

## Original article

## Ticks parasitizing cold-blooded animals from three different Brazilian biomes; with note for males of *Amblyomma rotundatum*

Hermes Ribeiro Luz<sup>a,\*</sup>, Carlos Eduardo Costa de Campos<sup>b</sup>, Livio Martins Costa-Junior<sup>c</sup>,  
 Ercileide Silva Santos<sup>b</sup>, Adriani Hass<sup>d</sup>, Dauana Mesquita Sousa<sup>c</sup>,  
 Ruth Myrian de Moares e Silva<sup>e</sup>, Josiane Moreira Rocha<sup>f</sup>, George Rego Albuquerque<sup>f</sup>,  
 Antonio Jorge Argolo<sup>g</sup>, Rogério Zacariotti<sup>h</sup>, Catia Dejuste de Paula<sup>i</sup>,  
 Luiz Cezar Machado Pereira<sup>j</sup>, Patricia Avello Nicola<sup>j</sup>, João Luiz Horacio Faccini<sup>a</sup>,  
 Mauricio Claudio Horta<sup>j</sup>, Rafael Michael Silva Nogueira<sup>k</sup>, Leonardo Teixeira DallAgnol<sup>k</sup>,  
 Marcelo Bahia Labruna<sup>l</sup>, Thiago Fernandes Martins<sup>l,m</sup>

<sup>a</sup> Parasite Control Laboratory, Post-Graduation Program in Northeast Biotechnology Network (RENORBIO), Biodiversity and Conservation, Health and Environment of Center of Biological and Health Sciences, Federal University of Maranhão, São Luís, MA 65085-580, Brazil

<sup>b</sup> Department of Biological and Health Sciences, Herpetology Laboratory, Bionorte Network Postgraduate Program in Biodiversity and Biotechnology, Federal University of Amapá, Rod. Juscelino Kubitschek, km 02 - Jardim Marco Zero, Macapá, AP 68903-419, Brazil

<sup>c</sup> Parasite Control Laboratory, Health and Environment of Center of Biological and Health Sciences, Federal University of Maranhão, São Luís, MA 65085-580, Brazil

<sup>d</sup> Vertebrate Ecology and Conservation Laboratory, Center of Biological and Health Sciences, Federal University of Maranhão, São Luís, MA 65085-580, Brazil

<sup>e</sup> Parasite Control Laboratory, Post-Graduation Program in Biodiversity and Conservation of Center of Biological and Health Sciences, Federal University of Maranhão, São Luís, MA 65085-580, Brazil

<sup>f</sup> Postgraduate Program in Animal Science, State University of Santa Cruz, Rod. Jorge Amado, Km 16 - Salobrinho, Ilhéus, BA 45662-900, Brazil

<sup>g</sup> Postgraduate Program in Zoology, State University of Santa Cruz, Rod. Jorge Amado, Km 16 - Salobrinho, Ilhéus, BA 45662-900, Brazil

<sup>h</sup> Wild cuesta institute, São Paulo, Brazil

<sup>i</sup> Wild Animal Medicine, Fluminense Federal University, Niterói, RJ 24220-900, Brazil

<sup>j</sup> Federal University of Vale do São Francisco, Juazeiro, BA 48902-300, Brazil

<sup>k</sup> Post-Graduation Program in Health and Environment, Federal University of Maranhão, São Luís, MA 65085-580, Brazil

<sup>l</sup> Department of Preventive Veterinary Medicine and Animal Health, Faculty of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, SP 05508-270, Brazil

<sup>m</sup> São Paulo State Department of Health, Pasteur Institute, São Paulo, SP 05402-000, Brazil

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## ABSTRACT

Ticks parasitize a wide variety of wild animals, including amphibians and reptiles. In addition to the possibility of microorganism transmission to these hosts, ticks can also cause severe bleeding, and high parasitism can lead to death. Therefore, knowing the diversity of ticks parasitizing amphibians and reptiles is important for conservation and preservation measures for these vertebrates. In the present study, we report parasitism by ticks in amphibians and reptiles from different Brazilian biomes (Amazon, Caatinga, Cerrado and Atlantic Forest). Ticks were collected from amphibians and reptiles deposited from the Herpetological Collection of the Federal University of Maranhão (UFMA), São Luís (Maranhão State), the State University Santa Cruz (UESC), Ilhéus (Bahia State), and the Federal University of São Francisco Valley (Univasf), Petrolina (Pernambuco State). Additionally, ticks were collected from amphibians and reptiles captured and road-killed in the Amazon biome, at Maranhão and Amapá States. Specimens of ticks were photographed under a Zeiss stereomicroscope (5.1 zoom). Map with the locations were made using the Qgis program. Overall, 1973 specimens of amphibians and reptiles were examined. A total of 927 ticks were collected: 98 larvae, 421 nymphs and 408 adults. Six species of ticks were identified: *Amblyomma rotundatum* and *Amblyomma dissimile* the most frequent, and *Amblyomma cajennense* sensu stricto, *Amblyomma sculptum*, *Amblyomma nodosum* and *Amblyomma humerale*, occasionally. Surprisingly, a total of twelve males of *A. rotundatum* were collected. Here we report new records of association between cold-blooded animals and ticks and reinforce the absence of *A. dissimile* in the Caatinga, Cerrado and Atlantic Forest biomes.

\* Corresponding author.

