UNIVERSIDADE ESTADUAL PAULISTA – UNESP

FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS (FCAV)

CÂMPUS DE JABOTICABAL

DETECÇÃO E CARACTERIZAÇÃO MOLECULAR DE *Bartonella* spp. EM MORCEGOS E MOSCAS STREBLIDAE ASSOCIADAS NA AMAZÔNIA BRASILEIRA

Eliz Oliveira Franco

Médica Veterinária

2024

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Orientador: Prof. Dr. Marcos Rogério André

Coorientadora: Adolorata Aparecida Bianco Carvalho

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Câmpus de Jaboticabal

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TÍTULO DA DISSERTAÇÃO: DETECÇÃO E CARACTERIZAÇÃO MOLECULAR DE *Bartonella spp*. EM MORCEGOS E MOSCAS STREBLIDAE ASSOCIADAS NA AMAZÔNIA BRASILEIRA

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Jaboticabal, 01 de março de 2024

DADOS CURRICULARES DA AUTORA

Eliz Oliveira Franco- Filha de Lúcia Maria Oliveira e Edmar Teodoro Pereira, casada com Mateus Pinheiro Sandoval, nasceu em 17 de dezembro de 1998, na cidade de Mineiros/GO. Em 2017 ingressou no Centro Universitário de Mineiros (UNIFIMES), onde em 2021 se formou em Medicina Veterinária. Em março de 2022 ingressou no Mestrado de Pós-Graduação em Ciências Veterinárias, na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho, Campus Jaboticabal, como bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) sob orientação do Prof. Dr. Marcos Rogério André.

EPÍGRAFE

“A bondade e a misericórdia me seguirão todos os dias da minha vida”

Salmos 23:6

AGRADECIMENTOS

A Deus, por me sustentar em cada obstáculo, por me livrar de todo, por me dar sabedoria e por me mostrar o seu amor incondicional todos os dias de minha vida.

À minha mãe, Lúcia Maria Oliveira por ter me educado para me tornar um ser humano de bem, pelo amor incondicional, pelo cuidado, pelos ensinamentos e por me proporcionar todas as oportunidades que tive, e que tenho.

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Ao meu Tio Sílvio Azarias de Oliveira (*in memoriam*) que, através dos seus atos de ajudar os que precisavam de forma gentil e acolhedora, que construí os meus valores e me tornei quem sou hoje através do seu exemplo.

À minha família e amigos, por serem meu lar.

Aos meus filhos de quatro patas, por serem acolhimentos durante todos os dias.

Ao Centro Universitário de Mineiros (UNIFIMES) e aos docentes do curso de Medicina Veterinária por toda a base do conhecimento.

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À Profa. Dra. Darci, pelos conhecimentos compartilhados

Ao Dr. André Luiz Rodrigues Roque, do Laboratório de Biologia de Tripanossomatídeos, Instituto Oswaldo Cruz (IOC/Fiocruz), Rio de Janeiro, RJ, por gentilmente ceder as amostras de baço de morcegos coletadas no estado do Acre e colaborar com nosso grupo de pesquisa.

À Dr. Taciana Fernandes Souza Barbosa do Instituto Evandro Chagas MS-SVS, São Brás, Pará, e Me. Laryssa Borges de Oliveira, por gentilmente cederem as amostras de baço de morcegos hematófagos coletadas nos estados da região Norte do Brasil.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil (CAPES) - Código de Financiamento 001, pelo suporte oferecido para o desenvolvimento do trabalho.

À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pelo Auxílio Regular à Pesquisa (Processo n. 2022/08543-2) concedido ao Prof. Marcos R. André, sem o qual não seria possível o desenvolvimento deste trabalho.

Às minhas amigas, Victória Valente Califre de Mello e Caroline Secato, por terem me acolhido em Jaboticabal e por terem tornado o meu aprendizado mais leve.Aos colegas do Vector-borne Bioagents Laboratory (VBBL): Anna Cláudia, Ana Carolina, Amir, Aamir, Daniel, Dália, Clara, Gabrielly, João Vitor, Jovêncio, Lorena, Paulo, Gustavo, Ricardo e Socorro, pela boa vivência do dia a dia. Em especial a Ana Calchi por toda ajuda dispensada nessa reta final.

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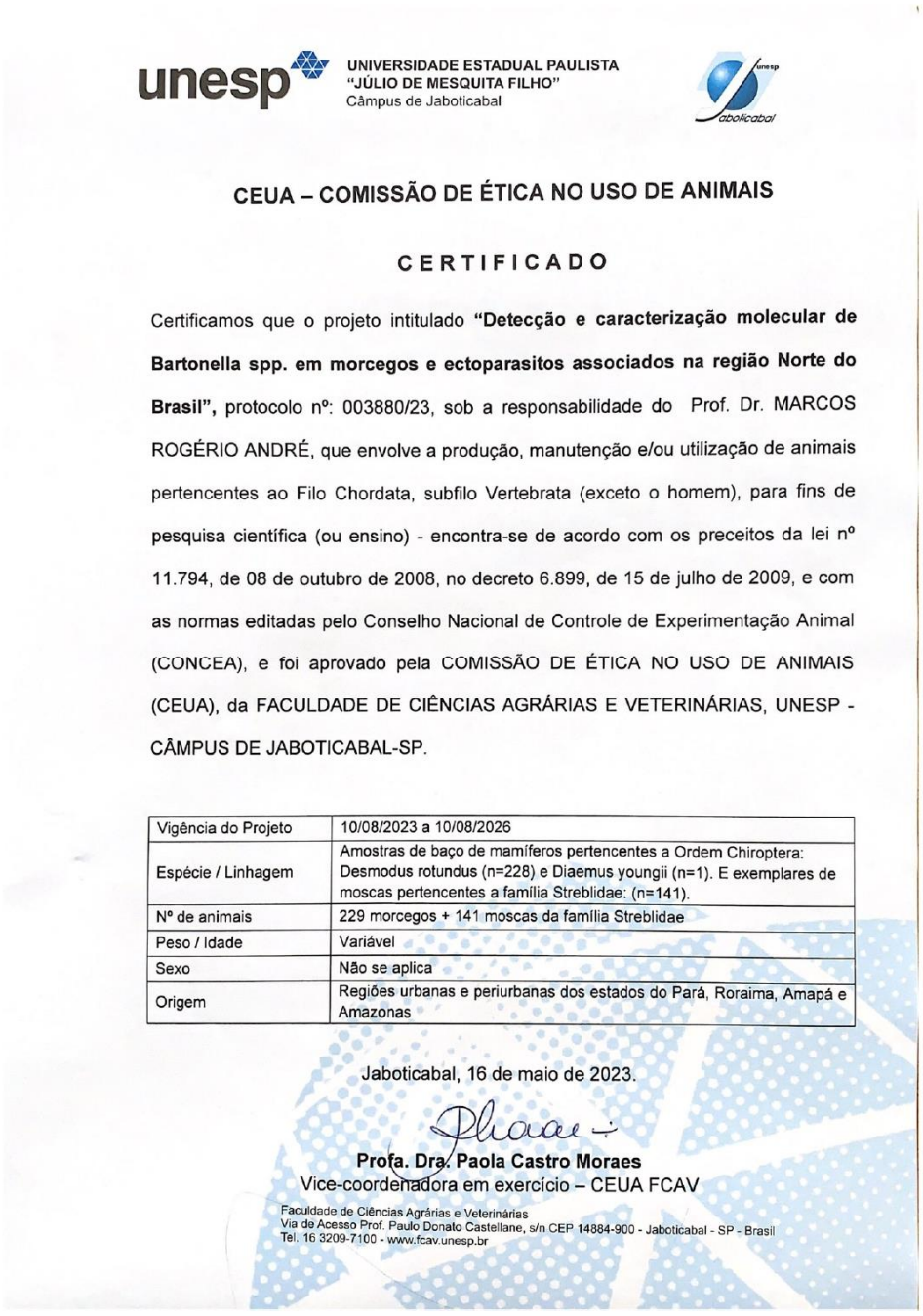
O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

SUMÁRIO

Certificado I de Comissão de Ética no Uso de Animais (CEUA)- Morcegos e ectoparasitos associados na Região Norte do Brasil



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto intitulado **"Detecção e caracterização molecular de Bartonella spp. em morcegos e ectoparasitos associados na região Norte do Brasil",** protocolo nº: 003880/23, sob a responsabilidade do Prof. Dr. MARCOS ROGÉRIO ANDRÉ, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP.

Vigência do Projeto	10/08/2023 a 10/08/2026
Espécie / Linhagem	Amostras de baço de mamíferos pertencentes a Ordem Chiroptera: Desmodus rotundus (n=228) e Diaemus youngii (n=1). E exemplares de moscas pertencentes a família Streblidae: (n=141).
Nº de animais	229 morcegos + 141 moscas da família Streblidae
Peso / Idade	Variável
Sexo	Não se aplica
Origem	Regiões urbanas e periurbanas dos estados do Pará, Roraima, Amapá e Amazonas

Jaboticabal, 16 de maio de 2023.

Profa. Dra. Paola Castro Moraes
Vice-coordenadora em exercício – CEUA FCAV

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209-7100 - www.fcav.unesp.br

Certificado II de Comissão de Ética no Uso de Animais (CEUA)- Morcegos do estado do Acre

unesp UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO" Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Detecção e caracterização molecular de Bartonella spp. em morcegos no estado do Acre"**, protocolo nº 3335/22, sob a responsabilidade do Prof. Dr. Marcos Rogério André, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 15 de junho de 2022.

Vigência do Projeto	01/08/2022 a 31/07/2024
Espécie / Linhagem	Ordem Chiroptera
Nº de animais	353
Peso / Idade	Variável
Sexo	Machos e fêmeas (com exceção de grávidas e /ou lactantes)
Origem	Estado do Acre: Rio Branco e Xapuri

Jaboticabal, 15 de junho de 2022.

Fabiana Pilarski

Profª Drª Fabiana Pilarski
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209-7500 - www.fcav.unesp.br

Autorização para atividades com finalidade científica (Ministério do Meio Ambiente- ICMBio/SISBIO) I

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A434E64

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A434E64**
Usuário:	**Marcos Rogério André**
CPF/CNPJ:	**302.435.148-59**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Desmodus rotundus
Diaemus youngii
Diphylla ecaudata

Título da Atividade:	**Detecção e caracterização molecular de agentes Anaplasmataceae, Hemoplasmas, Piroplasmideos, Hemosporideos e Coxiella burnetii em quirópteros hematófagos amostrados em diversas regiões do Brasil**

Equipe

Marcos Rogério André	**Campus de Jaboticabal**
Victória Valente Califre de Mello	**Universidade Estadual Paulista - UNESP / FCAV Campus**
Eliz Oliveira Franco	**Universidade Estadual Paulista - UNESP / FCAV Campus**
Laryssa Borges De Oliveira	**Universidade Federal do Paraná**
Ana Julia Vidal Placa	**Universidade Estadual Paulista "Julio de Mesquita Filho"**
Ana Cláudia Calchi	**Universidade Estadual Paulista "Julio de Mesquita Filho"**

Parceiras Nacionais

63.025.530/0005-38 / Instituto de Ciencias Biomedicas USP

00.394.544/0025-52 / INSTITUTO EVANDRO CHAGAS

Resultados Obtidos

Outros resultados

Data do Cadastro: **11/07/2022 15:55:06**

Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético

Situação cadastral conforme consulta ao SisGen em **13:35** de **05/02/2024**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

Autorização para atividades com finalidade científica (Ministério do Meio Ambiente- ICMBio/SISBIO) II

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº ACD062C

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**ACD062C**
Usuário:	**Marcos Rogério André**
CPF/CNPJ:	**302.435.148-59**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie
Anoura caudifer
Artibeus lituratus
Artibeus obscurus
Artibeus planirostris
Carollia benkeithi
Carollia brevicauda
Carollia perspicillata
Chiroderma villosum
Dermanura cinereus
Desmodus rotundus
Glossophaga soricina
Lasiurus blossevillii
Lonchophylla thomasi

Lophostoma silvicolum
Mesophylla macconnelli
Mimon crenulatum
Phyllostomus discolor
Phyllostomus hastatus
Platyrrhinus incarum
Platyrrhinus infuscus
Rhinophylla fischerae
Rhinophylla pumilio
Saccopteryx leptura
Sturnira lilium
Sturnira tildae
Tonatia saurophila
Trachops cirrhosus
Uroderma bilobatum
Vampyressa sp.

Título da Atividade:	**Detecção e caracterização molecular de Piroplasmídeos, agentes Anaplasmatacea, Bartonella spp., Hepatozoon spp. e Coxiella burnetii em quirópteros do Acre.**

Equipe

Marcos Rogério André	**Campus de Jaboticabal**
Ana Cláudia Calchi	**UNESP-FCAV**
Eliz Oliveira Franco	**UNESP-FCAV**

Parceiras Nacionais

33.781.055/0012-98 / Instituto Oswaldo Cruz

Data do Cadastro:	**19/01/2024 16:44:00**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **16:46** de **19/01/2024**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - **SISGEN**

DETECÇÃO E CARACTERIZAÇÃO MOLECULAR DE *Bartonella* spp. EM MORCEGOS E MOSCAS STREBLIDAE ASSOCIADAS NA AMAZÔNIA BRASILEIRA

RESUMO

Dentre os mamíferos, os morcegos se destacam como importantes reservatórios para *Bartonella* spp., ficando atrás apenas dos roedores. No Brasil são descritas 182 espécies de morcegos, sendo que três são hematófagas: *Desmodus rotundus*, *Diphylla ecaudata* e *Diaemus youngi.* A Amazônia brasileira detém grande biodiversidade destes mamíferos. Espécies de *Bartonella* têm sido cada vez mais relacionadas com doenças humanas e a busca de tais agentes em reservatórios animais e ectoparasitos mostra-se importante a fim de se elucidar a epidemiologia das bartoneloses. O presente estudo investigou a ocorrência e diversidade genética de *Bartonella* spp. em amostras de baço de morcegos da Amazônia Brasileira: Acre (n=208, de 31 diferentes espécies de morcegos), Pará (n = 206/*Desmodus rotundus*; n = 1/*Desmodus youngi*), Roraima (n = 18/*D. rotundus*), Amapá (n = 3/*D. rotundus*) e Amazonas (n = 1/*D. rotundus*), como também de 142 moscas Streblidae coletadas de 54 *D. rotundus* (23 de *Streblia wiedemanni* e 118 *Trichobius parasiticus*) e um 1 de *D. youngi* (1 *Trichobius diaemi*). Por meio de ensaio de PCR em tempo real quantitativo (qPCR) baseado no gene *nuoG* a partir de amostras de baço, DNA de *Bartonella* spp. foi detectado em 10,1% (21/208) de morcegos no estado do Acre, e em 31,9% (73/229) de morcegos hematófagos amostrados em quatro estados da região Norte do país (Pará, Roraima, Amapá e Amazonas). Ademais, 45 (50%) moscas Streblidae também foram positivas na qPCR para *Bartonella* spp. Os dois genótipos *gltA* de *Bartonella* spp. detectados em morcegos do Acre agruparam-se com aqueles previamente identificados em morcegos de outras localidades. Já as análises filogenéticas baseadas nos genes *gltA* e *rpoB* posicionaram as sequências de *Bartonella* spp. detectadas em morcegos hematófagos juntamente com genótipos previamente detectados em morcegos *D. rotundus* e moscas associadas a morcegos. Uma alta diversidade genotípica (4 genótipos *gltA* e 9 *rpoB*) de *Bartonella* spp. em *D. rotundus* na Amazônia brasileira foi encontrada. Os genótipos identificados em *D. rotundus* no presente estudo foram exclusivamente compartilhados com sequências de *Bartonella* spp. detectadas em morcegos hematófagos, não se sobrepondo com genótipos previamente detectados em morcegos não-hematófagos do Brasil. Demonstrou-se também alta ocorrência molecular (52,2%) de *Bartonella* spp. em moscas Streblidae, com alta diversidade genotípica (4 genótipos de *gltA* e 5 *rpoB*). A maioria das sequências detectadas em moscas Streblidae formaram genótipos únicos para cada espécie de díptero analisada. Por outro lado, dois genótipos *gltA* e um *rpoB* foram compartilhados por sequências detectadas em morcegos hematófagos e moscas Streblidae, sugerindo o possível papel de tais dípteros na transmissão de *Bartonella* spp. entre morcegos. O presente estudo forneceu a primeira evidência molecular da presença de *Bartonella* spp. em morcegos no Estado do Acre e em quirópteros das espécies *Lophostoma silvicolum*, *Vampyressa thyone*, *Tonatia saurophila* e *Phyllostomus elongatus.* Ainda, mostrou alta ocorrência e diversidade genética de *Bartonella* spp. em *D. rotundus* e moscas Streblidae associadas na Amazônia Brasileira.

Palavras-Chave: *Bartonella* spp., Floresta Amazônica, Chiroptera, Diversidade Genotípica

MOLECULAR DETECTION AND CHARACTERIZATION OF *Bartonella* spp. IN BATS AND ASSOCIATED STREBLIDAE FLIES IN THE BRAZILIAN AMAZON

ABSTRACT

Among mammals, bats stand out as important reservoirs for *Bartonella* spp., second only to rodents. In Brazil, 182 species of bats have been described, three of which are hematophagous: *Desmodus rotundus*, *Diphylla ecaudata* and *Diaemus youngi*. The Brazilian Amazon has great biodiversity of these mammals. *Bartonella* species have been increasingly associated to human diseases and the search for such agents in animal reservoirs and ectoparasites is important in order to elucidate the epidemiology of bartonelloses. The present study investigated the occurrence and genetic diversity of *Bartonella* spp. in spleen samples from bats from the Brazilian Amazon: Acre (n=208, from 31 different bat species), Pará (n = 206/*Desmodus rotundus*; n = 1/*Desmodus youngi*), Roraima (n = 18/*D. rotundus*), Amapá (n = 3/*D. rotundus*) and Amazonas (n = 1/*D. rotundus*), as well as 142 Streblidae flies collected from 54 *D. rotundus* (23 from *Streblia wiedemanni* and 118 *Trichobius parasiticus*) and 1 from *D. youngi* (1 *Trichobius diaemi*). Using a quantitative real-time PCR (qPCR) assay based on the *nuoG* gene on spleen samples, *Bartonella* DNA was detected in 10,1% (21/208) of bats in the state of Acre, and in 31,9% (73/229) of vampire bats sampled in four states in the northern region of the country (Pará, Roraima, Amapá and Amazonas). Furthermore, 45 (50%) Streblidae flies were also positive in qPCR for *Bartonella* spp. The two *gltA* genotypes of *Bartonella* spp. detected in bats from Acre grouped with those previously identified in bats from other locations. Phylogenetic analyzes based on the *gltA* and *rpoB* genes positioned the *Bartonella* sequences detected in vampire bats together with genotypes previously detected in *D. rotundus* bats and bat-associated flies. A high genotypic diversity (4 *gltA* and 9 *rpoB* genotypes) of *Bartonella* spp. in *D. rotundus* in the Brazilian Amazon was found. The genotypes identified in *D. rotundus* in the present study were exclusively shared with *Bartonella* sequences detected in vampire bats, not overlapping with genotypes previously detected in non-hematophagous bats from Brazil. A high molecular occurrence (52.2%) of *Bartonella* spp. was also demonstrated in Streblidae flies, with high genotypic diversity (4 *gltA* and 5 *rpoB* genotypes). The majority of sequences detected in Streblidae flies formed unique genotypes for each dipteran species analyzed. On the other hand, two *gltA* and one *rpoB* genotypes were shared by sequences detected in vampire bats and Streblidae flies, suggesting the possible role of these dipterans in the transmission of *Bartonella* spp. among bats. The present study provided the first molecular evidence of the presence of *Bartonella* spp. in bats in the State of Acre and in bats of the species *Lophostoma silvicolum*, *Vampyressa thyone*, *Tonatia saurophila* and *Phyllostomus elongatus*. It also demonstrated high occurrence and genetic diversity of *Bartonella* spp. in *D. rotundus* and associated Streblidae flies in the Brazilian Amazon.

Keywords: *Bartonella* spp., Amazon Rainforest, Chiroptera, Genotypic Diversity

CAPÍTULO 1 – Considerações gerais

1. Introdução

O Brasil é detentor de grande diversidade de quirópteros, com 182 espécies de morcegos distribuídas em nove famílias, compreendendo 12,5% do total mundial de espécies de morcegos. Tais mamíferos possuem papel fundamental na manutenção dos ecossistemas por atuarem na dispersão de sementes, polinização e controle de insetos. Por outro lado, devido à alta complexidade biológica, como capacidade de voo, alta longevidade, formação de colônias populacionais, vasta disseminação, e ampla diversidade de espécies, quirópteros são reconhecidos como importantes reservatórios naturais para diversos agentes patogênicos.

O gênero *Bartonella* sp. engloba α-proteobactérias Gram-negativas intracelulares facultativas que podem causar zoonoses emergentes. Tais agentes são transmitidos por vetores artrópodes e possuem mamíferos como principais reservatórios. Os quirópteros representam o segundo principal reservatório de *Bartonella* entre os mamíferos, ficando atrás apenas dos roedores. Até o momento, três espécies detectadas em morcegos (*Bartonella tamiae*, '*Candidatus* Bartonella mayotimonensis' e *'Candidatus* Bartonella rousetti') foram incriminadas como agentes zoonóticos.

Embora estudos prévios tenham sido conduzidos a respeito de *Bartonella* spp. em quirópteros no Brasil, inexistentes são os estudos no estado do Acre. Similarmente, poucos são os estudos acerca da ocorrência e diversidade de *Bartonella* spp. em morcegos hematófagos e moscas Hipposcoidea, possíveis vetores destes agentes entre morcegos, no mundo. No Brasil, 68 espécies de moscas Streblidae e 29 moscas Nycteribiidae parasitas de morcegos foram descritas até o momento.

O trabalho em tela investigou a ocorrência e diversidade genética de *Bartonella* spp. em quirópteros e moscas Streblidae na Amazônia brasileira, a fim de contribuir para o entendimento da diversidade de genótipos destes agentes em morcegos.

2. Revisão de Literatura

2.1 Ordem Chiroptera

A designação Chiroptera tem sua origem na combinação das palavras gregas “Cheir”, que significa mão, e “pteron”, que se traduz como asas. Embora compartilhem todas as características comuns aos demais mamíferos, são os únicos na classe Mammalia que possuem a capacidade de voar. Os membros anteriores passaram por modificações significativas, apresentando alongamento ósseo e a presença de uma membrana elástica conhecida como patágio. Em algumas espécies também possuem uma membrana nos membros posteriores, chamada de interfemural ou uropatágio (Peracchi et al., 2011).

