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OCCURRENCE OF *AMBLYOMMA ROTUNDATUM* (KOCH, 1844) IN *RHINELLA MARINA* (LINNAEUS, 1758) (ANURA: BUFONIDAE) FROM IQUITOS, PERU

OCURRENCIA DE *AMBLYOMMA ROTUNDATUM* (KOCH, 1844) EN *RHINELLA MARINA* (LINNAEUS, 1758) (ANURA: BUFONIDAE) DE IQUITOS, PERÚ

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ABSTRACT

The present study evaluated the influence of seasonality on the occurrence of ectoparasites in a population of *Rhinella marina* Linnaeus, 1758 from the region of Loreto, Iquitos, Peru. Hosts were collected during active night searches. Thirty individuals were collected in the dry season (September 2013) and 20 in the rainy season (March 2014). The collected hosts underwent morphometric evaluation and subsequently surveyed for the presence of ectoparasites through examination of the entire body surface under a stereomicroscope. Only one species of ectoparasite, *Amblyomma rotundatum* Koch, 1844, was found on the analyzed hosts. The prevalence and mean abundance of *A. rotundatum* on *R. marina* collected in the rainy season were higher than in the dry season. However, the mean intensity of infestation was similar in both seasons. The higher prevalence and abundance are likely related to the greater spatial distribution of *R. marina* in the environment during the rainy season because, at this time, more water bodies are available for reproduction. In addition, in the dry season, these organisms are buried and in estivation, which theoretically would decrease their encounter with ectoparasites. Our results demonstrate that the *R. marina* population presents a high prevalence of *A. rotundatum* in the rainy season and corroborates the hypothesis that seasonality is a factor that influences the occurrence of these ectoparasites in these hosts in the studied area. This is the first record that demonstrates the effect of seasonality on *A. rotundatum* infestation in *R. marina* in the Peruvian Amazon.

Keywords: Amazon – *Amblyomma rotundatum* – Peru – *Rhinella marina* – Seasonality

RESUMEN

El presente estudio evaluó la influencia de la estacionalidad en la ocurrencia de ectoparásitos en una población de *Rhinella marina* Linnaeus, 1758 de la región de Loreto, Iquitos, Perú. Los hospederos fueron colectados durante búsquedas nocturnas activas. Treinta individuos fueron recolectados en la estación seca (septiembre / 2013) y 20 en la estación lluviosa (marzo / 2014). Los hospederos recolectados fueron sometidos a evaluación morfométrica, y posteriormente, fueron examinados para la presencia de ectoparásitos a través del examen de la entera superficie corporal bajo un estereomicroscopio. Sólo una especie de ectoparásito, *Amblyomma rotundatum* Koch, 1844, se encontró en los hospederos analizados. La prevalencia y abundancia media de *A. rotundatum* en *R. marina* en la temporada de lluvias fue mayor que en la estación seca. Sin embargo, la intensidad media de infestación fue similar en ambas temporadas. La mayor prevalencia y abundancia probablemente se relaciona con la mayor distribución espacial de *R. marina* en el medio ambiente durante la estación lluviosa porque en este momento, hay más cuerpos de agua disponibles para la reproducción. Además, en la estación seca, estos organismos están enterrados y en estivación, que teóricamente disminuirían su encuentro con los ectoparásitos. Nuestros resultados demuestran que la población de *R. marina* presenta una alta prevalencia de *A. rotundatum* en la estación lluviosa y corrobora la hipótesis de que la estacionalidad es un factor que influye en la ocurrencia de estos ectoparásitos en estos hospederos en el área estudiada. Este es el primer registro que demuestra el efecto de la estacionalidad en la infestación de *A. rotundatum* en *R. marina* en la Amazonía peruana.

Palabras clave: Amazon – *Amblyomma rotundatum* – Estacionalidad – Perú – *Rhinella marina*

INTRODUCTION

The order Anura has the highest record of association with ticks among the amphibians, and the family Bufonidae (Gray, 1825) is associated with the highest number of reports in South America, represented mainly by the species of the genus *Rhinella* (Fitzinger, 1826) (Duellman & Trueb, 1986; Luz & Faccini, 2013).

Rhinella marina (Linnaeus, 1758) is a bufonid species of nocturnal and terrestrial habits and is found in dry and tropical forest environments. This species shows great adaptation to anthropogenic environments near human populations (Seebacher & Alford, 1999; Segalla *et al.*, 2014). Its geographical distribution extends throughout South and Central America, southern North America, and Australia (Zug & Zug, 1979; Frost, 2014).