E-mail address: [hermes.luz@ufma.br](mailto:hermes.luz@ufma.br) (H.R. Luz).

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**Table 1**  
Ticks collected from amphibians and reptiles in Brazil during the present study.

HOSTS			TICKS			LOCALITIES
Class, Family, Species	No. examined	No. infested (P)	Total No.	MI	Species (No. according to stages)	Municipality/State*
<b>AMPHIBIANS</b>						
<b>Bufoiidae</b>						
<i>Rhinella major</i>	8	4 (50.0)	10	2.5	<i>A. dissimile</i> (7F; 3 M)	Belém/PA
<i>Rhinella proboscidea</i> <sup>1, 2</sup>	3	2 (66.7)	6	3.0	<i>A. dissimile</i> (5F); <i>A. rotundatum</i> (1F)	Altamira/PA
<i>Rhinella marina</i> <sup>3</sup>	20	10 (50.0)	60	6.0	<i>A. dissimile</i> (30F; 18 M); <i>A. rotundatum</i> (11F); <i>A. nodosum</i> (1F)	Macapá/AP
<i>Rhinella margaritifera</i> <sup>4</sup>	3	1 (33.3)	5	5.0	<i>A. dissimile</i> (2F); <i>A. cajennense</i> s. (2F); <i>Ornithodoros</i> sp. (1 L)	Manaus/AM
<i>Rhinella jimi</i>	180	71 (39.4)	276	3.9	<i>A. rotundatum</i> (110 N; 88F); <i>Amblyomma</i> sp. (78 L)	Petrolina/PE; Ilha de Fernando de Noronha/PE
<b>REPTILES</b>						
<b>Teiidae</b>						
<i>Ameiva ameiva</i>	18	3 (16.7)	11	3.7	<i>A. rotundatum</i> (5 N; 4F); <i>Amblyomma</i> sp. (2 L)	Santo Amaro/MA, São Luís /MA, Urbano Santos/MA
<i>Kentropyx calcarata</i> <sup>1</sup>	5	1 (20.0)	1	1.0	<i>A. rotundatum</i> (1 N)	Unknown location
<i>Ameivula ocellifera</i> <sup>1</sup>	39	6 (15.4)	10	1.7	<i>A. rotundatum</i> (4 N; 2F), <i>Amblyomma</i> sp. (4 L)	São Luís/MA
<i>Cnemidophorus cryptus</i> <sup>1, 2</sup>	1	1 (100)	9	9.0	<i>A. rotundatum</i> (6 N; 1F); <i>A. dissimile</i> (2F)	Açailândia/MA
<i>Iguana iguana</i>	6	6 (100.0)	22	3.5	<i>A. rotundatum</i> (3 N; 6F; 9 M); <i>A. dissimile</i> (1 N); <i>Amblyomma</i> sp. (3 L)	São Luís/MA; Anajatuba/MA Marabá/PA
<i>Tupinambis teguixim</i>	9	5 (55.6)	14	2.8	<i>A. dissimile</i> (5 M; 9F)	Macapá/AP
<b>Scincidae</b>						
<i>Mabuya bistrata</i> <sup>1</sup>	2	2 (100)	3	1.5	<i>A. rotundatum</i> (2 N); <i>Ornithodoros</i> sp. (1 L)	Urbano Santos/MA
<b>Gymnophthalmidae</b>						
<i>Colobossaura modesta</i> <sup>1</sup>	1	1 (100)	1	1.0	<i>A. rotundatum</i> (1 N)	São José do Ribamar/MA
<b>Tropiduridae</b>						
<i>Tropidurus hispidus</i>	7	2 (28.6)	5	2.5	<i>A. rotundatum</i> (3 N); <i>Amblyomma</i> sp. (2 L)	Chapadinha/MA; Barreirinhas/MA
<b>Amphisbaenidae</b>						
<i>Leposternon polystergum</i>	1	1 (100)	1	1.0	<i>Amblyomma</i> sp. (1 L)	São Luís/MA
<b>Dipsadidae</b>						
<i>Thamnodynastes</i> sp. <sup>1</sup>	1	1 (100)	3	3.0	<i>A. rotundatum</i> (3F)	Salgueiro/PE
<i>Boiruna sertaneja</i> <sup>1</sup>	2	2 (100)	2	1.0	<i>A. rotundatum</i> (2F)	Unknown location
<b>Viperidae</b>						
<i>Crotalus durissus</i>	2	1 (50.0)	12	12.0	<i>A. rotundatum</i> (6 N; 4F); <i>Amblyomma</i> sp. (2 L)	Pinheiro/MA
<i>Bothrops jararacussu</i>	28	5 (17.9)	10	2.0	<i>A. rotundatum</i> (3 N; 7F)	Unknown location
<i>Bothrops leucurus</i> <sup>5</sup>	1368	49 (3.6)	73	1.5	<i>A. rotundatum</i> (4 N; 67F); <i>A. sculptum</i> (1 M; 1F)	Itajui; Itapetinga; Santa Cruz Cabralia; Itagibá; Aiquara; Itagi; Jequié; Itaquara; Mucuri; Aiquara; Valença; Almadina; Ibirataia; Mucuri; Itororó; Itamaraju; Jussari; Jequié; Iguaf; Ipiatú; Iguaf; Nova Viçosa; Coaraci; Barra do Rocha; Mucuri; Firmino Alvez; Guaratinga; Boa Nova; Ilhéus (all in BA)
<i>Bothrops erythromelas</i>	10	3 (30.0)	5	1.7	<i>A. rotundatum</i> (1F); <i>Amblyomma</i> sp. (4 L)	Cabrobó/PE
<i>Bothrops insularis</i>	78	25 (32.0)	335	13.4	<i>A. rotundatum</i> (260 N; 75F)	Ilha da Queimada Grande/SP
<i>Lachesis muta</i>	167	5 (3.0)	13	2.6	<i>A. rotundatum</i> (13F)	Itacaré/BA; Ibicarai/BA
<b>Boidae</b>						
<i>Eunectes murinus</i> <sup>2</sup>	2	2 (100)	6	3.0	<i>A. dissimile</i> (6F)	Macapá/AP
<i>Epicrates</i> sp.	1	1 (100)	2	2.0	<i>A. rotundatum</i> (2 N)	Macapá/AP, Juazeiro/BA
<i>Boa constrictor</i>	5	2 (40.0)	12	6.0	<i>A. rotundatum</i> (8 N; 1F; 3 M)	Santa Inês/MA; Anajatuba/MA; Petrolina/PE
<b>Colubridae</b>						
<i>Chironius bicarinatus</i> <sup>1</sup>	2	1 (50.0)	2	2.0	<i>A. rotundatum</i> (2 N)	Unknown location
<b>Testudinidae</b>						
<i>Chelonoidis denticulata</i>	3	3 (100)	17	5.7	<i>A. humerale</i> (5 M; 12F)	Serra do Navio/AP; Santa Inês/MA
<i>Chelonoidis carbonara</i>	1	1 (100)	1	1.0	<i>A. rotundatum</i> (1F)	Juazeiro/BA
<b>TOTAL</b>	1976	217 (10.8)	920	4.3	98 L, 421 N, 401A	

