

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL**

**MOLECULAR DETECTION AND GENETIC DIVERSITY OF
ARTHROPOD AND TREMATODE-BORNE AGENTS IN NON-
HEMATOPHAGOUS BATS FROM MIDWESTERN BRAZIL**

**Priscila Ikeda
Veterinarian**

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
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ARTHROPOD AND TREMATODE-BORNE AGENTS IN NON-
HEMATOPHAGOUS BATS FROM MIDWESTERN BRAZIL**

Doctorate candidate: Priscila Ikeda

Supervisor: Prof. Dr. Marcos Rogério André

Co-supervisor: Profa. Dra. Rosangela Zacarias Machado

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AUTORA: PRISCILA IKEDA

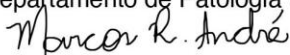
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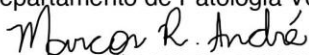
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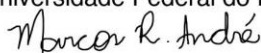
Prof. Dr. MARCOS ROGÉRIO ANDRÉ (Participação Virtual)
Departamento de Patologia Veterinária / FCAV / UNESP - Jaboticabal



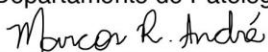
Profa. Dra. DARCI MORAES BARROS-BATTESTI (Participação Virtual)
Departamento de Patologia Veterinária / FCAV-UNESP / Câmpus de Jaboticabal



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Universidade Federal do Rio Grande do Sul-UFRGS / Porto Alegre/RS



Prof. Dr. ESTEVAM GUILHERME LUX HOPPE (Participação Virtual)
Departamento de Patologia, Reprodução e Saúde Única / FCAV / UNESP - Jaboticabal



Prof. Dr. GUSTAVO GRACIOLLI (Participação Virtual)
Universidade Federal de Mato Grosso do Sul - UFMS

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DADOS CURRICULARES DA AUTORA

Priscila Ikeda - nascida em 21 de fevereiro de 1992 na cidade de Guarapuava, Paraná, filha de Oscar Kunio Ikeda e Silvana Aparecida Ikeda, professor e servidora pública aposentados. Aprendeu a escrever e ler antes mesmo de iniciar na pré-escola, na qual ingressou em 1998 na Escola Municipal Cebula e cursou da 1ª a 4ª série primárias. Em 2002 ingressou no Colégio Estadual Ana Vanda Bassara, onde cursou de 5ª série ao 3º ano do ensino médio. Foi bolsista parcial de cursinho pré-vestibular e foi aprovada na Universidade Estadual do Centro-Oeste do Paraná (UNICENTRO) para o curso de Medicina Veterinária no qual se formou em fevereiro de 2014. Em março do mesmo ano, iniciou o curso de aprimoramento em Manejo e Medicina de Animais Selvagens pela mesma universidade, concluindo em fevereiro de 2015. Imediatamente, ingressou no mestrado acadêmico na Universidade Estadual Paulista, Campus de Jaboticabal, onde obteve título de Mestra em Medicina Veterinária com dissertação intitulada “Ocorrência e caracterização molecular de *Bartonella* spp. e *Mycoplasma* spp. em quirópteros amostrados no Brasil” defendida em fevereiro de 2017 sob orientação do Prof. Dr. Marcos Rogério André, sendo bolsista CAPES e FAPESP (#2015/04773-0) durante o período. Ao ingressar no doutorado em março de 2017, sob orientação do Prof. Dr. Marcos Rogério André e coorientação da Profa. Dra. Rosangela Zacarias Machado, obteve bolsa CAPES e então foi contemplada com bolsa FAPESP (#2017/14124-4) durante todo o curso de doutorado. Em novembro de 2019 iniciou período de pesquisa no exterior com bolsa FAPESP BEPE (#2019/13232-3) durante um ano com prorrogação de período até janeiro de 2021. Nesse período participou das atividades do laboratório na *Uniformed Services University of the Health Sciences* sob supervisão do Prof. Dr. John Stephen Dumler e desenvolveu o projeto intitulado “Microbiome of non-hematophagous bats’ ectoparasites, saliva and feces: a Metagenomic Approach”. Apesar de ter formação em inglês pelo Centro Cultural Brasil-Estados Unidos (CCBEU), desde a adolescência desfrutou de cursos ofertados pela rede pública sendo estes gratuitos ou valores irrisórios como: curso de francês básico, japonês básico, piano clássico, violão clássico, desenho e bordado. Essa autora é orgulhosamente um dos frutos que tiveram oportunidades na educação pública do Brasil.

CURRICULAR DATA OF THE AUTHOR

Priscila Ikeda - born on February 21st, 1992 at Guarapuava city, Paraná, daughter of Oscar Kunio Ikeda and Silvana Aparecida Ikeda. She learned to write and read even before entering in the preschool in 1998 at the Municipal School Cebula where she attended 1st to 4th grade. In 2002, she entered to Ana Vanda Bassara State School where she studied from the 5th grade to the 3rd year of high school. She received a pre-university half scholarship and was approved at the State University of the Midwest of Paraná (UNICENTRO) for the Veterinary Medicine bachelor course in which graduated in February 2014. In March of the same year, she started the improvement course in Medicine and Husbandry of Wild Animals by the same university concluding in February 2015. Right after, she started the master's degree at *Universidade Estadual Paulista, Campus of Jaboticabal* where she obtained the title of Master in Veterinary Medicine with a dissertation entitled "Occurrence and molecular characterization of *Bartonella* spp. and *Mycoplasma* spp. in chiropterans sampled in Brazil", which she presented in February 2017 under the guidance of prof. Dr. Marcos Rogério André being a CAPES and FAPESP fellow (#2015/04773-0) during the period. Upon entering the doctorate in March 2017, under the guidance of prof. Dr. Marcos Rogério André and prof. Dr. Rosangela Zacarias Machado, she obtained a CAPES scholarship for a month and was then awarded with a FAPESP scholarship (#2017/14124-4) throughout the doctoral course. In November 2019, she started a research period abroad with a FAPESP BEPE scholarship (#2019/13232-3) for one year with an extension until January 2021. During that period, she participated in the laboratory activities at Uniformed Services University of the Health Sciences under supervision of prof. Dr. John Stephen Dumler and developed the project entitled "Microbiome of non-hematophagous bats' ectoparasites, saliva and feces: a Metagenomic Approach". Despite being trained in English by the *Centro Cultural Brasil-EUA* (CCBEU), since her adolescence she enjoyed courses offered by the public educational system, these being free or for insignificant amounts such as basic French, basic Japanese, classical piano and guitar, drawing and embroidery. This author is proudly one of the many students that had opportunities inside the public education in Brazil.

*“There’s more to see than can ever be seen
More to do than can ever be done
There’s far too much to take in here
More to find than can ever be found”*

Composed by: Elton John / Tim Rice
The Lion King

I dedicate this work to all my professional colleagues and to my inner scientist. I wish we keep the science shining as a spark in our souls.

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DETECÇÃO MOLECULAR E DIVERSIDADE GENÉTICA DE AGENTES TRANSMITIDOS POR VETORES ARTRÓPODES E TREMATÓDEOS EM MORCEGOS NÃO HEMATÓFAGOS NO CENTRO-OESTE DO BRASIL

RESUMO- Estima-se que 75% das doenças emergentes compreendam zoonoses, cuja maioria tem como fontes de infecção animais selvagens; destas, cerca de 22,8% são veiculadas por vetores artrópodes. Desta forma, monitorar a presença de patógenos em animais selvagens em nichos compartilhados com seres humanos se torna um importante método preventivo de infecções zoonóticas. As famílias *Bartonellaceae* e *Anaplasmataceae* englobam Alphaproteobactérias Gram-negativas, intracelulares facultativas e obrigatórias, respectivamente, que vêm sendo identificadas em uma ampla variedade de mamíferos, incluindo seres humanos. Já os micoplasmas hemotróficos são bactérias Gram-negativas sem parede celular, de localização epieritrocitária, que podem causar desde infecções assintomáticas até anemia hemolítica severa, tanto em animais como em humanos. *Coxiella burnetii* é o principal representante da família *Coxiellaceae* (Gammaproteobactérias) e é conhecida por causar a Febre Q em humanos. Apesar de ser transmitido por aerossóis, esse agente vem sendo detectado em diversos ectoparasitos que talvez possam atuar em seu ciclo de vida. Já os piroplasmas são protozoários da ordem Piroplasmida e causam principalmente anemia hemolítica em animais de produção, domésticos e, eventualmente, em seres humanos. Embora esses agentes supracitados vêm sendo cada vez mais estudados em quirópteros, ainda pouco se sabe a respeito de sua ocorrência e diversidade genética em morcegos no Brasil. Os principais ectoparasitos encontrados nesses animais são representados por moscas das famílias *Strebliidae* e *Nycteribiidae*, carrapatos, e ácaros das famílias *Spinturnicidae* e *Macronyssidae*. Considerando os patógenos de interesse em Saúde Pública supracitados e a expressiva representatividade de animais da ordem Chiroptera no Brasil, objetivou-se obter informações sobre a ocorrência e diversidade genética de agentes *Anaplasmataceae*, *Bartonellaceae*, *Mycoplasmataceae*, *Coxiellaceae* e protozoários da ordem Piroplasmida em quirópteros e seus respectivos ectoparasitos coletados em área periurbana da cidade de Campo Grande, estado do Mato Grosso do Sul, no centro-oeste brasileiro. Um total de 418 amostras (135 amostras de baço, 133 de sangue e 150 ectoparasitos) foram coletados de 135 animais das famílias *Phyllostomidae*, *Vespertilionidae* e *Mollossidae*. Por meio de técnicas moleculares verificou-se positividade de 18,13% (34/418) para *Bartonella* spp. dentre todas as amostras coletadas. Dessas, 17 amostras foram clonadas para o gene *gltA* a fim de obter 3 clones de cada uma para acessar a diversidade genotípica dentro da mesma amostra biológica. Com os 51 *gltA*-clones obtidos foi possível observar 13 genótipos diferentes, sendo que pelo menos dois ocorreram dentro de uma mesma amostra. Análises filogenéticas baseadas nos genes *ftsZ*, *rpoB* e *nuoG* confirmaram a alta diversidade de genótipos de *Bartonella* spp. em quirópteros e seus ectoparasitos. Para agentes *Anaplasmataceae*, foi observada positividade de 1,67% (7/418), 11,96% (50/418) e 13,63% (57/418) das amostras testadas para *Anaplasma* spp. (16S rRNA), *Ehrlichia* spp. (*dsb*) e *Neorickettsia* spp. (16S rRNA), respectivamente. As sequências obtidas mostraram-se similares a *Anaplasma phagocytophilum*, *Ehrlichia minasensis*, *Neorickettsia risticii*, e *Neorickettsia findlayensis* por análise

pelo nBLAST, e filogeneticamente relacionado a *Ehrlichia ruminantium* baseado no gene *gltA*. Trata-se da primeira evidência molecular de agentes *Anaplasmataceae* em quirópteros e ectoparasitos associados no Brasil. Já para hemoplasmas, positividade de 13,6% (57/418) foi observada. Dentre as 24 sequências obtidas pelo gene 16S rRNA foi possível encontrar 12 genótipos diferentes que se distribuíram pelo macroclado do “grupo *haemofelis*”. As duas sequências 23S rRNA obtidas se posicionaram próximas a “*Candidatus* Mycoplasma haematohydrochaerus”, *M. haemofelis* e *M. haemocanis*. Todas as amostras de sangue e baço dos morcegos amostrados mostraram-se negativas na qPCR para *C. burnetii* baseada no gene *IS1111*. Para piroplasmídeos, 17 dos 135 (12,6%) morcegos amostrados mostraram-se positivos para agentes da ordem Piroplasmida (gene 18S rRNA). Trata-se do primeiro relato molecular de piroplasmídeos em quirópteros no Brasil. A análise filogenética mostrou a possível ocorrência de duas novas espécies: Piroplasmid n. sp. *Artibeus* spp., posicionado filogeneticamente próximo a *Babesia canis* e *B. vogeli*, e Piroplasmid n. sp. *P. discolor*, que formou um clado monofilético. Infelizmente, as amostras de ectoparasitos não puderam ser utilizadas na detecção de piroplasmídeos e *C. burnetii*. Enquanto moscas Streblidae mostraram positividade nos ensaios moleculares para *Bartonella* spp. (11/64), *Ehrlichia* spp. (7/64) e *Mycoplasma* spp. (1/64), carrapatos *Ornithodoros hasei* mostraram-se positivos para *Ehrlichia* spp. (3/19), *Anaplasma* spp. (1/19) e *Neorickettsia* spp. (1/19); por fim, ácaros Spinturniciidae e Macronyssidae apresentaram positividade para *Bartonella* spp. (4/21 Macronyssidae) e *Ehrlichia* spp. (18/21 Macronyssidae e 6/46 Spinturniciidae). Ainda, co-positividade para os diversos agentes pesquisados foi observada no presente estudo. Como conclusão, morcegos não-hematófagos de área periurbana do centro-oeste brasileiro atuam como hospedeiros para diversos genótipos de *Bartonella* spp. e hemoplasmas, para *Ehrlichia* spp. filogeneticamente associadas a *E. ruminantium* e para duas possíveis novas espécies de piroplasmídeos.

Palavras-chave: quirópteros, ectoparasitos, *Bartonella* spp., *Anaplasmataceae*, hemoplasmas, *Coxiella burnetii*, Piroplasmida.

MOLECULAR DETECTION AND GENETIC DIVERSITY OF ARTHROPOD AND TREMATODE-BORNE AGENTS IN NON-HEMATOPHAGOUS BATS FROM MIDWESTERN BRAZIL

ABSTRACT- It is estimated that 75% of emerging diseases comprise zoonoses, most of which have wild animals as sources of infection; of these, about 22.8% are carried by arthropod vectors. Thus, monitoring the presence of pathogens in wild animals in niches shared with humans becomes an important preventive method for zoonotic infections. *Bartonellaceae* and *Anaplasmataceae* families comprise Gram-negative, facultative and obligate intracellular Alphaproteobacteria, respectively, that have been identified in a wide variety of mammals, including humans. On the other hand, hemotrophic mycoplasmas are epierythrocytic Gram-negative bacteria lacking cell wall, which can cause from asymptomatic infections to severe hemolytic anemia, both in animals and in humans. *Coxiella burnetii* is the main representative of the *Coxiellaceae* family (Gammaproteobacteria) and is known to cause Q fever in humans. Despite being transmitted by aerosols, this agent has been detected in several ectoparasites that may act in its life cycle. Piroplasmids, on the other hand, are protozoa of the order Piroplasmida, which can cause hemolytic anemia in farm and domestic animals and, occasionally, in humans. Although the abovementioned agents have been increasingly studied in bats, little is known about their occurrence and genetic diversity in bats from Brazil. The main ectoparasites found in these animals are Streblidae and Nycteribiidae flies, soft ticks, and Spinturnicidae and Macronyssidae mites. Considering the abovementioned pathogens of interest in Public Health and the expressive representation of animals of the order Chiroptera in Brazil, this study aimed to investigate the occurrence and genetic diversity of *Anaplasmataceae*, *Bartonellaceae*, *Mycoplasmataceae*, *Coxiellaceae* agents and protozoa of the order Piroplasmida in chiropterans and their respective ectoparasites collected in a periurban area of the city of Campo Grande, state of Mato Grosso do Sul, in central-western Brazil. A total of 418 samples (135 spleen, 133 blood and 150 ectoparasites) were collected from 135 animals of the Phyllostomidae, Vespertilionidae and Molossidae families. Based on molecular assays, positivity of 18.13% (34/418) for *Bartonella* spp. was found among all collected samples. Out of these, 17 samples had their amplified *Bartonella gltA* amplicons cloned in order to obtain 3 clones of each one aiming at assessing the genotypic diversity within the same sample. Among the 51 *Bartonella gltA*-clones obtained, 13 different genotypes were found, with at least two occurring within individual samples. Phylogenetic analyses based on the *ftsZ*, *rpoB* and *nuoG* genes confirmed the high genotype diversity of *Bartonella* spp. in bats and their ectoparasites. For *Anaplasmataceae* agents, positivity of 1.67% (7/418), 11.96% (50/418) and 13.63% (57/418) was observed for *Anaplasma* spp. (16S rRNA), *Ehrlichia* spp. (*dsb*) and *Neorickettsia* spp. (16S rRNA), respectively. The sequences obtained were closely related to *Anaplasma phagocytophilum*, *Ehrlichia minasensis*, *Neorickettsia risticii*, and *Neorickettsia findlayensis* by BLASTn analyses, and phylogenetically related to *Ehrlichia ruminantium* based on *gltA* gene. This is the first molecular evidence of *Anaplasmataceae* agents in bats and associated ectoparasites from Brazil. Positivity of 13.6% (57/418) for hemoplasmas was found. Among the 24 sequences obtained by the 16S rRNA gene, it was possible to find 12 different

hemoplasma genotypes that were distributed into “*Haemofelis* group”. The two obtained 23S rRNA hemoplasma sequences were closely related to “*Candidatus* *Mycoplasma haematohydrochaerus*”, *M. haemofelis* and *M. haemocanis*. All bats’ blood and spleen samples were negative in the qPCR for *C. burnetii* based on the *IS1111* gene. Seventeen of the 135 (12.6%) bats were positive for Piroplasmida (18S rRNA gene). This is the first molecular report of piroplasmids in bats from Brazil. Phylogenetic analysis showed the possible occurrence of two new species: Piroplasmid n. sp. *Artibeus* spp., which was closely related to *Babesia canis* and *B. vogeli*, and Piroplasmid n. sp. *P. discolor*, which formed a monophyletic clade. Unfortunately, the ectoparasite samples could not be used in the PCR assays for piroplasmids and *C. burnetii*. While Streblidae flies showed positivity in molecular assays for *Bartonella* spp. (11/64), *Ehrlichia* spp. (7/64) and *Mycoplasma* spp. (1/64), *Ornithodoros hasei* ticks were positive for *Ehrlichia* spp. (3/19), *Anaplasma* spp. (1/19) and *Neorickettsia* spp. (1/19). Spinturnicidae and Macronyssidae mites were positive for *Bartonella* spp. (4/21 Macronyssidae) and *Ehrlichia* spp. (18/21 Macronyssidae and 6/46 Spinturnicidae). Also, co-positivity for the different agents studied was observed. In conclusion, non-hematophagous bats from a periurban area of central-western Brazil act as hosts for several genotypes of *Bartonella* spp. and hemoplasmas, *Ehrlichia* spp. closely related to *E. ruminantium*, and for two possible new species of piroplasmids.

Keywords: Chiroptera, ectoparasites, *Bartonella* spp., *Anaplasmataceae*, hemoplasmas, *Coxiella burnetii*, Piroplasmida.



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 agentes Anaplasmataceae, Bartonellaceae e Mycoplasmaceae em Quirópteros amostrados em região periurbana no Centro-Oeste Brasileiro"**, protocolo nº 010050/17, 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 06 de Julho de 2017.

Vigência do Projeto	01/09/2017 a 06/12/2020
Espécie / Linhagem	Ordem Chiroptera
Nº de animais	Aproximadamente 200
Peso / Idade	Variável
Sexo	Variável
Origem	Captura de vida livre

Jaboticabal, 06 de julho de 2017.


Profª Drª Lizandra Amoroso
Coordenadora – CEUA



Qualidade em Biossegurança para inclusão de áreas com nível de Biossegurança NBI para execução de atividades com organismos geneticamente modificados da classe de risco 1. As atividades a serem desenvolvidas pela instituição são: Pesquisa em regime de contenção e ensino com animais geneticamente modificados da classe de risco 1. Os organismos a serem manipulados são linhagens de camundongos (*Mus musculus*) nocautes para diversos genes. As instalações a serem credenciadas são denominadas de Biotério da UFSCPA, situadas à Rua Sarmiento Leite, nº 245, Prédio 3, Sala 415, Centro Histórico, Porto Alegre/RS. O responsável técnico pelas instalações será a Dra. Fernanda Basto de Mello e esta declara que o laboratório dispõe de infraestrutura adequada e pessoal técnico capaz de gerir o risco associado à atividade proposta. No âmbito das competências dispostas na Lei 11.105/05 e seu decreto 5.591/05, a Comissão concluiu que o presente pedido atende às normas da CTNBio e à legislação pertinente que visam garantir a biossegurança do meio ambiente, agricultura, saúde humana e animal.

A CTNBio esclarece que este extrato não exime a requerente do cumprimento das demais legislações vigentes no país, aplicáveis ao objeto do requerimento.

A íntegra deste Parecer Técnico consta do processo arquivado na CTNBio. Informações complementares ou solicitações de maiores informações sobre o processo acima listado deverão ser encaminhadas por escrito à Secretaria Executiva da CTNBio.

MARIA SUELI SOARES FELIPE

EXTRATO DE PARECER TÉCNICO Nº 6.012/2018

A Presidente da Comissão Técnica Nacional de Biossegurança - CTNBio, no uso de suas atribuições e de acordo com o artigo 14, inciso XIX, da Lei 11.105/05 e do Art. 5º, inciso XIX do Decreto 5.591/05, torna público que na 214ª Reunião Ordinária da CTNBio, realizada em 01 de agosto de 2018, a Comissão apreciou e emitiu parecer técnico para o seguinte processo:

Processo nº: 01250.024938/2018-73

Requerente: Universidade Federal do Rio Grande do Sul (UFRGS)

CQB: 060/98

Endereço: Av. Bento Gonçalves, nº 9.500, Prédio 43.431 - Campus do Vale/UFRGS C.P.15.005 - CEP 91.501-970, Porto Alegre-RS.

Assunto: Solicitação de extensão de CQB e atualização das informações do Laboratório de Radiobiologia Molecular do Centro de Biotecnologia no CQB da instituição.

Extrato Prévio: nº 6116/17, publicado no DOU em 19 de julho de 2018.

Decisão: Deferido

A CTNBio, após apreciação da Solicitação de parecer técnico para extensão de Certificado de Qualidade em Biossegurança para atividades de pesquisa em regime de contenção com OGM da classe de risco 1 em instalações com nível de biossegurança NB-1, concluiu pelo deferimento, nos termos deste Parecer Técnico. O Presidente da Comissão Interna de Biossegurança da Universidade Federal do Rio Grande do Sul - UFRGS, Dr. Giancarlo Pasquali, solicita à CTNBio parecer técnico para extensão do Certificado de Qualidade em Biossegurança (CQB) da instituição para atualização das informações do Laboratório de Radiobiologia Molecular do Centro de Biotecnologia (CBiot) da UFRGS. As instalações tiveram o nome modificado para Grupo de Estudos de Leveduras Cervejeiras (GELO) e estará sob a responsabilidade do Dr. Diego Bonatto e localizado no endereço: Centro de Biotecnologia - Lab. 107 - Av. Bento Gonçalves, 9.500, Prédio 43.421, Campus do Vale/UFRGS - Porto Alegre, RS, C.P. 15.005, CEP 91.501-970. As atividades a serem desenvolvidas nas instalações são: Pesquisa em regime de contenção e ensino com organismos geneticamente modificados da classe de risco 1. Os organismos a serem manipulados pela instituição nestas instalações são linhagens de comerciais da bactéria *Escherichia coli* derivadas de K12 e leveduras *Saccharomyces cerevisiae*, linhagens laboratoriais derivadas de BY e cepas industriais da classe de risco 1. O responsável pela unidade operativa declara que as instalações contam com salas e equipamentos úteis em nível de biossegurança adequado às atividades propostas. O processo descreve as condições de biossegurança das áreas a serem cadastradas, as medidas de biossegurança propostas para o laboratório e a qualificação da equipe de pesquisadores envolvida no projeto, bem como a declaração formal do responsável assegurando que as condições descritas no processo são apropriadas à realização dos projetos propostos. No âmbito das competências dispostas na Lei 11.105/05 e seu decreto 5.591/05, a Comissão concluiu que o presente pedido atende às normas da CTNBio e à legislação pertinente que visam garantir a biossegurança do meio ambiente, agricultura, saúde humana e animal.

A CTNBio esclarece que este extrato não exime a requerente do cumprimento das demais legislações vigentes no país, aplicáveis ao objeto do requerimento.

A íntegra deste Parecer Técnico consta do processo arquivado na CTNBio. Informações complementares ou solicitações de maiores informações sobre o processo acima listado deverão ser encaminhadas por escrito à Secretaria Executiva da CTNBio.

MARIA SUELI SOARES FELIPE

EXTRATO DE PARECER TÉCNICO Nº 6.013/2018

A Presidente da Comissão Técnica Nacional de Biossegurança - CTNBio, no uso de suas atribuições e de acordo com o artigo 14, inciso XIX, da Lei 11.105/05 e do Art. 5º, inciso XIX do Decreto 5.591/05, torna público que na 214ª Reunião Ordinária da CTNBio, realizada em 01 de agosto de 2018, a Comissão apreciou e emitiu parecer técnico para o seguinte processo:

Processo nº: 01250.026216/2018-53

Requerente: Instituto Oswaldo Cruz - Fiocruz

CQB: 105/99

Endereço: Av. Brasil, 4365. Pav. Gomes de Farias Sala 210, Mangueiras - Rio de Janeiro - RJ - CEP 21045-900.

Assunto: Solicitação de extensão de CQB para instalações para execução de atividades com OGMs da classe 1 de risco biológico.

Extrato Prévio: nº 6115/18, publicado no DOU em 21 de maio de 2018.

Decisão: Deferido

A CTNBio, após apreciação da Solicitação de parecer técnico para extensão de Certificado de Qualidade em Biossegurança para atividades de pesquisa em regime de contenção com OGM da classe de risco 1 em instalações com nível de biossegurança NB-1, concluiu pelo deferimento, nos termos deste Parecer Técnico. O Presidente da Comissão Interna de Biossegurança do Instituto Oswaldo Cruz, Dr. Ricardo Cunha Machado, solicita à CTNBio parecer técnico para extensão do Certificado de Qualidade em Biossegurança (CQB) da instituição para inclusão da área do Laboratório de Comunicação Celular (LCC) do Instituto Oswaldo Cruz para execução de atividades de pesquisa em regime de contenção com organismos geneticamente modificados da classe de risco biológico 1 nas instalações da instituição. As instalações a serem credenciadas são denominadas: Laboratório de Comunicação Celular (LCC), situado no Pavilhão 108, Sala 33 e 38B- Mangueiras - Av. Brasil, 4365, Rio de Janeiro. CEP: 21040360. A instituição solicita que as áreas sejam credenciadas para o nível de biossegurança 1 junto a CTNBio. Os organismos a serem manipulados pela instituição nestas instalações são linhagens de comerciais da bactéria *Escherichia coli* contendo genes de *Homo sapiens* da classe de risco 1. O projeto a ser desenvolvido denomina-se: "Receptor P2X7: análise estrutural e funcional a possíveis aplicações clínicas", sob a responsabilidade do Dr. Luiz Anastácio Alves. O responsável pela unidade operativa declara que as instalações contam com salas e equipamentos úteis em nível de biossegurança adequado às atividades propostas. O processo descreve as condições de biossegurança das áreas a serem cadastradas, as medidas de biossegurança propostas para o laboratório e a qualificação da equipe de pesquisadores envolvida no projeto, bem como a declaração formal do responsável assegurando que as condições descritas no processo são apropriadas à realização dos projetos propostos. No âmbito das competências dispostas na Lei 11.105/05 e seu decreto 5.591/05, a Comissão concluiu que o presente pedido atende às normas da CTNBio e à legislação pertinente que visam garantir a biossegurança do meio ambiente, agricultura, saúde humana e animal.

A CTNBio esclarece que este extrato não exime a requerente do cumprimento das demais legislações vigentes no país, aplicáveis ao objeto do requerimento.

A íntegra deste Parecer Técnico consta do processo arquivado na CTNBio. Informações complementares ou solicitações de maiores informações sobre o processo acima listado deverão ser encaminhadas por escrito à Secretaria Executiva da CTNBio.

MARIA SUELI SOARES FELIPE

EXTRATO DE PARECER TÉCNICO Nº 6.014/2018

A Presidente da Comissão Técnica Nacional de Biossegurança - CTNBio, no uso de suas atribuições e de acordo com o artigo 14, inciso XIX, da Lei 11.105/05 e do Art. 5º, inciso XIX do Decreto 5.591/05, torna público que na 214ª Reunião Ordinária da CTNBio, realizada em 01 de agosto de 2018, a Comissão apreciou e emitiu parecer técnico para o seguinte processo:

Processo nº: 01250.026233/2018-91

Requerente: Faculdade de Ciências Agrárias e Veterinárias - Universidade Estadual Paulista, UNESP, Câmpus de Jaboticabal.

CQB: 088/98

Endereço: Via de Acesso Prof. Paulo Donato Castellane, s/n. CEP 14884-900 - Jaboticabal - SP.

Assunto: Solicitação de extensão de CQB para instalações para execução de atividades com OGMs da classe 1 de risco biológico.

Extrato Prévio: nº 6115/18, publicado no DOU em 21 de maio de 2018.

Decisão: Deferido

A CTNBio, após apreciação da Solicitação de parecer técnico para extensão de Certificado de Qualidade em Biossegurança para atividades de pesquisa em regime de contenção com OGM da classe de risco 1 em instalações com nível de biossegurança NB-1, concluiu pelo deferimento, nos termos deste Parecer Técnico. O Presidente da Comissão Interna de Biossegurança da Faculdade de Ciências Agrárias e Veterinárias - Universidade Estadual Paulista, UNESP, Câmpus de Jaboticabal, Dr. Odair Aparecido Fernandes, solicita à CTNBio parecer técnico para extensão do Certificado de Qualidade em Biossegurança (CQB) da instituição para inclusão do Laboratório de Imunoparasitologia do Departamento de Patologia Veterinária para execução de atividades de pesquisa em regime de contenção, descarte, ensino e armazenamento com organismos geneticamente modificados da classe de risco biológico 1 nas instalações da instituição. As instalações a serem credenciadas são denominadas: Laboratório de Imunoparasitologia do Departamento de Patologia Veterinária, situado na Via de Acesso Prof. Paulo Donato Castellane, s/n. CEP 14884-900, Jaboticabal - SP. A instituição solicita que as áreas sejam credenciadas para o nível de biossegurança 1 junto a CTNBio. Os organismos a serem manipulados pela instituição nestas instalações são linhagens de comerciais da bactéria *Escherichia coli* contendo fragmentos dos genes codificadores de antígeno de superfície de merozoito msa-1, msa-2b

e msa-2c do protozoário *Babesia bovis* da classe de risco 1. O projeto a ser desenvolvido denomina-se: "Diversidade genética de *Babesia bovis* em bovinos de corte amostrados no pantanal sul matogrossense", sob a responsabilidade do Dr. Marcos Rogério André. O responsável pela unidade operativa declara que as instalações contam com salas e equipamentos úteis em nível de biossegurança adequado às atividades propostas. O processo descreve as condições de biossegurança das áreas a serem cadastradas, as medidas de biossegurança propostas para o laboratório e a qualificação da equipe de pesquisadores envolvida no projeto, bem como a declaração formal do responsável assegurando que as condições descritas no processo são apropriadas à realização dos projetos propostos. No âmbito das competências dispostas na Lei 11.105/05 e seu decreto 5.591/05, a Comissão concluiu que o presente pedido atende às normas da CTNBio e à legislação pertinente que visam garantir a biossegurança do meio ambiente, agricultura, saúde humana e animal.

A CTNBio esclarece que este extrato não exime a requerente do cumprimento das demais legislações vigentes no país, aplicáveis ao objeto do requerimento.

A íntegra deste Parecer Técnico consta do processo arquivado na CTNBio. Informações complementares ou solicitações de maiores informações sobre o processo acima listado deverão ser encaminhadas por escrito à Secretaria Executiva da CTNBio.

MARIA SUELI SOARES FELIPE

EXTRATO DE PARECER TÉCNICO Nº 6.017/2018

A Presidente da Comissão Técnica Nacional de Biossegurança - CTNBio, no uso de suas atribuições e de acordo com o artigo 14, inciso XIX, da Lei 11.105/05 e do Art. 5º, inciso XIX do Decreto 5.591/05, torna público que na 214ª Reunião Ordinária da CTNBio, realizada em 01 de agosto de 2018, a Comissão apreciou e emitiu parecer técnico para o seguinte processo:

Processo nº 01250.010280/2018-12

Requerente: Instituto Oswaldo Cruz.

CQB: 105/99

Endereço: Instituto Oswaldo Cruz. Av. Brasil, 4365 - Pav. Gomes de Farias - Sala 114. Mangueiras. Rio de Janeiro, RJ - CEP 21045-900. Tel. 21-2598-4440 - Fax: 21-2560-7864.

Assunto: Solicitação de parecer para execução de projeto com organismo geneticamente modificado da classe de risco 2.

Extrato Prévio: nº 5992/17, publicado no DOU em 21 de maio de 2018.

Decisão: Deferido

A CTNBio, após apreciação da Solicitação de parecer técnico para execução de projeto com organismo geneticamente modificado da classe de risco 2 em instalações com nível de biossegurança NB-2, concluiu pelo deferimento, nos termos deste Parecer Técnico. O Presidente da Comissão Interna de Biossegurança da Fundação Oswaldo Cruz, Dr. Ricardo Cunha Machado, solicita à CTNBio parecer técnico para execução de projeto com organismo geneticamente modificado da classe de risco 2. As instalações nas quais será conduzido o projeto são do Laboratório de Imunologia Clínica do Instituto Oswaldo Cruz, situado a Pavilhão Leônidas Deane, 4º andar, sala 409-C, Av. Brasil, 4365- Mangueiras- Rio de Janeiro - RJ, CEP. 21045-900. O projeto a ser executado é denominado "Avaliação in vitro do Delta BCG Moreau e Pasteur no Brasil", será coordenado pelo Dr. Paulo Renato Zuquim Antas, em nível de contenção NB-2. Os organismos a serem manipulados são *Cepas recombinantes de Mycobacterium bovis* Bacilo Calmette-Guerin (BCG) que já foram desprovidas dos genes BCG1419c (denominado BCGABCG1419c), e BCG1416c (denominado BCGABCG1416c). O responsável pela unidade operativa declara que o laboratório conta com equipamentos úteis para as atividades experimentais em nível de biossegurança adequado. O processo descreve as condições de biossegurança das áreas a serem cadastradas, as medidas de biossegurança propostas para o laboratório e a qualificação da equipe de pesquisadores envolvida no projeto, bem como a declaração formal do responsável assegurando que as condições descritas no processo são apropriadas à realização dos projetos propostos. No âmbito das competências dispostas na Lei 11.105/05 e seu decreto 5.591/05, a Comissão concluiu que o presente pedido atende às normas da CTNBio e à legislação pertinente que visam garantir a biossegurança do meio ambiente, agricultura, saúde humana e animal.

A CTNBio esclarece que este extrato não exime a requerente do cumprimento das demais legislações vigentes no país, aplicáveis ao objeto do requerimento.

A íntegra deste Parecer Técnico consta do processo arquivado na CTNBio. Informações complementares ou solicitações de maiores informações sobre o processo acima listado deverão ser encaminhadas por escrito à Secretaria Executiva da CTNBio.

MARIA SUELI SOARES FELIPE

EXTRATO DE PARECER TÉCNICO Nº 6.018/2018

A Presidente da Comissão Técnica Nacional de Biossegurança - CTNBio, no uso de suas atribuições e de acordo com o artigo 14, inciso XIX, da Lei 11.105/05 e do Art. 5º, inciso XIX do Decreto 5.591/05, torna público que na 210ª Reunião Ordinária da CTNBio, realizada em 08 de março de 2018, a Comissão apreciou e emitiu parecer técnico para o seguinte processo:



Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 56804-1	Data da Emissão: 27/06/2017 09:25	Data para Revalidação*: 27/07/2018
* De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Jaire Marinho Torres	CPF: 028.989.481-60
Título do Projeto: AVALIAÇÃO DO PAPEL DE MORCEGOS NA MANUTENÇÃO DE TRIPANOSSOMATÍDEOS EM REMANESCENTES FLORESTAIS URBANOS	
Nome da Instituição : MISSÃO SALESIANA DE MATO GROSSO	CNPJ: 03.226.149/0015-87

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Determinação dos fragmentos para amostragem	12/2016	01/2017
2	Coleta de morcegos e de material Biológico	04/2017	06/2018
3	Análise de Material Biológico	05/2017	06/2019
4	Análise de Dados	07/2018	10/2019
5	Elaboração de relatórios e Tese de Doutorado	08/2018	07/2020

Observações e ressalvas

1	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
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Equipe

#	Nome	Função	CPF	Doc. Identidade	Nacionalidade
1	Wanessa Teixeira Gomes Barreto	Pesquisador	013.418.161-17	001451636 ssp-MS	Brasileira
2	Gabriel Carvalho de Macedo	Médico Veterinário	008.981.562-97	001506668 SSP/MS-MS	Brasileira
3	Filipe Martins Santos	Pesquisador	032.292.371-96	001596796 SSP-MS	Brasileira
4	Grasiela Edith Porfírio	Pesquisador	003.126.181-74	001024081 ssp-MS	Brasileira
5	Heitor Miraglia Herrera	Pesquisador	444.869.871-87	049724503 IFP-RJ	Brasileira

Locais onde as atividades de campo serão executadas

#	Município	UF	Descrição do local	Tipo
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Nome: Jaire Marinho Torres	CPF: 028.989.481-60
Título do Projeto: AVALIAÇÃO DO PAPEL DE MORCEGOS NA MANUTENÇÃO DE TRIPANOSSOMATÍDEOS EM REMANESCENTES FLORESTAIS URBANOS	
Nome da Instituição : MISSÃO SALESIANA DE MATO GROSSO	CNPJ: 03.226.149/0015-87

1	CAMPO GRANDE	MS	Campo Grande	Fora de UC Federal
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Atividades X Táxons

#	Atividade	Táxons
1	Captura de animais silvestres in situ	Chiroptera
2	Coleta/transporte de amostras biológicas in situ	Chiroptera
3	Coleta/transporte de espécimes da fauna silvestre in situ	Chiroptera (*Qtde: 192)
4	Manutenção temporária (até 24 meses) de vertebrados silvestres em cativeiro	Chiroptera
5	Marcação de animais silvestres in situ	Chiroptera

* Quantidade de indivíduos por espécie, por localidade ou unidade de conservação, a serem coletados durante um ano.

Material e métodos

1	Amostras biológicas (Outros mamíferos)	Sangue, Ectoparasita, Regurgitação/conteúdo estomacal, Fragmento de tecido/órgão, Fezes
2	Método de captura/coleta (Outros mamíferos)	Rede de neblina
3	Método de marcação (Outros mamíferos)	Outros métodos de marcação (Anilha)

Destino do material biológico coletado

#	Nome local destino	Tipo Destino
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Táxon*	Qtde.	Tipo de amostra	Qtde.	Data

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* Identificar o espécime no nível taxonômico possível.

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CHAPTER 1 - General Considerations

1. Introduction

A representative number of emerging and re-emerging diseases comprises zoonoses, most of which have wild animals as sources of infection and/or are carried by arthropod vectors. Thus, monitoring the presence of pathogens in wild animals in niches shared with humans becomes an important preventive method for zoonotic infections.

The Order Chiroptera is the second largest and most unique in the Class Mammalia. The animals belonging to this order represent a great diversity of species that reflects in their complex biology and different behavior from one species to another. These mammals are highly adapted for flight, which facilitates their ecological task, which are, unfortunately, underappreciated, such as seed dispersal, pollination, and insect control. On the other hand, such complex biology behavior also makes these mammals unique pathogens' carriers and dispersers. In fact, bats are pointed out as the main reservoirs of zoonotic diseases, especially the viral ones, as we are seeing currently with Sars-Cov-2, which triggered the pandemic that started in 2019. Even though viral diseases have great relevance, little is known about the occurrence of bacterial and protozoan pathogens with zoonotic potential associated with these animals.

Bats have a great representativeness in Brazil, including the only three hematophagous species that occur only in South America. Generally, bat infestation by ectoparasites shows a close relationship, since the vast majority of these arthropods are bat-exclusive.

Among vector-borne bacteria with zoonotic potential, agents belonging to *Bartonellaceae*, *Mycoplasmataceae*, *Anaplasmataceae*, and *Coxiellaceae* stand out.

The family *Bartonellaceae* (order *Rhizobiales*) encompasses re-emerging facultative intracellular bacteria with zoonotic potential. Despite covering a wide range of hosts, this diverse group of bacteria is highly adapted to mammal reservoirs. In bats, bartonellae shows high occurrence and diversity, being detected in several countries in Europe, Africa, Asia and the Americas, including Brazil.

Hemotrophic mycoplasmas (hemoplasmas) (order *Mycoplasmatales*) are epierthrocytic bacteria that lack cell wall. This group of bacteria can cause asymptomatic to severe hemolytic anemia disease, whose outcome is mainly related to the hemoplasma species involved, patient condition and the possibility of co-infection with another pathogen. In addition to the transmission by arthropod vectors, grooming and aggressive interactions among animals have also been pointed out as important routes of infection, which gain great importance when animals that live in groups are involved, such as some species of bats. In bats, these agents have been reported in the USA, Spain, Australia, Chile, Peru, Belize, and Brazil.

The *Anaplasmataceae* family (order *Rickettsiales*) encompasses obligate intracellular bacteria that comprise the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia* and *Wolbachia*, the latter comprising endosymbionts of arthropods and nematodes. Among the most common diseases caused by this group of bacteria, the Human Granulocytic Anaplasmosis (HGA) and Human Granulocytic Ehrlichiosis (HME) affect humans. In animals, the main agents described are *Ehrlichia canis*, which causes canine monocytic ehrlichiosis, *Anaplasma marginale*, which affects cattle, and *Neorickettsia risticii*, which causes the Potomac Horse Fever (PHF). Even though diseases caused by *Anaplasmataceae* agents have not been described in bats in Brazil so far, these agents cause important diseases in animals throughout the Brazilian territory. While *Anaplasma phagocytophilum* has been detected in bats and/or associated ectoparasites in France, Hungary, Romania, and Russia, *Ehrlichia* spp. has already been detected in France, St. Kitts, and England. Regarding *N. risticii*, bats may play an important role in the life cycle of this agent, since these mammals are implicated on the maintenance and spread of the agent when ingesting the intermediary hosts of digenetic trematodes that harbors the bacteria. *N. risticii* has been detected in bats and/or associated trematodes in the USA, Argentina, Egypt, the Philippines, and the Netherlands.

Coxiella burnetii (order *Legionellales*) is the etiological agent of Q fever. It occurs almost worldwide and is mainly associated with abortive episodes in ruminants and anemia, fever and respiratory disorders in humans. Due to its sporulation capacity, this agent can remain in the environment for long periods. This agent has already been described in bats from Algeria, South Africa, Chile, and Brazil.

Finally, tick-borne protozoans belonging to Piroplasmida order comprise the genera *Babesia*, *Rangelia*, *Cytauxzoon*, and *Theileria*. These agents are generally associated with hemolytic anemia in animals and occasionally parasitize humans. In bats, *Babesia vesperuginis* has been described since the last millennium and have been detected in Europe, Asia, and Africa.

Given the importance of the studied agents in public and animal health, there is an interest in better understanding the occurrence and diversity of these pathogens in bats and associated ectoparasites.

2. Literature Review

2.1 Order Chiroptera

The Order Chiroptera (from the greek *Cheir*=hand and *pteron*=wings) represents the most unique group in Class Mammalia. It is believed that these flying mammals evolved from a small one that lived on top of trees and fed on insects, but did not have the ability to fly. With more than 60 million years of evolution, these animals evolved special structures for flying (Perachi et al., 2011; Laurindo and Novaes, 2015), with highly modified and flight specialized forelimbs, with bone elongation and presence of an elastic membrane called patagium. The membrane at the edge of the arm and forearm is called propatagium and, in many species there is also the uropatagium, a membrane between the hind limbs that can or cannot extend along the tail (Perachi et al., 2011).

This group used to be subdivided into two differentiated suborders, namely Megachiroptera and Microchiroptera (Murphy et al., 2001; Jones et al., 2002; Reis et al., 2007; Song et al., 2012). Megachiroptera, also called “megabats” or “Old World fruit bats”, used to be represented by a single family (Pteropodidae). However, after a new arrangement, the family Pteropodidae is part of the suborder Yinpterochiroptera, together with Rhinolophidae, Hipposideridae, Rhinopomatidae, Craseonycteridae, and Megadermatidae. The remaining families are grouped into the suborder Yangochiroptera (Perachi et al., 2011), previously known as part of Microchiroptera.

Bats are animals with great diversity of species, biological complexity and great ecological importance. With different eating habits, comprising hematophagy,

polynivory, nectarivory, piscivory, omnivory, insectivory, frugivory and carnivory, they are of great importance, especially in the dispersion of seeds, pollination and insect control (Peracchi et al, 2006; Reis et al., 2007, 2017). Also, the eating habits are not restricted to just one as seeds, since fruits and insects have already been found in same fecal samples collected from 35 different species (Bernard, 2002).

Known as nocturnal animals, they have a change in their activity in response to the increase in lunar luminosity. In the fragments where the area has greatest shading, the activity of these animals does not decrease in relation to the lunar cycle compared to the forest top without shading (Breviglieri, 2011). On the contrary that many people believe, bats are not blind and orient themselves mainly with high frequency sound waves, inaudible by humans, called echolocation that help to obtain food, even allowing them to find very small insects (Laurindo and Novaes, 2015).

Approximately 20% of known mammals are individuals of the Order Chiroptera, which comprises the second largest group of mammals in the world (Schipper et al., 2008). They are widely distributed throughout the planet and occupy almost all terrestrial environments, being present in all continents. They do not occur only in some islands of the Pacific Ocean, very cold regions, such as the Arctic and Antarctic, or regions of extreme altitude (Laurindo and Novaes, 2015). In the Neotropical region, chiropterans usually show the greatest diversity and abundance, which can show up to 120 species per 500 km² (approximately 310 square miles). Currently, there are 1,400 species described worldwide. From these, 181 bat species are officially registered in Brazil and distributed in 68 genera and 9 families (Garbino et al., 2020).

Besides the great diversity of species in Brazil, the greater knowledge of their distribution is reported in the Atlantic Forest. In the Amazon, for example, less than 25% of the region has records of the occurrence of chiropterans. However, it is estimated that almost 60% of the national territory does not have reports of the occurrence of chiropterans (Bernard, Aguiar and Machado, 2011). Following the same authors, the record of occurrence of bat species for the Brazilian Amazon is approximately 146 species, distributed in 64 genera, comprising the nine families of the neotropical region: Emballonuridae, Phyllostomidae, Mormoopidae, Noctilionidae, Furipteridae, Thyropteridae, Natalidae, Molossidae, and Vespertilionidae. The state of Pará is the one with the highest number of species registered.

In the south of Brazil, there are reports of a total of 70 species distributed in seven families: Emballonuridae, Noctilionidae, Phyllostomidae, Furipteridae, Thyropteridae, Vespertilionidae, and Molossidae. The state of Paraná is the richest, with 64 species, followed by Santa Catarina with 47, and Rio Grande do Sul with 40 (Passos et al., 2010). In other states it is reported the occurrence of 77 species in Rio de Janeiro (Peracchi and Nogueira, 2010), 79 in São Paulo (Vivo et al., 2011), and 80 in Minas Gerais (Tavares et al., 2010).

