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Microorganisms in the ticks *Amblyomma dissimile* Koch 1844 and *Amblyomma rotundatum* Koch 1844 collected from snakes in Brazil

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Abstract. Knowledge about ticks (Acari) and screening of ticks parasitizing various hosts are necessary to understand the epidemiology of tick-borne pathogens. The objective of this study was to investigate tick infestations on snakes (Reptilia: Squamata: Serpentes) arriving at the serpentarium at the Institute Vital Brazil, Rio de Janeiro. Some of the identified ticks were individually tested for the presence of bacteria of the genera *Rickettsia* (Rickettsiales: Rickettsiaceae), *Borrelia* (Spirochaetales: Spirochaetaceae), *Coxiella* (Legionellales: Coxiellaceae), *Bartonella* (Rhizobiales: Bartonellaceae), *Ehrlichia* (Rickettsiales: Anaplasmataceae), *Anaplasma* (Rickettsiales: Anaplasmataceae), and Apicomplexa protozoa of the genera *Babesia* (Piroplasmida: Babesiidae) and *Hepatozoon* (Eucoccidiorida: Hepatozoidae). A total of 115 hard ticks (Ixodida: Ixodidae) were collected from 17 host individuals obtained from four Brazilian states. Two species of tick were identified: *Amblyomma dissimile* Koch 1844 (four larvae, 16 nymphs, 40 adults), and *Amblyomma rotundatum* Koch 1844 (12 nymphs, 43 adults). *Rickettsia bellii* was found in *A. rotundatum* and *A. dissimile* ticks and *Rickettsia* sp. strain Colombianensi, *Anaplasma*-like and *Hepatozoon* sp. in *A. dissimile* ticks. Among the tested ticks, no DNA of *Borrelia*, *Bartonella*, *Coxiella* or *Babesia* was found. The present findings extend the geographic range of *Rickettsia* sp. strain Colombianensi in Brazil and provide novel tick–host associations.

Key words. *Amblyomma*, *Rickettsia*, ectoparasites, snakes.

Introduction

Ticks (Acari) are parasitic arthropods found in all terrestrial regions of the planet that feed on the blood of a variety of mammals, birds, reptiles and amphibians. Globally, approximately 900 species of tick are recognized, of which about 700 species are hard ticks (Ixodida: Ixodidae) and 200 species are soft ticks (Ixodida: Argasidae) (Guglielmone *et al*., 2014). Some of them are known to be vectors of a number of pathogens that affect both humans and animals (Jongean & Uilenberg, 2004). At present, the Brazilian tick fauna is represented by 70 species, of which 32 belong to the genus *Amblyomma* (Ixodida: Ixodidae) (Onofrio *et al*., 2006; Labruna *et al*., 2016).

The Brazilian snake fauna is represented currently by 392 species (Caldeira Costa & Bérnils, 2015). Snakes, like any other vertebrates, are susceptible to a wide array of parasites, including ticks. However, little is known about the ectoparasitic fauna of snakes in South America. The most common ticks found on snakes in Brazil are *Amblyomma dissimile* Koch 1844 and *Amblyomma rotundatum* Koch 1844. These species are similar and have vast geographic distributions across South and Central America (Guglielmone *et al*., 2014). Sporadically, other species

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are found, such as nymphs of *Amblyomma cajennense* (Fabricius, 1787) *sensu lato*, *Amblyomma fuscum* Neumann 1899 and immature *Amblyomma* spp. (Szabó *et al*., 2007; Dantas-Torres *et al*., 2008).

The most important zoonotic disease transmitted by ticks in Brazil is Brazilian spotted fever, caused by *Rickettsia rickettsii* and *Rickettsia* sp. strain Atlantic Forest, although several other *Rickettsia* species and strains have been described in recent years in Brazilian ticks (Labruna, 2009; Blanco *et al*., 2017) and the existence of new tick-borne human pathogens in Brazil is probable. Moreover, various other genera of bacteria and protozoa have been described among South American ticks and their vertebrate hosts. However, the subject of ticks parasitizing snakes and the pathogens found in them has been neglected.

