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Short Communication

Occurrence of *Bartonella* genotypes in bats and associated Streblidae flies from Maranhão state, northeastern Brazil

Ocorrência de genótipos de *Bartonella* em morcegos e moscas Streblidae no Estado do Maranhão, Nordeste do Brasil

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Abstract

Bartonella is a genus of emerging zoonotic bacteria that are mainly associated with mammalian erythrocytes and endothelial cells. Bats are natural reservoirs for a variety of important pathogens that impact human and animal health. Recent reports have highlighted the role of bats and bat flies in the maintenance of *Bartonella*. Here, we showed that none of the 29 bat DNA blood samples obtained from five bat species in São Luís Island, state of Maranhão, northeastern Brazil, were positive for *Bartonella* in qPCR assays targeting *nuoG*. On the other hand, three out of 15 DNA samples (20%) from flies in the family Streblidae were positive for *Bartonella*. The BLASTn results showed that the *gltA* and *rpoB* sequences shared identities ranging from 97.2% to 100%, with *Bartonella* sequences amplified from bats or bat flies from Costa Rica and Brazil. These findings were supported by phylogenetic analyses based on Bayesian inferences. The present study showed that *Bartonella* genotypes are present in bat flies, thus shedding some light on the distribution of bat fly-related *Bartonella* genotypes in South America.

Keywords: Bartonellosis, Chiroptera, Hippoboscoidea, PCR.

Resumo

Bartonella é um gênero de bactérias zoonóticas emergentes associadas principalmente a eritrócitos e células endoteliais de mamíferos. Morcegos são reservatórios naturais para uma variedade de patógenos importantes que afetam a saúde humana e animal. Além disso, estudos recentes destacaram o papel dos morcegos e de moscas associadas a morcegos na manutenção de *Bartonella*. No presente estudo, nenhuma das 29 amostras de DNA obtidas a partir do sangue de cinco espécies de morcegos amostrados na ilha de São Luís, estado do Maranhão, Nordeste do Brasil, foi positiva para *Bartonella* nos ensaios de qPCR direcionados ao gene *nuoG*. Por outro lado, três das 15 (20%) amostras de DNA de moscas da família Streblidae foram positivas para *Bartonella*. Os resultados do BLASTn mostraram que as sequências dos genes *gltA* e *rpoB* compartilharam identidade, variando de 97,2% a 100%, com as sequências de *Bartonella* amplificadas em morcegos ou moscas amostrados na Costa Rica ou Brasil. Tais resultados corroboraram as análises filogenéticas realizadas por Inferência Bayesiana. O presente estudo mostrou a ocorrência de *Bartonella* em moscas de morcegos, auxiliando a esclarecer a distribuição dos genótipos de *Bartonella* relacionadas a moscas Streblidae na América do Sul.

Palavras-chave: Bartonelose, Chiroptera, Hippoboscoidea, PCR.

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Introduction

The genus *Bartonella* (Rhizobiales: Bartonellaceae) comprises phylogenetically diverse facultative intracellular Gram-negative α-proteobacteria that mainly infect mammalian erythrocytes and endothelial cells (Eicher & Dehio, 2012). These bacteria are distributed throughout the world and are transmitted predominantly by blood-feeding arthropods, such as fleas, lice, flies and mosquitoes (Chomel et al., 2009).

Bats play an important role in the maintenance of ecosystem stability. Moreover, this mammal group provides important ecosystem services through pollinating flowers, dispersing seeds and consuming insects. Likewise, bats are recognized as reservoirs or carriers for many zoonotic pathogens (Mühldorfer, 2013).

Bartonella spp. have been reported in over 60 bat species worldwide. In addition, the diversification of bartonellae in bats seems to have followed the diversification of bats, with clustering of bartonellae restricted to single bat families (McKee et al., 2017). Moreover, studies have highlighted the role of bats as reservoirs for zoonotic *Bartonella* species (Veikkolainen et al., 2014; Bai et al., 2018).

