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Ticks parasitizing bats (Mammalia: Chiroptera) in the Caatinga Biome, Brazil

Carrapatos parasitando morcegos (Mammalia: Chiroptera) na Caatinga, Brasil

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Abstract

In this paper, the authors report ticks parasitizing bats from the Serra das Almas Natural Reserve (RPPN) located in the municipality of Crateús, state of Ceará, in the semiarid Caatinga biome of northeastern Brazil. The study was carried out during nine nights in the dry season (July 2012) and 10 nights in the rainy season (February 2013). Only bats of the Phyllostomidae and Mormoopidae families were parasitized by ticks. The species *Artibeus planirostris* and *Carollia perspicillata* were the most parasitized. A total of 409 larvae were collected and classified into three genera: *Antricola* (n = 1), *Nothoaspis* (n = 1) and *Ornithodoros* (n = 407). Four species were morphologically identified as *Nothoaspis amazoniensis*, *Ornithodoros cavernicolous*, *Ornithodoros fonsecai*, *Ornithodoros hasei*, and *Ornithodoros marinkellei*. *Ornithodoros hasei* was the most common tick associated with bats in the current study. The present study expand the distributional ranges of at least three soft ticks into the Caatinga biome, and highlight an unexpected richness of argasid ticks inhabiting this arid ecosystem.

Keywords: Ticks, *Nothoaspis*, *Ornithodoros*, *Antricola*, bat, Caatinga.

Resumo

Neste artigo, os autores relatam carrapatos parasitando morcegos da Serra das Almas Reserva Natural (RPPN), localizada no município de Crateús, Estado do Ceará, no bioma semi-árido da Caatinga no nordeste do Brasil. O estudo foi realizado durante nove noites, na estação seca (julho de 2012) e 10 noites, na estação chuvosa (fevereiro de 2013). Apenas morcegos das famílias Phyllostomidae e Mormoopidae estavam parasitados por carrapatos. As espécies *Artibeus planirostris* e *Carollia perspicillata* foram as mais parasitadas. Um total de 409 larvas foram coletadas e classificadas em três gêneros: *Antricola* (n = 1), *Nothoaspis* (n = 1) e *Ornithodoros* (n = 407). Quatro espécies foram identificadas morfológicamente como *Nothoaspis amazoniensis*, *Ornithodoros cavernicolous*, *Ornithodoros fonsecai*, *Ornithodoros hasei* e *Ornithodoros marinkellei*. *Ornithodoros hasei* foi a espécie de carrapato mais comum associada com morcegos no presente estudo. O presente estudo expande a distribuição de, pelo menos, três espécies de argasídeos para o bioma Caatinga, e destaca a inesperada riqueza de espécies habitando este árido ecossistema.

Palavras-chave: Carrapatos, *Nothoaspis*, *Ornithodoros*, *Antricola*, morcego, Caatinga.

Introduction

Ticks are obligate hematophagous ectoparasites and important vectors of agents that cause disease in wild and domestic animals, and humans worldwide (PAROLA & RAOULT, 2001; COLWELL et al., 2011). The family Argasidae, known as soft ticks, comprises more than 200 species worldwide with 91 representatives in the

Neotropics (NAVA et al., 2010; GUGLIELMONE et al., 2010; BARROS-BATTESTI et al., 2013, 2015; VENZAL et al., 2015; MUÑOZ-LEAL et al., 2016a; LABRUNA et al., 2016). While in Brazil Argasidae is represented by 24 species belonging to *Antricola*, *Argas*, *Nothoaspis*, and *Ornithodoros* genera, at least 19 species belong to the genus *Ornithodoros*: *Ornithodoros brasiliensis* Aragão, 1923; *Ornithodoros capensis* Neumann, 1901; *Ornithodoros fonsecai* (Labruna & Venzal, 2009); *Ornithodoros guaporensis* Nava, Venzal & Labruna, 2013; *Ornithodoros hasei* (Schulze, 1935); *Ornithodoros*