MI: mean intensity of infestation (Total No. of ticks/No. of infested hosts); L: larvae; N: nymphs; F: females; M: males; A: adults.

\*State abbreviations: PA: Pará; AP: Amapá, AM: Amazonas; PE: Pernambuco; MA: Maranhão; SP: São Paulo.

Superscript Nos. mean new host records for *A. rotundatum*<sup>1</sup>, *A. dissimile*<sup>2</sup>, *A. nodosum*<sup>3</sup>, *A. cajennense* s.s.<sup>4</sup> and *A. sculptum*<sup>5</sup>.

Additionally, we report new records of *A. rotundatum* males on reptiles in the Amazon biome. This last record allows us to speculate about a possible association of *A. rotundatum* males with reptiles and the Amazon biome.

### 1. Introduction

In Neotropics, cold-blooded animals (amphibians and reptiles) are parasitized by a variety of ticks, mainly *Amblyomma rotundatum* and *Amblyomma dissimile* (Ixodidae) (Guglielmone and Nava, 2010; Luz and Faccini, 2013; Guglielmone et al., 2014; Nava et al., 2017). In Brazil, *A. rotundatum* is generally present in all major biomes, whereas *A. dissimile* seems to be restricted to Amazon and Pantanal biomes (Polo et al., 2021). In addition to these two species of ixodids, new associations of cold-blooded animals and argasids (e.g., *Ornithodoros*) have drawn attention in the country, with two new species of *Ornithodoros* being recently described: *Ornithodoros faccinii* and *Ornithodoros saraivai* (Barros-Battesti et al., 2015; Muñoz et al., 2017; Alcantara et al., 2018).

While *A. dissimile* is known to be a bisexual species, *A. rotundatum* is known to reproduce only by parthenogenesis (Barros-Battesti et al., 2006; Nava et al., 2017). However, during the last two decades, male specimens of *A. rotundatum* have been occasionally recorded from different species of reptiles from a few locations in the Amazon biome of Brazil (Labruna et al., 2005; Martins et al., 2014; Silva et al., 2016; Gianizella et al., 2018; Costa et al., 2020).

Although there is an extensive list of studies of the parasitism by ticks on amphibians and reptiles in Brazil, additional data are needed if we consider its territorial extension (8516,000 km<sup>2</sup>), phytophysognomy

(six biomes and 74 distinct ecoregions) and its herpetological fauna (~1130 amphibian and 795 reptile species) (MMA, 2018; Renctas, 2023). In addition, many ticks associated with amphibians and reptiles have records of infection by bacterial and protozoal agents, some associated with diseases of animals and humans (Arthur, 1962; Petit et al., 1990; Khuo et al., 2000; Stenos et al., 2003; Adoh et al., 2015; Luz et al., 2018; Ogrzewalska et al., 2018; Sánhes-Montes et al., 2019). These ectoparasites also cause serious injuries, such as hemorrhages and abscesses that can lead to secondary infection (Luz et al., 2015). Therefore, new information of the association between ticks and cold-blooded animals, may help in future conservation measures for these hosts, particularly those most vulnerable to extinction (e.g., world amphibian extinction crisis). This study presents new records on the relationship ticks with amphibians and reptiles in Brazil, including males of *A. rotundatum*.

