Sloth’s giant tick (*Amblyomma varium*) parasitizing free-ranging maned sloth (*Bradypus torquatus*) in the Atlantic Forest biome, Brazil.

Carrapato gigante da preguiça (*Amblyomma varium*) parasitando preguiça-de-coleira (*Bradypus torquatus*) de vida livre no bioma Mata Atlântica, Brasil.

**Abstract**

*Amblyomma varium* is a neotropical tick popularly known as the sloth’s giant tick, during the adult stage is found almost exclusively on mammals of the Bradypodidae and Megalonychidae families of the superorder Xenarthra. The maned sloth (*Bradypus torquatus*) is the only sloth species in danger of extinction in Brazil. This species is arboreal and descends to the ground only to perform defecation behavior. This behavior can be the determining factor for infestation and infection by different parasitic agents, including *A. varium*. This study reported the occurrence of *A. varium* found parasitizing free-living maned sloths (*B. torquatus*) in the Atlantic Forest biome of Northeast and Southeast Brazil. A total of 36 individuals were evaluated and more than 50% of the individuals were parasitized by ticks of the species *A. varium*. This study confirmed the importance of *B. torquatus* as a host for adults and nymphs of *A. varium* and reported the variation in length of the spurs on coxa IV of *A. varium* males.

**Keywords:** *Amblyomma, Bradypus torquatus*, giant tick, parasite, sloth.

**Resumo**

O *Amblyomma varium* é um carrapato neotropical popularmente conhecido como carrapato gigante da preguiça, durante a fase adulta é encontrado quase que exclusivamente em mamíferos das famílias Bradypodidae e Megalonychidae da superordem Xenarthra. A preguiça-de-coleira (*Bradypus torquatus*) é a única espécie de preguiça ameaçada de extinção no Brasil. Esta espécie é arbórea e desce ao solo apenas para realizar comportamentos de defecação. Esse comportamento pode ser o fator determinante para infestações e infecções por diferentes agentes parasitários, incluindo *A. varium*. Este estudo relatou a ocorrência de *A. varium* encontrado parasitando preguiças-de-coleira (*B. torquatus*) de vida livre no bioma Mata Atlântica do Nordeste e Sudeste do Brasil. Foram avaliados 36 indivíduos e mais de 50% dos indivíduos estavam parasitados por carrapatos da espécie *A. varium*. Este estudo confirmou a importância de *B. torquatus* como hospedeiro para adultos e ninhas de *A. varium* e relatou a variação no comprimento dos espinhos da coxa IV de machos de *A. varium*.

**Palavras-chave:** *Amblyomma, Bradypus torquatus*, carrapato gigante, parasita, preguiça.
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Introduction

The species Amblyomma varium was first described by Koch (1844), in a male specimen, without data on host and locality. Over the years, several other researchers have published information (e.g. morphology, distribution, and hosts) that corroborates or adds to what was found by Koch (Aragão & Fonseca, 1961; Guimarães, 2001; Neumann, 1899). The redescription of the adults of A. varium, including a complete revision of the bibliography and clarification of the taxonomic status, was published by, Onofrio et al. (2008) published a redescription of both sexes of A. varium, clarifying the taxonomy and establishing the most recent and complete bibliography by Onofrio et al. (2008). Additionally, immature stages (larva and nymph) were described by Amorim and Serra-Freire (1996) and Martins et al. (2010), respectively.

Amblyomma varium is a neotropical tick, distributed in the following Brazilian states: Pará, Acre, Amazonas, Rondônia, Paraíba, Alagoas, Bahia, Mato Grosso, Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo (Acosta et al., 2016; Gruhn et al., 2019; Sanches et al., 2014; Witter et al., 2016). Popularly known as the sloth's giant tick, during the adult stage is found almost exclusively on mammals of the Bradypodidae and Megalonychidae families of the superorder Xenarthra (Marques et al., 2002; Onofrio et al., 2008).

Similarly to A. varium, sloths are found in tropical forests (Gilmour et al., 2000; Onofrio et al., 2008). The maned sloth (Bradypus torquatus) is the only species of sloth that is in danger of extinction in Brazil, and is listed as vulnerable by both the official list of Brazilian fauna in danger of extinction (Chiarello et al., 2018) and the International Union for Conservation of Nature and Natural Resources (Chiarello & Moraes-Barros, 2014). Its geographic distribution is limited to the Atlantic Forest coast (Chiarello et al., 2015), a biome that has already lost 72% of its area, mainly due to predatory hunting, illegal logging, invasion of exotic species (Rezende et al., 2018), and also infectious diseases, which could lead to the decline of a wild population or even lead to an increase in the risk of extinction of a wildlife (Pedersen et al., 2007; Woodroffe, 1999).