The Institute Vital Brazil, located in the city of Niteroi in the state of Rio de Janeiro, maintains snakes in a serpentarium in order to facilitate the extraction of venom for medical and scientific research. The Institute regularly receives individuals from throughout Brazil, mainly of venomous species. Before being submitted to the serpentarium, all specimens are examined and their health parameters, including the presence of ectoparasites, are verified. In this context, the current study aimed to evaluate the presence of ticks parasitizing these snakes and to screen the ticks for the presence of the following pathogens with potential impact on human and animal health: bacteria of the genera *Rickettsia*, *Ehrlichia*, *Anaplasma*, *Bartonella*, *Coxiella* and *Borrelia*, and Apicomplexa protozoa of the genera *Babesia* and *Hepatozoon*.

Materials and methods

During 2016–2018, during routine health evaluations of snakes arriving at the Institute Vital Brazil, ticks were collected from the animals. Snakes were evaluated for ticks immediately on arrival at the Institute and all ectoparasites were stored in plastic tubes in 96% ethanol.

Adults and nymphs of *Amblyomma* species were identified following dichotomous keys prepared by Onofrio *et al*. (2006) and Martins *et al*. (2010), respectively. Larvae of *Amblyomma* were identified by molecular analysis as previously described (Ogrzewalska *et al*., 2012). For this purpose, representative specimens of each tick species were submitted to DNA extraction using the QIAamp® DNA Mini Kit (Qiagen, Inc., Germantown, MD, U.S.A.) and tested by a polymerase chain reaction (PCR) assay targeting a portion of the tick mitochondrial 16S rRNA gene, as previously described (Mangold *et al*., 1998). Amplicons (~460 bp) were visualized on 1% agarose gels stained with GelRed Nucleic Acid Gel Stain™ (Biotium, Inc., Fremont, CA, U.S.A.) 10 000× in DMSO. All PCR products of the expected size were purified with GE Healthcare Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Chicago, IL, U.S.A.) and sequenced in a 96-capillary 3730xl DNA Analyzer® (Thermo Fisher Scientific Corp., Waltham, MA, U.S.A.) according to the protocols developed by Otto *et al*. (2008). Partial sequences obtained were submitted to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and were aligned with corresponding 16S rRNA sequences of different tick species available in GenBank. Some nymphs and adults in which part of the gnathosoma was destroyed, making taxonomic identification impossible, were submitted to the same molecular identification procedure.

Some of the ticks were individually tested by a battery of PCR assays targeting bacteria of the genera *Rickettsia*, *Borrelia*, *Ehrlichia*, *Anaplasma*, *Bartonella* and *Coxiella*, and protozoa of the genera *Babesia* and *Hepatozoon*. DNA extraction was performed as described above. Blank tubes containing water were always included for contamination control during DNA extraction. All PCR procedures were performed with the genus-specific primers shown in Table 1. All reactions, except those for *Bartonella* and *Coxiella*, were performed with 25 μL per reaction, which contained 12.5 μL of DreamTaq™ Green PCR Master Mix, 8.0 μL of nuclease-free water, 1 μL of each primer at 10 μM (Invitrogen Corp., Carlsbad, CA, U.S.A.), and 2.5 μL of template DNA. In nested PCRs, 9.0 μL of nuclease-free water and 1.5 μL of template DNA were used instead. Screening for *Coxiella*-like infection was performed using the 16S rRNA gene following the protocol described by Duron *et al*. (2015a). For *Bartonella* investigation, DNA samples were screened to target the *gltA* gene, following the technique developed by Rozental *et al*. (2017). In each PCR assay, negative controls (water) and an appropriate positive control sample (DNA of *R. rickettsii*, *Ehrlichia canis*, *Bartonella hanselae*, *Coxiella burnetii*, *Borrelia anserine* or *Babesia vogeli*) were run together with the tick samples. The protocols for purification and sequencing of products were as described above.