### 2. Materials and methods

Ticks were collected from amphibians and reptiles deposited in the Herpetological Collection of the Federal University of Maranhão (UFMA), São Luís (Maranhão State), the State University of Santa Cruz (UESC), Ilhéus (Bahia State), and the Federal University of São Francisco Valley (Univasf), Petrolina (Pernambuco State). Additionally, ticks were collected from amphibians and reptiles captured and road-killed in the

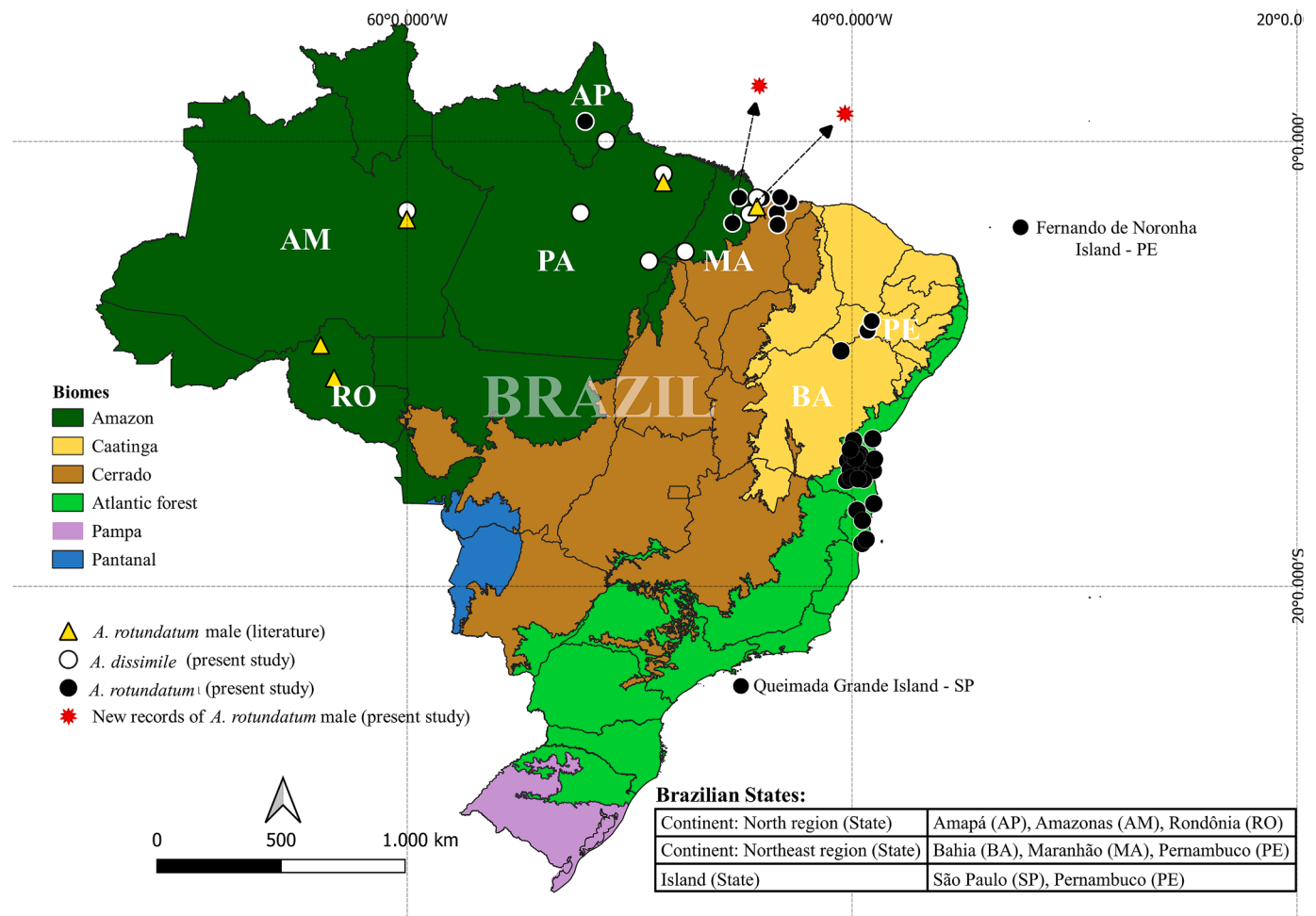
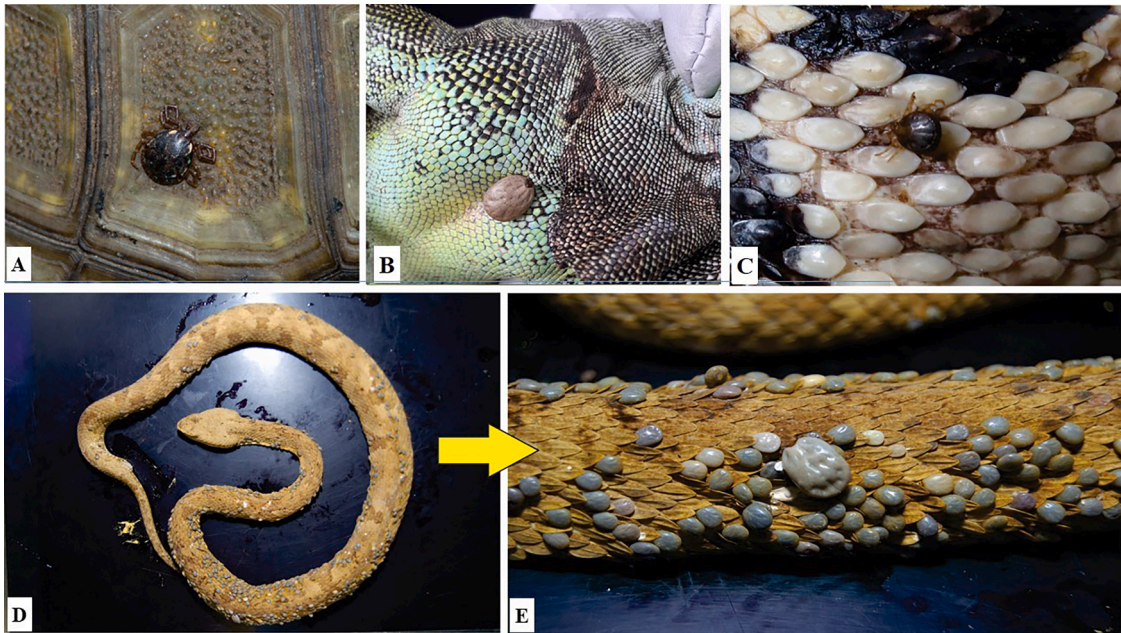


