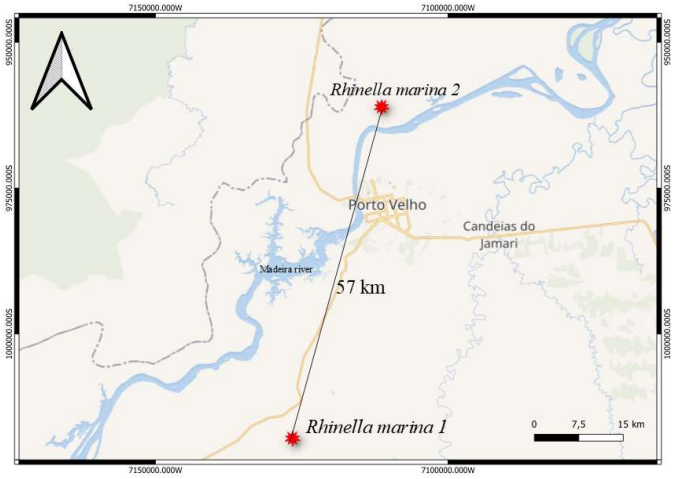

Figure 4: Distribution map of *R. marina* cases parasitized by *A. rotundatum*.

Like other anuran species, *Rhinella marina* also harbors a wide variety of parasites. This occurs mainly because of its large body size and because it occupies terrestrial environments, but also because it has direct contact with aquatic environments (larval and reproductive stages), thus presenting several opportunities for parasitic infections (AHO, 1990). Among the parasites most found in *R. marina*, are included viruses, bacteria, fungi, protozoa, and metazoans. Of the latter, organisms from the groups Trematoda, Nematoda, Pentastomida, Acari, Cestoda, Acantocephala, Culicidae and Monogenea are highlighted as the most representative parasites in *R. marina* (TEIXEIRA et al., 2017). The report in this paper highlights the occurrence of parasitism of the Acari group, represented by *A. rotundatum* in two different localities.

Ticks of the species *A. rotundatum* are arachnids with a diet based on hemophagy preferentially of the blood of cold-blooded animals such as some lizards, and amphibians (ARAGÃO, 1936; GUGLIELMONE et al., 2003). Previous studies point out that only 6 species of *Amblyomma* feed on frogs of the family Bufonidae (LUZ et al., 2013). The ticks of this species have a reproduction that does not require a male, although males of this species have been reported in previous studies (LABRUNA, 2005). They are vectors for several diseases such as viruses, bacteria and protozoa that can cause serious morbidities (MASSARD et al., 2004). This genus of ticks is one of the most important for Brazil because most 67% of ticks in the Brazilian region are composed of ticks of this genus (ARAGÃO, 1936). Although *A. rotundatum* is known to parasitize cold-blooded animals, there are few studies that report host-parasite interactions, especially the expansion of these interactions across a geographic barrier such as the Madeira River, which is notoriously responsible for causing endemism in the Amazon region.

The naturalist Wallace divided the Amazon into four areas of endemism, Brazil being one of them, besides Guiana, Ecuador, and Peru (CARVALHO et al., 2016), however, other researchers have proposed the

existence of eight zones of endemism, which would be: Belém, Guiana, Imeri, Napo, Inambari, Tapajós, Xingu and Rondônia (HAFFER, 1969; CRACRAFT, 1988; HALL et al., 2002). This phenomenon of endemism has the Amazonian rivers as its explanation because water bodies characterize geographical barriers (CARVALHO et al., 2016). The Madeira River has been pointed out as a geographical barrier for different species of Amazonian fauna, both upstream-downstream for mammals (GRAVENA, 2013), reptiles (SOUZA, 2007; MUNIZ, 2012; RANGEL, 2015) and fish (MARÍN, 2013), and on its opposite banks for birds (HAFT, 2007; SOUZA, 2014; NORES, 1999), primates (VAN ROOSMALEN et al., 1998), and Simões (2006) pointed differences a population delimitation of *Allobates femoralis* (Anura, Dendrobatidae). Despite acting as a causative agent for the phenomenon of endemism in the various species described, our report shows that for the case of *R. marina* and *A. rotundatum* this did not occur, thus expanding the occurrence of this host-parasite interaction.

CONCLUSION

This paper described the occurrences of host-parasite interactions between the species *Rhinella marina* (Linnaeus, 1758) and *Amblyomma rotundatum* (Koch, 1844) geographically expanding their occurrence in the Amazon rainforest through the Madeira River. It is possible to conclude that both species manage to cross this geographical barrier. With this study, it is possible to affirm the importance of these species. First, the Cane Toad because it is a species that can cause great impact on the environment in which it lives, given its distribution by human action worldwide and the environmental impact it causes; And the *A. rotundatum* for belonging to a known genus of ectoparasites with the ability to transmit diseases to wild animals and humans. The region described lacks further studies on the natural history of the species reported and their interaction, thus being an indication for other researchers to conduct future studies on this important topic.

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