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jul Schulze, 1940; *Ornithodoros kohlsi* (Guglielmone & Keirans, 2002); *Ornithodoros marinkellei* Kohls, Clifford & Jones, 1969; *Ornithodoros mimon* Kohls, Clifford & Jones, 1969; *Ornithodoros nattereri* Warburton, 1927; *Ornithodoros rondoniense* (Labruna, Terrasini, Camargo, Brandão, Ribeiro & Estrada-Peña, 2008); *Ornithodoros rostratus* Aragão, 1911; *Ornithodoros rudis* Karsh, 1880; *Ornithodoros setosus* Kohls, Clifford & Jones, 1969; *Ornithodoros stageri* Cooley & Kohls, 1941; *Ornithodoros talaje* (Guérin-Méneville, 1849); *Ornithodoros faccinii* Barros-Battesti, Landulfo & Luz, 2015; *Ornithodoros cavernicolous* Dantas-Torres, Venzal & Labruna, 2012 and *Ornithodoros rietcorrei* Labruna, Nava & Venzal, 2016 (GUGLIELMONE et al., 2010; DANTAS-TORRES et al., 2012; BARROS-BATTESTI et al., 2013, 2015; WOLF et al., 2016; LABRUNA et al., 2016). Of this diversity, nine species (47%) have been associated with bats or bat-frequented caves (LABRUNA & VENZAL, 2009; LABRUNA et al., 2011; DANTAS-TORRES et al., 2012; BARROS-BATTESTI et al., 2013; MARTINS et al., 2014; MUÑOZ-LEAL et al., 2016b).

The term “Caatinga” refers to the typical xeromorphic vegetation of the semiarid ecosystems in northeastern Brazil that covers an area of approximately 734,478 Km² (MARINHO-FILHO & SAZIMA, 1998), corresponding to 11% of Brazil’s territory. This region includes areas of semi-deciduous and cloud forests, and presents low rainfall and humidity regimes (COSTA et al., 2004). While ticks are associated with bats in other Brazilian biomes (LABRUNA & VENZAL 2009; LABRUNA et al., 2011; MUÑOZ-LEAL et al., 2016b), to date documented reports of ectoparasites on bats from the Caatinga Biome include only streblid flies (Diptera: Streblidae) (RIOS et al., 2008; BEZERRA et al., 2016) and spinturnicid mites (Acari: Spinturnicidae) (ALMEIDA et al., 2016). In this study we introduce the diversity of ticks parasitizing bats in the Caatinga biome, providing new records of hosts and localities for this understudied group of parasites.

Materials and Methods

Study site and capture of bats

The study was carried out during nine nights in the dry season (July 2012) and 10 nights in the rainy season (February 2013) at Serra das Almas Natural Reserve (RPPN), municipality of Crateús, state of Ceará (05°15’S, 41°00’W). This reserve covers an area of 6,146 hectares and is considered an outpost of the Caatinga Biosphere Reserve (ARAÚJO et al., 2011).

Bats were captured with five to ten mist nets (36 mm), 6 - 18 m long, 2.5 m height, placed in existing trails. Sampling period extended for six hours after sunset. Mist nets were examined at intervals of 10 min, and captured bats were kept in individualized and numbered cloth bags. These bags were used only once per day to avoid ectoparasite exchange among collected hosts. Voucher bat specimens were fixed in 10% formaldehyde and preserved in 70% alcohol, as previously described (VIZOTTO & TADDEI, 1973; HANDLEY, 1988), and deposited in the Adriano Lucio Peracchi (ALP) collection, at the Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil. Identification of bat species followed the descriptions of Silva et al. (2015) and the taxonomic

nomenclature of Nogueira et al. (2014). Accession numbers for bat vouchers are as follow: ALP 10157, 10132, 10138, 10150, 10165, 10167, 10168, 10184 and 10455.

The capture and collection of specimens was authorized by the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) of the Brazilian government (license number 32684-1).