Although the prevalence and genetic diversity of *Bartonella* have previously been assessed in bats and bat flies around the world (Morse et al., 2012; Bai et al., 2015), several biological aspects of this important bacterial group remain poorly assessed in Brazil. So far, *Bartonella* DNA has been detected in bats sampled in Brazil with prevalence ranging from 5.28% (17/322) to 24.51% (51/208) (Ikeda et al., 2017; Ferreira et al., 2018; André et al., 2019). In addition, *Bartonella* DNA has been amplified from flies in the family Streblidae (19.8% [40/202]) collected from bats in Brazil (Amaral et al., 2018). Thus, the current study aimed to verify the occurrence and the phylogenetic positioning of *Bartonella* in bats and associated flies sampled in northeastern Brazil. Additionally, flies collected from bats were molecularly characterized.

Material and Methods

Between September and July 2019, 29 bats belonging to five species were trapped in São Luís Island, state of Maranhão, northeastern Brazil (Table 1), during rabies virus surveillance performed by the Agricultural and Livestock Protection Agency of the State of Maranhão (Agência Estadual de Defesa Agropecuária do Maranhão, AGED-MA). These bats were then taken to a field laboratory to determine the species and to verify any presence of ectoparasites. Lastly, the bats were taken still alive in individual bags to the Virology Laboratory of the State University of Maranhão (Universidade Estadual do Maranhão - UEMA) for blood collection. All procedures were carried out according to the ethical guidelines for the use of animal samples permitted by the Institutional Animal Care and Use Committee (IACUC) of UEMA, São Luís, Maranhão (Protocol number: 04/2016).

Table 1. Number and species submitted to *Bartonella* screening, molecular characterization and BLASTn results.

Sample ID	qPCR	*gltA*	BLASTn	*rpoB*	BLASTn
Glossophaga soricina	0% (0/9)	–	–	–	–
Carollia perspicillata	0% (0/16)	–	–	–	–
Diaemus youngi	0% (0/1)	–	–	–	–
Pteronotus personatus	0% (0/1)	–	–	–	–
Pteronotus parnellii	0% (0/2)	–	–	–	–
***Trichobius* spp.**	20% (3/15)	66% (2/3)	99.4%-100% *Bartonella* sp. (KJ816691 and MH234352)	33% (1/3)	97.2% *Bartonella* sp. (MK578352)

Subsequently, EDTA-blood samples were subjected to DNA extraction using the InstaGene™ Matrix (Bio-Rad). Additionally, 15 Streblidae flies were individually subjected to DNA extraction using the lllustra Tissue and Cells Genomic Prep Mini Spin kit (GE Healthcare Life Sciences), in accordance with the manufacturer's instructions. To confirm the presence of amplifiable DNA, the DNA samples obtained from the bat blood samples and flies were initially subjected to conventional PCR assays targeting the endogenous mammals-*gapdh* and insects-*cox-1* (~600 bp) genes, respectively (Birkenheuer et al., 2003; Folmer et al., 1994). Endogenous gene-PCR positive DNA samples were subsequently subjected to a previously described broad-range qPCR assay based on the *nuoG Bartonella* gene

(André et al., 2016). Finally, the positive DNA samples in the above mentioned screening assay were subjected to conventional PCR assays targeting the *gltA* (750 bp) and *rpoB* (825 bp) genes, as previously described (Norman et al., 1995; Renesto et al., 2001).

Thereafter, the amplicons obtained, including insects-associated *cox-1*, were purified using the EXOSAP-IT® system (Applied Biosystems). Purified amplified DNA fragments were subjected to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems/ Perkin Elmer).

The *Bartonella* species were identified through BLASTn analysis using Megablast (NCBI, 2020). The phylogenetic analysis was performed using the Bayesian inference method, through MrBayes in XSEDE (3.2.7.a) and was performed in the CIPRES Science Gateway.

Results

All the bat-blood and fly DNA samples subjected to PCR assays targeting the endogenous mammals-*gapdh* and insects-*cox-1* genes, respectively, were positive. The BLASTn results from seven out of 13 *cox-1* fly sequences (two DNA samples showed weak band intensity, which precluded sequencing) shared identities ranging from 88.94% to 92.91% with *Trichobius parasiticus* (MH282310; sampled in Costa Rica). The other five *cox-1* fly sequences shared identities ranging from 97.28% to 97.79% with *Trichobius joblingi* (MH282259; sampled in Panama). Lastly, one sequence was 99.04% similar to *Trichobius yunkeri* (KY882244; sampled in Mexico).