A ordem Chiroptera é subdividida em duas subordens: (i.) Megachiroptera, encontrada exclusivamente no Velho Mundo e composta uma única família (Pteropodidae), conhecidos como “raposas-voadoras” (flying-fox), sendo o *Pteropus vampyrus* a maior espécie conhecida; e (ii.) Microchiroptera, representada pelos morcegos da Europa e Américas (Novo Mundo), com semelhanças nas famílias Vespertilionidae, Molossidae e Phyllostomidae, sendo o maior representante o *Vampyrum spectrum* (Simmons, 2001).

A ecolocalização, que consiste na emissão e percepção de sons de alta frequência, utilizada para orientação durante o voo, é distinta nas diferentes subordens. Os microchiropteros apresentam extraordinária capacidade de emitir e receber sons de alta frequência, enquanto os megachiropteros não são capazes de utilizar tal ferramenta, com exceção das espécies do gênero *Rousettus* (Simmons e Conway, 2003).

Além da subdivisão taxonômica, a ordem Chiroptera também é subdividida em duas subordens de acordo com as relações filogenéticas (Hutcheon e Kirsch, 2006). A subordem Yangochiroptera Koopman, 1984 que agrupa as superfamílias Emballonuroidae, Noctilionoidae (que inclui a família Phyllostomidae) e Vespertilionoidae. Já a Yinpterochiroptera Springer, Teeling, Madsen, Stanhope & Jong, 2001 compreende a família Rhinolophoidae (incluindo a antiga família Pteropodidae).

A principal característica que os diferem aos demais mamíferos está relacionada com o tipo de espécie e as necessidades de deslocamento. Por exemplo, morcegos frugíveros, que necessitam carregar frutos durante o voo, possuem asas largas e grandes. Já as espécies insetívoras, que para a caça precisam de manobras rápidas e alta velocidade, possuem asas curtas e estreitas (Vaughan et al., 2000).

Os hábitos alimentares e a ecologia dos morcegos são totalmente distintos dos demais grupos de mamíferos. Sendo reconhecidos por desempenharem funções essenciais na manutenção dos ecossistemas, devido a sua capacidade de: polinização (quiropterofilia), dispersão de sementes (quiropterocoria), controle de populações de insetos e bioengenheiros em ambientes cavernícolas (Fenton e Simmons, 2014). Além de apresentarem praticamente todos os hábitos alimentares (hematofagia, polinivoria, nectarivoria, piscivoria, onivoria, insetivoria, frugivoria e carnivoria) (Peracchi et al, 2006).

A reprodução dos morcegos varia de monoestria sazonal (com um único pico reprodutivo ao ano, mais comum em espécies tropicais) à poliestria sazonal, (com duas a três picos por ano). Normalmente as fêmeas parem um único filhote, porém alguns vespertilionídeos (família de morcegos pertencente à subordem Microchiroptera) podem parir de dois a cinco filhotes numa única parição (Fleming et al., 1972).

Além de desempenharem um papel crucial na ecologia por meio dos serviços ecossistêmicos que oferecem, os morcegos também são reconhecidos como reservatórios naturais significativos para diversos agentes patogênicos, como protozoários, vírus e bactérias, devido a características biológicas como: alta diversidade de espécies, distribuição geográfica, hábitos comportamentais, longevidade, capacidade de voo, formação de grandes colônias populacionais, alta dispersão e colonização de diversos nichos (Concannon et al. 2005; Wallau et al. 2023).

No Brasil foram registradas oficialmente 181 espécies de morcegos, as quais estão distribuídas em 68 gêneros e 9 famílias (Garbino et al., 2020), sendo o bioma amazônico aquele que abriga a maior parte desta riqueza de espécies, e o estado do Pará com o maior número de espécies registradas (Bernard, Aguiar e Machado,

2011). Tal cenário com exorbitante riqueza de espécies ressalta a importância do estudo de patógenos zoonóticos circulantes em quirópteros.

2.2 Gênero *Bartonella*

O gênero *Bartonella* (Hyphomicrobiales: Bartonellaceae) engloba bactérias Gram-negativas intracelulares facultativas que parasitam preferencialmente eritrócitos e células endoteliais de mamíferos, podendo também propagar-se em células dendríticas, macrófagos e monócitos (Eicher e Dehio, 2012).

A família Bartonellaceae compreende aproximadamente 45 espécies descritas e isoladas de humanos, animais selvagens e animais domésticos. Mais de 10 espécies são apontadas como zoonóticas (*B. bacilliformis*, *B. quintana*, *B. henselae*, *B. elizabethae*, *B. clarridgeiae*, *B. koehlerae*, *B. grahamii*, *B. rochalimae*, *B. tamiae*, *B. ancashensis*, *B. washoensis*) (Okaro et al., 2017), cujas manifestações clínicas variam de febre intermitente à inflamação de tecidos (Mogollon-Pasapera et al. 2009, Rao et al. 2021).

Devido à bacteremia intraeritrocítica de longa duração, os hospedeiros mamíferos podem desempenhar o papel de reservatórios para *Bartonella* spp., o que aumenta a probabilidade de transmissão desses agentes por meio de vetores, como por exemplo os ectoparasitos (Deng et al., 2012; Pitassi et al., 2015).

Dentre as espécies de *Bartonella* sp. de importância médica, destacam-se *Bartonella henselae*, *Bartonella quintana* e *Bartonella bacilliformis*. A primeira é responsável por causar a Doença da Arranhadura do Gato (DAG), principalmente em indivíduos imunocomprometidos. O contato com os gatos infectados é fator de risco para a infecção. A enfermidade pode cursar, em seres humanos, de forma típica (DAG típica) e atípica (DAG atípica). A primeira é benigna e autolimitante, com o aparecimento de pápulas eritematosas que podem evoluir para uma linfadenomegalia. Já a DAG atípica cursa com febre alta, anemia hemolítica, pneumonia, Síndrome Oculoglandular de Parinaud, encefalite, osteomielite, hepatoesplenomegalia, etc. (Guptill et al., 1997; Velho, 2001).

Bartonella henselae, assim como *B. quintana*, podem causar lesões de proliferação vascular na pele e no parênquima hepático, principalmente em indivíduos

infectados pelo vírus da imunodeficiência humana (HIV) (Velho, 2001). Além disso, *B. quintana* é o agente etiológico da Febre das Trincheiras, que afeta principalmente grupos sociais negligenciados, como populações com infestações de piolhos, sem-teto e dependentes químicos (Dehio, 2004). Já *B. bacilliformis* é causadora da Doença de Carrión, a qual apresenta duas fases de infecção: a febre de Oroya e "verruga peruana". A primeira é caracterizada por palidez, mialgia e desconforto; a segunda (chamada de eruptiva) está associada a sinais neurológicos, cardíacos e respiratórios, sendo endêmica no Peru, Equador e Colômbia, com casos esporádicos da doença na Bolívia, Chile e Guatemala (Maco et al., 2004; Lamas et al., 2008; Pitassi, 2015).

A maioria das espécies de *Bartonella* são adaptadas a determinadas espécies de mamíferos hospedeiros, como por exemplo, *Bartonella elizabethae*, *Bartonella grahamii*, *Bartonella washoensis* e *Bartonella vinsonii* subsp. *arupensis* associadas a roedores, *Bartonella henselae* associadas a gatos e *Bartonella vinsonii* subsp. *berkhoffii* a cães (Dehio, 2004).

A transmissão vetorial das espécies de *Bartonella* ainda é margem para estudos e pesquisas. Apesar de ser frequentemente associadas a pulgas e piolhos, o papel de carrapatos, ácaros, e moscas na transmissão vem sendo investigado (Breitschwerdt e Kordick, 2000).

2.3 *Bartonella* spp. em morcegos

2.3.1 Espécies zoonóticas de *Bartonella* associadas a morcegos

Os quirópteros representam o segundo principal reservatório de *Bartonella* entre os mamíferos, ficando atrás apenas dos roedores (Lin et al., 2012). Até o momento, dois Candidatus a espécies de *Bartonella* detectados em morcegos foram incriminados como agentes zoonóticos: '*Candidatus* Bartonella mayotimonensis' e '*Candidatus* Bartonella rousetti'.

Nos Estados Unidos, Lin et al. (2010) detectaram a presença de uma nova espécie de *Bartonella* ('*Candidatus* Bartonella mayotimonensis') em uma amostra de válvula aórtica de um paciente com endocardite. Lilley et al. (2017), ao investigarem a ocorrência e diversidade de *Bartonella* em 92 morcegos (73 *Myotis lucifugus* e 19 *M.*

grisescens) em Kentucky, Michigan, Pensilvânia e Tennessee, nos EUA, encontraram nove amostras de sangue positivas para o referido agente por meio de ensaio de PCR baseado no gene *gltA*. Três amostras de sangue de *M. lucifugus* continham DNA de *Bartonella* com sequências idênticas (100%) com '*Candidatus* Bartonella mayotimonensis' previamente detectada no paciente de Iowa, indicando, dessa forma, que morcegos na América do Norte podem atuar como hospedeiros para essa espécie de *Bartonella.*

'*Candidatus* Bartonella mayotimonensis' também foi detectada na Finlândia por em um estudo inicialmente conduzido visando a análise metagenômica da flora bacteriana fecal de morcegos. DNA de *Bartonella* foi encontrado nestes materiais fecais em espécimes de *Myotis daubentoni, Eptesicus nilssonii* e *Myotis brandtii.* DNA de '*Candidatus* Bartonella mayotimonensis' foi então detectada em sangue de *E. nilssonii* e *M. brandtii*, em uma pulga e em duas moscas Hiippoboscoidea (*Penicillidia monoceros* e *Nycteribia kolenatii)* (Veikkolainen et al., 2014). O referido agente também foi detectado em 11/109 tecidos cardíacos de morcegos insetívoros da França (2 *M. daubentonii*; 1 *M. mystacinus*; 2 *Nyctalus noctula*; 6 *Pipistrellus nathusii*) e 1/26 morcegos da Espanha (Não identificado) (Stuckey et al., 2017).*Bartonella tamiae* foi inicialmente isolada do sangue de três pacientes hospitalizados na província de Khon Kaen, Tailândia. Análises de sequências de seis marcadores moleculares *(*16S rRNA*, gltA, groEL, ftsZ, rpoB* e região espaçadora intergênica 16S-23S rRNA) e fenotípicas permitiram agrupar os três isolados dentro do gênero *Bartonella,* porém separado das demais espécies descritas (Kosoy et al., 2010). Leulmi et al. (2016) detectaram DNA (sequência do espaçador intergênico de RNA ribossômico 16S-23S) de *Bartonella tamiae* em 12/19 (63,2%) carrapatos *Ixodes vespertilionis,* 8/11 (72,7%) moscas Nycteribiidae e 6/10 (60%) de baços de morcegos da Argélia.

Na Ásia e África, seres humanos podem apresentar maior interação com morcegos frugíveros, devido a razões culturais ou de subsistência. Semestralmente, ocorre na Nigéria (Idanre Hills), um festival de morcegos no qual indivíduos, sem equipamentos de proteção, adentram em cavernas para a caça de quirópteros frugíveros, para posterior comércio ou consumo. Bai et al. (2018) detectaram a presença de uma nova espécie de *Bartonella*, nomeada de '*Candidatus* Bartonella rousetti', em morcegos frugíveros egípcios (*Rousettus aegyptiacus*) e moscas de

morcegos (*Eucampsipoda africana*). No referido trabalho, verificou-se que 8 (3,92%) de 204 pessoas que participaram do festival apresentaram a presença de anticorpos anti-*B. rousetti*, sem a ocorrência de reatividade cruzada com outras espécies de *Bartonella*.

Qiu et al. (2020) investigaram a presença de *Bartonella* spp. em 36 amostras de sangue de morcegos pertencentes a duas diferentes espécies (*Rousettus aegyptiacus* e *Macronycteris vittatus)* na Zâmbia. Seis colônias de *Bartonella* foram obtidas a partir de seis amostras. 3 isolados de *Rousettus aegyptiacus* eram de *Ca* . B. rousetti. Todas as sequências dos isolados apresentaram maior identidade molecular com '*Candidatus* Bartonella rousetti'.

2.3.2 *Bartonella* spp. em quirópteros no mundo

Kosoy et al. (2010) relataram grande diversidade de *Bartonella* spp. em amostras de sangue de 13 espécies de quirópteros insetívoros e frugíveros no Quênia. No total, 106/331 (30,2%) isolados foram obtidos para *Bartonella* spp.: *Eidolon helvum* (23/88 [26,1%]); *Rousettus aegyptiacus* (22/105 [21%]); *Coleura afra* (4/9 [44,4%); *Triaenops persicus* (7/8 [87,5%); *Hipposideros commersoni* (1/4 [25%]); e *Miniopterus* spp (49/87 [56,3%). Análise filogenética baseada no gene *gltA* mostrou uma grande variedade de genótipos de *Bartonella*, os quais mostraram especificidade de acordo com a espécie de morcego hospedeiro.

Bai et al. (2011) investigaram a diversidade de *Bartonella* spp. em amostras de sangue de morcegos hematófagos em cinco localidades no sul da Guatemala (San Lucas Tolimán, Oratorio, Conguaco, Taxisco e Santa Lucia Cotzumalguapa). Um total de 41 isolados de *Bartonella* foram obtidos de 39 (33,1%) dos 118 morcegos amostrados. Verificou-se prevalência variada do agente de acordo com as espécies de morcegos amostradas, a qual mostrou-se maior em quirópteros das espécies *Phyllostomus discolor* (88,8%, 9/8), *Phyllostomus davyi* (70%, 7/10) e *Desmodus rotundus* (48,4%, 15/31) quando comparadas àquelas observadas em *Sturnira lilium* (8,3%, 1/12) e *Glossophaga soricina* (13,3%, 2/15). Diversas variantes genéticas foram encontradas em diferentes espécies de morcegos, com uma variedade de filogrupos distintos. Tais resultados diferiram daqueles observados por Kosoy et al.

(2010) em morcegos no Quênia, nos quais verificou-se especificidade da variante genética de *Bartonella* de acordo com a espécie de hospedeiro. Bai et al. (2011) sugeriram, desta forma, que quirópteros podem compartilhar diversas variantes genéticas dos agentes sob estudo.

Brook et al. (2015) investigaram a presença de *Bartonella* spp. em amostras de sangue de quirópteros das espécies *Eidolon dupreanum* e *Pteropus rufus* e ectoparasitas das espécies *Cyclopodia dubia* e *Thaumapsylla* sp. em Madagascar. Apenas amostras de *E*. *dupreanum* (21/47 [44,68%]), com os seus respectivos ectoparasitas- *Cyclopodia dubia* (17/19 [89,47%) foram positivas para *Bartonella* spp.

Dietrich et al. (2016) detectaram *Bartonella* spp. em 13/238 (3,4%) amostras de sangue de quatro espécies de quirópteros: *Miniopterus natalensis*, *Nycteris thebaica*, *Epomophorus wahlbergi* e *Rousettus aegyptiacus* na África do Sul e Suazilândia. Além da detecção dos agentes sob estudo em morcegos, cinco moscas da família Nycteribiidae e uma espécie não identificada de ectoparasito também foram positivas para *Bartonella* spp. As sequências obtidas de quirópteros e seus ectoparasitas foram associadas à *B. grahamii* e *B. elizabethae*, anteriormente detectadas na África.

Na Guiana Francesa, Davoust et al. (2016) isolaram duas amostras de *Bartonella* spp. a partir do sangue de morcegos das espécies *Noctilius albiventris* e *Pteronotus parnellii*. As sequências genéticas dessas amostras baseadas nos genes 16S rRNA, *gltA*, *rpoB* e *ftsZ*, apresentaram diferenças em relação às espécies conhecidas de *Bartonella*, indicando a possível existência de duas novas espécies. Análise filogenética baseada em sequências concatenadas obtidas dos genes 16S rRNA e *gltA* posicionou os genótipos encontrados em clados únicos, porém próximos de *Bartonella rattaustraliani,* previamente detectada em roedores da Austrália, e *Bartonella australis,* detectada em *Macropus giganteus* da Austrália.

Han et al. (2017) relataram a ocorrência de *Bartonella* spp. em quirópteros das espécies *Rhinolophus ferrumequinum*, *Rhinolophus pusillus*, *Myotis fimbriatus*, *Myotis ricketti*, e *Myotis pequinius*, na China. Destes, 27/107 (25,2%) amostras de sangue foram positivas em ensaio de PCR baseado no gene *gltA.* A análise filogenética a partir do gene *gltA posicionou* as sequências detectadas em morcegos na China com genótipos previamente detectados em moscas associadas a morcegos da Coreia do Sul. Para o gene *rpoB* alguns dos genótipos encontrados no estudo ficaram próximos

daqueles detectados em morcegos da China, Geórgia e Japão. Já para o gene *ftsZ,* as sequências foram agrupadas com aquelas detectadas em morcegos da China, Geórgia, Japão e Quênia.

Corduneanu et al. (2018) detectaram a presença de DNA de *Bartonella* spp. em amostras de tecido cardíaco de morcegos coletados de quatro países da Europa Oriental e Central (Áustria, República Tcheca, Hungria e Romênia): 6/435 (1,38%): *Myotis cf. alcathoe* (3/12; 25%), *Nyctalus noctula* (2/228; 0,88%) e *Pipistrellus pipistrellus* (1/68; 1,47%). As sequências obtidas a partir do gene *gltA* mostraram similiaridade com diferentes sequências de genótipos de *Bartonella* spp. previamente detectados em morcegos na Europa, Ásia e África. Interessantemente, os genótipos detectados mostraram proximidade filogenética com sequências de *'Candidatus* Bartonella mayotimonensis'.

Na Guatemala, *Bartonella* spp. foi detectada em amostras biológicas (sangue, urina, saliva e fezes) de 396 morcegos da espécie *Desmodus rotundus*. Como resultado, 43 amostras biológicas de 39 indivíduos foram positivas para *Bartonella* spp., correspondendo a uma prevalência de 37,9%. DNA de *Bartonella* spp. foi detectado em 35/89 amostras de sangue (35/89), 3/89 amostras de soro, e 5/103 amostras de suabe fecal (5/103) (Wray et al., 2016).

McKee et al. (2021) descrevem a importância dos morcegos na disseminação de *Bartonella* no mundo. A partir da montagem de um banco de dados de sequências de cepas de *Bartonella*, consistindo de nove loci gênicos de 209 linhagens previamente caracterizadas, juntamente com 121 novos isolados cultivados de morcegos, foi realizada uma análise filogenética abrangente sobre o gênero. Inferiu-se que os morcegos representam os hospedeiros ancestrais de todas as bartonellas associadas a mamíferos, além de serem apontados como responsáveis pela expansão geográfica dos agentes em questão.

Recentemente, Yang et al. (2024) investigaram o estado de infecção e detecção molecular de patógenos transportados por ectoparasitas de morcegos *Miniopterus fuliginosus* em Yunnan, China. Foram analisados 37 *M. fuliginosus* juntamente com 388 ectoparasitas: 197 ácaros (*Steatonyssus nyctali*) e 191 moscas (*Nycteribia allotopa*). *Bartonella* spp. foi identificada em 7/37 (18,92%) *M. fuliginosus e* 71/191 (37,17%) moscas. A partir de análises filogenéticas das sequências do gene

gltA de *Bartonella* verificou-se proximidade filogenética com genótipos previamente detectados em morcegos e moscas associadas a morcegos na China e na Coreia do Sul.

Corduneanu et al. (2023) utilizaram a técnica de PCR em tempo real microfluidica para investigar a ocorrência de agentes transmitidos por vetores em 48 amostras de sangue de duas espécies diferentes de morcegos (39 *Miniopterus schreibersii* e 9 *Myotis capaccinii*) na Romênia. Como resultado, 16,66% foram positivos para *Bartonella* spp. As sequências obtidas foram agrupadas com sequências de *Bartonella* spp., *B. washoensis* e *B. henselae*. Além disso, DNA de *Bartonella* spp. foi dectado em duas espécies de moscas (*Nycteribia pedicularia* e *Nycteribia schmidlii*).

2.3.3 *Bartonella* spp. em quirópteros no Brasil

Ikeda et al. (2017) detectaram DNA de *Bartonella* em 17 (5,28%) amostras biológicas de morcegos não-hematófagos capturados nos Estados do Paraná, Pará, São Paulo, Mato Grosso e Tocantins. As amostras positivas foram provenientes de espécies de morcegos dos seguintes Estados: Paraná (*Sturnira lilium*), Pará (*Phyllostomus hastatus*, *Carollia perspicillata* e *Natalus espiritosantensis*) e Tocantins (*Carollia perspicillata* e *Glossophaga soricina*). Os genótipos de *Bartonella* detectados nos morcegos foram intimamente relacionados com aqueles previamente detectados em roedores, além de mostrarem-se filogeneticamente próximos a genótipos de *Bartonella* detectados em quirópteros amostrados em outras regiões do mundo.

Ferreira et al. (2018) analisaram a ocorrência de *Bartonella* spp. em morcegos de três regiões da Mata Atlântica Brasileira, nos Estados do Rio de Janeiro, Bahia e Santa Catarina. DNA de *Bartonella* spp. foi detectado em 22 (18,5%) amostras de baço. A análise filogenética baseada nas sequências do gene *gltA* identificou pelo menos dois clusters independentes, indicando a ocorrência de novos genótipos de *Bartonella*.

A diversidade genética de *Bartonella* em morcegos hematófagos no Brasil foi estudada por André et al. (2019) em 208 morcegos das espécies *Desmodus rotundus* (n= 167), *Diphylla ecaudata* (n= 32) e *Diaemus youngi* (n=9) em 15 estados diferentes.

Como resultado, 51 amostras de fígado (24,51%) foram positivas para a região intergênica 16S-23S rRNA (ITS) de *Bartonella*, dentre elas 40 (78,4%) amostras para *D. rotundus* e 11 (21,6%) amostras para *D. ecaudata*. Sequências ITS detectadas naquele estudo mostraram 80%-96% de identidade com *B. bacilliformis* e *B. ancachensis*. A inferência filogenética para o gene *gltA* posicionou as sequências obtidas em seis clados diferentes, os quais estavam relacionados com genótipos de *Bartonella* já detectados em *D. rotundus* e moscas de morcegos na América Latina. Já as sequências *rpoB* agruparam-se mais juntamente, ainda que mostrando um pouco de subclusterização.