Collection and identification of ticks

Ticks were removed with tweezers and kept in vials containing 70-90% ethanol for taxonomic identification. In the laboratory, ticks were slide-mounted in Hoyer’s medium, and photographed with an Olympus DP70 camera implemented in an Olympus BX40 optical microscope (Olympus Optical Co. Ltd., Japan) for morphological and morphometric analyses. Fully engorged specimens were measured using a stereoscope SteREO Discovery V12 (all measurements are given in mm). Ticks were identified at genus level according to Barros-Battesti et al. (2013), and species diagnoses followed Kohls et al. (1969), Jones & Clifford (1972), and original descriptions of other Neotropical Ornithodorinae (KEIRANS & CLIFFORD, 1975; NAVA et al., 2010; DANTAS-TORRES et al., 2012). Additional comparisons of immature specimens were made using part of the material deposited at the “Coleção Nacional de Carrapatos” (CNC) of the Faculty of Veterinary Medicine of the University of São Paulo, São Paulo, Brazil.

Molecular analyses were performed in order to confirm morphological identifications of ticks. For this purpose, larvae were individually submitted to DNA extraction by the guanidine isothiocyanate-phenol technique (SANGIONI et al., 2005). DNA of fully engorged larvae was extracted through a small incision in the posterior region of the idiosoma using a 23G needle. Extracted DNA was subjected to conventional polymerase chain reaction (PCR) targeting a fragment of approximately 460-bp of the mitochondrial 16S rRNA gene, as described elsewhere (MANGOLD et al., 1998). PCR products of the expected size were purified and sequenced using an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California, USA) with the same primers used in the PCR.

Prevalence of tick infestation (number of infested bats/number of examined bats x 100), and tick mean intensity of infestation (total number of collected ticks/total number of infested bats) were calculated according to Bush et al. (1997).

Results

A total of 347 bats belonging to six families and 23 species were captured: Phyllostomidae (n=265; 16 species), Mormoopidae (n=56; 2 species), Noctilionidae (n=2; 1 species), Natalidae (n=2; 1 species), Molossidae (n=9; 1 species), Vespertilionidae (n=13; 2 species). The species *Carollia perspicillata* (Linnaeus, 1758) (n=66), *Artibeus planirostris* Spix, 1823 (n=54) and *Pteronotus parnellii* Gray, 1843 (n=43) were the most abundant (SILVA et al., 2015).

Overall, 49 out of 260 (18.8%) bats, belonging to 12 species of the Phyllostomidae and Mormoopidae families were infested by ticks (Table 1). Of these, *Anoura geoffroyi*, *Artibeus planirostris* and

Table 1. Ticks collected on phyllostomid bats in the Private Reserve of the Natural Patrimony (RPPN) Serra das Almas, Ceará state, northeastern Brazil. NEB: number of examined bats; NIB: number of infested bats; L: larvae.

Bat species	NEB/ NIB	bat habitat*	Number of ticks					Overall prevalence (%)	MI#	
			<i>N. amazoniensis</i>	<i>O. cavernicolous</i>	<i>O. basei</i>	<i>O. marinikellei</i>	<i>O. fonsecai</i>			<i>Antricola</i> sp.
PHYLLOSTOMIDAE										
<i>Artibeus planirostris</i>	54/22	OC		2L	296L				40.7	13.5
<i>Carollia perspicillata</i>	66/10	OC		28L			7L		15.1	3.5
<i>Trachops cirrhosus</i>	18/3	OC	1L	10L			1L		16.6	4.0
<i>Anoura geoffroyi</i>	3/2	EC		4L					66.7	2.0
<i>Phyllostomus discolor</i>	4/2	OC		22L					50	11
<i>Tonatia</i> sp.	6/2	OC			4L				33.3	4.0
<i>Tonatia bidens</i>	4/1	OC		4L					25	4.0
<i>Tonatia saurophila</i>	14/1	NC			10L				7.1	10
<i>Glossophaga soricina</i>	17/1	OC		3L					5.9	3.0
<i>Sturnira lilium</i>	6/1	OC		1L					16.7	1.0
<i>Lophostoma brasiliense</i>	12/1	NC			6L				8.3	6.0
MORMOOPIDAE										
<i>Peronotus gymnonotus</i>	13/2	EC				10L			15.3	5.0
<i>Peronotus parnelli</i>	43/1	EC						1L	2.3	1.0
Total	260/49		1	74	315	10	8	1	18.8	8.3

*Cave use classification according to Guimarães & Ferreira (2014): essentially cave dweller (EC); opportunistic cave dweller (OC); not cave dweller (NC); #mean intensity of tick infestation.

