Investigation of *Bartonella* spp. in Brazilian mammals with emphasis on rodents and bats from the Atlantic Forest

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ABSTRACT

The *Bartonella* species are zoonotic agents that infect mammals and are transmitted by arthropod vectors. Approximately 18 distinct genotypes cause diseases in humans, and may be spread by both domestic and wild animals. In Brazil, *Bartonella* genotypes have been identified in several species of wild mammals, and in the present study, we analyzed samples from non-human primates (marmosets), marsupials, rodents, and bats, and compared them with the genotypes described in mammals from Brazil, to examine the distribution of *Bartonella* genotypes in two impacted areas of Rio de Janeiro state, in southeastern Brazil. We used polymerase chain reaction (PCR) methods to detect the *Bartonella* DNA using partial sequences of the *gltA, ftsZ*, and *groEL* genes. We generated Bayesian inference and maximum likelihood trees to characterize the positive PCR samples and infer the phylogenetic relationships of the genotypes. A total of 276 animals were captured, including 110 bats, 91 rodents, 38 marsupials, and 37 marmosets. The DNA of *Bartonella* was amplified from tissue samples collected from 12 (4.34%) of the animals, including eight rodents – *Akodon cursor* (5/44) and *Nectomys squamipes* (3/27) – and four bats, *Artibeus lituratus* (3/58) and *Carollia perspicillata* (1/15). We identified *Bartonella* genotypes closely related to those described in previous studies, as well as new genotypes in both the rodent and the bat samples. Considering the high diversity of the *Bartonella* genotypes and hosts identified in the present study, further research is needed to better understand the relationships between the different *Bartonella* genotypes and their vectors and host species. The presence of *Bartonella* in the wild rodents and bats from the study area indicates that local human populations may be at risk of infection by *Bartonella* due to the spillover of these strains from the wild environment to domestic and peri-domestic environments.

1. Introduction

*Bartonella* is a genus of Gram-negative bacteria intracellular facultative of the alpha proteobacteria group (Welch, 2015). These microorganisms present tropism for endothelial cells and erythrocytes, in particular in mammals (Buffet et al., 2013; Chomel et al., 2009; Mimnuck and Battisti, 2009). *Bartonella* is transmitted primarily by vectors, usually hematophagous arthropods, such as sandflies, lice, fleas, and ticks, although it has also been found in ants and honeybees (Breitschwerdt et al., 2010; Fritz et al., 2018; Kesnerova et al., 2016; Siener and Dehio, 2015). A total of 45 *Bartonella* species or genotypes have been described, all with a high degree of molecular diversity (Breitschwerdt et al., 2010; Breitschwerdt and Kordick, 2000; de Sousa et al., 2018; Okaro et al., 2017).

This pathogen can affect humans and both domestic and wild animals, may cause persistent intravascular infection, endocarditis, and...
other forms of vascular pathology (Chomel et al., 2003). Humans appear to be accidental hosts of 18 distinct genotypes and the principal reservoir of two species, *Bartonella quintana* and *B. bacilliformis*. Dogs and cats are hosts of six species, in particular, *B. henselae*, *B. koehlerae*, *B. rochalimae*, *B. claridgeiae*, and the *B. vinsonii* complex (Frank et al., 2018). Ruminants, such as cattle and sheep, are primary hosts of three *Bartonella* species, *B. melophagi*, *B. chomelli* and *B. bovis* (Breitschwerdt, 2014). Wild animals are known to be involved in the transmission cycle include rodents, rabbits, bats, and shrews (Breitschwerdt, 2014).

In Brazil, the occurrence of *Bartonella* in wild rodents was only confirmed by PCR in 2015, in samples obtained from seven species of sigmodontine rodents from the Cerrado region of central Brazil (Favacho 2018). Ruminants, such as cattle and sheep, are primary hosts of three *Bartonella* species, *B. rochalimae*, *B. clarridgeiae*, and the *B. melophagi*, *B. chomelli* and *B. bovis* (Breitschwerdt, 2014).

2. Methods

2.1. Study area

The study was conducted in two areas of Rio de Janeiro state, in southeastern Brazil. One area was in a peri-urban setting in the vicinity of Pedra Branca State Park (PBSP) in the municipality of Rio de Janeiro (southern Rio de Janeiro state), and the other in a rural landscape in three municipalities in the northwest of the state.

At the PBSP (22°53′04″S, 43°34′32″W), samples were collected between January 2015 and December 2018 in the area surrounding the park, over a gradient ranging from anthropogenic environments to preserved natural habitats. The rural study area encompassed parts of three municipalities in northwestern Rio de Janeiro state: Varre-Sai (20°55′52″S, 41°52′09″W), Cambuci (21°34′22″S, 41°54′35″W), and Miracema (21°24′53″S, 42°11′3″W). In the present study, we captured wild mammals in the areas surrounding forest fragments and in anthropogenic environments, in an attempt to identify possible trends in the circulation and transmission of the *Bartonella* genotypes in this interface (Daszk, 2000).