A great species diversity of neotropical bats is found in Latin American. Bernardi (2015) analyzed 83 chiropteran communities reported previously in the literature in Peru, Guiana and French Guiana, Venezuela, Mexico, Bolivia, Antilles, Nicaragua, Paraguay, Ecuador, Costa Rica, Colombia, Trinidad and Tobago, Argentina and a representative number of chiropterans reported in Brazil. The author found 214 species occurring in these communities, representing 53% of neotropical bats. The most representative species was *Artibeus lituratus* (Olfers, 1818), which occurred in 76 communities, followed by *Carollia perspicillata* (Linnaeus, 1758), *Desmodus rotundus* (Geoffroy, 1810), *Sturnira lilium* (Geoffroy, 1810), *Glossophaga soricina* (Pallas, 1766), *Myotis nigricans* (Schinz, 1821), *Artibeus planirostris* (Spix, 1823), *Phyllostomus hastatus* (Pallas, 1767), *Molossus molossus* (Pallas, 1766), *Chrotopterus auritus*, (Peters, 1856) and *Artibeus obscurus* (Schinz, 1821).

In the Mato Grosso do Sul state, where the present work was performed, Fischer et al. (2015) reported the occurrence of 74 bat species. Out of those, 60 were found in Cerrado area, 57 in Wetlands (Pantanal), and 17 in the Atlantic Forest.

Even with the great species richness of the Chiroptera order, specimens from the families Phyllostomidae, Vespertilionidae, and Molossidae were the most frequently found among chiropterans from Brazil (Passos et al., 2010; Bernard, Tavares and Sampaio, 2011; Bernardi, 2015). However, it is known that in the last 20 years, approximately two new bat species are reported per year in the Brazilian territory (Nogueira et al., 2014).

Regarding their importance to Public Health, bats are considered as reservoirs, hosts and sources of infection for various zoonotic agents (Mühldorfer, 2013), mainly rabies virus. However, the rates of rabies virus in bats vary between 0.5% and 0.8% in the largest Brazilian capitals, which corresponds to the normal standards established

by the World Health Organization. The spotlight in these animals is due to its high mobility, wide distribution, social behavior, and longevity (Bai et al., 2011). However, little is known about the occurrence of other zoonotic pathogens besides viruses in this group of animals (Pacheco et al., 2010). Thus, it is important to gather more epidemiological information about potential zoonotic pathogens circulating in chiropterans, which represent a large and diverse group of the Brazilian fauna.

2.2 Chiropteran's ectoparasites

Around 6,000 species of insects are known to parasitize vertebrates. Out of those, 687 species belonging to four Orders (Dermaptera, Hemiptera, Diptera, Siphonaptera) are known to parasitize bats; indeed, the majority of which are exclusive to chiropterans. The diversity of ectoparasites in chiropterans is influenced by the geographic distribution, behavior, size of the individual, type of housing and grouping of the bat species involved (Marshall, 1982).

The family Streblidae is composed of ectoparasite hematophagous flies of bats that include apterous, brachypterous and winged species. They are represented by three exclusive subfamilies of the New World: Trichobiinae with 19 genera, Streblinae with four genera, and Nycterophiliinae with two genera. The Nycteribiidae family, on the other hand, is formed by apterous and hematophagous flies having only two genera found in the New World: *Basilisa* (Miranda Ribeiro, 1903), which parasitizes mainly individuals of the families Phyllostomidae, Molossidae, and Vespertilionidae, and *Herskovitzia* (Guimarães and d'Andretta, 1957) that parasitizes individuals of the family Thyropteridae (Gracioli, 2010; Dick and Miller, 2010).

Mites of the family Spinturnicidae parasitize exclusively bats. They are found in all stages of their life cycle feeding on blood in the patagium of the host. The life cycle of Spinturnicidae includes five stages: egg, larva, protonymph, deutonymph, and adult. In animals that gather in groups, these mites can take advantage, as transfer among hosts occurs through direct contact among the animals. In contrast, mites of the Macronyssidae family parasitize several species of bats and can be transferred to other mammals, reptiles or birds. Fifteen Macronyssidae mite species had already been described parasitizing 19 different bat species in Brazil (Bassini-Silva et al., 2021). They feed on the blood and lymph of their hosts and their life cycle includes the

five stages described above for Spinturnicidae. However, Macronyssidae mites feed only on the protonymph and adult stages (Almeida, 2012).

In Brazil, several studies have shown that a great diversity of flies and mites can parasitize bats (Dornelles and Graciolli, 2017; Silva, Valim and Graciolli, 2017). Recently, Barbier and Bernard (2017) recorded 50 species of flies parasitizing 36 bat species and showed that the occurrence of these insects that are specific to the host may be directly related to the host availability.

Ticks of bats are usually host-specific and soft ticks (Ixodida: Argasidae) are the main ones reported parasitizing bats (Muñoz-Leal et al., 2018). The tick adaptation to the host usually differs depending on specificity, seasonality, prevalence, and intensity of infestation (Sándor et al., 2019).

Even though there are several reports of ticks in neotropical bats sampled in Brazil, the main species found is *Ornithodoros hasei* (Schulze, 1935), which has been frequently found parasitizing *Artibeus* spp. (Muñoz-Leal et al., 2016; Luz et al., 2016). Besides the identification of this tick species infesting *A. planirostris*, *Platyrrhinus lineatus* (Geoffroy, 1810), *P. hastatus*, *Gardnerycteris crenulatum* (Geoffroy, 1803), and *Noctilio albiventris* (Desmarest, 1818), Muñoz-Leal et al. (2016) also reported a new association of *Ornithodoros mimon* (Kohls, Clifford and Jones, 1969) and *A. planirostris*. The authors suggested that this bat species is the main host for larval stages of *O. hasei* and represent an important piece on the tick life cycle.

In Caatinga biome, Luz et al. (2016) reported *Ornithodoros* spp. (Koch, 1844) parasitizing bats from the Phyllostomidae and Mormoopidae families. Once again, *A. planirostris* appeared as one of the most infested species sampled. The authors found a total of 409 larvae of *Antricola* (Cooley and Kohls, 1942), *Nothoaspis* (Keiran and Clifford, 1975), and *Ornithodoros* genera parasitizing *A. planirostris*, *C. perspicillata*, *Trachops cirrhosis* (Spix, 1823), *Anoura geoffroyi* (Gray, 1838), *P. discolor*, *Tonatia* sp. (Gray, 1827), *Tonatia saurophila* (Koopman and Williams, 1951), *G. soricina*, *S. lilium*, *Lophostoma brasiliense* (Peters, 1867), *Pteronotus gymnonotus* (Natterer, 1843), and *Pteronotus parnelli* (Gray, 1843) individuals. The same ticks' genera were found by Muñoz-Leal et al. (2018) on 201 examined bats captured in Bahia, Pernambuco, Piauí, and Rondônia states. The authors also found a first stage nymph of *O. hasei* in two individuals. As the biological parameters of this tick life cycle are not known, it is

suggested that the larvae may moult to nymph during flight. However, it must be taken into consideration the time required for this change, which remains unknown.

Ticks from *Nothoaspis* genera are mainly related to hot and humid environments and their distribution is determined by the presence of caves showing these conditions and the presence of bats. Despite the report of *Nothoaspis amazoniensis* (Nava, Venzal and Labruna, 2010) in Brazil, Nava et al. (2010) described for the first time the presence of these ticks on bat caves situated in Brazilian Amazon, in their adult and immature forms (larva, nymph I, nymph II). Ticks from *Antricola* and *Ornithodoros* (*Carios*) genera have already been found on bat guano in caves in Rondônia state (Amazon region); free-living ticks have also been found in a cave in Mato Grosso do Sul state, and on cave walls and ceilings in Pernambuco state (Labruna et al., 2008; Labruna and Venzal, 2009; Barbier, Bernard and Dantas-Torres, 2020). The last authors also suggested that *Antricola guglielmonei* (Estrada-Peña, Barros-Battesti and Venzal, 2004) is commonly found in hot caves where bats can be found in Brazil and the abundance of the ticks of this genus can decrease according to the environment humidity, being influenced positively by the temperature.

The first record of the occurrence of the hard tick *Amblyomma sculptum* (Berlese, 1888) on bats has been recently reported (Novaes et al., 2020). These authors captured three different bats parasitized in the same locality (São João do Piauí, state of Piauí), but in different dates in the years of 2010 and 2012. The individuals from the species *Myotis lavalii* (Moratelli, Peracchi, Dias and de Oliveira, 2011), *Noctilio leporinus* (Linnaeus, 1758) and *N. albiventris* were captured and found infested by sub-adults of *A. sculptum*.

Although a great diversity of ectoparasites on chiropterans has been described, there is no certainty on the role of such arthropods in the cycles of transmission of pathogens among populations of chiropterans in Brazil.

2.3 *Bartonellaceae* agents

The *Bartonellaceae* family (order *Rhizobiales*) comprise at least 45 reemerging and zoonotic species, which can cause a wide range of clinical presentations from self-limiting manifestations to fatal systemic diseases (Kosoy et al., 2010, Pitassi, 2015; Vieira-Damiani et al., 2015, Breitschwerdt, 2015; Okaro et al., 2017). Most of these

agents have adapted to mammalian reservoirs in a unique way and in restricted ranges of host species (Kosoy et al., 2010; Bai et al., 2011; Veikkolainen et al., 2014) correlating predominantly to vectors involved, which also assumes preference for selected hosts. For instance, *Bartonella elizabethae*, *Bartonella grahamii*, *Bartonella washoensis* and *Bartonella vinsonii* subsp. *arupensis* are associated to rodents; *Bartonella henselae* is the species with the highest occurrence in cats, while *Bartonella vinsonii* subsp. *berkhoffii* is often associated with dogs. Depending on the occurrence and interactions with arthropod vectors, the prevalence of these bacteria in reservoir populations can be high (Dehio, 2004). Taking into consideration the large number of reservoirs and vectors, the transmission of these agents leads to challenging epidemiological scenarios from a zoonotic point of view (Breitschwerdt and Kordick, 2000; Breitschwerdt et al., 2010a).

Among the several species of *Bartonella* that infect humans, *Bartonella bacilliformis*, which is transmitted by *Lutzomyia verrucarum* (Townsend, 1913), was the first one to be described in 1909 (Dehio, 2004). This *Bartonella* species is known as the agent of the Carrión's disease, which is endemic in Peru, Ecuador and Colombia, with sporadic reports in Bolivia, Chile and Guatemala. Two distinct phases occur in the infection course: the first one, which is known as Oroya fever, is characterized by fever, pallor, discomfort and myalgia; the second phase, known as eruptive or “verruca peruana”, has a chronic character and is characterized by skin lesions, neurological and cardiac signs, with respiratory complications in severe cases. Most of deaths caused by this disease are associated with secondary infection due to immunosuppression (Maco et al., 2004; Lamas et al., 2008; Pitassi, 2013).

Cat Scratch Disease (CSD), which is caused by *B. henselae*, is one of the most important *Bartonella*-related zoonotic diseases. This agent was first isolated and identified in 1992 by Dolan and collaborators (Pitassi, 2013). The disease occurs more frequently in immunocompromised patients with a history of contact with domestic cats (Velho et al., 2003). The infection with *B. henselae* can show two main clinical presentations in humans: the typical CSD, which is characterized as an erythematous papule at the trauma site, which becomes vesicular and crusted evolving to a macula with lymphadenomegaly; and the atypical disease, characterized by high fever, Parinaud's oculoglandular syndrome, encephalitis, hemolytic anemia,

hepatosplenomegaly, glomerulonephritis, pneumonia, relapsing bacteremia, and osteomyelitis (Chomel, Boulois, Breitschwerdt, 2004). Also associated to CSD, *Bartonella clarridgeiae* (Dehio, 2005) is associated with clinical conditions characterized by fever, malaise, lymphadenopathy, and thoracopulmonary manifestations in humans, in addition to endocarditis and liver disease in dogs, and blindness and neuritis in cats (Vieira-Damiani et al., 2015).

Bartonella quintana and *B. henselae* are known as causative agents of bacillary angiomatosis, which is characterized by vascular proliferation lesions due to angiogenic proliferation induced by bartonellae. Although these lesions occur more frequently in the skin, vasoproliferation, and capillary dilation can also occur in the liver parenchyma, characterizing bacillary peliosis (Velho, 2001). *B. quintana* is also the etiological agent of Trench Fever (Velho, 2001; Pitassi, 2013) and had re-emerged in the twentieth century as a disease found among neglected social groups, such as homeless and drug addicts infested by body lice (*Pediculus humanus corporis* Linnaeus, 1758) (Dehio, 2004).

Although bartonellae transmission is often associated with blood-sucking arthropod vectors, mainly fleas and lice (Bai et al., 2011; Morse et al., 2012), little is known about the involvement of ticks, mites and flies in the transmission cycles of these pathogens. Nonetheless, it is known that the transmission of *B. bacilliformis* occurs through stings of *L. verrucarum*, *B. quintana* by *P. humanus corporis*' feces, and *B. henselae*, *B. clarridgeiae* and *Bartonella koehlerae* by *Ctenocephalides felis felis* (Bouché, 1835)' feces (reviewed by Chomel et al., 2006; Billeter et al., 2008).

Although species of *Bartonella* have been molecularly detected in ticks of the genera *Ixodes* (Latreille, 1795), *Dermacentor* (Koch, 1844), *Haemaphysalis* (Koch, 1844), *Rhipicephalus* (Koch, 1844) and *Argas* (*Carios*) (Latreille, 1796) (reviewed by Billeter et al., 2008), the vectorial competence of these arthropods in the transmission of bartonellae has not yet been proven experimentally (Telford and Wormser, 2010), except for *Ixodes ricinus* (Linnaeus, 1758), which showed to be a competent vector for *B. henselae* transstadially. Indeed, the authors observed proliferation of the bacteria in salivary glands and successfully transmitted *B. henselae* to blood with artificial feeding. After inoculation of tick's infected salivary glands in naïve cats, the latter showed bacteremia 7 and 14 days after inoculation (Cotté et al., 2008). Reis et al. (2011)

performed the first *in vivo* assay and demonstrated the vector competence of *I. ricinus* in the transmission of *Bartonella birtlesii*. More recently, Wechtaisong et al. (2020) showed that *B. henselae* might be transstadially transmitted from larvae to nymph of *Rhipicephalus sanguineus* (Latreille, 1806) *sensu lato* ticks. The authors fed naive tick larvae on *B. henselae*-infected blood through an artificial membrane feeding system. These larvae were tested in pools by PCR after five days. The positivity for *B. henselae* found was 57.1% (8/14) in engorged larvae; 66.7% (4/6) in semi-engorged larvae, and 66.7% (2/3) in the larvae feces pools. After molting, the nymph pools were also tested and 10% (1/10) were positive. Interestingly, *Bartonella* DNA was not found in the ticks' feces. Then, 60 molted nymphs were fed on non-infected mice from which blood was collected by 4 days. After feeding, the nymphs were counted for calculating the nymph attachment. *B. henselae* was detected by PCR in the blood samples obtained from the feeders, but the authors could not isolate colonies when plating the blood samples from feeders onto chocolate agar plates.

Besides the vector-related transmission, it has been described that *Bartonella* spp. may have other routes of transmission. In this context, Pitassi et al. (2015) reported the molecular detection of *B. henselae* and *B. clarridgeiae* from a blood donor human sample. Although transmission by this route in humans has not yet been confirmed, Silva et al. (2016) demonstrated the transmission of *Bartonella* through blood transfusion in rats, in which two out of four animals transfused experimentally were positive for *B. henselae* by molecular assays. Still, a possible perinatal transmission or by cesarean section procedures of *B. vinsonii* subsp. *berkhoffii* and *B. henselae* has been previously reported in the USA (Breitschwerdt et al., 2010b). Accidents with contaminated needles also may represent additional routes of transmission (Oliveira et al., 2010; Lin, Chen and Chang, 2011).

The infection by *Bartonella* spp. can be diagnosed by serological techniques as the indirect immunofluorescence reaction (RIFI), enzyme-linked enzyme immunoassay (ELISA), "Western blot", or microbiological and molecular techniques (Moreira, 2011; Pitassi, 2013). However, it is worth mentioning that the culture of these agents is not performed routinely in microbiology laboratories as it is a laborious process, since bartonellae have slow growth and requires controlled temperature and atmosphere (Kawasato, 2009; Moreira, 2011). In order to get a better detection of the

agent in blood and tissue samples, a pre-enrichment medium was developed for facilitating the isolation of these agents. The so-called *Alphaproteobacteria* Growth Medium (BAPGM) consists in a liquid medium formulated from insect growth medium that promotes effective growth of these bacteria before inoculation onto a solid medium. This medium can also be useful for fastidious bacteria besides *Bartonella* (Maggi et al, 2005; Duncan et al., 2007, Cadenas et al., 2007)

On the other hand, Gutiérrez et al. (2017) recommended diagnosis by molecular methods, as they are more practical and sensitive, mainly for those samples that are not suitable for culturing. In this sense, the authors indicate real-time PCR screening and, later, conventional PCR assays followed by sequencing to confirm the molecular identity or phylogenetic positioning. Recently, Maggi et al. (2020) showed a greater sensitivity of digital-droplet PCR (ddPCR) when compared to qPCR in the diagnosis of *Bartonella* spp.

The occurrence of *Bartonella* spp. has already been demonstrated in chiropterans sampled in the United Kingdom (Concannon et al., 2005), Kenya (Kosoy et al., 2010), Guatemala (Bai et al., 2011, Wray et al., 2016), Taiwan (Lin et al., 2012), Peru (Bai et al., 2012), Nigeria (Kamani et al., 2014), Puerto Rico (Olival et al., 2015), Finland (Lilley, Veikkolainen and Pulliainen, 2015), Madagascar (Brook and Dobson, 2015), Costa Rica (Judson, Frank and Hadly, 2015), French Guiana (Davoust et al., 2016), Ghana (Mannerings et al., 2016), Algeria (Leulmi et al., 2016), South Africa and Swaziland (Dietrich et al., 2016), China (Han et al., 2017), United States (Lilley et al., 2017), Republic of Georgia (Bai et al., 2017, Urushadze et al, 2017), Peru and Belize (Becker et al., 2018) and, more recently, in Brazil (Ikeda et al., 2017; Ferreira et al., 2018; André et al., 2019; Gonçalves-Oliveira et al., 2020) and Japan (Nabeshima et al., 2020).

In Brazil, *Bartonella* in bats was first reported by our research group (Ikeda et al., 2017). Seventeen (5.28%) out of 322 bat samples (107 spleen tissue, 56 heart tissue, and 40 blood samples) collected from 162 bats from 19 different species sampled in five different states of Brazil showed positivity by qPCR targeting the *nuoG* gene of *Bartonella* spp. The positive species were represented by three bats from the state of Paraná [*S. liliium* (n=3)]; six from the state of Pará [*P. hastatus* (n=1), *C. perspicillata* (n=4), and *Natalus espirosantensis* (Ruschi, 1951) (n=1)]; and seven from the state

of Tocantins [*C. perspicillata* (n=2) and *G. soricina* (n=5)]. Seven sequences were obtained from the molecular characterization targeting the *nuoG* (n=3), *gltA* (n=2), *rpoB* (n=1), *ftsZ* (n=1) genes. The obtained sequences showed to be closely related to genotypes previously described in bats sampled in Latin America.

Despite the great surveillance of the agent in bats, little is known about the involvement of mites and flies in the transmission cycles among these mammals. In this sense, Morse et al. (2012) identified *Bartonella* spp. in Nycteribiidae and Streblidae dipterans collected from chiropterans in 14 countries in Asia, the Americas, Europe and Africa. In Puerto Rico, Olival et al. (2015) detected four distinct genotypes of *Bartonella* spp. in *Monophyllus redmani* (Leach, 1821) and *Trichobius* spp. (Diptera: Streblidae), an ectoparasite commonly found on bats. The authors suggested that the load of ectoparasites and the specificity of the vectors for an individual influence on the diversity of *Bartonella*, as well as their prevalence among bat species.

In Madagascar, Brook and Dobson (2015) investigated the presence of DNA from *Bartonella* spp. in blood samples from chiropterans of the species *Eidolon dupreanum* (Pollen in Schlegel and Pollen, 1866) and *Pteropus rufus* (Geoffroyi, 1803) and ectoparasites *Cyclopodia dubia* (Westwood, 1835) and *Thaumapsylla* sp. (Rothschild, 1907) using conventional PCR assays, DNA of *Bartonella* spp. was detected only in specimens of *E. dupreanum* (44.68% [21/47]) and their associated ectoparasites of the species *C. dubia* (Nycteribiidae) (89.47% [17/19]). On the other hand, specimens of *P. rufus* were negative for *Bartonella* spp. and ectoparasites. The authors suggested that the bat flies may play an important role in the transmission and maintenance of *Bartonella* spp.

Judson, Frank and Hadly (2015) found an occurrence of 52.7% (29/55) of *Bartonella* spp. in bat associated flies and 33.3% (21/63) in bats sampled in Costa Rica, using conventional PCR. The obtained *gltA* genotypes of *Bartonella* spp. found were identical in different bats and their respective ectoparasites, suggesting that the same variants of *Bartonella* spp. can be shared among the sampled animals and the ectoparasites would act as vectors among these animals.

Bartonella spp. phylogenetically associated with *Bartonella tamiae* was detected by Leulmi et al. (2016) in 26 samples of ectoparasites and bat spleen from northeastern Algeria. Positive results were found in 63.2% (12/19) of specimens of *Ixodes*

vespertilionis (Koch, 1844), 72.7% (8/11) of Nycteribiidae flies and 60% (6/10) of bat spleen samples.

Thirteen out of 384 blood samples, five out of 14 Nycteribiidae flies and an unidentified ectoparasite were positive for *Bartonella* spp. in South Africa and Swaziland (Dietrich et al., 2016). The obtained *Bartonella gltA* sequences from the sampled chiropterans and ectoparasites showed to be phylogenetically associated, albeit distant from those previously described in Africa.

McKee and collaborators (2018) investigated the presence of *Bartonella* spp., *Brucella* spp. and *Yersinia* spp. in 55 insects collected from bats of the species *Eptesicus fuscus* (Palisot de Beauvois, 1796) from the USA. These insects were identified as *Cimex adjunctus* (Barber, 1939) according to sequences obtained from the 16S rRNA and COI genes. Only 4/55 (7.3%) were positive for *Bartonella* spp., and the phylogenetic analysis of the concatenated sequences of the *ftsZ* and *gltA* genes showed proximity to bartonellae previously detected in *Pipistrellus* sp. bats sampled in the United Kingdom and with a sequence of *Bartonella* detected in *Rhinolophus ferrumequinum* (Schreber, 1774) from the Republic of Georgia.

A high diversity and prevalence of *Bartonella* spp. in bat ectoparasites was found by Sándor and collaborators (2018), suggesting a broad co-evolution of *Bartonella* between Nycteribiidae flies and their hosts. The authors detected 18 *Bartonella* strains that were divided into 7 different *gltA* genotypes. The sequences were obtained from nine species of Nycteribiidae flies, namely *Basilia nana* (Theodor, 1954), *Basilia nattereri* (Kolenati, 1857), *Nycteribia kolenatii* (Theodor, 1954), *Nycteribia pedicularia* (Latreille, 1805), *Nycteribia schmidlii* (Schiner, 1853), *Nycteribia vexata* (Westwood, 1835), *Penicillidia conspicua* (Speiser, 1901), *Penicillidia dufourii* (Westwood, 1834), and *Phthiridium biarticulatum* (Hermann, 1804), which were collected from 11 species of bats (*Miniopterus schreibersii* (Kuhl, 1817), *Myotis bechsteinii* (Kuhl, 1817), *Myotis blythii* (Tomes, 1857), *Myotis capaccinii* (Bonaparte, 1837), *Myotis daubentonii* (Kuhl, 1817), *Myotis myotis* (Borkhausen, 1797), *Myotis nattereri* (Kuhl, 1817), *Rhinolophus blasii* (Peters, 1867), *Rhinolophus Euryale* (Blasius, 1853), *R. ferrumequinum*, and *Rhinolophus mehelyi* (Matschie, 1901)).

Among frugivorous and insectivorous bats, Moskaluk et al. (2018) found positivity by PCR for *Bartonella* targeting the *gltA* gene in an individual of *Paratrachobius*

longicrus (Ribeiro, 1907) collected from a specimen of *A. lituratus*. Among the flies collected from hematophagous bats known to be positive for *Bartonella* by isolation, 19/73 pools of *Trichobius parasiticus* (Gervais, 1844) and 3/5 pools of *Strebla wiedemanni* (Kolontai) were PCR-positive. Eleven out of 47 pools of *T. parasiticus* and 2/4 pools of *S. wiedemanni* collected from culture-negative bats were also PCR-positive for *Bartonella*. In contrast, the inverse was observed, since some ectoparasite pools collected from bacteremic bats were PCR-negative for *Bartonella*. Most of the *gltA* obtained sequences were in the same clade as *B. clarridgeiae* and *Bartonella rochalimae* or *Bartonella doshiae*. The authors found only one sequence that showed concordance between host-ectoparasite, showing that the bartonellae diversity can occur due to the frequent switch of hosts. Also, the authors demonstrated that the occurrence of *Bartonella* in bat flies is independent of the positivity of their bat hosts in Mexico.

Recently, Braga et al. (2020) found similar results when surveyed blood samples from bats and their associate-flies collected in São Luís Island, Maranhão state, northeastern Brazil. Despite none bat blood sample was positive for *Bartonella* spp., three out of 15 (20%) of DNA samples obtained from *Trichobius* sp. (Diptera: Streblidae) flies were positive for *Bartonella* by qPCR targeting the *nuoG* gene. From those, 2 were positive for the *gltA* gene and 1 for *rpoB* gene using a conventional PCR. The sequences were similar by nBLAST and phylogenetically related to sequences obtained previously from *C. perspicillata* or bat flies from Costa Rica (for the *gltA* gene) and with *Bartonella* detected in *D. rotundus* sampled in Brazil (for *rpoB* gene). The authors also suggested that the concentration of parasitized cells in the ectoparasite midgut may be higher when compared to the bats' bloodstream.

Also in Brazil, Do Amaral et al. (2018) found positivity for *Bartonella* in 40/202 (19.8%) Streblidae flies using qPCR targeting the *nuoG* gene, whereas all Spinturnicidae and Macronyssidae mites collected showed to be negative. The species that showed positivity were the following: 6/32 (18%) *P. longicrus*, 7/24 (29%) *Strebla guajiro* (Garcia and Casal, 1965), 2/5 (40%) *Aspidoptera phyllostomatis* (Perty, 1833), 5/43 (11%) *Aspidoptera falcata* (Wenzel, 1976), 1/10 (10%) *Trichobius anducei* (Guerrero, 1998), 1/4 (25%) *Megistopoda aranea* (Coquillett, 1899), and 18/55 (32%) *Trichobius joblingi* (Wenzel, 1966) collected from bats of the species: *Artibeus*

fimbriatus (Gray, 1838), *A. lituratus*, *C. perspicillata*, *S. lilium*, *A. obscurus* and *A. planirostris*. Most of the sequences obtained by the authors were closely related to *Bartonella* sequences previously described in bat species or associated ectoparasites.

In Europe, Hórnok et al. (2012) detected, by real-time PCR based on the *gltA* gene, DNA from *Bartonella* spp. in pools of Macronyssidae (*Steatonyssus occidentalis* Ewing, 1933) and Spinturnicidae (*Spinturnix myoti* Kolenati, 1856) mites collected from specimens of *M. myotis* in Hungary. More recently, *Bartonella* was molecularly detected in 28% of 134 pools of *S. myoti* blood-sucking mites and in 25% of 59 *M. myotis* bats in Poland (Szubert-Kruszyńska et al., 2018). Adult mites were three times more frequently positive when compared to immature stages, which might suggest transstadial transmission of the agent. Also, pools of mites collected from bats in caves showed greater positivity when compared to those collected in attics. Three genotypes were detected, with the most frequently detected being phylogenetically associated with '*Candidatus Bartonella hemsundetiensis*' previously detected in bats in Finland, but phylogenetically distant from those genotypes identified in bats in the UK and France. Interestingly, most of the sequences obtained were phylogenetically close related to *Bartonella* sp. detected in forest workers in Poland (Szubert-Kruszyńska et al., 2018).

Regarding the genotypic diversity of *Bartonella* spp. in bats and associated ectoparasites, it has been reported that these bacteria show to be diverse. For instance, Bai et al. (2011) found 21 *Bartonella gltA* genetic variants of 13 phylogroups obtained from 118 bats from different species sampled in Guatemala. The authors observed that different bat species shared the same *Bartonella* genotype. In the Amazon part of Peru, Bai et al. (2012) also found 21 *Bartonella gltA* genetic variants from 27 isolates of *Bartonella* spp. obtained from a total of 112 bats distributed in 19 species sampled. Olival et al. (2015) tested 51 sampled bats by culture and found 9 *Bartonella*-positive animals from *Artibeus jamaicensis* (Leach, 1821), *Brachyphylla cavernarum* (Gray, 1834), and *M. redmani* species collected in Porto Rico. The authors found six *Bartonella gltA* genotypes; *M. redmani* showed to have the greatest diversity with four genotypes that clustered with previously *Bartonella* spp. detected from *Trichobius* spp. bat flies and *P. discolor* from Guatemala.

Based on *Bartonella* isolation, McKee et al. (2017) detected seven *gltA* genogroups representing 42 isolates obtained from 34/93 (36.6%) individuals belonging to five bat species sampled in Thailand. The authors described that *Bartonella* are usually shared among bats from the same families, superfamilies and suborders, as each genogroup comprised bartonellae detected in bat species from the same genus. In fact, McKee et al. (2016) showed that the events of transitions and duplications occur more frequently within the same bat family than between families, superfamilies and suborders. Also, the exchange of *Bartonella* between bats and their ectoparasites are bidirectional, suggesting that the associated vectors may play a role on the patterns of difference among the bat-related *Bartonella*. These authors made a diverse of evolutionary assessment methods using a compiled data from the database available using *gltA* and *cytb* genes retrieved from 173 *Bartonella* genotypes from 66 bat species belonging to 41 genera and 11 families, respectively.

Taking into consideration vampire bats, Becker et al. (2018) analyzed 193 biological samples (blood, oral and rectal swabs) from *D. rotundus* collected in Peru and Belize. The *Bartonella* spp. sequences obtained (n=35) showed at least 11 paraphyletic *gltA* genotypes. In Brazil, our research group also found high diversity of bartonellae among hematophagous bats, with 11 genotypes found using *gltA* and *rpoB* genes among 51 positive animals from 208 bats sampled [*D. rotundus* (n=167), *Diphylla ecaudata* (Spix, 1823) (n=32) and *Diaemus youngii* (Jentink, 1893) (n=9)] throughout the country (André et al., 2019).

Since *Bartonella* appears to have high genotypic plasticity, it is important to gain knowledge on the circulation of such bacteria specially in bats, which have been pointed out as key hosts in the origin and spread of these important zoonotic bacteria. Indeed, “*Candidatus Bartonella mayotimonensis*”, which is a *Bartonella* species associated to a human case of endocarditis in the USA (Lin et al., 2012), has also been reported in bats from Finland, France, Spain and North America (Veikkolainen et al., 2014; Lilley, Veikkolainen and Pulliainen, 2015; Stuckey et al., 2017; Lilley et al., 2017). Moreover, the serological evidence of human exposure to “*Candidatus Bartonella roussetti*” together with its isolation and molecular characterization from *Rousettus aegyptiacus* (Geoffroyi, 1810) blood samples and their related ectoparasites (*Eucampsipoda Africana* Theodor, 1955) in Nigeria (Bai et al., 2018), as well as the

isolation of this agent from *R. aegyptiacus* bats from Zambia (Qiu et al., 2020), emphasizes once again that these animals can play a major role in the epidemiology of bartonellosis.

2.4 Hemoplasmas

Hemotrophic mycoplasmas or hemoplasmas are small Gram-negative bacteria belonging to the family *Mycoplasmataceae* (order *Mycoplasmatales*). Previously known as *Haemobartonella* and *Eperythrozoon* (Messick, 2004), these agents were reclassified by Neimark et al. (2001), after analyzing 16S rRNA sequences of four species, namely *Haemobartonella felis*, *Haemobartonella muris*, *Eperythrozoon suis* and *Eperythrozoon wenyonii*, which showed to be phylogenetically related to species belonging to the genus *Mycoplasma*. These bacteria have epierithrocytic localization and can cause from asymptomatic disease to severe hemolytic anemia, both in animals and humans (Tasker, 2010; Mascarelli et al, 2014; Millán et al., 2015). The immune-mediated anemia may lead to some clinical signs, such as lethargy, weakness, depression, tachycardia, dyspnea, tachypnea, cardiac murmur, hepatosplenomegaly, lymphadenopathy, fever, weight loss, and icterus (Barker, 2019). Infected animals can present anorexia, fever, icterus, and hypoglycemia in acute conditions (Neimark et al., 2001; Biondo et al., 2009; Tasker, 2010). Chronic infection, on the other hand, can occur in apparently healthy animals, mainly those that have undergone a splenectomy procedure, or are immunocompromised. The severity of the disease will depend on the strain or species of *Mycoplasma* involved (Messick, 2004).

Infection by hemoplasmas occurs mainly in animals, and reports in humans are rare. When reported, *Mycoplasma* spp. usually occurs in immunocompromised humans, although diseases associated with latent hemoplasma infection in healthy patients have been reported (Messick, 2004). Around the world, hemoplasmas have been detected in humans from China (Yang et al., 2000; Hu et al., 2009; Yuan et al., 2009), England (Steer et al., 2011), United States (Sykes et al., 2010; Maggi et al., 2013a), South Africa (Maggi et al., 2013b), Japan (Hattori et al., 2020), and New Caledonia (Descloux et al., 2020).

In Brazil, *Mycoplasma haemofelis* has already been molecularly detected in an HIV patient with signs of night sweating, loss of appetite, productive cough, muscle pain, and cervical, axillary and inguinal lymphadenomegaly in Porto Alegre, state of Rio Grande do Sul (dos Santos et al., 2008). The patient was positive in conventional PCR for hemoplasmas based on the 16SrRNA gene. The obtained sequence showed 99% identity with *M. haemofelis*. In addition, concomitant infection with *B. henselae* was also reported. In this case, the participation of cats as a possible source of infection for these agents to the patient was suggested, since all five 5 tested cats, kept as pets by the patient, were positive for *Bartonella* spp. and two for *M. haemofelis*.

Sykes et al. (2010) also reported in Texas, USA, the occurrence of co-infection by *B. henselae* and a hemoplasma closely related to *Mycoplasma ovis* in a patient with signs of progressive neurodegenerative disease. The patient was a veterinarian and had a history of the occurrence of bites and scratches by cats, dogs, small rodents and had previously worked with wild and zoo animals.

Hemoplasma infection was reported in a veterinarian showing neurological symptoms (Maggi et al., 2013b). The patient referred contact with fleas, ticks, flies, mosquitoes, spiders, mites, as well as scratches or bites by dogs, cats, birds, horses, reptiles, rabbits and rodents. After approximately two years of evolution of the condition without resolution, blood samples were collected from the patient and showed to be seropositive for *B. vinsonii* subsp. *berkhoffii* and *B. henselae*, albeit negative to molecular and isolation techniques for *Bartonella* spp. Based on PCR assays, positivity for *Anaplasma platys* and *Mycoplasma* spp. was detected. The hemoplasma sequences obtained from the 16S rRNA and RNaseP genes showed identity of 99.8% and 100%, respectively, to '*Candidatus Mycoplasma haematoparvum*'.

Steer et al. (2011) proposed a new species of *Mycoplasma*, which was identified in a patient with fever, abdominal and joint pain, weight loss and night sweats. The agent was identified by conventional PCR targeting the 16S rRNA gene from a bone marrow sample. The sequence showed identity with *Mycoplasma haemomuris* and '*Candidatus Mycoplasma turicensis*'. The authors therefore proposed the species '*Candidatus Mycoplasma haemohominis*', a hemoplasma distinct from those previously identified in animals, and associated mainly with signs of fever and hemolytic anemia in humans (Hattori et al., 2020).

Recently, Hornok et al. (2019) and Descloux et al. (2020) reported the occurrence of hemoplasmas closely related to the “*Ca. M. haemohominis*”, in bat-related *Ixodes simplex* (Neumann, 1906) (Acari: Ixodidae) and *Cyclopodia horsfieldi* (Meijere, 1899) (Diptera: Nycteribiidae), respectively. Descloux et al. (2020) also reported the positivity for this new agent in 15/23 hospitalized patients and 16/40 *Pteronopus* spp. bats from New Caledonia, emphasizing the need for additional studies on the genetic diversity of hemoplasmas in bats. Hemoplasmas closely related to “*Ca. M. haemohominis*” has also been detected in bats from Spain (Millán et al. 2015), Chile (Millán et al., 2019), and Nigeria (Di Cataldo et al., 2020).

Although the transmission of hemoplasmas seems to occur mainly by hematophagous arthropods or aggressive interactions among animals (Neimark et al., 2001; Biondo et al., 2009), few experimental studies have been carried out in this sense, given that such agents have not yet been cultivated *in vitro* (Biondo et al., 2009). Although the tick *R. sanguineus* has been incriminated as a vector and reservoir for *Mycoplasma haemocanis*, this assumption has been not yet totally elucidated (Novacco et al., 2010; Vieira et al., 2015). Additionally, it has been suggested the participation of *C. felis* fleas in the transmission of *M. haemofelis* and '*Candidatus Mycoplasma haemominutum*', as well as the role of lice, namely *Polyplax serrata* (Burmeister, 1839) and *Polyplax spinulosa* (Burmeister, 1839), in the transmission of *Mycoplasma coccoides*, and *Stomoxys calcitrans* (Linnaeus, 1758) and *Aedes aegypti* (Linnaeus, 1762) in the transmission of *Mycoplasma suis* (reviewed by Biondo et al., 2009). Regarding ectoparasites of wild animals in Brazil, despite de Sousa et al. (2017a) did not find positivity for hemoplasmas among ectoparasites collected from wild animals in the Brazilian Pantanal, Gonçalves et al. (2020) detected 16S rRNA hemoplasma sequences similar to those previously detected in synanthropic rodents from Brazil, Japan and Hungary in *Amblyomma* ticks and *P. spinulosa* lice collected from rodents.

Molecular tools help in the diagnosis of hemoplasma infection, since these bacteria have not been cultivated so far. Direct diagnosis by stained blood smears, in turn, does not bring reliable results, given its low sensitivity and specificity (Tasker and Lappin, 2002; Messick, 2004). Blood smear analysis during acute hemoplasmosis may be considered but it requires ability from the examiner (Barker, 2019). Although

serological tests have been developed using recombinant antigens (Messick and Santos, 2011; Liu et al., 2012), it is worth emphasizing that antibodies can be present in both asymptomatic and anemic animals. Tasker and Lappin (2002) suggested the standardization of serological assays that allow the detection of antibodies only against pathogenic species of hemoplasmas.

In wild animals, hemotropic *Mycoplasma* spp. have been detected in 21 countries mainly in the Americas and Europe. Hemoplasmas have already been detected in 19 Orders of mammals, more expressively in Rodentia, Carnivora, Artiodactyla, Primates, and Marsupialia (reviewed by Millán et al., 2020). In Brazil, these agents have been reported in wild carnivores (André et al., 2011a; de Sousa et al., 2017a; Guimarães et al., 2007; Cubilla et al., 2017a; Furtado et al., 2018; Carneiro et al., 2020), non-human primates (Bonato et al., 2015; Cubilla et al., 2017b; Ramalho et al., 2017; de Melo et al., 2019), wild boars (*Sus scrofa* Linnaeus, 1758) (Dias et al., 2019; Fernandes et al., 2021), wild and synanthropic rodents (Vieira et al., 2009; Gonçalves et al., 2015, 2020; de Sousa et al., 2017a; Valente et al., 2021), and opossums (*Didelphis albiventris* Lund, 1840) (Massini et al., 2019; Gonçalves et al., 2020; Pontarolo et al., 2021) as well as in their associate ectoparasites (Gonçalves et al., 2020).

In bats, *Mycoplasma* spp. have already been detected in the USA, Spain, Australia, Chile, Brazil, Peru and Belize, Germany, Costa Rica, Nigeria and New Caledonia (Mascarelli et al., 2014; Millán et al., 2015; Volokhov et al., 2017; Ikeda et al., 2017; Holz et al., 2018; Millán et al., 2019; Becker et al., 2020; dos Santos et al., 2020; Fritschi et al., 2020; Di Cataldo et al., 2020; Descloux et al., 2020). Indeed, a high genetic diversity of hemoplasmas has been reported in Neotropical bats (Volokhov et al., 2017; Becker et al., 2020).

In the USA, Mascarelli et al. (2014) evaluated the presence of *Mycoplasma* spp. DNA in 68 specimens of *Myotis lucifugus* (Le Conte, 1831) during hibernation in the states of Pennsylvania, Ohio, Kentucky, West Virginia, Tennessee, and North Carolina. A 47% positivity (32/68) was found using a conventional PCR targeting the 16S rRNA gene. In the phylogenetic analysis, the sequences were phylogenetically associated with *M. haemomuris*.

Millán et al. (2015) found 97% (29/31) of positivity for hemoplasmas by conventional PCR based on the 16S rRNA gene in chiropterans of the species *M.*

schreibersii and *M. capaccinii* sampled in Spain. In the phylogenetic analysis, the authors found two distinct groups: the first encompassed sequences closely related to 'Ca. *M. hemohominis*', and a second group composed of sequences with phylogenetic proximity with hemoplasmas previously detected in chiropterans in the USA by Mascarelli et al. (2014).

Volokhov et al. (2017) described a 67% (150/223) prevalence among *D. rotundus* sampled in Peru and Belize. The authors showed that three novel 16SrRNA genotypes were related to other sequences previously detected in non-hematophagous bats, rodents, humans and non-human primates. Also, their work also showed similar genotypes found in both blood and saliva, emphasizing the potential role of saliva in the transmission of this group of bacteria.

In fact, the similarity of bat-related hemoplasmas with previously identified hemoplasmas species in other bat species, ticks, rodents, primates, and carnivores seems to be common (Millán et al., 2020). In Chile, Millán et al. (2019) found positivity in 12/55 animals from the species *Histiotus macrotus* (Poeppig, 1835), *Histiotus montanus* (Philippi and Landbeck, 1861) and *Myotis chiloensis* (Waterhouse, 1840). The obtained 16S rRNA sequences were grouped in three genotypes closely related to sequences previously detected in other bats species, rodents and humans.

In Australia, Holz et al. (2018) found 8/83 positive samples collected from *Miniopterus schreibersii bassanii* (Cardinal and Christidis, 2000) and *Miniopterus fuliginosus* (Hodgson, 1835). The only sequence obtained showed similarity with bat-related hemoplasmas.

Hemoplasma sequences previously detected in vampire and Chilean bats were found in a study with free-living and captive bats from Phyllostomidae, Pteropodidae, Megadermatidae, Vespertilionidae, Rhinolophidae and Emballonuridae families. The 12 hemoplasma 16S rRNA sequences used in the phylogenetic tree were obtained from 15/462 animals sampled in Germany and Costa Rica (Fritschi et al., 2020).

In Nigeria, Di Cataldo et al. (2020) reported 35% (32/90) of positivity for hemoplasmas in bats captured near human settlements in Nigeria. Eight 16S rRNA hemoplasma sequences were obtained from the animals belonging to the families Pteropodidae, Rhinolophidae and Molossidae. The authors reported five genotypes

related to bat and rodent-associated hemoplasmas and/or to 'Ca. *M. haemohominis*' previously detected in humans.

Hemoplasma sequences obtained from humans, bats and their associated bat flies showed to be highly similar to 'Ca. *M. haemohominis*' in a survey performed by Descloux et al. (2020) in New Caledonia. The samples were retrieved from 23 hospitalized patients, 40 *Pteropus* spp. bats and one bat fly (*C. horsfieldi*) (Diptera: Nycteribiidae).

More recently, Becker et al. (2020) showed a high genetic diversity among hemoplasma 16S rRNA sequences obtained from 239/469 bats belonging to 23/33 species sampled. The authors describe 21 novel genotypes and point out the occurrence of strong host specificity.

In Brazil, two works were performed regarding the detection of hemotropic *Mycoplasma* in bats. The first evidence was reported by our research group, in which 13.97% (45/322) samples collected from bats belonging to the Vespertilionidae, Phyllostomidae, Mollossidae, and Natalidae families sampled in five states in Brazil (Mato Grosso, Pará, Paraná, São Paulo, and Tocantins). Five 16S rRNA sequences were obtained from *M. molossus* individuals and showed to be a monophyletic group in the phylogenetic analyses. The second report has been recently published by dos Santos et al. (2020) that survey hemoplasmas in ten animals belonging to the *D. rotundus*, *D. ecaudata* and *Molossus* sp. species sampled in Paraná state. Eight showed to be positive and the sequences were grouped accordingly with the host species.

Considering such scenario, the assessment of the occurrence and diversity of hemoplasmas in bats and their associate ectoparasites becomes important, since some genotypes detected in chiropterans showed to be closely related to those previously detected in humans.

2.5 *Anaplasmataceae* agents

The bacteria belonging to *Anaplasmataceae* family (order *Rickettsiales*) are mandatory intracellular agents that have been incriminated as pathogens of veterinary and human importance. The family comprises the genus *Anaplasma*, *Ehrlichia*, *Neorickettsia*, and *Wolbachia* (Dumler et al., 2001; Cabezas-Cruz et al., 2016; André,

2018), being the latter known to be an endosymbiont. The genus *Anaplasma* comprises the species *Anaplasma bovis*, *Anaplasma centrale*, *Anaplasma marginale*, *Anaplasma capra*, *Anaplasma phagocytophilum*, *A. platys*, *Anaplasma ovis*, and a number of unclassified genovariants and ‘*Candidatus Anaplasma spp.*’ (Silaghi et al., 2017; Rar, Tkachev and Tikunova, 2021). *Ehrlichia* genus comprises the following described species: *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia canis*, *Ehrlichia muris*, *Ehrlichia ruminantium* and *Ehrlichia minasensis* (Cabezas-Cruz et al., 2016; Ismail and McBride, 2017).

Ehrlichia and *Anaplasma* species are Gram-negative and small bacteria that can infect erythrocytes, endothelial cells, and granulocytes or mononuclear phagocytes (Ismail and McBride, 2017). These bacteria are known as etiological agents of human and animal ehrlichiosis and anaplasmosis. *A. phagocytophilum* is the agent of the Human Granulocytic Anaplasmosis (HGA), *E. chaffeensis* causes the Human Monocytic Ehrlichiosis (HME), *E. ewingii* is known as the agent of the Human Ewingii Ehrlichiosis (HEE), *E. canis* and *E. ruminantium* also are known as cause of human ehrlichiosis (Ismail, Bloch and McBride, 2010; Rar and Golovljova, 2011). Although *A. phagocytophilum* is the main *Anaplasma* species involved in human disease, *A. platys*, *A. ovis*, *A. bovis* and *A. capra* were already related as zoonotic agents (Silaghi et al., 2017; Rar, Tkachev and Tikunova, 2021). Similarly, *E. canis*, *E. ruminantium* and *E. muris*-like have already described in humans, but represent a major concern in animal health (Ismail and McBride, 2017).

The HGA is the most reported human disease caused by *Anaplasma* sp. Actually, it is reported by the CDC that the occurrence of this disease increased from 348 cases in 2000 to around 1800 cases in 2010 (Ismail, Bloch and McBride, 2010).

