

# Wildlife species, Ixodid fauna and new host records for ticks in an Amazon forest area, Rondônia, Brazil

Espécies de vida selvagem, fauna ixodídica e novos registros de hospedeiros de carrapatos em uma área de Floresta Amazônica, Rondônia, Brasil

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Received October 27, 2017

Accepted March 03, 2018

## Abstract

The objective of this work was to evaluate the diversity of ticks associated with free-living animals and to investigate new host records for ticks. Ticks were collected from animals rescued during the flood of the Jamari River in the municipality of Ariquemes, state of Rondônia, North Region of Brazil. A total of 39 animals were captured, out of which 10 were amphibians, 19 were reptiles and 10 were mammals. A total of 127 ticks of the *Amblyomma* genus were collected from these animals, distributed among seven species: *Amblyomma dissimile*, *Amblyomma geayi*, *Amblyomma humerale*, *Amblyomma longirostre*, *Amblyomma nodosum*, *Amblyomma rotundatum* and *Amblyomma varium*. In addition, one specimen of *Rhipicephalus (Boophilus) microplus* was collected. Among these specimens, 85 were adults and 42 were nymphs, with *A. rotundatum* being the most prevalent species. An *Amblyomma* spp. larvae was also collected from a lizard (*Uranoscodon superciliosus*), and one *Amblyomma calcaratum* and one *Amblyomma dubitatum* were recovered from the environment, thus totaling 130 ticks. Among the Ixodidae collected from different hosts, we provide the first report for the species *A. rotundatum* parasitizing *Rhinella major*, *U. superciliosus*, *Leptophis ahaetulla*, *Chironius multiventris*, and *Mastigodryas boddaerti*, as well as of *A. humerale* parasitizing *U. superciliosus*, *A. geayi* parasitizing *Choloepus didactylus*, and *Rhipicephalus (B.) microplus* parasitizing *Alouatta puruensis*.

**Keywords:** New hosts, tick fauna, wild animals.

## Resumo

O objetivo deste trabalho foi avaliar a diversidade de carrapatos associados a animais de vida livre e investigar novos registros de hospedeiros. Coletas foram feitas em animais resgatados durante a cheia do Rio Jamari, localizado no município de Ariquemes, estado de Rondônia, Região Norte do Brasil. Um total de 39 animais foi capturado, dos quais dez eram anfíbios, 19 eram répteis e dez eram mamíferos. 127 carrapatos do

gênero *Amblyomma* foram coletados destes animais, distribuídos em sete espécies: *Amblyomma dissimile*, *Amblyomma geayi*,

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*Amblyomma humerale*, *Amblyomma longirostre*, *Amblyomma nodosum*, *Amblyomma rotundatum* e *Amblyomma varium*. Adicionalmente, um exemplar de *Rhipicephalus (Boophilus) microplus* foi coletado. Dentre estes espécimes, 85 eram adultos e 42 eram ninfas, com *A. rotundatum* sendo a espécie mais prevalente. Uma larva de *Amblyomma* spp. também foi coletada de um lagarto (*Uranoscodon superciliosus*), um *Amblyomma calcaratum* e um *Amblyomma dubitatum* foram recuperados do ambiente, assim totalizando 130 carrapatos. Dentre os ixodídeos coletados de diferentes hospedeiros, este trabalho fornece o primeiro registro da espécie *A. rotundatum* parasitando *Rhinella major*, *U. superciliosus*, *Leptophis ahaetulla*, *Chironius multiventris* e *Mastigodryas boddaerti*, assim como da espécie *A. humerale* parasitando *U. superciliosus*, a espécie *A. geayi* parasitando *Choloepus didactylus* e *R. microplus* parasitando *Alouatta puruensis*.