Ikeda et al. (2020) avaliaram a diversidade genética de *Bartonella* spp. em morcegos não-hematófagos e ectoparasitas associados no centro-oeste do Brasil, por meio da análise de genótipos *gltA* em níveis intra e inter-hospedeiros. Para tal, foram coletadas um total de 418 amostras biológicas de 135 indivíduos, representadas por 135 tecidos de baço, 133 sangue total e 150 ectoparasitas (compreendendo 64 moscas de morcegos, 67 ácaros e 19 carrapatos). Como resultados, mostraram-se positivos para *Bartonella* spp.: 13,5% (18/133) amostras de sangue de morcegos, 17,18% (11/64) de moscas de morcegos e 23,8% (5/21) pools de ácaros de Macronyssidae. A partir das 11 sequências obtidas de 17 amostras, obtiveram-se sete e três sequências para *nuoG, rpoB e ftsZ*, respectivamente. Verificou-se a presença de pelo menos dois genótipos diferentes de bartonellas para cada amostra de mosca de morcego ou sangue de morcego. Observou-se também maior diversidade nas sequências *gltA* de *Bartonella* em amostras de sangue de morcegos quando comparadas àquelas de moscas de morcegos. A análise filogenética posicionou as sequências obtidas a genótipos de *Bartonella* identificados em roedores, morcegos e moscas de morcegos.

Gonçalves-Oliveira et al. (2020) coletaram 110 amostras de baços morcegos no estado do Rio de Janeiro. Destas, 4 (3,6%) (*Artibeus lituratus* (3/58) e *Carollia perspicillata* (1/15) mostraram-se positivas para *Bartonella*. A inferência filogenética para o gene *ftsZ* posicionou as sequências obtidas em três clados diferentes. Genótipos de *Bartonella groEL* e *ftsZ* descritos em *C. perspicillata* indicaram a existência de novas variantes de *Bartonella* nos morcegos amostrados.

Bernal et al. (2022) analisaram a ocorrência de *Bartonella* spp. no bioma Mata Atlântica em São Paulo. Foram analisadas 341 amostras de fígado de morcegos neotropicais pertencentes às famílias Molossidae, Phyllostomidae e Vespertilionidae. Como resultado, 2/341 (0,6%) amostras da espécie *Glossophaga soricina* do município de São Roque foram positivas para o gene *gltA.* As sequências obtidas formaram um caldo próximo a *Bartonella* spp. previamente detectados em *G. soricina* proveniente do bioma Cerrado no Tocantins (Ikeda et al., 2017).

Pacheco et al. (2024) investigaram a presença de DNA de *Bartonella* spp. em 139 morcegos no Centro-Oeste do Brasil, dos quais 23 (16,54%) mostraram-se positivos na qPCR baseada no gene *nuoG*. A análise filogenética baseada no gene *gltA* e *ftsZ* posicionou as sequências de *Bartonella* detectadas com genótipos previamente detectados em outras espécies de morcegos e moscas associadas a morcegos no Brasil e em outros países da América Latina e Geórgia. Já as sequências *rpoB de Bartonella* detectada em *Lophostoma silvicolum* agrupou-se com sequência de *Bartonella* spp. detectadas em *Noctilio albiventris* da Guiana Francesa.

2.4 *Bartonella* spp. em moscas de morcegos

2.4.1 *Bartonella* spp. em moscas associadas a morcegos no mundo

A primeira descrição e detecção de *Bartonella* spp. em moscas Streblidae ocorreu na Flórida por Reeves e colaboradores (2005). Os mesmos coletaram exemplares de *Basilia boardmani* (1 espécime) e *Trichobius major* (4 espécimes), no qual apenas um espécime de *T. major* foi positivo para *Bartonella* spp. A partir de sequências do gene *groEL* verificou-se 91% de identidade com *Bartonella* spp. previamente descritas em roedores (*Sciurus carolinensis*) no Reino Unido.

Moscas de morcegos (*Trichobius parasiticus* (533), *Strebla wiedemanni* (176), *Megistopoda aranea* (11), *Trichobius* spp. (4), *Aspidoptera* spp. (6), *Paratrichobius longicrus* (4) e *Trichobius caecus* (25)) foram coletadas no México por Moskaluk et al. (2018) de 5 espécies de morcegos (*Artibeus jamaicensis* (n=10), *Artibeus lituratus* (n=4), *Desmodus rotundus* (n=202), *Sturnira ludovici* (n=1), *Sturnira lilium* (n=1), e *Pteronotus parnellii* (n=15)). A presença de DNA de *Bartonella* foi maior em *T. parasiticus* (18,15%) em comparação com moscas *S. wiedemanni* (6%). A análise

filogenética revelou que sequências encontradas em morcegos e suas moscas apresentaram relação filogenética com *Bartonella clarridgeiae*, *Bartonella rochalimae* e *Bartonella doshiae,* porém nenhuma delas se aproximaram de '*Candidatus* Bartonella mayotimonensis'.

Nabeshima et al. (2020) realizaram a detecção e análise filogenética de espécies de *Bartonella* spp. em moscas associadas a *Miniopterus fuliginosus* no Japão. Foram coletadas 281 moscas de 114 morcegos. A prevalência de DNA de *Bartonella* foi de 47,1% (74/157) em *Nycteribia allotopa*, 15,2% (12/79) em novas espécies de *Nycteribia* e 6,7% (3/45) em *Penicilidia jenynsii*. A análise filogenética revelou que as sequências *gltA* obtidas mostraram-se próximas de genótipos de *Bartonella* previamente detectados em moscas *Nycteribia* e morcegos *Miniopterus*.

Mitchell et al. (2022) investigaram a diversidade genética de *Bartonella* spp. em 252 amostras de sangue de morcegos (18 espécies) e 62 pools de moscas associadas a morcegos, provenientes de 48 quirópteros capturados em cavernas na Costa Rica. A prevalência de *Bartonella* spp. foi de 10,7% (27/252) para morcegos e 29% (18/62) para pools de ectoparasitas. Os resultados sugerem que na Costa Rica as variantes *gltA* de *Bartonella* são compartilhadas entre morcegos e moscas associadas a quirópteros em diferentes partes do país, além de mostrarem proximidade filogenética com sequências de *Bartonella* previamente detectadas morcegos e moscas da Guatemala e México.

Han et al. (2022) identificaram a diversidade genética de *Bartonella* em morcegos (*Myotis adversus*, *Myotis davidii*, *Miniopterus schreibersii, Rhinolophus pusillus*) seus ectoparasitas (moscas *Penicillidia monoceros, Nycteribia* sp. e *Phthiridium sp.* e ácaros *Spinturnix* sp. e *Eyndhovenia* sp. na China. Morcegos, moscas e ácaros carregavam diversos novos genótipos de *Bartonella* com alta prevalência. Além disso, a espécie zoonótica *B. mayotimonesis* foi identificada tanto em morcegos quanto em seus ectoparasitas.

No Japão, Nabeshima et al. (2023) investigaram a prevalência de *Bartonella* spp. em 123 morcegos (*Eptesicus nilssonii*) e seus ectoparasitas (174 pulgas - *Ischnopsyllus needhami* e dois percevejos *Cimex japonicus*). Foi detectado DNA de *Bartonella* em 32 (26%) dos morcegos e em 79 (45,4%) das pulgas. A análise filogenética revelou que dois grupos de genótipos obtidos se agruparam com

sequências de *Bartonella* de morcegos *Eptesicus* da República da Geórgia e Finlândia, morcegos *Myotis* da Romênia e no Reino Unido, e uma pulga de morcego *Eptesicus* da Finlândia. Já o terceiro genótipo formou um caldo com *B. florencae*, *B. acomydis* e *B. birtlesii*. Este estudo sugere que duas espécies de *Bartonella* spp. circulam em morcegos no Norte do Japão.

Fagre et al. (2023) avaliaram a infeção por *Bartonella* em morcegos frugíveros e moscas associadas em Bangladesh. Foi detectada alta prevalência de DNA de *Bartonella* no sangue de morcegos (*Pteropus medius*- 35/55 [64%]) e moscasmorcego (*Cyclopodia sykesii*- 59/60 [98%]). As sequências *gltA* de *Bartonella* de morcegos e moscas agruparam-se em três clados: A (intimamente relacionadas com sequências previamente detectadas em *Pteropus hypomelanus* e *Cyclopodia horsfieldi* da Malásia), B (outra sequência de *Bartonella* identificada em *C. horsfieldi* coletada de *P. hypomelanus* na Malásia e distantes de sequências de *Eidolon* spp. e *Cyclopodia* spp. e sequências de outros nicteribiídeos) e C (*Bartonella* de morcegos vespertilionídeos e miniopterídeos e moscas e pulgas associadas). Interessantemente, foi evidenciado que as moscas se alimentavam de sangue de múltiplos morcegos hospedeiros.

2.4.1 *Bartonella* spp. em moscas associadas a morcegos no Brasil

A relação biológica de morcegos com diversos grupos de ectoparasitos, como carrapatos, pulgas, ácaros, percevejos e dípteros, está cada vez mais sendo estudada no que diz respeito ao papel desses artrópodes na dinâmica de disseminação de *Bartonella* e outros bioagentes patogênicos. Neste sentido, dípteros da família Streblidae (Diptera: Hippoboscoidea) são os ectoparasitos mais frequentemente registrados em morcegos (Frank et al., 2014, Barbier e Bernard 2017). Esta família compreende dípteros hematófagos, com cerca de 250 espécies conhecidas (Dick e Patterson 2006), sendo que pelo menos 110 delas são descritas no Brasil (Graciolli 2023, Barbier et al., 2018). Interessantemente, a partir de registros fósseis, acredita-se que esta família parasita morcegos há mais de 20 milhões de anos (Barbier e Graciolli 2016), sendo responsáveis pela maior carga parasitária em morcegos que usam abrigos permanentes (Barbier e Bernard 2017). Apesar do Brasil deter mais de

110 espécies destes dípteros, a grande maioria dos dados estão concentrados na Europa e América do Norte (Szentiványi et al., 2019).

A família Nycteribiidae também se encontra em destaque na transmissão de agentes entre morcegos. A sua distribuição se encontra principalmente no Velho Mundo (Europa, África e Ásia). No continente Americano existem apenas dois gêneros, *Basilia* e *Hershkovitzia* (Graciolli e Dick 2009, Graciolli 2010). No Brasil foram registradas 24 espécies de *Basilia* e cinco de *Hershkovitzia* (Graciolli e Hrycyna 2024).

Do Amaral et al. (2018) investigaram a presença de DNA de *Bartonella* spp. em 404 moscas (*Paratrichobius longicrus* (49), *Megistopoda aranea* (4), *Aspidoptera phyllostomatis* (9), *Trichobius joblingi* (109), *Trichubius anducei* (10), *Strebla guajiro* (29), *Megistopoda proxima* (77), *Aspidoptera falcata* (108), *Trichobius furmani* (4) e *Strebla wiedemanni* (5) de oito espécies de morcegos (*Artibeus lituratus* (16), *Sturnira lilium* (72), *Carollia perspicillata* (57), *Artibeus fimbriatus* (8), *Artibeus obscurus* (2), *Artibeus planirostris* (3), *Desmodus rotundus* (5) e *Diphyla ecaudata* (1)) no Rio de Janeiro. Positividade de 19,8% (40/202) para *Bartonella* spp. via qPCR baseada no gene *nuoG* foi encontrada entre as moscas Streblidae testadas. As sequências obtidas do gene *gltA* de *Bartonella* spp. formaram diversos grupamentos a partir da análise filogenética, a saber: (i) sequência detectada em *Strebla guajiro* coletado de *Carollia perspicillata* foi filogeneticamente associada a *Bartonella* spp. previamente detectados em *Sturnira lillium* do Paraná e com outros morcegos da Guatemala, México e Costa Rica (ii) sequências detectadas em *Strebla guajiro* coletado de *Carollia perspicillata* posicionou-se filogeneticamente próxima a genótipos de *Bartonella* spp. detectadas em roedores amostradas no Brasil e EUA, juntamente com *Bartonella* spp. detectada em pulga *Polygenis gwyni* dos EUA. Uma sequência de *rpoB* proveniente de um espécime de *M. aranea* coletado de *C. perspicillata* mostrou-se filogeneticamente relacionado à genótipo previamente detectado em morcego (*S. lillium*) no estado do Paraná. Já uma sequência *ribC* detectada em *M. aranea* coletada de *C. perspicillata* foi posicionada sozinho em um ramo, ainda que relacionado a *Bartonella tribocorum, Bartonella elizabetha*e*, Bartonella grahamii, Bartonella fuyuanensis e Bartonella rattimassiliensis.*

Braga et al. (2020), na Ilha de São Luís, estado do Maranhão, investigaram a presença de DNA de *Bartonella* spp. em 29 amostras de sangue de morcegos

(*Glossophaga soricina* (n=9), *Pteronotus parnellii* (n=2), *Carollia perspicillata* (n=16), *Diaemus youngi* (n=1), *Pteronotus personatus* (n=1)) e em 15 moscas *Trichobius* sp. (Diptera: Streblidae). Embora nenhuma amostra de sangue de morcegos tenha se mostrado positiva para o referido agente, DNA de *Bartonella* foi detectado em 3/15 espécimes de *Trichobius* sp., por meio de PCR em tempo real quantitativa (*qPCR)* baseada no gene *nuoG*. A análise filogenética a partir do gene *rpoB* agrupo as sequências obtidas com sequências previamente detectadas em *Desmodus rotundus* do Brasil (André et al., 2019). Já as sequências a partir do *gltA* agruparam-se com aquelas previamente detectadas em *Carollia perspicillata*, *Speiseria ambigua* e *Glossophaga soricina* na Costa Rica (Frank et al.,2018) e *Desmodus rotundus* do Brasil (André et al., 2019).

André et al. (2023) caracterizaram o microbioma bacteriano de morcegos não hematófagos e moscas Streblidae associadas e ácaros Macronyssidae e Spinturnicidae no estado de Mato Grosso do Sul, centro-oeste do Brasil. Foram analisados suabs orais e retais de 30 morcegos (*Artibeus lituratus* [13], *Artibeus planirostris* [9], *Eptesicus furinalis* [5], *Carollia perspicillata* [2] e *Platyrrhinus lineatus* [1]), juntamente com 58 ácaros (15 Macronyssidae e 43 Spinturnicidae) e 48 moscas-morcego Streblidae. *Spiroplasma*, *Wolbachia* e *Bartonella* representaram os gêneros mais abundantes em moscas Streblidae.

3. Objetivos

3.1 Objetivos gerais

O presente estudo teve como objetivo investigar a ocorrência molecular e diversidade genética de *Bartonella* spp. em morcegos e moscas Streblidae associadas na Amazônia brasileira.

3.2 Objetivos específicos

✧ Investigar, por meio de métodos moleculares, a ocorrência de *Bartonella* spp. em amostras de baço de morcegos amostrados de cinco estados brasileiros (Acre, Pará, Roraima, Amapá e Amazonas);

✧ Investigar, por meio de métodos moleculares, a ocorrência de *Bartonella* spp. em moscas Streblidae parasitas de morcegos hematófagos amostrados em quatro estados brasileiros (Pará, Roraima, Amapá e Amazonas);

✧ Traçar inferências filogenéticas com as sequências de *Bartonella* spp. obtidas em amostras de baço de morcegos da região norte do Brasil.

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CAPÍTULO 2- Molecular detection and characterization of *Bartonella* spp. in non-hematophagous bats from the Brazilian Amazon

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Abstract

Despite the great diversity of bats (64 species) in the State of Acre, northwestern Brazil, there are no studies on occurrence and diversity of *Bartonella* spp. in bats in this region. The present study investigated the occurrence and molecular identity of *Bartonella* spp. in spleen samples (n=271) from bats of 30 different species from this region, within the Amazon biome. Twenty-one out of 208 (10.1%) samples positive in the PCR for the mammalian *gapdh* endogenous genes were positive in the qPCR for *Bartonella* spp. based on the *nuoG* gene. The two *gltA Bartonella* genotypes detected grouped with those previously identified in bats from other locations, expanding the diversity of genotypes associated with bats. This study provided the first molecular evidence of the presence of *Bartonella* spp. in bats in the state of Acre and in bats of the species *Lophostoma silvicolum*, *Vampyressa thyone*, *Tonatia saurophila* and *Phyllostomus elongatus*.

Keywords: *Bartonella*, Chiroptera, Amazon, Acre

1. Introduction

The order Chiroptera comprises the second largest group of mammals in the world, representing approximately 25% of the species described in Brazil [1]. Due to high biological complexity, such as flight capacity, high longevity, formation of colonies, and wide distribution and diversity of species, bats play an important role in the dissemination of several zoonotic agents [2].

The genus *Bartonella* encompasses facultative intracellular Gram-negative bacteria that can cause emerging zoonoses. These agents preferentially parasitize

mammalian erythrocytes and endothelial cells, and can also be found in dendritic cells, macrophages and monocytes [3]. There are currently 38 *Bartonella* species validated according to the “List of prokaryotic names with position in the nomenclature database” (https://lpsn.dsmz.de/genus/bartonella - accessed January 25, 2024) [4]. Such agents are transmitted by arthropod vectors and mammals are reported as the main reservoirs [3]. Just after rodents, chiropterans represent the second mammalian group pointed as *Bartonella* spp. reservoirs [5]. The zoonotic species *Bartonella mayotimonensis* and ‘*Candidatus Bartonella rousetii*’ have bats as their main reservoirs [5,6].

Despite the existence of previous studies regarding the occurrence of *Bartonella* spp. in Brazilian bats [7,8,9,10,11,12,13], the diversity of *Bartonella* spp. is far from being clarified in this group of mammals. In Brazil, nine families, 69 genera and 182 species of bats have been described [14]. However, *Bartonella* genotypes have been described in only 15 host species (8.2%) so far.

In the state of Acre, located in the Northwest of Brazil, a rich biodiversity of bats has been documented, with around 64 species identified in forest and urban areas [15]. Among them, approximately 20 species stand out for their abundance, including the genera *Artibeus*, *Carollia* and *Phyllostomus* (Verde et al., 2021). Despite this, the diversity of *Bartonella* spp. infecting bats in this state is still unknown. The state of Acre borders Peru, a country where *Bartonella bacilliformis* [17] and *Bartonella ancashensis* [18], species that cause Carrión's disease and Peruvian wart, occur. Assessing the diversity of *Bartonella* spp. in the state of Acre is important to promote surveillance for zoonotic bartonella in this strategic region of Brazil. The present work aimed to investigate the occurrence and molecular identity of *Bartonella* spp. in Brazilian bats from Acre State.

2. Material and methods

2.1 Study area

Between 2014 and 2015, 367 bats were captured in two municipalities in the state of Acre, namely Rio Branco (09°58′29"S/67° 48′36"W) and Xapuri (10°10′95″S/ 68° 30′16″W) [19]. These samples come from a study already published with foccus on bat-associated-trypanosomatids [19]. Bats were identified based on morphological characteristics [20, 21], being classified into 23 genera and 30 species. The detailed methodology of bat sampling and tissue collection are described elsewhere [19].

Of the 367 bats captured [19], 271 from which spleen samples were available, were included in the present study: 192 from the municipality of Rio Branco and 79 from Xapuri (**Figure 1**). DNA spleen samples were obtained from the following species: *Anoura caudifer* (n=1), *Artibeus lituratus* (n=47), *Artibeus obscurus* (n=4), *Artibeus planirostris* (n=54), *Carollia benkeithi* (n=1), *Carollia brevicauda* (n=9), *Carollia perspicillata* (n=69), *Chiroderma villosum* (n=3), *Dermanura cinereus* (n=12), *Glossophaga soricina* (n=7), *Lasiurus blossevillii* (n=2), *Hysunycteris thomasi* (n=2), *Lophostoma silvicolum* (n=3), *Mesophylla macconnelli* (n=2), *Gardnerycteris crenulatum* (n=1), *Phyllostomus discolor* (n=5), *Phyllostomus elongatus* (n=9), *Phyllostomus hastatus* (n=7), *Platyrrhinus incarum* (n=1), *Platyrrhinus infuscus* (n=2), *Rhinophylla fischerae* (n=4), *Rhinophylla pumilio* (n=4), *Saccopteryx leptura* (n=1), *Sturnira giannae* (n=2), *Sturnira tildae* (n=2), *Tonatia maresi* (n=1), *Trachops cirrhosus* (n=1), *Uroderma bilobatum* (n=13), *Vampyressa thyone* (n=1) and *Vampyrum spectrum* (n=1).

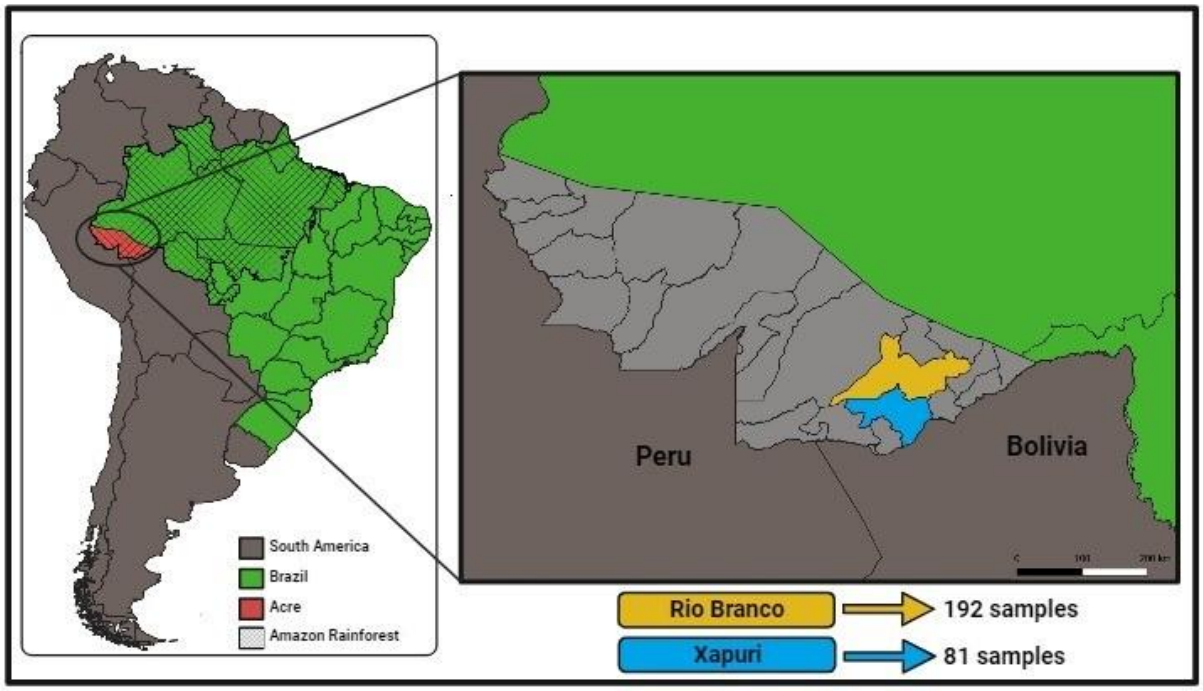

Figure 1. Representative image of Brazil (highlighted in green in South America) and location of the two municipalities in the State of Acre, northern Brazil: Rio Branco and Xapuri, where chiropterans were sampled. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

2.2 DNA extraction and PCR assay for endogenous mammalian gene

DNA extraction from bat spleen samples was performed using the BioPur Mini Spin Plus Extraction Kit (Mobius), according to the manufacturer's recommendations. The extracted DNA samples had their concentrations and ratios 260/280 and 260/230 measured in a spectrophotometer device (Nanodrop, Thermo Scientific®) and were stored at -20°C for subsequent analysis.