C. perspicillata were the most infested species, with a prevalence of 66.7%, 40.7% and 15.1%, respectively (Table 1).

A total of 409 larvae belonging to *Antricola*, *Nothoaspis* and *Ornithodoros* genera were collected (Table 1). The single larva of *Antricola* was identified to genus level by the presence of three postcoxal setae (KOHLS et al., 1965) (Figure 1). One fully engorged larva of *Nothoaspis amazoniensis* Nava, Venzal & Labruna 2010

was identified by the following combination of characters: dorsal plate elongate and triangular; dorsum with 12-13 setae pairs (5-6 anterolateral, 3 central, and 4 posterolateral); venter with 10 pairs of setae; posteromedian setae absent; 3 pairs of sternal setae; 1 pair of post-coxal setae; 5 pairs of circumanal setae; 1 pair of ventral posteromarginal setae and basis capituli pentagonal, following Nava et al. (2010) (Figure 2).

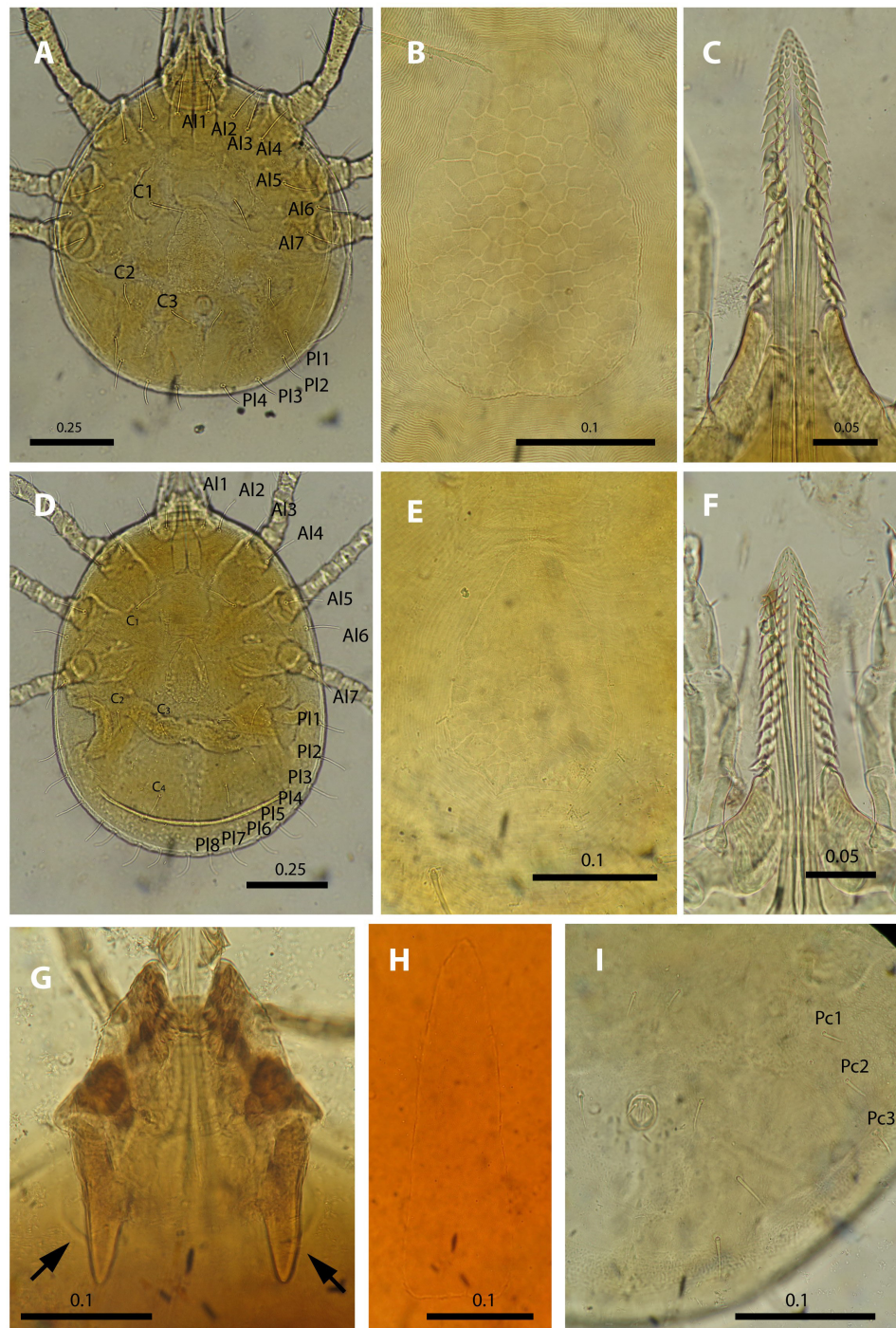


Figure 1. Micrographs of *Ornithodoros fonsecai*, *Ornithodoros hasei*, *Ornithodoros marinkellei* and *Antricola* sp. larvae. *Ornithodoros fonsecai*: dorsal idiosoma (A), dorsal plate (B), and hypostome (C). *Ornithodoros hasei*: dorsal idiosoma (D), dorsal plate (E), and hypostome (F). *Ornithodoros marinkellei*: cornua-like projection (black arrows) in basis capitulum (G) and dorsal plate (H). *Antricola* sp.: poscoxal setae (I). Abbreviations: Al, anterolateral seta; C, central seta; Pl, posterolateral seta; Pc, postcoxal seta.