The species B. torquatus is arboreal and descends to the ground only to perform defecation behavior (Voirin et al., 2013). However, when considering the extensive deforestation and fragmentation of its inhabited area, the animal's behavior of descending to the ground may also be related to the absence of connection between trees. Therefore, this behavior can be the determining factor for infestation and infection by different parasitic agents, including A. varium, either through contact with the soil, direct contact with the animal, or contact with vegetation shared among species (Chiarello, 1998; Pinto et al., 2011).

This study reports the new findings of the tick A. varium Koch, 1844 found parasitizing free-living maned sloths (B. torquatus) in the Atlantic Forest biome of Northeast and Southeast Brazil.

Material and methods

The study was carried out in three different locations (Figure 1), all with species of fauna and flora in danger of extinction: 1. Reserva Sapiranga, in the municipality of Mata de São João, state of Bahia, which was named an Environmental Protection Area (APA) through the State Decree 1.046 of March 17, 1992 and consists predominantly of 600 hectares of preserved secondary Atlantic Forest, 2. APA of Bacia do Rio São João/Mico Leão Dourado, which covers seven municipalities in the state of Rio de Janeiro. Among these municipalities are Silva Jardim and Rio das Ostras, which were created by the decree of June 27, 2002 and consist of 150,000 hectares of total area, including anthropized areas and preserved forest fragments, such as the 5,000 hectares referred to as the Reserva Biológica de Poço das Antas (Decree nº 73.791/1974 and Decree nº 76.534/1975); 3. Reserva Biológica União, in the state of Rio de Janeiro, which covers the municipalities of Macaé, Rio das Ostras, and Casimiro de Abreu, an Integral Protection Conservation Unit containing more than 7,000 hectares (Decree of April 22, 1998 and Decree of June 05, 2017).

The animals were captured/recaptured between the months of June 2019 and May 2021 under the approval and legal consent of the Brazilian Federal Authority. Authorization for activities with scientific purpose under numbers 67274-8 and 64635-5 of the Ministério do Meio Ambiente - MMA through Instituto Chico Mendes de Conservação da Biodiversidade - ICMBIO. These captures were completed in different locations and were georeferenced with a Global Positioning System (GPS).
The captures were conducted during an active search. Once an individual was spotted, a trained climber accessed the tree canopy to hand catch the animal. All of the animals’ claws were wrapped with Velcro tape to keep them closed during physical restraint. Thereafter, the animal was placed in a cotton bag and was lowered to the forest floor using a rope.

A combination of anesthetics (Ketamine Hydrochloride 4.0mg/kg and Medetomidine Hydrochloride 0.03mg/kg, intramuscularly, and Atipamezole Hydrochloride 0.1mg/kg, intravenously, as anesthetic antagonist) was used for sedation. These doses allowed safe handling of the animals for up to 60 minutes. During the anesthetic procedure heart rate, respiratory rate, and temperature were monitored every ten minutes. All information regarding chemical restraint was recorded on a pre-made anesthetic form.

The age of the maned sloths was established at both capture and recapture, and sexing was performed according to collar morphology, weight, and especially the morphology of the genitalia (Lara-Ruiz & Chiarello, 2005). The physical examination of the sloths was performed by a veterinarian and for the new captures, a nanochip was inserted subcutaneously for animal identification. The ticks found were collected, individually packaged in bottles containing 70% ethyl alcohol and sent to the Laboratory of Parasitic Diseases of the Department of Veterinary Preventive Medicine and Animal Health of the School of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ-USP).

The ticks were identified by stereomicroscope using taxonomic keys (Aragão & Fonseca, 1961; Barros-Battesti et al., 2006; Guimarães, 2001; Martins et al., 2010). Some specimens were deposited at the tick collection “Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva” of the FMVZ-USP, under the accession numbers CNC-4357 and 4358.