To evaluate the quality of the extracted DNA and avoid false negative results due to the presence of inhibitors, the DNA samples were subjected to conventional PCR (cPCR) aiming at the amplification of the mammalian endogenous

glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene (~450 bp) [22]. Samples positive in PCR for the endogenous gene were subsequently subjected to specific PCR assays for *Bartonella* spp.

2.3 Quantitative real-time PCR (qPCR) assays for *Bartonella* spp.

Only bat spleen DNA samples positive in PCR for the endogenous gene were subjected to a qPCR for *Bartonella* spp. based on the *nuoG* gene, using F (5'CAATCTTCTTTTGCTTCACC -3') and R (5'- TCAGGCTTTATGTGAATAC-3') primers and the TaqMan hydrolysis probe TexasRed-5'-TTYGTCATTTGAACACG-3'[BHQ2a-Q]3' (Integrated DNA Technologies®, Coralville, Iowa, United States) [23]. DNA samples were evaluated in duplicates and when the difference in Cq values was higher than 0.5, the samples were re-run in triplicates. To construct the standard curve for each reaction, serial dilutions were performed at different concentrations (2.0×10^7 to 2.0×10^0 copies) of a plasmid encoding an 83 bp fragment of the *Bartonella henselae nuoG* gene (pIDTSMART; Integrated DNA Technologies, Coralville, Iowa, United States), which was also used as positive controls. UltraPure™ DNase/RNase-Free Distilled Water (Thermo Scientific, Waltham, MA, USA) was used as a negative control. The number of plasmid copies was determined by the formula (XG/µL DNA/[Plasmid Length (BP) × 660]) × 6.22×10^{23} × plasmid copies/µL. The amplification efficiency (E) was calculated according to the slope of the standard curve using the formula $E= 10^{-1/slope}$ [24]. qPCR assays were performed on a C1000-CFX96 thermal cycler (BioRad, Hercules, California, USA).

Samples positive in the qPCR were also subjected to a qPCR based on the 16S-23S internal transcribed spacer (ITS) region, using the oligonucleotide primers F (5'-CAATCTTCTTTTGCTTCACC-3') and R (5'-TCAGGGCTTTATGTGAATAC-3') and

the TaqMan hydrolysis probe TexasRed-5'-TTYGTCATTTGAACACG-3'[BHQ2a-Q]3' [25]. Serial dilutions were performed at different concentrations (2.0 x 10^7 to 2.0 x 10^0 copies) of a plasmid encoding a 226 bp fragment of the *Bartonella henselae* ITS (pIDTSMART; Integrated DNA Technologies, Coralville, Iowa, United States), which were also used as positive controls. UltraPure™ DNase/RNase-Free Distilled Water (Thermo Scientific, Waltham, MA, USA) was used as a negative control.

2.4 Conventional PCR assays for molecular characterization of *Bartonella* spp.

In order to perform molecular characterization, samples positive in qPCR for the *nuoG* gene were subjected to conventional PCR protocols targeting six other molecular markers, namely *ribC* (~585-588 bp) [26], short *gltA* (~379 bp) [27], large *gltA* (~767 bp) [28], *rpoB* (~333 bp) [29], *pap-31* (~564 bp) [30], *groEL* (~752 bp) [31,32] e *ftsZ* (~515 bp) [32]. *Bartonella henselae* DNA (ON561953) [33] and sterilized ultrapure water were used as positive and negative controls, respectively, in all PCR assays.

The PCR products were subjected to electrophoresis on a 1% agarose gel stained with ethidium bromide (0.5 µL/mL) (Life Technologies™, Carlsbad, California, USA) in TBE pH 8.0 running buffer (44, 58 M Tris-base; 0.44 M boric acid; 12.9 mM EDTA) under 90 V/150 mA for 60 minutes. To visualize the results, a 100 base pair molecular weight marker (Kasvi, Campinas, São José do Pinhais, Paraná, Brazil) and a transilluminator coupled to the Image lab image analysis computer program (BioRad, Hercules, California, USA) were used.

2.5 Sequencing

PCR products were purified using the ExoSAP-IT™ PCR Product Cleanup Reagent kit (Thermo Scientific, San Jose, CA, USA), according to the manufacturer's recommendations. Sequencing of the amplified products was carried out using an automated technique based on the dideoxynucleotide chain termination method [34] on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) at the “Centro de Recursos Biológicos e Biologia Genômica - CREBIO” (Faculdade de Ciências Agrárias e Veterinárias/FCAV, UNESP, Jaboticabal, SP, Brazil).

2.6 Phylogenetic analysis

The analysis of the electropherograms generated in the sequencing and construction of consensus sequences were carried out using the Phred-Phrap version 23 program [35,36], respecting the minimum “Phred” quality value of 20 for the bases. The BLAST program [37] was used to compare the sequences obtained with those previously deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank). The sequences were saved in “FASTA” mode and aligned with other sequences retrieved from the “GenBank” database, using the BIOEDIT software version 7.0.5.3 [38]. Maximum likelihood (ML) phylogenetic analysis was inferred in IQ-TREE online server [39] using 1000 replicas of UFBoot2 – Ultrafast Bootstrap [40]. The evolutionary model was found using the jModelTest 2 software [41]. Editing of phylogenetic trees as well as rooting (via outgroup) was performed using Treegraph 2.0.56-381 beta software [42].

3. Results

3.1 Prevalence of *Bartonella* spp. in bats

Out of 271 bat spleen samples, 208 (76.8%) were positive for the mammalian endogenous gene (*gapdh*) and were further submitted to the qPCR assay for *Bartonella* spp. based on the *nuoG* gene. From these, 21/208 (10.1%) samples were positive (efficiency ranging from 95.1% to 100.2%; slope from -3.260 to -3.445; R^2 from 0.993 to 1; y-intercept from 36,143 to 37,685): 14/69 (20.3%) *Carollia perspicillata*, 3/54 (5.6%) *Artibeus planirostris*, 1/9 (11.2%) *Phyllostomus elongatus*, 1/3 (33.3%) *Lophostoma silviculum,* besides the unique examined individuals from species *Tonatia maresi* and *Vampyressa thyone*. The copy numbers of the *nuoG* fragment per microliter ranged from 1.830×10^{-1} to 2.8610×10^{10}. In 11/21 positive samples it was not possible to estimate quantification, since the Cq values of the triplicates were higher than 0.5 (**Table 1**).

Table 1. Species and origin of bats positive in qPCR for *Bartonella* spp., with quantification of the copy numbers of a fragment of the *nuoG* gene per microliter and molecular assay parameters.

Sixteen (57%) out of 21 qPCR-positive samples were also positive for at least one molecular marker in cPCR assays for *Bartonella* spp., including 2 (12.5%) for the *gltA* gene, 1 (6.3%) for the *rpoB* gene, 2 (12.5%) for the *groEL* gene, 1 (6.3%) for the

Bat species (ID)	Location	Quantification (*nuoG* copies /µL)	Efficiency	R^2 (Coefficient of Determination	Slope	y-intercept
Artibeus planirostis (73)	Xapuri	$4{,}433\times10^{0}$; $1{,}830\times10^{1}$	95,10%	0,993	-3,445	37,148
Artibeus planirostis (76)	Xapuri	$3{,}029\times10^{0}$	95,10%	0,993	-3,445	37,148
Carollia perspicillata (5)	Xapuri	$31{,}14\times10^{1}$; $1{,}09\times10^{2}$	100%	1	-3,323	37,685
Carollia perspicillata (71)	Xapuri	$4{,}277\times10^{1}$	95,10%	0,993	-3,445	37,148
Carollia perspicillata (215)	Xapuri	$4{,}351\times10^{0}$	102,70%	0,997	-3,26	36,298
Carollia perspicillata (225)	Xapuri	$4{,}697\times10^{1}$; $2{,}978\times10^{1}$	95,50%	0,996	-3,435	36,143
Lophostoma silviculum (2)	Xapuri	$3{,}835\times10^{0}$	100%	1	-3,323	37,685
Tonatia maresi (226)	Xapuri	$4{,}681\times10^{1}$; $3{,}510\times10^{1}$	95,50%	0,996	-3,435	36,143
Vampyressa sp. (223)	Xapuri	$3{,}029\times10^{0}$	102,70%	0,997	-3,26	36,298
Artibeus planirostis (90)	Rio Branco	$4{,}045\times10^{1}$; $7{,}862\times10^{1}$	95,10%	0,993	-3,445	37,148
Carollia perspicillata (233)	Rio Branco	$5{,}141\times10^{1}$	95,50%	0,996	-3,435	36,143
Carollia perspicillata (236)	Rio Branco	$1{,}468\times10^{1}$; $7{,}457\times10^{1}$	95,50%	0,996	-3,435	36,143
Carollia perspicillata (248)	Rio Branco	$5{,}371\times10^{1}$; $3{,}636\times10^{1}$	95,50%	0,996	-3,435	36,143
Carollia perspicillata (265)	Rio Branco	$9{,}729\times10^{2}$	95,50%	0,996	-3,435	36,143
Carollia perspicillata (266)	Rio Branco	$1{,}919\times10^{1}$	95,50%	0,996	-3,435	36,143
Carollia perspicillata (4)	Rio Branco	$31{,}14\times10^{1}$; $1{,}09\times10^{2}$	100%	1	-3,323	37,685
Carollia perspicillata (273)	Rio Branco	$1{,}919\times10^{1}$	95,50%	0,996	-3,435	36,143
Carollia perspicillata (126)	Rio Branco	$1{,}314\times10^{0}$; $1{,}59\times10^{0}$	96,30%	0,998	-3,314	36,478
Carollia perspicillata (147)	Rio Branco	$1{,}978\times10^{2}$; $1{,}365\times10^{2}$	96,30%	0,998	-3,314	36,478
Carollia perspicillata (168)	Rio Branco	$2{,}544\times10^{2}$; $1{,}474\times10^{2}$	96,30%	0,998	-3,314	36,478
Phyllostomus elongatus (255)	Rio Branco	$4{,}177\times10^{1}$	95,50%	0,996	-3,435	36,143

ftsZ gene, 5 (31.3%) for the *pap-31* gene and 6 (37.5%) for the 16S-23S rRNA intergenic region (ITS). Readable sequences were obtained for two molecular markers (*gltA* and *pap-31*) for sample #147. None of the 21 samples were positive in cPCR assays for the large fragment of the *gltA, ribC* and *nuoG* gene (**Table 2**).

Table 2. Bats sampled in the state of Acre and positive for *Bartonella* spp. in qPCR and cPCR assays targeting different molecular markers.

ID Sample	Location	Bat species	Quantification (*nuoG* copies /µL)	cPCR								qPCR
				gltA (~357 pb)	*gltA* (~767 bp)	*rpoB*	*nuoG*	*groEL*	*ribC*	*ftsZ*	*pap-31*	ITS
73	Xapuri	*Artibeus planirostis*	$4{,}433 \times 10^{0}$	-	-	-	-	-	-	NS	-	-
5	Xapuri	*Carollia perspicillata*	$31{,}14 \times 10^{-1}$	Seq	-	-	-	-	-	-	-	-
71	Xapuri	*Carollia perspicillata*	$4{,}277 \times 10^{-1}$	-	-	NS	-	-	-	-	-	Seq
215	Xapuri	*Carollia perspicillata*	$1{,}314 \times 10°$	-	-	-	-	-	-	-	NS	NS
90	Rio Branco	*Artibeus planirostris*	$4{,}045 \times 10^{1}$	-	-	-	-	-	-	-	-	NS
126	Rio Branco	*Carollia perspicillata*	$1{,}314 \times 10^{0}$	-	-	-	-	-	-	-	NS	-
147	Rio Branco	*Carollia perspicillata*	$1{,}978 \times 10^{2}$	Seq	-	-	-	-	-	-	NS	NS
168	Rio Branco	*Carollia perspicillata*	$2{,}544 \times 10^{2}$	-	-	-	-	-	-	-	NS	NS
236	Rio Branco	*Carollia perspicillata*	$1{,}468 \times 10^{1}$	-	-	-	-	-	-	-	NS	-
265	Rio Branco	*Carollia perspicillata*	$1{,}919 \times 10^{-1}$	-	-	-	-	-	-	-	-	Seq
255	Rio Branco	*Phyllostomus elongatus*	$4{,}177 \times 10^{-1}$	-	-	-	-	NS	-	-	-	-
275	Rio Branco	*Phyllostomus elongatus*	$4{,}177 \times 10^{1}$	-	-	-	-	NS	-	-	-	-

3.2 BLASTn analysis and phylogenetic inference

The partial sequence of the *gltA* gene from *Bartonella* spp. obtained from *C. perspicillata* (#5) showed 93% identity with *Bartonella* sp. previously detected in a bat from Costa Rica (accession number KJ816681). Another partial sequence of the

Bartonella gltA gene obtained from *C. perspicillata* (#147) showed 93% identity with *Bartonella* sp. previously detected in *Megistopoda* sp. collected from *C. perspicillata* from Costa Rica (KJ816681) (**Table 3**).

Partial sequences of the *ITS* gene from *Bartonella* spp. obtained from *C. perspicillata* (#71 and #265) showed 100% identity with Bartonella sp. previously detected in a bat from Guatemala (accession number MN504740).

In the Maximum Likelihood phylogenetic analysis based on the *gltA* gene and the GRT I+G evolutionary model, the sequences detected in *C. perspicillata* were positioned in a clade with genotypes previously detected in bats of the species *Lophostoma silvicolum*, from the State of Mato Grosso (central western Brazil) and *Sturnira parvidens*, from the province of Puntarenas (Costa Rica) (**Figure 2**).

Table 3. Maximum identity by BLASTn analysis of *Bartonella* sequences detected in bats sampled Rio Branco and Xapuri, in the state of Acre, northwerstern Brazil.

Sample	GenBank accession number	Sequence size (bp)	Bat species	Molecular marker	Query coverage	E-Value	GenBank Closest Match	Host/ Country
5	PP339717	350	*Carollia perspicillata*	*gltA*	99%	5e-137	93% *Bartonella* sp. clone SJ119 (KJ816681)	*Trichobius keenan*/Costa Rica
147	PP339718	151	*Carollia perspicillata*	*gltA*	99%	3e-139	93% *Bartonella* sp. isolate (MH234339)	*Megistopoda* sp./ Costa Rica
71	PP341332	194	*Carollia perspicillata*	ITS	84%	3e-77	100% *Bartonella* sp. strain (MN504740)	*Mimon cozumelae*/Guatemala
265	PP341333	183	*Carollia perspicillata*	ITS	83%	3e-71	100% *Bartonella* sp. strain (MN504740)	*Mimon cozumelae*/Guatemala

Figure. 2 Phylogenetic analysis of *gltA* sequences from *Bartonella* spp. (697 bp) based on the Maximum Likelihood method and GRT I+G evolutionary model. Node numbers correspond to bootstrap values with 1000 replicates. *Brucella abortus*, *Brucella ovis* and *Ochrobactrum* sp. were used as outgroups.

4. Discussion

Although the state of Acre has a large biodiversity of bats (around 64 species) [15], with 20 species being the most prevalent [16], the diversity of potentially vector-borne zoonotic pathogens in this group of mammals still remains unknown. Despite previous studies carried out in Brazil on the occurrence of *Bartonella* spp. in bats and associated ectoparasites [7,8,9,10,11,12,13], the diversity of *Bartonella* genotypes in this diverse group of mammals is far from being completely understood. In fact, the occurrence of these agents has been documented, to date, in only 15 (25.4%) of the 59 bat species investigated for *Bartonella* spp. in Brazil [7,8,9,10,11,13].

This study presents the first description of the occurrence and molecular characterization of *Bartonella* spp. in the state of Acre, located in the northwestern region of Brazil. This region is strategically important for bordering Peru, a country where *Bartonella bacilliformis* [17] and *Bartonella ancachensis* [18] occur, both of a zoonotic nature. Both species are zoonotic and are the main causative agents of Carrion Disease and Peruana Wart [17, 18].

The present study reveals a prevalence of 10.1% for *Bartonella* spp. in bats in the studied region. The positive bats belong to the species *C. perspicillata*, *L. silvicolum*, *A. planirostris*, *Vampyressa thyone*, *T. maresi* and *P. elongatus*. This is the first description of *Bartonella* spp. in bats of the species *L. silvicolum, Vampyressa thyone, T. maresi* and *P. elongatus*. Previous studies conducted in Brazil showed a molecular prevalence for *Bartonella* spp. ranging from 0.6 to 24.5% [7,8,9,11,12]. This variation in the molecular prevalence of *Bartonella* among the various studies conducted in the country and possible comparisons must be interpreted with caution, given that several factors may be involved, such as the level of susceptibility of bats

from different families/species to infection, habitat, behavior social status (solitary x gregarious species), degree of infestation by dipteran vectors, sex, age, molecular method and gene target used for screening, among others.

The results reveal that *Bartonella* spp. can infect bats regardless of their feeding habits or population density (e.g., *V. thyone*, with low population density, and *C. perspicillata*, with high population density). Generally, bats tend to form colonies, which brings them closer together and consequently increases the probability of pathogen transmission. In a study that aggregated published data, a positive association was observed between hematophagous/carnivorous diet and *Bartonella* infection compared to frugivorous and insectivorous bats of the Phyllostomidae family [43]. Other factors, such as shelter type and cohabitation in shelters, might likely contribute to interactions between bats, *Bartonella* and vectors, establishing the basis for a better epidemiological understanding of the pathogen's evolution, maintenance, and transmission in these systems. Further studies are needed to better understand the driving factors behind these potential associations at the geographic, ecological and host species levels.

In the phylogenetic analysis based on the *gltA* gene, the sequences detected in *C. perspicillata* sampled in the present study were grouped into a clade with genotypes previously found in bats of the species *L. silvicolum*, from the State of Mato Grosso (central-western region of Brazil) and *S. parvidens* from Costa Rica. This phylogenetic positioning corroborates previous findings, demonstrating the great diversity of genotypes of *Bartonella* species associated with chiropterans, comprising distinct clades, but specific to this group of mammals. Notably, both sequences found did not share the same clade with the two zoonotic species *Bartonella mayotimonensis* and

‘*Candidatus* Bartonella rousetti’. The zoonotic potential of the genotypes found in the present study remains unknown.

Interestingly, co-infection between *Bartonella* spp. and *Trypanosom*a sp. [19] was verified in bats captured in the present study: *T. maresi* (1/1), *V. thyone* (1/1) and *C. perspicillata* (1/69) were also positive for *T. cruzi*, and *L. silvicolum* (1/3) for *T. cruzi marinkellei.* Although it is not possible to establish a pattern, co-infection may reinforce the potential of bats to host both pathogens. The association of *Bartonella* spp. with other zoonotic agents must be taken into consideration. Corrêa and collaborators [44] verified the association between bacteremia due to *Bartonella* spp. and chagasic endocarditis, arrhythmia and cardiomyopathy. It was found that the probability of infection by *Bartonella* is 22 times greater in patients with endocarditis, 45 times greater in patients with arrhythmia, and 40 times greater for patients with Chagas cardiomyopathy, emphasizing a possible association between infections by *Bartonella* spp. and *Trypanosoma cruzi.*

5. Conclusion

This study provided the first molecular evidence of the presence of *Bartonella* in bats in the State of Acre. This is the first detection of *Bartonella* spp. in *L. silvicolum, Vampyressa thyone, T. maresi* and *P. elongatus*. The detected *Bartonella gltA* genotypes grouped with those found in bats from other locations, expanding knowledge about the diversity of genotypes associated with bats, especially in this Amazon area in the northern region of the country.

Ethics statement

Bat captures were approved by the Chico Mendes Institute for Brazilian Biodiversity Conservation (ICMBio – SISBIO), licenses 44089-1 and 47377-1. All procedures performed with bats followed protocols approved by the Animal Use Ethics Committee of Fiocruz (LW81-12) and by the Animal Use Ethics Committee of the Faculty of Agricultural and Veterinary Sciences of UNESP (CEUA FCAV/UNESP 3335/22).

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Data linking

Sequences generated during the study were submitted to NCBI Genbank (http://www.ncbi.nlm.nih.gov/genbank/). Sequences can be accessed by the following accession numbers: PP339717, PP339718, PP341332 and PP341333.

Authors' contributions

Eliz Oliveira Franco: Conceptualization, Methodology, Visualization, Formal analysis, Writing – original draft, Writing – review and editing. **Francisco CB dos Santos**: Methodology, Writing – review and editing. **Rair de Sousa Verde**- Methodology, Writing – review and editing. **Ana Cláudia Calchi**: Methodology, Visualization.

Victória Valente Califre de Mello: Methodology, Writing – review and editing. **Daniel Braga Lee**: Methodology, Writing – review and editing. **Clara Morato Dias**: Methodology, Writing – review and editing. **Rosangela Zacarias Machado**: Conceptualization, Writing – review and editing. **Adolorata Bianco Carvalho**: Writing – review and editing. **André Luiz Rodrigues Roque**: Conceptualization, Writing – review and editing. **Marcos Rogério André**: Conceptualization, Acquisition of financing, Supervision, Writing – original draft, Writing – review and editing.

Declaration of interest

The authors declare that there is no conflict of interest that may have influenced the results obtained and reported in this research.

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CAPÍTULO 3- Occurrence and genetic diversity of *Bartonella* spp. in vampire bats and associated Streblidae bat flies in the Brazilian Amazon

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Abstract: Among mammals, bats stand out as important reservoirs for *Bartonella* spp., second only to rodents. In Brazil, out of the 182 species of bats described, three are hematophagous, namely *Desmodus rotundus*, *Diphylla ecaudata* and *Diaemus youngii*. Considering that *Bartonella* species have been increasingly associated to human diseases, the search for such agents in animal reservoirs and ectoparasites is important in order to elucidate the epidemiology of bartonelloses. The present study aimed to investigate the occurrence and genetic diversity of *Bartonella* spp. in vampire bats and Streblidae bat flies in the Brazilian Amazon. For this purpose, 228 spleen samples of *D. rotundus* and 1 of *D. youngi* were collected from four states in the northern region of Brazil (Pará (n = 206/*D. rotundus*; n = 1/*D. youngii*), Roraima (n = 18/*D. rotundus*), Amapá (n = 3/*D.*

rotundus) and Amazonas (n = 1/*D. rotundus)*. Additionally, 142 Streblidae bat flies were collected from 54 *D. rotundus* (23 from *Streblia wiedemanni* and 118 *Trichobius parasiticus*) and one *D. youngii* (1 *Trichobius diaemi*). Seventy-three (31.9%) spleen samples of *D. rotundus* (62 (PA), 9 (RR) and 2 (AP) and 45 (50%) Streblide bat flies (1 *Trichobius diaemi*, 8 *Streblia wiedemanni* and 36 *Trichobius parasiticus*) were positive in qPCR for *Bartonella* spp. based on the *nuoG* gene. Phylogenetic analyses based on the *gltA* and *rpoB* genes grouped the sequences obtained with genotypes previously detected in *D. rotundus* and bat-associated flies. High genotypic diversity was found among sequences obtained from bats (4 *gltA* and 9 *rpoB* genotypes) and Streblidae bat flies (4 *gtlA* and 5 *rpoB* genotypes). The genotypes identified in *D. rotundus* in the present study were exclusively shared with sequences from *Bartonella* spp. detected in vampire bats, not overlapping with genotypes previously detected in non-hematophagous bats from Brazil. Most of the sequences detected in Streblidae bat flies formed unique genotypes for each dipteran species analyzed. The present study expanded the knowledge regarding the diversity of *Bartonella* genotypes in vampire bats and associated Streblidae flies.