Iquitos, Peru, which belongs to the district of Loreto, Maynas Province, is considered the capital of the Peruvian Amazon, and *R. marina* is one of six bufonid species recorded for the city (Medina Torres *et al.*, 2012).

Information about Ixodidae Murray, 1877 ectoparasites in *R. marina* and their seasonality is scarce for this Amazonian region, with the great majority of published reports being isolated records of occurrence. These records report a small number of hosts and ectoparasites in different localities, including Brazil (Antonucci *et al.*, 2011; Labruna *et al.*, 2010; Gambale *et al.*, 2014), Argentina (Rivas *et al.*, 2012), Guatemala (Bermudez *et al.*, 2013); India (Drake *et al.*, 2014), and the United States (Florida) (Goddard *et al.*, 2015).

Infestation occurs through the introduction of the oral apparatus of the tick, which penetrates the skin of the amphibian deeply, with the tick remaining fixed via the hypostomium and salivary secretion solidification. The action of parasitism causes the laceration of tissues and blood vessels, and the tick ingests blood and other tissue fluids from the hosts. During the feeding process, the ticks cause trauma, mechanical effects, direct spoliation, and toxic effects (Luz & Faccini, 2013). Thus, they can cause lesions in the hosts during the blood meal intake, resulting in the appearance of ulcerative and hemorrhagic lesions that can alter the behavior of the host animal, thereby affecting the host's

biological, behavioral, and reproductive interactions with the environment (Luz & Faccini, 2013).

The present study evaluated the influence of seasonality on the occurrence of *Amblyomma rotundatum* Koch, 1844 in a population of *R. marina* from the region of Loreto, Iquitos, Peru.

MATERIAL AND METHODS

Host collection

The collections were carried out in an urban area known as Boulevard (3°45'8.3" S 73°14'25" W) (Fig. 1) in the district of Loreto, Iquitos, through active night searching between 06:00 pm and 12:00 am in the dry season in September 2013 and in the rainy season in March 2014. Fifty *R. marina* individuals were collected: 30 in the dry season and 20 in the rainy season. The collection was authorized by Director's Resolution N°024-2014-SERFOR-DGGSPFFS.

The individuals collected were placed in individual plastic bags with a small amount of water and vegetation to maintain moisture and were transported immediately to the Laboratory of Parasitology of the National University of the Peruvian Amazon (Universidad Nacional de la Amazonía Peruana - UNAP). They were then anesthetized with Benzotop, a xylocaine derivative with local action, for the morphometric procedures and investigation of ectoparasites.

The species of the individuals was verified, and the snout-to-vent length (SVL) was measured with digital calipers with an accuracy of 0.01 mm.

Ectoparasite search

The analysis performed on all hosts consisted of observation of the dorsal, ventral, and lateral regions of the body; the orifices; and the limb extremities using a stereomicroscope. The ectoparasites were removed with tweezers to preserve the mouthpiece, identified according to the occupied sites, counted, and placed in bottles with 70% ethanol, properly labeled for each host.

The taxonomic identification of the ectoparasites was carried out at the Laboratory of Parasitology of

Wild Animals (Laboratório de Parasitologia de Animais Silvestres - LAPAS) of the Institute of Biosciences, São Paulo State University (Universidade Estadual Paulista - UNESP), Botucatu, São Paulo, Brazil, where ectoparasites were deposited.

Statistical analysis

The infestation patterns calculated for *R. marina* were estimated by prevalence (P, number of hosts infested divided by total number of hosts, expressed as a percentage), mean abundance (MA, number of collected ectoparasites divided by the total number of analyzed hosts), and mean infestation intensity (MII, number of collected ectoparasites divided by the number of parasitized hosts) according to Bush *et al.* (1997). The values presented for MA and MII are the mean \pm standard error, followed by the minimum and maximum values in parentheses.

The Mann-Whitney test was used to test the influence of seasonality on the occurrence of ectoparasites. The Z test was applied to compare the prevalences. The Spearman correlation test (r_s) was applied to test the correlation between the number of ectoparasites and the host size. All statistical tests were performed in SigmaStat 3.10, and the significance level was 5%.

RESULTS

The search for ectoparasites on *R. marina* specimens collected in Boulevard, Iquitos, Peru, during both seasons resulted in the identification of a single species of ectoparasite, *A. rotundatum*.

The *R. marina* population analyzed in this study had a total prevalence of 56% infestation with *A. rotundatum* in both seasons, and 315 ticks were recovered from the hosts. The mean total abundance was 6.30 ± 1.65 (0-58) ticks per individual, and the MII was 11.25 ± 2.61 (1-58) ticks per host.