Partial DNA sequences obtained from the amplified PCR products *gltA* and *ompA* were aligned with the corresponding sequences of other *Rickettsia* species available in the GenBank database. The dataset alignment was generated with the ClustalW algorithm [a tool integrated in MEGA Version 6.0 (Tamura *et al*., 2013)] and edited manually for optimization. The best-fit evolutionary model was determined using a Model test, another tool integrated in MEGA Version 6.0 (Tamura *et al*., 2013). The *Rickettsia* phylogenetic tree was estimated by maximum likelihood methods with MEGA Version 6.0. The supports for the tree nodes were calculated with 1000 bootstrap replicates.

All procedures involving snakes had been previously approved by the institution's ethics committee for animal research. Permits for field collection were granted by the Chico Mendes Institute for Biodiversity Conservation (ICMBIO Authorization 13373).

Results

A total of 115 hard ticks (Ixodida: Ixodidae) were collected from 17 hosts (Table 2). Two species of tick were identified: *A. dissimile* (four larvae, 16 nymphs, 40 adults), and *A. rotundatum* (12 nymphs, 43 adults). In total, 11 *A. dissimile* (four larvae, four nymphs, three adults) and 18 *A. rotundatum* (five nymphs, 13 adults) were identified by molecular analyses. The mitochondrial 16S rDNA gene partial sequences matched conspecific sequences for *A. rotundatum* available in the GenBank database with ≥99.5–100% similarity. In the case of *A. dissimile*, only one partial sequence was available in GenBank (*A. dissimile* from Colombia, MF353128), and the similarity was 98.0% (403/411 nt). The partial sequences of the 16S

Table 1. Primers used in polymerase chain reaction assays.

Organism	Gene	Primers (5′-3′)				T, °C∗	Cycles, *n*	Product size, bp	References
		Forward		Reverse					
Anaplasmataceae	16S rRNA	EHR16SD	GGTACCYACAGA AGAAGTCC	EHR16SR	TGCACTCATCG TTTACAG	55	35	345	Inokuma *et al.* (2000)
Rickettsia spp.	*gltA*	CS-78	GCAAGTATCGGT GAGGATGTAAT	CS-323	GCTTCCTTAAAA TTCAATAAATC AGGAT	48	35	401	Labruna *et al.* (2004)
Rickettsia spp.	*ompA*	190.70	ATGGCGAATAT TTCTCCAAAA	190.701	GTTCCGTTAATGG CAGCATCT	58	35	632	Roux *et al.* (1996)
Coxiella spp.	16S rRNA	Cox16SF1	CGTAGGAATCT ACCTTRTAGWGG	Cox16SR2	GCCTACCCGCTTC TGGTACAATT	56	40	1321–1429	Duron *et al.* (2015a)
Coxiella spp.	16S rRNA	Cox16SF1	CGTAGGAATCTAC CTTRTAGWGG	Cox16SR1	ACTYYCCAACAGC TAGTTCTCA	56	35	719–826	Duron *et al.* (2015a)
Bartonella spp.	*gltA*	*gltA* F1	GCTATGTCT GCVTTCTATCAYGA	*gltA* R1	AGAACAGTAAAC ATTTCN GTHGG	58	40	731	Rozental *et al.* (2017)
Babesia and *Hepatozoon* spp.	18S rRNA	Bab18F1	GCGGTAATTCCAGC TCCAATAGCGTATAT	Bab18R1	TCCGAATAATTCA CCGGATCACTCGAT	63	25	1150	Blanco *et al.* (2017)
Babesia and *Hepatozoon* spp.	18S rRNA	Bab18F2	AGACGATCAGATAC CGTCGTAGTCCTA	Bab18R2	ATCACTCGATCGG TAGGAGCGACG	66	30	670	Blanco *et al.* (2017)
Borrelia spp.	*fla*	BorFlaF1	TACATCAGCTATTA ATGCTTCAAGAA	BorFlaR1	GCAATCATWGCCAT TGCRGATTG	65	25	729	Blanco *et al.* (2017)
Borrelia spp.	*fla*	BorFlaF2	CTGATGATGCTG CTGGWATGG	BorFlaR2	TCATCTGTCATTRT WGCATCTT	61	30	410	Blanco *et al.* (2017)

∗T, annealing temperature.

rDNA gene obtained in the present study were deposited in GenBank under accession numbers MG023141–MG023152.