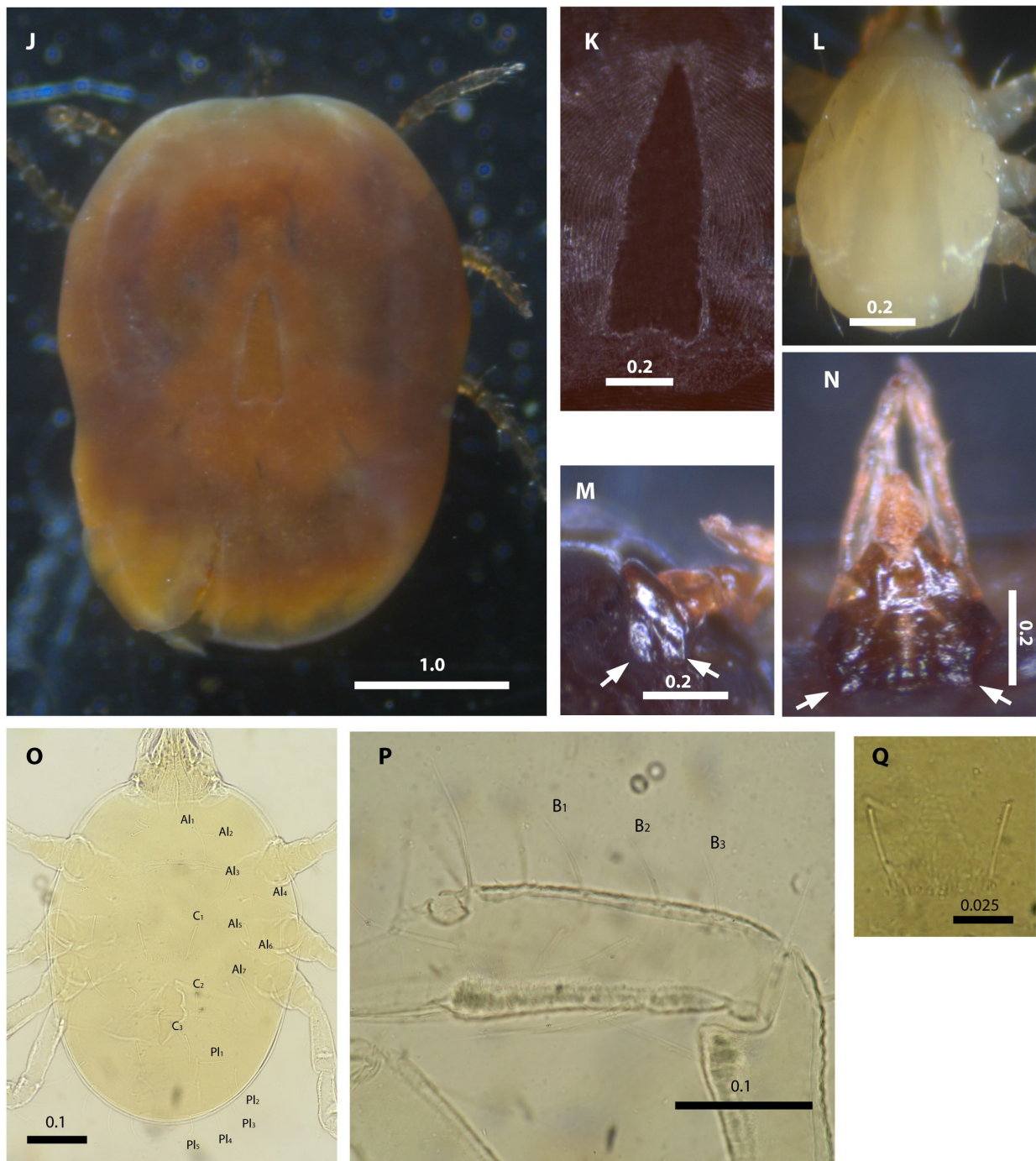


Figure 2. Micrographs of *Nothoaspis amazoniensis* and *Ornithodoros cavernicolous* larvae collected in the present study and paratype of *N. amazoniensis* (NAVA et al., 2010). *Nothoaspis amazoniensis*: dorsal idiosoma (J), dorsal plate of the collected specimen (K), dorsal plate of paratype. (L), triangular spurs (white arrows) in coxa I (M), auricle-like projections (white arrows) in posterior margin of basis capitulum (N). *Ornithodoros cavernicolous*: dorsal idiosoma (O), tarsus I (P), and dorsal plate (Q). Abbreviations: Al, anterolateral seta; C, central seta; Pl, posterolateral setae; B, basiventral seta.

Four hundred and seven larvae of the genus *Ornithodoros* were classified in the following four species: *O. cavernicolous* (n = 74), *O. fonsecai* (n = 8), *O. hasei* (n = 315), and *O. marinkellei* (n = 10) (Table 1). The morphological characters for the identification of larvae of these species are as follows: 1) *O. cavernicolous*: dorsal plate with a sub-equilateral triangular shape, dorsum provided with 7 anterolateral, three central and 5 posterolateral pairs of setae, and

three pairs of basal setae on tarsus I (DANTAS-TORRES et al., 2012); 2) *O. fonsecai*: dorsal plate elongate, pyriform, broadest posteriorly, dorsum with 13–14 pairs of setae, with 10–11 dorsolateral pairs and 3 central pairs; hypostome long, pointed apically with dental formula 3/3 in the anterior half, 2/2 posteriorly almost to base; dentition: file 1 with 17 to 18 denticles, 2 with 15 to 17, and 3 with 9 to 10 (LABRUNA & VENZAL, 2009); 3) *O. hasei*:

dorsal plate pyriform, 15 pairs of dorsolateral setae and 4 pairs of central setae; hypostome apically pointed with 3 rows of denticles (17-19 denticles in row I; 16-17 in row II, and 11-12 in row III); dentition formula 3/3 in the anterior two-thirds and 2/2 towards the base; hypostome arising from a triangular median extension (KOHLS et al., 1965); 4) *O. marinkellei*: dorsal plate long and narrow, triangular, surface smooth, posterior margin slightly concave; dorsum with 13 pairs of setae, 7 anterolateral, 3 central, and 3 posterolateral, 3 first anterolateral setae short and thick; venter with 8 pairs of setae plus pair on anal valves; posteromedian setae absent; hypostome with dental formula 2/2 throughout length, file 1 with 21 denticles and file 2 with 20-21 denticles, additional denticles around apex, apex pointed, presence of two cornua-like projections in the posterior margin of basis capitulum (LABRUNA et al., 2011) (Figures 1, 2). In addition, morphological identifications of 20 larvae were confirmed by molecular analyses. GenBank accession numbers for the obtained sequences and their respective similarities with other conspecific ticks are shown in Table 2. Attempts to amplify and generate a confident sequence of the mitochondrial 16S rRNA gene from the *N. amazoniensis* were unsuccessful.