**Palavras-chave:** Novos hospedeiros, fauna ixodídica, animais selvagens.

## Introduction

The Rondônia state, located in the North Region of Brazil, possesses wide reserves of natural Amazon forest, as well as many degraded areas. This state has been the focus of several tick-related studies, putting it in a prominent position since 34 (52%) of the 65 species in the country had already been catalogued here by 2014 (MARTINS et al., 2014). However, the *Amblyomma sculptum* Berlese, 1888 species was recently reported for the first time in Rondônia, bringing the total to 35 species in the state (MARTINS et al., 2016). Although an already large number of Ixodidae species have been reported, that number will tend to increase due to new discoveries. Notably, reports regarding Brazilian tick fauna in recent years have been gradually increasing for both the Ixodidae and Argasidae families. Brazil today has 71 tick species, with 46 belonging to the Ixodidae family and 25 belonging to the Argasidae family (MARTINS et al., 2014, 2016; BARROS-BATTESTI et al., 2015; KRAWCZAK et al., 2015; LABRUNA et al., 2016; WOLF et al., 2016; MUÑOZ-LEAL et al. 2017).

Many of these ectoparasites have been getting closer to and interacting more frequently with domestic animals and, consequently, human beings. Because of this interaction, current research studies have focused on Ixodidae populations as well as their interaction with hosts and the environment since these ticks have the vectorial capacity to transmit agents that can cause diseases in human beings (LABRUNA et al., 2005, 2010; MARTINS et al., 2014).

Interventions in natural environments provide favorable conditions for infectious diseases due to both human interference, such as deforestation, and the constant changes made by man with the construction of roads, barrages, agriculture, and other activities (PATZ et al., 2004). Some tick species in Brazil, mainly from the *Amblyomma* genus, are pathogen vectors and are directly involved in the transmission of infectious agents to human beings, including spotted fever (Brazilian Spotted Fever) caused by bacteria of the genus *Rickettsia* (LABRUNA et al., 2011; OGRZEWALSKA & PINTER, 2016).

The objective of this study was to identify the tick fauna on wild animals captured in a degraded area and to investigate new records of tick-host relationships.

## Material and Methods

### *Location and period of collection*

The capture of hosts was performed during the rescue of wild animals from the flood of the Jamari River after the construction of the Jamari Hydroelectric Central (PCH – Jamari) (10° 53' 7" S

and 61° 57' 6" W), located in the municipality of Ariquemes, state of Rondônia, North Region of Brazil. The captures started in the beginning of November 2014 and lasted until February 2015.

### *Animal capture*

All animals were captured and physically contained according to the methods in Rodrigues et al. (2016) before being transported to the wild animal triage center located near PCH - Jamari. The animal capture, triage, and translocation were executed by a team hired for the task and composed of field workers, biologists and veterinarians with the necessary technical abilities. During triage, each animal was identified by species, sex and weight. Afterwards, tick collections were performed.

### *Collection, identification and processing of ticks*

Ticks were collected during the clinical examination and inspection of captured animals. The parasites were removed from hosts by holding ticks by their bodies and applying force against the host, turning the tick body 90° until the hypostome was completely removed from the skin. Afterwards, they were put in plastic receptacles with pierced covers for subsequent identification.

Later, the ticks were taken to the laboratory and identified with a stereomicroscope using dichotomous keys from Onofrio et al. (2006) for adults and Martins et al. (2010) for nymphs. For confirmation of the species, the *Amblyomma humerale* Koch, 1844 nymph was subjected to DNA extraction and sequencing, as this specimen had breakdowns that made identification by dichotomous key difficult. The ticks were deposited into the collection of the Tick Laboratory, Embrapa Gado de Corte, Campo Grande, Mato Grosso do Sul, Brazil. (Access numbers: 28-30, 35).

### *DNA extraction, PCR and sequencing*

Tick DNA was extracted using a protocol based on guanidine isothiocyanate and phenol/chloroform extraction (SANGIONI et al., 2005 - adapted). The DNA was quantified with a spectrophotometer, and its integrity was verified by agarose gel electrophoresis at 0.8%.

PCR using a 16S rDNA primer for molecular identification of the *A. humerale* species was performed according to Mangold et al. (1998).

The product was analyzed on a 1.5% agarose gel, stained with ethidium bromide, visualized, and photographed under ultraviolet light exposure.

Purification of the amplified fragment was done using the PureLink kit (Invitrogen). The fragment was cloned in a pGEM® T-Easy (Promega) vector and sequenced using the Sanger method (SANGER et al., 1977) with an ABI 3130 Genetic Analyzer (Applied Biosystems®) for species verification.

Sequencing analysis was obtained with the Geneious Pro 4.8.5 software. Sequence similarity of the obtained sequence was compared using BLAST (NCBI, 2017).