Samples of nine females and one nymph of 31 (32.3%) *A. rotundatum* and one nymph sample of 32 (3.1%) *A. dissimile* yielded positive results in PCRs targeting the *gltA* gene. Further analyses showed the sequences obtained shared 99.5% (376/378 nt) sequence identity with *Rickettsia bellii* (CP015010, CP000087) (Table 2).

A total of 18 (56.2%) *A. dissimile* specimens collected from *Bothrops atrox* (Linnaeus 1758) (Squamata: Viperidae), captured in the Brazilian Amazon (Pará state), yielded positive results in PCRs targeting both the *gltA* and *ompA* genes. The PCR products from four samples were sequenced and the *gltA* and *ompA* partial sequences were 100% (379/379 nt) and 99.8% (586/587 nt), respectively, identical to partial sequences of *Rickettsia* sp. strain Colombianensi found in ticks in Colombia (MF034493, MF034497) (Table 2). Inference analysis of the two rickettsial genes showed that this *Rickettsia* sp. strain Colombianensi is in the same cluster as *Rickettsia* sp. strain Colombianensi found in *A. dissimile* and *A. cajennense* from Colombia, a finding supported by a high bootstrap value (100%) (Fig. 1).

Two specimens of *A. dissimile*, collected from *B. atrox* captured in the municipality of Santarém (Pará state), yielded positive results in PCRs targeting a fragment of the 16S rDNA gene specific for Anaplasmataceae. After sequencing of the PCR products, the partial sequences were 99.1% (340/343 nt) similar to a sequence for '*Candidatus* Cryptoplasma californiense' (KP276587, KP276586) recently found in *Ixodes pacificus* Cooley and Kohls 1943 (Ixodida: Ixodidae) ticks in North America, and 98.8% (339/343 nt) similar to various uncultured *Anaplasma* spp. found in *Haemaphysalis longicornis* Neumann 1901 (Ixodida: Ixodidae) in Asia (JN715833, GU075704).

Two other *A. dissimile* collected from *B. atrox* sampled in Santarém (Pará state) yielded positive results in PCRs targeting a fragment of the 18S rRNA gene. After sequencing of the PCR products, the partial sequences were 98.2% (614/625 nt) identical to *Hepatozoon* spp. previously detected in various birds of prey in Israel (MF541372) and marsupials in Chile (FJ719813), and 98.1% (613/625 nt) similar to *Hepatozoon* spp. found in the rattlesnake *Crotalus durissus terrificus* (Laurenti 1768) (Squamata: Viperidae) (KC342523) in Brazil.

Among the tested ticks, no DNA of *Borrelia*, *Bartonella*, *Coxiella* or *Babesia* was found. The partial sequences of agents detected are: MG437271 (18S rDNA of *Hepatozoon* sp.); MG437272 (16S rDNA of *Anaplasma*-like); MG550957 (*gltA* of *R. bellii*), and MG563768 and MG563769 (*gltA* and *ompA*, respectively, of *Rickettsia* sp. strain Colombianensi).

Discussion

In the current study, two tick species were found parasitizing snakes: *A. dissimile* and *A. rotundatum*. The present findings are supported by the current literature, which states that these ectoparasites are commonly found on cold-blooded vertebrates, including reptiles (Squamata) (Guglielmone *et al.*, 2014), and amphibians, especially toads (Anura: Bufonidae). Other hosts

Table 2. Tick species collected from snakes in the states of Pará (PA), Rio de Janeiro (RJ), Minas Gerais (MG) and São Paulo (SP), Brazil, 2016–2018.

