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Microhabitat determines uneven distribution of *Amblyomma parvum* but not of *Amblyomma sculptum* ticks within forest patches in the Brazilian Pantanal

Vanessa do Nascimento Ramos[1] · Vinicius da Silva Rodrigues[1] · Ubiratan Piovezan[2] · Matias Pablo Juan Szabó[1]

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Abstract
Environmental distribution of the two most abundant ticks in forest areas in the Brazilian Pantanal was evaluated by CO_2 traps methodology in the wet season (peak of adult ticks) of 2012 and 2013. Adults of *Amblyomma parvum* were concentrated inside agglomerates of *Bromelia balansae*, in the border of forest patches. Adults of *Amblyomma sculptum* occurred in similar numbers both in bromeliad clumps and in bromeliad-free areas. Differential distribution of ticks in this habitat could be associated to the frequent use of bromeliad clumps by wild animals (potential hosts) and to the microclimate conditions inside this vegetation in the Pantanal. It is important to verify whether larvae and nymphs of *A. parvum* have a similar pattern of distribution in the same areas, during the dry season. These stages are more susceptible to desiccation and their principal hosts, non-volant small mammals, also use these bromeliad areas.

Keywords Bromeliads · Ixodidae · Host-seeking · Tick ecology

Introduction

Although ticks are an important parasite group of domestic and wild animals, they spend most of their lifetime off-hosts (Needham and Teel 1991) and along non-parasitic time within tick's life cycle is related to a high environmental specificity (Klompen et al. 1996). These invertebrates are, among others, sensible to desiccation and they need suitable habitat for molting, laying eggs and questing for hosts (Lane et al. 1995; Randolph 2004; Schulze et al. 1995; Sonenshine et al. 2002; Tälleklint-Eisen and Lane 2000).

✉ Vanessa do Nascimento Ramos
vanvanecologia@gmail.com

[1] Laboratório de Ixodologia, Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Av. Mato Grosso, 3289/Campus Umuarama – Bloco 2S, Uberlândia, Minas Gerais 38405-314, Brazil

[2] Empresa Brasileira de Pesquisa Agropecuária – Embrapa Tabuleiros Costeiros, Av. Governador Paulo Barreto de Menezes 3250, Aracaju, SE 49025-040, Brazil

Previous studies in the Pantanal have reported that *Amblyomma sculptum* and *Amblyomma parvum* are the tick species more commonly observed inside forest formations (Cançado et al. 2008; do Nascimento Ramos et al. 2016). Both ticks were collected using visual search and CO_2 traps (Cançado et al. 2008; Martins 2016; do Nascimento Ramos et al. 2014; Szabó et al. 2007, 2009; Veronez et al. 2010). Attraction to CO_2 traps indicates a hunter tick behavior (Fourie et al. 1993), despite exhibition of ambush host-seeking behavior as well, by *A. sculptum* in this area (do Nascimento Ramos et al. 2017).

Agglomerates of *Bromelia balansae* Mez are common in the Brazilian Pantanal. Dense clumps of this spiny bromeliad are frequently localized at borders of forest fragments, indicating the limits of flood (Pott and Pott 1994). *Bromelia balansae* is a large bromeliad, with leaves reaching 2.5 m length (Romero and Vasconcellos-Neto 2005). The spines of this plant are distributed along all the leaves, offering protection to animals which take refuge in the clumps for resting, nesting and mainly for protection from predators (Antunes 2009; Araújo and Mourão 2012; Medri and Mourão 2005; Pott and Breyer 1986). As a local of permanence or transient passage for many vertebrates, clumps of *B. balansae* should propitiate host encounter by ticks over adjacent bromeliad-free areas, where the passage of hosts may be more random. Besides, clumps could provide differential microhabitat conditions and, in consequence, favor the maintenance of non-parasitic stages of ticks. In this sense, we verified if agglomerates of *B. balansae* are more infested by ticks in relation to bromeliad-free areas within forest fragments in the Brazilian Pantanal.

Methods

This study was carried out in the Brazilian Pantanal, at the sub-region of Nhecolândia, Mato Grosso do Sul state (18º 59′ 15″ S; 56º 37′ 03″ W). Free-living ticks were collected using dry ice traps (Oliveira et al. 2000) in two consecutive wet seasons (February 2012 and February 2013). We used a CO_2 attraction method for capture of ticks since the spines of *B. balansae* are numerous and highly trenchant, thus making impossible an efficient use of techniques as dragging, flagging or visual search. CO_2 traps are known to attract adults of several tick species of the Pantanal (Cançado et al. 2008; do Nascimento Ramos et al. 2016) and therefore wet season was chosen because it is the adult tick peak period in this region (do Nascimento Ramos et al. 2016). For tick collection 10 CO_2 traps were installed in each forest fragment (n = 4), five at the center of clumps of bromeliads and five in bromeliad-free areas, with a distance of 5 m between traps, and kept for 1 h until the inspection for ticks. Ticks were identified according Onofrio et al. (2006), Martins et al. (2010) and Nava et al. (2014). Number of ticks collected in each zone of the forest fragment was compared by Friedman test for repeated measures (Zar 1999). In addition, vegetation along trails inside the forest fragments was visually inspected in both wet and dry seasons and height of host-seeking ticks on the vegetation measured. Methodology for measuring of questing height is detailed in do Nascimento Ramos et al. (2017).