None of the 29 bat-blood DNA samples were positive in qPCR assays for *Bartonella* spp. targeting the *nuoG* gene. On the other hand, three out of 15 DNA samples (20%) from Streblidae flies were positive for *Bartonella* **(**Table 1**)**. The amplified sequences shared identities ranging from 97.2% to 100% with sequences previously detected in *Carollia perspicillata* (MH234352) or bat flies (*Trichobius joblingi*; KJ816691) from Costa Rica; and in bats (*Desmodus rotundus*; MK578352) from Brazil (Table 1).

Moreover, and in agreement with phylogenetic analyses, the sequences clustered with other sequences detected in bats and bat flies from the countries mentioned above, and they were supported by high posterior probability values (98%) in the Bayesian inference analysis (Figure 1 and 2). The sequences amplified in the current study were deposited in GenBank under accession numbers MT275628 and MT275629 for the *gltA* gene and MT275630 for the *rpoB* gene.

Discussion

Bats and associated ectoparasites have been distinguished as important sources of new *Bartonella* species/ genotypes (McKee et al., 2017; Sándor et al., 2018). Here, none of the 29 bat blood samples were positive in qPCR assays for *Bartonella*. In a recent report, André et al. (2019) found high prevalence (24.5% [51/208]) of *Bartonella* in vampire bat liver samples from 15 different states in Brazil. However, the three bats caught in the state of Maranhão were negative in real-time PCR assays for *Bartonella* spp. (André et al., 2019), thus corroborating the results found in the present study.

On the other hand, low prevalence (5.28% [17/322]) of bartonellae was previously reported in non-hematophagous bats from Brazil that were sampled in the states of São Paulo, Pará, Tocantins and Mato Grosso (Ikeda et al., 2017). These differences in the prevalence of *Bartonella* in bats may be attributed to distinct factors, such as sample type (e.g., blood, spleen and heart), bat guilds, assay type (e.g. culturing, qPCR assays or conventional PCR assays), number of samples analyzed and distribution of sampled animals.

However, three flies (20%) that were identified as *Trichobius* spp. through *cox-1* sequencing, were found to be positive for *Bartonella*. Similar prevalence of *Bartonella* (19.8% [40/202]) had previously been reported in Streblidae flies sampled in northeastern Nova Iguaçu, Rio de Janeiro, Brazil (Amaral et al., 2018). On the other hand, high prevalence of *Bartonella* spp. was reported in bat flies from western Africa (66%) (Billeter et al., 2012) and Costa Rica (up to 100%) (Judson et al., 2015). Additional studies aiming to evaluate the factors affecting *Bartonella* prevalence in bats and associated bat flies are needed.

The presence of amplifiable DNA in the bat blood samples was strikingly evident and was confirmed through an endogenous control PCR assay targeting the mammals-*gapdh* gene in all the bat samples analyzed. This excluded the possibility of false negative results due to PCR inhibitors.