Keywords: *Bartonella*; Chiroptera; *Desmodus rotundus*; Amazon Forest; genotypic diversity

1. Introduction

The genus *Bartonella* (Hyphomicrobioales: Bartonellaceae) encompasses facultative intracellular Gram-negative α-proteobacteria that primarily infect mammalian erythrocytes and endothelial cells, as well as macrophages, monocytes and dendritic cells (Eicher; Dehio, 2012). These agents have a cosmopolitan distribution and are transmitted by hematophagous arthropod vectors, such as fleas, ticks, mosquitoes, sandflies and lice (Breitschwerdt; Kordick, 2000; Chomel et al., 2006).

Biological characteristics such as abundance, species diversity, geographic distribution, behavioral habits and longevity make bats as ideal for several pathogens

(Bai et al., 2011). Among mammals, bats stand out as important reservoirs for *Bartonella* spp., second only to rodents (Stuckey et al., 2017). In Brazil, 182 bat species were recorded, distributed in 68 genera and 9 families. Of these, three species are hematophagous, namely *Desmodus rotundus*, *Diphylla ecaudata* and *Diaemus youngii* (Garbino et al., 2022). The former is distributed throughout the Brazilian territory (Paglia et al., 2012), with its diet based mainly on the blood of large mammals (Sánchez-Cordero et al., 2011), in addition to livestock, such as cattle, horses and pigs (Mialhe et al., 2014). It is the most abundant bat species in the Desmodontinae subfamily and has a social habit with the formation of colonies ranging from 5 to 20,000 individuals (Mialhe et al., 2014). They have social behavior of cleaning, sharing food and self-cleaning when infested by ectoparasites (Wilkinson et al., 1986).

Although *D. youngii* occurs in several states in Brazil, it is considered locally rare. Unlike those of *D. rotundus*, this bat species is found in small colonines (up to 30 individuals) (Greenhall et al., 1993), with blood of birds as the main food source, although they can occasionally feed on the blood of mammals (Uieda et al., 1996). Similarly to *D. youngii*, *D. eucaudata* can also be found in several states in Brazil, feeding on the blood of birds, albeit may occasionally also feed on the blood of mammals. It generally forms small colonies, with groups of up to 12 individuals (Rocha et al., 2014).

Since *Bartonella* species have been increasingly associated with human diseases (Breitschwerdt, 2017), the search for these agents in animal reservoirs and ectoparasites is important in order to elucidate the epidemiology of bartonelloses. '*Candidatus* Bartonella mayotimonensis' (Lin et al., 2012), detected in bats in the Northern Hemisphere, and '*Candidatus* Bartonella rousetti' (Bai et al., 2018), described in fruit bats of the species *Rousettus aegyptiacuse* and nycteribiid bat flies (*Eucampsipoda africana)* in the African continent, were incriminated as zoonotic agents. Although numerous studies have been conducted regarding *Bartonella* spp. in non-hematophagous bats (Han et al., 2017; Ikeda et al., 2017; Ferreira et al., 2018; Corduneanu et al., 2018; Ikeda et al., 2020; Gonçalves-Oliveira et al., 2020; McKee et al., 2021; Bernal et al., 2022; Han et al., 2023; Fagre et al., 2023), few have investigated the occurrence and genetic diversity of these agents in vampire bats (Bai

et al., 2011; Bai et al., 2012; Wray et al., 2016; Stuckey et al., 2017; Becker et al., 2018; André et al., 2019).

Bats can host a wide variety of ectoparasites, including Hippoboscoidea flies (Diptera: Nycteribiidae and Streblidae), bedbugs (Hemiptera: Cimicidae and Polyctenidae), fleas (Siphonaptera: Ischnopsyllidae), ticks (Ixodida: Ixodidae and Argasidae) and mites (Mesostigmata: Spinturnicidae and Macronyssidae) (Szentiványi et al., 2019). Among the ectoparasites abovementioned, Streblidae and Nycteribiidae bat flies have been incriminated as carriers and possible vectors of a wide diversity of genotypes of *Bartonella* spp. among bats (Reeves et al., 2005; Morse et al., 2012; Reeves et al., 2016; do Amaral et al., 2018; Moskaluk et al., 2018; Braga et al., 2020; Ikeda et al., 2020; Lee et al., 2021; André et al., 2023). Although 101 species of Streblidae (Graciolli, 2024) and 28 Nycteribiidae bat flies (Graciolli & Hrycyna, 2024) have been reported in Brazil, few studies have investigated the diversity of bartonellae in these dipterans (do Amaral et al., 2018; Ikeda et al., 2020; Braga et al., 2020).

The present work investigated the occurrence and genetic diversity of *Bartonella* spp. in vampire bats and Streblidae bat flies in the Brazilian Amazon, in order to expand the knowledge on the diversity of bartonellae in bats and associated dipterans.

2. Material and merthods

2.1 Ethics statement

The conduct of this work was approved by the “National Council for the Control of Animal Experimentation (CONCEA) and Committee for the Use of Animals—CEUA-FCAV/UNESP” under protocol number 003880/23 and also registered in the National Genetic Heritage Management System and Associated Traditional Knowledge (SISGEN) accession number A434E64.

2.2 Animals and ectoparasites sampled and studied areas

Between 2017 and 2019, carcasses of vampire bats (*Desmodus rotundus* and *Diaemus youngii*) collected in different states in the northern region of Brazil (Pará, Roraima, Amapá and Amazonas) were received at the Rabies Diagnostic Laboratory of the Evandro Chagas Institute (Belém, Pará). Streblidae bat flies, which were

collected from 55 individuals, were identified using a stereoscopic microscope and previously described dichotomous keys (Wenzel et al., 1966; Guerrero, 2019).

2.3 DNA extraction and PCR for endogenous mammalian and insect genes

DNA extraction from spleen samples from 229 vampire bats was performed using the BIOPUR Mini Spin Plus extraction kit (Mobius), following the protocol recommended by the manufacturer. DNA from 142 Streblidae flies was extracted with TRIzol™ (Invitrogen™) following the protocol recommended by the manufacturer. The concentration (µg/µL) and purity (260/280 ratio) of the DNA samples were evaluated by optical spectrophotometry (Nanodrop, Thermo Scientific). Extracted DNA samples were subsequently stored at −20°C until molecular analyses.

DNA samples from bat spleens were initially subjected to a conventional PCR (cPCR) assay based on the endogenous mammalian glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) gene (Birkenheuer et al., 2003) to verify the absence of PCR inhibitors and the presence of amplifiable DNA in the samples. DNA samples from Streblidae flies were subjected to a cPCR based on the subunit I gene of the insect Cytochrome Oxidase (*COI*) gene (Folmer et al., 1994). Samples that tested positive for endogenous genes were subsequently subjected to specific PCR assays for *Bartonella* spp.

2.4 Real-time quantitative PCR (qPCR) and conventional assays for *Bartonella* spp.

Bat spleen DNA samples positive in PCR for the endogenous gene (*gapdh*) and Streblidae flies DNA samples positive in the PCR for *COI* gene were subjected to a qPCR based for *Bartonella* spp. based on the *nuoG* gene, using primer (Integrated DNA Technologies®, Coralville, Iowa, Estados Unidos) F (5'CAATCTTCTTTTGCTTCACC-3') and R (5'- TCAGGGCTTTATGTGAATAC-3') and the TaqMan hydrolysis probe TexasRed-5'-TTYGTCATTTGAACACG-3'[BHQ2a-Q]3' previously designed (André et al., 2016). DNA samples were evaluated in duplicates and when differences in Cq values were greater than 0,5, samples were retested in triplicates. The mean quantification of replicates of a certain positive sample was only

performed when the difference in Cq values was less than or equal to 0,5. To construct the standard curve for each reaction, serial dilutions were performed at different concentrations (2,0 × 10^7 a 2,0 × 10^0 cópias) of a plasmid encoding an 83 bp fragment of the *Bartonella henselae nuoG* gene (pIDTSMART; Integrated DNA Technologies, Coralville, Iowa, Estados Unidos), which were also used as positive controls. UltraPure™ DNase/RNase-Free Destilled Water (Thermo Scientific, Waltham, MA, EUA) were used as negative controls. Plasmid copy number was determined by the formula (XG/µL DNA/ [Plasmid Length (bp) × 660]) × 6. 22 × 10^{23} × plasmid copies/µL Amplification efficiency (E) was calculated according to the slope of the standard curve using the formula E= $10^{-1/slope}$ (Bustin et al., 2009). qPCR assays were performed in a C1000-CFX96 thermocycler (BioRad, Hercules, California, USA).

DNA samples from vampire bat spleen and Streblidae bat flies positive in the qPCR for the *nuoG* gene were subjected to cPCR assays for *Bartonella* spp. targeting six molecular markers, namely *ribC* (~585-588 bp) (Johnson et al., 2003), *gltA* (~379 bp) (Norman et al., 1995), (~767 bp) (Billeter et al., 2011), *rpoB* (~800 bp) (Renesto et al., 2001), *pap-31* (~564 bp) (Maggi; Breitschwerdt, 2005), *groEL* (~752 bp) (Zeaiter et al., 2002; Paziewska et al., 2011) and *ftsZ* (~515 bp) (Paziewska et al., 2011). *Bartonella henselae* DNA (ON561953) (Dias et al., 2023) and sterilized ultrapure water were used as positive and negative controls, respectively, in all PCR assays.

PCR products were revealed by electrophoresis in a 1% agarose gel stained with ethidium bromide (0,5 µL/mL) (Life Technologies™, Carlsbad, California, EUA) in running buffer TBE pH 8,0 (44,58 M Tris-base; 0,44 M boric acid; 12,9 mM EDTA) under 90 V/150 mA for 60 minutes. A 100 base pair molecular weight marker (Kasvi, Campinas, São José do Pinhais, Paraná, Brasil) and a transilluminator coupled to the Image Lab computer program for image analysis (BioRad, Hercules, California, USA) were used to visualize the results.

2.5 Amplicon purification and Sanger Sequencing

PCR products were purified using the kit ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Scientific, San Jose, CA, USA), according to the manufacturer's recommendations. Sequencing of the amplified products was carried out using an automated technique based on the dideoxynucleotide chain termination method

(Sanger et al., 1997) on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems) at the DNA Sequencing Sector, Center for Genome Studies, Human and Cells (Institute of Biosciences – USP, São Paulo, SP, Brazil).

2.6 BLASTn analyses and phylogenetic inferences

The analysis of the electropherograms generated in the Sanger sequencing and construction of consensus sequences were carried out using the Phred-Phrap version program (Ewing and Green, 1998; Ewing et al., 1998) respecting the minimum “Phred” quality value of 20 for the read bases. The BLAST program (Altschul et al., 1990) was used to compare the sequences obtained with those previously deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank). The sequences were saved in “FASTA” mode and aligned with other sequences previously deposited in the GenBank database, using the BIOEDIT software version 7.0.5.3 (Hall, 1999). Maximum likelihood (ML) phylogenetic analysis was inferred on the IQ-TREE online server using 1000 replicates of UFBoot2 – Ultrafast Bootstrap (Hoang et al., 2018). The evolutionary model was found using the jModelTest 2 software (Darriba et al., 2012). Editing of phylogenetic trees as well as rooting (via outgroup) was performed using Treegraph 2.0.56-381 beta software (Stover et al., 2010).

2.7 Genotype diversity analysis

The aligned sequences for each locus were analyzed using the DnaSP v5 software (Librado; Rozas, 2009) to identify the number of genotypes (h), nucleotide diversity (π), the level of polymorphism (genotype diversity - [dh]) and nucleotide difference (k). Furthermore, the sequences were subjected to the construction of the genotype network using the TCS v.1.21 software (Clement et al., 2001) and the figures were generated with Population Analysis with Reticulate Trees (PopART, Allan Wilson Center Imaging Evolution Initiative - http: //popart.otago.ac.nz) (Leigh and Bryant, 2015).

3. Results

3.1 Sampled bats and ecrtoparasites

Carcasses of vampire bats belonging to the species *Desmodus rotundus* (n = 228) and *Diaemus youngii* (n = 1) collected in different states in the northern region of Brazil (Pará (n = 206/*D. rotundus*; n = 1/*D. youngii*), Roraima (n = 18/*D. rotundus*), Amapá (n = 3/*D. rotundus*) and Amazonas (n = 1/*D. rotundus*) were received at the Rabies Diagnostic Laboratory of the Instituto Evandro Chagas (Belém, Pará) (**Figure 1**). In addition, 142 Streblid bat flies were collected from 55 bats, of which 23 *Streblia wiedemanni* collected from 17 *D. rotundus* from PA, 1 *Trichobius diaemi* from 1 *D. youngii* from PA, and 118 *Trichobius parasiticus* from 48 *D. rotundus* from PA and 1 *D. rotundus* from AP.

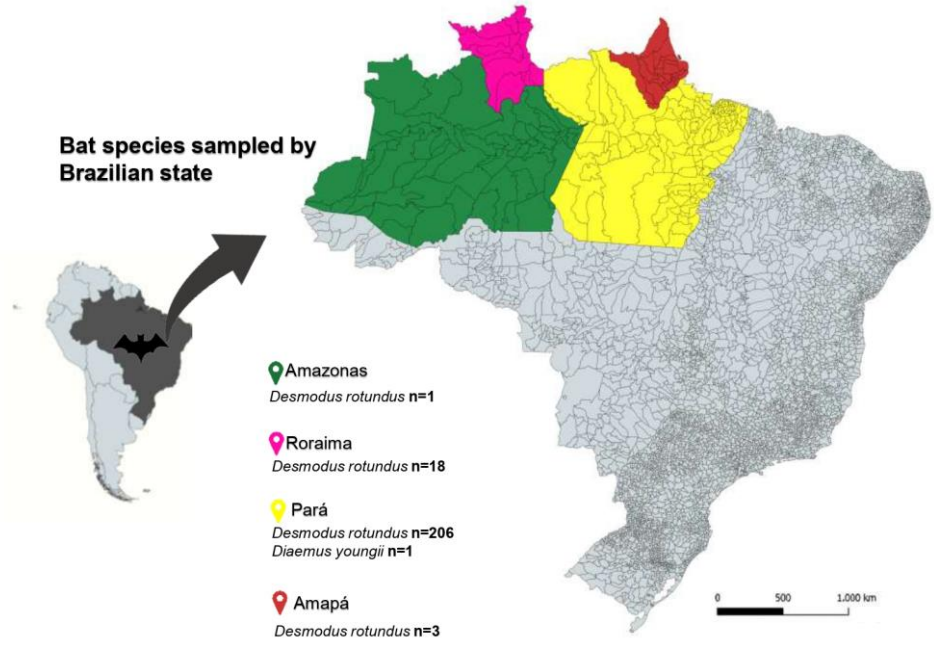


Figure 1. Geographic distribution of vampire bat capture sites. Map created by MapChart (https://www.mapchart.net/index.html)

3.2 Molecular occurrence of *Bartonella* spp. in bat spleen samples and Streblidae bat flies

All 229 bat spleen DNA samples were positive in the cPCR targeting the mammalian *gapdh* gene. The mean DNA concentration and absorbance ratios (260/280 nm and 260/230 nm) of bat spleen DNA samples were 81.3 ng/µL (SD 19.5), 1.94 and 2.25 (SD ± 3.87 and ± 2.50), respectively. The mean DNA concentration and

absorbance ratios (260/280 nm and 260/230 nm) of the Streblidae bat fly DNA samples were 4 ng/µL (±4.39), 0.4 and 1.0 (SD ± 1 and ± 1.03), respectively.

In the qPCR assays for *Bartonella* spp. based on the *nuoG* gene, 73/229 (31.9%) vampire bat spleen samples were positive (Pará 61/206 *D. rotundus*; 1/1 *D. youngii*), Roraima (9/18 *D. rotundus*), Amapá (2/3 *D. rotundus*) and Amazonas (1/1 *D. rotundus*). The qPCR parameters were: efficiency ranging from 97.7% to 105.7%; slope from -3.193 to -3.466; R^2 from 0.995 to 0.999; y-intercept from 36.100 to 38.015. The number of copies of a fragment of the *nuoG* gene per microliter of DNA ranged from 1.830×10^{-1} to 2.8610×10^{10}. In 37/73 positive samples, it was not possible to estimate quantification, since the Cq values of the replicates were higher than 0.5, even after repeating in triplicates.

Of the 141 Streblidae bat flies, 90 (64.5%) were positive in cPCR targeting the COI gene. In qPCR assays for Bartonella spp. based on the nuoG gene, 45/90 (50%) were positive (efficiency ranging from 94% to 108.6%; guidance from -3.131 to -3.470; R^2 from 0.944 to 0.997; intercept and from 36.330 to 38.350). Only four samples (8.9%) were quantifiable **(Table 1)**. Positivity rates for *Bartonella* spp. of 8/23 (24.8%) was obtained for *Streblia wiedemanni*, 36/118 (30.5%) for *T. parasiticus* and 1/1 (100%) for *T. diaemi*.

Table 1. Streblidae bat flies positive in qPCR for *Bartonella* spp. based on the *nuoG* gene and parasitized vampire bats, with respective quantification values.

Streblidae bat fly species and ID	**Quantification (*Bartonella nuoG* copies /*µL*) in the bat fly specimen**	**Host bat species (ID/Locality/P-N)**	**Quantification (*Bartonella nuoG* copies/*µL*) in the spleen sample of bat hosts**
Trichobius parasiticus (69-A)	N/Q	*Desmodus rotundus* (69/PA/P)	N/Q
Trichobius parasiticus (69-B)	N/Q		
Trichobius diaemi (70)	N/Q	*Diaemus youngii* (70/PA/N)	*
Trichobius parasiticus (71-A)	N/Q	*Desmodus rotundus* (71/PA/N)	*
Trichobius parasiticus (71-B)	N/Q		
Trichobius parasiticus (75-A)	N/Q	*Desmodus rotundus* (75/PA/P)	$3,851\times10^2$; $4,320\times10^2$
Streblia wiedemanni (75-B/S)	N/Q		
Streblia wiedemanni (76-A/S)	N/Q	*Desmodus rotundus* (76/*PA/P)*	NQ
Trichobius parasiticus (88-B)	N/Q	Desmodus rotundus (88/PA/P)	NQ

Trichobius parasiticus (90-B)	N/Q	*Desmodus rotundus* (90/PA/N)	*
Trichobius parasiticus (105)	N/Q	*Desmodus rotundus* (105/PA/N)	*
Trichobius parasiticus (120-D)	$5,52\times10^{-1}$	*Desmodus rotundus* (120/PA/P)	NQ
Trichobius parasiticus (120-E)	N/Q		
Trichobius parasiticus (141)	N/Q	*Desmodus rotundus* (141/PA/N)	*
Streblia wiedemanni (144-A/S)	N/Q	Desmodus rotundus (144/PA/P)	NQ
Trichobius parasiticus (148)	N/Q	*Desmodus rotundus* (148/PA/N)	*
Streblia wiedemanni (154-S)	N/Q	*Desmodus rotundus* (154/PA/P)	$6,67\times10^{1}$
Trichobius parasiticus (162-A)	N/Q	*Desmodus rotundus* (162/PA/P)	$3,72\times10^{1}$
Trichobius parasiticus (162-B)	N/Q		
Streblia wiedemanni (166-A/S)	N/Q	*Desmodus rotundus* (166/PA/P)	$3,71\times10^{1}$
Trichobius parasiticus (166-B)	N/Q		
Streblia wiedemanni (167-S)	N/Q	*Desmodus rotundus* (167/PA/N)	*
Trichobius parasiticus (169-B)	N/Q	*Desmodus rotundus* (169/PA/N)	*
Trichobius parasiticus (177-A)	N/Q	Desmodus rotundus (177/PA/N)	*
Trichobius parasiticus (177-D)	N/Q		
Trichobius parasiticus (195-B)	N/Q	*Desmodus rotundus* (195/PA/N)	*
Trichobius parasiticus (202)	N/Q	*Desmodus rotundus* (202/PA/N)	*
Trichobius parasiticus (203-A)	N/Q	*Desmodus rotundus* (203/PA/N)	*
Trichobius parasiticus (212-C)	N/Q	*Desmodus rotundus* (212/PA/N)	*
Trichobius parasiticus (213-B)	N/Q	*Desmodus rotundus* (213/PA/N)	*
Streblia wiedemanni (2018-S)	N/Q	*Desmodus rotundus* (218/PA/N)	*
Trichobius parasiticus (219-A)	N/Q	*Desmodus rotundus* (219/PA/N)	*
Trichobius parasiticus (219-D)	N/Q		
Trichobius parasiticus (221)	N/Q	*Desmodus rotundus* (221/PA/N)	*
Trichobius parasiticus (222-A)	N/Q	*Desmodus rotundus* (222/PA/N)	*
Trichobius parasiticus (222-C)	N/Q		
Streblia wiedemanni (224-A/S)	N/Q		*

Trichobius parasiticus (224-D)	2,30x10°	*Desmodus rotundus* (224/PA/N)	
Trichobius parasiticus (224-F)	NQ		
Trichobius parasiticus (224-H)	5,04×10°		
Trichobius parasiticus (225)	1,33×10¹	*Desmodus rotundus* (225/PA/N)	*
Trichobius parasiticus (229-A)	N/Q	*Desmodus rotundus* (229/PA/N)	*
Trichobius parasiticus (229-C)	N/Q		
Trichobius parasiticus (231-A)	N/Q	*Desmodus rotundus* (231/PA/N)	*
Trichobius parasiticus (231-B)	N/Q		

P= bat spleen sample positive in the qPCR for *Bartonella* spp. based on the *nuoG* gene. **N**= bat samples negative for *Bartonella* spp.. **NQ**: positive sample in the qPCR for *Bartonella* spp. based on the *nuoG* gene but non-quantifiable. ***** negative sample in the qPCR for *Bartonella* spp. based on the *nuoG* gene.