Of the total number of hosts analyzed in the dry season, 36% were positive for *A. rotundatum*, with ticks recovered mainly from the dorsal trunk and plantar regions (Fig. 2A-C). In the rainy season, *A. rotundatum* exhibited an infestation level of 85%,

with all ticks located on the dorsal trunk (Fig. 2B-C). The level of infestation was significantly higher in the rainy season ($Z = 3.12$, $p = 0.002$). We also found that the MA was higher in the rainy season, but the MII was similar in the two seasons (Table

1). We did not observe a correlation between the host body size and the number of *A. rotundatum* recovered in the dry season ($r_s = -0.04$, $p = 0.84$) or in the rainy season ($r_s = -0.03$; $p = 0.83$).

Table 1. Number of collected hosts (N), snout-to-vent length (SVL) (mm), prevalence (P%), mean abundance (MA), mean infestation intensity (MII), and total number of *Amblyomma rotundatum* recovered from *Rhinella marina* from Iquitos, Peru, Peruvian Amazon.

Variable	Dry (n = 30)	Rainy (n = 20)	Statistic
SVL (mm)	76	85	
Number of ticks collected	93	222	
Prevalence (%)	36.67 ^a	85.00 ^b	$Z = 3.12$; $p = 0.002$
Mean abundance	3.10 ± 1.13^a (0-27)	11.1 ± 3.56^b (0-58)	$U = 658$; $p = 0.003$
Mean intensity of infestation	8.46 ± 2.36^a (2-27)	13.06 ± 4.01^a (1-58)	$U = 156.5$; $p = 0.90$



Figure 1. A) Overview of the collection area, Boulevard, municipality of Iquitos, Peru (yellow circle); B) view of the area in the dry season; C) view of the area in the rainy season. Source: A) Google Earth; B) Fonseca, 2013; and C) Silva, 2014.