2.2. Capture methods

2.2.1. Capture of non-human primates

The marmosets (*Callithrix spp.*) were captured only in the PBSP study area, in three areas: (i) a residential area (peridomicile); (ii) transition zone (secondary forest), and (iii) more preserved areas of forest in the PBSP, where contact with domestic animals is less frequent. Marmosets were captured in January 2015, November 2016, and September 2018. Each trapping platform was first baited with bananas for approximately 10 days to attract the local marmosets and habituate them to the presence of the traps. After this habituation period, depending on the frequency of occurrence of the marmosets at the platform, 10 Tomahawk™ model 201 traps (16° x 5″ x 5″) were set on the platform to capture the animals. The marmosets captured were transported inside the traps to the field laboratory at the Oswaldo Cruz Atlantic Forest Foundation (FMA) for the collection of biological samples.

2.2.2. Capture of rodents and marsupials

Rodents and marsupials were captured in both study areas (peri-urban and rural) of Rio de Janeiro state (Gonçalves et al., 2016b). In the Pedra Branca State Park (PBSP), these mammals were captured on 10 linear transects, which traversed areas of varying degrees of impact. Three expeditions were conducted in July, October, and November 2017. Each transect consisted of 20 trapping stations arranged at 20 m intervals. Two of the transects were established in the residential area (i), and four transects each in the transition zone (ii) and within the PBSP (iii). Two traps were set at each trapping station, one Tomahawk™ model 201 (16° x 5″ x 5″) and one Sherman™ trap (3″ x 3.75″ x 12″). In addition, four transects of pitfall traps were installed, two in area (ii) and two inside the park (iii).

Seven transects were installed in the rural study area in northwestern Rio de Janeiro state, four in forest fragments and three in an altered matrix (with pasture), with traps being set both on the ground and in the understory. Each transect consisted of 15 trapping stations arranged at 20 m intervals. Two expeditions were carried out to each study area, one in the dry season (from April to August) and the other in the rainy season (from October to March). In both regions, the captured specimens were taken to a field laboratory for the collection of the biological samples. The small mammals were identified by their morphology, karyotypes, and a molecular analysis (Gonçalves et al., 2016b).

2.2.3. Capture of bats

Bats were captured in four areas (P1–P4) of the PBSP, representing different degrees of habitat integrity, during both the dry and the rainy seasons. One capture was conducted per night in each area. Five expeditions were carried out in October and November 2017 and again in February, July, and December 2018. The sampling areas were established along an anthropogenic gradient with P1 being the most degraded area, with intense anthropogenic pressure, P2 having an intermediate degree of conservation and anthropogenic pressure, P3 being more conserved with less pressure, and P4 the most conserved area, with little anthropogenic pressure. The bats were captured in 10 sets of polyester mist-nets (9 m x 3 m; 20 mm mesh) were deployed near food sources, bodies of water, and along flight routes, such as existing trails in the forest, along roads, and in clearings. The mist-nets were opened at twilight (around 6 p.m.) and reviewed at 15-min intervals over the next 4 h (Esberard and Bergallo, 2008). The captured bats were placed in cotton bags and transported to the field laboratory for the collection of biological samples.

2.2.4. Ethical statement

All the specimens were captured, anesthetized, and euthanized following standard protocols based on the health and safety procedures recommended for procedures of this type (Lemos and D’Andrea, 2014). The marmoset specimens were anesthetized using Ketamine Hydrochloride (100 mg/ml) at a dose of 10 mg/kg, while the marsupials received Ketamine associated (1:1) with Xylazine Hydrochloride (20 mg/ml) at a dose of 0.1 ml/100 g. For the rodents and bats, the Ketamine was associated (9:1) with Acepromazine (10 mg/ml) at a dose of 0.15 ml/100 g. The specimens were euthanized by total exsanguination or
intracardiac injection of 19.1% Potassium Chloride (KCL), at a dose of 2 mL/kg, for the rodents, marsupials and bats or of Sodium Thioridain (30 mg/kg) for the marmosets. All the procedures applied to each animal group were approved previously by the Ethics Committee for Animal Research of the Oswaldo Cruz Foundation under license numbers LW-63/14 (marmosets), LW39/14 (rodents and marsupials), and LM-6/18 (bats).