Fig. 1. Distribution of *A. rotundatum* and *A. dissimile* parasitizing cold-blooded animals in the present study, Brazil.

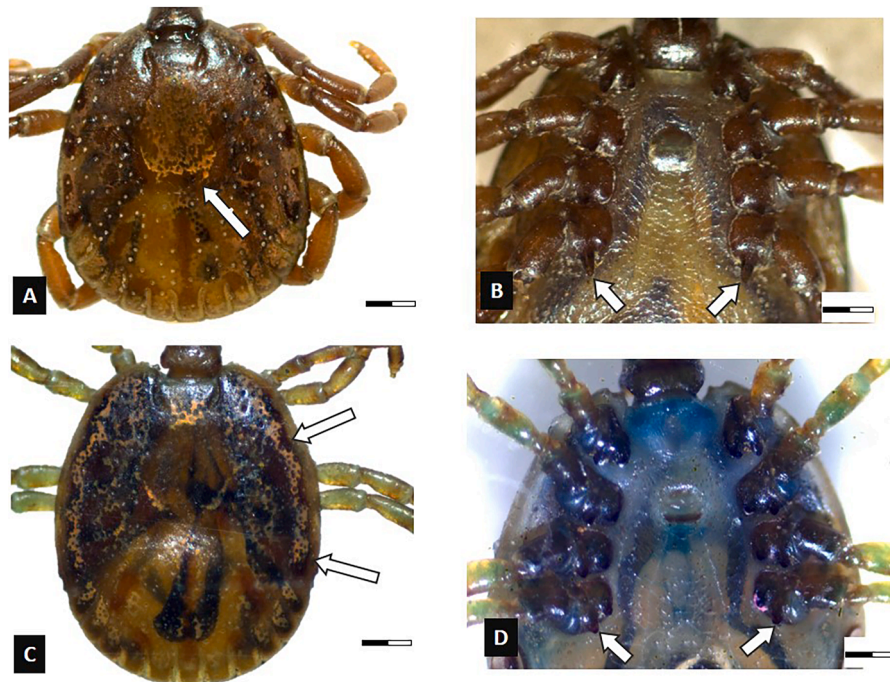


**Fig. 2.** *Amblyomma* spp. parasitizing cold-blooded animals in the present study. *Amblyomma humerale* on *Chelonoidis denticulata* (A); *Amblyomma rotundatum* on *Iguana iguana* (B), *Lachesis muta* (C), *Bothrops insularis* (D and E).

Amazon biome, at Maranhão and Amapá States. In the municipality of São Luís, some tick specimens were collected directly from an *Iguana iguana* specimen killed by a domestic dog in the UFMA campus. The locations and geographic positions according to Brazilian states are shown in Table 1 and Fig. 1.

Amphibians and reptiles were inspected for the presence of ticks, which were collected with tweezers and preserved in labelled vials containing 70% ethanol at room temperature. Ticks from herpetological collections were collected only when attached to hosts. Free ticks in

alcohol or formaldehyde were identified but not included in statistical analyses. Ticks were collected and stored in 70 % alcohol for later morphological identification (nymphs and adults), following the taxonomic keys and literature of Barros-Battesti et al. (2006), Martins et al. (2010), Luz et al. (2018) and Dantas Torres et al. (2019), under the Zeiss stereomicroscope (50x). A total of 96 larvae of *Amblyomma* were identified at the genus level due to the absence of a specific key for this *Amblyomma* stage. Although there are specific morphological keys for a variety of *Ornithodoros* spp. larvae, little is known about the taxonomy of



**Fig. 3.** Morphology of *Amblyomma dissimile* and *Amblyomma rotundatum* males: (A) dorsal view of *A. dissimile*: scutum with whitish, copper-colored ornamentation, covering most of its surface, and formation of a pseudoscutum (arrow); (B) ventral view of *A. dissimile*: thigh IV with a medium-sized, external pointed spur (arrow). (C) dorsal view of *A. rotundatum*: scutum densely punctuated (especially on the periphery), ornamented with bright paleo-orange spots present along the margins (arrow); (D) Coxae I to IV with two short, rounded spine (arrow). Scale bar 500 µm.

this group associated with cold-blooded animals in Brazil, with descriptions only for the species *O. faccinii* (Barros-Battesti et al., 2015) and *O. saravai* (Muñoz-Leal et al., 2017). Thus, *Ornithodoros* larvae in the present study were identified at the genus level only. Specimens of *A. rotundatum* (male and female) and *A. dissimile* (male and female) were photographed under a Zeiss stereomicroscope (Fig. 2).