Results

We collected, on the CO_2 traps, 984 adult ticks of two species: *A. parvum* (n = 377) and *A. sculptum* (n = 607). *Amlyomma sculptum* presented a similar distribution between the two compared zones ($F = 34.5$; $p > 0.05$), whereas *A. parvum* ticks were concentrated inside

the clumps of bromeliads ($F = 53.0$; $p < 0.05$) (Table 1). The proportion of the two species did not differ inside the agglomerates ($F = 29.0$; $p > 0.05$), but in bromeliad-free zones, *A. sculptum* was more abundant than *A. parvum* ($F = 51.0$; $p < 0.05$). For *A. parvum*, six specimens (adults) were observed questing on vegetation along trails in the wet season (concomitant with the CO_2 traps), and the questing height ranged from 12 to 31 cm (mean ± SD = 23.3 ± 6.5). In the dry season, 12 adults were observed from 5 to 62 cm (23.6 ± 16.2). For *A. sculptum*, data on questing height in the study area were presented in do Nascimento Ramos et al. (2017).

Discussion

Amblyomma sculptum is an abundant tick both in agglomerates of bromeliads and in bromeliad-free areas inside forest fragments of the Nhecolândia Pantanal. The dominance of this tick species in forest fragments of this region was demonstrated before (do Nascimento Ramos et al. 2016; Cançado et al. 2008). In a different way, *A. parvum* was more frequent inside clumps of *B. balansae*. This result suggests that agglomerates of bromeliads provide a more suitable environment to encounter of *A. parvum* adults inside forest formations. The causes of this concentration are uncertain, but one hypothesis is related to usage of the bromeliad clumps by hosts, particularly for immature stages of *A. parvum*. Many potential hosts for *A. parvum* in the Pantanal use this microenvironment as shelter, as before mentioned. Immature stages of *A. parvum* are commonly found on small mammals in other regions (Barbieri et al. 2019; Nava et al. 2006). Particularly, in this Brazilian region, larvae and nymphs of *A. parvum* are habitually found in echimyid rodents (Caviomorpha) (do Nascimento Ramos 2013). Thus, the adults observed in this study could be originated from engorged larvae and nymphs in animals using the bromeliads clumps. In addition, these non-fed adults should easily encounter adequate hosts in this environment. *Amblyomma parvum* adults are commonly associated to carnivores (Labruna et al. 2005; Sousa et al. 2018), but they parasitize many others groups, as feral pigs, cattle and dogs

Table 1 Number of free-living ticks collected by dry ice traps in forest fragments (inside clumps of bromeliads and in bromeliad-free areas) in the wet season (February of 2012 and 2013) at Nhecolândia subregion, Brazilian Pantanal

	Amblyomma parvum	*Amblyomma sculptum*	Total of ticks
	Inside clumps of bromeliads		
Total	287	296	583
Range	11–89	1–140	12–151
Median	29.0[a]	22.0[a]	66.5
1st–3rd quartiles	14.0–50.5	16.5–39.0	41.5–102.0
	Bromeliad-free areas		
Total	90	311	411
Range	0–44	107–311	3–135
Median	2.0[b]	23.5[a]	26.0
1st–3rd quartiles	0.0–21.0	11.5–65.5	1.5–97.0

Medians followed by a different letter are significantly different (Friedman test for repeated measures, between tick species and between areas: $p < 0.05$)

(Cançado et al. 2008; Costa et al. 2017; do Nascimento Ramos et al. 2014, 2016), animals regularly observed in that vegetation. A second hypothesis is about the microhabitat in these dense clumps. Differential conditions of microclimate might favor the survival of ticks, especially of immature stages, which are susceptible to desiccation, similarly to many bromeliad-dwelling invertebrates which take advantages in the usage of these plants (Frank and Lounibos 2009). Despite the occurrence of adults on animals and environment, low numbers of larvae and nymphs of *A. parvum* were collected from environment using three different techniques in wet and dry seasons in the Pantanal, in the study area (do Nascimento Ramos et al. 2014). This result is similar to the other studies in areas of *A. parvum* occurrence in Brazil (Martins 2016; Barbieri et al. 2019). In the Pantanal, it is noteworthy that echimyids and other small mammals parasitized by larvae and nymphs of *A. parvum* use bromeliad clumps as a preferential microhabitat (Antunes 2009). In this sense, it could be possible that larvae and nymphs also use this vegetation because it is an adequate microenvironment for non-parasitic periods and for encountering hosts. Unfortunately, our methodology does not appoint which factors (environmental variables or host presence) drive the uneven distribution of *A. parvum* ticks in the studied area. Data on temperature and humidity and tick collections in these bromeliads during the dry season, when the immature forms of *Amblyomma* species peak in the Pantanal and Southeastern Brazil should clarify such issue (Labruna et al. 2002; do Nascimento Ramos et al. 2016). At the same time, questing heights of *A. parvum* adults along trails within the forest fragments indicate search for middle sized- hosts and decreased possibility to attach to small vertebrates. Such observation underscores common hosts for adults of this species, as above mentioned. Finally, our data corroborates that *A. sculptum* has multiple strategies for find hosts, exploiting various habitat types in off-hosts periods. On the other hand, *A. parvum* has a more concentrated distribution in specific microenvironments, represented by clumps of bromeliads in the Brazilian Pantanal. Further studies shall investigate the ecology of larvae and nymphs of *A. parvum* in non-parasitic periods to verify whether they have a heterogeneous distribution, similar to that of adults inside the forest fragments, or not.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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