Das 73 amostras de DNA de baço de morcegos hematófagos positivas na qPCR, 60 (82%) foram positivas para, pelo menos, um gene em ensaios de PCR para *Bartonella* spp., incluindo 41 (56,1%) para o gene *gltA* (~379 pb), 22 (30%) para o gene *rpoB,* 10 (13,7%) para o gene *ftsZ*, 13 (18%) para o gene *pap-31* e 25 (34,2%) para o gene *gltA* longo (~767 pb). Nenhuma das 73 amostras mostrou-se positiva nos ensaios de PCR para os genes *groEL* e *ribC* (**Tabela 2**).

Table 2. *Desmodus rotundus* spleen DNA samples positive in PCR assays for *Bartonella* spp. targeting different molecular markers.

	***nuoG* qPCR**	**cPCR assay**						
Bat species/sample ID	**Mean quantification(copies/µL)**	***gltA* (~379 bp)**	***gltA* (~767 bp)**	***rpoB* (~333 bp)**	***groEL* (~752 bp)**	***ribC* (~585 bp)**	***ftsZ* (~515 bp)**	***pap-31* (~564 bp)**
Desmodus rotundus/71	NQ	-	-	-	-	-	-	-
Desmodus rotundus/11	NQ	-	-	-	-	-	-	Seq
Desmodus rotundus/15	1,36×10¹; 1,346×10¹	NS	-	-	-	-	-	-
Desmodus rotundus/19	6,348×10¹; 5,081×10¹	NS	-	Seq	-	-	-	NS
Desmodus rotundus/20	2,19×10²	Seq	-	NS	-	-	-	-
Desmodus rotundus/22	NQ	-	-	-	-	-	-	NS
Desmodus rotundus/23	NQ	NS	-	-	-	-	-	-
Desmodus rotundus/27	4,186×10¹; 4,456×10¹	NS	-	-	-	-	-	-
Desmodus rotundus/32	7,479×110¹	-		-	-	-	-	Seq
Desmodus rotundus/33	2,416×10	NS	-	-	-	-	-	-
Desmodus rotundus/35	NQ	NS	-	-	-	-	-	-
Desmodus rotundus/36	4,753×10¹; 4,482×10¹	Seq	-	-	-	-	-	-

Desmodus rotundus/39	2,959×10¹; 3,825×10¹	NS	-	Seq	-	-	-	-
Desmodus rotundus/40	1,245×10¹	Seq	-	-	-	-	-	-
Desmodus rotundus/42	3,898×10¹; 4,482×10¹	NS	NS	NS	-	-	-	NS
Desmodus rotundus/55	2,471×10°; 2,334×10°	NS	-	-	-	-	-	-
Desmodus rotundus/61	NQ	NS	-	-	-	-	-	-
Desmodus rotundus/66	6,270×10¹; 5,530×10¹	NS	NS	-	-	-	-	Seq
Desmodus rotundus/69	NQ	-	NS	-	-	-	-	Seq
Desmodus rotundus/75	3,851×10²; 4,320×10²	Seq	NS	-	-	-	-	Seq
Desmodus rotundus/76	NQ	NS	NS	Seq	-	-	-	-
Desmodus rotundus/85	4,403×10¹; 3,388×10¹	NS	-	-	-	-	-	-
Desmodus rotundus/87	6,458×10¹; 7,040×10¹	NS	-	-	-	-	-	-
Desmodus rotundus/92	NQ	-	-	Seq	-	-	-	-
Desmodus rotundus/94	NQ	-	-	-	-		NS	-
Desmodus rotundus/107	5,176×10°; 3,882×10°	NS	-	-	-	-	-	-
Desmodus rotundus/108	5,143×10²; 3,480×10°	NS	NS	Seq	-	-	Seq	Seq
Desmodus rotundus/110	2,352×10°; 3,480×10°	NS	-	-	-	-	-	-
Desmodus rotundus/112	NQ	NS	NS	Seq	-	-	-	-
Desmodus rotundus/114	6,962×10¹; 7,228×10¹	NS	NS	-	-	-	Seq	-
Desmodus rotundus/119	5,154×10¹; 4,202×10¹	NS	-	NS	-	-	-	-
Desmodus rotundus/120	NQ	-	NS	-	-		NS	-
Desmodus rotundus/123	8,327×10²; 9,317×10²	NS	Seq	-	-	-	-	-
Desmodus rotundus/123	NQ	NS	-	NS	-	-	-	-
Desmodus rotundus/126	NQ	-	-	-	-	-	-	NS
Desmodus rotundus/137	NQ	NS	-	NS	-	-	-	-
Desmodus rotundus/140	NQ	NS	-	NS	-	-	-	-
Desmodus rotundus/144	NQ	-	Seq	-	-	-	-	-
Desmodus rotundus/145	NQ	NS	Seq	-	-	-	-	-
Desmodus rotundus/147	NQ	-	-	-	-	-	-	NS
Desmodus rotundus/149	NQ	NS	NS	Seq	-	-	NS	-
Desmodus rotundus/153	NQ	-	NS	-	-	-	-	-
Desmodus rotundus/154	6,518×10¹; 6,282×10¹	NS	Seq	NS	-	-	NS	-
Desmodus rotundus/156	3,703×10¹; 2,676×10¹	NS	NS	-	-	-	-	-
Desmodus rotundus/158	NQ	NS	-	-	-	-	-	-
Desmodus rotundus/160	NQ	-	-	NS	-	-	-	-
Desmodus rotundus/162	3,533×10¹; 3,913×10¹	-	Seq	-	-	-	-	-
Desmodus rotundus/166	3,411×10¹; 4,015×10¹	-	NS	-	-		NS	-
Desmodus rotundus/168	NQ	-	-	-	-	-	-	NS
Desmodus rotundus/172	NQ	Seq	Seq	-	-	-	NS	-
Desmodus rotundus/174	1,696×10°; 5,527×10°	Seq	-	Seq	-	-	-	-
Desmodus rotundus/179	NQ	NS	-	Seq	-	-	-	-

Desmodus rotundus/180	NQ	-	-	Seq	-	-	Seq	-
Desmodus rotundus/183	NQ	Seq	NS	-	-	-	-	-
Desmodus rotundus/184	NQ	-	NS	Seq	-	-	-	-
Desmodus rotundus/186	NQ	NS	NS	NS	-	-	NS	-
Desmodus rotundus/190	NQ	-	NS	-	-	-	-	-
Desmodus rotundus/191	$3{,}780\times10^{2}$; $4{,}165\times10^{2}$	NS	Seq	Seq	-	-	-	-
Desmodus rotundus/201	NQ	NS	-	-	-	-	-	-
Desmodus rotundus/215	NQ	-	-	-	-	-	-	NS
Desmodus rotundus/220	NQ	NS	-	-	-	-	-	-
Desmodus rotundus/222	$1{,}353\times10^{2}$; $6{,}761\times10^{1}$	-	NS	Seq	-	-	-	-
Desmodus rotundus/236	NQ	-	-	-	-	-	-	-

NS = positive sample in cPCR, but not sequenced due to the low intensity of the amplified products**; Seq =** Sequences obtained**; NQ:** positive sample in the qPCR for *Bartonella* spp. based on the *nuoG* gene but not non-quantifiable.

Out of the 90 Streblidae bat flies DNA samples positive in qPCR, 20 (22.2%) were positive in at least one cPCR assay for *Bartonella* spp., including 15 (16.7%) for the gene *gltA* (~379 bp), 8 (8.9%) for the *rpoB* gene, 1 (1.1%) for the *pap-31* gene and 5 (5.6%) for the *gltA* gene (~767 bp). None of the 90 Streblidae bat flies DNA were positive in the PCR assays for the *groEL*, *ribC* and *ftsZ* genes (**Table 3**).

Table 3. Streblidae bat fly DNA samples positive in PCR assays for *Bartonella* spp. targeting different molecular markers.

		cPCR						
Streblidae fly species (sample ID)	**Bat host species (ID/Locality/P-N)**	***gltA* (~379 bp)**	***gltA* (~767 bp)**	***rpoB* (~333 bp)**	***groEL* (~752 bp)**	***ribC* (~585 bp)**	***ftsZ* (~515 bp)**	***pap-31* (~564 bp)**
Trichobius diaemi (70)	*Diaemus youngii* (70/PA/N)	Seq	-	-	-	-	-	-
Trichobius parasiticus (71B)	*Desmodus rotundus* (71/PA/N)	NS	-	-	-	-	-	-
Trichobius parasiticus (88B)	*Desmodus rotundus* (88/PA/P)	NS	NS	-	-	-	-	-
Trichobius parasiticus (90B)	*Desmodus rotundus* (90/PA/N)	NS	-	-	-	-	-	-
Trichobius parasiticus (162A)	*Desmodus rotundus* (162/PA/P)	Seq	Seq	Seq	-	-	-	-
Trichobius parasiticus (165B)	*Desmodus rotundus* (165/PA/P)	-	Seq	-	-	-	-	-

Trichobius parasiticus (203A)	*Desmodus rotundus* (203/PA/N)	**NS**	-	-	-	-	-	-
Trichobius parasiticus (213B)	*Desmodus rotundus* (213/PA/N)	**Seq**	-	-	-	-	-	-
Trichobius parasiticus (219A)	*Desmodus rotundus* (219/PA/N)	**NS**	-	-	-	-	-	-
Trichobius parasiticus (222A)	*Desmodus rotundus* (222/PA/N)	**NS**	-	-	-	-	-	-
Trichobius parasiticus (229C)	*Desmodus rotundus* (229/PA/N)	**NS**	-	-	-	-	-	-
Trichobius parasiticus (213B)	*Desmodus rotundus* (231/PA/N)	**NS**	-	-	-	-	-	-
Trichobius parasiticus (69B)	*Desmodus rotundus* (69/PA/P)	-	-	**Seq**	-	-	-	-
Trichobius parasiticus (231B)	*Desmodus rotundus* (231/PA/N)	-	-	-	-	-	-	-
Streblia wiedemanni (166AS)	*Desmodus rotundus* (166/PA/P)	**NS**	-	**Seq**	-	-	-	-
Streblia wiedemanni (218S)	*Desmodus rotundus* (218/PA/N)	**NS**	**NS**	**Seq**	-	-	-	-
Streblia wiedemanni (224AS)	*Desmodus rotundus* (224/PA/N)	**NS**	-	-	-	-	-	-
Streblia wiedemanni (154S)	*Desmodus rotundus* (154/PA/P)	**NS**	**NS**	**Seq**	-	-	-	-
Streblia wiedemanni (144AS)	*Desmodus rotundus* (144/PA/P)	-	-	**Seq**	-	-	-	**NS**
Streblia wiedemanni (167S)	*Desmodus rotundus* (167/PA/N)	-	-	**Seq**	-	-	-	-
Streblia wiedemanni (221AS)	*Desmodus rotundus* (221/PA/N)	-	-	**Seq**	-	-	-	-

NS = positive sample in cPCR, but not sequenced due to the low intensity of the amplified products; **Seq** = Sequences obtained; **NQ**: positive sample in the qPCR for *Bartonella* spp. based on the *nuoG* gene but not quantifiable.

3.3 BLASTn results

The results of BLASTn analyses of *Bartonella* sequences detected in *Desmodus rotundus* spleen samples and associated Streblidae bat flies are shown in **Tables 4** and **5**, respectively.

Table 4. Maximum identity by BLASTn analysis of *Bartonella* sequences detected in spleen samples of *Desmodus rotundus* sampled in the Brazilian Amazon.

Genbank accession number (#sample)	Target gene	Size of sequence obtained (bp)	Query coverage	E-value	GenBank Closest Match	Host/Country
PP715665 (#20)	*gltA*	378	99%	0.0	99% *Bartonella* sp. strain NE03_7 (MF467787)	*Desmodus rotundus*/ Mexico
PP715668 (#36)	*gltA*	372	94%	0.0	100% *Bartonella* sp. strain JU03_7 (MF467790)	*Desmodus rotundus*/ Mexico
PP715667 (#40)	*gltA*	404	96%	0.0	100% *Bartonella* sp. strain JU03_7 (MF467790)	*Desmodus rotundus*/ Mexico
PP715666 (#75)	*gltA*	359	97%	0.0	100% *Bartonella* sp. strain JU03_7 (MF467790)	*Desmodus rotundus*/ Mexico
PP715669 (#172)	*gltA*	372	93%	0.0	99% *Bartonella* sp. strain NE03_7 (MF467787)	*Desmodus rotundus*/ Mexico

PP715671 (#174)	*gltA*	379	99%	0.0	100% *Bartonella* sp. strain D216 LR3 (MG799416)	*Desmodus rotundus/* Peru
PP715670 (#183)	*gltA*	452	99%	0.0	98% *Bartonella* sp. strain JU03_7 (MF467790)	*Desmodus rotundus/* Mexico
PP764588 (#123)	*gltA*	735	100%	0.0	96% *Bartonella* sp. (MT874197)	*Carollia perspicillata/* Brazil
PP764589 (#144)	*gltA*	566	100%	0.0	91% *Bartonella* sp. (MT874189)	*Platyrrhinus lineatus/* Brazil
PP764590 (#145)	*gltA*	327	100%	2-141	95% *Bartonella* sp. (MH234369)	*Sturnira parvidens/* Costa Rica
PP764591 (#154)	*gltA*	694	99%	0.0	97% *Bartonella* sp. (MH234357)	*Glossophaga soricina/* Costa Rica
PP764592 (#160)	*gltA*	532	99%	0.0	92% *Bartonella* sp. (MH234319)	*Artibeus lituratus/* Costa Rica
PP764593 (#162)	*gltA*	676	94%	0.0	99% *Bartonella* sp. (MH204891)	*Desmodus rotundus/* Brazil
PP764594 (#172)	*gltA*	695	98%	0.0	97% *Bartonella* sp. (MH234357)	*Glossophaga soricina/* Costa Rica
PP764595 (#191)	*gltA*	534	100%	0.0	91% *Bartonella* sp. (MT874189)	*Platyrrhinus lineatus/* Brazil
PP715678 (#19)	*rpoB*	667	100%	0.0	98% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715680 (#39)	*rpoB*	360	100%	0.0	95% *Bartonella* sp. strain B43871 (MN529444)	*Diphylla ecaudata/* Guatemala
PP715675 (#76)	*rpoB*	628	100%	0.0	99% *Bartonella* sp. strain B43871 (MN529444)	*Desmodus rotundus/* Guatemala
PP715679 (#92)	*rpoB*	668	100%	0.0	95% *Bartonella* sp. strain B43871 (MN529444)	*Diphylla ecaudata/* Guatemala
PP715677 (#108)	*rpoB*	550	100%	0.0	99% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715676 (#112)	*rpoB*	769	100%	0.0	100% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715681 (#149)	*rpoB*	598	99%	0.0	91% *Bartonella* sp. strain B32855 (MN270139)	*Desmodus rotundus/* Peru
PP715674 (#180)	*rpoB*	773	100%	0.0	99% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715672 (#184)	*rpoB*	668	99%	0.0	87% *Bartonella sp.* isolate (GU143438)	*Rattus rattus brunneusculus/* Nepal
PP715673 (#191)	*rpoB*	674	100%	0.0	99% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715682 (#222)	*rpoB*	672	100%	0.0	99% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala

Table 5. Maximum identity by BLASTn analysis of *Bartonella* sequences detected in Streblidae bat flies found parasiziting *Desmodus rotundus* sampled in the Brazilian Amazon.

Genbank accession number (#sample)	Streblidae fly species	Bat host/ State	Target gene	Size of sequence obtained (bp)	Query coverage	E-Value	GenBank Closest Match	Host/Country

PP715685 (#162A)	*Trichobius parasiticus*	*Desmodus rotundus*/PA	*gltA*	275	97%	8e-180	100% *Bartonella* sp. strain *D141 (MG799424)*	*Desmodus rotundus/* Belize
PP715686 (#213B)	*Trichobius parasiticus*	*Desmodus rotundus*/PA	*gltA*	244	94%%	0.0	100% *Bartonella* sp. strain *JU03_7 (MF467790)*	*Desmodus rotundus/* Mexico
PP715684 (#70)	*Trichobius diaemi*	*Diaemus youngii* /PA	*gltA*	278	99%	4,00E-137	99% *Bartonella* sp. strain *OK75 (PP317863)*	*Tadarida brasiliensis*/USA
PP715683 (#166AS)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*gltA*	620	100%	0.0	95% *Bartonella* sp. strain *TS_202 (MT882404)*	*Lophostoma silvicolum/* Brazil
PP715687 (#231B)	*Trichobius parasiticus*	*Desmodus rotundus*/PA	*gltA*	649	99%	0.0	92% *Bartonella sp. 24_3 (MT874189)*	*Platyrrhinus lineatus/* Brazil
PP715694 (#69B)	*Trichobius parasiticus*	*Desmodus rotundus*/PA	*rpoB*	674	100%	0.0	99% *Bartonella* sp. strain B32576 (MN529455)	*Desmodus rotundus/* Guatemala
PP715688 (#144AS)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*rpoB*	563	100%	0.0	*92%* Bartonella sp. *strain B32855 (MN270139)*	*Desmodus rotundus/* Peru
PP715689 (#154S)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*rpoB*	528	99%	0.0	91% *Bartonella* sp. strain B32855 (MN270139)	*Desmodus rotundus/* Peru
PP715695 (#162A)	*Trichobius parasiticus*	*Desmodus rotundus*/PA	*rpoB*	785	100%	0.0	98.7% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715690 (#166AS)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*rpoB*	774	100%	0.0	99% *Bartonella* sp. strain B41407 (MN529421)	*Desmodus rotundus/* Guatemala
PP715691 (#167S)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*rpoB*	786	100%	0.0	96% *Bartonella* sp. strain B40889 (MN529436)	*Desmodus rotundus/* Guatemala
PP715692 (#218S)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*rpoB*	822	100%	0.0	99% *Bartonella* sp. strain B41407 (MN529421)	*Desmodus rotundus/* Guatemala
PP715693 (#221AS)	*Streblia wiedemanni*	*Desmodus rotundus*/PA	*rpoB*	819	100%	0.0	99% *Bartonella* sp. strain B41407 (MN529421)	*Desmodus rotundus/* Guatemala

3.4 Phylogenetic Inferences

In the phylogenetic analysis based on a 420 bp alignment of the *gltA* gene and inferred by the Maximum Likelihood method and the GRT+I+G evolutionary model, the *Bartonella* sequences detected in *D. rotundus* grouped into a single clade, together with a genotype detected in *T. parasiticus* from the present study. The sequence detected in a specimen of *T. parasiticus* collected from *D. rotundus* from Pará grouped with a genotype detected in *Strebla mirabilis* from Mexico. Another genotype detected in *D. rotundus* from Pará grouped with *Bartonella* sp. previously detected in *Tadarida brasiliensis* from the USA (**Figure 2**).

In the phylogenetic analysis based on an alignment of 890 bp of the *rpoB* gene and inferred by the Maximum Likelihood method and the TN+I+G evolutionary model, the sequences detected in *D. rotundus* (#222, #180, #191, #76, #112, #108, #19) were positioned in a clade with genotypes previously detected in bats of the species *D. rotundus*, from Brazil and Guatemala, together with genotypes previously detected in Streblidae bat flies found parasitizing *D. rotundus* in the present study. The sequences detected in *D. rotundus* (#92, #39 and #149) were positioned in a clade with genotypes previously detected in *Trichobius* sp. from Brazil and *Streblia wiedemanni* collected from *D. rotundus* in the present study. The sequence (#184) detected in *D. rotundus* detected in the present study was positioned close to two distinct clades, one being a genotype of *Bartonella* sp. detected in a bat *Platyrrhinus lineatus* from Brazil and another one represented by a genotype detected in *Eidolon helvum* from Tanzania (**Figure 3**).

3.5 Genotype diversity analysis

Among the 7 *Bartonella gltA* sequences detected in *D. rotundus* (π=0.127; Hd=0.965), 4 genotypes were found; additionally, 9 genotypes were found among the 13 *Bartonella rpoB* sequences detected in *D. rotundus* from the present study (π=0.100; Hd = 0.951) **(Table 6)**.

The 7 *Bartonella gltA* sequences detected in spleen samples from vampire bats in the present study were distributed into four genotypes (**2, 5, 8, 24**): **(I) genotype 2** (sample #75 (PA), #40 (RR) and #36 (RR) and vampire bats from Guatemala, Mexico and Peru); **(II) genotype 5** (samples #20 (AP) and #172 (PA), and vampire bats from

Guatemala); **(III) genotype 8** (sample #183 (PA)) and **(IV) genotype 24** (samples #174 (PA) and vampire bats from Mexico) **(Table 6; Figure 4)**.

The 13 *Bartonella rpoB* sequences detected in spleen samples from vampire bats in the present study were distributed across 9 genotypes **(1, 4, 5, 6, 7, 8, 22, 29 and 30)**: (**I**): **genotype 1** (sample #184 (PA)); (**II**): **genotype 4** (samples #191, #76, #180 and #222 (from PA)) and sequences from vampire bats sampled in previous studies in Tocantins and Pará (**III**): **genotype 5** (samples #112, #108 (both from PA) and sequences from vampire bats sampled in previous studies in Espírito Santo (**IV**): **genotype 6** (sample #19 (PA)); **(V) genotype 7:** (sample #92 (PA)); (**VI**): **genotype 8** (sample #39 (PA)); (**VII**): **genotype 22** (sample #149 (PA)); (**VIII**) **genotype 29** (sample #174 (PA)) and (**IX**) **genotype 30** (sample #179 (PA)) (**Figure 5**).

Among 5 *Bartonella gltA* sequences detected in Streblidae flies parasitizing *D. rotundus* (π=0.126; Hd=0.998) in the present study, 4 genotypes were found (**Table 7; Figure 6**). Two sequences obtained from *T. parasiticus* (samples #162A and #231B) were represented by two genotypes with unique sequences (**6** and **46**), while two sequences from Streblidae bat flies, one from *S. wiedemanni* (sample #166AS) and one from *T. parasiticus* (sample #162B), comprised a single genotype (**53**). The sequence obtained from *T. diaemi* (sample #70) grouped into a genotype (**45**) shared with a sequence previously detected in *Streblia diaemi* from Panama.

Among 8 *Bartonella rpoB* sequences detected in Streblidae flies parasitizing *D. rotundus* (π=0.115; Hd=0.994) in the present study, 5 genotypes were found (**Table 7; Figure 7**). Two sequences obtained from *T. parasiticus* were distributed across two distinct genotypes (**19** (sample #69B) and **24** (sample #162A)), while the other sequences obtained from *S. wiedemanni* were distributed across three genotypes (**21** (Samples #218S, #221AS), **22** (Sample #166AS, #144AS and #154S) and **25** (Sample #167S).