2.3. Laboratory methods for detection of Bartonella DNA

The DNA was extracted from samples of the liver and spleen using a commercial extraction kit (QiAamp DNA Mini Kit, Qiagen®), according to the manufacturer’s instructions. Samples of the spleen are preferred for molecular testing (Kosoy et al., 2017), but when unavailable, liver samples were used. The quality of the extracted DNA was assessed in agarose gel. The DNA of Bartonella detected in wild rodents and bats in previous studies (Ferreira et al., 2018; Rozental et al., 2017) was used as the positive control for the molecular assays, while nuclease-free water (UltraPure™ DNase/RNase-Free – Invitrogen) was used as the negative control. The DNA samples were submitted to conventional PCR for the gltA (731 bps - Rozental et al., 2017), ftsZ and groEL (present study) genes. The assay was run for each gene containing 0.5 μL of each primer (10 μM), 0.5 μL of 20 mM dNTP (Invitrogen™), 4.0 μL of 50 mM MgCl2 (Applied Biosystems®), 2.5 μL of 10x PCR buffer (Applied Biosystems®), 0.2 μL of AmpliTag Gold® DNA Polymerase (5U/μL, Applied Biosystems®), 13.8 μL of nuclease-free water (UltraPure™ DNase/RNase-Free – Invitrogen) and 3 μL of the sample DNA in a final volume of 25 μL. The cycle conditions and steps applied for each gene are listed in Table 1. For the sequencing reaction, the amplified products were purified using the illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare®) and were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction®3.1kit (Thermo Fisher Scientific™, Waltham, MA, USA). Sequences were deposited in GenBank database (MN613434-MN613442).

2.4. Bioinformatics

The nucleotide sequences, their chromatograms, the consensus sequence, and the divergence in the nucleotides between sequences, were all elucidated by a contig analysis run in Geneious® (Kearse et al., 2012). The sequences in this study and those retrieved from GenBank were aligned with the species described by Okaro and collaborators (2017). Sequences of Brucella abortus IVRI/95 and Ca. Toxoplasma gondii (Segers et al., 2017) were used as the outgroups. The final alignments of the gltA (718 bps), ftsZ (788 bps), and groEL (798 bps), and the Brazilian genotypes of each gene were included in the analyses (Gonçalves et al., 2020; Calchi et al., 2020; Andre et al., 2019a; Andre et al., 2019b; Pedrassani et al., 2019; Silva et al., 2019; do Amaral et al., 2018; de Sousa et al., 2018; Ferreira et al., 2018; Rozental et al., 2017; Ikeda et al., 2017; Gonçalves et al., 2016a; Diniz et al., 2016; Andre et al., 2014; Miceli et al., 2013; Filoni et al., 2012). All the sequences were aligned using the MAFFT v7.388 algorithm for each sequen
cent gene (Katoh and Standley, 2013). These alignments were used to calculate the number of haplotypes, in the DNASp v5 software (Librado and Rozas, 2009). The best evolutionary model for each gene was determined by the Bayesian Inference Criterion (BIC) using the Smart model selection algorithm of the Montpellier Bioinformatics Platform (Lefort et al., 2017). The phylogenetic analysis of each gene was based on (a) the Maximum Likelihood (ML) approach, run in the Montpellier Bioinformatics Platform. The statistical support for the different clades was determined by a heuristic search with 1000 bootstrap replicates (Guindon and Gascuel, 2003) and (b) Bayesian Inference (BI) run in Mr.Bayes 3.1.2 (Huelshenbeck and Ronquist, 2005) with the uncorrelated relaxed clock (Drummond et al., 2006) and constant population size (Kingman, 1982). The Markov Chain Monte Carlo (MCMC) simulations were run for 10^6 generations and sampled at every 10^3 generations, with a burn-in of 10%, in BEAST v1.8.4 (Drummond and Rambaut, 2007). The nodal support of the topology of each gene was calculated by bootstrap replication and the posterior probability (PP) with a cutoff of 50%.

3. Results

A total of 276 animals were captured during the present study, including 110 bats, 91 wild rodents, 38 marsupials, and 37 marmosets (Table 2). The most abundant species captured was the rodent Akodon cursor, followed by the marmosets. The largest number of specimens were captured in the urban study area at the PBSP (161) and in the rural area of Varre-Sai municipality (72). The DNA of Bartonella was amplified from the tissue samples obtained from 12 animals (4.34% of the total). These animals included eight rodents – five A. cursor (n = 44) and three Nectomyys squamipes (n = 27) – and four bats – three Artibeus lituratus (n = 58) and one Carollia perspicillata, of the 15 captured (Table 2).

The PCRs indicated that the ftsZ target gene was amplified in all 12 samples of rodents and bats, while the gltA gene was amplified in 10 samples, and the groEL gene in seven samples (Supplementary File, S1; Table S1). The genus Bartonella formed a monophyletic group, and the principal lineages were reconstructed with high nodal support. The ML and BI tree were congruent for most species and lineages. The best evolutionary model identified for the gltA and ftsZ genes was the GTR model with invariable sites (I) and gamma distribution (G), while the best model for the groEL gene was the HKY + I + G model. The effective sample sizes were over 200 for all the Bayesian trees.