Prevalence of infestation (P) and mean intensity of infestation (MI) were calculated following the recommendations of Bush et al. (1997). Map with the locations were made using the Qgis program. The samplings were authorized by SISBIO under number 83,484–1, 48,102–2.

### 3. Results

Overall, 214 amphibians and 1762 reptiles were examined, totaling 1976 specimens. Of this total, 217 (10.8 %) vertebrate specimens were parasitized by ticks distributed in five species of amphibians and 25 species of reptiles. The prevalence of infestation ranged from 3.0 to 100 %, and the overall MI was 4.3 ticks/infested host (Table 1). Among the amphibians, *Rhinella jimi* was the most captured (180 specimens) and also the most parasitized by ticks (71 specimens). However, the highest MI was registered on *Rhinella marina* (60 ticks and MI: 6.0). Among the reptiles, *Bothrops leucurus* was the most parasitized (49 specimens) and was also the species with the highest number of specimens examined, totaling 1368 specimens. The reptiles *Bothrops insularis* and *Crotalus durissus* had the highest MI values, 13.4 and 12.0, respectively (Table 1).

A total of 927 ticks were collected: 98 larvae, 421 nymphs and 408 adults. After morphological analysis, two genera were identified, *Amblyomma* (918 specimens) and *Ornithodoros* (two larvae). Six species of ticks were identified: *A. rotundatum* (420 nymphs, 287 females, and twelve males) and *A. dissimile* (one nymph, 61 females, and 26 males) as the most frequent and abundant, and then occasionally the species *Amblyomma cajennense* sensu stricto (two females), *Amblyomma sculptum* (one female, one male), *Amblyomma nodosum* (one female) and *Amblyomma humerale* (12 females, five males) (Table 1).

Twelve males of *A. rotundatum* were collected, all from reptiles captured in the Amazon biome: nine on *Iguana iguana* and three on *Boa constrictor* (Table 1). The males of *A. rotundatum* were identified according to the following morphological characters: oval body shape; absent marginal groove; scutum densely punctuated (especially on the periphery), ornamented with bright paleo-orange spots present along the margins of the scutum; dorsal base of capitulum rectangular, with short cornua; hypostome with dental formula 3/3; coxae I to IV with two short, rounded spine (Fig. 3).

Co-infestations occurred in one specimen of *Rhinella proboscidea* (*A. rotundatum* and *A. dissimile*), one of *Rhinella margaritifera* (*A. rotundatum*, *A. dissimile*, *A. cajennense* s.s. and *Ornithodoros* sp.), one *Cnemidophorus cryptus* (*A. rotundatum* and *A. dissimile*), one *I. iguana* (*A. rotundatum* and *A. dissimile*), one *Mabuya bistriata* (*A. rotundatum* and *Ornithodoros* sp.), one *B. leucurus* (*A. rotundatum* and *A. sculptum*) (Table 1).

New tick-host associations occurred on both reptiles and amphibians for *A. rotundatum*, *A. dissimile*, *A. nodosum*, *A. cajennense* s.s. and *A. sculptum* (Table 1).

### 4. Discussion

As already observed in numerous studies on the association of cold-blooded animals in the Neotropics (Guglielmo and Nava, 2010; Luz et al., 2018; Orgzewaska et al., 2018; Polo et al., 2021), *A. rotundatum* and *A. dissimile* were the most frequent species in the present work.

*Amblyomma dissimile* has a wide distribution that extends from northern Argentina to southern Mexico, the Caribbean Islands, and southern Florida (Nava et al., 2017). This tick has a restricted distribution in Brazil (Amazon and Pantanal biomes) and is constantly confused with *A. rotundatum* due to its morphological similarity (Luz et al., 2018; Polo et al., 2021). Therefore, reports outside these biomes in Brazil must

be viewed with caution as they could represent misidentifications (Luz et al., 2018, 2021). As mentioned above, this ixodid parasitizes almost exclusively amphibians and reptiles, but there are some reports parasitizing mammals: *A. dissimile* parasitizing Bovidae, Tayassuidae, Didelphidae, Homnidae, Leporidae, Capromyidae, Caviidae, Cricetidae, Dasyproctidae and Echimyidae; *Amblyomma rotundatum* parasitizing Suidae, Procyonidae, Phyllostomidae, Dasypodidae, Homnidae, Bradypodidae, Myrmecophagidae and Caviidae (Guglielmo and Nava, 2010; Luz and Faccini, 2013; Nogueira et al., 2022). Seven species of amphibians have records associated with *A. dissimile*, all *Rhinella* spp.: *Rhinella bergi*, *Rhinella granulosa*, *Rhinella jimi*, *Rhinella major*, *R. margaritifera*, *R. marina*, and *Rhinella schneideri* (Guglielmo and Nava, 2010; Kelehear et al., 2017; Pedroso-Santos et al., 2020). Here we present the first record of *A. dissimile* on *Rhinella proboscidea*. Reptiles are one of the main groups of vertebrates associated with *A. dissimile*. For example, in the Amazon biome, *A. dissimile* has reports parasitizing only three species of amphibians (*R. marina*, *R. major* and *R. margaritifera*), with the largest number of records (26 species) on reptiles Boidae, Viperidae, Elapidae, Colubridae, Dipsadidae, Testudinidae, Teiidae, Iguanidae, Dactyloidae, Dactyloidae and Alligatoridae (Kelehear et al., 2017; Luz et al., 2021). The parasitism of *A. dissimile* on *C. cryptus* and *Eunectes murinus* is unprecedented.