Host (verified hosts, *n*)		Locality		Tick specimens collected by species and developmental stage, *n*		Pathogen infection			
Family	Species	State	Municipality	*A. dissimile*	*A. rotundatum*	*R. bellii*	*Rickettsia* sp. strain Colombianensi	*Anaplasma* sp.	*Hepatozoon* sp.
Boidae	*Boa constrictor* (1)	PA	Belém	5 N, 2 M		0/4	0/4	0/4	0/4
Viperidae	*Bothrops atrox* (1)	PA	Belém	4 L		1/4	0/4	0/4	0/4
	Bothrops atrox (3)	PA	Santarém	11 N, 14 F, 24 M		0/23	18/23	2/23	2/23
	Bothrops jararaca (1)	RJ	Niterói		3 F	0/3	0/3	0/3	0/3
	Bothrops jararaca (1)	MG	Governador Valadares		13 F, 1 N	3/7	0/7	0/7	0/7
	Bothrops jararaca (1)	MG	Teófilo Otoni		3 N	0/0	0/0	0/0	0/0
	Bothrops jararaca (1)	RJ	Cachoeiras de Macacu		1 F, 1 N	0/0	0/0	0/0	0/0
	Bothrops moojeni (1)	SP	Itapecerica da Serra		1 F	0/1	0/1	0/1	0/1
	Crotalus durissus (1)	MG	Curvelo		11 F	1/5	0/5	0/5	0/5
	Crotalus durissus (1)	MG	Itaúna		1 F	1/1	0/1	0/1	0/1
	Crotalus durissus (1)	MG	Pouso Alegre		13 F	5/12	0/12	0/12	0/12
Dipsadidae	*Xenodon merremi* (1)	MG	Curvelo		6 N	0/4	0/4	0/4	0/4
Elapidae	*Micrurus corallinus* (1)	RJ	Cachoeiras de Macacu		1 N	0/0	0/0	0/0	0/0
Total	**17**	**4**		**4 L, 16 N, 14 F, 26 M**	**12 N, 43 F**	**11/63**	**18/63**	**2/63**	**2/63**

A. dissimile, *Amblyomma dissimile*; *A. rotundatum*, *Amblyomma rotundatum*; *R. bellii*, *Rickettsia bellii*; F, female; L, larva; M, male; N, nymph.

such as Aves and Mammalia, including humans, are considered exceptional (Guglielmone *et al*., 2014). In the present study, *A. dissimile* ticks were collected only from snakes from the Amazon (Pará state) and *A. rotundatum* from snakes from the Cerrado (Minas Gerais state) and Atlantic Forest (São Paulo and Rio de Janeiro states) biomes. However, the present results may have been influenced by the low number of evaluated animals because both species occur in Nearctic and Neotropical regions according to the literature (Pontes *et al*., 2009; Guglielmone *et al*., 2014; Luz *et al*., 2018). Unlike *A. dissimile*, *A. rotundatum* is a parthenogenetic tick and few records of males exist (Santos da Silva *et al*., 2016), which is in concordance with the current results, in which only females and nymphs of *A. rotundatum* were observed.

Although it was not possible to calculate the prevalence of ticks in the present study as the total number of snakes evaluated was not available, it seems that tick parasitism is not very common. These results may have been influenced by the fact that snakes captured in the field sometimes spend several weeks in regional serpentaria and only later are sent to the Institute Vital Brazil, by which time ticks may have detached. However, the rare field studies that exist report that only a few individual snakes are parasitized by ticks in nature (Pontes *et al*., 2009; Viana *et al*., 2012).