The genotypes showed in trees are defined by haplotypic analysis of Bartonella DNA sequences. Each terminal taxon represents a species and/or genotypes included for each gene separately. The gene gltA showed 41
variable sites of polymorphism, \textit{groEL} showed 35 variable sites and \textit{ftsZ} showed 40 variable sites. The genotypes found in this study were associated to genotypes already described to other Brazilian mammals and vectors and represent the diversity of \textit{Bartonella} hosts from Brazil to these genes (Fig. 1 – 3).

The phylogenetic tree of the \textit{gltA} gene had eight genotypes arranged in four clades (A-D), which were closely-related to the genotypes found in other rodents and bats, recorded in Brazil (Fig. 1). The \textit{Bartonella} \textit{gltA} genotypes have been described in many wild mammal hosts, such as rodents, bats and armadillos, as well as in domestic animals, including dogs, cats, and cattle, from Brazil (Fig. 1). In addition to these vertebrate hosts, some genotypes have been described in hematophagous vectors, such as fleas, flies, lice, and ticks (Fig. 1).

Clade A, dominated by rodent genotypes, presented high nodal support in both the ML and BI analyses (85/1), and contain genotypes which were shared with those recorded in previous studies, all in Rio de Janeiro state. The \textit{Bartonella} genotype found in \textit{A. cursor} (18118) from Cambuci was shared with that recorded in \textit{A. cursor} from the municipality of Valença, in Rio de Janeiro state. The \textit{A. cursor} genotype (13,321) was shared with two other species from Rio de Janeiro, \textit{Akodon montensis} and \textit{Euryoryzomys russatus}. The \textit{A. cursor} genotype (18074) from Varre-Sai was also shared with \textit{A. cursor} from Valença, and with \textit{A. montensis} from the municipality of Teresópolis, in Rio de Janeiro state. In this clade, the \textit{N. squamipes} genotypes from Varre-Sai and Miracema were shared with those of \textit{N. squamipes} and \textit{E. russatus}, from the municipalities of Cantagalo and Angra dos Reis (Rio de Janeiro) and were closely related to the genotype described in \textit{Oecomys mamorae}, from Corumbá, in the Brazilian state of Mato Grosso do Sul.

Clade B, composed of bat genotypes, did not have nodal support in either the ML or the BI analyses, although the \textit{A. lituratus} genotype (FMA9) from Rio de Janeiro did group with the genotypes of \textit{Sturnira lilium} and another unidentified bat, both described from Brazil, with high nodal support in both the ML and the BI analyses (98/1). This genotype was similar to that found in the dipteran vector, \textit{Sребла guajiro}, collected from \textit{C. perspicillata} in Rio de Janeiro. Clades A and B were grouped with high nodal support in both the ML and BI analyses (95/1), and with species of the \textit{B. vinsonii} complex, although differently in the ML and BI analyses. Clade C, also represented by bat genotypes, had high nodal support in both the ML and BI analyses (65/0.94), and combined two clades. One \textit{A. lituratus} genotype (RM1612) grouped with the genotypes of \textit{S. lilium} and two \textit{Artibeus} species, \textit{Artibeus fimbriatus} and \textit{Artibeus obscurus}. The second \textit{A. lituratus} genotype grouped with the genotypes of \textit{Phyllostomus discolor} and another unidentified bat, both described in Brazil. In clade D, the last genotype described in the tree (FMA31), which was found in \textit{C. perspicillata}, did not group with the other \textit{Bartonella} species, however, it was close to \textit{Bartonella anchashensis} and \textit{Bartonella bacilliformis}.

The phylogenetic tree of the \textit{groEL} gene had seven genotypes grouped in three clades (E-G), which were close to other rodent and bat genotypes described in Brazil (Fig. 2). \textit{Bartonella groEL} genotypes have