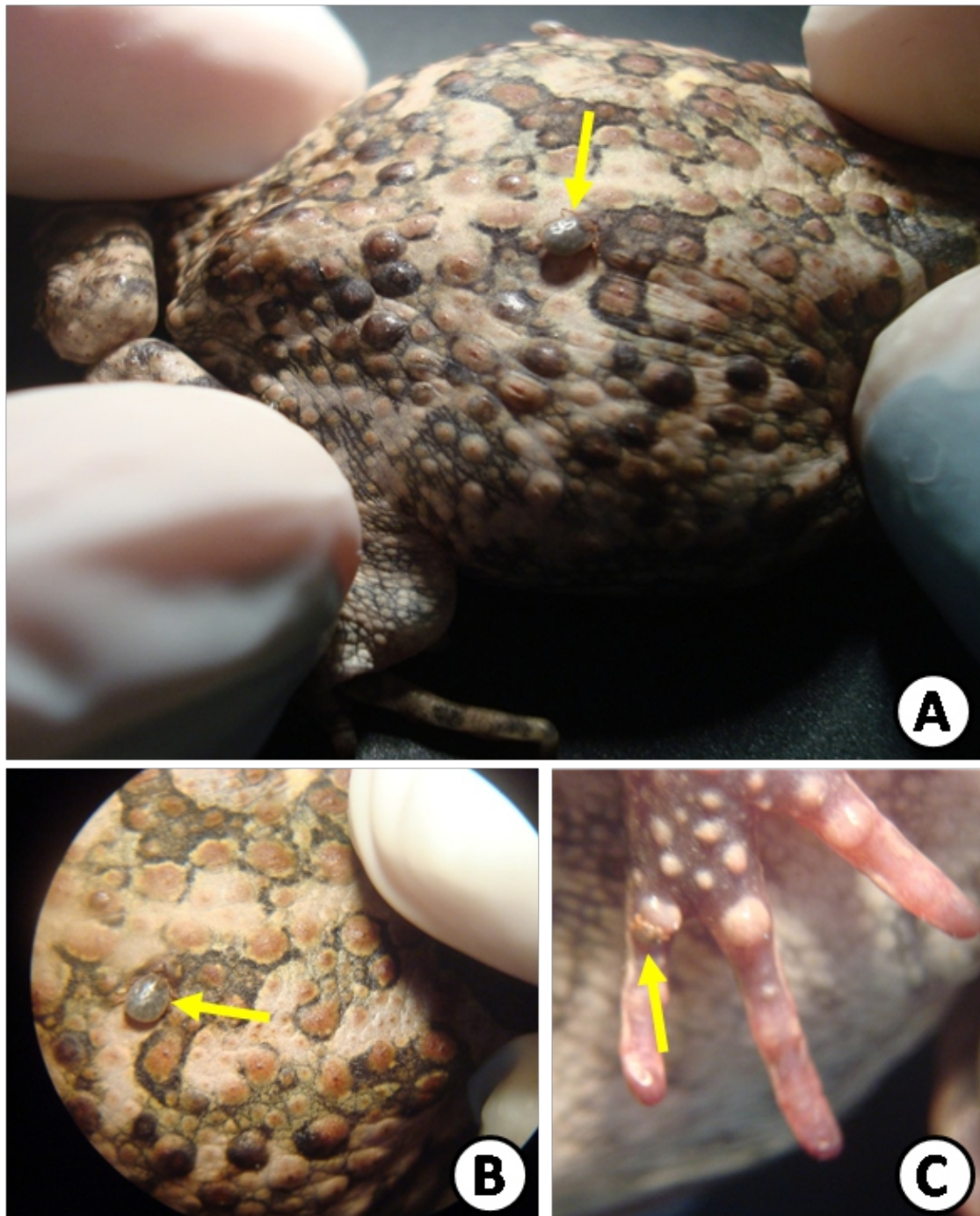


Figure 2. Location of *Amblyomma rotundatum* collected from *Rhinella marina* in the municipality of Iquitos, Peru: A-B) dorsal trunk region; C) limb extremities.

DISCUSSION

In the present study, we found that seasonality influenced the incidence of ectoparasites in the population of *R. marina*, specifically for *A. rotundatum*, which parasitizes ectothermic animals and reproduces exclusively by parthenogenesis. This tick is often reported infesting reptiles and amphibians under natural conditions and sometimes in captive animals. It has been reported in Brazil and in several other countries in South, Central, and North America (Labruna *et al.*, 2010, Antonucci *et al.*, 2011, Rivas *et al.*, 2012, Bermúdez *et al.*, 2013; Drake *et al.*, 2014; Gambale *et al.*, 2014; Goddard *et al.*, 2015; Silva Araújo *et al.*, 2017).

The significant difference found for the sampled period is probably due to the greater spatial distribution of *R. marina* in the environment because a greater number of water bodies are available for reproduction in the rainy season. In addition, in the dry season, these organisms are buried and in estivation, which theoretically would decrease their encounters with ectoparasites.

Our results demonstrate that the population of *R. marina* has a high incidence of ectoparasites in the rainy season and corroborate the hypothesis that seasonality influences the incidence of ectoparasites for this species in the Peruvian Amazon.

According to Luz & Faccini (2013), various endo- and ectoparasites have been recorded in anurans: bacteria, fungi, protozoa, helminths, and arthropods. However, in spite of the large number of tick species, the most common ones recorded parasitizing anurans of the genus *Rhinella* in South America were *Amblyomma dissimile* Koch, 1844 and *Amblyomma rotundatum* Koch, 1844, which agrees with the species found for the Peruvian Amazon in the present study. In addition, this is the first record of occurrence of *A. rotundatum* infesting *R. marina* during the dry and rainy seasons for the region studied.

Amblyomma rotundatum has three life cycle developmental stages (larva, nymph, and adult), all of which are active and depend on the blood supply for full performance of their biological functions

(Terassini, 2010; Viana *et al.*, 2012; Drake *et al.*, 2014). Studies have reported that *A. rotundatum* is a parthenogenetic species, but a male obtained from a natural infestation has been described, and another was raised in the laboratory (Keirans & Oliver, 1993; Labruna *et al.*, 2005).

Infestation by these ticks is extremely pathogenic to hosts, and even at small scales, can lead to death through blood spoliation, transmission of hemoparasites, and inoculation with toxins (Terassini, 2010). The occurrence of *A. rotundatum* infesting *R. marina* in the dry and the rainy seasons is worrisome because the possible spoliative lesions caused by the ticks can alter the reproductive behavior of the individuals in this important phase of their life cycle.

According to Duellman & Trueb (1989), bufonids of the genus *Rhinella* are the main hosts for Ixodidae ticks, especially *A. rotundatum* in South America (Luz *et al.*, 2015). However, most studies are limited to records of *A. rotundatum* infesting other species of bufonids, such as *R. icterica* (Woehl Jr, 2002) and *R. jimi* (Ferreira & Faria, 2011).

Thus, further studies are needed to evaluate in the short and long term how the populations of these hosts and their descendants interact with the spoliation effect of the ectoparasites and the environment in which they live.

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