Ornithodoros hasei was the most abundant tick followed by *O. cavernicolous*. *O. fonsecai* was collected only in *C. perspicillata* and *Trachops cirrhosus*, and *O. marinkellei* only in *Pteronotus gymnonotus*. Less frequent *N. amazoniensis* and *Antricola* sp. larvae were only collected in *T. cirrhosus* and *P. parnelli*, respectively. The occurrence of co-infestations was observed for *N. amazoniensis*, *O. cavernicolous* and *O. fonsecai* parasitizing *T. cirrhosus*; *O. cavernicolous* and *O. hasei* parasitizing *A. planirostris*, and *O. cavernicolous* and *O. fonsecai* parasitizing *C. perspicillata*. Detailed data is presented in Table 1. Overall, the mean intensity of tick infestation was 18.8 ± 3.6 ticks per bat, ranging from 1 to 61 ticks per bat (Table 1). Ticks were found attached on the tail membrane, legs, around eyes and mouth of the bats.

Seventeen slide-mounted ticks were deposited at the CNC tick collection under accession numbers CNC-3291, CNC-3353 (*O. cavernicolous*), CNC-3358 (*O. hasei*), CNC-3363 (*O. fonsecai*), CNC-3365 (*O. marinkellei*) and CNC-3367 (*Antricola* sp.), and 340 ethanol preserved specimens CNC-3292 (*N. amazoniensis*), CNC-3354, CNC-3355, CNC-3356, CNC-3357 (*O. cavernicolous*), CNC-3359, CNC-3360, CNC-3361, CNC-3362 (*O. hasei*), CNC-3364 (*O. fonsecai*), and CNC-3366 (*O. marinkellei*).

Discussion

In this study, the majority of tick-infested bats constitute species associated with caves, where bats live in colonies or use the caves as opportunistic roosting places (GUIMARÃES & FERREIRA, 2014).

Ornithodoros hasei was the most common tick found in association with bats from the Caatinga area prospected in the current study, and *A. planirostris* corresponded to the most parasitized host. In a recent study from the Pantanal biome, Muñoz-Leal et al. (2016b) also evidenced high prevalence of *O. hasei* larvae on *A. planirostris*. As suggested by these authors, and reinforced by the data from the Caatinga biome, *A. planirostris*, a bat with wide distribution in Brazil, might play an important role in the biological cycle of *O. hasei*, contributing to the dispersion of this tick.

Ornithodoros cavernicolous, the second most common species, was collected only in cave-roosting bats. This soft tick was described from free-living specimens from caves in the Caatinga biome, and from bat-associated larvae in the Cerrado Biome of central-western Brazil (DANTAS-TORRES et al., 2012). To date only *Desmodus rotundus* (E. Geoffroy, 1810), *Anoura caudifer* (E. Geoffroy, 1818), and *C. perspicillata* were reported as hosts of this tick species; therefore, the present study adds seven host species: *A. planirostris*, *Phyllostomus discolor*, *T. cirrhosus*, *Tonatia bidens* (Spix, 1823), *Glossophaga soricina* (Pallas, 1766), *Sturnira lilium* (E. Geoffroy, 1810) and *A. geoffroyi*.

In contrast to *O. hasei* and *O. cavernicolous*, the other two *Ornithodoros* species collected in this study, *O. fonsecai* and *O. marinkellei*, presented low prevalence. From a biogeographic point of view, *O. fonsecai* was exclusively known from a sole cave in the state of Mato Grosso do Sul within the Cerrado biome, and by parasitizing cave-dweller bats such as *Peropteryx macrotis* (Wagner, 1843) and *D. rotundus* (E. Geoffroy, 1810) (LABRUNA & VENZAL, 2009). Here we report new records of this tick in the Caatinga biome, parasitizing the bats *C. perspicillata* and *T. cirrhosus*. The occurrence of *O. fonsecai* in these new localities expands its actual distribution towards the north of Brazil in ≈ 2400 km, and adds two new hosts.