Wang et al. (2020) evaluated data from 56 studies available on database that used IFA to detect anti-*A. phagocytophilum* antibodies in the studied populations. The authors divided the populations in healthy, febrile, high-risk (the ones that have contact with ticks daily), tick-bitten, and presenting clinical signs related to tick-borne diseases. The authors observed that the seroprevalence ranged from 0-37.26% based on reports acquired from 28,927 individuals retrieved from the 56 studies analyzed. The Europe and the USA showed to have a higher number of tick-borne disease reports than Asian countries. On the other hand, the highest seroprevalence to *A. phagocytophilum* was

found in a high-risk population in Mongolia. Nine cases of HGA were reported in children, whose age ranged from 9 days to 14 years old (Sigurjonsdottir, Feder Jr. and Wormser, 2017).

Both HGA and HME cause similar clinical symptoms that usually start on day nine after a tick bite event. The most common manifestations are fever, headache, and myalgia. The main hematological and biochemical abnormalities are thrombocytopenia, leukopenia, and an increase of liver enzymes. Neurologic manifestations seem to be more frequent in HME than HGA. Less commonly, anorexia, nausea, arthralgias, cough, and abdominal pain have also been described in HGA cases (Baken and Dumler, 2006; Ismail and McBride, 2017; Rar, Tkachev and Tikunova, 2021).

In animals, *A. phagocytophilum* is known to cause the tick-borne fever (TBF) that is characterized by fever, weakness, anorexia, abortion, and decreased production. Equine granulocytic anaplasmosis (EGA) is characterized by high fever, anorexia, ataxia, icterus, and edema in the lower limbs. While granulocytic anaplasmosis appears as mild fever, lethargy, and anorexia in dogs, infected cats can also present conjunctivitis and dehydration in cats in addition to those signs reported in dogs (Rar, Tkachev and Tikunova, 2021).

The main *Ehrlichia* species described causing disease in veterinary medicine is *E. canis* that causes the canine monocytic ehrlichiosis. The disease can be presented as acute, subclinical and chronic forms, in which the clinical signs are represented by fever, anorexia, vomiting, ocular/nasal discharge, ocular lesions, hepatosplenomegaly and dyspnea until death in chronic cases, where hemorrhage can occur due to thrombocytopathy (reviewed by Vieira et al., 2011).

While *A. marginale* can be transmitted biologically by Ixodidae ticks, mechanically by hematophagous dipterans and fomites, and transplacentally, other species of *Anaplasma* and *Ehrlichia* are mainly transmitted biologically by Ixodidae ticks and their occurrence is correlated with the presence of competent tick vectors and a reservoir host. The distribution of vectors and reservoirs also predicts the occurrence of the human disease (Vieira et al., 2011; Ismail and McBride, 2017).

Even though transovarial transmission of *A. phagocytophilum* in ticks of the specie *Dermacentor albipictus* (Packard, 1869) is reported, the same does not happen

nor with *Ixodes* spp. neither for *Ehrlichia* species (only transstadial from nymphs to adults), which leads to a major role of vertebrate hosts when compared to arthropods in the maintenance of the epidemiological cycle (Bakken and Dumler, 2006; Ismail and McBride, 2017; Rar, Tkachev and Tikunova, 2021).

Up to 20 tick species are involved in the transmission of *A. marginale* and *A. phagocytophilum* and the main tick genera are represented by *Ixodes* and *Haemaphysalis* (for *A. phagocytophilum*), *Dermacentor* (for *A. phagocytophilum* and *A. marginale*), *Rhipicephalus* and *Hyalomma* (for *A. marginale*). *A. centrale* and *A. platys* are mainly transmitted by *Rhipicephalus* spp.; *A. bovis* by *Haemaphysalis* spp. and less frequent by *Dermacentor* spp. and *Rhipicephalus* spp.; and *A. ovis* can be transmitted by *Dermacentor* spp., *Haemaphysalis* spp., *Hyaloma* spp., *Ixodes* spp. and *Rhipicephalus* spp. ticks (Silaghi et al., 2017; Rar, Tkachev and Tikunova, 2021).

While *E. canis* is known to be transmitted by the tropical lineage of *R. sanguineus sensu lato* (Moraes-Filho et al., 2015), *Amblyomma* spp. (Koch, 1844) ticks are related to the transmission of *E. ewingii*, *E. chaffeensis* and *E. ruminantium*. The ticks from genera *Dermacentor* and *Ixodes* can also have a role in the transmission of *E. chaffeensis*. *E. muris* is mainly transmitted by *Ixodes* spp. and *Haemaphysalis* spp. ticks (Ismail, Bloch and McBride, 2010; Rochlin and Toledo, 2020). *E. minasensis* was primarily isolated from *Rhipicephalus microplus* (Canestrini, 1888) and naturally infected cattle (Cabezas-Cruz et al., 2016). Differently from *E. canis* that causes disease in dogs, *E. minasensis* is incriminated to cause disease in cattle, but recently *Ehrlichia* sp. *dsb* sequences closely related to *E. minasensis* was detected in mammals from the superorder Xenarthra from Brazil (Calchi et al., 2020).

The diagnosis of *Anaplasma* and *Ehrlichia*-related diseases, specially HME and HGA is mainly done by clinical suspicion as rapid diagnostic tests are limited and early treatment is fundamental for a better prognosis. However, the infection can be confirmed by blood smears and PCR analysis at the onset of clinical signs; serological tests can be used at least four days after clinical illness or in a convalescent patient (Bakken and Dumler, 2006; Ismail and McBride, 2017). The blood smear examination by light microscopy is inexpensive and quick to be performed, but it is less sensitive, which may be a bias in early diagnosis. The diagnosis by blood smear examination depends not only on the experience of the examiner, but also on the bacteremia level

and number of infected cells. Indeed, molecular assays (PCR) has been increasingly used for surveillance and clinical diagnosis; for that purpose, whole blood and buffy coat represent the optimal samples for molecular screening. Even though it represents a more accurate tool, the sensitivity of such assays will depend on the type and quality of sample, bacterial load, number of copies of the targeted genes, and the fragment length. It is important to highlight that the species of each agent may have to be confirmed with additional assays, such as sequencing or specific Taq-Man based real-time PCR assays. Finally, *in vitro* cultures, except for *E. ewingii*, showed to be a useful tool for studying the biology of these bacteria, mainly *A. phagocytophilum*. Taking into consideration the laborious procedure that requires time and expertise to perform the isolation of these fastidious agents, this latter technique has been mainly used for research purposes (Dumler et al., 2007; Silaghi et al., 2017).

A sister clade to *Ehrlichia* has been described and named as “*Candidatus Neoehrlichia* spp.”. Despite it has not been considered a new genus, due to difficulty of culturing, five species belonging to this “group” have been reported in literature so far. “*Candidatus Neoehrlichia mikurensis*” was the first described in wild rats and in *Ixodes ovatus* (Neumann, 1899) ticks (Kawahara et al., 2004); “*Candidatus Neoehrlichia lotoris*” was described by Yabsley et al. (2008) in raccoons (*Procyon lotor* Linnaeus, 1758); “*Candidatus Neoehrlichia australis*” and “*Candidatus Neoehrlichia arcana*” were described in *Ixodes* ticks from Australia (Gofton et al., 2016); and “*Candidatus Neoehrlichia chilensis*” has been more recently described in rodents from Chile (Müller et al., 2018).

A wide description of uncultured agents has also been described in literature, such as “*Candidatus Ehrlichia walkerii*”, “*Candidatus Ehrlichia occidentalis*”, “*Candidatus Ehrlichia khabarensis*”, “*Candidatus Ehrlichia urmitei*” (provisionally), and “*Candidatus Ehrlichia ornithorhynchid*” (Brouqui et al., 2003; Gofton et al., 2017; Rar et al., 2015; Dahmani et al., 2017a; Gofton et al., 2018). In addition, despite authors did not propose a ‘Candidatus’ denomination (onward *Ehrlichia* sp. Magellanica), Muñoz-Leal et al. (2019) also detected a novel *Ehrlichia* spp. in penguins (*Spheniscus magellanicus* J.R.Forster, 1781) sampled in Chile. Similarly, regarding *Anaplasma* genus, “*Candidatus Anaplasma boleense*”; “*Candidatus Anaplasma mediterraneum*”, “*Candidatus Anaplasma corsicanum*”, “*Candidatus Anaplasma dongdaense*”;

“*Candidatus Anaplasma zhouziense*”, “*Candidatus Anaplasma sphenisci*”, and recently reported in Brazil “*Candidatus Anaplasma amazonensis*” and “*Candidatus Anaplasma brasiliensis*” have also been reported (Kang et al., 2014; Dahmani et al., 2017b; Guo et al., 2018; Vanstreels et al., 2018; Dahmani et al., 2019; Calchi et al., 2020).

Neorickettsia genus encompasses obligatory intracellular bacteria that, differently from the other *Anaplasmataceae* agents, are endosymbiotic within digeneans and depend on trematodes for their life cycle (Greiman, 2015).

The genus comprises essentially three species, namely *Neorickettsia risticii*, *Neorickettsia helminthoeca*, and *Neorickettsia sennetsu*, and seven genotypes described as SF (sennetsu fever-like) agent; EFF (Elokomin Fluke Fever) agent; Rainbow trout agent, *Neorickettsia* sp. from needlefish, *Neorickettsia* sp. from notothenoid, *Neorickettsia* sp. from catfish agent 1 and *Neorickettsia* sp. from catfish agent 2 (Vaughan, Tkach and Greiman, 2012). However, four diseases are reported caused by *N. risticii*, *N. helminthoeca*, *N. sennetsu*, and EFF agent. Recently, Teymournejad et al. (2020) has described a new species of *Neorickettsia* (i.e., *Neorickettsia findlayensis*) in Ontario, Canada, causing PHF disease based on serological evidence, whole genome sequencing and experimental infection of horses.

Holland et al. (1985) isolated the first *Neorickettsia* strain incriminated to cause an outbreak of a disease (known as equine monocytic ehrlichiosis or Potomac horse fever [PHF]) in horses in 1979, in the surroundings of the Potomac River that borders the Montgomery County in Maryland, USA. After the first outbreak, the number of cases increased, being reported in the states of Virginia, Pennsylvania, New Jersey, and Idaho. The authors described a disease in horses characterized by fever, depression, anorexia, edema in the hind limbs, laminitis, colic with mild to severe diarrhea, and leukopenia. Back then, the authors also reported more than 30% rate of mortality. They named this bacterium as *Ehrlichia risticii*, which would be re-classified later as *N. risticii* (Dumler et al., 2001). Even though Holland et al. (1985) suggested a possible arthropod vector for this agent, Barlough et al. (1998) suggested that the vector of this agent had an aquatic reservoir instead, by showing the presence of *N. risticii* DNA in freshwater operculate snails. In fact, the main transmission route occurs through aquatic snails that act as an intermediary host to a *Neorickettsia*-infected

digenean trematode. However, the epidemiological cycle of this agent turns out to be much more complex cycle as nine terrestrial and/or aquatic digenean families are implied on the *N. risticii* life-cycle, namely Dicrocoeliidae, Echinostomatidae, Fasciolidae, Heronimidae, Lecithodendriidae, Macroderoididae, Microphallidae, Plagiorchiidae and Schistosomatidae (Vaughan, Tkach and Greiman, 2012).

Recently, Paulino et al. (2020) detected *N. risticii* (16S rRNA) DNA in horses' blood samples sampled in Rio de Janeiro, Brazil. The positive animals presented hypoalbuminemia and high levels of creatine kinase and lactate dehydrogenase in the biochemical analysis. Previously, cases of the equine monocytic ehrlichiosis had also been reported in the state of Rio Grande do Sul (Dutra et al., 2001). In fact, snails collected from Rio Grande, Santa Vitoria and Arroio Grande, Rio Grande do Sul state, showed to be potentially involved in the PHF epidemiology. Based on PCR targeting the 16S rRNA gene, the authors found 13.33% (4/30) *N. risticii* positive snails belonging to the *Heleobia* genus (Stimpson, 1865) (*Heleobia piscium*, *Heleobia parchappii* d'Orbigny, 1835, *Heleobia davisii* Silva and Thomé, 1985). Also, cercariae of *Parapleurolophocercous cercariae*, which were identified in 16.66% (18/108) dissected snails from *H. piscium* snails, showed 20% positivity to *N. risticii* (Coimbra et al., 2006).

The sennetsu fever, which is caused by *N. sennetsu*, can cause an acute mononucleosis-like disease in humans with high fever, lymphadenopathy, malaise, anorexia and hepatosplenomegaly can occur (Vaughan, Tkach and Greiman, 2012). This agent has been frequently described in Japan and Asia. After a very long period of time in which none research had been done regarding the occurrence of *N. sennetsu* and associated disease, Newton et al. (2009) showed high seroprevalence to this agent among humans in Laos (17%) and Thailand (4%). More recently, Dittrich et al. (2015) identified and confirmed 0.2% of *N. sennetsu* in humans from Laos by culturing followed by molecular confirmation, suggesting that hospitalization with acute disease is rare.

It has been suggested that sennetsu fever is acquired by eating raw grey mullet fish (*Mugil cephalus* Linnaeus, 1758) containing *N. sennetsu*-infected metacercariae (Fukuda and Yamamoto, 1981). A digenean trematode species is probably involved in the transmission cycle of this agent, albeit other routes of transmission cannot be rule

out (Vaughan, Tkach and Greiman, 2012). Indeed, the main reservoirs, the zoonotic cycle and pathogenicity of *N. sennetsu* remain unclear (Dittrich et al., 2015).

N. helminthoeca causes an acute febrile and fatal disease known as “salmon poisoning disease” (SPD) in canids in the USA, Canada and Brazil (Headley et al., 2006; 2011). The Elokomin Fluke Fever (EFF), which is potentially caused by a specific *N. helminthoeca* strain, causes a similar disease regarding the clinical signs and pathological manifestations in canids and bears. *Nanophyetus salmincola* (Chapin, 1926) is a digenean trematode, vector of the SPD. It requires three hosts on the life-cycle: the snail *Oxytrema silicula*, an intermediate host that can be a salmonid or non-salmonid fish, and a definitive host represented by mammals and birds that feed on fish. The diagnosis of neorickettsiosis can be performed by the detection of *N. salmincola* infection searching for trematode eggs in the feces or the adult fluke within the intestine, direct microscopy, isolation, serology and molecular assays (Headley et al., 2011).

In Brazil, *Anaplasma* sp. closely related to *A. phagocytophilum*, *A. marginale*, *A. bovis*, *A. platys*, *E. canis*, *Ehrlichia* spp. closely related to *E. chaffeensis* and *E. minasensis*, and new potential species have been detected in different species of wild animals, such as deer (*Blastocerus dichotomus* Illiger, 1815, *Ozotoceros bezoarticus* subsp. *leucogaster* Goldfuss, 1817, and *Mazama gouazoubira* G.Fischer) (Machado et al., 2006; Picoloto et al., 2010; Sacchi et al., 2012; Silveira et al., 2012, 2013, 2014; Mongrue et al., 2016), wild canids (André et al., 2012; Almeida et al., 2013; de Sousa et al., 2017b; André, 2018), wild felids (André et al., 2010, 2012; Widmer et al., 2011; André, 2018); coatis (*Nasua nasua* Linnaeus, 1766) (de Sousa et al., 2017b; André, 2018); wild and synanthropic rodents (de Sousa et al., 2017b; Wolf et al., 2016; Benevenuto et al., 2017; Braga et al., 2018), caititus and peccaries (*Pecari tajacu* Linnaeus, 1758, and *Tayassu pecari* Link, 1795) (Soares et al., 2017a), opossums (*D. didelphis aurita* Wied-Neuwied, 1826) (Lopes et al., 2018; Guimarães et al., 2019), birds (Machado et al., 2012; Werther et al., 2017; Sacchi et al., 2021), and more recently in Xenarthra mammals (Calchi et al., 2020).

Regarding the occurrence of *Anaplasmataceae* agents in bats, the studies are scarce. For instance, Afonso and Goydadin (2018) found 22.7% (63/278) of molecular positivity for *A. phagocytophilum* (*msp-2* gene) in fecal samples collected from

Rhinolophus hipposideros (Bechstein, 1800) individuals in rural villages in France. Based on a qPCR based on *msp-2* gene, Hornok et al. (2019) detect *A. phagocytophilum* in bat-related ticks. Despite the authors did not show any sequencing or phylogenetic data, they reported the occurrence of positivity for *A. phagocytophilum* in 2 out of 138 *Ixodes simplex* ticks collected from *M. schreibersii* sampled in Hungary and Romania. Zabashta et al. (2019) reported the molecular evidence of DNA of *A. phagocytophilum* in 1/7 brain samples collected from *Nyctalus noctula* (Schreber, 1774), and *Ehrlichia* spp. in 9/19 and 1/6 brain samples collected from *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus kuhlii* (Kuhl, 1817), respectively, in Russia. Unfortunately, the authors did not show any sequencing or phylogenetic inference.

In bat-associated ticks, Socolovschi et al. (2012) found positivity for *Ehrlichia* sp. in three out of five *Argas vespertilionis* (Latreille, 1796) ticks collected from an attic infested with bats in France. From those, two were also positive for *Borrelia* sp., and one of these was also positive for *Rickettsia* sp. The obtained 16S rRNA and *gltA* sequences were positioned within the *E. canis* group. In Saint Kitt islands, Reeves et al. (2016) found 16% (4/25) of positivity for *Ehrlichia* sp. by PCR in whole blood samples collected from *B. cavernarum* bat species. Even though the authors were not able to compare the sequences obtained with other bat-related previously described due to the short length, they showed that the 4 obtained sequences were identical between each other and showed 95% identity with *Ehrlichia* sp. detected in ticks collected from mammals from Asia. More recently, Lv et al. (2018) collected ticks from bats sampled in different counties of England. They found DNA of *Ehrlichia/Anaplasma* in five out 120 *A. vespertilionis* ticks collected from three *P. pipistrellus* bats. The obtained sequences showed 99% identity with *Ehrlichia* sp. Yunnan and in the phylogenetic inference based on 16S rRNA gene clustered those sequences into a clade composed by *E. canis* and uncultured *Ehrlichia* sp. previously detected in *A. vespertilionis* in France.

Regarding the occurrence of *Neorickettsia* spp., bats have been implicated as important hosts in the *N. risticii* cycle (Pusterla et al., 2003; Gibson et al., 2005; Cicuttin et al., 2013). Pusterla et al. (2003) suggested bats play a role as possible reservoirs for *N. risticii* when investigated the occurrence of such agent in bats and digenetic

trematodes recovered from the sampled animals. Lung, heart, liver, spleen, kidney and intestine tissues were collected from five *Myotis yumanensis* (H.Allen, 1864) individuals in California, USA, which represents an endemic area for the PHF disease. Digenetic trematodes (*Acantharium* sp. and *Lecithodendrium* sp. Looss, 1896) were recovered from the sampled bats' gastrointestinal tract. PCR positivity for *N. risticii* was found in intestine and spleen from three and two bats, respectively. Also, two pools of the trematode *Acantharium* sp. (recovered from two bats) and one pool of *Lecithodendrium* sp. (recovered from one bat) tested positive for *N. risticii*. The sequences obtained showed to be identical to *N. risticii* strain Shasta identified in the same region of California state. The authors suggested that bats not only act as a host for trematodes but also as a possible natural reservoir for *N. risticii*.

Gibson et al. (2005), by using a nested PCR for *N. risticii*, found positivity in blood, liver or spleen samples of 23 out of 53 bats individuals (*E. fuscus* and *M. lucifugus* species) as well as trematodes (*Acantharium oregonense* Dana, 1853) collected from six *E. fuscus* bats sampled in Pennsylvania, USA. The sequences obtained clustered in the same clade of *N. risticii*. Also, the authors investigated the vertical transmission of *N. risticii* in the infected trematodes testing individual embryonated eggs randomly collected and found that three out of five eggs from one bat individual and four out of five eggs recovered from another bat. Not only the eggs were PCR positive for the agent, but also showed a positive reaction when incubated with anti-*N. risticii* horse serum, suggesting the occurrence of vertical transmission of *N. risticii* in the trematodes.

In South America, Cicuttin et al. (2013) collected liver, spleen and lungs fragments from 30 Brazilian free-tailed bats (*Tadarida brasiliensis* I.Geoffroy, 1824) sampled in urban areas of Buenos Aires city, Argentina. From those, three (10%) were positive on the PCR targeting the 16S rRNA gene for *Ehrlichia*, *Anaplasma* and *Neorickettsia* genera. The sequences obtained showed to be identical to *N. risticii* strain Illinois, which was confirmed by the phylogenetic analyses performed by the authors. Also in Argentina, Cicuttin et al. (2017) found 8.2% (5/61) positivity in tissue samples (pool of liver, spleen and lungs) collected from *T. brasiliensis* individuals. The authors obtained four *p51* gene sequences that showed to be identical to those previously detected in Argentina as described above and 99.7% with *N. risticii* strain

Illinois. Based on the phylogenetic assessment, the authors reported that the obtained sequences clustered with *N. risticii* found in the West Coast of the USA.

Greiman et al. (2017) surveyed digenean trematodes collected from 50 bats in Egypt and Mindoro Island, Philippines. The authors found the occurrence of eight unique genotypes of *Neorickettsia* sp. by qPCR assays based on *groEL* gene and confirmed by nested PCR also targeting the *groEL* gene. The sequences obtained from the digeneans (*Lecithodendrium* sp.) collected from bats clustered as a monophyletic group between *N. helminthoeca* and *N. sennetsu*, and within the *N. risticii* clade.

In Europe, Hornok et al. (2018) evaluated 196 individuals and 25 pooled fecal samples were collected from *Myotis* spp., *Pipistrellus* spp., *Nyctalus* spp., *Barbastella* spp., *Plecotus* spp., *Rhinolophus* spp., *Miniopterus* spp. bats from Hungary and Netherlands. The authors found positivity in 2.96% (4/135) of *Myotis dasycneme* (Boie, 1825) individuals sampled in Netherlands for *N. risticii* using PCR targeting the 16S rRNA gene. The identity and phylogenetic analyses showed that the sequences obtained were similar and clustered with *N. risticii* detected in horses in the USA, and *N. risticii* identified in bat-associated flukes and other bats previously described.

Even though the only two works that aimed to investigate the occurrence of *Anaplasmataceae* agents in bats from Brazil did not find positive results (dos Santos et al., 2013; Ferreira et al., 2018), there is a need for additional surveys considering the huge diversity of bats in the Brazilian territory.

2.6 Coxiellaceae agents

The *Coxiellaceae* family (*Legionellales* order) comprises Gammaproteobacteria, including *Coxiella burnettii*, *Coxiella cheraxi* and *Coxiella*-like microorganisms that have been described in mammals, birds and nonvertebrate species (Bielawska-Drózd et al., 2013; Duron et al., 2015; Mori et al., 2017). Previously described as a rickettsiae agent, *C. burnettii*, which is the etiological agent of Q fever, is a pleomorphic Gram-negative coccobacillus with capacity of pseudosporulation, favoring a great resistance and survival in the environment (Bielawska-Drózd et al., 2013). The bacteria present two forms that are related to a biphasic cycle: Large Cell Variant (LCV), that represents the replicating form, and Small Cell Variant (SCV), that is the non-replicating form (Bielawska-Drózd et al., 2013; Eldin et al., 2016).

Q Fever is worldwide reported except in New Zealand and represents an important zoonotic disease. Even though the disease was firstly described in Australia in 1935 and called as Query Fever, only in 1948 it was possible to identify *Coxiella* as the causal agent of this illness (Marrie, 2003; Damasceno and Guerra, 2018). Cattle, sheep and goats are incriminated as the main reservoirs for *C. burnetii*. The human infection is associated with inhalation of aerosols as the main route of transmission. The aerosolization of the bacteria can happen when an infected animal delivers or aborts. Considering that transmission from person-to-person or by contaminated blood rarely occurs, the epidemiology of the disease shows a strong association with the presence of this agent in primary reservoirs (Marrie, 2003; Eldin et al., 2016). Although the presence of *C. burnetii* is reported in wildlife, wild animals do not play an important role as the main source of infection, albeit contributing for the maintenance of this agent in the ecosystems (Kazar, 2005).

Q Fever can show different clinical manifestations depending on the transmission route. While acute Q fever is characterized by fever, pneumonia, hepatitis, anorexia, headache, cough, chills, chest pain, rigors, myalgia, arthralgia and confusion, the chronic phase appears as endocarditis or hepatitis in humans (Marrie, 2003). In domestic ruminants, which represent the main reservoirs, *C. burnetii* can lead to reproductive disorders, such as infertility, abortion, endometritis and mastitis, with subsequent production lost. The infected animals shed the bacteria in urine, vaginal mucus, feces, milk or birth products (Eldin et al., 2016).

Even though the main and most important transmission route is represented by aerosol/inhalation, *Coxiella* has been isolated/detected from more than 40 Ixodidae tick species of the following genera: *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma* (Koch, 1844), *Ixodes*, *Rhipicephalus*; and at least 14 Argasidae tick species of the genera *Argas*, *Ornithodoros* and *Otobius* (Banks, 1812); as well as from bed bugs (*Cimex lectularius* Linnaeus, 1758); flies (*Musca domestica* DeGeer, 1776); Mesostigmata mites; fleas (*C. felis*); and mosquitoes (*A. aegypti*) whose role in the transmission is not clear (Duron et al., 2015; Eldin et al., 2016). González et al. (2019) suggested strong evidence of transstadial and transovarial transmission of this agent by *Hyalomma lusitanicum* (Koch, 1844), but emphasized that more assays should be performed to confirm such vector competence. Despite that, it is known that the

majority of these isolates are endosymbionts, which are able to enhance or reduce the infection by other tick-borne pathogens (Machado-Ferreira et al., 2011, 2016; Almeida et al., 2012; Boumedine et al., 2015; Guizzo et al., 2017). Indeed, the vector capacity of ticks can be substantially different under laboratory conditions when compared to what really happens in the environment. In fact, the role of ticks in the transmission of *C. burnetii* seems to serve as a bridge between the bacteria and the mammal reservoirs (Boumedine et al., 2015).

After 80 years from the first report of a Q Fever case, the diagnosis of this disease is still challenging. The main surveillance method is based on serological assays, such as indirect immunofluorescence (IFA) or immunoenzymatic assay (ELISA). Despite that, molecular techniques have also been used for diagnosis, allowing differentiation of different lineages. Isolation *in vitro* should be performed in safety level 3 laboratories using cell lines. Also, embryonated hen egg inoculation diagnosis can be used for detection of *C. burnetii* (Bielawska-Drózd et al., 2013; Mori et al., 2017).

In Brazil, the first report of Q fever occurred in 1953, but since then publications on the disease in the country remain scarce (Damasceno and Guerra, 2018). In fact, evidence shows that the occurrence of *C. burnetii* in Brazil has a public health concern that deserves more attention. Lemos et al. (2018) reported five cases of Q fever in cadets participating in a Firefighters Military Academy training program in the Rio de Janeiro state. The patients were 20-25 years old males with historical of tick bites and participation in slaughtering animals for self-consumption. They presented febrile illness and one of them had severe pneumonia. The authors confirmed molecularly the presence of *C. burnetii* in serum samples and highlighted the concern on this matter.

According to the Centers for Disease Control and Prevention (CDC), people who work directly with animals are at higher risk to be infected with *C. burnetii*. The high-risk jobs include farmers, slaughterhouse workers, veterinarians and animal researchers. In fact, serological evidence in slaughterhouse employees has been reported in Brazil (Rieman et al., 1975). Recently, Mioni et al (2020) surveyed nine slaughterhouses for assessing the prevalence of *C. burnetii* using an immunofluorescence assay (IFA) and quantitative real-time PCR (qPCR) in São Paulo state. Out of 1,515 cattle, 360 (23.8%) were serologically reactive to the agent and 44

(12.2%) were qPCR-positive. Impressively, 83.3% out of the 54 cities that were surveyed had at least one seropositive animal. This work showed the exposure that workers are subject and point out the need of good preventive measures in these places.

Even more concerning, *C. burnetii* DNA has been detected in raw cow's milk artisanal cheeses sold to human consumption (Mioni et al., 2019; Rozental et al., 2020; Nascimento et al., 2021). In the Goiás state, Brazil, Mioni et al. (2019) collected a total of 112 raw milk samples from grocery stores, bars, farmer's markets and small farms in 13 municipalities. Using real-time qPCR assay targeting IS1111 gene, the authors detected 3.57% (4/112) of positivity. Strikingly, population consumes these products sold without official inspection or pasteurization. Rozental et al. (2020) evaluated 53 cheese samples from manufacturing agroindustries and five (9.43%) were positive for *C. burnetii* based on a molecular assay. The authors estimated that 1.62 tons/day of cheese made from the raw-milk produced daily (16.2 tons) in the region may be contaminated. Nascimento et al. (2021) evaluated not only the occurrence of *C. burnetii* DNA in artisanal cheeses, but also the consumers' profile. Out of 87 samples of artisanal cheese made from unpasteurized raw milk, 4.6% (4/87) showed to be positive by qPCR based on the IS1111 gene. Two of those samples also amplified by nested PCR targeting the 16S rRNA gene and the obtained sequences were 99.84% similar with *C. burnetii*. The samples were collected in Minas Gerais state, southeastern Brazil, which is known for the appreciation for dairy products evidenced in the consumers profiling. Out of 150 interviewed individuals, 95.3% (143/150) consumed milk and dairy products; 50% (75/150) consumed fresh "Minas artisanal cheese" and milk at least once a day; 64.58% (93/144) did not check if the product was inspected, which is shown with stamps on the packaging; and 81.56% (122/146) did not know the meaning of these stamps.

Even though the first molecular evidence of *C. burnetii* circulation in humans in Brazil occurred in 2011 (Lemos et al., 2011), serological evidence of exposure to *C. burnetii* has been reported in veterinary colleges staff (Rieman et al., 1974), rural community (Costa, Brigatte and Greco, 2006), patients with febrile illness, patients with culture-negative endocarditis (Siciliano et al., 2008; Lamas et al., 2013; 2016), in HIV-positive patients (Lamas et al., 2009), in people with clinical suspicion of Dengue

(Mares-Guia et al., 2016), and more recently in drug addict individuals (Rozental et al., 2018).

Even with all these previous studies, the disease still does not require mandatory notification to the authorities. So it is believed that Q fever is more common than it is actually recognized in Brazil (Damasceno and Guerra, 2018).

In Brazil, Rozental et al. (2017) detected *C. burnetii* DNA in 4.6% (6/131) of wild rodents sampled in Atlantic Forest in Rio de Janeiro. In Caatinga biome, de Oliveira et al. (2020) found one rodent (*Wiedomys pyrrhorhinos* Wied-Neuwied, 1821) and one marsupial (*D. albiventris*) seroreactive to *C. burnetii* but negative when tested by PCR. Similarly, Zanatto et al. (2019) found 5% (9/169) of seropositivity for *C. burnetii* in neotropical deer from two different species: 8% (2/25) among *M. gouazoubira* individuals, and 5.43% (7/129) among *B. dichotomus* individuals, despite the negative results in nested PCR assays performed on deer blood samples.

When it comes to bats, *C. burnetii* DNA has already been detected in *I. verpertilionis* ticks collected from bats in northeastern Algeria. A fragment of the *C. burnetii* IS1111 gene was amplified in 15.8% (3/19) of tick samples collected from a total of 10 bats (Leulmi et al., 2016).

Diettrich et al. (2017) evidenced the occurrence of *Coxiella* in feces of one *Nycteris thebaica* (E.Geoffroy, 1818) bat when investigating the bacterial community in saliva, urine and feces collected from insectivorous bats, namely *N. thebaica*, *Miniopterus natalensis* (A.Smith, 1833), *Rhinolophus simulator* (K.Andersen, 1904) and *Neoromicia capensis* (A.Smith, 1829). Additionally, to previous studies with gut microbiota, the information about other excretion routes, such as saliva and urine, can contribute to understanding the microbiome diversity and the transmission of potential pathogenic agents.

In Brazil, a study carried out with 23 (20 nymphs and 3 adult females) specimens of *Ornithodoros rostratus* (Aragão, 1911) ticks (adults, nymphs, and eggs) collected in the Pantanal region showed 100% of positivity in the PCR assays for *Coxiella* based on 16S rRNA, *pyrG* and *cap* genes. The phylogenetic analysis showed that the organism detected was related to *Coxiella* symbionts of soft ticks (Almeida et al., 2012). However, in another study performed with 20 *O. mimon* tick nymphs, commonly found parasitizing bats and collected from bat-infested attics from the state of Minas Gerais,

showed no positivity when submitted to PCR assays targeting three genes of *C. burnetii* (16S rRNA, *pyrG* and *cap*) (Labruna et al., 2014).

In Chile, the first report of *C. burnetii* in bats has been recently published. Müller et al. (2020) tested a total of 55 bats belonging to the species *M. chiloensis* (n=27), *H. montanus* (n=8), *Lasiurus varius* (Poeppig, 1835) (n=7), *H. macrotus* (n=3), *Lasiurus cinereus* (Palisot de Beauvois, 1796) (n=1), and *T. brasiliensis* (n=9). By real-time PCR (qPCR) targeting the IS1111 gene, DNA from *C. burnetii* was found in 5 individuals (9%) of *T. brasiliensis*. The authors also emphasized the lack of research on this agent as it is the first report of *C. burnetii* in wild animals in Chile.

The first and unique study reporting the occurrence of *Coxiella* spp. in bats specimens from Brazil was conducted by Ferreira et al. (2018), in which a positivity of 3.4% (4/119) for the *htpAB* gene of *C. burnetii* was found in spleen (n=2), liver (n=1) and heart (n=1) tissues. The obtained sequences showed 100% identity with *C. burnetii*. The positive animals belonged to *A. lituratus* (3/4) and *A. fimbriatus* (1/4) species collected in Rio de Janeiro and Santa Catarina states. In agreement with the authors, the role of bats in the *Coxiella* cycle and epidemiology is poorly accessed and neglected in Brazil.

2.7 Piroplasmids

Piroplasmids are apicomplexan protozoa transmitted by ticks that comprise the genera *Babesia*, *Rangelia*, *Cytauxzoon* and *Theileria* (Yabsley and Shock, 2013) and were named as “piroplasms” due to their pear-shaped appearance (Uilenberg, 2018). Depending on the species, they parasitize blood cells from wild and domestic vertebrates, including erythrocytes, lymphocytes, and monocytes, as well as endothelial cells. Such agents can cause serious diseases in farm or companion animals, wild mammals and, occasionally, humans (Alvarado-Rybak et al., 2016).

Babesiosis represents a zoonotic disease transmitted by hard ticks (Ixodidae) from animal reservoirs to humans. Although there is a report of the deer-related *Babesia odocoilei* infecting humans (Scott et al., 2021), the main species involved in human babesiosis are *Babesia microti*, *Babesia divergens*, *Babesia duncani* and *Babesia venatorum* (Krause, 2019; Young et al., 2019). Besides ticks' bite transmission, blood transfusion and transplacental can also represent routes of

infection. In fact, the pathogen that is most commonly transmitted by blood transfusion in the USA is *B. microti* (Vannier et al., 2015; Krause, 2019). However, most of the cases match with summer in areas with endemic tick vectors and reservoirs (Vannier et al., 2015). Since there is an important tick route of transmission, co-infection with other vector-borne pathogens should be considered. Lyme disease, ehrlichiosis, anaplasmosis, bartonellosis and rickettsioses are commonly found together with babesiosis (Young et al., 2019). Clinical signs can range from mild to severe starting with fatigue followed by fever and then anorexia, chills, headache, myalgia, nausea, cough, and arthralgia. Complicated cases can involve respiratory syndrome, pulmonary edema, intravascular coagulation, multiple organs failure, until coma and death. The mortality rate is high in immunocompromised patients or people with comorbidities (Vannier et al., 2015; Krause, 2019).

In dogs, babesiosis is caused by *Babesia canis*, *Babesia vogeli*, *Babesia conradae*, *Babesia rossi* and *Babesia gibsoni*. Recently, Baneth et al. (2020) described *Babesia negevi* n. sp. parasitizing dogs with fever, anemia, thrombocytopenia and icterus. While *B. vogeli* is more widespread in Latin America, *B. rossi* and *B. canis* are not reported in this area, which can be related to the absence of the tick vectors *Haemaphysalis elliptica* (Koch, 1844) / *Haemaphysalis leachi* (Audouin, 1826) and *Dermacentor reticulatus* (Fabricius, 1794), respectively (Ayoob et al., 2010; Panti-May and Rodríguez-Vivas, 2020). *B. vogeli* has been reported in Brazil affecting dogs since early 2000's, being probably introduced with its vector, *R. sanguineus* (Dantas-Torres and Figueredo, 2006; Maggi and Krämer, 2019; Panti-May and Rodríguez-Vivas, 2020). *B. gibsoni* has been scarcely reported in dogs in Brazil. While *B. vogeli* is mainly transmitted by tick bites, *B. gibsoni* can be transmitted by animal aggressive interactions, blood transfusion and transplacental (Maggi and Krämer, 2019). The infected animals usually show regenerative hemolytic anemia, leukocytosis, monocytosis, and thrombocytopenia (Dantas-Torres and Figueredo, 2006). The disease causes a plethora of clinical signs, such as fever, pallor, anorexia, splenomegaly, and vascular disturbances, which can be mild to severe. Some clinical signs can appear without association with hemolytic anemia, such as cardiovascular, respiratory, hepatic, renal, gastrointestinal, neurologic, and coagulopathic dysfunctions (Ayoob et al., 2010).

Another significant disease associated with severe anemia in dogs is caused by the piroplasmid known as *Rangellia vitalii*. Firstly, called as “Nambiuuvú”, which means bloody ears, rangellosis is a common disease in rural areas and has been reported only in South America (Loretti and Barros, 2005; França et al., 2014). It is known that *R. vitalii* is naturally transmitted by the tick *Amblyomma aureolatum* (Pallas, 1772) (Soares et al., 2018). However, the occurrence of the agent in a specimen of *A. sculptum* collected from a wild canid (*Cerdocyon thous* Linnaeus, 1766) has been recently reported, although it was not possible to evaluate if the host was positive as well (Campos et al., 2018). The clinical signs caused by *R. vitalii* in dogs are persistent bleeding, especially in the pinnae surface, in the oral cavity, nares, and eyes, with petechiation, mucous palled and yellowish, fever, apathy, dehydration, weight loss, lymphadenomegaly, hepatosplenomegaly, haematemesis and dark diarrhea, which indicates the presence of digested blood. The laboratory findings are characterized by regenerative anemia, spherocytosis, and icteric plasma with bilirubinuria (Loretti and Barros, 2005; França et al., 2014).

The Equine piroplasmosis is known to be caused by *Theileria equi* and *Babesia caballi*. Even though more than 30 species of ticks are described as possible vectors, the transovarian transmission is recorded in ticks only for *B. caballi*, which implies in the maintenance of this parasite in the environment. For *T. equi*, iatrogenic transmission is also possible through needle sharing, blood transfusion or surgical instruments, whereas *B. caballi* is only transmitted by tick vectors (Tirosh-Levy et al., 2020). Transstadially and intrastadially transmission occurs in *T. equi* infected ticks as the parasites move from midgut to the salivary glands. However, in the case of *B. caballi* it is more common to be transmitted transovarially, which gives it a longer permanence in the environment (Scoles and Ueti, 2015). The clinical signs are derived from the hemolytic anemia, such as fever, inappetence, icterus, hemoglobinuria, pale mucous, tachycardia and tachypnea. Usually, thrombocytopenia is found and edema with hemorrhage can occur (Tirosh-Levy et al., 2020).

In bovines, babesiosis, which is caused by *Babesia bovis* and *Babesia bigemina*, is the most important tick-borne disease, especially due the economic losses on cattle industry. Both are transmitted by *R. (Boophilus) microplus* and worldwide distributed mainly in Australia, Africa, South and Central Americas. Even though the disease

represents a challenge to cattle breeders, it is known that *Bos indicus* in *Babesia*-endemic region can have a natural resistance. When the exposure to *Babesia* spp. occurs in early ages in areas with a high vector population, an endemic stability phenomenon may take place (Bock et al., 2005). Also, *Babesia orientalis*, which is transmitted by *Rhipicephalus haemaphysaloides* (Supino, 1897), is known to cause disease in water buffaloes; *Babesia motasi* and *Babesia ovis* are known to infect sheep and are transmitted by *Rhipicephalus bursa* (Canastrini and Fanzago, 1878) and *Haemophysalis* sp. ticks, respectively (Uilenberg, 2018).

Feline babesiosis occurs mostly in South Africa, where *Babesia felis*, *Babesia leo* (firstly described in lions [*Panthera leo* Linnaeus, 1758]), and *Babesia lengau* (firstly described in cheetahs [*Acinonyx jubatus* Schreber, 1775]) comprise the three main pathogenic babesias for cats (Penzhorn and Oosthuizen, 2020). Recently, a new piroplasm associated to feline babesiosis was identified in Western Cape Town (Bosman et al., 2019). *B. lengau* is associated to severe clinical cases in domestic cats, mainly cerebral and hemolytic babesiosis (Bosman et al., 2013). Moreover, other species of *Babesia* can affect cats but usually are not associated with clinical disease, such as *Babesia vulpes* in cats from Portugal and France, and *B. microti* in cats from Pakistan, South Africa and Italy. *Babesia lohiae* was already identified in *Ixodes holocyclus* (Neumann, 1899) from a cat, but not associated with disease in Australia (Greay et al., 2018). In Hong Kong, *Babesia hongkongensis* was detected in a stray cat (Wong et al., 2012). The canid-associated *B. canis* and *B. vogeli* is also described in cats eventually (Dantas-Torres and Figueredo, 2006; Maggi and Krämer, 2019; Penzhorn and Oosthuizen, 2020). Domestic cats from Brazil can be infected by *B. vogeli* (André et al., 2015; Malheiros et al., 2016) and *Theileria* spp. closely related to *T. equi* and ruminant-associated *Theileria* spp. (André et al., 2014; 2015). The clinical outcome of *Babesia/Theileria* infection in cats is still poorly understood in Brazil (André et al., 2015).

Cytauxzoon felis is the etiological agent of feline cytauxzoonosis. In the USA, it is transmitted by *Dermacentor variabilis* (Say, 1821) and *Amblyomma americanum* (Linnaeus, 1758). The wild bobcat *Lynx rufus* (Schreber, 1777) is considered the main reservoir in the USA (Boes and Durham, 2017). In Brazil, wild felids have been incriminated as potential reservoirs for *Cytauxzoon* sp. closely related to *C. felis* (André

et al., 2009). *Cytauxzoon manul* parasitizes Pallas cats (*Otocolobus manul* Pallas, 1776) in Mongolia (Reichard et al., 2005). Following the same pattern of other vector-borne diseases, cytauxzoonosis also occurs seasonally related with the tick activity. In domestic cats, the disease shows to be highly fatal and the clinical signs include fever, pale mucous membranes, icterus, and hepatosplenomegaly, mainly due to the intravascular hemolysis. Ataxia, nystagmus, abnormal vocalization and dyspnea can also occur (Meinkoth and Kocan, 2005; Cunningham and Yabsley, 2012). The laboratory findings show non regenerative anemia, leukopenia, thrombocytopenia, hyperbilirubinemia, and hypoalbuminemia (Meinkoth and Kocan, 2005). Recently, three new species of *Cytauxzoon*, namely, *Cytauxzoon europaeus* n. sp., *Cytauxzoon otrantorum* n. sp., and *Cytauxzoon banethi* n. sp., have been described in wild felids in Europe (Panait et al., 2021).

The infections caused by piroplasmids can be diagnosed by the historical contact with possible vectors, clinical signs and laboratory findings. The agent can be identified by microscopy in blood smears stained with Wright-Giemsa, serologically by Immunofluorescence Assay (IFA) and Enzyme-Linked Immunosorbent Assay (ELISA) with specific standardized protocols, or by molecular approaches (Dantas-Torres and Figueredo, 2006; França et al., 2014; Krause, 2019; Panti-May and Viva, 2020; Tirosh-Levy et al., 2020).

In Brazil, studies have been conducted aiming to verify the occurrence and molecularly characterize piroplasmids in wild animals. *C. thous* and *Lycalopex gymnocercus* (G.Fischer, 1814) are pointed out as wild reservoirs for *R. vitalii* (Soares et al., 2014, Fredo et al, 2015; Silva et al., 2018; de Souza et al., 2019), even though there were reports of mortality related to this disease in *Chrysocyon brachyurus* (Illiger, 1815) and *C. thous* individuals (Silveira et al., 2016; Copat et al., 2019). *Theileria cervi* has been detected in *Mazama americana* (Ercleben, 1777), *M. gouazoubira*, and *B. dichotomus* (da Silveira, Rabelo and Ribeiro, 2011; Soares et al., 2017b), whereas *Theileria* sp. closely related to *T. equi* has been reported in wild rodents, *N. nasua*, and *Tapirus terrestris* (Linnaeus, 1758) (da Silveira et al., 2017; de Sousa et al., 2018; de Souza-Gonçalves et al., 2020). *Theileria* spp. has also been molecularly detected in *Dasypus novemcinctus* (Linnaeus, 1758), *Dasyprocta* sp. (Illiger, 1811), and *Cuniculus paca* (Linnaeus, 1766) (Soares et al., 2017b), and *Babesia* spp. in *Didelphis*

marsupialis (Linnaeus, 1758) and *T. pecari* (Soares et al., 2017b). *Babesia* sp. closely related to *B. leo* was molecularly detected in pampas cat (*Leopardus colocolo* Molina, 1782) and genets (*Genetta genetta* Linnaeus, 1758) maintained in captivity (André et al., 2011b). *B. vogeli*-related 18S rRNA sequences have been detected in goose (*Neochen jubata* Spix, 1825) and wild rodents (Werther et al., 2017; de Sousa et al., 2018). A piroplasmid closely related to *B. caballi* was detected in *C. thous* in the Brazilian Pantanal (de Sousa et al., 2018). *B. bovis* and *B. bigemina* were already detected in *M. gouazoubira* and *B. dichotomus* (da Silveira, Rabelo and Ribeiro, 2011; de Sousa et al., 2018). Finally, *Cytauxzoon* sp. has been detected in captive and wild felids (André et al., 2009; Soares et al., 2017b; Furtado et al., 2017; de Sousa et al., 2018; Antunes et al., 2018).

In bats, *Babesia* sp. was firstly described in blood smears from *N. noctula* and *P. pipistrellus* individuals in Italy in the years of 1898 and 1899 by Dionisi, who called it as *Babesia vesperuginis*. Gardner and Molyneux (1987) showed the infectivity of the parasite in *P. pipistrellus* bats by inoculation of infected blood to 5 naïve animals. In the necroscopic analysis, the authors observed anemia, higher levels of white blood cells and esplenomegaly, indicating the pathogenicity of *B. vesperuginis* in bats.

Almost one century after the first description of *B. vesperuginis*, Marinkelle (1996) was the first to morphologically characterize this *Babesia* in the Americas, by examining blood smear obtained from *Mormoops megalophylla* (Peters, 1864) bats sampled in Colombia.

The first molecular characterization of piroplasmids in bats was performed by Concannon et al. (2005) that investigated the presence of DNA from hemoparasites of the order Piroplasmida in spleen samples from 60 bats belonging to seven different species in Cornwall, UK. Six (10%) specimens of *Pipistrellus* spp. were positive for *B. vesperuginis*.

Hornok et al. (2015) collected 221 bat feces samples belonging to 19 different species in Hungary and the Netherlands. Such samples had their DNA extracted and were tested for the presence of piroplasmids DNA by PCR assays based on 18S rRNA gene. Five species of bats sampled in Hungary were PCR positive, namely *N. noctula* (1/21), *M. daubentonii* (1/49), *Pipistrellus pygmaeus* (Leach, 1825) (1/1), and *Myotis*

alcathoe (von Helversen and Heller, 2001) (1/23). Sequencing of an 18S rRNA gene fragment from piroplasmids showed 100% identity to *B. canis*.