When grouping the *Bartonella* sequences obtained in the present study from *D. rotundus* and associated Streblidae bat flies, 8 *gltA* (π=0.112; Hd=0.892) and 12 (π=0.919; Hd=0.071) genotypes were found (**Table 8, Figures 8 and 9**). Among *gltA* sequences, four genotypes grouped only sequences detected in *D. rotundus* (**4** [samples #20 and #172]), **5** [samples #183], **7** [samples #123, #154 and #162] and **8** [sample #172]), while two genotypes grouped only sequences detected in Streblidae

bat flies (**2** [#sample 70], **6** [sample #162A]). On the other hand, some sequences detected in Streblidae flies and vampire bats shared the same genotypes: **1** (*D. rotundus*: samples #144 and #191; *T. parasiticus*: samples #231B); **3** (*D. rotundus*: sample #40, #36 and #75; *T. parasiticus*: samples #213B) (**Figure 8**).

Among the 12 *rpoB* genotypes obtained from Streblidae flies and vampire bats in the present study, three genotypes were represented by sequences detected only in Streblidae bat flies (**1** - sample #69B; **2** - sample #166AS; and **3** - samples # 218S and #221AS). On the other hand, eight genotypes were represented by sequences detected only in *D. rotundus* (**5** - sample #112; **6** - sample #108; **7** - sample #19; **8** - sample #92; **9** - sample #39; **10** - sample #149; **11** - sample #148; and **12** - sample #179. Only the genotype **4** was shared by sequences detected in Streblidae flies and vampire bats, being detected in samples #76, #180, #191 and #76 of *D. rotundus* and in *T. parasiticus* sample #162A (**Figure 9**).

Table 6. Polymorphisms detected in *gltA* and *rpoB* sequences of *Bartonella* spp. obtained in the present study from *Desmodus rotundus* spleen samples in the Brazilian Amazon.

Gene	bp	N	VS	GC%	g	gd (mean ± SD)	π (mean ± SD)	K
gltA	352	7	50	34.3	4	0.810±0.130	0.04703±0.02761	15.23810
rpoB	620	13	130	44.3	9	0.910±0.068	0.07573±0.001790	42.41026

N= Number of sequences analyzed; VS= number of variable sites; GC%= G+C content; g= number of genotypes; gd= genotype diversity; SD= standard deviation; π= nucleotide diversity (per sites); K= number of nucleotide differences.

Table 7. Polymorphisms detected in the *gltA* and *rpoB* sequences of *Bartonella* spp. obtained in the present study from samples of Streblidae bat flies in the Brazilian Amazon.

Gene	bp	N	VS	GC%	g	gd (mean ± SD)	π (mean ± SD)	K
gltA	306	5	59	34.6	4	1±0.177	0.11275±0.02895	34.5

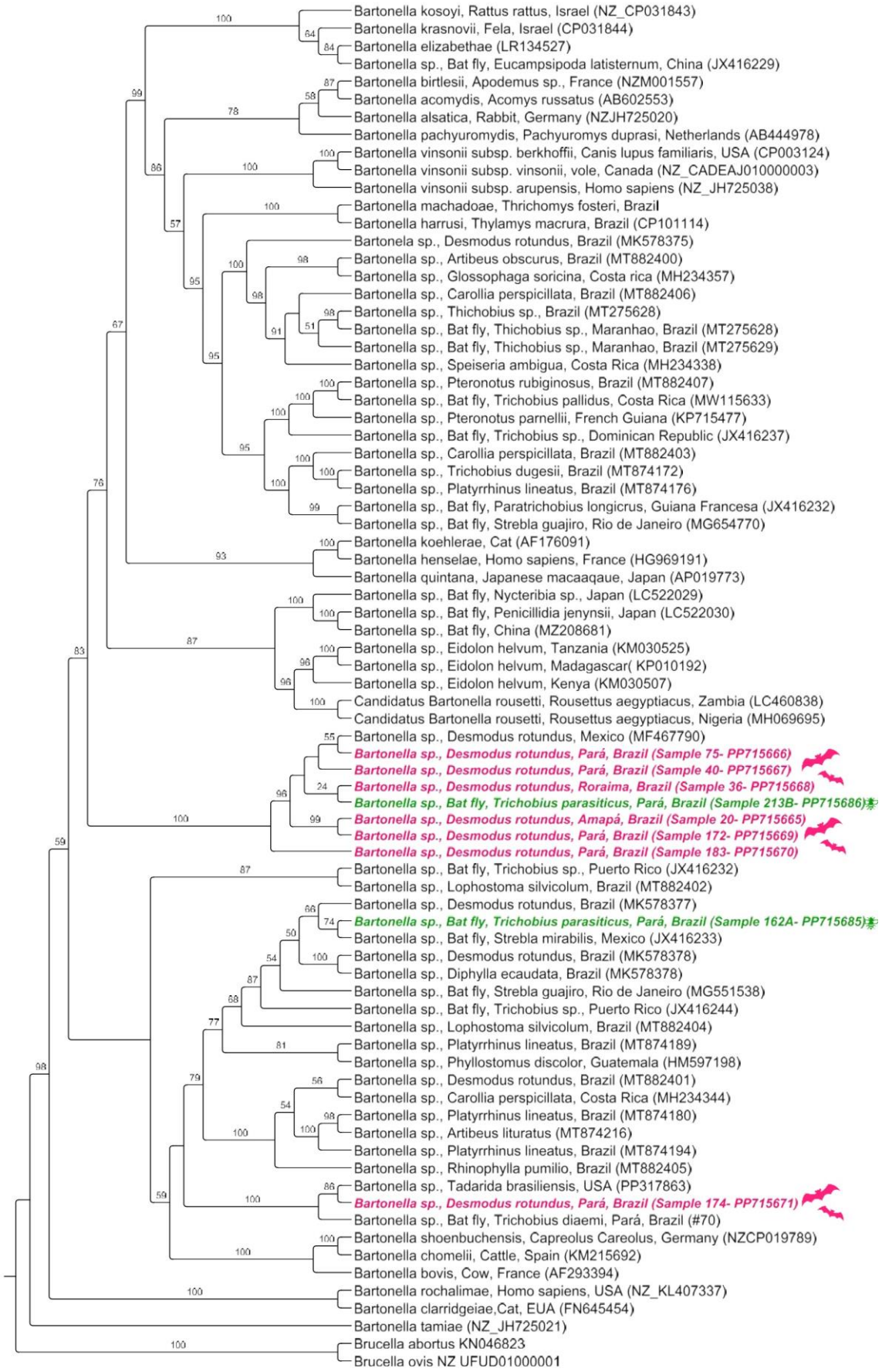
rpoB	587	8	52	44.6	5	0.9±0.161	0.03884±0.01657	22.8

N= Number of sequences analyzed; VS= number of variable sites; GC%= G+C content; g= number of genotypes; gd=genotype diversity; SD= standard deviation; π= nucleotide diversity (per sites); K= number of nucleotide differences.

Table 8. Polymorphisms detected in *gltA* and *rpoB* sequences of *Bartonella* spp. obtained in the present study from *Desmodus rotundus* (spleen samples) and associated Streblidae bat flies.

Gene	bp	N	VS	GC%	g	gd (mean ± SD)	π (mean ± SD)	K
gltA	306	16	75	35.4	8	0.892±0.048	0.11239±0.00998	39.81243
rpoB	587	17	148	44.0	12	0.919±0.057	0.07163±0.01357	42.04412

N= Number of sequences analyzed; VS= number of variable sites; GC%= G+C content; g= number of genotypes; gd= genotype diversity; SD= standard deviation; π= nucleotide diversity (per sites); K= number of nucleotide differences.


Bartonella kosoyi, Rattus rattus, Israel (NZ_CP031843)
Bartonella krasnovii, Fela, Israel (CP031844)
Bartonella elizabethae (LR134527)
Bartonella sp., Bat fly, Eucampsipoda latisternum, China (JX416229)
Bartonella birtlesii, Apodemus sp., France (NZM001557)
Bartonella acomydis, Acomys russatus (AB602553)
Bartonella alsatica, Rabbit, Germany (NZJH725020)
Bartonella pachyuromydis, Pachyuromys duprasi, Netherlands (AB444978)
Bartonella vinsonii subsp. berkhoffii, Canis lupus familiaris, USA (CP003124)
Bartonella vinsonii subsp. vinsonii, vole, Canada (NZ_CADEAJ010000003)
Bartonella vinsonii subsp. arupensis, Homo sapiens (NZ_JH725038)
Bartonella machadoae, Thrichomys fosteri, Brazil
Bartonella harrusi, Thylamys macrura, Brazil (CP101114)
Bartonela sp., Desmodus rotundus, Brazil (MK578375)
Bartonella sp., Artibeus obscurus, Brazil (MT882400)
Bartonella sp., Glossophaga soricina, Costa rica (MH234357)
Bartonella sp., Carollia perspicillata, Brazil (MT882406)
Bartonella sp., Thichobius sp., Brazil (MT275628)
Bartonella sp., Bat fly, Thichobius sp., Maranhao, Brazil (MT275628)
Bartonella sp., Bat fly, Thichobius sp., Maranhao, Brazil (MT275629)
Bartonella sp., Speiseria ambigua, Costa Rica (MH234338)
Bartonella sp., Pteronotus rubiginosus, Brazil (MT882407)
Bartonella sp., Bat fly, Trichobius pallidus, Costa Rica (MW115633)
Bartonella sp., Pteronotus parnellii, French Guiana (KP715477)
Bartonella sp., Bat fly, Trichobius sp., Dominican Republic (JX416237)
Bartonella sp., Carollia perspicillata, Brazil (MT882403)
Bartonella sp., Trichobius dugesii, Brazil (MT874172)
Bartonella sp., Platyrrhinus lineatus, Brazil (MT874176)
Bartonella sp., Bat fly, Paratrichobius longicrus, Guiana Francesa (JX416232)
Bartonella sp., Bat fly, Strebla guajiro, Rio de Janeiro (MG654770)
Bartonella koehlerae, Cat (AF176091)
Bartonella henselae, Homo sapiens, France (HG969191)
Bartonella quintana, Japanese macaaqaue, Japan (AP019773)
Bartonella sp., Bat fly, Nycteribia sp., Japan (LC522029)
Bartonella sp., Bat fly, Penicillidia jenynsii, Japan (LC522030)
Bartonella sp., Bat fly, China (MZ208681)
Bartonella sp., Eidolon helvum, Tanzania (KM030525)
Bartonella sp., Eidolon helvum, Madagascar(KP010192)
Bartonella sp., Eidolon helvum, Kenya (KM030507)
Candidatus Bartonella rousetti, Rousettus aegyptiacus, Zambia (LC460838)
Candidatus Bartonella rousetti, Rousettus aegyptiacus, Nigeria (MH069695)
Bartonella sp., Desmodus rotundus, Mexico (MF467790)
Bartonella sp., Desmodus rotundus, Pará, Brazil (Sample 75- PP715666)
Bartonella sp., Desmodus rotundus, Pará, Brazil (Sample 40- PP715667)
Bartonella sp., Desmodus rotundus, Roraima, Brazil (Sample 36- PP715668)
Bartonella sp., Bat fly, Trichobius parasiticus, Pará, Brazil (Sample 213B- PP715686)
Bartonella sp., Desmodus rotundus, Amapá, Brazil (Sample 20- PP715665)
Bartonella sp., Desmodus rotundus, Pará, Brazil (Sample 172- PP715669)
Bartonella sp., Desmodus rotundus, Pará, Brazil (Sample 183- PP715670)
Bartonella sp., Bat fly, Trichobius sp., Puerto Rico (JX416232)
Bartonella sp., Lophostoma silvicolum, Brazil (MT882402)
Bartonella sp., Desmodus rotundus, Brazil (MK578377)
Bartonella sp., Bat fly, Trichobius parasiticus, Pará, Brazil (Sample 162A- PP715685)
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Bartonella sp., Desmodus rotundus, Brazil (MK578378)
Bartonella sp., Diphylla ecaudata, Brazil (MK578379)
Bartonella sp., Bat fly, Strebla guajiro, Rio de Janeiro (MG551538)
Bartonella sp., Bat fly, Trichobius sp., Puerto Rico (JX416244)
Bartonella sp., Lophostoma silvicolum, Brazil (MT882404)
Bartonella sp., Platyrrhinus lineatus, Brazil (MT874189)
Bartonella sp., Phyllostomus discolor, Guatemala (HM597198)
Bartonella sp., Desmodus rotundus, Brazil (MT882401)
Bartonella sp., Carollia perspicillata, Costa Rica (MH234344)
Bartonella sp., Platyrrhinus lineatus, Brazil (MT874180)
Bartonella sp., Artibeus lituratus (MT874216)
Bartonella sp., Platyrrhinus lineatus, Brazil (MT874194)
Bartonella sp., Rhinophylla pumilio, Brazil (MT882405)
Bartonella sp., Tadarida brasiliensis, USA (PP317863)
Bartonella sp., Desmodus rotundus, Pará, Brazil (Sample 174- PP715671)
Bartonella sp., Bat fly, Trichobius diaemi, Pará, Brazil (#70)
Bartonella shoenbuchensis, Capreolus Careolus, Germany (NZCP019789)
Bartonella chomelii, Cattle, Spain (KM215692)
Bartonella bovis, Cow, France (AF293394)
Bartonella rochalimae, Homo sapiens, USA (NZ_KL407337)
Bartonella clarridgeiae,Cat, EUA (FN645454)
Bartonella tamiae (NZ_JH725021)
Brucella abortus KN046823
Brucella ovis NZ UFUD01000001

Figure 2. Phylogenetic analysis based on a 420 bp alignment of the *gltA* gene and inferred by the Maximum Likelihood method and the GRT+I+G evolutionary model. The sequences obtained in the present study from *D. rotundus* spleen samples and Streblidae bat flies were highlighted in pink and green, respectively. Node numbers correspond to bootstrap values with 1,000 replicates. *Brucella abortus* and *Brucella ovis* were used as outgroups.

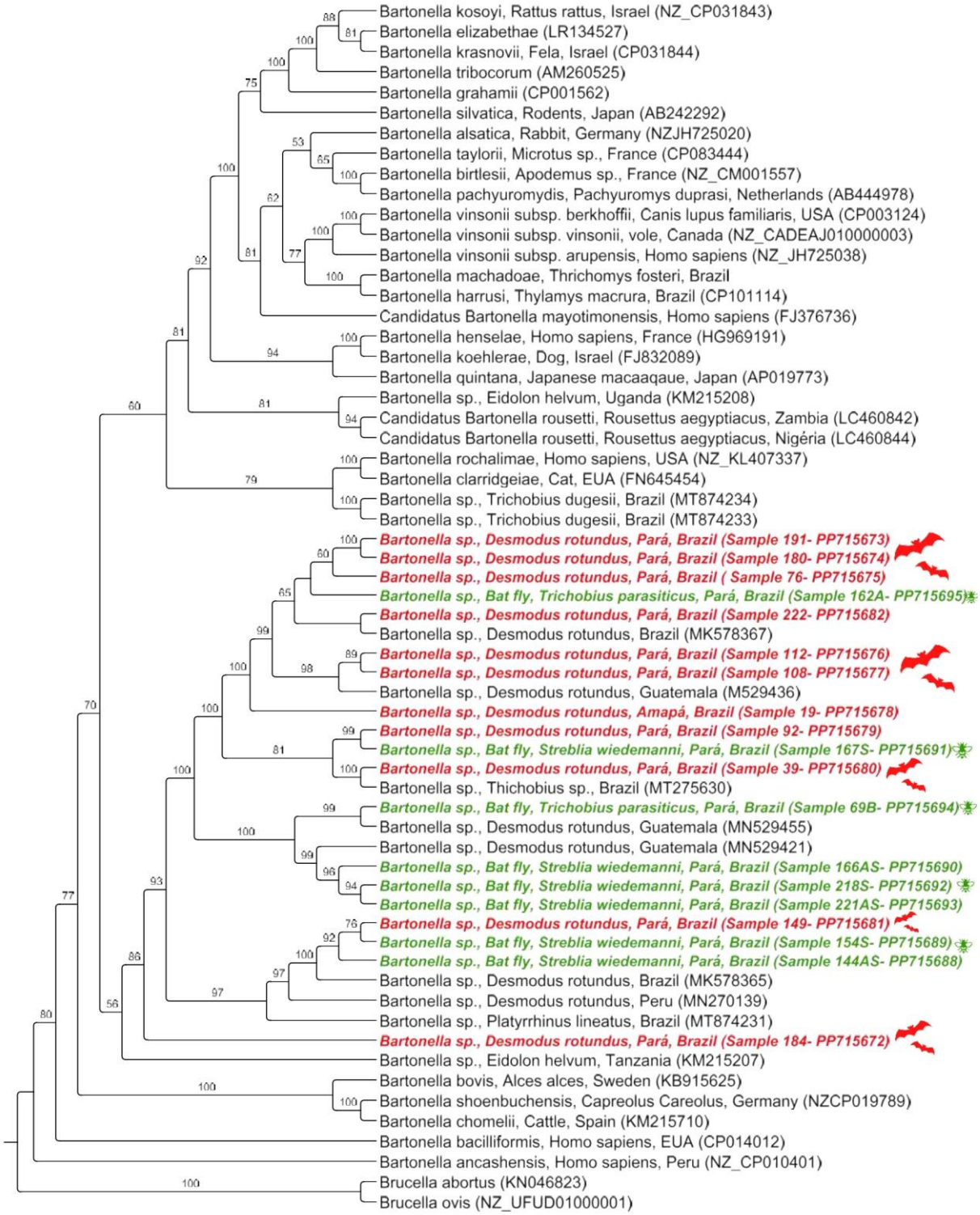

Figure 3. Phylogenetic analysis based on an 890 bp alignment of the *rpoB* gene and inferred by the Maximum Likelihood method and the TN+I+G evolutionary model. The sequences obtained in the present study from *D. rotundus* spleen samples and Streblidae bat flies were

highlighted in red and green, respectively. Node numbers correspond to bootstrap values with 1,000 replicates. *Brucella abortus* and *Brucella ovis* were used as outgroups.

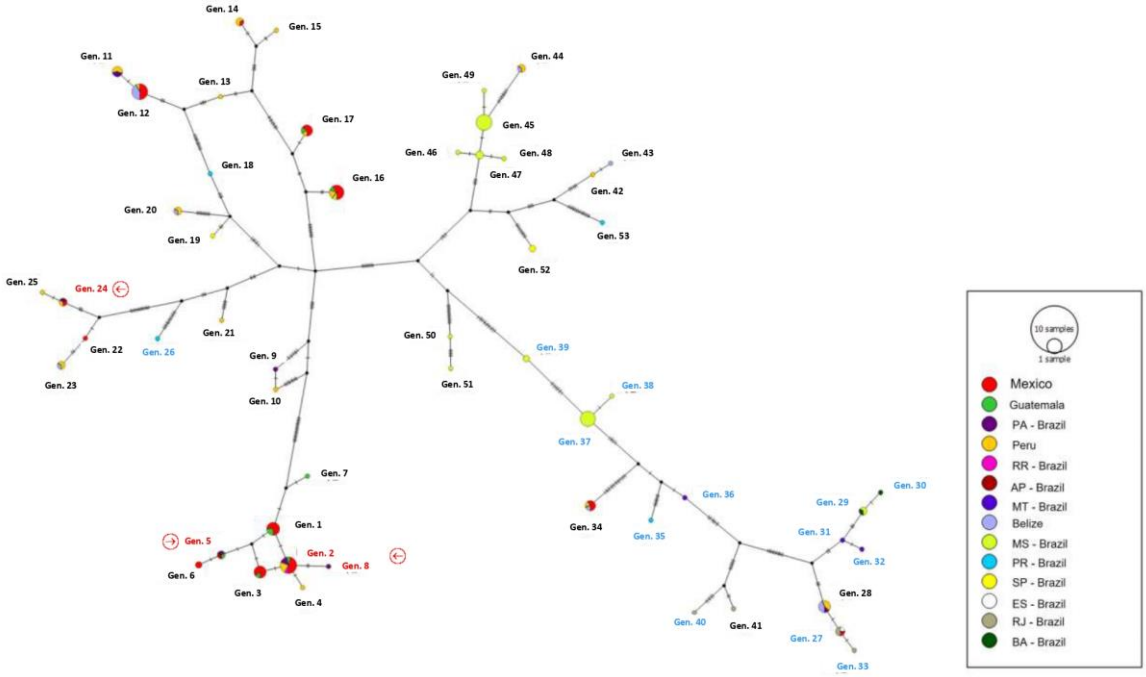

Figure 4. Genotypic network formed between *Bartonella gltA* sequences detected in spleen samples of *Desmodus rotundus* from the Brazilian Amazon, vampire bats from other locations in Brazil and Latin America and non-hematophagous bats from Brazil. The size of the circles varies according to the number of sequences belonging to each genotype. Each color represents the location where each sequence was detected. The black dots represent median vectors and the vertical black lines represent the mutational events that occurred between each genotype. The genotypes corresponding to sequences obtained in the present study were identified in red and sequences from non-hematophagous bats in blue. *PA: Pará; RR: Roraima; AP: Amapá; MT: Mato Grosso; MS: Mato Grosso do Sul; PR: Paraná; SP: São Paulo; ES: Espírito Santo; RJ: Rio de Janeiro; BA: Bahia.

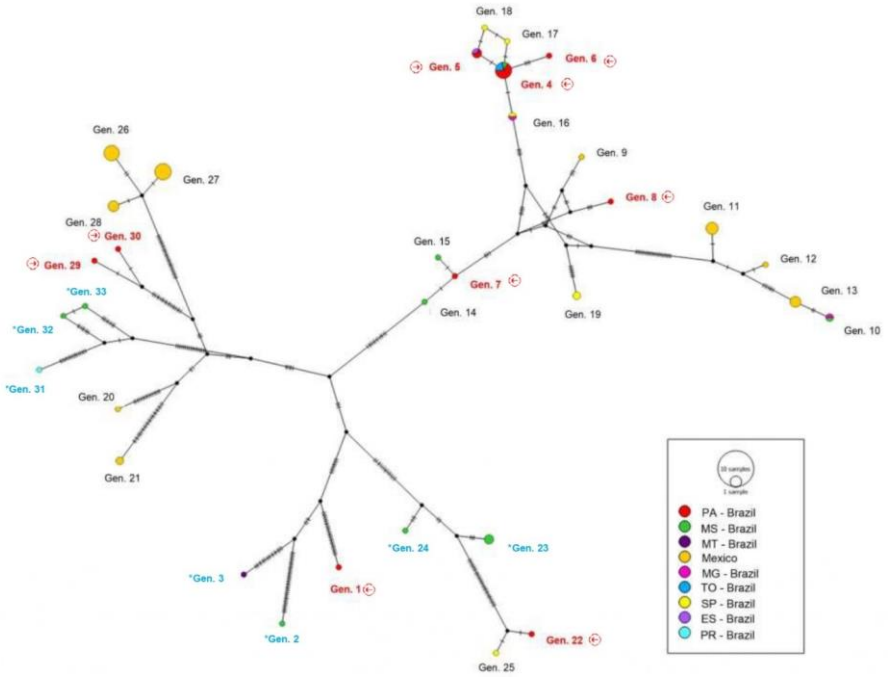

Figure 5. Genotype network formed among *Bartonella rpoB* sequences detected in *Desmodus rotundus* spleen samples from the Brazilian Amazon, vampire bats from other locations from Brazil and Latin America and non-hematophagous bats from Brazil. The size of the circles varies according to the number of sequences belonging to each genotype. Each color represents the location where each sequence was detected. The black dots represent median vectors and the vertical black lines represent mutational events that occurred between each genotype. The genotypes corresponding to sequences obtained in this work were identified in red and sequences from non-hematophagous bats in blue. *PA: Pará; RR: Roraima; AP: Amapá; MT: Mato Grosso; MS: Mato Grosso do Sul; PR: Paraná; SP: São Paulo; ES: Espírito Santo; RJ: Rio de Janeiro; BA: Bahia.