In Brazil, *O. marinkellei* was previously reported in caves from eastern (LABRUNA et al., 2011) and western (HENRIQUE-SIMÕES et al., 2012) Amazonian biome, in association with three bat species of the genus *Pteronotus* (LABRUNA et al., 2011). In this way it is not surprising that in the current study larval stages of this tick

Table 2. Mitochondrial 16S rDNA sequences for the analyzed ticks, and their closest similarities in GenBank.

Tick species	GenBank - accession number	Number of larvae submitted to PCR	Host	Closest similarity in GenBank for the mitochondrial 16S rDNA sequence
<i>O. cavernicolous</i>	KX781697	13	<i>C. perspicillata</i> , <i>P. discolor</i>	100% (428-pb) <i>O. cavernicolous</i> , Ceará State (JF714964)
<i>O. marinkellei</i>	KX781700	4	<i>P. gymnonotus</i>	99% (423/426-pb) <i>O. marinkellei</i> , Brazilian Amazon (HM582439)
<i>O. hasei</i>	KX781698	2	<i>A. planirostris</i>	99% (425/427-pb) <i>O. hasei</i> , Mato Grosso do Sul State (KT894588)
<i>O. fonsecai</i>	KX781699	1	<i>C. perspicillata</i>	96% (406/425-pb) <i>O. fonsecai</i> , Mato Grosso do Sul State (KC769597)
Total		20		

were also collected from the already known host *P. gymnonotus* (LABRUNA et al., 2011); however, at the Caatinga biome. Similarly to *A. planirostris* for *O. hasei*, *Pteronotus* bats might play an essential role in the biological cycle of *O. marinkellei*, as emphasized in literature (KOHLS et al., 1969; JONES & CLIFFORD, 1972; VENZAL et al., 2006).

Less abundant collected ticks corresponded to one larva of *N. amazoniensis* and one of *Antricola* sp.. Previous records of *N. amazoniensis* were documented only from the Amazonian biome and exclusively from the bat *P. parnellii* (NAVA et al., 2010). Here we report for the first time this tick on *T. cirrhosus* and in the Caatinga biome. Both, *P. parnellii* and *T. cirrhosus* have overlapping distributional ranges and use caves as shelter in the Amazonian, Savannah and Caatinga biomes (BREDDT et al., 1999; GUIMARÃES & FERREIRA, 2014). In this sense, the occurrence of *N. amazoniensis* on either *T. cirrhosus* or *P. parnellii* might be explained by the sharing of caves as roosting places. As both bat species are also distributed in the Savannah, further prospections might find this tick species also in this biome.

All three *Antricola* species that occurs in Brazil, *Antricola guihelmonei* Estrada-Peña, Barros-Battesti & Venzal 2004, *Antricola delacruzi* Estrada-Peña, Barros-Battesti & Venzal 2004, and *Antricola inexpectata* Estrada-Peña, Barros-Battesti & Venzal 2004 were described from mature specimens collected in caves from the Sergipe and Ceará states (ESTRADA-PÉÑA et al., 2004), located in a transition area between the Atlantic rainforest and the Caatinga biomes. Additional reports included the collection of adults from caves in the Rondônia state within the Amazonian biome (LABRUNA et al., 2008). Although a detailed description of the larvae from both species has not been published, morphological characters of *Antricola* larvae presented by Cooley & Kohls (1944), and posteriorly by Kohls et al. (1965), coincide with the general morphology of the larva found parasitizing *P. parnellii* in the present study. This host-parasite association is not unexpected since both *Antricola* and *Pteronotus* bats live in caves (ESTRADA-PÉÑA et al., 2004; GUIMARÃES & FERREIRA, 2014; LABRUNA et al., 2011), and because *Antricola* larvae have been reported on *Pteronotus* bats in the Amazon biome (LABRUNA et al., 2011).

This study provides new geographical ranges for five Brazilian bat-associated soft tick species, expanding the distribution of *N. amazoniensis*, *O. marinkellei* and *O. fonsecai* to the Caatinga biome.

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