In Europe, Corduneanu et al. (2017) confirmed the presence of *B. vesperuginis* in cardiac tissue samples obtained from bats. In this study, 461 bat carcasses belonging to 24 species and three families (Miniopteridae, Rhinolophidae and Vespertilionidae) were collected from four different countries in Eastern and Central Europe, namely Austria, Czech Republic, Hungary and Romania. The PCR assays based on 18SrRNA and *cox-1* genes revealed the presence of piroplasmid DNA in 20 of the 461 bats (4.34%). *B. vesperuginis* was detected in specimens of *M. alcathoe* (1/12), *M. bechsteinii* (1/4), *M. myotis* (1/6), *N. noctula* (4/246), *Pipistrellus nathusii* (Keyserling and Blasius, 1839) (3/28), *P. pipistrellus* (6/71) and *Vespertilio murinus* (Linnaeus, 1758) (4/23).

In China, a total of 146 bats belonging to six species (*R. ferrumequinum*, *Rhinolophus pusillus* Temminck, 1834, *Eptesicus serotinus* Schreber, 1774, *Myotis fimbriatus* Peters, 1871, *Myotis ricketti* Thomas, 1894 and *Myotis pequinius* Thomas, 1908) belonging to the Rhinolophidae and Vespertilionidae families were captured. Of these, 107 had DNA samples obtained from aliquots of blood tested for the presence of *Babesia* spp. DNA. Two of 15 *E. serotinus* blood samples (2/15, 13.3%) were positive for *Babesia* spp. BLAST analysis of 18S rRNA sequences showed 99.4% identity with *B. vesperuginis* (Hui-Ju et al., 2018). Also in China, Han et al. (2018) sampled insectivorous bats from six species belonging to two families in the Shandong Province. A positivity of 13.3% (2/15) in *E. serotinus* was found in PCR assays based on 18S rRNA and *cox-1* genes. The sequences obtained showed to be similar with *B. vesperuginis*. Also in China, 98 *P. pipistrellus* bat carcasses (45 from Shihezi city and the remaining from Xinyuan county) were tested for the presence of a fragment of the 18S rRNA gene from piroplasmids. *B. vesperuginis* was detected in 10% (5/48) of bats from Shihezi City, northwestern China. Additionally, four (4/21; 19%) *A. vespertilionis* ticks collected from the positive bats sampled in Shihezi were also positive for *B. vesperuginis* (Liu et al., 2018).

More recently, Ranaivoson et al. (2019) reported the unprecedented *Babesia* detection in pteropodid fruit bats. A total of 203 *P. rufus* were sampled in Madagascar.

Based on PCR assays targeting 18S rRNA gene, nine (4.43%) were found infected with piroplasmids. The sequences obtained showed to be closely related to *B. microti*.

Regarding bat-related ectoparasites, Hornok et al. (2016) detected piroplasmids DNA (18S rRNA) in 20 (15 larvae, four nymphs and one adult female) out of 308 ticks collected in 200 bats belonging to 17 different species. Fifteen percent (4/26) of *Ixodes ariadnae* (Hornok, 2014) larvae were positive only for *B. vesperuginis*. Three percent of *I. vespertilionis* larvae (3/89) were positive for *B. vesperuginis* (2/89; 2.24%) and *B. crassa* (1/89; 1.12%). Ten percent of *I. simplex* larvae (8/79) were positive for *Babesia* spp., *B. crassa* (1/79; 1.26%), *B. venatorum* (1/79; 1.26%), *B. canis* (1/79; 1.26%), *Theileria capreoli* (1/79; 1.26%) and *Theileria orientalis* (4/79; 5.06%). Eight percent of the nymphs of *Ixodes simplex* ticks (4/50) were positive for *B. crassa* (1/50; 2%) and *B. canis* (3/50; 6%). Eleven percent of female of *Ixodes simplex* (1/9) were positive for *Theileria* spp. The piroplasmid DNA sequences detected in ticks possibly originated from the blood of their respective chiropteran hosts, emphasizing the fact that bats appear to be susceptible to infection by a wide range of piroplasmid species.

Hornok et al. (2017) collected 321 *A. vespertilionis* larvae from 15 bat species in Hungary, Romania, Italy, Kenya, and Vietnam and 11 *V. murinus* in northwestern China (Xinjiang province). Twelve out of 193 DNA extracts obtained from the tick samples showed to be positive for piroplasmids, ten collected from bats in Hungary and two from bats collected in China. In Hungary, PCR-positive *A. vespertilionis* larvae based on a fragment of the 18S rRNA gene for piroplasmids were collected from specimens of *E. serotinus* (5/12; 41.66%), *Plecotus austriacus* (J.Fischer, 1829) (4/6; 66, 6%) and *P. pipistrellus* (1/1; 100%). In China, two *A. vespertilionis* larvae (2/11; 18.18%) collected from specimens of *V. murinus* bats were PCR positive for piroplasmids. The sequencing of the amplicons obtained showed 100% identity with *B. vesperuginis*.

In England, Lv et al. (2018) collected 120 bat ticks, which were molecularly tested for the presence of zoonotic pathogens. The bats captured belonged to the species *M. daubentonii*, *P. pipistrellus*, *Plecotus auratus* (Linnaeus, 1758), *M. natteri*, *E. serotinus*, *Myotis mystacinus* (Kuhl, 1817), *N. noctula* and *R. hipposideros*. Piroplasmid DNA fragments (18S rRNA) were detected in three (2.5%) ticks of the species *A. vespertilionis*. Two ticks collected from *P. pipistrellus* showed the presence of a

piroplasmid DNA with 100% identity with *B. vesperuginis*. On the other hand, a tick collected from *P. auritus* showed the presence of DNA from a piroplasmid with 99% identity with *B. venatorum*.

Hornok et al. (2017) investigated the phylogenetic positioning of *B. vesperuginis* analyzing the *cox-1* gene separately and concatenated with 18S rRNA gene. The results showed that *B. vesperuginis* is more phylogenetic related to piroplasmids from the Theileriidae group than to those from the *Babesia sensu stricto* group. *B. vesperuginis* was placed in a separate clade, but closely related to *C. felis* and *B. conradae*. The *cox1* sequence of *B. vesperuginis* showed greater identity with *C. felis* (79.1%; 709/896 bp) and lower identity with *Babesia* spp. (74.9 for *B. bigemina* and 77.6% for *B. canis*) and *Theileria* spp. (74.2 for *Theileria sinensis* and 77.5% for *Theileria luwenshuni*).

3. Objectives

3.1 General objectives

The present study aimed to investigate the molecular occurrence and diversity of bacteria from the families *Anaplasmataceae*, *Bartonellaceae*, *Coxiellaceae* and *Mycoplasmataceae*, as well as protozoa from Piroplasmida order in chiropterans and associated ectoparasites sampled in the periurban region of Campo Grande city (Mato Grosso do Sul, Brazil).

3.2 Specific objectives

 To investigate, using molecular methods, the occurrence of agents from the families *Anaplasmataceae*, *Mycoplasmataceae* and *Bartonellaceae* in blood and tissue samples from chiropterans and associated ectoparasites sampled in a perirurban region in the state of Mato Grosso do Sul;

 To investigate, using molecular methods, the occurrence of agents from the *Coxiellaceae* family and Piroplasmida order in blood and tissue samples from chiropterans sampled in a perirurban region in the state of Mato Grosso do Sul;

 To isolate *Bartonella* spp. from chiropteran blood samples, using pre-enrichment liquid and solid culture media;

 To assess the phylogenetic positioning of genotypes of *Anaplasmataceae*, *Bartonellaceae*, and *Coxiellaceae* agents, hemoplasmas and piroplasmids circulating in chiropterans and ectoparasites sampled in Mato Grosso do Sul;

 To investigate the diversity of haplotypes of the studied agents in chiropterans and ectoparasites.

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CHAPTER 2 - Intra and inter-host assessment of *Bartonella* diversity with focus on non-hematophagous bats and associated ectoparasites from Brazil.¹

Priscila Ikeda^{1*}; Jaire Marinho Torres²; Livia Perles¹; Elizabete Captivo Lourenço³; Heitor Miraglia Herrera²; Carina Elisei de Oliveira²; Rosangela Zacarias Machado¹; Marcos Rogério André^{1*}

¹ Laboratório de Imunoparasitologia, Departamento de Patologia, Reprodução e Saúde Única, Universidade Estadual “Júlio de Mesquita Filho”, Jaboticabal, Brazil.

² Laboratório de Biologia Parasitária, Programa de Pós Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brazil.

³ Laboratório de Ecologia de Mamíferos, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

Abstract: The relationship among bats, ectoparasites and associated microorganisms is important to investigate how humans can become exposed to zoonotic agents. The diversity of *Bartonella* sp. in bats and ectoparasites have been observed, but the occurrence of *gltA* genotypes within hosts has not been assessed so far. We aimed to investigate the genetic diversity of *Bartonella* spp. in non-hematophagous bats and associated ectoparasites assessing cloned *gltA* *Bartonella* genotypes in intra and inter-hosts levels and other eight genes. We found 8.1% (34/418) of positivity for *Bartonella* sp. and seventeen samples were submitted to *gltA*-cloning and three clones were sequenced for each sample. We also obtained 11, seven and three sequences for *nuoG*, *rpoB* and *ftsZ* genes respectively. None were positive for the other studied genes. We found at least two genotypes among the three *gltA*-cloned sequences from each sample, and 13 between all the 51 sequences. Among the *nuoG*, *rpoB* and *ftsZ* sequences we found eight, five and three genotypes, respectively. In the phylogenetic analysis, the sequences were positioned mainly in groups related to *Bartonella* identified in rodents, bats and batflies. Therefore, we showed the genetic diversity of *Bartonella* in bats blood and their associated ectoparasites samples at both intra and inter-hosts levels.

Keywords: genetic diversity, chiroptera, *gltA*, *Bartonella*

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1. Introduction

The interest in emerging and reemerging diseases has increased since the late 20th century. It is estimated that 75% of the emerging infectious diseases are zoonoses, which are mainly transmitted by vectors. The main cause of zoonoses emergence is the human encroachment on natural habitats changing parasite-host relationships due to the fragmentation of and close contact with wildlife, domestic animals, and humans [1].

Bartonella spp. are Gram-negative, fastidious, and facultative intracellular bacteria that can be transmitted by arthropods vectors. These bacteria primarily parasitize erythrocytes and endothelial cells, but can also colonize dendritic cells, macrophages, and monocytes of a wide variety of mammals and humans [2, 3].

The order Chiroptera represents the second larger group of mammals in the world [4]. In Brazil, 9 families, 69 genera and 182 species of bats have been described so far in all morphoclimatic regions [5]. Due to their high mobility, wide distribution, social behavior, longevity [6] and unique immunological system [7], these animals are frequently associated to several emerging infectious diseases and pointed out as reservoir hosts and source of infection for potential zoonotic agents [8, 9]. Indeed, it has been suggested that bats are the most probable ancestral hosts for eubartonellae and that their ability to fly may have contributed to make these mammals ideal hosts for the spread infectious agents, such as those caused by arthropod-borne bartonellae [10].

Due to the high abundance of bat ectoparasites in Brazil, which comprises so far 96 Streblidae species [11], 26 Nycterebiidae species [12], and 32 species of mites [13], it is important to achieve a better understanding of the relationship among bats, ectoparasites and associated microorganisms in order to understand the epizooetiology of such agents [14].

Genotypic diversity of *Bartonella* sp. in bats and associated ectoparasites have been widely reported [6,15,16,17,18,19,20]. Even though bartonellae genotypes have been detected in bats [21,22] and ectoparasites [23] in Brazil, the occurrence of multiple citrate synthase *gltA* genotypes in both vertebrate and invertebrate hosts infected by *Bartonella* spp. has not been assessed so far, since most studies in the

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literature has focused on the analysis of genetic diversity by targeting different *Bartonella* housekeeping genes, as previously proposed [24].

Therefore, this work aimed to investigate the genetic diversity of *Bartonella* spp. in non-hematophagous bats and associated ectoparasites from central-western Brazil. For this purpose, two different strategies were used: while the first one was based on the assessment of the genetic diversity of cloned *gltA* *Bartonella* genotypes at both intra and inter-hosts levels, the second one investigated such diversity using a set of eight genes.

2. Material and Methods

All experimental procedures were approved by the “Instituto Chico Mendes de Biodiversidade” (ICMBio) (SISBIO 57450-1) and the Ethics Committee on Animal Use of the School of Agricultural and Veterinary Sciences, UNESP (CEUA FCAV/UNESP 010050/17).

Between June/2017 and March/2018, bats sampling was performed quarterly in two distinct places in the periurban region of Campo Grande city, Mato Grosso do Sul state, central-western Brazil, namely “Centro de Educação Ambiental Polonês” (CEA Pol.; 54°34'49.816"W and 20°26'50.673"S) and “Florestinha” (CEA Flor.; 54°33'43.352"W and 20°24'11.614"S). Lactating and/or pregnant females were released after field screening, as well as individuals who reached the maximum number of species collected per location as license permit. The animals and their ectoparasites were identified using previously described identification keys for bats [25,26] and ectoparasites [27,28,29]. The bats were then taken to the Laboratory of Parasitic Biology of the Catholic University Dom Bosco. Bats were euthanized using 10% ketamine and 1% acepromazine association at 9:1 ratio and 1 mL/kg injected intramuscularly or intraperitoneally. From each bat specimen, 0.2-2 mL blood samples and spleen fragments were collected by intracardiac puncture and necropsy in EDTA and absolute ethanol (Merck®, Darmstadt, Alemanha)–containing RNase and DNase-free microtubes (Kasvi®, São José dos Pinhais, Brazil) and maintained at -80°C and -20°C, respectively, until their use in molecular assays. All samples were collected in laminar flow, using appropriate and sterilized instruments, to avoid environmental contamination.

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Bats' blood and spleen samples were submitted to DNA extraction using the Illustra Tissue and Cells Genomic Prep Mini Spin Kit (GE Healthcare), following the manufacturer's recommendations. DNA samples were evaluated by optical spectrophotometry (Nanodrop, Thermo Scientific) to assess the concentration ($\mu\text{g}/\mu\text{L}$) and 260/280 - 260/230 ratios.

The DNA samples were firstly tested by conventional (c) PCR assays based on endogenous genes to verify the absence of PCR inhibitors. In this sense, for the DNA samples obtained from blood and spleen tissue, the cPCR protocol based on the mammals-glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) gene was performed as previously described [30]. For the ectoparasites collected from the animals, two different cPCR protocols were used. While a PCR protocol based on cytochrome c oxidase subunit I (*cox-1*) gene [31] was used for DNA samples obtained from bat flies and mites, a protocol based on the 16S rRNA gene [32] was used for DNA samples obtained from tick larvae. All the obtained 16S rRNA tick larvae amplicons were submitted to dideoxynucleotide chain termination [33] sequencing to check the molecular identity by nBlast analysis.

The positive samples for the endogenous genes protocols were then submitted to a screening quantitative (q) PCR for *Bartonella* spp. based on the NADH dehydrogenase gamma subunit (*nuoG*) gene, as previously described [34]. All positive samples on the qPCR assay were tested by cPCR assays based on the following genes: *nuoG* [35], riboflavin synthase (*ribC*) [36], citrate synthase (*gltA*) [37], β -subunit of RNA polymerase (*rpoB*) [38], hemin binding protein (*pap-31*) [39], heat-shock chaperonin protein (*groEL*) [40,38], cell division protein (*fstZ*) [38], 16S rRNA [38] and intergenic region 16S-23S rRNA (ITS) [41], following the protocols previously described.

Products amplified in the cPCR assays were submitted to horizontal electrophoresis on ethidium bromide stained- agarose gels (1.0%) at 100 V / 150 mA for 50 minutes.

Bartonella-gltA amplicons were submitted to the cloning protocol using the vector pGEM-T Easy (Promega), following the manufacturer's recommendations. The ligation products were inserted into competent *Escherichia coli* One Shot Match 1T1R Chemically Competent Cells (Invitrogen Cat # C8620-03) (10^9 - 10^{10} cfu / ng of DNA) by

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thermal shock method. After seeding on a solid Luria Bertani (LB) medium prepared with 100 µg / mL ampicillin, 40 µL of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) (Sigma-Aldrich, St. Louis, Missouri, USA) and 20 µL IPTG (isopropylthio-β-galactoside) (Sigma-Aldrich, St. Louis, Missouri, USA), the plates were incubated at 37°C overnight. Three individual white colonies of bacteria were chosen for each sample and transferred into tubes containing LB liquid medium and 100 µg / mL ampicillin. After incubation at 37°C for up to 24 hours, plasmid DNA was extracted from the transforming clones by the alkaline lysis method [42].

The obtained amplicons were purified using the Silica Bead DNA gel extraction kit (Thermo Fisher Scientific™, Waltham, Massachusetts, USA) and submitted to sequencing by dideoxynucleotide chain termination [33], performed in ABI PRISM 3700 DNA Analyzer sequencer (Applied Biosystems®, Foster City, CA, USA) at the “Centro de Recursos Biológicos e Biologia Genômica (CREBIO -FCAV - UNESP)”. All the sequences obtained were evaluated and a consensus sequence was generated using the Phred-Phrap software version 23 [43,44]. Then, all obtained consensus sequences were compared with sequences previously deposited in GenBank database by nBLAST [45].

The cloned sequences were aligned to evaluate the nucleotide polymorphism within each bat blood/spleen and ectoparasite sample and among all the cloned samples. The number of genotypes (h), genotype diversity (Hd) and nucleotide diversity (π) were determined using the DnaSP 5 program, version 5.10.01 [46]. The genotype networks were plotted using PopART (<http://popart.otago.ac.nz>) with TCS networks [47]. Additionally, for the *gltA* gene, a genealogy network was created using the software Splitstree v4.11.3 [48] using Neighbor-net method and bootstrap of 1000 replicates.

Phylogenetic inferences were performed for all four molecular markers from which sequences were obtained. The sequences were aligned with other sequences from the GenBank database through BioEdit [49]. The best evolutionary model was chosen by the jModelTest2 program (version 2.1.6) on 11 XSEDE [50] under the Bayesian Information Criterion (BIC) [51]. The Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 [52] using the CIPRES Science Gateway [53]. The phylogenetic tree edition and rooting (outgroup) were performed using the Treegraph

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2.0 beta software [54]. The evolutionary models used for Bayesian Inferences were TPM1uf, TIM2ef, TIM1 and HKY for *gltA*, *nuoG*, *ftsZ*, and *rpoB* genes respectively. All of them were settled with proportion of invariable sites and gamma rates.

The sequences were deposited in GenBank database and the accession numbers have not yet been obtained at the time of submission and will be provided during review.

3. Results

3.1 PCR assays and sequencing

All the DNA samples were positive in the cPCR assay based on the endogenous genes (*gapdh* for the blood and spleen samples, 16S rRNA to the ticks' samples, and *cox-1* to mite and fly samples). In qPCR assays for *Bartonella* spp. based on the *nuoG* gene, 8.1% (34/418) blood samples showed positive results (efficiency ranging from 100.5% to 104.7%; slope from -3.311 to -3.215; R^2 from 0.969 to 0.997; y-intercept from 35.955 to 37.425). The positive samples were represented by 18 (52.9%) bats: *Platyrrhinus lineatus* n=12/23; *Artibeus planirostris* n=3/33; *Artibeus lituratus* n=2/37; and *Carollia perspicillata* n=1/5; and 16 (47.0%) from the following ectoparasites species: *Trichobius dugesii* complex n=6/10; *Trichobius costalimai* n=4/29; *Megistopoda aranea* n=1/18; and *Steatonyssus* sp. n=5/20. One *P. lineatus* (#79) and one *C. perspicillata* (#42) had both blood sample and associated ectoparasites (*T. dugesii* complex bat flies – one from animal #79 and two from the animal #42) positives in qPCR assay for *Bartonella* spp. None spleen sample were positive for the studied agent. All the 34 positive samples were tested by cPCR to *Bartonella* spp. based on *gltA*, *nuoG*, *rpoB* and *ftsZ* genes. Due to the low volume of DNA eluted obtained in the nucleic acids' extraction procedure from ectoparasites, they were not submitted to the cPCR protocols for *ribC*, *groEL*, *pap-31* and 16S rRNA genes and ITS region.

Even though 23 samples were positive in the cPCR assay based on *gltA* gene, 17 (14 from bats and 3 from flies) were submitted to cloning due to the quality of the amplicons observed in agarose gel electrophoresis. For this specific gene, three clones were sequenced from each sample, totaling 51 sequences that were used to determine the genotypic diversity among the different species of bats and flies studied as well as

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within each individual host. Similarly, although 13 and 15 samples were positive in the cPCR assays for the *rpoB* and *ftsZ* genes, seven (5 from bats and 2 from flies) and three sequences (2 from bats, and 1 from flies) were obtained from each gene, respectively. All the samples submitted to the *nuoG* cPCR protocol were positive, except for the DNA samples from mites (Macronyssidae). Only those samples that did not show positive results for any other gene but yielded *nuoG* amplicons were submitted to sequencing, totalizing 11 sequences (3 from bats and 8 from flies). None of the 18 *Bartonella* *nuoG* qPCR-positive bat blood samples were positive in the cPCR protocols based *ribC*, *groEL*, *pap-31* and *16S* rRNA genes and ITS region (Supplementary Table 1).

3.2 Sequences homology analysis

In the nBLAST analysis, three out of 51 *gltA* cloned showed 88,20-88,55% identity (100% of query coverage) with *Bartonella vinsonii* strain NCTC12905 (GenBank Access Number LR134529). The 48 remaining *gltA* cloned sequences showed 91.29%-100% identity (query coverage value of 90%-100%) to *Bartonella* spp. sequences previously detected in bats and their ectoparasites sampled in Costa Rica (17/51 sequences showed identity to KJ816676 - *Myotis keaysi*; 14/51 similar to MH234380 - *Glossophaga soricina*; 5/51 were similar to KJ816682 - *Artibeus jamaicensis*–*Aspidoptera phyllostomatis*; 3/51 sequences similar to MH234319 – *A. lituratus*; 3/51 similar to MH234346 – *C. perspicillata*; and 1/51 were similar to MH234347 – *C. perspicillata*; 1/51 to MH234333 - *Artibeus glaucus watsoni*; 1/51 to MH234322 – *A. lituratus*; 1/51 to KJ816687 – *A. lituratus*–*Paratrachobius longicrus*; 1/51 to KJ816666 - *Anoura geoffroyi*; and 1/51 to KJ816673 - *Sturnira lillium*–*Aspidoptera delatorrei*) (Supplementary Table Table 2).

Regarding the *nuoG* gene, nine sequences showed 92.7-98.98% identity (query coverage of 86-100%) to *Bartonella* sp. previously detected in blood from vampire bats *Desmodus rotundus* (GenBank Access Number: MK314980 / MK314982) and *Diphylla ecaudata* (GenBank Access Number: MK314979), and non-hematophagous bats *C. perspicillata* (GenBank Access Number: KY356757) and *S. lillium* (GenBank Access Number: KY356755) from Brazil. The remaining two sequences showed 91.72-

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91.81% identity (query coverage of 99-100%) to *Bartonella* sp. detected in *Cervus nippon* from Japan (GenBank Access Number: CP019781).

The five *rpoB* sequences obtained showed 85.66-90.08% identity (query coverage of 99-100%) to *Bartonella* spp. detected in a blood sample from *Eidolon helvum* bat (GenBank Access Number: KM215201) and to *Bartonella taylorii* detected in *Apodemus flavicollis* from Lithuania (GenBank Access Number: MH547315).

Finally, the three *ftsZ* sequences obtained showed 91.42% to 92.48% identity (query coverage of 100%) to *Bartonella* spp. detected in rodents (*Meriones libycus*) and bats (*Rhinolophus euryale*) from Georgia (GenBank Access Numbers: KT327035 e KX300105) and in the bat *E. helvum* from Tanzania (GenBank Access Number: KJ999693).

3.3 Genotype analysis

The results found for the 51 *gltA*-cloned sequences, which were obtained from 17 *Bartonella*-positive samples, showed the presence of at least two different genotypes among the three cloned sequences from each bat fly or bat blood sample (Table 3). However, the number of variable sites found among the genotypes was high (at least 20 variable sites) in only 6/17 cloned samples (blood samples), while the others (8 from blood samples and 3 from batflies) showed 1 to 9 variable sites. Thus, even with more than one genotype per sample, the sequences obtained from the blood of animals #05, #20, #24, #32, #81 and #114 (all from the same bat species, namely *P. lineatus*) showed a greater number of nucleotide differences among them. When all the 51 cloned *gltA* sequences obtained were analyzed together, 13 different genotypes were observed (Figure 1a). In the genotype network generated for this gene fragment, a higher diversity was observed among the sequences obtained from bat blood samples when compared to those obtained from bat flies. Among the nine sequences obtained from three bat flies (*T. dugesii* complex), two different genotypes (1 and 2) were found. Only one ectoparasite had the cloned sequences separate in the two genotypes. These two abovementioned genotypes were also represented by sequences obtained from bat blood samples. The genotype 1 was found in blood samples from bats #05 with two clones, #20 with one clone, and #32 with one clone, as well as in bat flies #79 and #83, both with the three clones. The genotype 2 was

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represented by sequences found in blood samples from bats #18 with three clones, #114 with one clone, and in bat fly #30 with one clone.

Table 3. Polymorphism found among the three *Bartonella gltA* cloned sequences obtained from each bat blood and fly sample.

Sample	(pb)	N	VS	h	Hd (mean $\pm$ SD)	π (mean $\pm$ SD)	K
Bat fly #30	716	3	1	2	0,667 $\pm$ 0,314	0,00093 $\pm$ 0,00044	0,66667
Bat fly #79	731	3	2	3	1,000 $\pm$ 0,272	0,00182 $\pm$ 0,00061	1,33333
Bat fly #83	734	3	2	2	0,667 $\pm$ 0,314	0,00182 $\pm$ 0,00086	1,33333
Blood #05	601	3	94	2	0,667 $\pm$ 0,314	0,10427 $\pm$ 0,04915	62,66667
Blood #15	734	3	1	2	0,667 $\pm$ 0,314	0,00091 $\pm$ 0,00043	0,66667
Blood #18	528	3	3	3	1,000 $\pm$ 0,272	0,00379 $\pm$ 0,00133	2
Blood #20	650	3	102	2	0,667 $\pm$ 0,314	0,10462 $\pm$ 0,04932	68
Blood #24	662	3	87	2	0,667 $\pm$ 0,314	0,08761 $\pm$ 0,04130	58
Blood #25	739	3	4	3	1,000 $\pm$ 0,272	0,00361 $\pm$ 0,00134	2,66667
Blood #32	549	3	90	3	1,000 $\pm$ 0,272	0,10929 $\pm$ 0,04870	60
Blood #42	674	3	1	2	0,667 $\pm$ 0,314	0,00099 $\pm$ 0,00047	0,66667
Blood #77	568	3	2	2	0,667 $\pm$ 0,314	0,00235 $\pm$ 0,00111	1,33333
Blood #79	734	3	1	2	0,667 $\pm$ 0,314	0,00091 $\pm$ 0,00043	0,66667
Blood #81	782	3	86	3	1,000 $\pm$ 0,272	0,07332 $\pm$ 0,02460	57,33333
Blood #114	377	3	25	2	0,667 $\pm$ 0,314	0,04421 $\pm$ 0,02084	16,66667
Blood #125	719	3	1	2	0,667 $\pm$ 0,314	0,00093 $\pm$ 0,00044	0,66667
Blood #126	674	3	9	3	1,000 $\pm$ 0,272	0,00890 $\pm$ 0,00339	6

N: number of sequences analyzed; VS: number of variable sites; h: number of genotypes; Hd: genotype diversity; π : nucleotide diversity; K: nucleotide difference number; SD: standard deviation.

In addition, polymorphism divergence analysis was performed between the group of sequences obtained from ectoparasites and bat blood samples and no significant difference between the two groups was found (Table 4).

Table 4. Polymorphism found within each sample population (ectoparasite and bat blood samples) for *gltA Bartonella* sequences.

Population analyzed	N	PS	π	k
Ectoparasites	9	1	0	0,389
Blood Samples	42	39	0	12,668

N: number of sequences analyzed; PS: number of polymorphic sites; π : nucleotide diversity; K: nucleotide difference number.

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The genotype diversity analysis of the *nuoG* (Figure 1b), *rpoB* (Figure 1c) and *ftsZ* (Figure 1c) sequences demonstrated the presence of eight, five and three distinct genotypes, respectively, among 11 sequences for the *nuoG* gene, seven for the *rpoB* gene and three for the *ftsZ* gene. In the genotype network generated for these gene fragments, the sequences obtained from bat flies were grouped in different groups from those obtained from bat blood samples as previously observed among *gltA* sequences.

All the results from the genotype analyses are shown in Table 5.

Table 5. Polymorphism found among the sequences obtained from ectoparasite and bat blood samples for each gene analyzed in the present study.

Gene	N	(pb)	VS	h	Hd (mean $\pm$ SD)	π (mean $\pm$ SD)	K
<i>gltA</i>	51	154	39	13	0,887 $\pm$ 0,022	0,09499 $\pm$ 0,00536	14,53412
<i>nuoG</i>	11	283	72	8	0,945 $\pm$ 0,054	0,11147 $\pm$ 0,00709	31,54545
<i>rpoB</i>	7	518	115	5	0,857 $\pm$ 0,138	0,10195 $\pm$ 0,02417	53,80952
<i>ftsZ</i>	3	239	35	3	1 $\pm$ 0,272	0,09902 $\pm$ 0,04410	23,66667

N: number of sequences analyzed; VS: number of variable sites; h: number of genotypes; Hd: genotype diversity; π : nucleotide diversity; K: nucleotide difference number; SD: standard deviation.

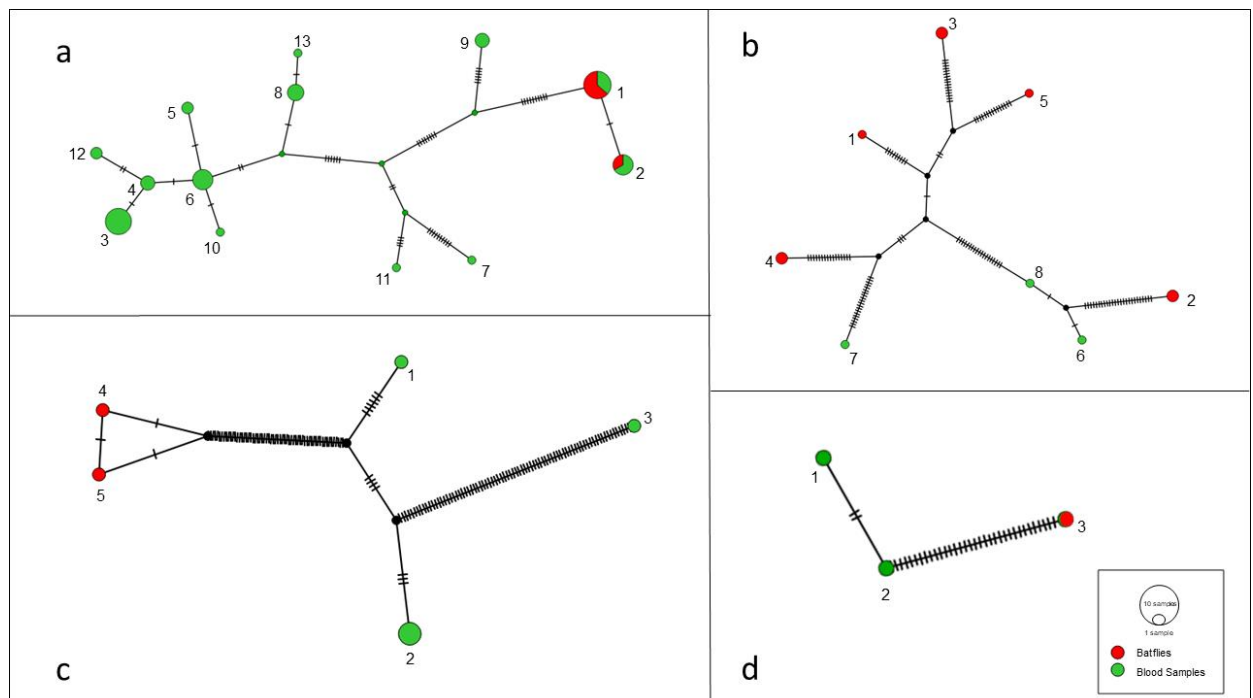


Figure 1. Genotype network with *gltA* (a), *nuoG* (b), *rpoB* (c) and *ftsZ* (d) sequences obtained from bat blood and ectoparasite samples obtained in the present study.

3.4 Phylogenetic analysis

For the *gltA* gene fragment (792 bp), the *Bartonella* sequences were allocated in four groups into two large clades (Figure 2). The first one, containing only *Bartonella* sequences obtained from bats and including nine genotypes found in the present work, comprised two paraphyletic groups: one containing eight genotypes (3, 4, 5, 6, 8, 10, 11 and 13) closely related to each other, whereas the other one contained the genotype 7 and represented by only one clone from the bat blood sample #24. The other sequences obtained from this same bat specimen (blood sample #24) were positioned in the first paraphyletic group and represented by genotype 6, thus showing that the same bat blood sample contained more than one *Bartonella* genotype.

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Figure 2. Phylogenetic tree based on an alignment of 792 bp fragment of *Bartonella* spp. *gltA* sequences, using Bayesian Inference method and TPM1uf+I+G as evolutionary model. Numbers at nodes correspond to the posterior probability value support. Each sequence-clone or group of sequences-clone are highlighted according to their respective genotype. The colored bats are positioned according to the groups found in the genealogy network.

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For the *nuoG* gene (368 pb) (Figure 4), seven different clusters were observed: the first and second ones are monophyletic and comprise three genotypes: genotype 2 with two sequences obtained from *T. dugesii* complex (#42M1 and #42M2) collected from the same animal (#42), and the other two genotypes (6 and 8) with sequences obtained from blood samples from the same animal species (*A. planirostris*) but different individuals (#16 and #44) that were positioned as monophyletic groups with 86% of posterior probability. Two *nuoG* *Bartonella* sequences from *C. perspicillata* and *S. liliun* previously sampled in Brazil [21] (Ikeda et al., 2017) were also positioned in the same branch. Two other genotypes (4 and 7), which comprised a sequence detected in *P. lineatus* (#34) and two sequences from *T. costalimai* (#59 and #56F1), were positioned as polytomic branches related to *Bartonella clarridgeiae*, *B. rochalimae*, *B. bacilliformis*, ruminant-related *Bartonella* and sequences previously detected in bats from Brazil and Ghana. The genotypes 1 and 5 were positioned as basal groups and comprised one sequence from *T. dugesii* complex (#23) and one from *M. aranea* (#120) that appeared to be closely related to *Bartonella* identified in vampire bats from Brazil [22] (André et al., 2019). The last genotype comprising two sequences from *T. costalimai* flies (#55 and #57M) were positioned as one group basal from the others.

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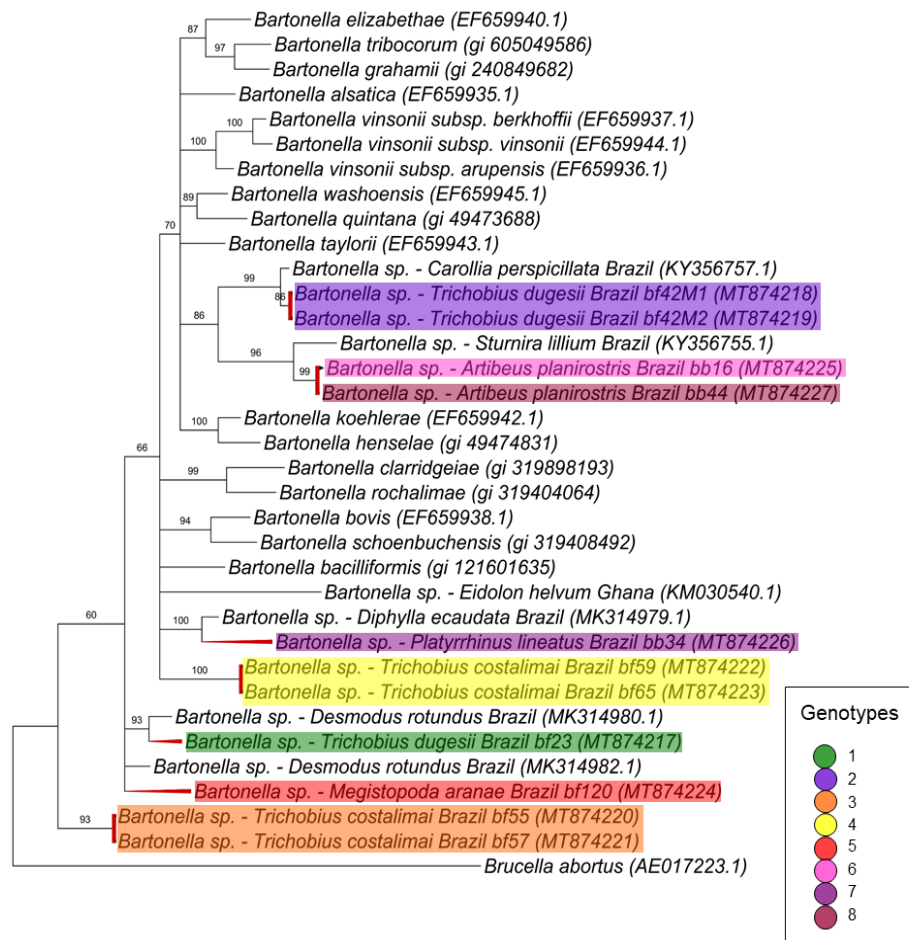


Figure 4. Phylogenetic tree based on an alignment of 368 bp fragment of *Bartonella* spp. *nuoG* sequences, using Bayesian Inference method and TIM2ef +I+G as evolutionary model. Numbers at nodes correspond to the posterior probability value support. Each sequence is highlighted according to its respective genotype.

In the phylogenetic analysis based on the *rpoB* gene (760 pb), the sequences were positioned in three separate clades (Figure 5). The first clade comprises two genotypes (4 and 5) with one sequence each and obtained from *T. dugesii* flies (#79 and #83) that were closely related to *Bartonella* genotypes previously detected in a bat and in a bat fly from Brazil, supported by 100% of posterior probability. The second clade, which was supported by 100% of posterior probability, was composed by four sequences detected in blood samples from *P. lineatus* (#05, #24, #25 and #81) and represented by two different genotypes (1 and 2). The most closely related sequences in this clade were represented by sequences obtained from bats from French Guiana and Brazil. The last sequence represented by genotype 3 and obtained from the blood

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sample of an *A. lituratus* individual appeared in a polytomic branch with *Bartonella chomellii*.

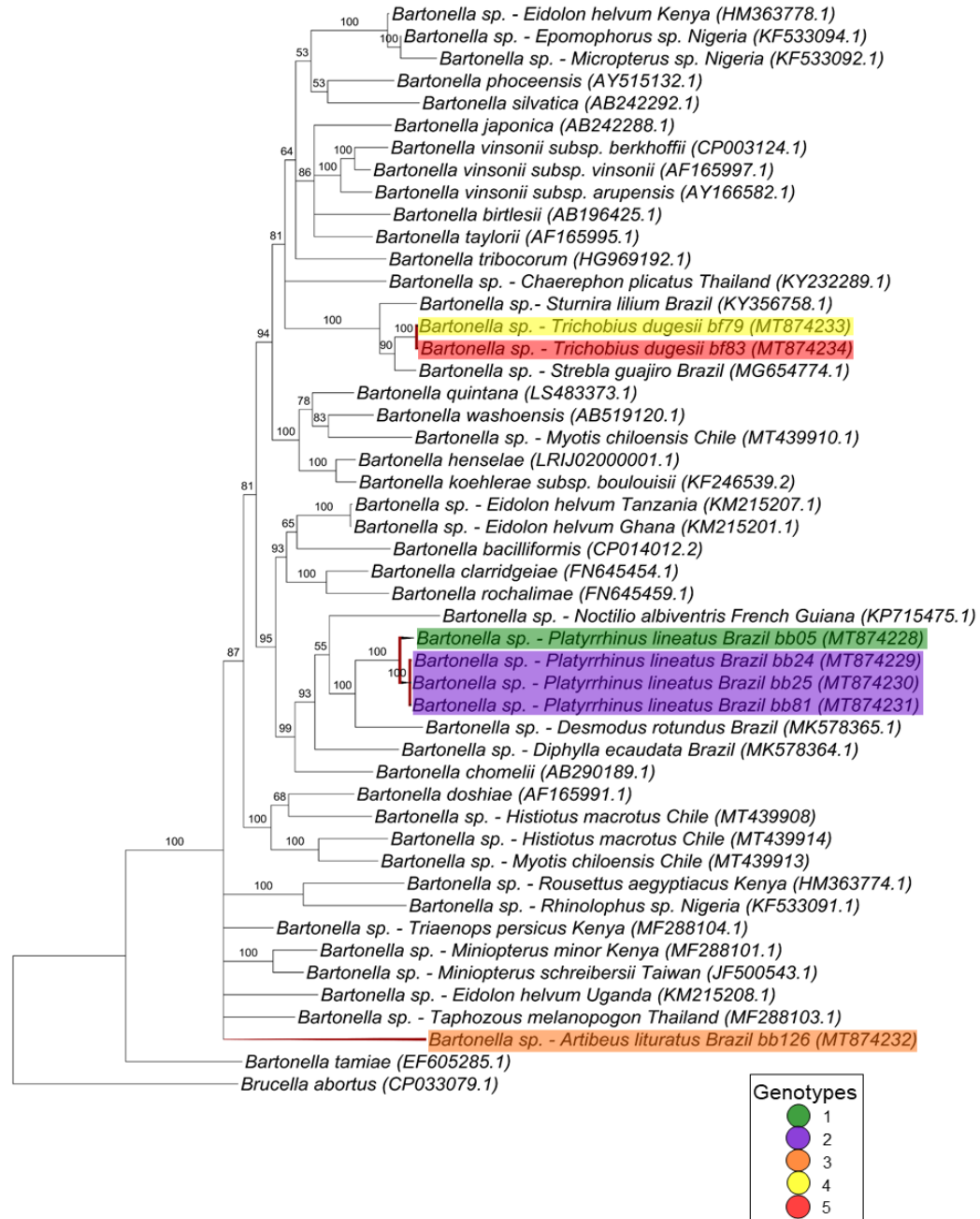


Figure 5. Phylogenetic tree based on an alignment of 760 bp fragment of *Bartonella* spp. *rpoB* sequences, using Bayesian Inference method and HKY+I+G as evolutionary model. Numbers at nodes correspond to the posterior probability value support. Each sequence is highlighted according to its respective genotype.

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Finally, for the *ftsZ* gene fragment (789 bp), the three sequences corresponding to one genotype each were positioned in two different branches (Figure 6). The first branch comprised the genotype 1 (represented by a sequence detected in a bat blood sample [*C. perspicillata* #42]) that clustered together with rodents related *Bartonella* species, with 64% of posterior probability. The other two sequences, which were represented by genotypes 2 (represented by a sequence detected in a blood sample from *P. lineatus* #77) and 3 (represented by a sequence detected in *M. aranea* sample #120), clustered together as a basal group and supported by 86% of posterior probability.

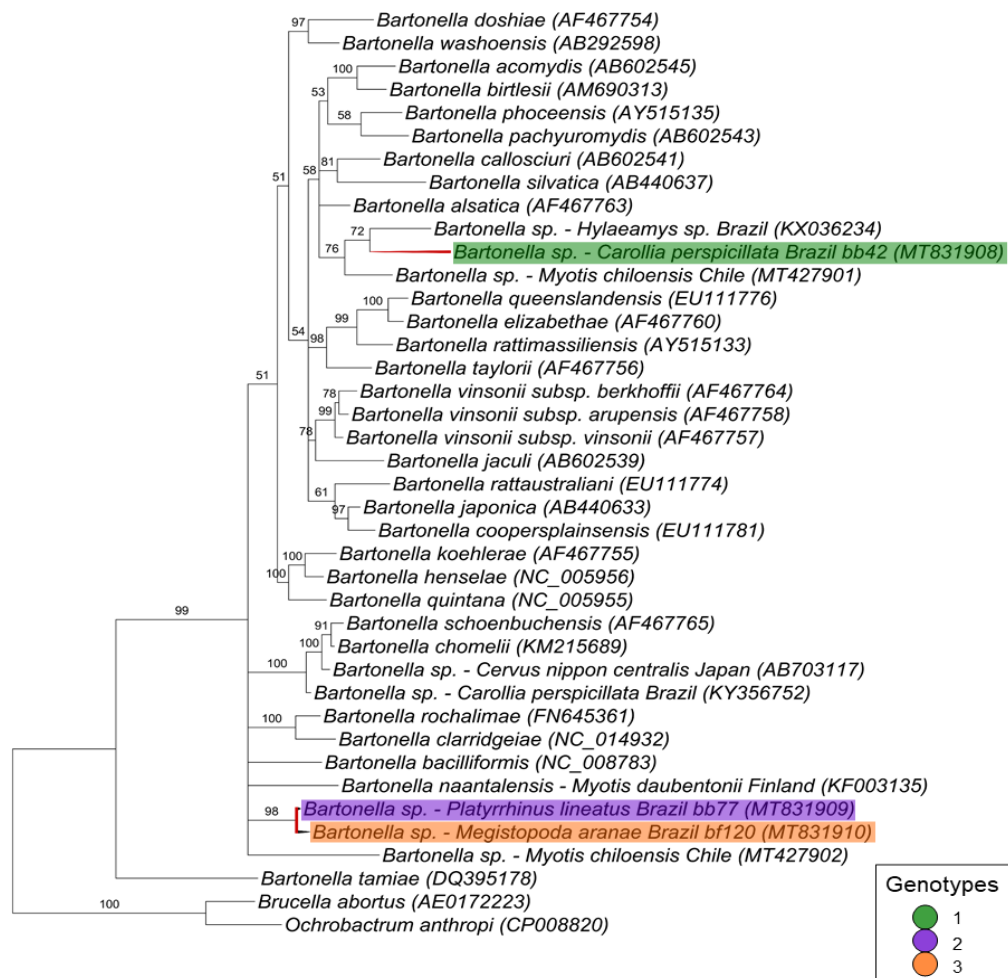


Figure 6. Phylogenetic tree based on an alignment of 789 bp fragment of *Bartonella* spp. *ftsZ* sequences, using Bayesian Inference method and TIM1+I+G as evolutionary model. Numbers at nodes correspond to the posterior probability value support. Each sequence is highlighted according to its respective genotype.

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4. Discussion

Genotypic diversity of *Bartonella* sp. in bats and associated ectoparasites have been widely observed and reported [6,15,16,17,18,19,20]. In Brazil, previous studies showed the genetic diversity of bartonellae among non-hematophagous bats [21] and Streblidae flies [23] by phylogenetic analyses based on *gltA* gene as well among vampire bats by phylogenetic and genotypic analysis based on *gltA* and *rpoB* genes [22]. However, while the majority of studies in the literature has focused on the analysis of genetic diversity of bartonellae in bats and their associated ectoparasites by targeting different *Bartonella* housekeeping genes, as previously proposed [24], the present work aimed to investigate such diversity within both vertebrate and invertebrate hosts at an intra-host perspective. When it comes to *Bartonella* genotyping, the *gltA* gene is the most frequently used molecular marker and is considered a good tool to evaluate the genotype diversity [24,55]. Even more, the number of *Bartonella* *gltA* sequences in GenBank is high and most frequently updated. La Scola et al (2003) [55] also indicated the *gltA* together with the *rpoB* gene as suitable molecular markers for *Bartonella* differentiation and characterization. Besides, in parallel, we also assessed the genetic diversity by targeting three additional molecular markers, namely *rpoB*, *ftsZ* and *nuoG*.