Two distinct *Rickettsia* agents were found among the ticks: *R. bellii* and *Rickettsia* sp. strain Colombianensi. *Rickettsia bellii* has been found in 28 American tick species from distinct ecological regions, with diverse life histories (Parola *et al*., 2013; Costa *et al*., 2017; Krawczak *et al*., 2018; Santodomingo *et al*., 2018). Although there have been several previous reports of *R. bellii* infecting *A. rotundatum* ticks parasitizing toads in the Caatinga and Amazon biomes in Brazil and pet tortoises imported to Israel from Florida, U.S.A. (Labruna *et al*., 2004; Santos da Silva *et al*., 2016; Costa *et al*., 2017), there is only one report of *R. bellii* infecting *A. dissimile* in Colombia (Santodomingo *et al*., 2018). Although there has been serological evidence of domestic and wild animal exposure to *R. bellii* (Costa *et al*., 2017), at present, *R. bellii* is classified as an agent of unknown pathogenicity to humans and has never been detected in vertebrate hosts (Parola *et al*., 2013).

The spotted fever group *Rickettsia* sp. strain Colombianensi has been found in *A. dissimile* collected from various reptiles,

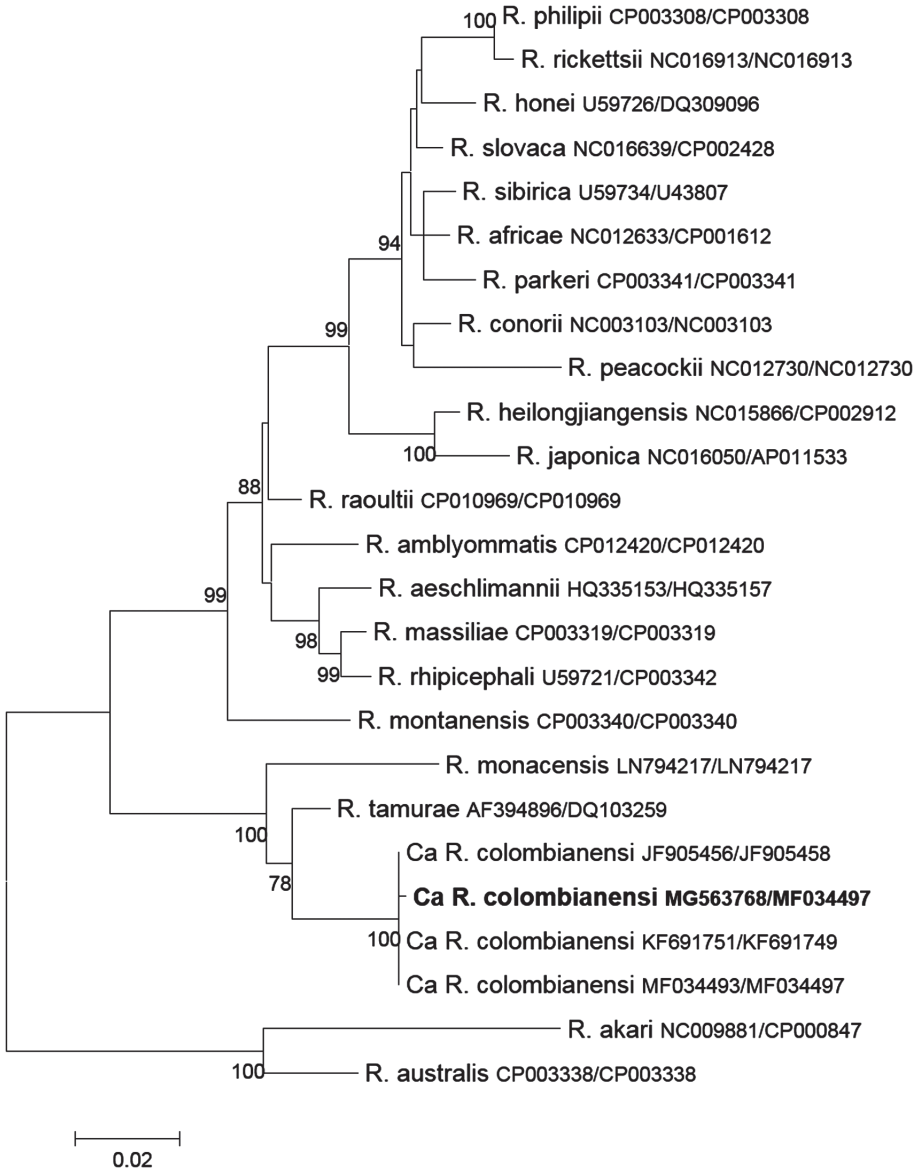

Fig. 1. Phylogenetic relationships among *Rickettsia* species based on maximum likelihood (ML). The tree is based on concatenated datasets of *gltA* and *ompA* partial nucleotide sequences (836 bp). The scale bars indicate an evolutionary distance of substitutions per position in the sequence. The numerical value ≥ 70 at the node indicates the bootstrap replicates that supported the interior branch. The Tamura 3-parameter model with gamma-distributed rate heterogeneity (T92 + G) was selected as the best fitted evolutionary model according to the Bayesian information criterion, calculated with JModel Test. The branch labels include GenBank accession numbers.

free-living *Rhipicephalus microplus* (Canestrini 1888) (Ixodida: Ixodidae), unidentified immature *Amblyomma* spp. and *A. cajennense s.l.*, collected from *Hydrochoerus hydrochaeris* (Linnaeus 1766) (Rodentia: Caviidae) in Colombia (Miranda *et al.*, 2012; Miranda & Mattar, 2014; Santodomingo *et al.*, 2018), nymphs of *A. dissimile* collected from *Ctenosaura bakeri* Stejneger, 1901 (Squamata: Iguanidae) and *Iguana iguana* (Linnaeus 1758) (Squamata: Iguanidae) in Honduras (Novakova *et al.*, 2015), and recently in larvae of *A. dissimile* parasitizing *Rhinella marina* (Linnaeus 1758) (Anura: Bufonidae) in the northern Brazilian Amazon (Luz *et al.