| Table 2 |
|---|
| Captured animals in disturbing areas of Rio de Janeiro, number of positive for \textit{Bartonella} spp. and prevalence of infection. |
| Species | FMA | Cambuci | Miracema | Varre-Sai | Total capture | Bartonella positive | Prevalence by species |
|---|---|---|---|---|---|---|---|
| Order Primates | | | | | | | |
| Callithrix jacchus/penicillata | 37 | – | – | – | 37 | – | – |
| Order Didelphimorphia | | | | | | | |
| Didelphis aurita | 7 | 3 | 7 | 5 | 22 | – | – |
| Grotsimuss microtarsus | – | 1 | – | – | 1 | – | – |
| Marmousis incanus | – | – | – | 8 | 8 | – | – |
| Marmosa (Micoressus) paragrayana | – | – | 2 | – | 2 | – | – |
| Monodelphis americana | 3 | – | – | – | 3 | – | – |
| Philander frenatus | – | – | – | 2 | 2 | – | – |
| Order Rodentia | | | | | | | |
| Subfamily Sigmodontinae | | | | | | | |
| Akodon cursor | – | 10 | 12 | 22 | 44 | 5 | 11,36% |
| Calomyx tener | – | 1 | 1 | 1 | 3 | – | – |
| Calomyx ccerqueiri | – | 1 | – | – | 3 | – | – |
| Nectomyx squamipes | – | 1 | 3 | 23 | 27 | 3 | 11,11% |
| Oligoryzomys nigripes | 4 | – | 1 | 1 | 6 | – | – |
| Oxymycterus davystrichus | – | – | – | 2 | 2 | – | – |
| Family Echymyidae | | | | | | | |
| Triomomyx setosus | – | – | – | 5 | 5 | – | – |
| Order Chiroptera | | | | | | | |
| Artibeus fimbriatus | 5 | – | – | – | 5 | – | – |
| Artibeus lituratus | 58 | – | – | – | 58 | 3 | 5,17% |
| Artibeus obscurus | 1 | – | – | – | 1 | – | – |
| Carollia perspicillata | 15 | – | – | – | 15 | 1 | 6,66% |
| Chiroderma villianum | 1 | – | – | – | 1 | – | – |
| Dexamodous rotundus | 1 | – | – | – | 1 | – | – |
| Epiteicus brasiliensis | 1 | – | – | – | 1 | – | – |
| Glossophaga soricina | 2 | – | – | – | 2 | – | – |
| Lonchophylla peracchi | 1 | – | – | – | 1 | – | – |
| Micronycteris minutu | 1 | – | – | – | 1 | – | – |
| Molossus molossus | 2 | – | – | – | 2 | – | – |
| Myotis ischshohni | 5 | – | – | – | 5 | – | – |
| Myotis nigricans | 6 | – | – | – | 6 | – | – |
| Phyllostomus hastatus | 3 | – | – | – | 3 | – | – |
| Playzrrhinus lineatu | 1 | – | – | – | 1 | – | – |
| Playzrrhinus recifitus | 1 | – | – | – | 1 | – | – |
| Starnira lilium | 3 | – | – | – | 3 | – | – |
| Vampyressa pusilla | 3 | – | – | – | 2 | – | – |
| Total | 161 | 17 | 26 | 72 | 276 | 12 | 4,34% |
been described in rodents, bats, and domestic cats from Brazil. Clade E had high nodal support, and was congruent in the ML and BI analyses (95/1). It was composed of the genotypes found in wild rodents in Brazil, and was closely-related to the B. vinsonii complex, as in the case of the gltA gene, with 0.99 nodal support in the BI analysis. In this topology, the genotypes of N. squamipes from Miracema and Varre-Sai were grouped with the Bartonella genotypes found in Hylaemys megacephalus from Novo Jardim, in the Brazilian state of Tocantins. In clade F, the A. lituratus genotype (FMA9) from Miracema and Varre-Sai was grouped with those described in another four rodents from four Brazilian states: O. nigripes, Rhipidomys gr. macrurus, Oligoryzomys nigripes, Necromys lasiurus, and Trichomys fosteri. These genotypes were close to a flea collected from the wild rodent Polygenis (P.) bohaii, in the municipality of Corumbá, in the state of Mato Grosso do Sul. In the clade H, it had high nodal support in both the ML and BI analyses (99/0.98).

The phylogenetic tree of Bartonella ftsZ genotypes had nine genotypes grouped in five clades (H-L, Fig. 3). The ftsZ gene has been described in many wild mammal hosts, such as rodents, felds, armadillos, and tamarins. Some Bartonella genotypes and their occurrence localities, have been described only for the ftsZ gene. These genotypes include those associated with wild rodents (Rhipidomys gr. macrurus, Oligoryzomys nigripes, Necromys lasiurus, and Trichomys fosteri) and larger mammal hosts, such as Leopardus wiedii and Tamandua tetradactyla.

Clade H, which included mostly wild rodents, had high nodal support, unlike the gltA and groEL genes, these genotypes did not group together with the B. vinsonii complex, but rather, formed a distinct clade (Fig. 3). The Bartonella genotypes found in Akodon cursor (18069) were grouped with those described in N. lasiurus and O. nigripes from the municipalities of Luziânia (Goiás state), Cassilândia (Mato Grosso do Sul), and Ribeirão Grande and Capão Bonito, in the Brazilian state of São Paulo. This group had good nodal support in the ML and BI analyses (50/1). The Bartonella genotype of A. cursor (13,321/18,074) from Varre-Sai grouped with the A. montensis genotypes from Teresópolis, a municipality in the state of Rio de Janeiro, and Capão Bonito in São Paulo, and also with a genotype described from a flea collected from the wild rodent Polygenis (P.) bohaii, in the municipality of Corumbá, in the state of Mato Grosso do Sul. In the clade H, it had high nodal support in both the ML and BI analyses (99/0.98).

The clade H, N. squamipes genotypes from Varre-Sai and Miracema, in the state of Rio de Janeiro, formed a clade with the other genotypes described in wild rodents, with high nodal support in the ML and BI analyses (81/0.97). In this group, the N. squamipes genotypes were close to those described in another four rodents from four Brazilian states: Oecomys mamorae, from Corumbá, in the state of Mato Grosso do Sul, Hylaemys megacephalus from Novo Jardim, in Tocantins, N. lasiurus from São Paulo, in Mato Grosso, and O. nigripes from Ribeirão Grande and Capão Bonito, in São Paulo.