Considering that Nycteribiidae and Streblidae flies have been suggested as putative vectors for *Bartonella* among bats [56,57,58], the assessment of the genetic diversity of bartonellae among vertebrate hosts and associated ectoparasites has been increasingly addressed.

Despite not presenting data on nucleotide sequence polymorphisms, Dietrich et al. (2016) [59] showed that *Bartonella* genotypes detected in bats and their ectoparasites (Nycteribiidae) from South Africa and Swaziland proved to be phylogenetically associated to each other, albeit distant from those previously described in Africa, showing a possible exchange of genotypes between bats and related ectoparasites. Such findings were corroborated by Judson, Frank and Hadly (2015) [56], who detected the same genetic variants of *Bartonella* in both bats and associated ectoparasites (Streblidae and Nycteribiidae) sampled in Costa Rica, Central America. In addition, McKee et al. (2016) [60], when evaluating the host-parasite interaction of *Bartonella* in bats, pointed out the existence of frequent and

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bidirectional exchange of this group of bacteria between bats and associated ectoparasites, highlighting the influence of arthropod vectors on the pattern of genetic variants of *Bartonella* spp. in vertebrate hosts. Herein, we observed such matched relationship only when we considered the genotypic analysis based on the *gltA* gene. In this sense, we found two *Bartonella* genotypes (1 and 2) that comprised sequences obtained from both the vertebrate host and ectoparasites.

However, when analyzing the other targeted gene fragments, the detected genotypes showed to be exclusive only to the vertebrate host or only to the sampled flies. In the phylogenetic analysis based on the *rpoB* and *nuoG* genes, we also observed that sequences obtained from ectoparasites samples clustered together among themselves and in different branches from those obtained from blood samples collected from their respective bat hosts. Even though one sequence from a bat blood sample (*P. lineatus* #77) and one sequence from *M. aranea* (#120) clustered together in the *ftsZ* gene phylogeny, both represent one genotype each. In fact, all the three sequences obtained from this marker were separated in three different genotypes. These findings might be explained by the symbiotic character of some *Bartonella* spp. in arthropods that are not present in vertebrate hosts, as previously shown [19, 60].

Interestingly, in the *nuoG* phylogenetic tree, four sequences obtained from bat flies were positioned as more ancient groups. Previously, McKee et al. (2020) [10] raised the hypothesis that *Bartonella* species evolved from symbionts found in blood-feeding ectoparasites once these arthropods depend on symbionts for additional nutrients [61].

Despite the high genetic diversity of *Bartonella* sp. previously reported in bat ectoparasites [16], the present work found a lower genetic diversity among the *gltA* sequences obtained from flies when compared to those found in blood samples from bats. Only one bat fly sample showed more than one *Bartonella gltA* genotype among the three clones obtained from the same sample, while eight bat blood samples showed two or three different genotypes. In addition, *Bartonella* sequences detected in bat blood samples showed a higher number of variable sites, but without significant statistical difference when compared to sequences obtained from bat flies. Sandór et al. (2018) [62] proposed that a lower genetic diversity of *Bartonella* in bat flies could be related to a high exchange of hosts, since they would not be able to maintain a diversity

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of genotypes, despite having more access to it. On the other hand, flies with a lower host exchange rate would present a greater capacity to maintain the acquired genotypes since they would maintain them for a longer time. Conversely, some authors pointed out the possibility that a greater diversity of bartonellae in bat flies would be the opposite and related to the frequent change of hosts [19] as well as their lack of specificity [60], since this kind of behavior would allow the infection with different genotypes. Finally, we cannot rule out the possibility of a greater *Bartonella* diversity among bat than in flies due to the number of samples analyzed, i.e., *gltA* amplicons for cloning were obtained from only three flies whereas 14 amplicons were obtained from bat blood samples. In addition, Becker et al. (2018) [20] suggested that the different levels of bartonellae genetic diversity between bats and their associated ectoparasites may also be related to the dispersion of this group of bacteria by other arthropod vectors or other bat species involved in the dynamic transmission cycles. However, as pointed out by McKee et al. (2020) [10], while strict ectoparasites vectors may maintain enzootic *Bartonella* infections, generalist vectors can carry the bacteria to other phylogenetically distant hosts. Moreover, the same authors incriminated bats as likely the key for *Bartonella* diversification and spreading.

McKee et al. (2019; 2020) [10,14] has shown that bat-associated *Bartonella* genotypes were scattered positioned in the phylogenetic trees than those described in other mammal orders. Even though the *Bartonella* phylogeny is congruent with the host phylogeny, bat-related *Bartonella* clusters appeared to be polyphyletic, which is consistent to the topology observed in the phylogenetic analyses from the present work and from previous studies performed by our research group involving non-hematophagous bats [21], vampire bats [22] and Streblidae bat flies [23]. Herein, the sequences obtained from ectoparasites and bat samples appeared as branches distributed throughout the tree, showing that bartonellae detected in both bats and flies are very diverse. Also, we could observe that the found *Bartonella* sequences usually clustered together or closely related to sequences previously obtained from bats and ectoparasites from Peru, Guatemala, French Guiana and Brazil. This pattern was observed previously when Stuckey et al. (2017) [63] analyzed *Bartonella* sequences from bats from Mexico, which were positioned along with sequences obtained from other works performed in Latin America. Besides that, our research group has already

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observed relatedness between *Bartonella* sequences obtained from bats and rodent-associated genotypes [21,23]. Herein, only one sequence (from a bat *P. lineatus* blood sample #77) was closely positioned to rodent-related *Bartonella* sequences in the *ftsZ* phylogenetic analysis.

Although *Bartonella* have already been detected in Macronyssidae mites from rodents [64], few works have reported the occurrence of bartonellae in bat-associated mites. Herein, we found positivity to *Bartonella* sp. in five out of 21 pooled samples from Macronyssidae mites (*Steatonyssus* spp.) and none of 46 pooled Spinturnicidae mites. Reeves et al. (2006) [65] reported positivity for a *Bartonella* sp. showing 96% identicalness to *B. grahamii* in a *Steatonyssus* sp. mite collected from a bat. In Hungary, Hornok et al. (2012) [66] detected *Bartonella* sp. in 25% (1/4) Macronyssidae (*Steatonyssus occidentalis*) and 100% (3/3) Spinturnicidae (*Spinturnix myoti*) mite pooled samples collected from *Myotis myotis* specimens. Recently, 28% of the *S. myoti* (Spinturnicidae) mite pooled samples collected from bats from Poland were positive to *Bartonella* sp. [67]. Previously in Brazil, do Amaral et al. (2018) [23] did not found positivity among 200 Macronyssidae and Spinturnicidae collected from bats in the state of Rio de Janeiro. It is important to highlight that the pooled mite samples that were positive for *Bartonella* were collected in PCR negative animals and did not yield positive results in the additional conventional PCR assays.

None of the *Ornithodoros* tick larvae samples were positive to *Bartonella* spp. in the present study. Similarly, Tahir et al. (2016) [68] found all 107 larvae of *Ornithodoros hasei* from *Noctilio albiventris* collected in French Guiana to be negative for *Bartonella* spp. On the other hand, Davoust et al. (2016) [69] found four out of 105 *O. hasei* ticks positive for *Bartonella* spp. The ticks were collected from 4 *N. albiventris* also in French Guiana. Noteworthy, zoonotic-related *Bartonella* has already been detected in ticks associated to bats previously. Lofti et al (2005) [70] detected *Bartonella henselae* 16S-23S ITS in a single *Carios kelleyi* nymph sampled in residential and community buildings in Iowa, and Leulmi et al. (2016) [71] detected *Bartonella tamiae* in 12/19 *Ixodes vespertilionis* ticks collected from bats in the Algeria. Even though *Bartonella* sp. has been detected in several tick species [72,73], there is no strong evidence that ticks can be competent vectors for *Bartonella* sp. [74,75]. So far, it has been shown that *Ixodes ricinus* tick may act as competent vectors for *B. henselae* in laboratory [76].

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We showed the genetic diversity of *Bartonella* genotypes in bats blood and their associated ectoparasite samples at both intra and inter-hosts levels. Based on the *gltA* molecular marker, we found a high genotypic diversity in both bat and fly hosts, which may lead to a complex host-pathogen interaction and displays a new perspective on the role of these animals in the maintenance of *Bartonella* spp. in the environment. Our results obtained for *rpoB*, *nuoG* and *ftsZ* markers highlight the consistency with previously works that showed the genetic diversity of *Bartonella* in bats and their related ectoparasites. Since bats represent a large and widespread group of mammals around the world and *Bartonella* appears to have high genotypic plasticity, these flying mammals have been pointed out as key hosts in the origin and spread of these important zoonotic bacteria.

Supplementary Material: The following are available online at <https://www.mdpi.com/2076-2607/8/11/1822/s1>, **Table S1.** Results of quantification and positivity of bat blood and ectoparasite samples tested for *Bartonella* spp. by qPCR (*nuoG* gene) and conventional PCR assays for the *gltA*, *rpoB*, *ftsZ* and *nuoG* genes used in the present work. The sequenced samples are highlighted in dark yellow, **Table S2.** Results of nBLAST analysis with correspondent GenBank accession number found for each sequenced clone obtained from bat blood and fly samples with the correspondent genotype for each sample.

Author Contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Priscila Ikeda; Jaire Marinho Torres; Lívia Perles and Elizabete Captivo Lourenço. The first draft of the manuscript was written by Priscila Ikeda and Marcos Rogério André and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Supplementary Material:

Table S1. Results of quantification and positivity of bat blood and ectoparasite samples tested for *Bartonella* spp. by qPCR (*nuoG* gene) and conventional PCR assays for the *gltA*, *rpoB*, *ftsZ* and *nuoG* genes used in the present work. The sequenced samples are highlighted in dark yellow.

Local	Species	Ectoparasite Host	Sample	qPCR quantification (<i>nuoG</i> copies/ μ L)	cPCR <i>gltA</i>	cPCR <i>rpoB</i>	cPCR <i>ftsZ</i>	cPCR <i>nuoG</i>
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #05	$5.19 \times 10^0 \pm 5.00 \times 10^{-1}$	+	+	-	+NS
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #15	$3.02 \times 10^0 \pm 7.78 \times 10^{-1}$	+	-	-	+NS
CEA Polônês	<i>Artibeus planirostris</i>		Blood #16	$1.86 \times 10^0 \pm 2.40 \times 10^{-1}$	+NS	+ F	+ F	+
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #18	$4.73 \times 10^1 \pm 1.08 \times 10^0$	+	+ F	+ F	+NS
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #20	$1.20 \times 10^0 \pm 1.55 \times 10^{-1}$	+	-	-	+NS
CEA Polônês	<i>Trichobius dugesii</i> complex	<i>Eptesicus furinalis</i>	Batfly #23	$3.36 \times 10^1 \pm 6.28 \times 10^0$	-	+ F	+ F	+
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #24	$1.80 \times 10^1 \pm 4.13 \times 10^0$	+	+	-	+NS
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #25	$9.57 \times 10^1 \pm 1.78 \times 10^1$	+	+	+ F	+NS
CEA Polônês	<i>Trichobius dugesii</i> complex	<i>Platyrrhinus lineatus</i>	Batfly #30	$3.28 \times 10^2 \pm 6.49 \times 10^0$	+	+ F	-	+NS
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #32	$1.40 \times 10^0 \pm 1.59 \times 10^{-1}$	+	-	-	+NS
CEA Polônês	<i>Platyrrhinus lineatus</i>		Blood #34	$1.20 \times 10^0 \pm 6.35 \times 10^{-2}$	+ F	-	-	+
CEA Florestinha	<i>Carollia perspicillata</i>		Blood #42	$2.91 \times 10^3 \pm 1.77 \times 10^2$	+	-	+	+NS
CEA Florestinha	<i>Trichobius dugesii</i> complex	<i>Carollia perspicillata</i>	Batfly #42 M1	$1.87 \times 10^0 \pm 2.98 \times 10^{-1}$	-	-	-	+
CEA Florestinha	<i>Trichobius dugesii</i> complex	<i>Carollia perspicillata</i>	Batfly #42 M2	$1.04 \times 10^0 \pm 2.36 \times 10^{-1}$	-	-	-	+
CEA Polônês	<i>Artibeus planirostris</i>		Blood #44	$1.09 \times 10^1 \pm 9.88 \times 10^{-1}$	+ F	+ F	+ F	+
CEA Polônês	<i>Artibeus planirostris</i>		Blood #51	$8.14 \times 10^{-1} \pm 2.60 \times 10^{-2}$	+NS	-	+ F	+NS
CEA Florestinha	<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Batfly #55	$9.84 \times 10^{-1} \pm 2.03 \times 10^{-1}$	-	-	-	+

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CEA Florestinha	<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Batfly #57 M	$1.69 \times 10^{-1} \pm 4.53 \times 10^{-2}$	+ F	-	+ F	+
CEA Florestinha	<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Batfly #59	$1.94 \times 10^0 \pm 2.39 \times 10^{-1}$	+ F	-	+ F	+
CEA Florestinha	<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Batfly #65 F1	$1.77 \times 10^0 \pm 2.85 \times 10^{-1}$	-	-	-	+
CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Bat mite #74	$1.07 \times 10^0 \pm 2.31 \times 10^{-1}$	-	-	+ F	-
CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Bat mite #75	$3.51 \times 10^{-1} \pm 7.8 \times 10^{-2}$	-	-	+ F	-
CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood #77	$2.95 \times 10^0 \pm 3.98 \times 10^{-1}$	+	-	+	+NS
CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood #79	$3.65 \times 10^{-1} \pm 8.27 \times 10^{-3}$	+	-	-	+NS
CEA Polonês	<i>Trichobius dugesii complex</i>	<i>Platyrrhinus lineatus</i>	Batfly #79	$1.29 \times 10^3 \pm 2.24 \times 10^2$	+	+	-	+NS
CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood #81	$1.97 \times 10^0 \pm 3.03 \times 10^{-1}$	+	+	-	+NS
CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Bat mite #82	$9.75 \times 10^{-2} \pm 3.51 \times 10^{-3}$	-	-	+ F	-
CEA Polonês	<i>Trichobius dugesii complex</i>	<i>Platyrrhinus lineatus</i>	Batfly #83	$6.38 \times 10^2 \pm 2.40 \times 10^1$	+	+	+ F	+NS
CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood #114	$9.38 \times 10^1 \pm 1.58 \times 10^{-1}$	+	+ F	+ F	+NS
CEA Florestinha	<i>Megistopoda aranea</i>	<i>Artibeus planirostris</i>	Batfly #120	$9.20 \times 10^{-1} \pm 2.23 \times 10^{-1}$	-	-	+	+
CEA Polonês	<i>Artibeus lituratus</i>		Blood #125	$6.43 \times 10^0 \pm 1.36 \times 10^0$	+	-	-	+NS
CEA Polonês	<i>Artibeus lituratus</i>		Blood #126	$6.34 \times 10^0 \pm 1.37 \times 10^0$	+	+	-	+NS
CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus cf. furinalis</i>	Bat mite #132	$4.27 \times 10^0 \pm 8.38 \times 10^{-1}$	-	-	+ F	-

+ = positive and sequenced; +NS = positive, but not sequenced; +F = positive with weak band in electrophoresis agarose-gel; - = negative.

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Table S2. Results of nBLAST analysis with correspondent GenBank accession number for each sequenced clone obtained from bat blood and fly samples with the correspondent genotype for each sample.

Animal	Local	Specie	Sample	Clone 1	Clone 2	Clone 3	Query Coverage	Identity	Genotypes
MCG 05	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (MH234319.1)	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	98-100%	93-95%	1 and 3
MCG 15	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	95-99%	92%	4
MCG 18	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (KJ816687.1)	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	95-97%	95%	2
MCG 20	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (MH234347.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	96-98%	92-96%	1 and 5
MCG 24	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (MH234333.1)	89-97%	92-99%	6 and 7
MCG 25	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	94-98%	92%	8
MCG 30	CEA Polônês	<i>Trichobius dugesii</i> complex	Bat fly 30	<i>Bartonella vinsonii</i> (LR134529.1)	<i>Bartonella vinsonii</i> (LR134529.1)	<i>Bartonella vinsonii</i> (LR134529.1)	100%	88%	1 and 2
MCG 32	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (MH234319.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (MH234380.1)	98-99%	92-95%	1, 3 and 6
MCG 42	CEA Florestinha	<i>Carollia perspicillata</i>	Blood	<i>Bartonella</i> sp. (MH234346.1)	<i>Bartonella</i> sp. (MH234346.1)	<i>Bartonella</i> sp. (MH234346.1)	90-91%	99-100%	9
MCG 77	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (MH234319.1)	<i>Bartonella</i> sp. (KJ816682.1)	<i>Bartonella</i> sp. (KJ816682.1)	99-100%	93%	3 and 6
MCG 79	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	95-97%	92-93%	3
		<i>Trichobius dugesii</i> complex	Bat fly 79	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	95-97%	95%	1
MCG 81	CEA Polônês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (KJ816673.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (MH234322.1)	90-96%	91-96%	6, 10 and 11

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MCG 83	CEA Polonês	<i>Trichobius dugesii complex</i>	Bat fly 83	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	96-98%	95%	1
MCG 114	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	<i>Bartonella</i> sp. (MH234380.1)	95-97%	92-96%	2 and 12
MCG 125	CEA Polonês	<i>Artibeus lituratus</i>	Blood	<i>Bartonella</i> sp. (KJ816682.1)	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816676.1)	97-99%	92%	3
MCG 126	CEA Polonês	<i>Artibeus lituratus</i>	Blood	<i>Bartonella</i> sp. (KJ816676.1)	<i>Bartonella</i> sp. (KJ816682.1)	<i>Bartonella</i> sp. (KJ816682.1)	97-99%	92%	6, 8 and 13

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CHAPTER 3 - Molecular detection and genotype diversity of hemoplasmas in non-hematophagous bats and associated ectoparasites sampled in peri-urban areas from Brazil ¹

Priscila Ikeda^a, Jaire Marinho Torres^b, Elizabete Captivo Lourenço^c, Gregory F. Albery^d, Heitor Miraglia Herrera^b, Carina Elisei de Oliveira^b, Rosangela Zacarias Machado^a, Marcos Rogério André^{a*}

^a Laboratório de Imunoparasitologia, Departamento de Patologia, Reprodução e Saúde Única, Universidade Estadual “Júlio de Mesquita Filho”, Jaboticabal, São Paulo, Brazil;

^b Laboratório de Biologia Parasitária, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brazil;

^c Laboratório de Ecologia de Mamíferos, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

^d Department of Biology, Georgetown University, Washington D.C., United States of America.

Abstract: Hemoplasmas have already been detected in bats in the United States of America, Spain, Australia, Chile, Brazil, Peru, Belize, Nigeria, Costa Rica, Germany, Switzerland and New Caledonia. The recent detection of hemoplasmas closely related to *Mycoplasma haematomonis*, an agent causing disease in humans, emphasizes the need for additional studies on the diversity of hemoplasmas in bats. The present work aimed to investigate the occurrence and assess the phylogenetic positioning and genetic diversity of hemoplasmas in bats and associated ectoparasites sampled in central-western Brazil. Overall, 43% (58/135) sampled bats and 1.56% (1/64) bat flies (*Megistopoda aranea*) were positive for hemoplasmas; however, 24 and 2 hemoplasma sequences were obtained from PCR assays targeting 16S and 23S rRNA genes, respectively, since the majority of the obtained amplicons showed faint bands in agarose gel electrophoresis. The obtained 16S rRNA sequences showed to be broadly distributed along the phylogenetic tree, albeit positioned within the ‘*Haemofelis* group’ and clustering with other bat-associated hemoplasmas. Twelve 16S rRNA hemoplasma genotypes were found

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among the 24 obtained sequences. When compared to other bat-related hemoplasmas sequences retrieved from the Genbank, 52 genotypes were found. The two 23S rRNA sequences obtained were positioned as a sister clade to “*Candidatus Mycoplasma haematohydrochaerus*”, *M. haemofelis* and *M. haemocanis*. High genetic diversity was found among 16S rRNA hemoplasma sequences detected in non-hematophagous bats from central-western Brazil and previously detected in other regions of the world. Even though the phylogenetic and genotype analysis showed that hemoplasmas from a same genus tend to group together, the results from the unipartite and bipartite analyses did not robustly support the hypothesis. Further studies addressing the specificity of hemoplasma genotypes according to bat species should be performed.

Keywords: genetic diversity, Chiroptera, hemoplasmas, 16S rRNA, 23S rRNA.

1. Introduction

Hemotropic mycoplasmas (hemoplasmas) are small Gram-negative erythrocytic bacteria that can cause hemolytic anemia (Tasker, 2010; Mascarelli et al., 2014; Millán et al., 2015). The severity of the disease depends on the genotype or species of *Mycoplasma* involved. The recrudescence of chronic infection can occur in animals apparently healthy and submitted to a splenectomy procedure or when immunocompromised, as previously reported in many human cases (Messick, 2004).

The order Chiroptera comprises the second largest group of mammals in the world (Schipper et al., 2008). These animals have been pointed as reservoirs, hosts and sources of infection for several agents with zoonotic potential (Mühldorfer, 2013; Brook and Dobson, 2015; Letko et al., 2020). Currently in Brazil, 181 bat species belonging to 68 genera and 9 families have been found distributed throughout the national territory (Garbino et al., 2020). Considering that several species of Chiroptera are present in urban and periurban areas, it is important to achieve a better understanding on the importance of these animals in the epidemiological cycles of (re)-emerging diseases of veterinary and public health importance.

Hemoplasmas have been detected in humans in China (Yang et al., 2000; Hu et al., 2009; Yuan et al., 2009), Brazil (dos Santos et al., 2008), England (Steer et al.,

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2011), United States (Sykes et al., 2010; Maggi et al., 2013b), South Africa (Maggi et al., 2013c), Japan (Hattori et al., 2020), and New Caledonia (Deslcloux et al., 2020).

In wild animals, *Mycoplasma* spp. have been detected all over the world (Millán et al., 2020). In Brazil, these agents have been reported in wild carnivores (Guimarães et al., 2007; André et al., 2011; de Sousa et al., 2017; Cubilla et al., 2017a; Sousa et al., 2017; Furtado et al., 2018; Carneiro et al., 2020), non-human primates (Bonato et al., 2015; Cubilla et al., 2017b; Ramalho et al., 2017; de Melo et al., 2019), wild boars (Dias et al., 2019), wild and synanthropic rodents (Vieira et al., 2009; Gonçalves et al., 2015; Sousa et al., 2017; Gonçalves et al., 2020), and opossums (Massini et al., 2019; Gonçalves et al., 2020; Pontarolo et al., 2020) as well as in their associate ectoparasites (Gonçalves et al., 2020).

In bats, *Mycoplasma* spp. have already been detected in the United States of America, Spain, Australia, Chile, Brazil, Peru, Belize, Nigeria, Costa Rica, Germany, Switzerland and New Caledonia (Mascarelli et al., 2014; Millán et al., 2015; Volokhov et al., 2017; Ikeda et al., 2017; Holz et al., 2019; Millán et al., 2019; Becker et al., 2020; Descloux et al., 2020, Di Cataldo et al., 2020, Fritschi et al., 2020, dos Santos et al., 2020). Indeed, a high genetic diversity of hemoplasmas has been reported in Neotropical bats (Volokhov et al., 2017; Becker et al., 2020).

Even though these agents are suspected to be transmitted by hematophagous arthropods, alternative routes, such as social interactions among individuals, seem to be more significantly important (Neimark et al., 2001; Biondo et al., 2009; Cohen et al., 2018). However, little is known about the occurrence of such agents in bat-associated ectoparasites. In this sense, Hornok et al. (2019) and Descloux et al. (2020) reported the occurrence of hemoplasmas closely related to *Mycoplasma haematomominis*, which has been incriminated as an agent causing hemolytic anemia and pyrexia in humans (Steer et al., 2011; Hattori et al., 2020), in *Ixodes simplex* (Acari: Ixodidae) and *Cyclopodia horsfieldi* (Diptera: Nycteribiidae).

The recent detection of *M. haematomominis* in *Pteropus* bats in New Caledonia (Deslcloux et al., 2020) emphasizes the need for additional studies on the genetic diversity of hemoplasmas in bats. Considering such scenario, the present work aimed to investigate the occurrence and assess the phylogenetic positioning and genetic diversity of hemoplasmas focusing in non-hematophagous bats and

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associated ectoparasites that were sampled in human-shared area within the Campo Grande city, central-western Brazil.

2. Material and Methods

2.1 Bat capture and sampling

A total of 135 spleen, 133 blood and 150 ectoparasites samples were collected from 135 non-hematophagous bats belonging to 12 species.

Capture procedures were performed every three months from July 2017 to March 2018, comprising a total of 24 days of sampling. Nets were placed at sunset (around 6 p.m.) and checked every 30 minutes, during 6 hours. Two spots were sampled: “Centro de Educação Ambiental Polonês” (CEA Pol. – GPS: 54° 34' 49.816" W / 20° 26' 50.673" S) and “Centro de Educação Ambiental Florestinha” (CEA Flor. – GPS: 54° 33' 43.352" W / 20° 24' 11.614" S), located in the peri urban area of Campo Grande city, Mato Grosso do Sul state, central-western Brazil. Considering the captures sites, only non-hematophagous were captured for the present work. It is interesting to highlight that these two spots are frequented by children and teenagers who participate on environmental education programs. The species of captured animals were identified and measures from forearm size, reproductive status, sex, and age of each animal were recorded at the field laboratory. At this point, the non-hematophagous bats were screened for ectoparasites that, when present, were collected using tweezers and kept in alcohol 70%. After the animals were captured, they were taken to the laboratory for euthanasia and necropsy. The blood samples were kept in EDTA-containing microtubes for blood smears confection and PCR assays. Spleen samples were kept in absolute alcohol. Both were maintained at -20°C until use for molecular analysis. Sampling procedures and all experiments were approved by the “Instituto Chico Mendes de Biodiversidade” (ICMBio) (SISBIO 57450-1) and by the Ethics Committee of School of Agricultural and Veterinary Sciences - UNESP (CEUA FCAV/UNESP 010050/17). While the captured bats species were identified following identification keys proposed by Reis et al. (2007, 2017), the ectoparasites were identified following Herrin and Tripton (1975), Wenzel (1976) and Guimarães et al. (2001).

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2.2 DNA purification

DNA was extracted using the Illustra Tissue Mini Kit (GE Healthcare®) for the 10 mg of each bat spleen tissue and ectoparasites. The Illustra Blood Mini Kit (GE Healthcare®) was used for 50-200 µL blood samples, according to manufacturer's instructions. The DNA concentration and absorbance ratio (260/280 nm) were measured using a spectrophotometer (Nanodrop, Thermo Scientific, USA).

2.3 PCR assays

2.3.1 PCR for mammals and ectoparasites' endogenous genes

To check the quality of the extracted DNA and avoid the presence of false results, each spleen and blood DNA extracted was tested by a conventional (c)PCR targeting the mammal *gapdh* (Glyceraldehyde 3-phosphate dehydrogenase) gene (Birkenheuer et al., 2003). For the ectoparasites collected, two different cPCR protocols were used: one based on cytochrome c oxidase subunit I (*cox-1*) gene (Folmer et al., 1994) for DNA samples obtained from bat flies and mites, and another protocol based on the 16S rRNA gene (Mangold, Bargues and Mas-Coma, 1998) for DNA samples obtained from tick larvae.

2.3.2 PCR assays for hemoplasmas

Positive samples in *gapdh*, *cox-1* and 16S rRNA PCR protocols were submitted to two different PCR protocols to amplify *Mycoplasma* spp. based on 16S rRNA gene using two sets of primers (HemMycop16S-41S / HemMyco16S-938AS (~1000 bp) and HemMycop16S-322S / HemMycop16S-1420AS (~800 bp) (Maggi et al., 2013a). Only the second protocol was used for the ectoparasites samples due to the small amount of DNA obtained from these specimens. For each reaction, *Mycoplasma suis* DNA obtained from a naturally infected swine (Gatto et al., 2019) and ultra-pure sterile water (Nuclease-Free Water, Promega™, Madison, Wisconsin, USA) were used as positive and negative controls, respectively.

The 16S rRNA-*Mycoplasma* spp. positive samples were additionally submitted to PCR protocols targeting the RNaseP gene- *Mycoplasma* sp. (165 bp) and the 23S rRNA gene (800 pb), as described previously (Maggi et al., 2013a; Mongruel et al., 2020).

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The products obtained in all the cPCR assays were separated by electrophoresis on a 1% agarose gel stained with ethidium bromide (Life Technologies™, Carlsbad, California, USA) at 100 V/150 mA for 50 min. The gels were imaged under ultraviolet light (ChemiDoc MP Imaging System, Bio Rad™, Hercules, California, USA) using the Image Lab Software version 4.1.

2.4 Sequencing

Amplified products were purified using the ExoSAP-IT™ PCR Product Cleanup Reagent (Applied Biosystems™, Foster City, California, USA) and submitted to sequencing, which was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific™) and ABI PRISM 310DNA Analyzer (Applied Biosystems™, Foster City, California, USA) (Sanger et al., 1997).

2.5 Sequence analysis and phylogeny

The obtained sequences were firstly submitted to a screening test using Bioedit version 7.0.5.3 (Hall, 1999) to evaluate the electropherogram quality and generate the consensus sequences. The samples that were positive for both 16S rRNA-based protocols were aligned in order to construct one single sequence from the two consensus sequences obtained previously. The BLAST program (Altschul et al., 1990) was used to analyze the sequences of nucleotides (BLASTn), aiming to browse and compare with sequences from an international database (GenBank) (Benson et al., 2018). The consensus sequences obtained in the current study and those retrieved from GenBank were aligned using the Clustal/W software (Thompson et al., 1994) via Bioedit version 7.0.5.3 (Hall, 1999). Phylogenetic inferences were based on Maximum Likelihood (ML) method submitted to IQ-TREE in the online server (Nguyen et al., 2015) using 1000 replicates of UFBoot2 - Ultrafast Bootstrap (Hoang et al., 2018). The best model of evolution was selected by the ModelFinder (Kalyaanamoorthy et al., 2017). All the trees were examined in Treegraph 2.0 beta software (Stover and Muller, 2010). The phylogenetic analyses were performed using the obtained sequences from 16S rRNA and 23S rRNA-based PCR protocols. All the sequences obtained from the present work were submitted to GenBank database

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under accession numbers MZ048285-308 (16S rRNA sequences) and MZ047476-477 (23S rRNA sequences).

2.6 Genetic diversity assessment

The genetic diversity was performed using only the obtained sequences from 16S rRNA gene. In order to calculate nucleotide diversity (π), polymorphism level (haplotype diversity - [dh]), number of haplotypes (h) and the average number of nucleotide differences (K) between the sequences obtained, we used the DnaSP program v5 (Librado and Rozas, 2009). The Genotype network was constructed in PopART (<http://popart.otago.ac.nz>), using the TCS inference method (Clement et al., 2002). Additionally, a genotype network was constructed using the sequences obtained from the present work and those detected from bats in other countries that were available in the database GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). Sequences that did not show good alignment length or degenerated nucleotides were excluded from analysis.

2.7 Phylogenetic relatedness

To investigate whether phylogenetic relatedness affected bat species' tendency to share hemoplasmas, a bipartite host-parasite network was constructed and examined. We collapsed the bipartite network into an unipartite (host-host) parasite sharing network, where nodes represented bat species and edges represented the number of hemoplasma genotypes that each bat species pair shared, following a previously described methodology (Albery et al., 2020).

3. Results

A total of 135 non-hematophagous bats were sampled and identified as: *Artibeus lituratus* (n= 37), *Artibeus planirostris* (n= 33), *Carollia perspicillata* (n= 5), *Chiroderma villosum* (n= 1), *Eptesicus furinalis* (n= 9), *Eumops perotis* (n= 4), *Glossophaga soricina* (n= 1), *Molossops temminckii* (n= 1), *Molossus molossus* (n= 4), *Myotis nigricans* (n= 2), *Phyllostomus discolor* (n= 15), and *Platyrrhinus lineatus* (n= 23). The ectoparasites were identified as *Paratrachobius longicrus* (n=1), *Megistopoda aranea* (n=18), *Trichobius complexo parasiticus* (n=2), *Trichobius*

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complexo dugesii (n=10), *Trichobius joblingi* (n=2), *Strebla hertigi* (n=3), *Trichobius costalimai* (n=28), *Periglischrus iheringi* (n=29), *Periglischrus acutisternus* (n=4), *Periglischrus torrealbai* (n=5), *Steatonyssus* spp. (n=20). The tick larvae (n=19) were identified molecularly as *Ornithodoros hasei*.

A total of 418 DNA samples were obtained from 135 spleen tissues, 133 blood samples and 150 ectoparasites that showed to be positive for the endogenous control PCR assays and were used in the PCR assays for hemoplasmas.

Overall, 43% (58/135) sampled non-hematophagous bats were positive for hemoplasmas, belonging to the following species: *A. planirostris* (n=17/33), *P. discolor* (n=15/15), *A. lituratus* (n=11/37), *P. lineatus* (n=5/23), *C. perspicillata* (n=3/5), *E. furinalis* (n=3/9), *M. molossus* (n=2/4), *C. villosus* (n=1/1), and *G. soricina* (n=1/1). Only one out of 64 bat flies, represented by a specimen of *Megistopoda aranea* collected from an *A. planirostris* individual, was positive for hemotropic mycoplasmas (Table 1).

Despite the high positivity for hemoplasmas, a total of 24 sequences were obtained; the majority of positive samples showed faint bands in agarose gel electrophoresis, including the only positive ectoparasite. From those, 3 (12.5%) were only obtained from the first 16S rRNA-based PCR protocol (HemMycop16S-322S/ HemMyco16S-1420AS) from blood samples of *A. lituratus* and *P. discolor* species, 14 were only obtained from the second 16S rRNA-based PCR protocol (HemMycop16S-41S / HemMycop16S-938AS) from 10 spleen samples from *A. planirostris*, *G. soricina*, *E. furinalis*, *P. lineatus*, and *P. discolor*, and four blood samples from *A. planirostris* and *P. discolor* species. Seven consensus sequences were obtained from blood samples that showed to be positive for both 16S rRNA-based PCR protocols (from *A. lituratus*, *A. planirostris* and *P. discolor* species).

A total of 75 samples (17.94%) out of the 418 samples tested (represented by 47/133 blood; 27/135 spleen; 1/150 ectoparasite) that showed to be positive in 16S rRNA-based PCR protocols were also submitted to the 23SrRNA-based PCR protocol and only two samples showed to be positive and were sequenced.

nBLAST results for the sequences obtained for 16S rRNA and 23S rRNA genes are presented in Table 2.

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Table 1. Positivity for each bat biological sample (blood/spleen) with correspondent Animal ID according to the two PCR protocols used targeting the 16S rRNA gene) for hemotropic *Mycoplasma* spp.

Animal #	Capture site	Specie	Sample type	16S rRNA gene First protocol (322S / 1420AS)	16S rRNA gene Second protocol (938AS / 41S)
MCG 02	CEA Polonês	<i>Artibeus lituratus</i>	Spleen	+ (weak band)	
MCG 05	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	+	
MCG 06	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+	+ (weak band)
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>	Spleen	+ (weak band)	Positive and sequenced
			Blood	+ (weak band)	+ (weak band)
MCG 11	CEA Polonês	<i>Artibeus planirostris</i>	Spleen		+ (weak band)
			Blood	+ (weak band)	+ (weak band)
MCG 13	CEA Polonês	<i>Artibeus planirostris</i>	Spleen		Positive and sequenced
			Blood	+ (weak band)	+ (weak band)
MCG 14	CEA Polonês	<i>Artibeus planirostris</i>	Spleen		+ (weak band)
MCG 15	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	+ (weak band)	
MCG 23	CEA Polonês	<i>Eptesicus furinalis</i>	Spleen		Positive and sequenced
MCG 25	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	+ (weak band)	
MCG 27	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+ (weak band)	+ (weak band)
BF 27		<i>Megistopoda aranea</i>	Bat fly		+ (weak band)
MCG 35	CEA Polonês	<i>Artibeus planirostris</i>	Spleen	+ (weak band)	+
			Blood	Positive and sequenced	
MCG 37	CEA Florestinha	<i>Carollia perspicillata</i>	Spleen	+	+
MCG 38	CEA Florestinha	<i>Artibeus planirostris</i>	Spleen	+ (weak band)	+ (weak band)
			Blood	+	+ (weak band)

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MCG 39	CEA Florestinha	<i>Artibeus planirostris</i>	Spleen	+ (weak band)	+ (weak band)
			Blood	+	+
MCG 42	CEA Florestinha	<i>Carollia perspicillata</i>	Spleen	+ (weak band)	
			Blood	+ (weak band)	+ (weak band)
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>	Spleen	+ (weak band)	
			Blood	+ (weak band)	
MCG 47	CEA Polonês	<i>Platyrrhinus lineatus</i>	Spleen		Positive and sequenced
MCG 48	CEA Polonês	<i>Artibeus planirostris</i>	Spleen		+ (weak band)
			Blood	Positive and sequenced	
MCG 51	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+	+
MCG 54	CEA Florestinha	<i>Molossus molossus</i>	Blood	+	
MCG 55	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		+
			Blood	Positive and sequenced	
MCG 56	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	Positive and sequenced (no contig)	
MCG 57	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		Positive and sequenced
			Blood	+	+ (weak band)
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	+	+
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		+ (weak band)
			Blood	Positive and sequenced.	
MCG 62	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		Positive and sequenced
			Blood	+	Positive and sequenced
MCG 63	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	+	+
MCG 64	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	Positive and sequenced	
MCG 65	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	+	Positive and

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					sequenced
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	+	+
MCG 67	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		Positive and sequenced
			Blood	+	+
MCG 68	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	Positive and sequenced	+
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>	Spleen		+ (weak band)
MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		Positive and sequenced
			Blood	+ (weak band)	+ (weak band)
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	Positive and sequenced	
MCG 72	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen		Positive and sequenced
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>	Spleen		Positive and sequenced
MCG 76	CEA Polonês	<i>Artibeus lituratus</i>	Blood	Positive and sequenced	+
MCG 94	CEA Florestinha	<i>Molossus molossus</i>	Blood	+ (weak band)	
MCG 98	CEA Polonês	<i>Artibeus lituratus</i>	Blood	+ (weak band)	+ (weak band)
MCG 99	CEA Polonês	<i>Chiroderma villosum</i>	Spleen	+ (weak band)	
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>	Blood	Positive and sequenced	
MCG 101	CEA Polonês	<i>Platyrrhinus lineatus</i>	Spleen	+ (weak band)	
MCG 105	CEA Polonês	<i>Artibeus lituratus</i>	Spleen	+ (weak band)	
			Blood	+	+ (weak band)
MCG 107	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+ (weak band)	+ (weak band)
MCG 108	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+	+ (weak band)
MCG 109	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+	+
MCG 112	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+	Positive and sequenced
MCG 115	CEA Florestinha	<i>Artibeus planirostris</i>	Blood	+ (weak band)	+ (weak band)

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MCG 117	CEA Florestinha	<i>Artibeus lituratus</i>	Blood	+ (weak band)	+ (weak band)
MCG 125	CEA Polonês	<i>Artibeus lituratus</i>	Spleen		+ (weak band)
			Blood	+ (weak band)	
MCG 126	CEA Polonês	<i>Artibeus lituratus</i>	Blood	+ (weak band)	
MCG 128	CEA Florestinha	<i>Artibeus lituratus</i>	Blood	Positive and sequenced	
MCG 132	CEA Polonês	<i>Eptesicus furinalis</i>	Blood	+ (weak band)	
MCG 134	CEA Florestinha	<i>Artibeus lituratus</i>	Blood	+ (weak band)	
MCG 135	CEA Florestinha	<i>Artibeus lituratus</i>	Blood	+ (weak band)	

Table 2. Results of nBLAST analysis for obtained sequences from each bat biological sample (blood/spleen) with correspondent GenBank accession according to two targeted genes (16S rRNA and 23S rRNA) for hemotropic *Mycoplasma* spp.

Animal #	Capture Site	Species	Biological Sample	16S rRNA gene First protocol (322S / 1420AS)	16S rRNA gene Second protocol (938AS / 41S)	Query Coverage	Identity	E-value	Acc. Number	23S rRNA gene	QC	ID	E-value	Acc. Number
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>	Spleen	+	<i>Mycoplasma</i> sp. (<i>Glossophaga commissarisi</i> from Costa Rica)	100%	99.30%	0.0	LR699022.1	Uncultured <i>Mycoplasma</i> sp. (<i>Hydrochaeris hydrochaeris</i> from Brazil)	100%	84.41%	2e-85	MW617219.1
MCG 13	CEA Polonês	<i>Artibeus planirostris</i>	Spleen	negative	Uncultured <i>Mycoplasma</i> sp. (<i>Platyrrhinus helleri</i> from Belize)	100%	98.92%	0.0	MK353883.1	negative				
MCG 23	CEA Polonês	<i>Eptesicus furinalis</i>	Spleen	negative	Uncultured <i>Mycoplasma</i> sp. (<i>Hisiotus montanus</i> from Chile)	100%	99.41%	0.0	MK295631.1	negative				
MCG 35	CEA Polonês	<i>Artibeus planirostris</i>	Blood	Uncultured <i>Mycoplasma</i> sp. (<i>Platyrrhinus helleri</i> from Belize)		100%	98.05%	0.0	MK353883.1	Uncultured <i>Mycoplasma</i> sp. (<i>Coendou villosus</i> from Brazil)	100%	84.22%	2e-147	MN692881.1
MCG 47	CEA Polonês	<i>Platyrrhinus lineatus</i>	Spleen	negative	<i>Mycoplasma</i> sp. (<i>Glossophaga</i>	100%	95.42%	0.0	LR699022.1	negative				

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					commissarisi from Costa Rica)					
MCG 48	CEA Polônês	Artibeus planirostris	Blood	Uncultured Mycoplasma sp. (Platyrrhinus helleri from Belize)		100%	98.31%	0.0	MK353883.1	negative
MCG 55	CEA Florestinha	Phyllostomus discolor	Blood	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)		100%	94.62%	0.0	KY932679.1	negative
MCG 56	CEA Florestinha	Phyllostomus discolor	Blood	w/o consensus	Uncultured Mycoplasma sp. (Desmodus rotundus from Belize)	97%	97.37%	0.0	KY932680.1	negative
MCG 57	CEA Florestinha	Phyllostomus discolor	Spleen	negative	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)	100%	94.34%	0.0	KY932679.1	negative
MCG 61	CEA Florestinha	Phyllostomus discolor	Blood	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)		100%	95.19%	0.0	KY932679.1	negative
MCG 62	CEA Florestinha	Phyllostomus discolor	Spleen	negative	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)	100%	94.54%	0.0	KY932679.1	negative
			Blood	+ not sequenced	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)	100%	94.54%	0.0	KY932679.1	negative
MCG 64	CEA Florestinha	Phyllostomus discolor	Blood	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)		100%	94.22%	0.0	KY932679.1	negative
MCG 65	CEA Florestinha	Phyllostomus discolor	Blood	+ not sequenced	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)	100%	94.51%	0.0	KY932679.1	negative
MCG 67	CEA Florestinha	Phyllostomus discolor	Spleen	negative	Uncultured Mycoplasma sp. (Desmodus rotundus from Peru)	100%	94.32%	0.0	KY932679.1	negative
MCG 68	CEA Florestinha	Phyllostomus discolor	Blood	Uncultured Mycoplasma sp. (Lophostoma evotis from Belize)	+ not sequenced	100%	98.41%	0.0	MK353891.1	negative

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MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen	negative	Uncultured <i>Mycoplasma</i> sp. (<i>Desmodus rotundus</i> from Peru)	100%	94.20%	0.0	KY932679.1	negative
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>	Blood	Uncultured <i>Mycoplasma</i> sp. (<i>Desmodus rotundus</i> from Peru)		99%	94.80%	0.0	KY932679.1	negative
MCG 72	CEA Florestinha	<i>Phyllostomus discolor</i>	Spleen	negative	Uncultured <i>Mycoplasma</i> sp. (<i>Desmodus rotundus</i> from Peru)	100%	94.66%	0.0	KY932678.1	negative
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>	Spleen	negative	Uncultured <i>Mycoplasma</i> sp. (wild rodent from Brazil)	100%	99.74%	0.0	KT215642.1	negative
MCG 76	CEA Polonês	<i>Artibeus lituratus</i>	Blood	Uncultured <i>Mycoplasma</i> sp. (<i>Artibeus lituratus</i> from Belize)	+ not sequenced	100%	100%	0.0	MK353857.1	negative
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>	Blood	Uncultured <i>Mycoplasma</i> sp. (<i>Artibeus intermedius</i> from Belize)	negative	95%	99.74%	0.0	MH245186.1	negative
MCG 112	CEA Polonês	<i>Artibeus planirostris</i>	Blood	+ not sequenced	Uncultured <i>Mycoplasma</i> sp. (<i>Platyrrhinus helleri</i> from Belize)	100%	98.93%	0.0	MK353883.1	negative
MCG 128	CEA Florestinha	<i>Artibeus lituratus</i>	Blood	Uncultured <i>Mycoplasma</i> sp. (<i>Platyrrhinus helleri</i> from Belize)		100%	98.91%	0.0	MK353883.1	negative

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Based on Maximum Likelihood (ML) method and GTR+F+I+G4 as evolutionary model, the phylogenetic analysis of an alignment of 625 pb of the 16S rRNA gene showed that the sequences obtained in the present work were distributed along the phylogenetic tree and within the '*Haemofelis* group' (Figure 1). Thirteen sequences obtained from *P. discolor* specimens, four sequences from *A. planirostris*, two from *A. lituratus* and one from *E. furinalis* clustered in a group with the same common ancestral to other bat-related sequences, with 97 of bootstrap. Thirteen sequences obtained from *P. discolor* individuals grouped with sequences of hemotropic *Mycoplasma* sp. previously detected in *Lophostoma evotis* from Belize, with 89 of bootstrap, and closely related to sequences from *Desmodus rotundus* and *Sturnira pavidens* from Belize and Peru (bootstrap=99). The second sub-clade was positioned near the first one and comprised sequences obtained from *A. lituratus* and *A. planirostris* together with hemotropic *Mycoplasma* sp. previously detected in *Platyrrhinus helleri* (bootstrap=100). The third subclade was composed by one sequence of hemotropic *Mycoplasma* sp. detected in *E. furinalis*, appearing as a polytomic branch in this cluster. A second big clade comprised the four remaining sequences of hemotropic *Mycoplasma* sp. detected in *A. lituratus*, *P. lineatus*, *G. soricina* and *E. furinalis*. The first three sequences clustered together in a group with sequences from two *Artibeus intermedius* from Belize and one *Glossophaga commissarisi* from Costa Rica (bootstrap=95) and closely related to hemotropic *Mycoplasma* sp. detected in *Diphylla ecaudata* from Brazil (bootstrap=100) and *Mycoplasma haemofelis*, *Mycoplasma haemocanis* and carnivore-related hemotropic *Mycoplasma* previously detected in Brazil (bootstrap=53). Finally, a sequence obtained from *E. furinalis* clustered far from the other sequences detected in the present work, and related to hemotropic *Mycoplasma* sp. detected in *Histiotus montanus*, *Vespertilio murinus* and *Nyctalus noctule* from Germany and Chile, with 100 of bootstrap.