All amplicons were sequenced, and individual sequences were aligned with each other and with the corresponding *Amblyomma* sequences available in GenBank using the BioEdit Sequence Alignment Editor (HALL, 1999) with the CLUSTAL W program (THOMPSON et al., 1994). Pair-wise comparisons of sequences were made using MEGA version 5 (TAMURA et al., 2011). Phylogenetic relationships were then explored in terms of neighbor-joining distances (NJ). An NJ tree was generated using the Tamura–Nei model with any gaps in the pair-wise comparisons excluded, and support for the NJ topology was tested by bootstrapping over 1,000 replications.

## Results

A total of 39 animals were captured (Table 1), including 10 amphibians (eight *Rhinella marina*, and two *Rhinella major*), 19 reptiles (14 *Uranoscodon superciliosus*, as well as *Bothrops atrox*, *Leptophis abaetulla*, *Chironius multiventris*, *Mastigodryas boddaerti* and *Boa constrictor* species, with one specimen each) and 10 mammals (three specimens of *Choloepus hoffmanni* and three *Choloepus didactylus*, two specimens of *Tamandua tetradactyla*, and one specimen each of *Coendou prehensilis* and *Alouatta puruensis*). A total of 127 ticks from the *Amblyomma* genus were collected from these animals, distributed among seven species (disregarding one

*Amblyomma* larva, the species of which could not be identified): *Amblyomma dissimile* Koch, 1844, *Amblyomma geayi* Neumann, 1899, *A. humerale*, *Amblyomma longirostre* (Koch, 1844), *Amblyomma nodosum* Neumann, 1899, *Amblyomma rotundatum* Koch, 1884 and *Amblyomma varium* Koch, 1844. Additionally, one specimen of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1888) was also collected.

During the field activities, the authors found one specimen of *Amblyomma calcaratum* Neumann, 1899 and one of *Amblyomma dubitatum* Neumann, 1899 in free-living stages in the environment. A total of 87 adult ticks (10 males and 77 females), 42 nymphs and one larva were collected. Among the nymphs, only one was from the *A. humerale* species, while the rest were *A. rotundatum*. Among adults, the most abundant species was *A. rotundatum*, with 59 specimens, followed by *A. nodosum*, *A. geayi*, *A. longirostre* and *A. varium*, with 13, five, three and three samples each, respectively. Only one specimen of *A. calcaratum*, one *A. dissimile*, one *A. dubitatum*, and one *R. (B.) microplus* were present, reaching a total of 130 specimens (Table 1).

The present study reports, for the first time, the parasitism by *A. rotundatum* of *R. major*, *U. superciliosus*, *L. abaetulla*, *C. multiventris* and *M. boddaerti*, as well as *A. humerale* parasitizing *U. superciliosus*, *A. geayi* parasitizing *C. didactylus*, and *R. (B.) microplus* parasitizing *A. puruensis*.

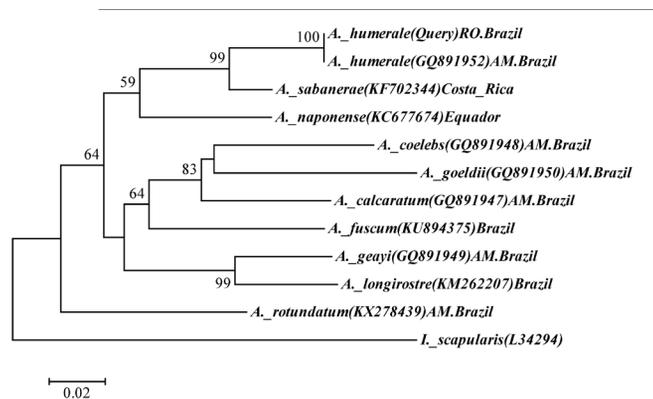
BLAST analysis showed that the obtained *A. humerale* DNA sequence is close (99% identity) to the corresponding sequence of *A. humerale* from the state of Amazonas, North Region of Brazil (GQ891952), as well as 95% similar to the *Amblyomma sabanerae* Stoll, 1894 sequence from Costa Rica (KF702455), as illustrated in Figure 1.