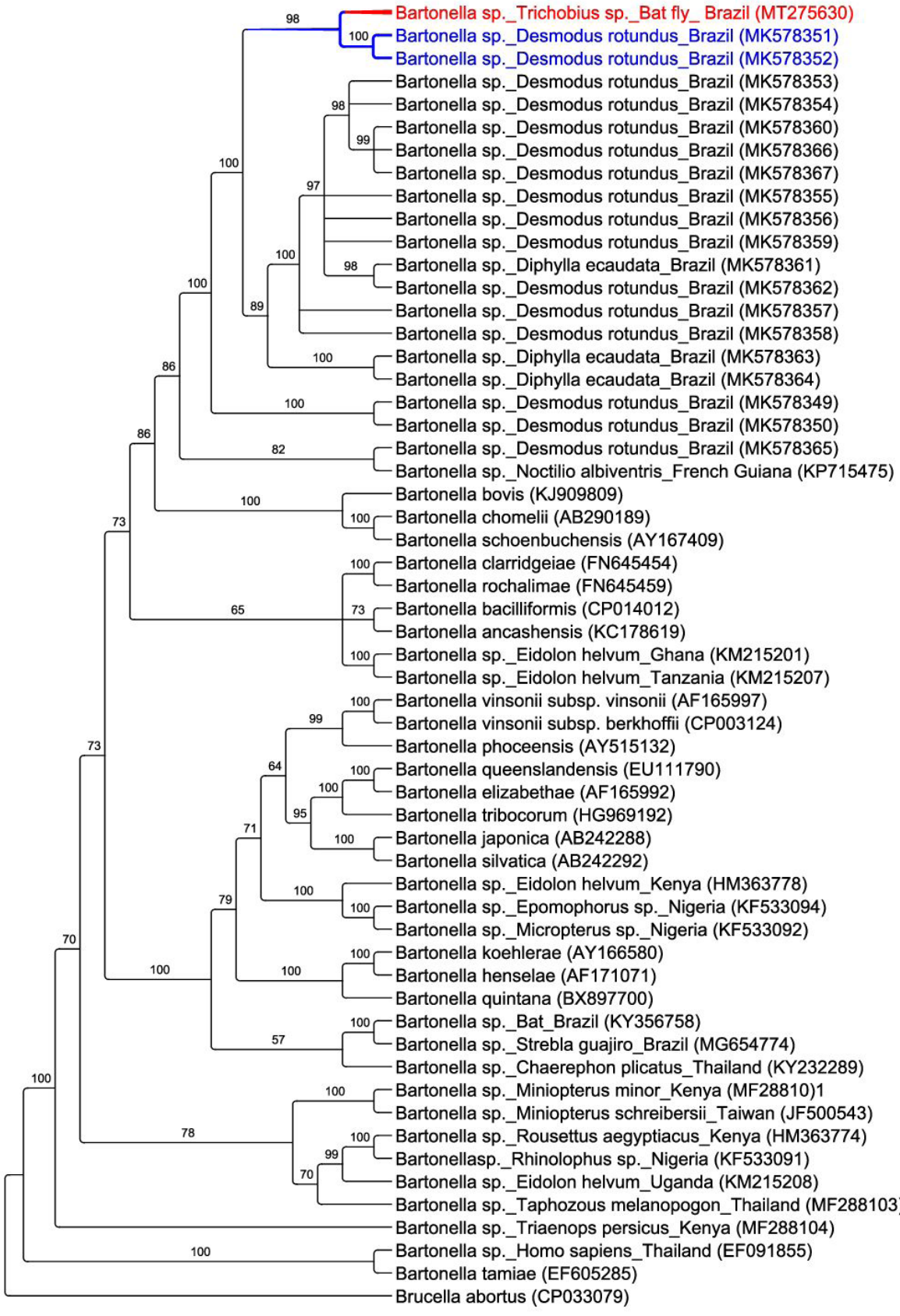

Figure 1. Phylogenetic tree constructed with 800-bp *Bartonella rpoB* sequences, using Bayesian method and GTR+G+I evolutionary model. Numbers at nodes correspond to posterior probability over 50%. *Brucella abortus* sequence was used as outgroup. The sequence amplified in the current study is highlighted in red. The blue color highlighted sequences belonging to the cluster in which the amplified sequences grouped with.