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Regarding the ML phylogenetic analysis (K2P+G4 as evolutionary model) based on an alignment of 230 pb of the 23S rRNA gene (Figure 2), the two sequences obtained, one from *G. soricina* specimen and another one from an *A. planirostris* individual, clustered closely related to hemotropic *Mycoplasma* sp. detected in *A. lituratus* and *Coendou villosus* from Brazil and ‘*Candidatus Mycoplasma haematohydrochaerus*’, with bootstrap of 69. The clade containing these sequences showed to be related to *M. haemofelis* and *M. haemocanis* sequences (bootstrap = 92).

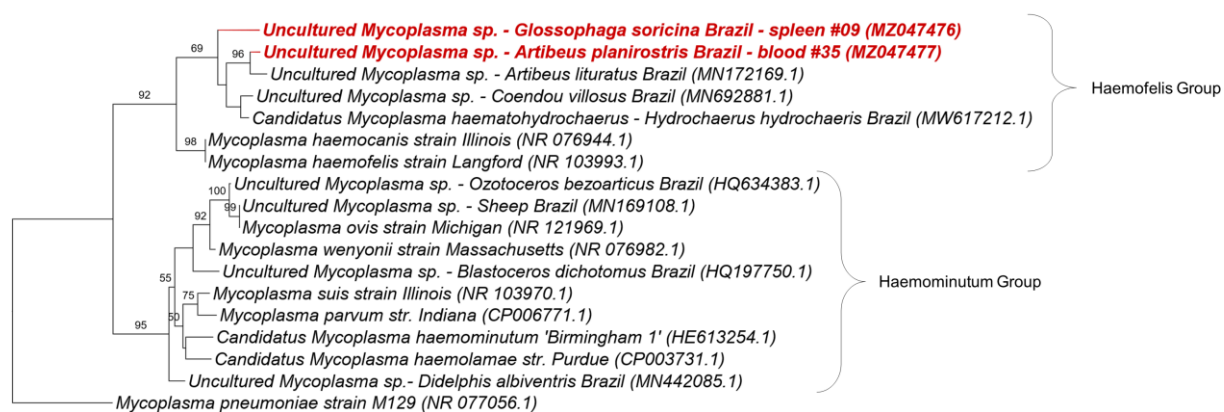


Figure 2. Phylogenetic tree based on an alignment of 230 bp of the 23S rRNA gene for hemoplasmas, using Maximum Likelihood method and K2P+G4 as evolutionary model. Numbers at nodes correspond to the bootstrap (1000 replicates). Each sequence obtained in the present work is highlighted in red. *Mycoplasma pneumoniae* was used as outgroup.

When using only sequences from the present study, 12 genotypes among the 24 sequences were found, based on an alignment of 401 pb of 16S rRNA. The genotype network (Figure 3) showed that each genotype corresponded to one or more sequences obtained exclusively from the same bat species. The genotypes #5 and #10; #9 and #6; #1, #8 and #11; and #2, #3 and #12 were composed by sequences obtained from individuals from the same species, namely *A. planirostris*, *E. furinalis*, *P. discolor* and *A. lituratus* respectively.

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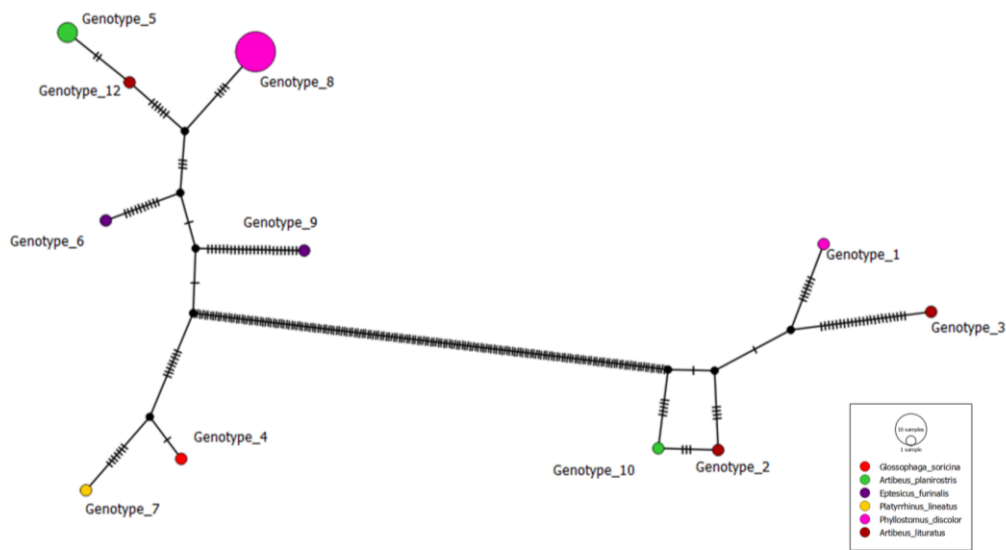


Figure 3. Genotype network with hemoplasmas 16S rRNA sequences (401 pb) obtained from blood and spleen samples obtained from non-hematophagous bats sampled in the present study.

The genotype network constructed based on the hemoplasmas sequences retrieved from the Genbank database included non-hematophagous and hematophagous bats and 52 genotypes were found among 135 sequences used based on a 271 bp alignment (Figure 4). Despite most of the genotypes were composed by only one sequence (37 genotypes), each genotype corresponded mainly to sequences obtained from the same host genus. Indeed, only two of the genotypes showed to be formed by sequences obtained from different host genus: the genotype #3 was composed by sequences obtained from *E. furinalis* and *Histiotus montanus*, and genotype #8 was composed by sequences obtained from *D. rotundus*, *D. ecaudata*, and *Carollia sowellii* individuals. When we took into consideration the capture sites of these animals, it was possible to observe that more than one genotype occurs in each locality (Figure 5). Also, among the 15 genotypes composed by two or more sequences, seven genotypes occurred in more than one site. The sequences used for the genotype analysis are presented in Supplementary Table S1.

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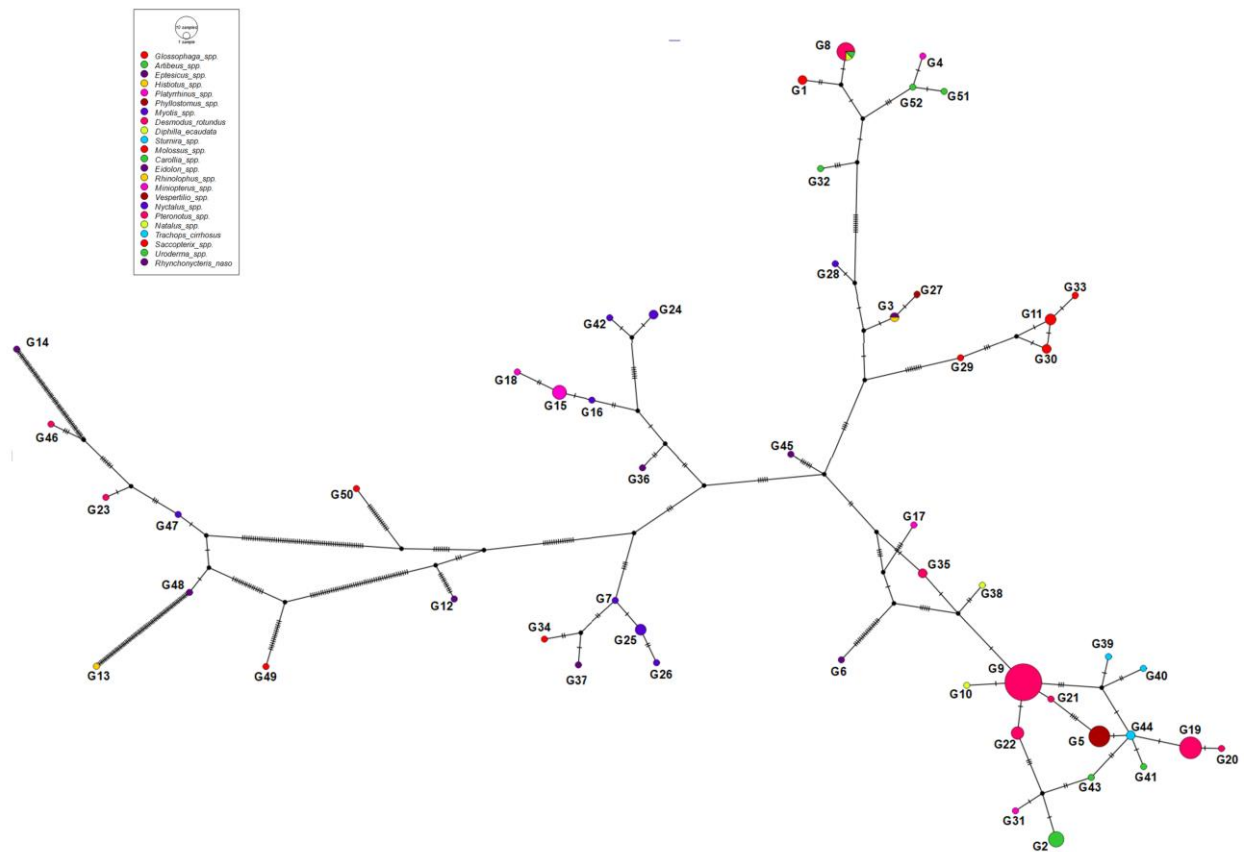


Figure 4. Genotype network with hemoplasma 16S rRNA sequences (271 pb) obtained from non-hematophagous bats sampled in the present study and compared to those retrieved from GenBank database (including sequences from non-hematophagous and vampire bats).

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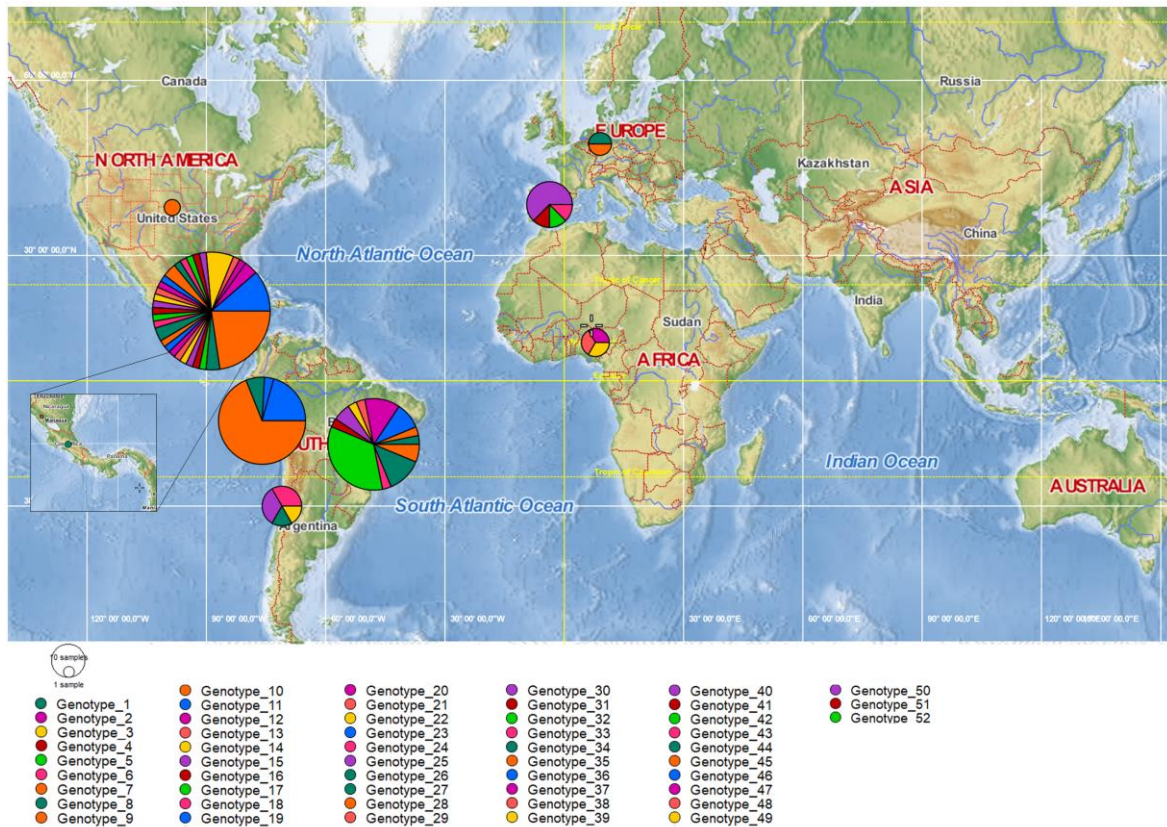


Figure 5. Genotype network with hemoplasmas 16S rRNA sequences (271 pb) obtained from bats samples from the present study and compared to those retrieved from GenBank database according to each capture site.

The bipartite network constructed to investigate whether phylogenetic relatedness affected bat species' tendency to share hemoplasmas (Figure 6) showed that, although it appeared to be a slight phylogenetic pattern where species in the same genus tended to be infected with the same genotypes (Figure 7), the sharing network was too sparse to effectively fit a species-level effect to empirically identify a phylogenetic pattern.

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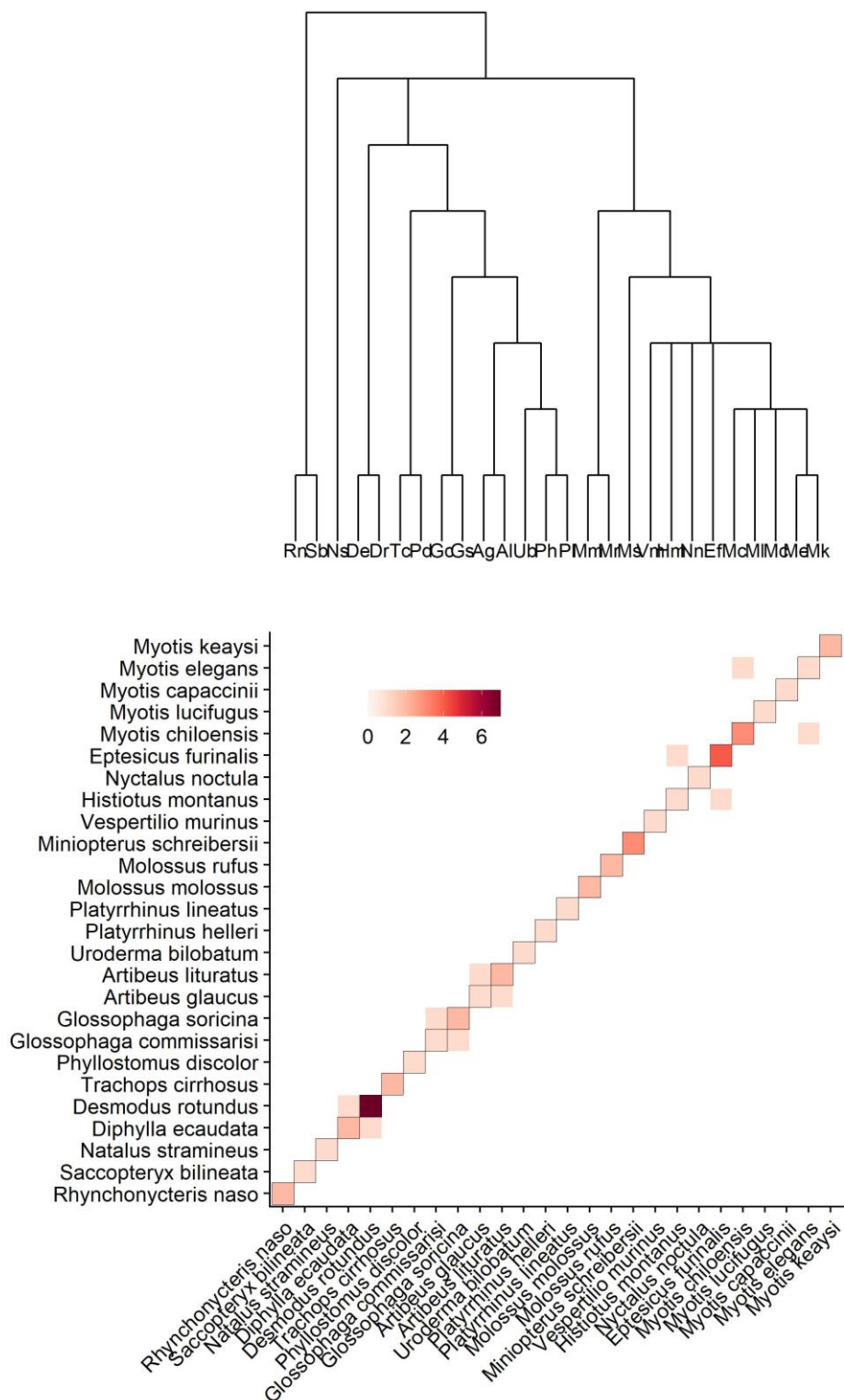


Figure 6. Phylogenetic tree and sharing matrix of the unipartite bat-bat hemoplasma sharing network. Darker colors represent the number of genotypes shared by a given host species pair. Tiles on the diagonal (linking a species with itself) denote that species' hemoplasma richness -- i.e., the number of genotypes that that species had been identified with.

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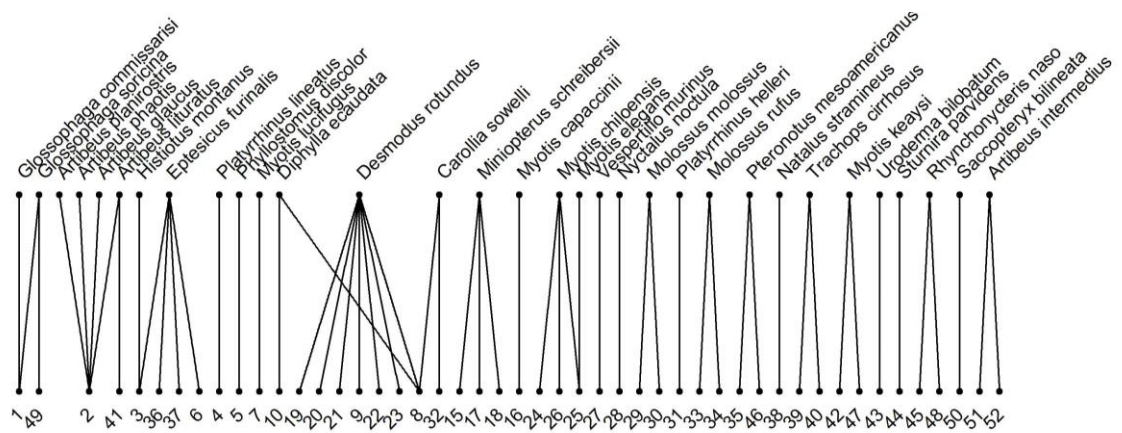


Figure 7. Depiction of the bipartite host-parasite network for bat-hemoplasma links. The names on the top row denote species' names; the numbers on the bottom row denote the names of the hemoplasma genotypes. Edges represent binary bat-hemoplasma associations.

4. Discussion

The high occurrence of hemoplasmas among non-hematophagous bats sampled in the current study is in accordance with previous works. For instance, 47% (32/68) of positivity for hemoplasmas was reported in bats sampled in the USA (Mascarelli et al., 2014), 96,8% (30/31) in *Miniopterus schreibersii* bats sampled in Spain (Millán et al., 2015), and 67% (150/223) in *D. rotundus* vampire bats sampled in Peru and Belize (Volokhov et al., 2017).

Even though 75 biological samples (among blood, spleen tissues and ectoparasites) were positive in the PCR assays for hemoplasmas, only 24 sequences were obtained, since the majority of the obtained amplicons showed faint bands in agarose gel electrophoresis, precluding obtaining high quality sequences. This finding has already previously reported by our research group (Ikeda et al., 2017), representing a difficulty to obtain good samples for sequencing. It is probably mainly due to hemoplasmas low concentration in the biological samples. Indeed, it has been previously shown a variation in bacteria number throughout time, which is observed with *M. haemofelis* in cats, suggesting a possible fluctuation in hemoplasma bacteremia (Tasker et al., 2018), turning to potentially interference in the PCR results. Also, even though tissue sequestration can happen as reported before in '*Candidatus Mycoplasma*

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turicensis' infection (Novacco et al., 2013), it seems to be controversy as, for example, no significant evidence of tissue sequestration of *M. haemofelis* has been described before (Tasker et al., 2009).

Previously, sequences of hemoplasmas detected in non-hematophagous bats sampled in the Brazilian states of Paraná (south) and Pará (north) grouped in a monophyletic group (Ikeda et al., 2017), differently from the phylogenetic pattern found in the present work. Herein, the obtained sequences were broadly distributed, albeit still clustering with bat-related hemoplasmas similarly to previous works (Millán et al., 2015, 2019; Holz et al., 2019; Fritschi et al., 2020). In fact, hemoplasmas sequences found in bats usually cluster with genotypes previously described in bats from several species, rodents, carnivores, humans and non-human primates (Millán et al., 2020).

A great diversity of genotypes was found among the 24 sequences obtained in the current work and among those sequences retrieved all over the world from GenBank database. A greater diversity was also found when analyzed by locality, since several genotypes can occur in a certain place. The same pattern has already been observed by Becker et al. (2020), who showed a high genetic diversity among bat-related hemoplasmas when identified 21 novel genotypes among bats sampled in Belize. Those authors also observed strong host specificity when considered the found hemoplasma genotypes.

Even though such sort of genotype assessment has not been performed with bat-associated hemoplasmas in Brazil so far, 16S rRNA hemoplasma sequences obtained from individuals belonging to the same species (*Molossus molossus*) grouped into a monophyletic group in a previous work (Ikeda et al., 2017). Also, Santos et al. (2020), when surveying hemoplasmas in hematophagous bats from south Brazil, showed that hemoplasmas from vampire bats grouped together. Endorsing it, all 16S rRNA hemoplasmas sequences obtained from *P. discolor* individuals sampled herein grouped together. Such relatedness of the sequences obtained from *P. discolor* is likely associated with the fact that they were captured in the same site and mist net. Knowing that this species is usually found as small harems within large colonies and have close contact with one another including grooming between females (Kwiecinski, 2006), animal interaction in this case might have favored the

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transmission of genotypes as previously described in other animal species (Biondo et al., 2009). Also, among hematophagous bats, hemoplasma genotypes identified in blood samples showed to be similar to those detected in saliva, evidencing the potential transmission of these agents by social interactions (Volokhov et al., 2017). Even though the phylogenetic and genotype analysis showed that hemoplasmas from a same genus tend to group together, the results from the unipartite and bipartite analyses did not robustly support the hypothesis.

In fact, direct contact and environmental exposure may be a potential transmission route for hemoplasmas between bats as previously observed for *Bartonella* spp. in hematophagous bats for example (Becker et al., 2018). The only Streblidae bat fly (*M. aranae*) showing positivity for hemoplasmas was collected from a positive animal that had blood sample positive for both 16S rRNA-based PCR protocols. However, the positivity observed in this ectoparasite might represent remnant hemoplasma DNA acquired from blood ingestion of the bat host. As previously pointed out for the dynamics of transmission of *Mycoplasma haemomuris*-like and wild rodents (Cohen et al., 2018), the role of arthropods in the transmission of hemoplasmas may not represent the most important transmission route. However, Hornok et al. (2019) detected hemotropic *Mycoplasma* sp. closely related to *M. haematohominis* in *I. simplex* ticks collected from a bat in Hungary. More recently, Descoux et al. (2020) reported the occurrence of hemoplasmas also closely related to the *M. haematohominis* in bat-related *C. horsfieldi* (Diptera: Nycteribiidae). In face with this scenario, future studies based on more sensitive approaches, such as deep sequencing, will shed some light on the presence of hemoplasmas in saliva, feces, and bat-associated ectoparasites will share light on the transmission of hemoplasmas among bats (Volokhov et al., 2017; Becker et al., 2018).

In conclusion, high genetic diversity was found among 16S rRNA hemoplasma sequences detected in non-hematophagous bats from central-western Brazil and previously detected in non-hematophagous and vampire bats from other regions of the world. Moreover, ectoparasites seem to have a minor or no role in the maintenance and transmission of hemoplasmas between bats.

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CRedit author statement: All authors contributed to the study conception and design. Material preparation, data collection and analysis: Priscila Ikeda; Jaire Marinho Torres, Elizabete Captivo Lourenço and Gregory F Albery. First draft of the manuscript, reviewing: Priscila Ikeda and Marcos Rogério André. Supervision: Marcos Rogério André, Heitor Miraglia Herrera, Carina Elisei de Oliveira, Rosangela Zacarias Machado. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Supplementary material

Table 1. Hemoplasmas 16S rRNA sequences obtained from bats worldwide used in the genotype analysis.

Accession number	Bat host specie	Country	Reference
KF713538	<i>Myotis lucifugus</i>	USA	Mascarelli et al., 2014
KM538691-92/ KM538694-98	<i>Miniopterus schreibersii</i>	Spain	Millán et al., 2015
KM5386993	<i>Myotis capaccini</i>		
KY3567447-51 / MN710412	<i>Molossus molossus</i>	Brazil	Ikeda et al., 2017 / dos Santos et al., 2020
MN715763 / MN710404/ MN710406/ MN710410 / KX722541	<i>Desmodus rotundus</i>		dos Santos et al., 2020
MN687571 / MN710405	<i>Diphylla ecaudata</i>		
MN398576 / MN398576	<i>Eidolon</i> sp.		
MN398577	<i>Rhinolophus</i> sp.	Nigeria	Di Cataldo et al., 2018
MK295626-30	<i>Myotis chiloensis</i>	Chile	Millán et al., 2019
LR699022	<i>Glossophaga commissarisi</i>	Costa Rica	Frittschi et al., 2020
LR699021	<i>Vespertilio murinus</i>	Germany	
LR699020	<i>Nyctalus noctule</i>		
KY932701-21 / KY932723-24/ KY932677-79 / KY932682-84	<i>Desmodus rotundus</i>	Peru	Volokhov et al., 2017 / Becker et al., 2020
MK353807 / MH245119/ MH245130 / KY932700/ KY932722 / KY932674-76 / 80-81 / 85-99	<i>Desmodus rotundus</i>		
MK353883	<i>Platyrrhinus helleri</i>		
MH245174 / MH245151	<i>Molossus rufus</i>		
MH245159 / MK353864	<i>Pteronotus parnellii mesoamericanus</i>		
MK353844	<i>Pteronotus americanus</i>	Belize	
MH245147/ MH245131	<i>Eptesicus furinalis</i>		
MK353818	<i>Natalus stramineus mexicanus</i>		
MH245145 / MK353860	<i>Trachops cirrhosus</i>		
MH245153 / MK353862	<i>Myotis keaysi</i>		
MH245187	<i>Artibeus lituratus</i>		
MK353871	<i>Uroderma bilobatum</i>		
MH245168 / MH245141	<i>Sturnira pavidens</i>		

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MK353871 / MH245146	<i>Rhynchonycteris naso</i>
MH245140	<i>Glossophaga soricina</i>
MH245138	<i>Saccopterix bilineata</i>
MH245186 / MK353876	<i>Artibeus intermedius</i>
MH245132	<i>Artibeus phaeotis</i>
MH245194	<i>Artibeus glaucus</i> <i>watsoni</i>
MK353833 / MH245134	<i>Carollia sowelli</i>

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CHAPTER 4 - Molecular Survey of *Anaplasmataceae* Agents and *Coxiellaceae* in non-hematophagous Bats and Associated Ectoparasites from Brazil¹

Priscila Ikeda¹, Jaire Marinho Torres², Ana Julia Vidal Placa¹, Victória Valente Califre de Mello¹, Elizabete Captivo Lourenço³, Heitor Miraglia Herrera², Carina Elisei de Oliveira², Claudia Hemsley⁴, Rick W. Titball⁴, Rosangela Zacarias Machado¹, Marcos Rogério André^{1*}

¹ Laboratório de Imunoparasitologia, Departamento de Patologia, Reprodução e Saúde Única, Universidade Estadual “Júlio de Mesquita Filho”, Jaboticabal, São Paulo, Brazil;

² Laboratório de Biologia Parasitária, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brazil;

³ Laboratório de Ecologia de Mamíferos, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil;

⁴ College of Life and Environmental Sciences – Biosciences, University of Exeter, Exeter, EX4 4QD, United Kingdom

Abstract: The *Anaplasmataceae* family (order *Rickettsiales*) encompasses obligately intracellular bacteria of the genera *Anaplasma*, *Ehrlichia*, and *Neorickettsia*. Together with *Coxiella burnetii* (*Coxiellaceae* family, order *Legionellales*), these bacteria represent important causative agents of diseases in humans and animals. The scarcity of studies that investigated the occurrence of these agents in bats and their associated ectoparasites, emphasizes the need to achieve a better understanding of the role of these animals in the maintenance of such bacteria. Herein, 418 samples (133 blood, 135 spleen, and 150 ectoparasites) are collected from 135 non-hematophagous bats belonging to 12 species in a periurban area of Campo Grande city, Mato Grosso do Sul state, midwestern Brazil. In the results, 1.65% (7/418), 12.04% (50/418), and 13.63% (57/418) of samples are positive in PCR assays for *Anaplasma* spp. (16S rRNA gene), *Ehrlichia* spp. (*dsb* gene), and *Neorickettsia* spp. (16S rRNA gene), respectively. *Anaplasma* spp. and *Neorickettsia* spp. are detected in one (5.26%) *Ornithodoros hasei* tick larva. *Ehrlichia* spp. is detected in 14% of bat

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flies (represented by *Megistopoda aranea*, *Trichobius costalimai*, and *Strebla hertigi*), 6% of tick larvae (*O. hasei*), 12% of Spinturnicidae mites (represented by *Periglischrus* sp., *P. torrealbai*, and *P. acutisternus*), and 38% of Macronyssidae mites (*Steatonyssus* sp.). The obtained sequences are observed to be similar to *Anaplasma phagocytophilum* (97.42–97.6% identified), *Ehrlichia minasensis* (96.73–100% identified), *Neorickettsia risticii* (96.7–100% identified), and *Neorickettsia findlayensis* (95.07–100% identified) by BLASTn analyses, and closely related to *Ehrlichia ruminantium* by phylogenetic analyses based on the *gltA* gene. No bat samples (blood/spleen) are positive in the qPCR assay for *C. burnetii* based on the *IS1111* gene. The present work shows, for the first time, the occurrence of *Anaplasmataceae* in bats and associated ectoparasites (ticks, mites, and bat flies) from Brazil.

Keywords: *Anaplasma* spp.; *Ehrlichia* spp.; Chiroptera; *Coxiella burnetii*; *Neorickettsia* spp.

1. Introduction

Approximately 20% of known mammal species belong to the order Chiroptera, which composes the second largest group of mammals in the world [1]. Currently, there are 1400 species described worldwide. From those, 181 bat species are officially registered in Brazil and distributed in 68 genera and nine families [2]. Even though bats are considered as important reservoirs, hosts, and sources of infection for various zoonotic agents (mainly viruses [3]), little is known about their role in the maintenance of vector-borne bacteria, such as *Ehrlichia* spp., *Anaplasma* spp., *Neorickettsia* spp., and *Coxiella burnetii*.

The *Anaplasmataceae* family (order *Rickettsiales*) comprises mandatory intracellular agents of veterinary and medical importance. The family comprises the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Neoehrlichia*, and *Wolbachia* [4–6]. Among the most common diseases caused by this group of bacteria, human granulocytic anaplasmosis (HGA) and human granulocytic ehrlichiosis (HME) affect humans and cause similar clinical symptoms that usually start after a tick bite event [7–9].

The main *Anaplasmataceae* agents associated with animal diseases are *Ehrlichia canis* and *Anaplasma platys*, which cause canine monocytic ehrlichiosis and

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canine cyclic thrombocytopenia; *Anaplasma marginale*, which causes bovine anaplasmosis; and *Neorickettsia risticii*, which causes the Potomac horse fever (PHF).

Up to 20 tick species are involved in the transmission of *A. marginale* and *A. phagocytophilum*; the main tick genera are represented by *Ixodes* spp. and *Haemophysalis* spp. (for *A. phagocytophilum*), *Dermacentor* spp. (for *A. phagocytophilum* and *A. marginale*), and *Rhipicephalus* spp. and *Hyalomma* spp. (for *A. marginale*) [9,10]. While *E. canis* is transmitted by the tropical lineage of *Rhipicephalus sanguineus* sensu lato [11] and *Amblyomma* spp., ticks are involved in the transmission of *E. ewingii*, *E. chaffeensis*, and *E. ruminantium* [12,13]. *Ehrlichia minasensis* has been primarily isolated from *Rhipicephalus microplus* and naturally infected cattle [5].

Despite being a sister clade to *Ehrlichia*, '*Candidatus Neoehrlichia* spp.' is not recognized as a new genus, mainly due to the difficulty of isolation [14–17]. Additionally, a plethora of uncultured agents was also described in the literature with the '*Candidatus*' status [18–29].

Neorickettsia genera encompasses obligatory intracellular bacteria that, differently from the other *Anaplasmataceae* agents, are endosymbiotic within digeneans and depend on trematodes for their life cycle [30]. Four diseases are related to these agents: Potomac horse fever (PHF) caused by *N. risticii*; salmon poisoning disease in dogs, which is mainly caused by *N. helminthoeca*; Elokomin fluke fever (EFF) in canids and bears, which is caused by *N. elokominica*; Sennetsu fever in humans caused by *N. sennetsu* agent. Recently, Teymournejad et al. (2020) [31] described a new species of *Neorickettsia* (i.e. *Neorickettsia findlayensis*) in Ontario, Canada, causing PHF disease based on serological evidence, whole genome sequencing, and experimental infection of horses. In Brazil, *N. risticii* was molecularly detected in horses from Rio de Janeiro, which presented with hypoalbuminemia, high levels of creatine kinase, and lactate dehydrogenase [32], as well in equids in the state of Rio Grande do Sul, where outbreaks of equine monocytic ehrlichiosis were reported [33,34].

Studies on the occurrence of *Ehrlichia* spp. and *Anaplasma* spp. in bats are scarce. For instance, *A. phagocytophilum* DNA was already reported in bat fecal samples in France [35] and in *Ixodes simplex* parasitizing bats in Hungary and

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Romania [36]. The DNA of *A. phagocytophilum* and *Ehrlichia* spp. was found in *Pipistrellus pipistrellus* and *P. kuhlii* bats, respectively, in Russia [37]. *Ehrlichia* spp. was also molecularly detected in bat-related *Argas vespertilionis* ticks in France [38] and *Brachyphylla cavernarum* bats in Saint Kitts Island [39]. *A. vespertilionis* ticks collected from bats in different counties of England were also positive for *Ehrlichia/Anaplasma* [40]. The detection of these agents in bats and associated ectoparasites in human-shared sites [35,38] contributed to an increased concern about the role of these animals as reservoirs and in the spread of potentially zoonotic diseases.

On the other hand, bats species have been implicated as important hosts for *N. risticii*. This agent was detected in bat tissues and/or associated trematodes in the USA, Argentina, Egypt, Philippines, and the Netherlands [41–45]. Additionally, this agent was detected in fecal samples collected from a variety of bats from Europe [46].

Coxiella burnetii (order *Legionellales*), the etiological agent of Q fever in humans, is a Gram-negative bacterium that parasites monocytes and macrophages [47]. It can be found in feces, milk, urine, and abortion products, such as placenta, where it is possible to find a high concentration of the bacteria [48]. This agent can cause abortion and infertility in cattle and goats [49]. In humans, while the acute phase is characterized by hepatitis or, more unusually, pneumonia, the most frequent clinical manifestation in the chronic phase is endocarditis [47,50]. This agent was already described in bats and related ectoparasites from Algeria, South Africa, Chile, and Brazil [51–53].

Due to the importance of *Anaplasmataceae* agents and *C. burnetii* in animal and human health and the high representativeness of the order Chiroptera in Brazil, the present study aims to molecularly survey these agents in non-hematophagous bats and their ectoparasites sampled in midwestern Brazil.

2. Results

A total of 135 bats were captured and DNA samples obtained from 135 spleen tissues, 133 blood, and 150 ectoparasites (including 64 bat flies, 46 Spinturnicidae mites, 21 Macronyssidae mites and 19 tick larvae) were positive in the PCR assays for the endogenous genes (Supplementary Table S1).

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All DNA samples obtained from spleen fragments, whole blood samples, and ectoparasites, totaling 418 samples, were negative for *Ehrlichia* spp. and *Anaplasma* spp. when tested for the *groEL* gene by a multiplex real-time PCR. Seven (1.67%) out of the 418 samples submitted to the protocol, using conventional nested PCR targeting the 16S rRNA gene for *Anaplasma* spp., were considered positive, presenting a single band with low intensity (Supplementary Table S2). Out of these, three samples were represented by spleen fragments from *Platyrrhinus lineatus* individuals, three from blood samples of *Phyllostomus discolor* specimens, and one from the *Ornithodoros hasei* tick larvae, which was collected from a negative *P. discolor* individual. Out of all positive samples, only one amplicon obtained from *O. hasei* was sequenced, due to the low intensity of bands in agarose gel electrophoresis. When analyzed by BLASTn analysis, the obtained sequence (542 bp) showed 97.6% identity (100% query coverage; E-value = 0.0) with the *A. phagocytophilum* detected in a deer (*Hydropotes inermis*) from the Republic of Korea (Accession number: KR611598.1) and 97.42% of identity with *A. phagocytophilum* and *A. bovis* detected in *Haemaphysalis longicornis* (100% of query coverage; E-value = 0.0), sampled in South Korea (Accession numbers: GU046565.1 / GU064901.1).

Out of the seven samples showing positivity in the nested PCR for *Anaplasma* spp., none showed positivity neither when submitted to quantitative real time PCR (qPCR) for *A. phagocytophilum* based on the *msp-2* gene nor in PCR assays targeting ITS 23S-5S and *groEL*. On the other hand, one blood sample obtained from a *P. discolor* individual was positive in the PCR targeting the *gltA* gene and sequenced. However, when tested by BLASTn, the sequence obtained (620 bp) showed to be similar (83.15% of identity) to *Ehrlichia* sp. identified in a tick from China (KJ410275.1) (100% of query coverage; E-value = 4×10^{-155}).

Regarding the PCR targeting the *dsb* gene for *Ehrlichia* spp., 50 (11.96%) out of the 418 samples were positive: ten (20%) blood samples (collected from five *Artibeus planirostris*, four *P. discolor*, and one *Eptesicus furinalis*), eight (16%) spleen fragments (collected from three *A. planirostris*, two *Artibeus lituratus*, two *P. lineatus*, and one *Carollia perspicillata*), seven (14%) bat flies [namely *Megistopoda aranea* (n = 3), *Trichobius costalimai* (n = 2), and *Strebla hertigi* (n = 2)], three (6%) tick larvae (*O. hasei*), six (12%) Spinturnicidae mites (three *Periglischrus* sp., two *Periglischrus*

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torrealbai and one *Periglischrus acutisternus*), and 19 (38%) Macronyssidae mites (*Steatonyssus* sp.). Six animals had more than one positive sample, while animal #7 (*A. planirostris*) showed positivity in both spleen and blood samples. All the remaining five individuals were represented by *P. discolor* specimens and showed positivity for blood and/or one or more ectoparasites associated. Out of these positive samples, one bat blood sample, two Spinturnicidae mite samples, and nine Macronyssidae mite samples were chosen for sequencing (Supplementary Table S3). The sample obtained from one Macronyssidae mite (#75) (353 bp) showed to be 100% identical to a sequence of *E. minasensis* detected in *R. microplus* (MT135771) from Brazil (100% of query coverage; E-value = 0.0). The remaining sequences (399–416 bp) were similar (identity ranging from 96.73 to 99.26%) to a sequence of *E. minasensis* detected in *Bradypus variegatus* (MT212414) from Brazil (query coverage ranging from 99–100%; E-value = 0.0). None of the 50 *Ehrlichia* (dsb)-positive samples showed to be positive neither in qPCR protocols for *Ehrlichia canis* (dsb gene) and *E. chaffeensis* (vlpt gene) nor in cPCR protocols for *Ehrlichia* spp. based on the *omp-1*, *sodB*, and *groEL* genes.

Fifty-seven (13.63%) out of 418 samples were positive in the nested PCR assay for *N. risticii*, based on the 16S rRNA gene: 11 (19.3%) were from animals of the species *A. lituratus* (five from spleen fragments and six from whole blood), 11 (19.3%) of the species *P. lineatus* (four from spleen fragments and seven from whole blood), 10 (17.54%) of the species *A. planirostris* (six from spleen fragments and four from whole blood), nine (15.79%) of the species *P. discolor* (two from spleen fragments and seven from whole blood), four (7.01%) of the species *E. furinalis* (one from spleen fragments and three from whole blood), three (5.26%) of the species *C. perspicillata* (two from spleen fragments and one from whole blood), two (3.5%) of the species *Molossus molossus* (one from spleen fragments and one from whole blood), two (3.5%) of the species *Glossophaga soricina* (one from spleen fragments and one from whole blood), and one (1.57%) positive blood sample of the species *Myotis nigriscans*, *Molossops temminckii*, *Eumops perotis*, and *Chiroderma villosum*. The only ectoparasite sample that was positive for this agent was an *O. hasei* tick larva collected from a negative specimen of *P. discolor* (Supplementary Table S4). Out of the 34 positive blood samples for the 16S rRNA-based PCR assay, 11 (32.35%) were chosen for sequencing. The samples were chosen for convenience, but in such a way as to

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represent an individual of each bat species that showed positivity in this protocol. When analyzed by BLASTn, eight sequences (426–524 bp) obtained from blood samples showed similarity (97.9–100%) with *N. risticii* (Accession numbers MK760560 and CP001431.1) (100% of query coverage; E-value = 0.0); three sequences obtained from a tick larvae (304 bp), one bat spleen (421 bp) and one bat blood sample (385 bp) was observed to be 95.07–100% identical to *N. findlayensis* (Accession number MH476282) (100% of query coverage; E-value = 7×10^{-131} to 0.0); finally, two sequences obtained from bat blood samples (212–282 bp) were observed to be similar (96.7–100%) to *N. risticii* and *N. findlayensis* (Accession numbers MK580530 and MH476282) (100% of query coverage ; E-value = 1×10^{-143} to 1×10^{-96}). All the positive samples were negative in the additional PCR protocols targeting the *p51kDa*, *groEL*, and near-full 16S rRNA genes.

Moreover, *Ehrlichia* DNA was detected in 7/7 bat flies, 3/3 ticks, 18/19 Macronyssidae, and 4/6 Spinturnicidae mites, whose bat hosts were negative. The ticks that were positive for *Neorickettsia* spp. and *Anaplasma* spp. were also collected from a negative animal.

All the BLASTn results are summarized and presented in the Supplementary Table S5.

A total of 268 DNA samples (133 from whole blood and 135 from spleen fragments) were screened for the presence of *C. burnetii* DNA and none were positive. Unfortunately, the DNA samples from ectoparasites did not yield sufficient volume for this assay.

One phylogenetic tree was constructed based on a 522 bp alignment of the *gltA* gene (Figure 1), using the Maximum Likelihood method and TIM3 + F + I + G4 as the evolutive model. The unique sequence obtained from a blood sample from *P. discolor* (GenBank Accession Number OK338001) was positioned closely to *E. ruminantium*. The remaining sequences were not further analyzed as the final alignment length was not suitable for robust inferences.

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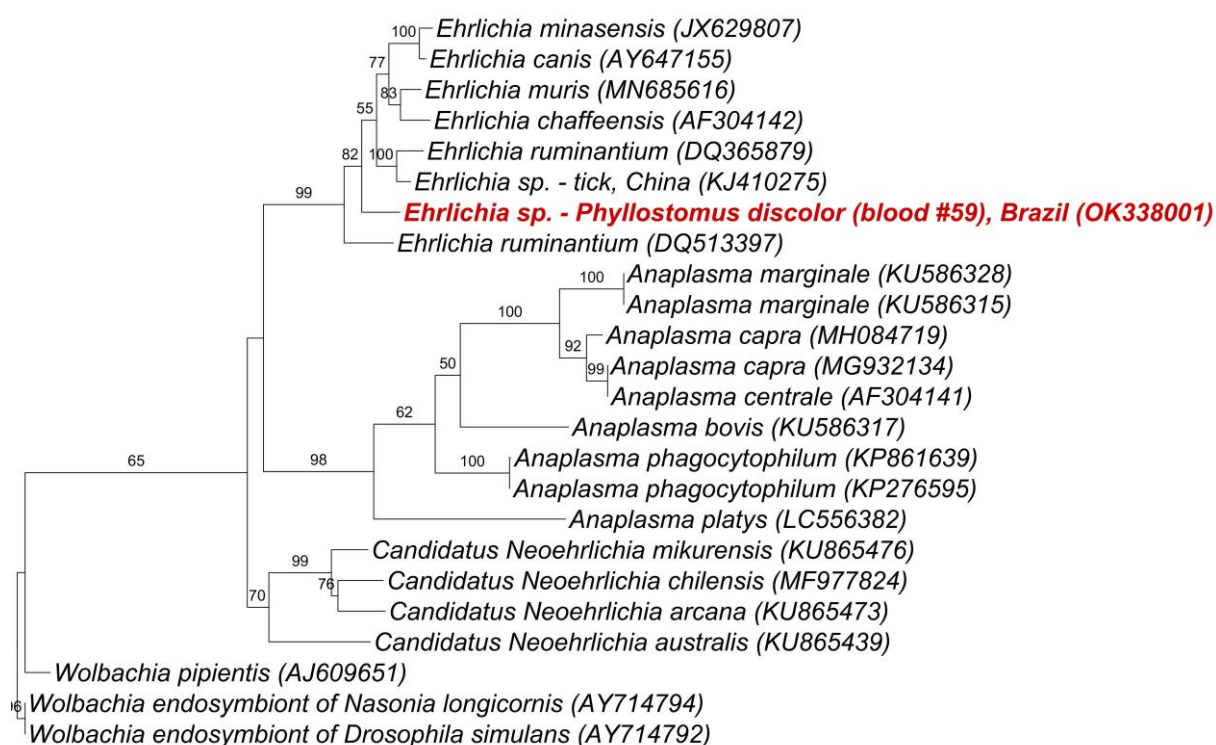


Figure 1. Phylogenetic tree based on an alignment of 522 bp of *Ehrlichia gltA* gene, using Maximum Likelihood method and TIM3 + F + I + G4 as evolutive model. Sequence from the present study is highlighted in red. *Wolbachia* spp. was used as outgroup.

3. Discussion

Among all the seven positive samples for *Anaplasma* spp., only the amplicon obtained from *O. hasei* tick larva was sequenced, obtaining 97.6% similarity with *A. phagocytophilum*, which was previously detected in deer of the species *Hydropotes inermis* from the Republic of Korea. Previously, the occurrence of *A. phagocytophilum* was described in 22.6% (63/278) of fecal samples of *Rhinolophus hipposideros* in France [35], 1.7% (1/59) of blood samples of *Myotis myotis* [54] in Poland, 4.3% (6/138) of *I. simplex* ticks collected from bats from Hungary and Romania [36], and 14.28% (1/7) of *Nyctalus noctula* species from Russia [37]. Despite the high occurrence of *A. phagocytophilum* in bats from France, the remaining abovementioned studies showed low positivity for this agent among bats or associated ectoparasites, corroborating the results found in the present work for *Anaplasma* spp. (1.67%; 7/418). Noteworthy, even though sequences similar to *A. phagocytophilum* have been detected in several wild animals from Brazil, negative results have been reported when a qPCR specific for this

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agent and based on *msp-2* gene was used [6,55]. Together, these findings suggest that a putative new *Anaplasma* species is circulating among wild animals from Brazil. Indeed, new ‘*Candidatus Anaplasma* spp.’, namely ‘*Candidatus Anaplasma brasiliensis*’ and ‘*Candidatus Anaplasma amazonensis*’ have been recently detected in mammals from the Superorder Xenarthra from Brazil [29]. Future studies aiming at obtaining larger fragments of different molecular markers are needed in order to check the phylogenetic relatedness of bat-associated *Anaplasma* spp. to these newly described ‘*Candidatus Anaplasma* spp.’