*, 2018). The prevalence of infection found in the present study (56.2%) is very high in comparison with previous reports of less than 4% (Luz *et al.*, 2018; Santodomingo *et al.*, 2018). Although *Rickettsia* sp. strain Colombianensi is a spotted fever group rickettsia, its role as a human pathogen is unknown. If *Rickettsia* sp. strain Colombianensi is a pathogenic rickettsia, exposure to infected *A. dissimile* ticks may pose a health risk. However, the high prevalence found here may suggest a symbiotic role of this bacterium.

In the tick *A. dissimile*, an *Anaplasma*-like organism closely related to '*Ca*. Cryptoplasma californiense' was found. '*Candidatus* Cryptoplasma californiense' is a recently described Anaplasmataceae species found in *I. pacificus* in California, U.S.A. (Eshoo *et al.*, 2015). However, the fragment obtained was

relatively short (343 nt) and longer sequences of 16S rDNA and sequences of other genes would be necessary to better characterize the organism.

In *A. dissimile* collected from *B. atrox*, DNA of a novel *Hepatozoon* sp. was detected, closely related to various *Hepatozoon* spp. found previously in birds of prey, marsupials and reptiles. *Hepatozoon* spp. are commonly found infecting Brazilian snakes (Moço *et al*., 2012; O'Dwyer *et al*., 2013), which become infected after ingestion of the definitive invertebrate host, which contains the oocysts in its hemocoel, or after ingestion of infected intermediate hosts. However, the mechanism of the pathogenic changes of *Hepatozoon* spp. in snakes is not clearly understood (O'Dwyer *et al*., 2013). The present authors were unable to fully characterize the potential pathogen because the description of a new *Hepatozoon* species should also be supported by morphologic and morphometric characterization (O'Dwyer *et al*., 2013). To the present group's knowledge, this is the first report of *A. dissimile* infection by *Hepatozoon*, which suggests that this tick species may play a role in *Hepatozoon* circulation among populations of snakes in Brazil. Nevertheless, this hypothesis must be tested. No DNA of other protozoa, namely *Babesia* spp., was detected in the present study. Hard ticks are the primary source of transmission of *Babesia* to a large number of vertebrates worldwide (Vannier *et al*., 2015). In Brazil, a few *Babesia* species, transmitted by *Rhipicephalus* ticks, are known to affect domestic animals, but information on infection by ticks of wild animals is very scarce (Alvarado-Rybak *et al*., 2016).