Phylogenetic analysis indicated that the specimens collected from Amazon forest area, Rondônia, Brazil, correspond to *A. humerale*

**Table 1.** Number, instars and tick species identification include of the genera *Amblyomma* and *Rhipicephalus* collected from wild animals rescued from a forest area in the municipality of Ariquemes, Rondônia, Brazil.

Species of captured animals	Number of captured animals	Tick species
<b>Amphibians</b>		
<i>Rhinella marina</i>	8	27N and 50F <i>A. rotundatum</i>
<i>Rhinella major</i>	2	2F <i>A. rotundatum</i>
<b>Reptiles</b>		
<i>Uranoscodon superciliosus</i>	14	1L <i>Amblyomma</i> spp.; 7N and 6F <i>A. rotundatum</i> ; 1N <i>A. humerale</i>
<i>Bothrops atrox</i>	1	1F <i>A. rotundatum</i>
<i>Leptophis abaetulla</i>	1	3N <i>A. rotundatum</i>
<i>Chironius multiventris</i>	1	3N <i>A. rotundatum</i>
<i>Mastigodryas boddaerti</i>	1	1N <i>A. rotundatum</i>
<i>Boa constrictor</i>	1	1F <i>A. dissimile</i>
<b>Mammals</b>		
<i>Choloepus hoffmanni</i>	3	1F and 1M <i>A. geayi</i> ; 1F <i>A. varium</i>
<i>Choloepus didactylus</i>	3	1M and 2F <i>A. geayi</i> ; 1M and 1F <i>A. varium</i>
<i>Tamandua tetradactyla</i>	2	4M and 9F <i>A. nodosum</i>
<i>Coendou prehensilis</i>	1	3M <i>A. longirostre</i>
<i>Alouatta puruensis</i>	1	1F <i>R. (B.) microplus</i>
<b>Free-living stages</b>	-	1F <i>A. calcaratum</i> ; 1F <i>A. dubitatum</i>
<b>Total</b>	<b>39</b>	<b>130</b>

M male; F female; N nymph; L larva.



**Figure 1.** Neighbor-joining (NJ) tree built from *Amblyomma humerale* 16S rDNA sequence collected in the municipality of Ariquemes, Rondônia, Brazil. The numbers represent the identity generated from 1,000 repetitions. These were used when identity is >50%. The *Ixodes scapularis* Say, 1821 sequence was used as an external group (out group).

(Figure 1). The Amazonian specimens clustered with sequences from Amazonas, Brazil, which may correspond to the geographic area of *A. humerale*, and is located closed to Rondônia, sharing the Amazon rainforest.

## Discussion

*Amblyomma rotundatum* ticks were present on all captured species of amphibians and reptiles, with reports of nymph and adult instars; however, only females were found among the adults. This is due to the characteristic fact that this species parasitizes different cold-blooded hosts and reproduces through parthenogenesis, despite the presence of a male specimen on a *Tropidurus* sp. lizard and another one on a *B. constrictor* snake (ARAGÃO, 1936; LABRUNA et al., 2005; MARTINS et al., 2014).

Witter et al. (2016) reported *A. rotundatum* on *R. marina* and *B. atrox* in the state of Mato Grosso; however, according to Guglielmo & Nava (2010), this tick species has never been reported on *R. major*, *U. superciliosus*, *L. abaelulla*, *C. multiventris* or *M. boddaerti*. Therefore, this study reports, for the first time, these new hosts for the *A. rotundatum* species.

Among reptiles, the presence of *A. dissimile* on *B. constrictor* was also reported. Similar results were obtained by Fiorini et al. (2014) and Witter et al. (2016), who reported this tick species parasitizing *B. constrictor*. *Amblyomma dissimile* ticks are known to prefer parasitizing reptiles (BARROS-BATTESTI et al., 2006; SCOFIELD et al., 2011).

This study reports, for the first time in Brazil, an *A. humerale* tick parasitizing a lizard of the *U. superciliosus* species, with the *A. humerale* nymph identified morphologically and then confirmed through DNA extraction and sequencing. A clade was demonstrated a posteriori, with a 100% probability, between *A. humerale* (Query) RO, Brazil and *A. humerale* (GQ891952), within the phylogenetic tree. *Amblyomma sabanerae* from Costa Rica, showed 99% of similarity with the *A. humerale* clade demonstrated in this study

(Figure 1). The other clades did not demonstrate any similarity with *A. humerale* species.