The unique *Anaplasmataceae gltA* sequence obtained was observed to be similar to *Ehrlichia* spp. with low identity obtained when compared to other sequences (<90%). The phylogenetic analysis evidenced that the sequence was closely related to *E. ruminantium* and *Ehrlichia* spp., detected in a tick from China. Even though there are reports of the occurrence of *E. canis*, *E. ruminantium*, and *E. muris*-like in humans [8], these agents represent a major concern in animal health. *E. ruminantium*, which is known to cause heartwater or cowdriosis in wild and domestic ruminants, is mainly transmitted by *Amblyomma* spp. ticks in Africa and the Caribbean [12,56]. Interestingly, a genotype (Strain Jaguar) related to, albeit distantly, called *E. ruminantium*, was detected in jaguars and in associated ticks (*A. triste*, *A. sculptum*, and *Amblyomma* spp.) sampled in Pantanal wetland, in midwestern Brazil, and is based on *rrs* and *dsb* sequences [57]. Additionally, a similar genotype (fox-ES-1) was detected in crab-eating foxes (*Cerdocyon thous*) in southeastern Brazil [58].

Herein, 11.96% (50/418) of samples were positive in PCR assays for *Ehrlichia* spp. based on the *dsb* gene. Out of these positive samples, 35 [70% (35/50)] were obtained from ectoparasites. In fact, few reports have shown the occurrence of *Ehrlichia* sp. in bats and associated ectoparasites. Socolosvich et al. (2012) [38] detected *Ehrlichia* DNA in *A. vespertilionis*, an Argasidae species often associated with bats and humans in places cohabitated by them. In Saint Kitts Island, Reeves et al. (2016) [39] found 16% (4/25) of positivity for *Ehrlichia* spp. in blood samples collected from bats (*Brachyphylla caverum*). The obtained sequences showed 95% identity with *Ehrlichia* sp. detected in ticks collected from mammals from Asia. More recently, Lv et al. (2018) [40] detected DNA of *Ehrlichia/Anaplasma* in five out of 120 *A. vespertilionis* ticks collected from three *P. pipistrellus* bats in England. Zabashta et al. (2019) [37]

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also detected *Ehrlichia* DNA (without sequencing) in *P. pipistrellus* and *P. kuhlii* bats from Russia.

Interestingly, the obtained *Ehrlichia dsb* sequences were observed to be related to *E. minasensis*. This agent has been described in cattle and *R. microplus* from Brazil [5,59,60], potentially causing disease in cattle [61]. Recently, *Ehrlichia dsb* genotypes closely related to *E. minasensis* were detected in Xenarthra mammals in Brazil [29]. These results suggest that *E. minasensis* may infect a broader range of hosts than previously recognized.

Even though the multiplex qPCR assay, used herein for detecting a fragment of *groEL* gene for *Ehrlichia* and *Anaplasma* species with high sensitivity and specificity [62], was originally designed for amplifying DNA from the main *Ehrlichia* (namely *E. canis*, *E. chaffeensis*, *E. muris*, and *Ehrlichia* sp., Anan strain/IOE agent), and *Anaplasma* (namely *A. phagocytophilum*, *A. platys*, *A. marginale*, *A. centrale*, *A. ovis*, and *A. bovis*) species. Considering that bats and associated ectoparasites likely harbor DNA of genetically diverse *Ehrlichia* and *Anaplasma* species, the used qPCR assays, based on the *groEL* gene, which is less conserved than 16S rRNA gene, were not able to catch these putative new genotypes/species of *Ehrlichia* and *Anaplasma*. This incongruence between the results of the *groEL*-based multiplex qPCR and 16S rRNA-based cPCR assays was already reported previously [29,62,63], when working on the molecular detection of *Ehrlichia* and *Anaplasma* in biological samples from wild animals from Brazil, which emphasizes the occurrence of putative new genotypes or even species of *Anaplasmataceae* in the Brazilian wildlife.

High prevalence of *N. risticii* in bats has been reported in the USA (e.g., 60% (3/5) in *Myotis yumanensis*, 44.7% (17/38) in *Myotis lucifugus*, 40% (6/15) in *Eptesicus fuscus*) [41,42] and in the Netherlands (100% (4/4) in *Myotis dasycneme*) [46]. In South America, while the present work found 38.51% (52/135) of occurrence for *Neorickettsia* spp., Ciccutin et al. [43,44] found 8.2% (5/61)–10% (3/30) prevalence of *N. risticii* in *Tadarida brasiliensis* sampled in Argentina.

N. risticii is known to cause the equine monocytic neorickettsiosis (formerly ehrlichiosis) or Potomac horse fever (PHF) in horses [64], being reported primarily in the USA. The main transmission route occurs through ingestion of aquatic snails that act as intermediary hosts for *Neorickettsia*-infected digenean trematodes [65,66].

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Recently, Paulino et al. (2020) [32] detected *N. risticii* (16S rRNA) DNA in horse blood samples, sampled in Rio de Janeiro, Brazil. Previously, an outbreak of equine monocytic neorickettsiosis has also been reported in the state of Rio Grande do Sul [33]. In addition, *N. risticii* DNA has been detected in *Heleobia* snails (*H. piscium*, *H. parchappei*, *H. davisii*) collected in the state of Rio Grande do Sul [34]. Recently, *N. findlayensis* was described as a new species that also causes a disease similar to PHF [31].

It is known that bats not only act as a host for *N. risticii*-infected trematodes but also as a possible natural reservoir for *N. risticii*, as this agent has been detected in tissues (e.g., intestine, lung, liver, and spleen) of naturally infected bats [41–44]. The detection of *Neorickettsia* spp. in digenean trematodes found parasitizing bats [45] emphasized their contribution to the maintenance of these agents. In addition, vertical transmission of *N. risticii* from adult trematodes (*Acanthatrium oregonense*) to eggs was evidenced when these helminthes were collected from *E. fuscus* bats in the USA [42]. In Europe, Hornok et al. (2018) [46] found 2.96% (4/135) positivity for *N. risticii* in *M. dasycneme* individuals sampled in the Netherlands. Future studies should be performed in order to investigate the bat-associated trematodes involved in the epidemiology of *N. risticii* in Brazil. Considering that it was not possible to confirm which *Neorickettsia* species is circulating among the sampled bats, epidemiological studies should be performed in an attempt to establish a possible correlation between infected bats and the occurrence or neorickettsiosis in horses in Brazil.

Herein, *Ehrlichia* DNA was detected in 14% bat flies (*M. aranea*, *T. costalimai*, and *S. hertigi*), 6% tick larvae (*O. hasei*), 12% Spinturnicidae mites, and 38% Macronyssidae mites. Out of those, *Ehrlichia* DNA was detected in seven bat flies, three ticks, 18 Macronyssidae and four Spinturnicidae mites, collected from bats that showed to be negative in the PCR assay for this agent. Similarly, the *O. hasei* tick larva that was positive for *Neorickettsia* spp. was collected from a specimen of *P. discolor* that showed to be negative for this agent. Moreover, DNA of *Anaplasma* spp. was detected in *O. hasei* larvae collected from a negative *P. discolor* individual. Considering the impossibility that the larval stage had ever fed on another animal, the transovarial transmission of bat-associated *Anaplasma* spp. cannot be rule out. Indeed, while the transovarial transmission of non-pathogenic *A. phagocytophilum* variants has been

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already reported in *Dermacentor albipictus* ticks, transovarial transmission of *Ehrlichia* spp. has not been proved [8,67]. Also, a possible explanation for these results might rely on a false-negative result with the bat samples (blood or spleen). It may occur due to the higher concentration of the host blood meal in the arthropod digestive tract, which may have increased the chances of detection of *Ehrlichia* DNA in the ectoparasite sample, as previously reported [68]. Lastly, the efficiency of the PCR assays should also be considered in this case. Nevertheless, the real role of ticks, mites, and bat flies in the transmission of *Anaplasmataceae* agents among bats should be further investigated.

Despite the lack of positivity for *C. burnetii* among bats sampled in the present work, this agent has already been reported in 3.4% (4/119) of bats in the Atlantic Forest in the states of Santa Catarina and Rio de Janeiro, Brazil, by conventional PCR and nested PCR assays based on the *IS1111* gene in which the positive animals belonged to the species *A. lituratus* (3/4) and *A. fimbriatus* (1/4) [53]. Furthermore, this agent was detected in 15.8% (3/19) of *I. verpertilionis* ticks collected from bats in northeastern Algeria using real-time PCR based on the *IS1111* marker [51]. More recently, *C. burnetii* was detected in five individuals (9%) of *T. brasiliensis* from Chile, also using a qPCR targeting the *IS1111* gene [69]. However, it is worth mentioning that *Coxiella*-like endosymbionts are commonly found in ticks and, recently, it was shown that endosymbionts and *C. burnetii* are closely related [70]. The authors also suggested that the lineages might have evolved independently. Therefore, the possible participation of bats and specially their ectoparasites in the transmission of *C. burnetii* should be further investigated, since molecular and serological evidence of infection by *C. burnetii* has been reported in humans from Brazil [71–73].

In conclusion, the present work shows, for the first time, the occurrence of *Ehrlichia* spp. related to *E. minasensis*, *E. ruminantium*, and *Anaplasma* spp., similar to *A. phagocytophilum* and *Neorickettsia* spp., in non-hematophagous bats and associated ectoparasites (ticks, mites, and bat flies) from Brazil.

4. Materials and Methods

A total of 135 spleen, 133 blood and 150 ectoparasites samples were collected from 135 non-hematophagous bats captured and sampled in two different sites

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(“Centro de Educação Ambiental Polonês” and “Centro de Educação Ambiental Florestinha”) situated in the Campo Grande city, Mato Grosso do Sul state, Brazil as described elsewhere [74]. The bats’ capture occurred quarterly between June 2017 and March 2018.

The DNA was purified from 50–200 µL blood samples using the Illustra Blood Mini Kit (GE Healthcare®, Chicago, IL, USA) and from 10 mg of each bat spleen tissue and ectoparasites using the Illustra Tissue Mini Kit (GE Healthcare®), according to manufacturer’s instructions. The DNA concentration and absorbance ratio (260/280 nm) were measured using a spectrophotometer (Nanodrop, Thermo Scientific, Waltham, MA, USA). To check the quality of the extracted DNA and avoid the presence of PCR inhibitors, each spleen and blood DNA extracted was tested by a conventional PCR targeting the mammal *gapdh* (Glyceraldehyde 3-phosphate dehydrogenase) gene [75]. For the ectoparasites collected from the animals, two different conventional PCR protocols were used. While a PCR protocol based on cytochrome c oxidase subunit I (*cox-1*) gene [76] was used for DNA samples obtained from bat flies and mites, a protocol based on the 16S rRNA gene [77] was used for DNA samples obtained from tick larvae.

Bat blood and spleen samples positive for the mammals’ endogenous gene (*gapdh*) PCR protocol were submitted to PCR assays for *Anaplasmataceae* agents and *C. burnetii*. Ectoparasites positive in PCR assays based on *cox-1* and 16S rRNA genes were only submitted to PCR assays for *Anaplasmataceae* agents, due to the low volume of DNA obtained.

For *Anaplasmataceae* agents, quantitative real-time (q), nested (n), and conventional (c) PCR screening protocols were performed for *Anaplasma* spp. and *Ehrlichia* spp. A multiplex qPCR for *Anaplasma* spp. and *Ehrlichia* spp. based on the *groEL* gene was performed as previously described [62]. Additionally, a nested PCR for *Anaplasma* spp., based on the 16S rRNA gene [78] and a conventional PCR for *Ehrlichia* spp., based on the *dsb* gene [79], were also used as screening protocols. All the positive samples found in the above-mentioned protocols were submitted to qPCR assays for *E. canis* based on the *dsb* gene [79], *E. chaffeensis* based on the *vlpt* gene [80], and *A. phagocytophilum* based on *msh-2* gene [81]. Conventional and nested PCR assays, based on *groEL* [17,82], *gltA* [16], *sodB* [83], and *omp-1* [84] genes as

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well as based on the intergenic region ITS 23S-5S [85], were performed for molecular characterization.

For molecular detection of *N. risticii*, DNA samples were submitted to a nested PCR based on the 16S rRNA gene, as previously described [86]. Additionally, the positive samples for the 16S rRNA were submitted to PCR assays targeting the nearly full-length 16S rRNA [87], *p51kDa*, and *groEL* [43,63,88] genes.

A quantitative real-time PCR targeting the repetitive element *IS1111* was used for *C. burnetii* detection, as previously described [89].

As positive controls for the PCR assays, DNA samples from *A. phagocytophilum* and *N. risticii* kindly provided by Professor John Stephen Dumler (Uniformed Services University of the Health Sciences, Bethesda, MD, USA), and the *E. canis* Jaboticabal-strain (maintained in DH82 cell culture) were used. The *C. burnetii* DNA positive control was kindly provided by Professor Dr. Renato Arruda Mortara (Universidade Federal de São Paulo, SP, Brazil).

Firstly, the obtained sequences were submitted to a screening test using the Bioedit version 7.0.5.3 [90] to evaluate the electropherogram quality and to obtain consensus sequences from the alignment of the sense and antisense sequences. The BLAST program [91] was used to analyze the sequences of nucleotides (BLASTn), aiming to compare with sequences from an international database (GenBank) [92]. The consensus sequences obtained in the present study and those retrieved from GenBank were aligned using the ClustalW software [93] via Bioedit version 7.0.5.3 [89]. Phylogenetic inferences were based on Maximum Likelihood (ML). The best evolutionary model was selected by ModelFinder [94] and the trees were constructed using IQTree web server [95] with ultrafast Bootstrap of 1000 replicates [96]. All the trees were examined in Treegraph 2.0 beta software [97].

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Description of the 418 samples (spleen, blood or ectoparasites) collected from bats in Campo Grande, Mato Grosso do Sul, Brazil, and used in the present work for molecular screening of *Anaplasmataceae* agents and *Coxiella burnetii*, Table S2: Bat samples positive for *Anaplasma* spp. according to PCR assays targeting five different molecular markers, Table S3: Bat samples positive for

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Ehrlichia spp. according to PCR assays targeting six different molecular markers, Table S4: Bat samples positive for *Neorickettsia* spp. according to PCR assays targeting three different molecular markers, Table S5: BLASTn results obtained after sequencing of amplicons obtained from PCR assays for *Ehrlichia* spp., *Anaplasma* spp. and *Neorickettsia* spp.

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Supplementary Material

Supplementary Table 1. Description of the 418 samples (spleen, blood or ectoparasites) collected from bats in Campo Grande, Mato Grosso do Sul, Brazil, and used in the present work for molecular screening of *Anaplasmataceae* agents and *Coxiella burnetii*.

ID	Capture site	Bat or ectoparasite species	Forearm size	Sex	Age	Reproductive status	Sample type
MCG 01	CEA Polonês	<i>Artibeus lituratus</i>	73,6	M	Adult	Active	Spleen and Blood
SM 01		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 02	CEA Polonês	<i>Artibeus lituratus</i>	73,8	F	Adult	Inactive	Spleen and Blood
BF 02		<i>Paratrichobius longicrus</i>					Bat fly
SM 02		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 03	CEA Polonês	<i>Artibeus planirostris</i>	60,7	F	Adult	Inactive	Spleen and Blood
MCG 04	CEA Polonês	<i>Myotis nigricans</i>	37,8	M	Adult	Active	Spleen
MM 04		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 05	CEA Polonês	<i>Platyrrhinus lineatus</i>	49,8	F	Adult	Inactive	Spleen and Blood
SM 05		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MM 05		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 06	CEA Polonês	<i>Artibeus planirostris</i>	67,2	M	Adult	Active	Spleen and Blood
MCG 07	CEA Polonês	<i>Artibeus planirostris</i>	59,6	F	Juvenile	Inactive	Spleen and Blood
BF 07.1		<i>Megistopoda aranea</i>					Bat fly
BF 07.2		<i>Megistopoda aranea</i>					Bat fly
MCG 08	CEA Polonês	<i>Artibeus lituratus</i>	73,7	M	Adult	Active	Spleen and Blood
BF 08		<i>Megistopoda aranea</i>					Bat fly
SM 08		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>	36,7	M	Adult	Active	Spleen and Blood

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BF 09		<i>Trichobius parasiticus</i> (complex)					Bat fly
MCG 10	CEA Polonês	<i>Artibeus lituratus</i>	73,4	M	Adult	Active	Spleen and Blood
BF 10		<i>Megistopoda aranea</i>					Bat fly
MCG 11	CEA Polonês	<i>Artibeus planirostris</i>	57,8	M	Adult	Active	Spleen and Blood
BF 11		<i>Megistopoda aranea</i>					Bat fly
SM 11		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 12	CEA Polonês	<i>Artibeus planirostris</i>	62,4	F	Juvenile	Inactive	Spleen and Blood
BF 12		<i>Megistopoda aranea</i>					Bat fly
MCG 13	CEA Polonês	<i>Artibeus planirostris</i>	63	M	Juvenile	Inactive	Spleen and Blood
MCG 14	CEA Polonês	<i>Artibeus planirostris</i>	65,6	M	Juvenile	Inactive	Spleen and Blood
SM 14		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 15	CEA Polonês	<i>Platyrrhinus lineatus</i>	50	F	Juvenile	Inactive	Spleen and Blood
MCG 16	CEA Polonês	<i>Artibeus planirostris</i>	64,1	F	Juvenile	Inactive	Spleen and Blood
BF 16		<i>Megistopoda aranea</i>					Bat fly
MCG 17	CEA Polonês	<i>Artibeus planirostris</i>	62,3	F	Adult	Pos-lactating	Spleen and Blood
BF 17		<i>Megistopoda aranea</i>					Bat fly
MCG 18	CEA Polonês	<i>Platyrrhinus lineatus</i>	49,1	F	Juvenile	Inactive	Spleen and Blood
SM 18		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 19	CEA Polonês	<i>Artibeus planirostris</i>	61,6	F	Juvenile	Inactive	Spleen and Blood
BF 19.1		<i>Megistopoda aranea</i>					Bat fly
BF 19.2		<i>Megistopoda aranea</i>					Bat fly
MCG 20	CEA Polonês	<i>Platyrrhinus lineatus</i>	48,9	M	Juvenile	Inactive	Spleen and Blood
BF 20		<i>Trichobius dugesii</i> (complex)					Bat fly
MCG 21	CEA Polonês	<i>Platyrrhinus lineatus</i>	49,9	F	Juvenile	Inactive	Spleen and Blood
SM 21		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 22	CEA Polonês	<i>Platyrrhinus lineatus</i>	47,4	M	Juvenile	Inactive	Spleen and Blood
MCG 23	CEA Polonês	<i>Eptesicus furinalis</i>	39,9	F	Juvenile	Inactive	Spleen and Blood

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BF 23		<i>Trichobius dugesii</i> (complex)					Bat fly
MM 23		<i>Steatonyssus</i> sp.					Macronyssidae mite
MCG 24	CEA Polonês	<i>Platyrrhinus lineatus</i>	48,7	F	Juvenile	Inactive	Spleen and Blood
MCG 25	CEA Polonês	<i>Platyrrhinus lineatus</i>	50,9	F	Juvenile	Inactive	Spleen and Blood
BF 25		<i>Trichobius dugesii</i> (complex)					Bat fly
SM 25		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 26	CEA Polonês	<i>Artibeus planirostris</i>	64,3	F	Juvenile	Inactive	Spleen and Blood
SM 26		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 27	CEA Polonês	<i>Artibeus planirostris</i>	60,7	F	Juvenile	Inactive	Spleen and Blood
BF 27		<i>Megistopoda aranea</i>					Bat fly
SM 27		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 28	CEA Polonês	<i>Artibeus lituratus</i>	69,5	F	Adult	Inactive	Spleen and Blood
SM 28		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 29	CEA Polonês	<i>Artibeus planirostris</i>	63,4	M	Juvenile	Inactive	Spleen and Blood
MCG 30	CEA Polonês	<i>Platyrrhinus lineatus</i>	50,7	F	Juvenile	Inactive	Spleen and Blood
BF 30		<i>Trichobius dugesii</i> (complex)					Bat fly
MCG 31	CEA Polonês	<i>Artibeus lituratus</i>	78,4	M	Adult	Inactive	Spleen and Blood
MCG 32	CEA Polonês	<i>Platyrrhinus lineatus</i>	50,3	F	Juvenile	Inactive	Spleen and Blood
SM 32		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 33	CEA Polonês	<i>Artibeus planirostris</i>	64,9	F	Juvenile	Inactive	Spleen and Blood
SM 33		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 34	CEA Polonês	<i>Platyrrhinus lineatus</i>	50,4	F	Juvenile	Inactive	Spleen and Blood
BF 34		<i>Trichobius parasiticus</i> (complex)					Bat fly
SM 34		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 35	CEA Polonês	<i>Artibeus planirostris</i>	62,4	F	Adult	Inactive	Spleen and Blood

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SM 35		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 36	CEA Florestinha	<i>Artibeus planirostris</i>	63,7	F	Adult	Inactive	Spleen and Blood
MCG 37	CEA Florestinha	<i>Carollia perspicillata</i>	45,4	M	Adult	Active	Spleen and Blood
BF 37		<i>Trichobius joblingi</i>					Bat fly
MCG 38	CEA Florestinha	<i>Artibeus planirostris</i>	61,9	F	Juvenile	Inactive	Spleen and Blood
BF 38		<i>Megistopoda aranea</i>					Bat fly
MCG 39	CEA Florestinha	<i>Artibeus planirostris</i>	62,8	F	Juvenile	Inactive	Spleen and Blood
BF 39		<i>Megistopoda aranea</i>					Bat fly
MCG 40	CEA Florestinha	<i>Artibeus planirostris</i>	63,4	F	Adult	Inactive	Spleen and Blood
BF 40		<i>Megistopoda aranea</i>					Bat fly
MCG 41	CEA Florestinha	<i>Platyrrhinus lineatus</i>	51,4	M	Juvenile	Inactive	Spleen and Blood
SM 41		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 42	CEA Florestinha	<i>Carollia perspicillata</i>	44,3	M	Juvenile	Inactive	Spleen and Blood
BF 42.1		<i>Trichobius dugesii</i> (complex)					Bat fly
BF 42.2		<i>Trichobius dugesii</i> (complex)					Bat fly
MCG 43	CEA Florestinha	<i>Artibeus planirostris</i>	62	M	Juvenile	Inactive	Spleen and Blood
BF 43.1		<i>Megistopoda aranea</i>					Bat fly
BF 43.2		<i>Megistopoda aranea</i>					Bat fly
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>	66,3	M	Adult	Active	Spleen and Blood
BF 44		<i>Megistopoda aranea</i>					Bat fly
MCG 45	CEA Polonês	<i>Myotis nigricans</i>	38,5	F	Juvenile	Inactive	Spleen and Blood
MCG 46	CEA Polonês	<i>Artibeus lituratus</i>	72,4	M	Adult	Active	Spleen and Blood
MCG 47	CEA Polonês	<i>Platyrrhinus lineatus</i>	46,4	M	Adult	Active	Spleen and Blood
MCG 48	CEA Polonês	<i>Artibeus planirostris</i>	60,5	M	Juvenile	Inactive	Spleen and Blood
MCG 49	CEA Polonês	<i>Artibeus lituratus</i>	73,4	M	Adult	Active	Spleen and Blood
MCG 50	CEA Polonês	<i>Artibeus lituratus</i>	76,1	M	Adult	Active	Spleen and Blood
MCG 51	CEA Polonês	<i>Artibeus planirostris</i>	64,1	M	Adult	Active	Spleen and Blood
MCG 52	CEA Polonês	<i>Molossus molossus</i>	36,4	F	Juvenile	Inactive	Spleen and Blood

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MCG 53	CEA Polonês	<i>Artibeus lituratus</i>	73,1	M	Adult	Active	Spleen and Blood
MCG 54	CEA Florestinha	<i>Molossus molossus</i>	40,4	M	Juvenile	Inactive	Spleen and Blood
MCG 55	CEA Florestinha	<i>Phyllostomus discolor</i>	63,6	M	Adult	Active	Spleen and Blood
BF 55		<i>Trichobius costalimai</i>					Bat fly
MCG 56	CEA Florestinha	<i>Phyllostomus discolor</i>	63,7	M	Adult	Active	Spleen and Blood
BF56.1		<i>Strebla hertigi</i>					Bat fly
BF 56.2		<i>Trichobius costalimai</i>					Bat fly
BF 56.3		<i>Trichobius costalimai</i>					Bat fly
BF 53.4		<i>Trichobius costalimai</i>					Bat fly
BF 56.5		<i>Trichobius costalimai</i>					Bat fly
SM 56.1		<i>Periglischrus torrealbai</i>					Spinturnicidae mite
SM 56.2		<i>Periglischrus acutisternus</i>					Spinturnicidae mite
MM 56		<i>Steatonyssus sp.</i>					Macronyssidae mite
TL 56.1		<i>Ornithodoros hasei</i>					Tick larvae
TL 56.2		<i>Ornithodoros hasei</i>					Tick larvae
TL 56.3		<i>Ornithodoros hasei</i>					Tick larvae
TL 56.4		<i>Ornithodoros hasei</i>					Tick larvae
TL 56.5		<i>Ornithodoros hasei</i>					Tick larvae
TL 56.6		<i>Ornithodoros hasei</i>					Tick larvae
MCG 57	CEA Florestinha	<i>Phyllostomus discolor</i>	60,8	M	Adult	Active	Spleen and Blood
BF 57.1		<i>Trichobius costalimai</i>					Bat fly
BF 57.2		<i>Trichobius costalimai</i>					Bat fly
BF 57.3		<i>Trichobius costalimai</i>					Bat fly
BF 57.4		<i>Trichobius costalimai</i>					Bat fly
BF 57.5		<i>Trichobius costalimai</i>					Bat fly
SM 57		<i>Periglischrus torrealbai</i>					Spinturnicidae mite
TL 57.1		<i>Ornithodoros hasei</i>					Tick larvae
TL 57.2		<i>Ornithodoros hasei</i>					Tick larvae

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MCG 58	CEA Florestinha	<i>Artibeus lituratus</i>	74,6	M	Adult	Active	Spleen and Blood
SM 58		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>	61,2	M	Adult	Inactive	Spleen and Blood
BF 59		<i>Trichobius costalimai</i>					Bat fly
SM 59		<i>Periglischrus sp.</i>					Spinturnicidae mite
MCG 60	CEA Florestinha	<i>Molossops temminckii</i>	30	M	Adult	Active	Spleen and Blood
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>	68,7	M	Adult	Active	Spleen and Blood
SM 61		<i>Periglischrus torrealbai</i>					Spinturnicidae mite
TL 61		<i>Ornithodoros hasei</i>					Tick larvae
MCG 62	CEA Florestinha	<i>Phyllostomus discolor</i>	62,3	M	Adult	Active	Spleen and Blood
BF 62		<i>Trichobius costalimai</i>					Bat fly
SM 62.1		<i>Periglischrus sp.</i>					Spinturnicidae mite
SM 62.2		<i>Periglischrus sp.</i>					Spinturnicidae mite
TL 62.1		<i>Ornithodoros hasei</i>					Tick larvae
TL 62.2		<i>Ornithodoros hasei</i>					Tick larvae
MCG 63	CEA Florestinha	<i>Phyllostomus discolor</i>	64,7	M	Adult	Active	Spleen and Blood
BF 63.1		<i>Trichobius costalimai</i>					Bat fly
BF 63.2		<i>Trichobius costalimai</i>					Bat fly
MM 63		<i>Steatonyssus sp.</i>					Macronyssidae mite
TL 63.1		<i>Ornithodoros hasei</i>					Tick larvae
TL 63.2		<i>Ornithodoros hasei</i>					Tick larvae
TL 63.3		<i>Ornithodoros hasei</i>					Tick larvae
TL 63.4		<i>Ornithodoros hasei</i>					Tick larvae
TL 63.5		<i>Ornithodoros hasei</i>					Tick larvae
TL 63.6		<i>Ornithodoros hasei</i>					Tick larvae
TL 63.7		<i>Ornithodoros hasei</i>					Tick larvae
MCG 64	CEA Florestinha	<i>Phyllostomus discolor</i>	63,7	M	Adult	Active	Spleen and Blood
BF 64.1		<i>Trichobius costalimai</i>					Bat fly

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BF 64.2		<i>Trichobius costalimai</i>					Bat fly
BF 64.3		<i>Trichobius costalimai</i>					Bat fly
MM 64		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 65	CEA Florestinha	<i>Phyllostomus discolor</i>	60,4	M	Adult	Active	Spleen and Blood
BF 65.1		<i>Trichobius costalimai</i>					Bat fly
BF 65.2		<i>Trichobius costalimai</i>					Bat fly
BF 65.3		<i>Trichobius costalimai</i>					Bat fly
BF 65.4		<i>Trichobius costalimai</i>					Bat fly
SM 65		<i>Periglischrus acutisternus</i>					Spinturnicidae mite
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>	63,2	M	Adult	Active	Spleen and Blood
BF 66.1		<i>Trichobius costalimai</i>					Bat fly
BF 66.2		<i>Trichobius costalimai</i>					Bat fly
SM 66		<i>Periglischrus acutisternus</i>					Spinturnicidae mite
MCG 67	CEA Florestinha	<i>Phyllostomus discolor</i>	63,5	M	Adult	Active	Spleen and Blood
SM 67		<i>Periglischrus torrealbai</i>					Spinturnicidae mite
MCG 68	CEA Florestinha	<i>Phyllostomus discolor</i>	62,8	M	Adult	Active	Spleen and Blood
BF 68.1		<i>Trichobius costalimai</i>					Bat fly
BF 68.2		<i>Trichobius costalimai</i>					Bat fly
BF 68.3		<i>Trichobius costalimai</i>					Bat fly
SM 68		<i>Periglischrus torrealbai</i>					Spinturnicidae mite
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>	45	F	Juvenile	Inactive	Spleen and Blood
BF69		<i>Trichobius joblingi</i>					Bat fly
MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>	64,3	M	Adult	Active	Spleen and Blood
BF 70.1		<i>Strebla hertigi</i>					Bat fly
BF 70.2		<i>Strebla hertigi</i>					Bat fly
SM 70.1		<i>Periglischrus sp.</i>					Spinturnicidae mite
SM 70.1		<i>Periglischrus sp.</i>					Spinturnicidae mite
SM 70.2		<i>Periglischrus sp.</i>					Spinturnicidae mite

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MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>	59,3	M	Juvenile	Inactive	Spleen and Blood
SM 71		<i>Periglischrus sp.</i>					Spinturnicidae mite
MM 71		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 72	CEA Florestinha	<i>Phyllostomus discolor</i>	64,7	M	Juvenile	Inactive	Spleen and Blood
BF 72.1		<i>Trichobius costalimai</i>					Bat fly
BF 72.2		<i>Trichobius costalimai</i>					Bat fly
SM 72		<i>Periglischrus acutisternus</i>					Spinturnicidae mite
TL 72		<i>Ornithodoros hasei</i>					Tick larvae
MCG 73	CEA Florestinha	<i>Molossus molossus</i>	41,8	M	Adult	Active	Spleen and Blood
MCG 74	CEA Polonês	<i>Eptesicus furinalis</i>	36,8	M	Juvenile	Inactive	Spleen and Blood
MM 74		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>	39,7	F	Juvenile	Inactive	Spleen and Blood
MM 75		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 76	CEA Polonês	<i>Artibeus lituratus</i>	73	M	Adult	Active	Spleen and Blood
MCG 77	CEA Polonês	<i>Platyrrhinus lineatus</i>	47	M	Juvenile	Inactive	Spleen and Blood
MCG 78	CEA Polonês	<i>Artibeus lituratus</i>	77,8	M	Adult	Active	Spleen and Blood
SM 78		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 79	CEA Polonês	<i>Platyrrhinus lineatus</i>	50,2	F	Juvenile	Inactive	Spleen and Blood
BF 79		<i>Trichobius dugesii</i> (complex)					Bat fly
MCG 80	CEA Polonês	<i>Platyrrhinus lineatus</i>	46,8	F	Juvenile	Inactive	Spleen and Blood
MCG 81	CEA Polonês	<i>Platyrrhinus lineatus</i>	46,9	F	Juvenile	Inactive	Spleen and Blood
MCG 82	CEA Polonês	<i>Eptesicus furinalis</i>	36,5	M	Juvenile	Inactive	Spleen and Blood
MM 82		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 83	CEA Polonês	<i>Platyrrhinus lineatus</i>	49,8	F	Juvenile	Inactive	Spleen and Blood
BF 83		<i>Trichobius dugesii</i> (complex)					Bat fly

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SM 83		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 84	CEA Polonês	<i>Artibeus lituratus</i>	66,9	F	Juvenile	Inactive	Spleen and Blood
SM 84		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 85	CEA Polonês	<i>Artibeus lituratus</i>	71	F	Adult	Pos-lactating	Spleen and Blood
MCG 86	CEA Polonês	<i>Platyrrhinus lineatus</i>	49,1	M	Adult	Inactive	Spleen and Blood
MCG 87	CEA Polonês	<i>Artibeus lituratus</i>	73	M	Juvenile	Inactive	Spleen and Blood
SM 87		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 88	CEA Polonês	<i>Platyrrhinus lineatus</i>	46,8	F	Juvenile	Inactive	Spleen and Blood
MCG 89	CEA Polonês	<i>Platyrrhinus lineatus</i>	46,2	M	Juvenile	Inactive	Spleen and Blood
BF 89		<i>Trichobius dugesii</i> (complex)					Bat fly
MCG 90	CEA Polonês	<i>Artibeus lituratus</i>	69,3	M	Juvenile	Inactive	Spleen and Blood
SM 90		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MM 90		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 91	CEA Florestinha	<i>Eumops perotis</i>	80,3	F	Adult	Pos-lactating	Spleen and Blood
MM 91		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 92	CEA Florestinha	<i>Eumops perotis</i>	82,5	F	Adult	Pos-lactating	Spleen and Blood
MM 92		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 93	CEA Florestinha	<i>Eumops perotis</i>	82,9	M	Adult	Inactive	Spleen and Blood
MM 93		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 94	CEA Florestinha	<i>Molossus molossus</i>	40	M	Juvenile	Inactive	Spleen and Blood
MM 94		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 95	CEA Florestinha	<i>Eumops perotis</i>	81,9	M	Adult	Active	Spleen and Blood
MM 95		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 96	CEA Polonês	<i>Artibeus lituratus</i>	73,7	M	Adult	Active	Spleen and Blood

¹This chapter corresponds to the scientific paper accepted in the *Parasitologia (MDPI)* in September, 2021.

MCG 97	CEA Polonês	<i>Artibeus lituratus</i>	77,5	M	Adult	Active	Spleen
SM 97		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 98	CEA Polonês	<i>Artibeus lituratus</i>	72,9	M	Adult	Active	Spleen and Blood
MCG 99	CEA Polonês	<i>Chiroderma villosum</i>	52,2	F	Adult	Pos-lactating	Spleen and Blood
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>	72,5	M	Adult	Active	Spleen and Blood
MCG 101	CEA Polonês	<i>Platyrrhinus lineatus</i>	48,1	M	Juvenile	Inactive	Spleen and Blood
MCG 102	CEA Polonês	<i>Artibeus lituratus</i>	72,8	M	Juvenile	Inactive	Spleen and Blood
SM 102		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 103	CEA Polonês	<i>Artibeus planirostris</i>	58,1	M	Juvenile	Inactive	Spleen and Blood
MCG 104	CEA Polonês	<i>Artibeus lituratus</i>	71,1	M	Juvenile	Inactive	Spleen and Blood
SM 104		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 105	CEA Polonês	<i>Artibeus lituratus</i>	70	M	Adult	Active	Spleen and Blood
MCG 106	CEA Polonês	<i>Eptesicus furinalis</i>	38,4	F	Juvenile	Inactive	Spleen and Blood
MM 106		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 107	CEA Polonês	<i>Artibeus planirostris</i>	65	F	Adult	Pos-lactating	Spleen and Blood
MCG 108	CEA Polonês	<i>Artibeus planirostris</i>	66,5	F	Adult	Inactive	Spleen and Blood
MCG 109	CEA Polonês	<i>Artibeus planirostris</i>	61,7	F	Juvenile	Inactive	Spleen and Blood
MCG 110	CEA Polonês	<i>Artibeus planirostris</i>	64,6	F	Juvenile	Inactive	Spleen and Blood
MCG 111	CEA Polonês	<i>Carollia perspicillata</i>	44,5	F	Juvenile	Inactive	Spleen and Blood
MCG 112	CEA Polonês	<i>Artibeus planirostris</i>	66	M	Juvenile	Inactive	Spleen and Blood
MCG 113	CEA Polonês	<i>Artibeus planirostris</i>	66,1	F	Juvenile	Inactive	Spleen and Blood
MCG 114	CEA Polonês	<i>Platyrrhinus lineatus</i>	48	F	Juvenile	Inactive	Spleen and Blood
MCG 115	CEA Florestinha	<i>Artibeus planirostris</i>	64,7	F	Adult	Pos-lactating	Spleen and Blood
MCG 116	CEA Florestinha	<i>Artibeus lituratus</i>	77,3	F	Adult	Pos-lactating	Spleen and Blood
MCG 117	CEA Florestinha	<i>Artibeus lituratus</i>	70,9	M	Adult	Active	Spleen and Blood
MCG 118	CEA Florestinha	<i>Artibeus lituratus</i>	72,6	M	Adult	Active	Spleen and Blood
MCG 119	CEA Florestinha	<i>Artibeus lituratus</i>	77,7	M	Adult	Active	Spleen and Blood
MCG 120	CEA Florestinha	<i>Artibeus planirostris</i>	64,9	F	Adult	Pos-lactating	Spleen and Blood

¹This chapter corresponds to the scientific paper accepted in the *Parasitologia (MDPI)* in September, 2021.

BF 120		<i>Megistopoda aranea</i>					Bat fly
MCG 121	CEA Florestinha	<i>Artibeus lituratus</i>	78,1	F	Adult	Pos-lactating	Spleen and Blood
MCG 122	CEA Polonês	<i>Artibeus planirostris</i>	60,7	M	Juvenile	Inactive	Spleen and Blood
MM 122		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 123	CEA Polonês	<i>Eptesicus furinalis</i>	36,8	M	Juvenile	Inactive	Spleen and Blood
MM 123		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 124	CEA Polonês	<i>Eptesicus furinalis</i>	39	F	Juvenile	Inactive	Spleen and Blood
SM 124		<i>Periglischrus sp.</i>					Spinturnicidae mite
MCG 125	CEA Polonês	<i>Artibeus lituratus</i>	73,3	M	Adult	Inactive	Spleen and Blood
MCG 126	CEA Polonês	<i>Artibeus lituratus</i>	77,5	F	Adult	Pos-lactating	Spleen and Blood
BF 126		<i>Trichobius dugesii</i> (complex)					Bat fly
MCG 127	CEA Polonês	<i>Carollia perspicillata</i>	45,8	M	Juvenile	Inactive	Spleen and Blood
MCG 128	CEA Florestinha	<i>Artibeus lituratus</i>	74,9	F	Adult	Inactive	Spleen and Blood
SM 128		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 129	CEA Florestinha	<i>Artibeus lituratus</i>	73,5	M	Adult	Active	Spleen and Blood
MCG 130	CEA Florestinha	<i>Artibeus lituratus</i>	67,2	M	Adult	Active	Spleen and Blood
MCG 131	CEA Polonês	<i>Eptesicus furinalis</i>	36,3	F	Juvenile	Inactive	Spleen and Blood
MM 131		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 132	CEA Polonês	<i>Eptesicus furinalis</i>	40,2	F	Adult	Inactive	Spleen and Blood
MM 132		<i>Steatonyssus sp.</i>					Macronyssidae mite
MCG 133	CEA Florestinha	<i>Artibeus lituratus</i>	73,8	M	Juvenile	Inactive	Spleen and Blood
SM 133		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 134	CEA Florestinha	<i>Artibeus lituratus</i>	72,6	M	Adult	Active	Spleen and Blood
SM 134		<i>Periglischrus iheringi</i>					Spinturnicidae mite
MCG 135	CEA Florestinha	<i>Artibeus lituratus</i>	74,3	M	Adult	Active	Spleen and Blood

¹This chapter corresponds to the scientific paper accepted in the *Parasitologia (MDPI)* in September, 2021.

Supplementary Table 2. Bat samples positive for *Anaplasma* spp. according to PCR assays targeting five different molecular markers.

ID	Capture site	Bat or ectoparasite species	Host species (if applicable)	Sample type	16S rRNA (cPCR) - Screening	<i>msp-2 A. phagocytophilum</i> (qPCR)	<i>gltA</i> (cPCR)	ITS (cPCR)	<i>groEL</i> (cPCR)
MCG 22	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos (unique band)	neg	neg	neg	pos
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos (unique band)	neg	pos + sequenced	neg	pos + sequenced
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos (unique band)	neg	neg	neg	pos
MCG 62	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos (unique band)	neg	neg	neg	pos
TL 72	CEA Florestinha	<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae	pos (unique band)	neg	pos + sequenced	neg	pos + sequenced
MCG 88	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos (unique band)	neg	neg	neg	pos
MCG 89	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos (unique band)	neg	neg	neg	neg

neg = negative; pos: positive; pos + sequenced: positive and submitted to Sanger sequencing.

Supplementary Table 3. Bat samples positive for *Ehrlichia* spp. according to PCR assays targeting six different molecular markers.

ID	Capture site	Bat or ectoparasite species	Host specie (if applicable)	Sample type	<i>dsb</i> (cPCR) - Screening	<i>dsb E.canis</i> (qPCR)	<i>vlpt E. chaffeensis</i> (qPCR)	<i>groEL</i> (cPCR)	<i>sodB</i> (cPCR)	<i>omp-1</i> (cPCR)
MCG 01	CEA Polonês	<i>Artibeus lituratus</i>		Spleen	pos	neg	neg	neg	neg	neg
MCG 03	CEA Polonês	<i>Artibeus planirostris</i>		Blood	pos	neg	neg	neg	neg	neg
MM 04	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Myotis nigricans</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 05	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Platyrrhinus lineatus</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
MCG 07	CEA Polonês	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg	neg	neg
MCG 07				Blood	pos	neg	neg	neg	neg	neg
BF 10	CEA Polonês	<i>Megistopoda aranea</i>	<i>Artibeus lituratus</i>	Bat fly 10	pos	neg	neg	neg	neg	neg
BF 12	CEA Polonês	<i>Megistopoda aranea</i>	<i>Artibeus planirostris</i>	Bat fly 12	pos	neg	neg	neg	neg	neg
MCG 14	CEA Polonês	<i>Artibeus planirostris</i>		Blood	pos	neg	neg	neg	neg	neg
MM 23	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
MCG 25	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos	neg	neg	neg	neg	neg
MCG 29	CEA Polonês	<i>Artibeus planirostris</i>		Blood	pos	neg	neg	neg	neg	neg
BF 38	CEA Florestinha	<i>Megistopoda aranea</i>	<i>Artibeus planirostris</i>	Bat fly	pos	neg	neg	neg	neg	neg
MCG 40	CEA Florestinha	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg	neg	neg
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg	neg	neg

¹This chapter corresponds to the scientific paper accepted in the *Parasitologia (MDPI)* in September, 2021.

MCG 55	CEA Florestinha	<i>Phyllostomus</i> <i>discolor</i>		Blood	pos	neg	neg	neg	neg	neg
BF 56.5	CEA Florestinha	<i>Trichobius</i> <i>costalimai</i>	<i>Phyllostomus</i> <i>discolor</i>	Bat fly	pos	neg	neg	neg	neg	neg
SM 56.1		<i>Periglischrus</i> <i>torrealbai</i>	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	pos	neg	neg	neg	neg	neg
SM 56.2		<i>Periglischrus</i> <i>acutisternus</i>	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	pos	neg	neg	neg	neg	neg
MM 56		<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
TL 56.2		<i>Ornithodoros</i> <i>hasei</i>	<i>Phyllostomus</i> <i>discolor</i>	Tick larvae	pos	neg	neg	neg	neg	neg
BF 57.5	CEA Florestinha	<i>Trichobius</i> <i>costalimai</i>	<i>Phyllostomus</i> <i>discolor</i>	Bat fly	pos	neg	neg	neg	neg	neg
MCG 59	CEA Florestinha	<i>Phyllostomus</i> <i>discolor</i>		Blood	pos	neg	neg	neg	neg	neg
MCG 62	CEA Florestinha	<i>Phyllostomus</i> <i>discolor</i>		Blood	pos	neg	neg	neg	neg	neg
SM 62.1		<i>Periglischrus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	pos + sequenced	neg	neg	neg	neg	neg
SM 62.2		<i>Periglischrus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	pos + sequenced	neg	neg	neg	neg	neg
MM 63	CEA Florestinha	<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
TL 63.1		<i>Ornithodoros</i> <i>hasei</i>	<i>Phyllostomus</i> <i>discolor</i>	Tick larvae	pos	neg	neg	neg	neg	neg
TL 63.4		<i>Ornithodoros</i> <i>hasei</i>	<i>Phyllostomus</i> <i>discolor</i>	Tick larvae	pos	neg	neg	neg	neg	neg
MM 64	CEA Florestinha	<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
SM 67	CEA Florestinha	<i>Periglischrus</i> <i>torrealbai</i>	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	pos	neg	neg	neg	neg	neg
MCG 69	CEA Florestinha	<i>Carollia</i> <i>perspicillata</i>		Spleen	pos	neg	neg	neg	neg	neg

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BF 70.1	CEA Florestinha	<i>Strebla hertigi</i>	<i>Phyllostomus discolor</i>	Bat fly	pos	neg	neg	neg	neg	neg
BF 70.2		<i>Strebla hertigi</i>	<i>Phyllostomus discolor</i>	Bat fly	pos	neg	neg	neg	neg	neg
SM 70.1		<i>Periglischrus sp.</i>	<i>Phyllostomus discolor</i>	Spinturnicidae mite	pos	neg	neg	neg	neg	neg
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos + sequenced	neg	neg	neg	neg	neg
MM 71		<i>Steatonyssus sp.</i>	<i>Phyllostomus discolor</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
MM 74	CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
MM 75	CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
MM 82	CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Macronyssidae mite	pos + sequenced	neg	neg	neg	neg	neg
MM 90	CEA Polonês	<i>Steatonyssus sp.</i>	<i>Artibeus lituratus</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 91	CEA Florestinha	<i>Steatonyssus sp.</i>	<i>Eumops perotis</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 92	CEA Florestinha	<i>Steatonyssus sp.</i>	<i>Eumops perotis</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 93	CEA Florestinha	<i>Steatonyssus sp.</i>	<i>Eumops perotis</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 94	CEA Florestinha	<i>Steatonyssus sp.</i>	<i>Molossus molossus</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 95	CEA Florestinha	<i>Steatonyssus sp.</i>	<i>Eumops perotis</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MCG 101	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos	neg	neg	neg	neg	neg
MCG 102	CEA Polonês	<i>Artibeus lituratus</i>		Spleen	pos	neg	neg	neg	neg	neg
MM 106	CEA Polonês	<i>Steatonyssus sp.</i>	<i>Eptesicus furinalis</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg

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MCG 113	CEA Polonês	<i>Artibeus planirostris</i>		Blood	pos	neg	neg	neg	neg	neg
MM 122	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Artibeus planirostris</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MM 123	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Macronyssidae mite	pos	neg	neg	neg	neg	neg
MCG 124	CEA Polonês	<i>Eptesicus furinalis</i>		Blood	pos	neg	neg	neg	neg	neg

neg = negative; pos: positive; pos + sequenced: positive and submitted to Sanger sequencing.