The absence of bacteria of the genus *Borrelia* in *Amblyomma* ticks was expected and is in concordance with other studies conducted in Brazil. *Borrelia* spp. are responsible for causing numerous diseases that may affect humans and animals worldwide. Among these diseases are relapsing fevers, caused by bacteria transmitted by soft ticks, and Lyme disease caused by bacteria transmitted by hard ticks, mainly *Borrelia burgdorferi s.l.* The group *B. burgdorferi s.l.* comprises genetically related spirochetes, mostly associated with tick species belonging to the *Ixodes ricinus* complex in the Northern Hemisphere. Only a few studies have been carried out in South America (Barbieri *et al*., 2012; Dall'Agnol *et al*., 2017) and all show the presence of *Borrelia* in the genus *Ixodes* and a lack of infection in the genus *Amblyomma* (Blanco *et al*., 2017; Dall'Agnol *et al*., 2017).

Among bacteria of the genus *Coxiella*, the best known is *C. burnetii*, an obligate intercellular bacterium causing Q fever in almost all countries of the world. The aerosol route is the primary mode of transmission in humans and domestic animals. Although *C. burnetii* has been isolated from various species of tick, it is unlikely that this route of transmission to humans is significant (Pacheco *et al*., 2013; Duron *et al*., 2015b). However, ticks may play a critical role in the transmission of *C. burnetii* among wild vertebrates (Maurin & Raoult, 1999). Moreover, many *Coxiella*-like bacteria, including endosymbionts, are widespread in ticks (Machado-Ferreira *et al*., 2016). In the present study, no *Coxiella* infection was found among tested ticks, although, according to some authors, *Coxiella*-like bacteria are widespread in *Amblyomma* ticks in Brazil, including *A. rotundatum* (Machado-Ferreira *et al*., 2016).

The present study did not find any *Bartonella*-infected ticks. *Bartonella* species are transmitted predominantly by arthropods and infect erythrocyte and endothelial cells of a wide range of animal species, including humans (Breitschwerdt *et al*., 2010). It remains controversial whether ticks are involved in the transmission of pathogenic *Bartonella* spp. to vertebrates under natural conditions (Angelakis *et al*., 2010; Telford & Wormser, 2010). However, findings in Europe of questing *I. ricinus* ticks infected with *B. henselae* and *Bartonella grahamii* highlight the need for public awareness and draw attention to the possibility of infection with zoonotic *Bartonella* spp. after a tick bite (Janecek *et al*., 2012). In Brazil, the circulation of the agent has been demonstrated in humans, cats, dogs and wild animals using serological and molecular techniques, but only a few ticks were tested for this pathogen and all were shown to be negative for the presence of DNA of *Bartonella* spp. (Fontalvo *et al*., 2017).

Conclusions

Current knowledge concerning tick infestation of wild reptiles remains limited and hence the present study makes a contribution by reporting parasitism by ticks on snakes. The current findings extend the geographic range of *Rickettsia* sp. strain Colombianensi, which until now was limited to Colombia, Honduras and the state of Amapá in Brazil. Among the ticks tested, no DNA of *Babesia*, *Borrelia*, *Bartonella* or *Coxiella* was found, although this does not rule out the possible circulation of these pathogens among reptiles and ticks in Brazil. Further studies are required to characterize their ecology and pathogenic potential for humans and animals.

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