In clade I, the Bartonella genotype found in A. lituratus (FMA9) from Rio de Janeiro, grouped closely with Bartonella pachyuromydis, although it did not have nodal support in the ML analysis, and the support was limited to 0.59 in the BI analysis. In clade J, the Bartonella genotype found in C. perspicillata formed a distinct clade, closely to Bartonella chomelii, Bartonella schoenbuchensis, Bartonella melophagi, Bartonella
capreoli, and Bartonella bovis, although there was no congruence or nodal support in the ML and BI analyses. The genotype of clade K, described in A. cursor (18069) from Varre-Sai was related to B. rochalimae as shown by the topology of the groEL gene, with high nodal support in both the ML and the BI analyses (99/1). The genotypes of clade L, in A. lituratus (RM1612/RM1618), formed a unique clade, distinct from all other species, but without nodal support in the ML or BI analyses.

4. Discussion

There are many Bartonella genotypes described in Brazilian mammals, primarily rodents and bats (André et al., 2019b; do Amaral et al., 2018; de Sousa et al., 2018; Ferreira et al., 2018; Rozental et al., 2017; Ikeda et al., 2017; Gonçalves et al., 2016a). In the present study, we analyzed not only the Bartonella genotypes found in the rodents and bats specimens collected, but also included the data from the other genotypes described in these and in other mammals from wild and domestic environments in Brazil (André et al., 2014, 2019a; Gonçalves et al., 2020; Pedrassani et al., 2019; Silva et al., 2019; Calchi et al., 2020; Miceli et al., 2013; Filoni et al., 2012).

The available data on the gltA, groEL, and ftsZ genes indicate that 26 mammal species are known to be hosts of Bartonella genotypes, including 12 rodents (Oxymycterus dasytrichus, A. cursor, A. montensis, Delomys dorousis, Euryzonus russatus, N. squamipes, Oecomys moratus, Hylaeamys megacephalus, Necromys lasiurus, O. nigripes, Rhipidomys gr. macrurus and Thrichomys fosteri), seven bats (A. lituratus, A. fimbriatus, A. obscurus, C. perspicillata, Desmodus rotundus, Phyllostomus discolor, Sturira lilium), one felid (Leopardus wiedii), one armadillo (Euphractus sexguttatus), and the tamandua (Tamandua tetradactyla). Four domesticated mammals, dogs (Canis lupus familiaris), cats (Felis catus), cattle (Bos taurus), and buffalo (Bos bubalis), have also been described as hosts of Bartonella DNA.

The genotypes associated with the genus Akodon have already been described in previous studies (Gonçalves et al., 2016a; Rozental et al., 2017). This genus is widely distributed in eastern Brazil, where A. cursor and A. montensis occur in sympatry in some areas of the Atlantic Forest biome (Bonvicino et al., 2009). These rodents are common in anthropogenic environments, farmland, residential landscapes, and the edge of forest fragments (Bonecker et al., 2009; Feliciano et al., 2002; Geise, 2012; Jordão et al., 2010), and may thus act as hosts of Bartonella between wild and domestic environments.

In the specific case of the gltA gene, we recorded Bartonella genotypes that are closely related to those described in previous studies, mainly of the B. vinsonii complex, in clade A (Figs. 1 and 2; Gonçalves et al., 2016a; Rozental et al., 2017). The genotypes described for the gltA and ftsZ gene (clade A and clade H) are widely distributed in the Atlantic Forest, in both the northern and southern regions of Rio de Janeiro state. These findings indicate that the same Bartonella genotype is shared by rodents of the same genus, that is, A. cursor and A. montensis, as well as those of different genera, such as A. cursor and Euryzonus russatus (Gonçalves et al., 2016a; Rozental et al., 2017). This sharing of Bartonella genotypes may be due to the sympatry of A. cursor with the other two species in the respective study areas (Rozental et al., 2017).

The analysis of groEL and ftsZ genes in A. cursor identified a genotype closely related to B. rochalimae (Figs. 2 and 3; Clade G and K). Bartonella rochalimae is the second most prevalent clade in wild carnivores and can also be found in domestic cats and dogs and their vectors (Bai et al., 2016; Fleischman et al., 2015; Frye et al., 2015; Gerrikagotia et al., 2012; Hen et al., 2009; Kosoy and Goodrich, 2019; Saisongkorh et al., 2009; Schott et al., 2019). Akodon is known to be preyed on by carnivores in the Brazilian Atlantic Forest (Facure et al., 2003; Gatti et al., 2006b, 2006c; Santos et al., 2003). As we found B. rochalimae in a new host group, that is, the rodents, the contact between the wild carnivores and this rodent (A. cursor) may be important for the transmission of B. rochalimae. This pattern is similar to the tick-borne disease food chain given that generalist rodents, such as A. cursor, which occur in both forest fragments and anthropogenic environments, may increase the prevalence of Bartonella infection in its carnivore predators (Ostfeld et al., 2018).