Labruna et al. (2002a) described the presence of *A. humerale* nymphs on *Plica plica* and *Plica umbra* lizards belonging to the Tropicuridae family, which is the same family to which *U. superciliosus* belongs, and *Kentropyx calcarata*, belonging to the Teiidae family, captured along the same river featured in this study. Labruna et al. (2002a) also described for the first time the presence of *A. humerale* in the state of Rondônia, as well as immature stages parasitizing a variety of small vertebrates.

Adult *A. varium* and *A. geayi* ticks found parasitizing sloths (*C. hoffmanni* and *C. didactylus*) in this study are associated primarily with the Xenarthra order, according to Marques et al. (2002) and Labruna et al. (2009). However, the first report of *A. geayi* parasitism on *C. didactylus* was made in this study, since descriptions of this tick species on sloths refer to the *C. hoffmanni*, *Bradypus tridactylus*, and *Bradypus variegatus* species (FAIRCHILD et al., 1966; LABRUNA et al., 2009; MARTINS et al., 2013).

*Amblyomma nodosum* adult ticks were found parasitizing *T. tetradactyla*. This tick species, when in its adult phase, prefers mammals from the Xenarthra order, which includes this host species. However, immature phases are involved in bird parasitism (GUIMARÃES et al., 2001; BARROS-BATTESTI et al., 2006). Witter et al. (2016) noted the presence of *A. nodosum* on free *T. tetradactyla* individuals. Different research studies have also reported adult *A. nodosum* present on *T. tetradactyla* (GARCIA et al., 2013; MARTINS et al., 2014).

Parasitism by *A. longirostre* adults is commonly associated with some *Coendou* species (GUIMARÃES et al., 2001), such as *Coendou prehensilis*, as registered in this study. Immature phases of *A. longirostre* are usually associated with bird parasitism (OGRZEWALSKA & PINTER, 2016).

This is also the first report of an *R. (B.) microplus* semi-engorged female parasitizing an *A. puruensis*. This possibly occurred accidentally, since this female tick was attached to a young *A. puruensis* found separated from its mother and on the ground, where it possibly contacted *R. (B.) microplus*. Other Ixodidae species, especially *Amblyomma* sp. ticks, were reported parasitizing animals of the *Alouatta* genus (LABRUNA et al., 2002b; MARTINS et al., 2006; LAVINA et al., 2011; MARTINS et al., 2013, 2015). Labruna et al. (2002b) also rescued animals from the flood caused by the construction of the Porto Primavera hydroelectric plant and reported the presence of 18 *A. sculptum* nymphs parasitizing three *Alouatta caraya*.

Other studies have reported the presence of *A. aureolatum* (Pallas, 1772) on *Alouatta guariba* specimens (MARTINS et al., 2006), *A. aureolatum* and *A. ovale* Kock, 1844 on *Alouatta clamitans* (LAVINA et al., 2011) and parasitism by *A. parkeri* Fonseca e Aragão, 1952 and *A. sculptum* nymphs of *A. guariba* (MARTINS et al., 2013, 2015). As observed in the present study, which reported a young debilitated animal on the ground, other studies show that parasitism by Ixodidae of primates of the *Alouatta* genus are usually associated with the presence of stressful conditions for these animals, either because they are hurt, young, or due to their permanent residence on the ground caused by some physical debility (MARTINS et al., 2006; LAVINA et al., 2011; MARTINS et al., 2015).

This study observed and presented Ixodidae fauna parasitizing several animal species in a region of the state of Rondônia, in the North Region of Brazil. *Amblyomma rotundatum* was described, for the first time, parasitizing amphibians and reptiles of the *R. major*, *U. superciliosus*, *L. abaetulla*, *C. multiventris* and *M. boddaerti* species. Also unprecedented were the parasitism reports of *A. humerale* on *U. superciliosus* lizards, *A. geayi* on *C. didactylus* sloths, and *R. (B.) microplus* on *A. puruensis* monkeys.

## Acknowledgements

The research was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul - FUNDECT-MS, Governo do Estado de Mato Grosso do Sul, Fundação de Amparo a Pesquisa do Estado de Rondônia - FAPERO-RO and Fiocruz – Rondônia.

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