Results

Between 2019 and 2021, 36 specimens of B. torquatus were captured or recaptured, of which 64% (n=23) were parasitized by A. varium ticks. Of the 23 parasitized maned sloths, 13 and 8 were females and males, respectively. Two specimens were of undetermined sex. The ages stipulated on the different capture dates along with the data for each individual are shown in Table 1.

A total of 70 ticks were collected, 4 nymphs, 12 females, and 54 males. The length of the spurs on coxa IV can be seen in Table 1. Among the 54 males of the species A. varium collected in this
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study, 37 specimens were from the State of Rio de Janeiro (municipalities of Silva Jardim and Rio das Ostras) in the Southeast region of Brazil. A total of 8 and 29 males had the spur on coxa IV short and long, respectively. Of the three individuals of *B. torquatus* captured in the municipality of Rio das Ostras, only one had two specimens of *A. varium* with spurs of both sizes on coxa IV, while the other two individuals had three ticks with long spurs. The same variation in the length of the spur on coxa IV was observed in individuals of *B. torquatus* captured in the municipality of Silva Jardim.

The remaining 17 male specimens of *A. varium* collected were from the state of Bahia (municipality of Mata de São João), Northeast region of Brazil. Of these males, 8 specimens had a short spur and 9 had a long spur on coxa IV.

### Table 1. *Amblyomma varium* ticks collected on free-ranging *Bradypus torquatus* from the Atlantic Forest biome between 2019 and 2021.

| Individual Identification | Capture Date   | Municipality (Federative Unit) | Age   | Sex | Number of ticks collected per host | Number of ticks per stage and sex | Length of the spurs on coxa IV of males |
|---------------------------|----------------|-------------------------------|-------|-----|-----------------------------------|-----------------------------------|----------------------------------------|
| BT 03                     | July/2019      | RJ (RJ)                       | Adult | Male | 6                                 | 6 ♂                             | Long                                   |
| BT 03                     | November/2019  | RJ (RJ)                       | Adult | Male | 3                                 | 1 ♂ 2 ♀                         | Long                                   |
| BT 04                     | June/2019      | RJ (RJ)                       | Adult | Female | 2                               | 2 ♂                             | Long                                   |
| BT 04                     | July/2019      | RJ (RJ)                       | Adult | Female | 1                               | 1 ♂                             | Long                                   |
| BT 05                     | June/2019      | RJ (RJ)                       | Subadult | Male | 2                               | 2 ♂                             | Long                                   |
| BT 05                     | July/2019      | RJ (RJ)                       | Subadult | Male | 1                               | 1 ♂                             | Short                                   |
| BT 05                     | November/2019  | RJ (RJ)                       | Adult | Male | 2                               | 1 ♂ 1 ♀                         | Long                                   |
| BT 07                     | July/2019      | RJ (RJ)                       | Adult | Female | 3                               | 3 ♂                             | 2 Long 1 Short                           |
| BT 07                     | January/2021   | RJ (RJ)                       | Adult | Female | 2                               | 2 ♂                             | Long                                   |
| BT 08                     | July/2019      | RJ (RJ)                       | Adult | Female | 1                               | 1 ♂                             | Long                                   |
| BT 09                     | July/2019      | RJ (RJ)                       | Adult | Female | 3                               | 3 ♂                             | 2 Long 1 Short                           |
| BT 09                     | January/2021   | RJ (RJ)                       | Adult | Female | 2                               | 2 ♂                             | Long                                   |
| BT 10                     | January/2021   | RJ (RJ)                       | Adult | Female | 6                               | 3 ♂ 2 ♀ 1 N                       | 2 Long 1 Short                           |
| BT 11                     | December/2019  | RJ (RJ)                       | Adult | Female | 2                               | 2 ♂                             | 1 Long 1 Short                           |
| BT 12                     | December/2019  | RJ (RJ)                       | Subadult | Female | 5                               | 3 ♂ 2 ♀                         | Long                                   |
| BT 12                     | January/2021   | RJ (RJ)                       | Subadult | Female | 1                               | 1 ♂                             | Long                                   |
| BT 13                     | January/2021   | RJ (RJ)                       | Adult | Female | 1                               | 1 ♀                             | -                                      |
| BT 14                     | January/2021   | RJ (RJ)                       | Adult | Female | 2                               | 2 ♂                             | Short                                   |
| BT 15                     | January/2021   | RJ (RJ)                       | Subadult | Female | 1                               | 1 ♂                             | Short                                   |
| BTPF 02                   | July/2020      | BA (BA)                       | Adult | Male | 4                               | 3 ♂ 1 ♀                         | Short                                   |
| BTPF 02                   | May/2021       | BA (BA)                       | Adult | Male | 2                               | 2 ♂                             | 2 Long                                   |
| BTPF 04                   | May/2021       | BA (BA)                       | Adult | Female | 2                               | 1 ♂ 1 ♀                         | Long                                   |
| BTPF 10                   | March/2021     | BA (BA)                       | Adult | Male | 1                               | 1 ♂                             | Long                                   |
| BTPF 11                   | March/2020     | BA (BA)                       | Adult | Male | 1                               | 1 ♂                             | Short                                   |
| BTPF 13                   | March/2020     | BA (BA)                       | Subadult | Male | 2                               | 2 ♂                             | 1 Long 1 Short                           |
| BTPF 15                   | March/2021     | BA (BA)                       | Subadult | Male | 1                               | 1 N                             | -                                      |
| BTPF 30                   | March/2021     | BA (BA)                       | Adult | Male | 2                               | 2 N                             | -                                      |
| BTPF 31                   | May/2021       | BA (BA)                       | Adult | Female | 2                               | 1 ♂ 1 ♀                         | Long                                   |
| BTPF 35                   | May/2021       | BA (BA)                       | Adult | Undetermined | 1 | 1 ♀                             | -                                      |
| BTPF 36                   | May/2021       | BA (BA)                       | Adult | Female | 4                               | 4 ♂                             | 3 Long 1 Short                           |
| BTPF 37                   | May/2021       | BA (BA)                       | Adult | Undetermined | 2 | 2 ♂                             | Short                                   |
Of the 38 ticks with long spurs, 76% (n=29) come from the state of Rio de Janeiro. In addition, of the 37 ticks from Rio de Janeiro, 78% had long spurs, while in Bahia the distribution between ticks with long and those with short spurs was closer, 53% and 47% respectively.