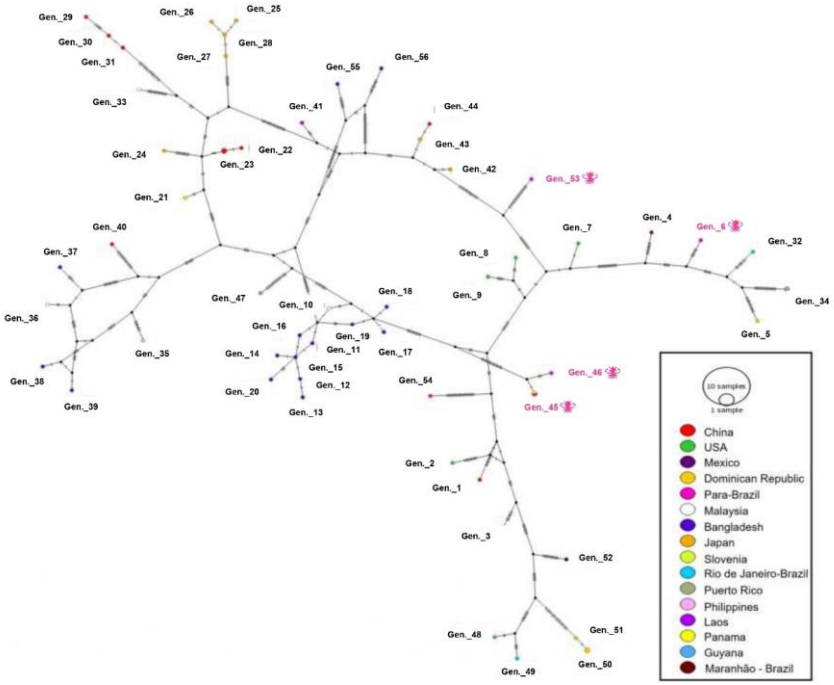

Figure 6. Genotype network formed among *Bartonella gltA* sequences detected in Streblidae bat flies collected from *Desmodus rotundus* from the Brazilian Amazon and in bat flies around the world. The size of the circles varies according to the number of sequences belonging to each genotype. Each color represents the location where each sequence was detected. The black dots represent median vectors and the vertical black lines represent mutational events that occurred between each genotype. The genotypes representing the sequences obtained in the present study were identified in pink.

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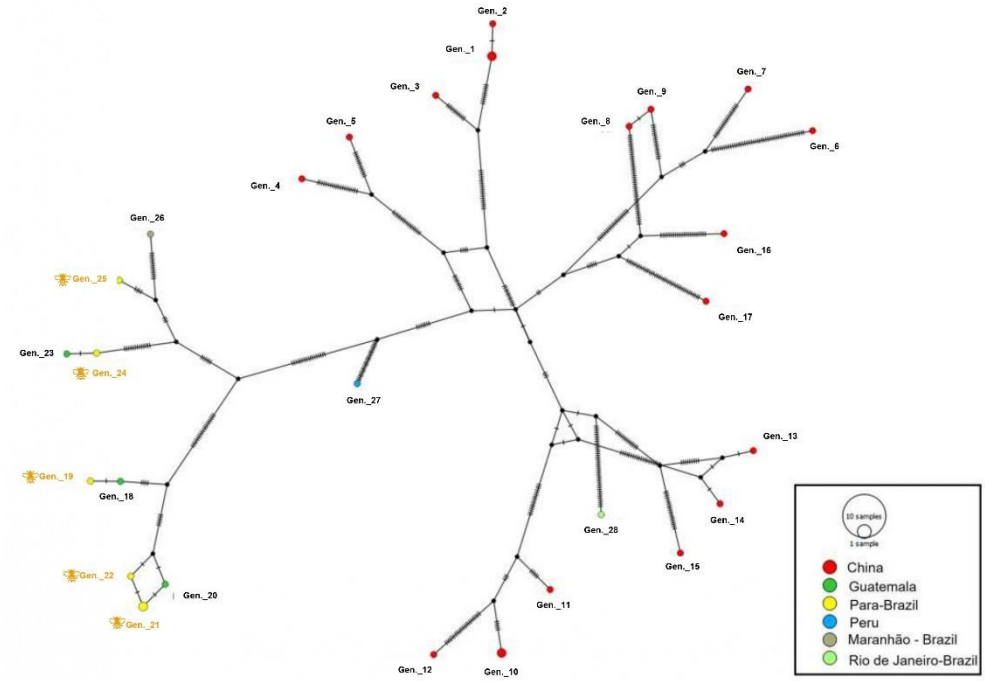

Figure 7. Genotype network formed among *Bartonella rpoB* sequences detected in Streblidae bat flies collected from *Desmodus rotundus* in the Brazilian Amazon and bat flies worldwide. The size of the circles varies according to the number of sequences belonging to each genotype. Each color represents the location where each sequence was detected. The black dots represent median vectors and the vertical black lines represent mutational events that occurred between each genotype. The genotypes representing the sequences obtained in the present study were identified in orange.

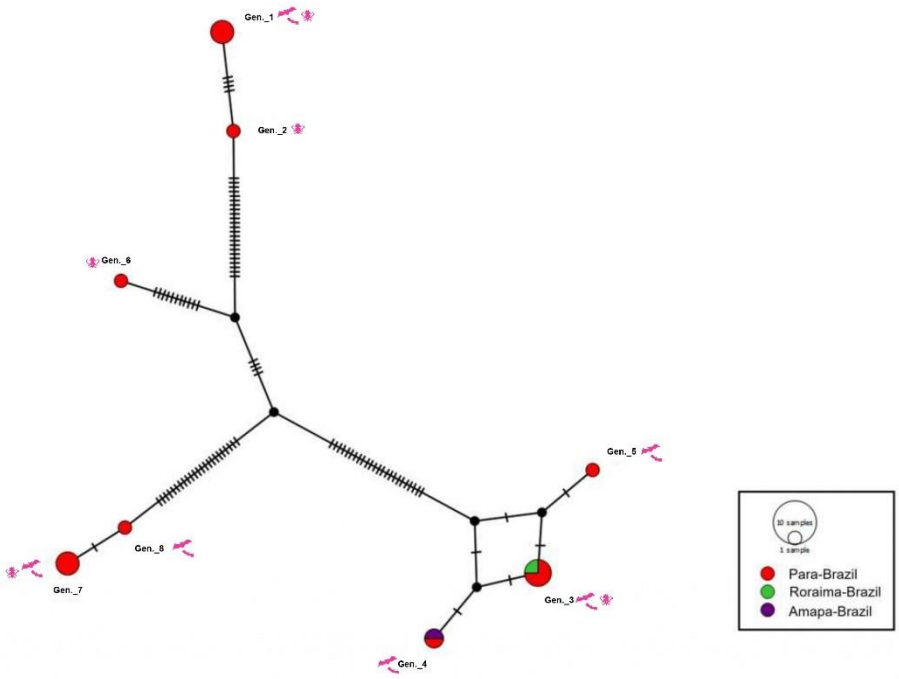

Figure 8. Genotype network formed among *gltA Bartonella* sequences detected in Streblidae bat flies and *Desmodus rotundus* spleen samples in the present study. The size of the circles varies according to the number of sequences belonging to each genotype. Each color represents the location where each sequence was detected. The black dots represent median vectors and the vertical black lines represent mutational events that occurred between each genotype. The genotypes representing the *Bartonella* sequences detected in Streblidae bat fly in the present work were identified with (), while those detected in *Desmodus rotundus* spleen samples were identified with ().

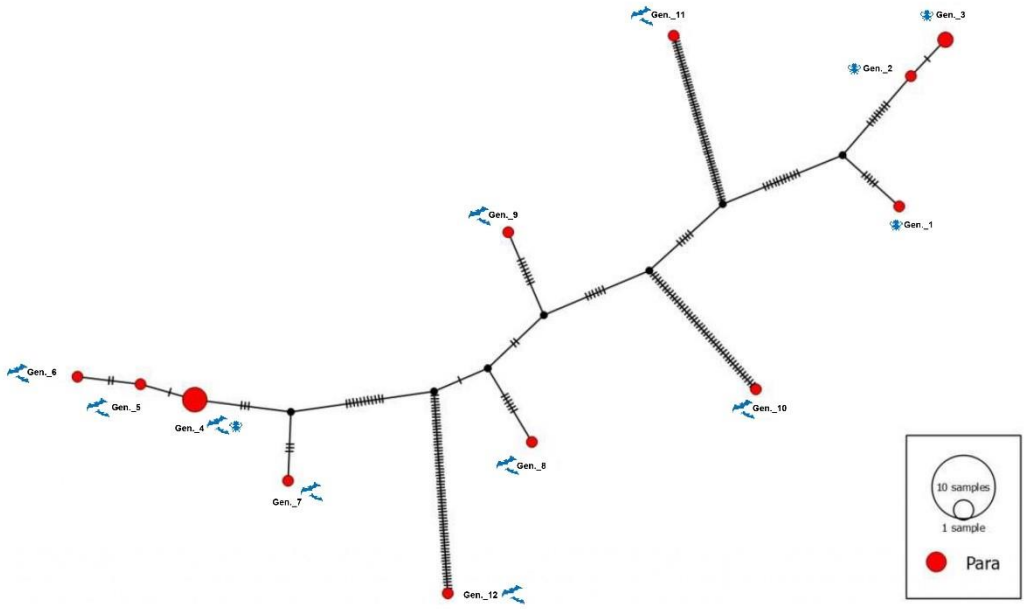

Figure 9. Genotype network formed among *Bartonella rpoB* sequences detected in Streblidae bat flies and in *Desmodus rotundus* spleen samples in the present study. The size of the circles varies according to the number of sequences belonging to each genotype. Each color represents the location where each sequence was detected. The black dots represent median vectors and the vertical black lines represent mutational events that occurred between each genotype. The genotypes representing the *Bartonella* sequences detected in Streblidae bat sequences obtained in this work were identified with (🕷), while those detected in *Desmodus rotundus* spleen samples were identified with (🦇).

4. Discussion

The biological characteristics of bats, including their behavioral habits, longevity, wide geographic distribution and species diversity make them ideal reservoirs for the spread of *Bartonella* spp. In this study, a prevalence of 31.9% was found for *Bartonella* spp. in vampire bats sampled in four states in the northern region of Brazil, which was higher than that found (24.5%) by André et al. (2019), who sampled vampire bats in 15 different Brazilian states. In previous studies conducted in Latin America, reported prevalences were 37.9%-38% in Guatemala (Bai et al., 2011; Wray et al., 2016), 38% in Mexico (Stuckey, et al., 2017) and 67% in Belize and Peru (Becker et al., 2018).

The variation in the molecular prevalence of *Bartonella* spp. in vampire bats in Latin America should be interpreted with caution, since factors such as the type of tissue sampled need to be considered. In this study, spleen samples were used, which is considered the tissue of choice for detecting *Bartonella* spp. when blood samples are not available, due to the presence of bartonellae in erythrocytes and splenic macrophages (Gutiérrez et al., 2017). The lowest molecular prevalence for *Bartonella* spp. found in the study conducted by André et al. (2019) is likely due to the use of liver samples. Previous studies conducted in America used blood (Bai et al., 2011), serum, urine, blood and oral and fecal swabs (Wray, et al. 2016), heart tissue and blood (Stuckey et al., 2017) and oral, rectal and blood swabs (Becker et al., 2018), which would explain the higher prevalence of *Bartonella* spp. in previous studies conducted in other Latin American countries.

Of a total of 230 spleen samples from vampire bats available, only one belonged to the species *Diaemus youngii*, which was not positive for *Bartonella* spp. Future studies are needed aiming at performing more representative sampling of this bat species to investigate the prevalence and diversity of *Bartonella* spp. in *D. youngii*. On the other hand, the high occurrence of *Bartonella* in *D. rotundus* might be related to the biological characteristics of this bat species, such as the formation of large colonies, wide geographic distribution and social habits (sharing blood meals, grooming and co-roosting with other bat species) (Bai et al., 2011).

The *gltA* and *rpoB* sequences from *Bartonella* spp. detected in the present study grouped with other genotypes previously detected in bats and bat-associated flies. The results obtained in the present study corroborate those found by Bai et al. (2011) and Stuckey et al. (2017), who also detected genotypes of *Bartonella* spp. in vampire bats that clustered with bartonellae previously identified in bats from the United Kingdom and Kenya (Bai et al., 2011), Guatemala, Costa Rica, Puerto Rico and Peru (Stuckey et al., 2017).

Despite the regular interaction between vampire bats and cattle and horses, with a potential risk of pathogen transmission (Wray et al., 2016), the genotypes detected in bats in the present study were not related to those found in ruminants. On the other hand, genotypes of *Bartonella* spp. detected in vampire bats (André et al., 2019) and in non-hematophagous bats (Ikeda et al., 2017) in Brazil and Mexico (Raya

et al., 2018) were positioned close to ruminant- associated bartonellae (*B. bovis*, *B. capreoli*, *B. chomelii* and *B. schoenbuchensis*), which may suggest that bat and ruminant-associated bartonellae might have a common ancestor (André et al., 2019). Interestingly, ruminants sampled in an area with a history of vampire bat bites were found to be infected with ruminant-associated *Bartonella* species but not with bat-associated genotypes (Raya et al., 2018). Since *Bartonella* DNA has been detected in oral swabs of bacteremic *D. rotundus* sampled in Belize and Peru (Becker et al., 2018), but not in those sampled in Guatemala (Wray et al., 2016) and Mexico (Raya et al., 2018), future studies are needed aiming at deeply investigate the possible transmission of *Bartonella* spp. for ruminants and horses through bat blood feeding habits.

Phylogenetic inferences based on the *gltA* and *rpoB* genes of *Bartonella* spp. in Streblid bat flies in the present study corroborate the findings found by do Amaral et al. (2018), Ikeda et al., (2020) and Braga et al. (2020), since the sequences detected in *T. parasiticus* and *S. wiedemanni* collected from *D. rotundus* were closely related to *Bartonella* genotypes previously detected in bats from Latin America and Central America.

The present study revealed a wide diversity of genotypes (4 *gltA* and 9 *rpoB* genotypes) of *Bartonella* spp. in *D. rotundus* bats, highlighting broad genetic diversity and geographic distribution of *Bartonella* in bats in Brazil. These results are in line with previous studies carried out in Latin America, which also investigated *Bartonella gltA* genetic variants in *D. rotundus*. André et al. (2019) found 11 *gltA* and 11 *rpoB* genotypes of *Bartonella* spp. in vampire bats sampled in different Brazilian geographic regions, reporting genotypic diversity values higher than 0.81, as in the present study. On the other hand, 7 *gltA* genotypes were reported in vampire bats in Guatemala (Bai et al., 2011), and 11 genotypes in Peru and Belize (Becker et al., 2018).

Based on genotype network analysis, genotypes detected in *D. rotundus* in the present study were shared exclusively with those previously detected in vampire bats, not overlapping with genotypes detected in non-hematophagous bats from Brazil. According to McKee et al. (2016), the sharing of *Bartonella* genotypes between bats belonging to different families, superfamilies and suborders is not frequent.

The diversity and distribution of bartonellae in vampire bats may be related to their dissemination by arthropod vectors. Bats harbor a diversity of ectoparasites, such as Streblidae and Nycteribiidae, which have been pointed out as possible vectors of different *Bartonella* genotypes among these mammals (Reeves et al., 2005; Morse et al., 2012; Reeves et al., 2016; do Amaral et al., 2018; Moskaluk et al., 2018; Braga et al., 2020; Ikeda et al., 2020; Lee et al., 2021; André et al., 2023). Herein, we observed a 50% prevalence for *Bartonella* spp. in Streblidae bat flies collected from vampire bats in the Brazilian Amazon, which was higher than the prevalence rates reported in previous studies conducted in the country, which were 17.18% in the state of Mato Grosso do Sul (Ikeda et al., 2020), 19.8% in the state of Rio de Janeiro (do Amaral et al., 2018) and 20% in the state of Maranhão (Braga et al., 2020).

In the present study, the presence of *Bartonella* DNA was relatively higher in *T. parasiticus* (30.5%) when compared to *S. wiedemanni* flies (24.8%), which corroborates the findings of Moskaluk et al. (2018), who reported a prevalence of 18.15% in *T. parasiticus* when compared to 6% detected in *S. wiedemanni*. Based on multilayer analysis networks, Alcântara et al. (2023) identified associations between bartonellae and Streblidae bat flies, as well as high specificity of Streblidae flie species with Phyllostomidae bats. These findings suggest that bat flies may play a significant role in the transmission of *Bartonella* spp. between bats. Interestingly, most of the sequences detected in Streblidae bat flies formed unique genotypes for each dipteran species analyzed, which could represent the presence *Bartonella* genotypes specific to Streblidae bat flies, acting as endosymbionts of this group of dipterans.

5. Conclusion

The present study demonstrated high occurrence (31.9%) and genetic diversity (4 *gltA* and 9 *rpoB* genotypes) of *Bartonella* spp. in *D. rotundus* in the Brazilian Amazon. Furthermore, a high molecular occurrence (52.2%) of *Bartonella* spp. and genetic diversity (4 *gltA* and 5 *rpoB* genotypes) were reported in Streblidae bat flies found parasitizing *D. rotundus*, suggesting a potential role of these dipterans in the transmission of *Bartonella* spp. among bats. Phylogenetic analyses based on the *gltA* and *rpoB* genes reinforce previous findings by demonstrating that the *Bartonella* genotypes circulating in vampire bats and associated Streblidae bat flies grouped with

those previously detected in bats, regardless of the blood feeding habit on ruminants and horses by these mammals. The *Bartonella* genotypes identified in *D. rotundus* in the present study were exclusively represented by *Bartonella* sequences detected in vampire bats, not overlapping with genotypes previously detected in non-hematophagous bats in Brazil. Most of the sequences detected in Streblidae bat flies formed unique genotypes for each dipteran species analyzed. On the other hand, two *gltA* and one *rpoB* genotypes were represented by sequences detected in vampire bats and Streblidae bat flies, suggesting the possible role of these dipterans in the transmission of *Bartonella* spp. among bats.

Ethics Statement

All procedures performed with bats followed protocols approved by the Ethics Committee on the Use of Animals of the Faculty of Agricultural and Veterinary Sciences at UNESP (CEUA FCAV/UNESP 3880/23). They were registered in the National Genetic Heritage and Associated Traditional Knowledge Management System (SisGen) with accession number ACD062C.

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Research data

Sequences generated during the study were submitted to NCBI Genbank (http://www.ncbi.nlm.nih.gov/genbank/). The sequences can be accessed by the following accession numbers: PP715665, PP715666, PP715667, PP715668, PP715669, PP715670, PP715671, PP715672, PP715673, PP715674, PP715675, PP715676, 15677, PP715678, PP715679, PP715680, PP715681, PP715682,

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Declaration of interest

The authors declare that there is no conflict of interest that might have influenced the results obtained and reported in this research.

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CAPÍTULO 4- Considerações finais

O presente trabalho investigou a ocorrência e diversidade genética de *Bartonella* spp. em morcegos e moscas Streblidae associadas no bioma Amazônico, em estados da região Norte do país.

O estudo realizado no estado do Acre, evidenciou molecularmente, pela primeira vez, a ocorrência de *Bartonella* spp. em morcegos deste estado da região Norte do Brasil. Este estudo traz a evidência molecular de *Bartonella* spp. em morcegos das espécies *Lophostoma silvicolum*, *Vampyressa thyone*, *Tonatia saurophila* e *Phyllostomus elongatus*. Os dois genótipos *gltA* de *Bartonella* spp. detectados nos morcegos sob estudo mostraram-se filogeneticamente relacionados àqueles encontrados em morcegos de outras regiões geográficas, expandindo o entendimento acerca da diversidade genética deste grupo de bactérias em quirópteros do bioma Amazônico.

Alta ocorrência (31,9%) e diversidade genética de *Bartonella* spp. foi encontrada no presente estudo em espécimes de *Desmodus rotundus* amostrados em 4 estados da região Norte do Brasil. Paralelamente, alta ocorrência molecular (64,5%) para *Bartonella* spp. foi também encontrada em moscas Streblidae coletadas de *D. rotundus*, sugerindo um potencial papel na transmissão destes dípteros na transmissão de *Bartonella* spp. entre os morcegos.

As análises filogenéticas baseadas nos genes *gltA* e *rpoB* reforçam achados anteriores ao demonstrarem que os genótipos de *Bartonella* spp. circulantes em morcegos hematófagos e moscas Streblidae associadas agrupam-se com aqueles previamente detectados em quirópteros, independentemente do hematofagismo realizado em ruminantes e equídeos por parte destes mamíferos. Os genótipos identificados em *D. rotundus* no presente estudo foram exclusivamente compartilhados com àqueles previamente detectados em morcegos hematófagos, não se sobrepondo com genótipos previamente detectados em morcegos não-hematófagos do Brasil. A maioria das sequências detectadas em moscas Streblidae formaram genótipos únicos para cada espécie de díptero analisada. Por outro lado, dois genótipos *gltA* e um *rpoB* foram compartilhados por sequências detectadas em morcegos hematófagos e moscas Streblidae, sugerindo o possível papel de tais dípteros na transmissão de *Bartonella* spp. entre morcegos.

Futuros estudos devem ser conduzidos objetivamente o isolamento de *Bartonella* spp. a partir de amostras de sangue de quirópteros, para que tais agentes bacterianos possam ser caracterizados molecularmente (por meio de sequenciamento do genoma completo e inferências filogenômicas), microscopicamente (por meio de microscopia de luz e de transmissão), bioquimicamente e metabolicamente. Tal metodologia expandirá o conhecimento acerca da diversidade de bartonelas associadas a quirópteros.