*Amblyomma rotundatum* occurs from Florida to Argentina, and in Brazil it is the most common tick parasitizing amphibians and reptiles (Labruna et al., 2005; Guglielmo and Nava, 2010; Guglielmo et al., 2014; Luz and Faccini, 2013), may eventually parasitize mammals (Luz and Faccini, 2013; Guglielmo et al., 2014). This ixodid has high ecological adaptability, being found in all Brazilian regions (Polo et al., 2021), therefore adapted to different microclimatic conditions. In the present study, specimens of *A. rotundatum* were recorded in the four biomes studied (Amazon, Cerrado, Caatinga and Atlantic Forest), parasitizing both amphibians and reptiles. So far, this ixodid has parasitic association with 14 species of amphibians in Brazil, mostly of the genus *Rhinella*: *Rhinella arenarum*, *R. bergi*, *Rhinella crucifer*, *Rhinella diptycha*, *Rhinella gildae*, *Rhinella granulosa*, *Rhinella icterica*, *R. jimi*, *Rhinella major*, *R. margaritifera*, *R. marina*, *Rhinella ornate*, *Rhinella pygmaea*, and *R. schneideri* (Guglielmo and Nava, 2010; Luz and Faccini, 2013; Alcantara et al., 2018; Luz et al., 2018; Oda et al., 2018; Oliveira et al., 2018; Costa et al., 2020; Fonseca et al., 2020; Luz et al., 2021). Therefore, the record of *A. rotundatum* on *R. proboscidea* in our study is unprecedented. Parasitizing reptiles, *A. rotundatum* has been recorded on ~ 64 species of reptiles being snakes of the genus *Bothrops*. The most frequently parasitized by this ixodid (Guglielmo and Nava, 2010; Alcantara et al., 2018; Oda et al., 2018; Costa et al., 2020; Fonseca et al., 2020; Luz et al., 2021). In the present study, reptiles *Kentropyx calcarata*, *A. ocellifera*, *C. cryptus*, *Mabuya bistriata*, *Colobossaura modesta*, *Thamnodynastes* sp., *Boiruna sertaneja*, *Bothrops insularis* and *Chironius bicarinatus* are reported for the first time in association with *A. rotundatum*.

Twelve males of *A. rotundatum* were collected in our study, three on *B. constrictor* and nine on *I. iguana*. The males of *A. rotundatum* were identified according with Labruna et al. (2005). As mentioned earlier, the tick *A. rotundatum* is an obligate parthenogenetic species, with rare records of males in nature. The first report of a male of this species in Brazil occurred in the municipality of Monte Negro, Rondonia state, one specimen parasitizing *Tropidurus* sp. (Squamata: Tropiduridae) (Labruna et al., 2005). The second also occurred in Rondônia state in the municipality of Porto Velho, a male specimen parasitizing *B. constrictor* (Martins et al., 2014). Later, Silva et al. (2016) reported a male of *A. rotundatum* on *I. iguana* in the municipality of Baião (Pará state) and Gianizella et al. (2018) found two males on *Chelonoidis denticulatus* in the municipality of Manaus, Amazonas state. Recently, Costa et al. (2020) reported one male of *A. rotundatum* on *Bothrops atrox* municipality of São Luís, Maranhão state. Here we report twelve more records of *A. rotundatum* males parasitizing reptiles in the Maranhão state. Therefore, indicating the possibility of a bisexual population of *A. rotundatum* in the Amazon biome associated with reptiles. It is

noteworthy that all records to date, including the current study, were on reptiles and the Amazon biome, indicating a possible relationship with type of setting (e.g., environment and host). Although studies are needed, here we hypothesize that small bisexual populations (males and females) of *A. rotundatum* may be parasitizing reptiles in restricted areas of the Amazon, and exclusively parthenogenetic populations would be in the rest of the country parasitizing amphibians and reptiles, and occasionally mammals. This hypothetical view resembles the behavior of the parthenogenetic tick *Haemaphysalis longicornis* in Asia and Oceania, which exhibits a heterogeneous spatial distribution between its bisexual and parthenogenetic populations (Chen et al., 2014). Additionally, parthenogenetic populations of *H. longicornis* are less demanding about the type of habitat, easily invading and establishing in new areas (USDA, 2019). In fact, exclusively parthenogenetic populations of *A. rotundatum* are widely distributed in Brazil.