Discussion
In this study, adults and nymphs of *A. varium* parasitizing *B. torquatus* were found in the Southeast and Northeast regions of Brazil, corroborating Marques et al. (2002)’s study which also found the same tick-host association.

Of the 54 males of *A. varium* collected, 16 had short spurs while 38 had long spurs on coxa IV. The difference in length between spurs on coxa IV has already been described in Brazil by Onofrio et al. (2008), who studied this intraspecific polymorphic characteristic in populations from the North (states of Amazonas, Pará, and Rondônia), Southeast (states of Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo), and Northeast (states not reported). The authors reported long length of the spur on coxa IV only in the states of Amazonas, Rio de Janeiro, and São Paulo, making this the first study to report this feature in the state of Bahia.

Since the spurs primary function is to assist in attaching to the host hair, perhaps the longer spurs can fixate deeper or more securely in the host hair, which could support the fact that more animals are parasitized in Rio de Janeiro than in Bahia. However, further studies are strongly recommended for any conclusive statement on this topic.

Conclusion
In the present study, more than 50% of the captured individuals were parasitized by adults or nymphs of *A. varium*, and only one specimen of *B. torquatus* showed simultaneous parasitism by both stages of this tick species. This study confirms the importance of *B. torquatus* as a host for adults of *A. varium*. Additionally, the results report the variation in the length of the spurs on coxa IV in male specimens of the species *A. varium*, confirming previous findings in the literature of this intraspecific polymorphic characteristic in populations from the Southeast region, and recording this characteristic for the first time in Northeast Brazil.

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Ethics statement
The animals were captured under the approval and legal consent of the Brazilian Federal Authority. Authorization for activities with scientific purpose under numbers 67274-8 and 64635-5 of the Ministério do Meio Ambiente - MMA through Instituto Chico Mendes de Conservação da Biodiversidade - ICMBIO.

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Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. FCSB, TFM, SSF, BFR, CRRM, GAFG, FLS, FRM - No conflict of interest.
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Authors’ contributions

FCSB, TFM - Writing, review and editing manuscript. SSF, BFR, CRRM, GAFG, FLS - Review and editing manuscript. FRM - Review and editing manuscript, acquisition of the financial support for the project leading to this publication.

Availability of complementary results

Open access. The work was carried out at Laboratório de Biodiversidade & Saúde, Universidade Estadual de Santa Cruz - UESC, Ilhéus, Bahia, Brazil.

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