The water rat, N. squamipes, has semiaquatic habits and is widely distributed along watercourses in eastern Brazil, in the Atlantic Forest and in its transition zone with the Cerrado, savanna (Bonvicino et al., 2008; D’Andrea et al., 2007). This species has already been found to be infected with a number of Bartonella genotypes in previous studies. Gonçalves et al. (2016a) found that N. squamipes was PCR positive for Bartonella in Minas Gerais and Mato Grosso do Sul states, although they...
did not describe its phylogenetic relationships with other genotypes. Rozental et al. (2017) found *Bartonella* DNA in *N. squamipes* in southern and western Rio de Janeiro state. Given the topology of the gltA gene (Fig. 1; clade A), the genotypes found in our study are the same as those identified in the study areas surveyed by Rozental et al. (2017).

The *Bartonella* genotypes found in *N. squamipes* are closely related to those described in previous studies, in *Oecomys mamorae* (clade A; de Sousa et al., 2018), *Hylaeamys megacephalus*, *N. lasiurus* and *O. nigripes* (clade E and clade I; Gonçalves et al., 2016a). The *Bartonella* genotypes found in *N. squamipes* were closely related to those found in rodents from three biomes— the Atlantic Forest (*O. nigripes*: Agrellos et al., 2012; Weksler et al., 2003), the Cerrado (*N. lasiurus* and *H. megacephalus*: Becker et al., 2007; Pires et al., 2011; Weksler et al., 2006) and Pantanal (*Oecomys mamorae*: de Sousa et al., 2018; Carleton et al., 2009). These findings indicate a lack of specificity in this *Bartonella* genotype, given its occurrence not only in different tribes of cricetid rodents, but also in different Brazilian biomes.

The diversity of *Bartonella* found in bats in recent studies reveals the importance of this mammal group as hosts of the different *Bartonella* genotypes (Bai et al., 2012; Corduneanu et al., 2018; do Amaral et al., 2018; Ferreira et al., 2018; Ikeda et al., 2017; McKee et al., 2016; Stuckey et al., 2017).

The *Bartonella* genotypes of the gltA and ftsZ genes (clades D and J), described from *C. persicicillata* in the present study, indicated the existence of a variant that requires further attention, given that the phylogenetic relationships are not consistent in the two analyses. This is the first report of *Bartonella* in *C. persicicillata* from Rio de Janeiro state, based on the analysis of tissue samples. In clade D, the *Bartonella gltA* genotypes of *C. persicicillata* from Rio de Janeiro clustered between two species, *Bartonella anchashensis* and *B. bacilliformis*. André et al. (2019b) obtained similar results based on a BLAST analysis of the *Bartonella* ITS sequences found in two species of vampire bat. In clade J, the *Bartonella* ftsZ genotypes of *C. persicicillata* from Rio de Janeiro clustered near the *Bartonella* species found primarily in ruminants. This genotype had already been detected in *C. persicicillata* from the Brazilian state of Pará, in a similar phylogenetic arrangement (Ikeda et al., 2017). Based on the topology of the *Bartonella rpoB* genotypes, André et al. (2019) found *Bartonella* associated with three species of vampire bat close to the *Bartonella* ruminant clade. This reflects the importance of the feeding behavior of the mammals for the transmission of this zoonotic agent, and that bats with different feeding adaptations may host a genetic diversity of *Bartonella* that is still underestimated.

The dipterans of the family Streblidae include a large number of flies that are obligatory hematophagous ectoparasites of bats (Reeves and Lloyd, 2019; Szentiványi et al., 2019). Some of these streblid species are known to be vectors of *Bartonella* in a number of different regions of the

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**Fig. 3.** Phylogenetic relationships within the *Bartonella* genus based on the ftsZ gene. The tree was inferred by using the Maximum Likelihood (ML) and Bayesian inference (BI) with the GTR + I + G model. The nodal support is described at the left by bootstrap replicates and at the right by posterior probability to each node represented. The symbol of one asterisk (*) indicates low nodal support in ML or BI, and the symbol of two asterisk (**) indicates incongruence between ML and BI. The sequences detected in the present study are described in red and the sequences of previous studies of Brazilian genotypes in blue. The highlighted clades represented the genotypes described to Brazil. The clade H, I, J, K and L represent the genotypes obtained in this study. *Brucella abortus* and *Ca. Tokpelaia hoelldobleri* was used as an outgroup. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
world (do Amaral et al., 2018; Judson et al., 2015; Morse et al., 2012; Stuckey et al., 2017) and, considering the enormous diversity of fly species found in Brazil (Lourenço et al., 2016), they are potential vectors of Bartonella in Neotropical bat communities. Interestingly, a Bartonella genotype has already been recorded in Streblia guajiro from Rio de Janeiro state (do Amaral et al., 2018). This dipteran species is a common ectoparasite of C. perspicillata (Dornelles and Gracioli, 2017; Lourenço et al., 2020). In our analysis, this genotype (clade B) was closely related to the one found in A. lituratus (Clade B; FM9A). These results indicate that the Bartonella genotypes found in A. lituratus may also occur in C. perspicillata and the ectoparasites of both bats in the Atlantic Forest of Rio de Janeiro state.