A total of five males and twelve females of *A. humerale* were collected on *Chelonoidis denticulata* in the Amazon biome, corroborating Luz et al. (2021) who mention adults of this ixodid on reptiles in this biome. Occasional records were made for ticks that preferentially parasitize warm-blooded animals (birds and mammals): *A. sculptum* on *B. leucurus*, *A. cajennense* s.s. on *R. margaritifera*, *A. nodosum* on *R. marina* and *Ornithodoros* sp. on *R. margaritifera* and *M. bistrata*. Although there are reports of *A. sculptum* and *A. nodosum* parasitizing reptiles in Brazil (Mendoza-Roldan et al., 2020), in our study these species were reported for the first time parasitizing *B. leucurus* and *R. marina*, respectively. Furthermore, this is the first record of the association of *A. nodosum* with amphibians in a natural environment. Parasitism by *A. cajennense* s.s. on *R. margaritifera* was also unprecedented.

All the species of ticks parasitizing amphibians and reptiles in the present study have records of infection by at least one bacterial or protozoan agent in the literature, with unknown pathogenicity for these hosts: *Rickettsia bellii* detected in *A. dissimile*, *A. rotundatum*, *A. nodosum* and *A. humerale*; ‘*Candidatus Rickettsia colombianensi*’ in *A. dissimile*; *Rickettsia amblyommatis* in *A. humerale* and *A. cajennense* s.s.; *Anaplasma* sp. and *Hepatozoon* sp. in *A. dissimile*; *Hemolivia stellata* in *A. rotundatum* and *A. dissimile*; *Rickettsia parkeri* in *A. nodosum* and *A. sculptum*; and *Rickettsia rickettsii* and *Borrelia* sp. in *A. sculptum* (Labruna et al., 2004; Ogrzewalska et al., 2009, 2018; Miranda et al., 2012; Horta et al., 2015; Luz et al., 2018; Cotes-Perdomo et al., 2018; Higa et al., 2020). As far as we know, none of the above agents have been proven to cause disease in amphibians and reptiles, except for *H. stellata*, which can cause damage in toads. Other tick species reported to parasitize amphibians and reptiles in Brazil are infected with agents of unknown pathogenicity for vertebrates, for example, infection by *Rickettsia* sp. strain Itinguçú on *O. faccinii*, *Rickettsia* sp. and *Borrelia* sp. in *Ornithodoros mimon*, *Hepatozoon* sp. in *Amblyomma fuscum* and *R. bellii* in *Amblyomma goeldii* (Blanco et al., 2017; Peixoto et al., 2021; Muñoz-Leal et al., 2021).

Finally, with the exception of *A. nodosum* all species in the present study have records parasitizing humans in Brazil (Nogueira et al., 2022), with highlight for vectors of *Rickettsia* from the spotted fever group *A. cajennense* s.s. and *A. sculptum*. The Amazonian tick *A. cajennense* s.s. is the vector of *R. amblyommatis* in this biome, but its pathogenicity for humans is still controversial (Richardson et al., 2023). Therefore, understanding of the relationship between ticks, agents (bacteria, protozoa, etc.) and cold-blooded animals can be a very useful tool for the conservation and/or preservation of these vertebrates and in preventing the spread and introduction of disease to animals and man. For example, studies show that ticks can regulate toad populations (Lampo and Bayliss, 1996; Smith et al., 2008). According to Aragão (1912), under experimental conditions, parasitism with 10 adult ticks can be fatal for a toad.

In that regard, Brazil has one of the main amphibian and reptile trafficking routes in the world, where 70 % are destined for local trade and ~30 % for other countries. Among the taxonomic groups, *Crotalus* sp., *Micrurus* sp., *Bothrops* sp., *Boa* sp., *Tupinambis* sp. and *Lachesis* sp. are among the most coveted worldwide (Renctas, 2023).

## 5. Conclusion

Here we report new records of association between cold-blooded animals and ticks and reinforce the absence of *A. dissimile* in the Caa-tinga, Cerrado and Atlantic Forest biomes. Additionally, we report new records of *A. rotundatum* males on reptiles in the Amazon biome. This last record allows us to speculate about a possible bisexual population of *A. rotundatum* in the Amazon biome associated with reptiles.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## CRediT authorship contribution statement

**Hermes Ribeiro Luz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Carlos Eduardo Costa de Campos:** Methodology, Data curation, Conceptualization. **Livio Martins Costa-Junior:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Ercileide Silva Santos:** Methodology, Conceptualization. **Adriani Hass:** Methodology, Investigation, Conceptualization. **Dauana Mesquita Sousa:** Methodology, Formal analysis, Conceptualization. **Ruth Myrian de Moares e Silva:** Methodology, Conceptualization. **Josiane Moreira Rocha:** Methodology, Conceptualization. **George Rego Albuquerque:** Methodology, Conceptualization. **Antonio Jorge Argolo:** Methodology, Conceptualization. **Rogério Zacariotti:** Methodology, Conceptualization. **Catia Dejuste de Paula:** Methodology, Conceptualization. **Luiz Cezar Machado Pereira:** Methodology, Conceptualization. **Patricia Avello Nicola:** Methodology, Conceptualization. **João Luiz Horacio Faccini:** Writing – original draft, Validation, Methodology. **Mauricio Claudio Horta:** Validation, Methodology, Formal analysis, Conceptualization. **Rafael Michael Silva Nogueira:** Methodology, Conceptualization. **Leonardo Teixeira Dallagnol:** Writing – original draft, Methodology, Conceptualization. **Marcelo Bahia Labruna:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Thiago Fernandes Martins:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization.

## Declaration of competing interest

The authors declare no conflict of interest.

## Data availability

All relevant data generated during this study are included in the article.

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