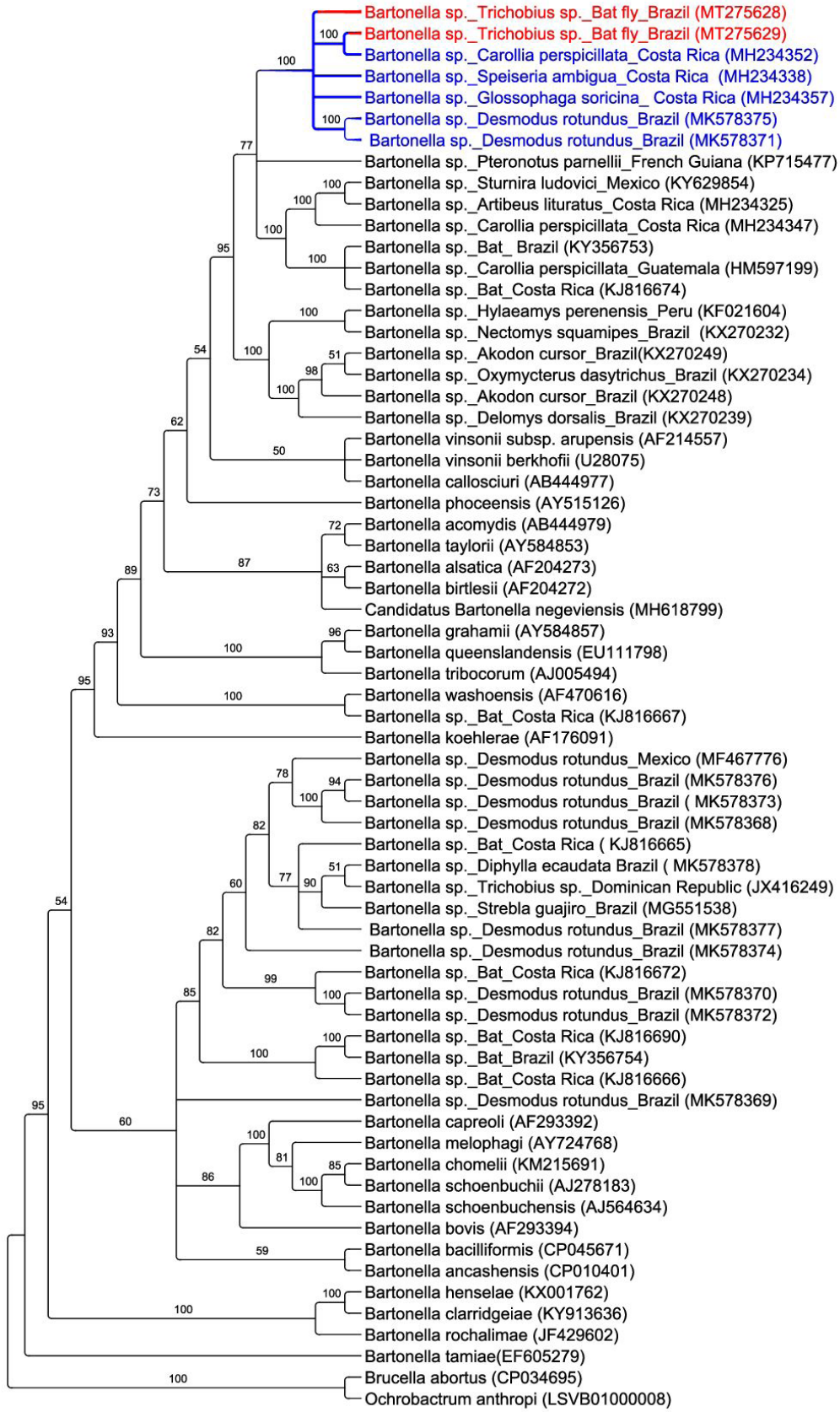

Figure 2. Phylogenetic tree constructed with 700-bp *Bartonella gltA* sequences, using Bayesian method and TIM+G+I evolutionary model. Numbers at nodes correspond to posterior probability over 50%. *Ochrobactrum anthropi* and *Brucella abortus* sequences were used as outgroup. The sequences amplified in the current study are highlighted in red. The blue color highlighted sequences belonging to the cluster in which the amplified sequences grouped with.

Even though none of the bat-blood DNA samples analyzed were positive for *Bartonella*, three out of the 15 flies were positive in the screening assay. This result can possibly be partially explained by the higher concentration of parasitized red blood cells in the fly's midgut, compared with the erythrocyte levels in the bats' bloodstream.

Interestingly, the *gltA* and *rpoB* genotypes detected in Streblidae flies in the present study were shown to be phylogenetically related to those previously detected in bats and bat flies from Brazil and Costa Rica. This highlights the fact that bats and bat fly-related *Bartonella* genotypes are widespread in Latin America. Further studies are required to elucidate the role of bat flies in the transmission of *Bartonella* among bat species.

Conclusions

The present study showed the occurrence of new *Bartonella* genotypes in *Trichobius* spp. from northeastern Brazil, despite the absence of bartonellae DNA in the bats that were sampled.

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