The phylogenetic relationships of the Bartonella genotypes found in A. lituratus (FM9A) varied in their topology (clade B, F and I). Based on the topology of the gltA gene, clade B is composed exclusively by genotypes that have been described in Brazilian bats, such as Sturnira lilium, the vampire bat, Desmodus rotundus, and another unidentified bat (Andre et al., 2019b; do Amaral et al., 2018; Ferreira et al., 2018; Ikeda et al., 2017). Clade B may thus possibly represent a Bartonella species group adapted specifically to bat hosts. In the other topologies, however, there are divergences in the phylogenetic relationships, as observed in the groEL tree, where this genotype grouped with R. rattouaustaliani and B. japonica (clade F), and in the ftsZ tree, where it grouped with P. phalium (clade I). There is a lack of data on occurrence of the Bartonella genotypes (of genes other than gltA) in bat hosts from Brazil. It is thus possible that the variation in the phylogenetic arrangement is a result of the occurrence of more than one Bartonella species in these hosts, but possibly also by the incomplete lineage sorting of the genotypes. Bartonella DNA has been recorded in A. lituratus from Central and South America (Bai et al., 2012, 2011; Judson et al., 2015; Olival et al., 2015). This bat is common in both urban areas and forest fragments. Ferreira et al. (2018) recorded Bartonella in A. fimbristylus, a sympatric congener of A. lituratus, in the same area surveyed in the present study. They also recorded Bartonella in A. lituratus from the Brazilian state of Bahia.

Although all the marmoset (non-human primate) samples were negative, it is important to monitor these animals for Bartonella infection, given that some Callithrix species, such as C. jacchus and C. penicillata, are invasive in Rio de Janeiro, where they have been introduced from other regions of Brazil through the illegal animal trade (Cezar et al., 2017). This finding, and the lack of records of Bartonella infection in other Brazilian studies of non-human primates, may be related to low bacteremia in these animals, which would hamper molecular detection (Bonato et al., 2015; Breitschwerdt, 2017).

As for the marmosets, the marsupial samples collected in the present study all tested negative. Gonçalves et al. (2020b) challenged the idea that Brazilian marsupials are natural hosts of Bartonella infection, given that some Callithrix species, such as C. jacchus and C. penicillata, are invasive in Rio de Janeiro, where they have been introduced from other regions of Brazil through the illegal animal trade (Cezar et al., 2017). This finding, and the lack of records of Bartonella infection in other Brazilian studies of non-human primates, may be related to low bacteremia in these animals, which would hamper molecular detection (Bonato et al., 2015; Breitschwerdt, 2017).

The fact that Bartonella genotypes have been recorded in 26 mammalian host species from Brazil emphasizes the diversity of this bacterium in this country. However, these genotypes are represented by different loci, and may not always be detected in the assays, possibly because of the low bacteremia in the hosts, as shown by de Sousa et al. (2018) and Gonçalves et al. (2016a). This may hamper the understanding of the epidemiological dynamics of Bartonella, especially given the general lack of data on many of the housekeeping genes.

5. Conclusions

In the present study, we recorded new Bartonella genotypes in rodents and bats from distinct landscapes in the Brazilian state of Rio de Janeiro. These findings emphasize the need for further research, based on alternative approaches, such as the culture of isolates and pathogenicity strategies, in order to better comprehend the relationships of the Bartonella genotypes with their vectors and host species. The considerable diversity of mammalian hosts and Bartonella genotypes already found in Brazil suggests that the genetic diversity of these microorganisms has been underestimated, up to now, in Brazil, given that many other mammals occur in sympathy with these host species and share many of their ectoparasites, which would bring them into potential contact with Bartonella. Our findings are consistent with the previous studies that have shown that Bartonella genotypes associated with specific host lineages may act as zoonotic agents. Given the considerable diversity of Bartonella genotypes identified in the present study, the spillover of Bartonella strains from wild environments to urban and residential areas is highly likely, and may represent a risk for many human populations. Further research is clearly required to understand the interactions among wildlife and domestic animals, and their ectoparasites, in order to better define the dynamics of Bartonella infection patterns.

Declaration of competing interest

Authors have seen and approved this version of the manuscript and have no conflict of interest or disclosures concerning this paper. On behalf of the co-authors, I declare that it is not currently submitted for publication elsewhere.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.07.004.

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