Supplementary Table 4. Bat samples positive for *Neorickettsia* spp. according to PCR assays targeting three different molecular markers.

ID	Capture site	Bat or ectoparasite species	Host specie (if applicable)	Sample type	16S rRNA (cPCR) Screening	Additional 16S rRNA (cPCR)	p51kDa (cPCR)	groEL (cPCR)
MCG 01	CEA Polonês	<i>Artibeus lituratus</i>		Spleen	pos	neg	neg	neg
MCG 02	CEA Polonês	<i>Artibeus lituratus</i>		Blood	pos + sequenced	neg	neg	neg
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>		Spleen	pos	neg	neg	neg
MCG 09				Blood	pos + sequenced	neg	neg	neg
MCG 15	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos	neg	neg	neg
MCG 16	CEA Polonês	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg
MCG 20	CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood	pos	neg	neg	neg
MCG 21	CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood	pos	neg	neg	neg
MCG 22	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos	neg	neg	neg
MCG 26	CEA Polonês	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg
MCG 30	CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood	pos	neg	neg	neg
MCG 32	CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood	pos	neg	neg	neg
MCG 34	CEA Polonês	<i>Platyrrhinus lineatus</i>		Spleen	pos	neg	neg	neg

¹This chapter corresponds to the scientific paper accepted in the *Parasitologia (MDPI)* in September, 2021.

MCG 35	CEA Polonês	<i>Artibeus planirostris</i>		Blood	pos	neg	neg	neg
MCG 39	CEA Florestinha	<i>Artibeus planirostris</i>		Blood	pos + sequenced	neg	neg	neg
MCG 40	CEA Florestinha	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg
MCG 43	CEA Florestinha	<i>Artibeus planirostris</i>		Blood	pos	neg	neg	neg
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>		Spleen	pos	neg	neg	neg
MCG 45	CEA Polonês	<i>Myotis nigricans</i>		Blood	pos	neg	neg	neg
MCG 46	CEA Polonês	<i>Artibeus lituratus</i>		Spleen	pos	neg	neg	neg
MCG 51	CEA Polonês	<i>Artibeus planirostris</i>		Spleen	pos + sequenced	neg	neg	neg
MCG 52	CEA Polonês	<i>Molossus molossus</i>		Spleen	pos	neg	neg	neg
MCG 53	CEA Polonês	<i>Artibeus lituratus</i>		Blood	pos	neg	neg	neg
MCG 54	CEA Florestinha	<i>Molossus molossus</i>		Blood	pos + sequenced	neg	neg	neg
MCG 55	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos	neg	neg	neg
MCG 57	CEA Florestinha	<i>Phyllostomus discolor</i>		Spleen	pos	neg	neg	neg
MCG 57				Blood	pos	neg	neg	neg
MCG 58	CEA Florestinha	<i>Artibeus lituratus</i>		Spleen	pos	neg	neg	neg
MCG 58				Blood	pos	neg	neg	neg
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos	neg	neg	neg
MCG 60	CEA Florestinha	<i>Molossops temminckii</i>		Blood	pos + sequenced	neg	neg	neg
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos	neg	neg	neg
TL 62	CEA Florestinha	<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae	pos + sequenced	neg	neg	neg
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos + sequenced	neg	neg	neg
MCG 68	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos	neg	neg	neg
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>		Spleen	pos	neg	neg	neg
MCG 69				Blood	pos + sequenced	neg	neg	neg
MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>		Spleen	pos	neg	neg	neg
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	pos	neg	neg	neg
MCG 74	CEA Polonês	<i>Eptesicus cf. furinalis</i>		Blood	pos	neg	neg	neg
MCG 75	CEA Polonês	<i>Eptesicus cf. furinalis</i>		Blood	pos + sequenced	neg	neg	neg

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MCG 79	CEA Polonês	<i>Platyrrhinus lineatus</i>	Spleen	pos	neg	neg	neg
MCG 82	CEA Polonês	<i>Eptesicus cf. furinalis</i>	Blood	pos	neg	neg	neg
MCG 83	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	pos + sequenced	neg	neg	neg
MCG 84	CEA Polonês	<i>Artibeus lituratus</i>	Blood	pos	neg	neg	neg
MCG 88	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	pos	neg	neg	neg
MCG 89	CEA Polonês	<i>Platyrrhinus lineatus</i>	Blood	pos	neg	neg	neg
MCG 90	CEA Polonês	<i>Artibeus lituratus</i>	Blood	pos	neg	neg	neg
MCG 92	CEA Florestinha	<i>Eumops perotis</i>	Blood	pos + sequenced	neg	neg	neg
MCG 96	CEA Polonês	<i>Artibeus lituratus</i>	Blood	pos	neg	neg	neg
MCG 99	CEA Polonês	<i>Chiroderma villosum</i>	Blood	pos + sequenced	neg	neg	neg
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>	Spleen	pos	neg	neg	neg
MCG 103	CEA Polonês	<i>Artibeus planirostris</i>	Blood	pos	neg	neg	neg
MCG 108	CEA Polonês	<i>Artibeus planirostris</i>	Spleen	pos	neg	neg	neg
MCG 111	CEA Polonês	<i>Carollia perspicillata</i>	Spleen	pos	neg	neg	neg
MCG 123	CEA Polonês	<i>Eptesicus cf. furinalis</i>	Spleen	pos	neg	neg	neg
MCG 129	CEA Florestinha	<i>Artibeus lituratus</i>	Spleen	pos	neg	neg	neg

neg = negative; pos: positive; pos + sequenced: positive and submitted to Sanger sequencing.

Supplementary Table 5. BLASTn results obtained after sequencing of amplicons obtained from PCR assays for *Ehrlichia* spp., *Anaplasma* spp. and *Neorickettsia* spp.

ID	Capture site	Bat or ectoparasite species	Host Specie (if applicable)	Sample type	Targeted gene	BLASTn result	Query coverage (%)	Identity (%)	Accession Number
TL 72	CEA Florestinha	<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae	16S rRNA (<i>Anaplasma</i> spp.)	<i>Anaplasma phagocytophilum</i>	100	97.6	KR611598.1
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	<i>gltA</i> (<i>Anaplasma</i> spp.)	<i>Ehrlichia</i> spp.	100	83.15	KJ410275.1
TL 72	CEA Florestinha	<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae	<i>gltA</i> (<i>Anaplasma</i> spp.)	none	-	-	-

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MCG 59	CEA Florestinha	<i>Phyllostomus</i> <i>discolor</i>		Blood	<i>groEL</i> (<i>Anaplasma</i> spp.)	none	-	-	-
TL 72	CEA Florestinha	<i>Ornithodoros</i> <i>hasei</i>	<i>Phyllostomus</i> <i>discolor</i>	Tick larvae	<i>groEL</i> (<i>Anaplasma</i> spp.)	none	-	-	-
MM 05	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Platyrrhinus</i> <i>lineatus</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	97.74	MT212414.1
MM 23	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Eptesicus</i> <i>furinalis</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	99.03	MT212414.2
MM 56		<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	98.3	MT212414.3
SM 62.1		<i>Periglischrus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	98.75	MT212414.4
SM 62.2		<i>Periglischrus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Spinturnicidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	98.54	MT212414.5
MM 63	CEA Florestinha	<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	97.35	MT212414.6
MM 64	CEA Florestinha	<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	99.02	MT212414.7
MCG 71	CEA Florestinha	<i>Phyllostomus</i> <i>discolor</i>		Blood	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	100	99.26	MT212414.8
MM 71		<i>Steatonyssus</i> sp.	<i>Phyllostomus</i> <i>discolor</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	96.73	MT212414.9
MM 74	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Eptesicus</i> <i>furinalis</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	99	98.29	MT212414.10
MM 75	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Eptesicus</i> <i>furinalis</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	Uncultured <i>Ehrlichia</i> sp.	100	100	MT135771.1
MM 82	CEA Polonês	<i>Steatonyssus</i> sp.	<i>Eptesicus</i> <i>furinalis</i>	Macronyssidae mite	<i>dsb</i> (<i>Ehrlichia</i> spp.)	<i>Ehrlichia</i> <i>minasensis</i>	100	97.76	MT212414.10
MCG 02	CEA Polonês	<i>Artibeus</i> <i>lituratus</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> <i>risticii</i> isolate	100	100	MK760560.1
MCG 09	CEA Polonês	<i>Glossophaga</i> <i>soricina</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> <i>risticii</i> str. Illinois	100	99.42	CP001431.1
MCG 39	CEA Florestinha	<i>Artibeus</i> <i>planirostris</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> <i>risticii</i>	100	99.77	MK760560.1
MCG 51	CEA Polonês	<i>Artibeus</i> <i>planirostris</i>		Spleen	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> <i>risticii</i>	100	98.57	MK760560.1
MCG 54	CEA Florestinha	<i>Molossus</i> <i>molossus</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> sp. strain Ohio	100	100	MK281366.1

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MCG 60	CEA Florestinha	<i>Molossops temminckii</i>	<i>Phyllostomus discolor</i>	Spleen	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia risticii</i> str. Illinois	100	100	CP001431.1
TL 62.2	CEA Florestinha	<i>Ornithodoros hasei</i>		Tick larvae	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia risticii</i>	100	95.07	MK760560.1
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia risticii</i> str. Illinois	100	100	CP001431.1
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> sp. strain Ohio	100	96.7	MK281366.1
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia risticii</i> str. Illinois	100	97.9	CP001431.1
MCG 83	CEA Polonês	<i>Platyrrhinus lineatus</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia risticii</i> str. Illinois	100	100	CP001431.1
MCG 92	CEA Florestinha	<i>Eumops perotis</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia</i> sp. strain Ohio	100	100	MK281366.1
MCG 99	CEA Polonês	<i>Chiroderma villosum</i>		Blood	16S rRNA (<i>Neorickettsia</i> spp.)	<i>Neorickettsia risticii</i> str. Illinois	100	99.42	CP001431.1

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CHAPTER 5- First molecular detection of piroplasmids in non-hematophagous bats from Brazil, with evidence of putative novel species.¹

Priscila Ikeda¹; Taline Revollo Menezes¹; Jaire Marinho Torres²; Carina Elisei de Oliveira², Elizabete Captivo Lourenço³; Heitor Miraglia Herrera², Rosangela Zacarias Machado¹, Marcos Rogério André^{1*}

¹Laboratório de Imunoparasitologia, Departamento de Patologia Veterinária, Universidade Estadual “Júlio de Mesquita Filho”, Jaboticabal, Brazil.

²Laboratório de Biologia Parasitária, Programa de Pós Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, Mato Grosso do Sul, Brazil.

³Laboratório de Ecologia de Mamíferos, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil.

Abstract: Piroplasmida is an order of the phylum Apicomplexa that comprises the *Babesia*, *Cytauxzoon*, and *Theileria* genera. These hemoparasites infect vertebrate blood cells and may cause serious diseases in animals and humans. Even though previous studies have shown that bats are infected by different species of piroplasmids, the occurrence and diversity of these hemoparasites have not been investigated in this group of mammals in Brazil. Therefore, the present work aimed to investigate the occurrence and assess the phylogenetic placement of piroplasmids infecting bats sampled in a peri-urban area from Central-Western Brazil. Seventeen (12.6%) out of 135 animals were positive by nested PCR assay for the detection of *Babesia/Theileria* targeting the 18S rRNA gene. Eleven sequences of the 17 positive samples could be analyzed and showed an identity of 91.8–100% with *Theileria bicornis*, *Babesia vogeli*, a *Babesia* sp. identified in a small rodent (*Thrichomys pachyurus*) from the Brazilian Pantanal and a *Babesia* sp. identified in a dog from Thailand as assessed by nBLAST. A phylogenetic tree was constructed from an alignment of 1399 bp length using analyzed and known piroplasmid 18S rRNA sequences. In this tree, piroplasmid 18S rRNA sequences detected in three specimens of *Phyllostomus discolor* (Piroplasmid n. sp., *P. discolor*) were placed as a sister taxon to *Theileria* sensu stricto (Clade V) and *Babesia* sensu stricto (Clade VI). An additional phylogenetic tree was generated from a shorter alignment of 524 bp length including

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analyzed piroplasmid 18S rRNA sequences of bat species *Artibeus planirostris* and *A. lituratus* (Piroplasmid sp., *Artibeus* spp.). The two 18S rRNA sequences detected in *Artibeus* spp. (Piroplasmid n. sp., *Artibeus* spp.) were placed within *Babesia* sensu stricto (Clade VI) into a strongly supported clade (bootstrap: 100) that included *Babesia vogeli*. The two 18S rRNA sequences of Piroplasmid sp., *Artibeus* spp. showed a single and a two-nucleotide differences, respectively, with respect to *B. vogeli* in a 709 pb length alignment. For the first time, the present study shows the occurrence of putative new piroplasmid species in non-hematophagous bats from Brazil.

Keywords: Piroplasmida. *Babesia*, 18S rRNA. Chiroptera.

1. Introduction

Piroplasmids are tick-borne apicomplexan protozoa comprising of the genera *Babesia*, *Cytauxzoon* and *Theileria* (Schnittger et al. 2012; Yabsley and Shock, 2012). These agents parasitize red blood cells, lymphocytes or monocytes of wild and domestic vertebrates and can cause serious diseases in livestock, domestic and wild mammals (Alvarado-Rybak et al. 2016; Hunfeld et al. 2008), and, accidentally, humans (Kjemtrup and Conrad 2000; Zintl et al. 2003; Hunfeld et al. 2008; Leiby 2011; Vannier et al. 2015). Piroplasmids are transmitted by ixodid tick vectors (*Rhipicephalus*, *Ixodes*, *Haemaphysalis*, and *Hyalomma*) to a mammalian or avian host (Homer et al., 2000; Yabsley and Shock 2013; Antunes et al., 2017).

Bats are among the most geographically dispersed and abundant vertebrates, being distributed worldwide except for the Antarctic Continent (Schipper et al. 2008). They are considered reservoirs for several protozoa, bacteria and viruses of zoonotic potential (Correa et al. 2013; Brook and Dobson 2015). In addition, they host a variety of ectoparasites with significant importance for the transmission of pathogens (Dantas-Torres et al. 2012, do Amaral et al. 2018).

In morphoclimatic regions of Brazil, 9 families, 69 genera and 182 species of bats have been described (Nogueira et al. 2018 updated in <<http://www.sbeq.net/updatelist>>). The diversity of habitats combined with the wide geographic distribution, flight capacity, high longevity, dispersion over long distances, formation of colonies and adaptation to anthropic environments, facilitate the

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maintenance and the dispersal of zoonotic pathogens that are found as natural reservoirs in bats and are of great relevance for human health (FAO 2011).

Piroplasmids have been described in chiropterans since the end of the 19th century. Their first report was in 1899 by Dionisi, who found structures similar to malaria parasites in red blood cells of *Nyctalus noctula* and *Pipistrellus* sp. bats and named them *Achromaticus vesperuginus*. Since then, piroplasmids have been identified in blood smears of different bat species in Germany, the Netherlands, the United Kingdom and Colombia (Goedbloed et al. 1964; Gardner and Molyneux 1987; Gardner et al. 1987; Marinkelle 1996). The bat-infecting piroplasmid *Babesia vesperuginis* was first detected in tissue samples from *Pipistrellus* spp. bats in the United Kingdom using molecular diagnostics (Concannon et al. 2005). Since then, 18S rRNA sequences of *B. vesperuginis*, *B. venatorum*, *B. canis*, *B. crassa* (Clade VI – *Babesia* sensu stricto) and *Theileria* spp. (Clade V – *Theileria* sensu stricto) have been detected in bats and associated ticks in Hungary, Romania, Austria, Czech Republic (Hornok et al. 2015, 2016, 2017 and 2018; Corduneanu et al. 2017), United Kingdom (Lv et al. 2018) and China (Han et al. 2018; Liu et al. 2018). Most recently, Ranaivoson et al. (2019) described a previously unknown 18S rRNA gene sequence of a piroplasmid isolate from blood samples of *Pteropus rufus* from Madagascar. Analyzed sequences clustered into a monophyletic clade within -group (Clade I, which comprises piroplasmid 18S rRNA sequences detected in Madagascan lemurs, *Babesia microti*, *B. rodhaini* and *B. felis* and clearly distinct from *B. vesperuginis*, which places in the Western group (Clade II).

Although piroplasmid infections of bats have been shown in previous studies, there are no molecular evidence on the occurrence of such agents in this group of mammals in the neotropical region so far. Therefore, the present study aimed to investigate the occurrence and assess the phylogenetic position of piroplasmids in bats sampled in a peri-urban area from Central-Western Brazil.

2. Material and Methods

2.1 Bats sampling

Animal sampling was performed quarterly between June/2017 and March/2018. The animals were captured in two distinct places in the periurban region of Campo

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Grande city, Mato Grosso do Sul state, central-western Brazil: “Centro de Educação Ambiental Polonês” (CEA Pol.; 54°34'49.816"W and 20°26'50.673"S) and “Florestinha” (CEA Flor.; 54°33'43.352"W and 20°24'11.614"S) (**Fig 1**). The animals and their ectoparasites (Diptera: Streblidae and Nycteribiidae; Acarina: Macronyssidae, Spinturnicidae and Argasidae) were identified using the identification keys for bats (Reis et al. 2017) and parasites (Herrin and Tripton 1975; Wenzel 1976; Guimarães et al. 2001). The bats were then taken to the laboratory and euthanized using 10% ketamine and 1% acepromazine association at 9:1 ratio and 0.1mL/kg injected intramuscularly. From each bat specimen 0.2-2 mL blood samples were collected by intracardiac puncture in EDTA-containing microtubes while spleen fragments were maintained in absolut ethanol (Merck®, Darmstadt, Alemanha) both in RNase and DNase-free microtubes (Kasvi®, São José dos Pinhais, Brazil) at -20°C until use. All experimental procedures were approved by the “Instituto Chico Mendes de Biodiversidade” (ICMBio) (SISBIO 57450-1) and by the Ethics Committee on Animal Use of the School of Agricultural and Veterinary Sciences, UNESP (CEUA FCAV/UNESP 010050/17).

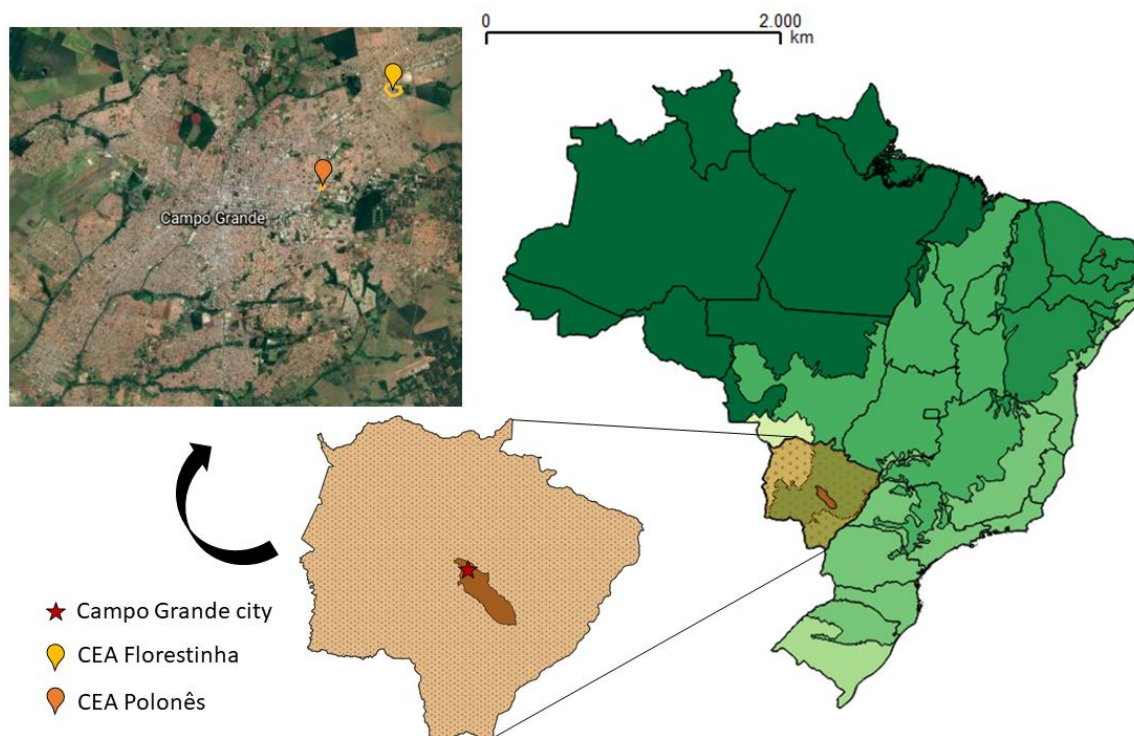


Fig 1. Map showing the sites where the bats were captured between June/2017 and March/2018.

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2.2 DNA extraction

Blood and spleen bat samples were submitted to DNA extraction using the Illustra Tissue and Cells Genomic Prep Mini Spin Kit (GE Healthcare), following the manufacturer's recommendations. The DNA samples were evaluated by optical spectrophotometry (Nanodrop, Thermo Scientific®) and showed concentration of $9.48 \pm 5.05 \mu\text{g}/\mu\text{L}$ for the blood samples and 64.4 ± 42.26 for spleen samples.

2.3 PCR assays

To verify the absence of PCR inhibitors, DNA samples were first tested by a conventional PCR assay based on the endogenous glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) gene as previously described (Birkenheuer et al. 2003).

Positive samples obtained using the abovementioned protocol were used in nested PCR assays for *Babesia/Theileria* spp. based on the 18S rRNA gene (~ 800 pb) (Jefferies et al. 2007). The positive samples in the nested PCR protocol for *Babesia/Theileria* were submitted to additional PCR assays based on the 18S rRNA comprising ~ 600 pb (Yabsley et al. 2006) and ~1500 pb (Greay et al., 2018), *hsp70* (~ 740 pb) (Soares et al. 2011), β -*tubulin* (~ 1500 pb) (Zamoto et al. 2011) genes, and intergenic region ITS2 (~ 300 pb) (Shock et al. 2012). Ultrapure water and *Babesia vogeli* DNA (Jaboticabal strain) were used as positive and negative controls in all PCR assays.

Products amplified in PCR assays were submitted to horizontal agarose electrophoresis on ethidium bromide stained 1.0% agarose gel at 100 V / 150 mA for 50 minutes and the results were visualized and analyzed using an ultraviolet light transilluminator (ChemiDoc MP Imaging System, BIO RAD®).

2.4 Sequencing and phylogenetic analyses

The positive samples that showed high intensity amplicons in the agarose gel electrophoresis were submitted to sequencing by dideoxynucleotide chain termination (Sanger et al. 1977), performed in an ABI PRISM 3700 DNA Analyzer sequencer (Applied Biosystems®, Foster City, CA, USA) at the “Centro de Recursos Biológicos e Biologia Genômica (CREBIO -FCAV - UNESP)”. Obtained sequences were aligned and a consensus sequence generated for each sample using BioEdit (Hall, 1999) and

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Phred-Phrap software version 23 (Ewing and Green, 1998; Ewing et al., 1998). Then, analyzed sequences were compared with sequences previously deposited in GenBank database by nBLAST (Benson et al. 2018). Two alignments with the obtained sequences and those retrieved from the Genbank database were obtained through MAFFT software, version 7 (Katoh et al. 2019) using gap penalty default on EMBL-EBI analysis tools (Madeira et al, 2019) and progressive method for the large (1,399 bp-length) and short (524 bp-length) sequence alignments, respectively. For constructing the phylogenetic trees, the best evolution model (GTR + G4 + I for both analysis) was selected by the ModelFinder (Kalyaanamoorthy et al. 2017), using the Bayesian Information Criterion (BIC). Maximum Likelihood (ML) analysis was performed using W-IQ-Tree (available *online*: <http://iqtree.cibiv.univie.ac.at/>) (Trifinopoulos et al. 2016) using ultrafast bootstrap of 1000 replicates (Hoang et al. 2017). The consensus tree was edited using TreeGraph 2.14.0-771 Beta version (Stöver and Müller 2010).

Additionally, an analysis of DNA divergence was performed based on the 18S rRNA sequences of interest. The sequences were aligned using the BioEdit (Hall, 1999) and the analysis was performed using the DnaSP 5 program, version 5.10.01 (Librado and Rozas 2009).

3. Results

A total of 268 samples (135 spleen tissues and 133 whole blood) were collected from 135 individuals. All the DNA samples were positive in the *gapdh* gene PCR assay and submitted to subsequent PCR protocols as described previously. Seventeen (12.6%) of 135 animals were positive using the *Babesia/Theileria* nPCR assay protocol based on 18S rRNA gene (~ 800 bp) described by Jefferies et al. (2007) (**Table 1**). The positive bats belonged to the following species: *Artibeus planirostris*, *Artibeus lituratus*, *Phyllostomus discolor*, and *Platyrrhinus lineatus*. Three animals (*Phyllostomus discolor* species) showed positive results for both spleen and blood samples in the nPCR assay targeting the 18S rRNA sequence of piroplasmids. Out of these, 2 blood samples from *P. discolor* individuals also yielded positive results in two additional nPCR protocols for *Babesia/Theileria* based on 18S rRNA gene previously described by Yabsley et al. (2006) (~ 600 bp) and described by Greay et al. (2018) (~1500 pb). Applying the latter protocol, an extra blood sample from *P. discolor* was

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also positive. All positive samples in the PCR assays for piroplasmids targeting 18S rRNA gene were negative in PCR assays targeting the ITS-2 region and *hsp-70* and *β-tubulin* genes (**Supplementary table 1**).

Table 1. Piroplasmid-positive blood and spleen samples from different bat species.

Species	Number of spleen samples (positive samples / total samples)	Number of blood samples (positive samples / total samples)	Total
<i>Artibeus lituratus</i>	0 / 37	1 / 36	1 / 73
<i>Artibeus planirostris</i>	3 / 33	1 / 33	4 / 66
<i>Phyllostomus discolor</i>	3 / 15	7 / 15	10 / 30
<i>Platyrrhinus lineatus</i>	2 / 23	0 / 23	2 / 46
<i>Eptesicus furinalis</i>	0 / 09	0 / 09	0 / 18
<i>Carollia perspicillata</i>	0 / 05	0 / 05	0 / 10
<i>Eumops perotis</i>	0 / 04	0 / 04	0 / 08
<i>Molossus molossus</i>	0 / 04	0 / 04	0 / 08
<i>Myotis nigricans</i>	0 / 02	0 / 01	0 / 03
<i>Glossophaga soricina</i>	0 / 01	0 / 01	0 / 02
<i>Molossops temminckii</i>	0 / 01	0 / 01	0 / 02
<i>Chiroderma villosus</i>	0 / 01	0 / 01	0 / 02
Total	8 / 135	9 / 133	17 / 268

In total, 11 amplicons were sequenced: nine showed to be Piroplasmid n. sp. – *P. discolor* and two were named Piroplasmid sp. – *Artibeus* spp. For those samples that were positive for more than one nPCR protocol targeting the 18S rRNA gene, a consensus sequence was created from all obtained and analyzed amplicon sequences. In BLAST analysis, eight sequences (deposited in GenBank under accession numbers MT002346-50 and MT002352-55) showed 91.8% to 93.6% identity (query coverage = 98-100%) with *Theileria bicornis* (MF536661.1). The other three sequences (deposited in GenBank under accession numbers MT002345, MT002356 and MT002351) showed 99.9% identity (query coverage = 100%) with *Babesia vogeli* (MH100819.1), 97.2% identity (query coverage = 94%) with *Babesia* sp. identified in a rodent (*Thrichomys pachyurus*) from the Brazilian Pantanal (KP757840.1), and 100% identity (query coverage = 100%) with a *Babesia* sp. identified in a dog from Thailand (MK830995.1) (**Supplementary table 2**).

For the phylogenetic analysis, two trees were constructed: one containing only 18S rRNA large sequences obtained from the PCR protocol described by Greay et al. (2018), and another one with all sequences obtained in the present study. The first tree, based on a 1,399 pb-alignment (**Fig. 2**), showed that the three sequences detected in *Phyllostomus discolor* bats and named herein as Piroplasmid n. sp. – *P. discolor* were placed into a strongly supported sister clade (bootstrap: 100) with respect to the *Theileria* s.s. Clade V and the *Babesia* s.s. Clade VI (Schnittger et al. 2012). Bat-related piroplasmid 18S rRNA sequences available in GenBank database were too short (448 pb, 514 pb, and 624 pb) and therefore not included in this phylogenetic analysis. In the second tree, based on a 524 pb-alignment (**Fig. 3**), the sequences of Piroplasmid sp. – *Artibeus* spp. detected in *Artibeus literatus* and *Artibeus platirostris* bats clustered together with *Babesia vogeli* into a single well supported clade (bootstrap 100). Interestingly, 18S rRNA piroplasmid sequences previously obtained from other bat species (*Nyctalus noctula* and *Pipistrellus pygmaeus*) and from a bat tick (*Ixodes simplex*) from Hungary and Romania were positioned into a strongly supported clade (bootstrap: 100) with *Babesia canis*. The DNA divergence analysis, based on a 709 pb-length alignment showed that sequence #40 (*Artibeus planirostris*) and #87 (*Artibeus lituratus*) differed from *B. vogeli* (AY072925) by two and one nucleotide differences and from *B. canis* (AY072925) by 18 and 17 nucleotides respectively.

All the sequences used for the analysis are presented in **Supplementary Table 3**.

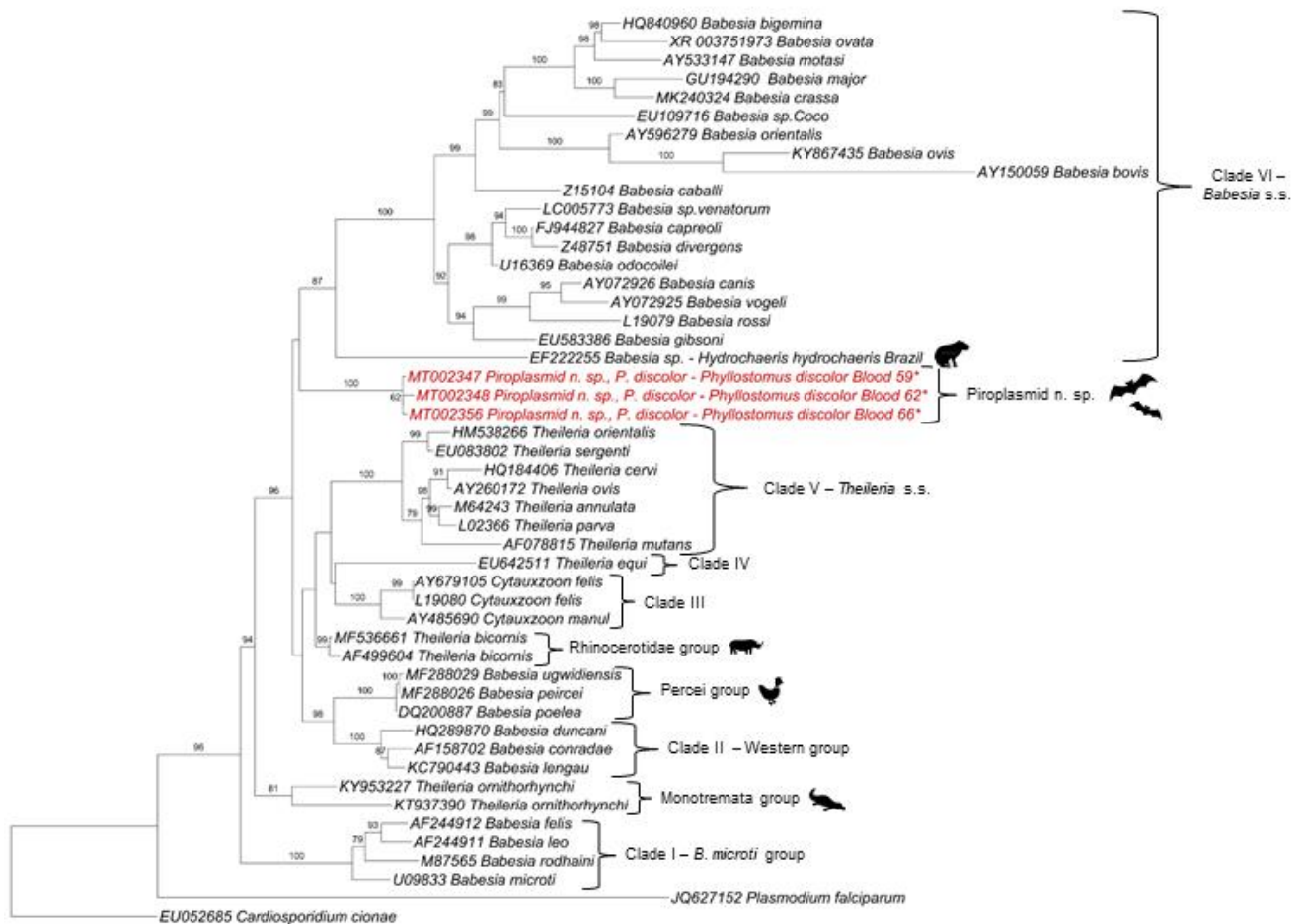


Fig 2. Phylogenetic tree of nearly complete piroplasmid 18S rRNA sequences using maximum likelihood (ML). The bootstrap supports were estimated by 1000 replicates and are presented at nodes. 18S rRNA sequences of Piroplasmid n sp. *P. discolor* isolates are highlighted in red.

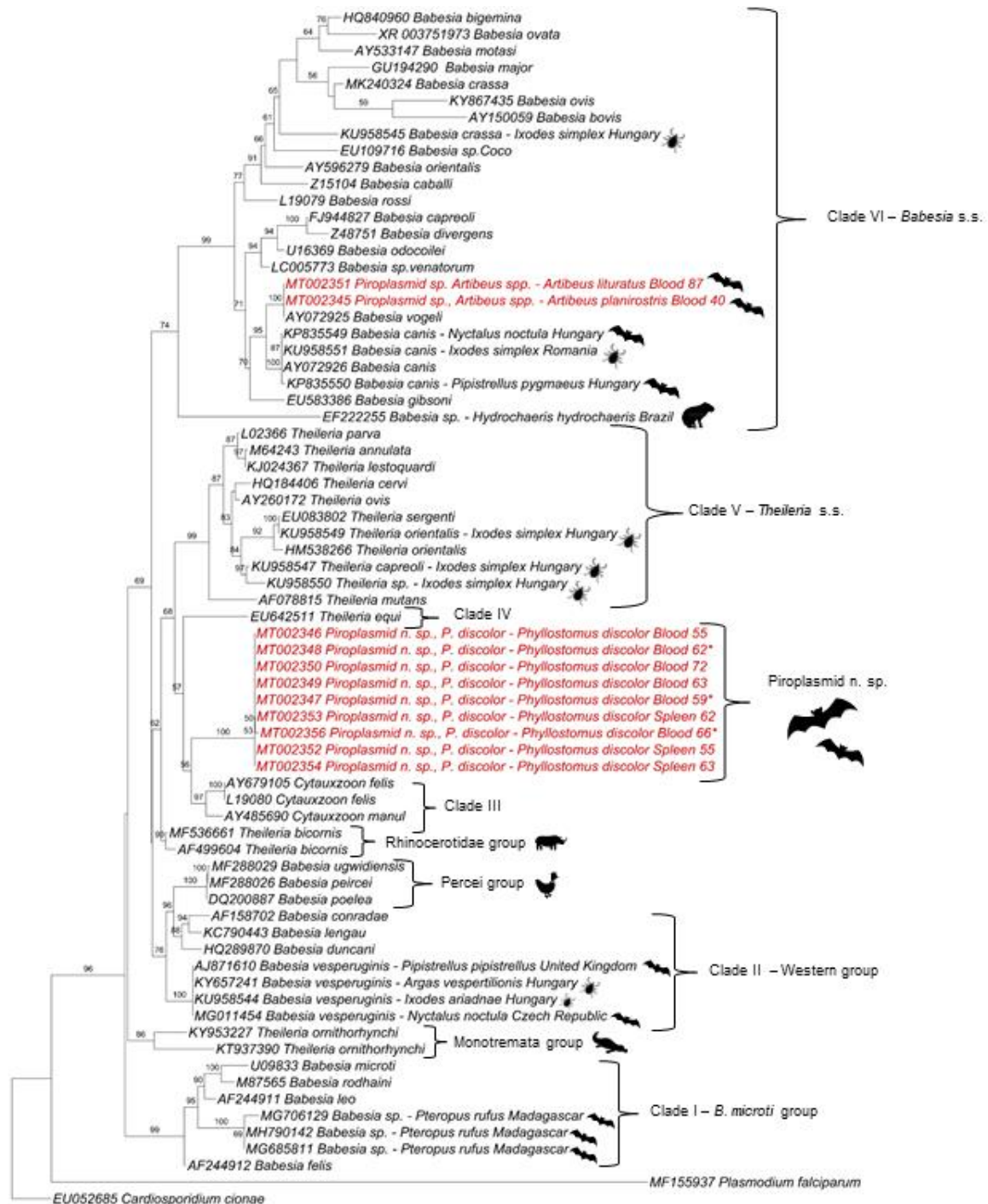


Fig 3. Phylogenetic tree based on an alignment of partial 18S rRNA sequences using maximum likelihood (ML). Bootstrap supports were estimated by 1000 replicates and are presented at nodes. Sequences of piroplasmids detected in the present study are highlighted in red and place in two different clades. One clade includes nine sequences (Piroplasmid n. sp., *P. discolor*) and the remaining two sequences (Piroplasmid sp., *Artibeus* spp. bats) placed into Clade VI (*Babesia* s.s.).

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4. Discussion

It has long been known that Brazil hosts a great diversity of bat species. These animals are of importance in the transmission of several parasites, bacteria and viruses of which some cause diseases in animals and/or humans (Nunes et al. 2017). Given that these mammals also inhabit urban and rural areas and can live very close to humans and domestic animals (Reis et al. 2007; Nunes et al. 2017), the study of their pathogen reservoirs, as well as of potential pathogen transmitters, becomes of great importance for the elucidation of disease risks of veterinary and medical relevance. This work showed the first molecular detection of piroplasmids in bats from the Neotropical region.

Herein, a 12.6% frequency of positivity for piroplasmids was found among the sampled bats (17/135). Previously, frequencies of positivity of 2.7% was described among bats sampled in Hungary and in the Netherlands (Hornok et al., 2015), 4.3% in Austria, Czech Republic, and Romania (Corduneanu et al. 2017), 4.4% in Madagascar (Ranaivoson et al. 2019), 10% in the United Kingdom (Concannon et al. 2005), and 13.3% in China (Han et al. 2018). Considering the different molecular techniques used in each of the abovementioned studies, a comparison of the prevalence rates cannot be performed.

Piroplasmids have been detected using molecular methodology in bats and/or associated ectoparasites in several European countries (Concannon et al. 2005; Hornok et al. 2017; Corduneanu et al. 2017) and China (Han et al. 2018), where identified as *Babesia vesperuginis*, which has been exclusively found in bats. Recently, Ranavaison et al. (2019) reported the detection of a putative new piroplasmid species in *Pteropus rufus* bats sampled in Madagascar. Interestingly, the corresponding 18S rRNA sequences placed into the *B. microti*-group (Clade I, Schnittger et al 2012) demonstrating that piroplasmids species infecting bats pertain to diverse piroplasmid lineages. Phylogenetic analysis based on 1,399 bp alignment of 18S rRNA sequences positioned Piroplasmid n. sp. *Phyllostomus discolor* into a highly supported novel clade and placed between *Babesia* s. s. and *Theileria* s. s. clades (bootstrap=100) (Fig. 2). On the other hand, the phylogenetic analysis based on a 524 bp alignment of 18S rRNA sequences positioned Piroplasmid sp. *Artibeus* spp. near *B. vogeli*.

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Babesia spp. can probably parasitize the majority of wild mammals and usually cause subclinical infections (Schnittger et al. 2012). In this study, we identified two putatively novel piroplasmid species in bats: one seems to specifically infect *Phyllostomus* bats (Piroplasmid n. sp. *P. discolor*), whereas the other species has been exclusively been detected in bats of the genus *Artibeus*. Future studies sampling different bat species from diverse geographic regions of Brazil would be necessary to confirm the existence of the proposed host specificity of the two putative novel piroplasmid species identified in this study. Noteworthy, additional studies should combine molecular and other methods (blood smear analysis and clinical examination) in order to better characterize piroplasmids occurring in bats (Uilenberg, Gray and Kahl, 2018). Despite Ranaivoson et al. (2019) had observed no health impact on piroplasmid-infected bats, the authors reported an incomplete health assessment from those animals and further investigations should be performed. Unfortunately, clinical and laboratory examination were not performed in the present work.

Babesia canis sequences have been detected in bats and associated ectoparasites from Romania (Hornok et al. 2016) and Hungary (Hornok et al. 2015). Interestingly, while 18S rRNA piroplasmid sequences closely related to *B. canis* were detected in bats from the Northern Hemisphere, sequences obtained in the present study were closely related to *B. vogeli*. Indeed, while *B. canis* is highly prevalent in Central Europe and the main vector is the tick *Dermacentor reticulatus* (Solano-Gallego et al., 2016), *B. vogeli* is the most widespread piroplasmid species found in dogs in Brazil and the main vector is the brown dog tick (*Rhipicephalus sanguineus sensu lato*) (Maggi and Krämer 2019). Even though *R. sanguineus* has been described parasitizing *Myotis capaccinii*, *Myotis punicus*, *Miniopterus schreibersii* bats in Algeria (Bendjeddou et al., 2013), *Nothoaspis*, *Ornithodoros* and *Antricola* argasid ticks represent the main ticks parasitizing bats in Brazil (Luz et al., 2016; Muñoz-Leal et al., 2016, 2018).

Despite several attempts, we could not obtain nearly complete 18S rRNA sequences for Piroplasmid sp. 2 detected in *Artibeus* bats in the present study. Possibly the low parasitemia displayed by the parasite in the sampled bats precluded amplification by simple PCR (Greay et al., 2018). Amplification based on previously described PCR protocols were unsuccessful in our hands and precluded phylogenetic

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analysis based on other target genes or intergenic regions. Future studies using whole genome sequencing (WGS) of bat-associated piroplasmids may aid to define the expanding diversity of Piroplasmida in bats and contribute to untangling the unclear phylogenetic positioning of new described taxa.

Although *B. vogeli* is a piroplasmid whose main hosts are dogs (Maggi and Krämer 2019), our research group has detected 18S rRNA sequences closely related to this species in cats from the states of São Paulo (André et al. 2014), Mato Grosso do Sul (André et al. 2015) and Rio Grande do Sul (Malheiros et al. 2016), in Orinoco geese (*Neochen jubata*) sampled in the state of Goiás (Werther et al. 2017), and wild rodents from the wetlands of Mato Grosso do Sul state (de Sousa et al. 2018). Furthermore, our group found a high seroprevalence of *B. vogeli* in dogs sampled in the city of Campo Grande (de Sousa et al. 2013), where the bats in the present study were captured. These findings suggest a *B. vogeli* spill over phenomena from dogs to other vertebrate hosts (André et al. 2015). The possible routes for *B. vogeli* transmission from dogs to non-hematophagous bats are still unknown and the role of immature stages of ticks with low specificity needs to be further investigated.

Although piroplasmid vectors of bats are as yet undefined, DNA specific for piroplasmids infecting bats has already been detected in *Ixodes* ticks collected from bats in Hungary and Romania (Hornok et al. 2016) and *Argas verspertilionis* collected from bats in Hungary, China (Hornok et al. 2017; Liu et al. 2018) and England (Lv et al. 2018). However, these reports should be interpreted with caution, since engorged ectoparasites may contain piroplasmid DNA acquired during their blood meal. Ideally, vector competence experiments should be performed to unveil the role of ticks in the transmission of piroplasmids among bats (Uilenberg, Gray and Kahl, 2018). In the present study, only two positive animals had no ectoparasites at the time of sampling. The remaining 15 piroplasmid-positive animals presented infestation by at least one bat fly (Diptera: Streblidae) and/or mite (Acari: Macronyssidae and Spinturnicidae) and/or tick larvae (Acari: Argasidae) represented by the following species *Megistopoda aranae*, *Trichobius costalimai*, *Periglischrus iheringi*, *Periglischrus torrealbai*, *Periglischrus acutisternus*, *Steatonyssus* sp. and *Ornithodoros hasei* (data shown in Perles et al. 2020). Even though *Ornithodoros erraticus* has been incriminated as a possible argasid vector for *Babesia meri* (Homer et al. 2000; Yabsley and Shock 2013)

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and *Ornithodoros moubata* seemed to be involved in the transmission of *B. gibsoni*, the role of soft ticks as vectors for piroplasmids should be further investigated (Schnittger et al., 2012). Recently, Baneth et al. (2020) suggested the role of *Ornithodoros tholozani* in the transmission of a new piroplasmid species infecting dogs in Israel. Since argasid ticks represent the main ticks found in birds, Schnittger et al. (2012) suggested that soft ticks might play a role in the *Babesia* transmission among these vertebrates. In this study, *Ornithodoros* larvae were collected from four *P. discolor* individuals that were positive for piroplasmids but, unfortunately, ectoparasites were not available for PCR amplification followed by direct sequencing as they were needed for other tests. However, it is worth mentioning that signs of human parasitism by *Ornithodoros* ticks have been associated with the presence of bats in human dwellings in Brazil, which can be a threat to human health (Labruna et al. 2014; de Oliveira et al. 2018).

All piroplasmid-positive animals (*P. discolor*, *P. lineatus*, *A. planirostris* and *A. lituratus* species) belonged to the Phyllostomidae family, which comprises predominantly of frugivorous animals whereas remaining bat species belonging to this family feed on insects, nectar, pollen and flies (Reis et al. 2007; Martins et al. 2014 a, b; Porfirio and Bordignon 2015; Torres et al. 2018). It is possible that grooming behavior among bats, including eating of piroplasmid-infected ticks, represent a transmission route between of piroplasmids between chiropterans (Kerth, 2008). Recently, Corduneanu et al. (2020) detected *B. canis* DNA in tissues of rodents following ingestion of *Dermacentor reticulatus* infected ticks, raising questions about alternative transmission routes for piroplasms. Moreover, Hornok et al. (2015) detected *B. canis* DNA in fecal samples from five bats in Hungary, which also might be associated with ingestion of infected vectors by the host. Unfortunately, the authors did not investigate the presence of possible invertebrate vectors in the bat fecal samples. In conclusion, a possible transmission of bat-infecting piroplasmid species by grooming and consequent ingestion of piroplasmid-infected ticks should be taken into account in future studies as an alternative route of piroplasmid transmission between individual bats.

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The present work pointed out the scarcity of knowledge about bat-related piroplasmid species worldwide and provided additional evidence for the high diversity of this group of apicomplexan hemoparasites in bats.

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Declaration of interest The authors declare that they have no conflict of interest.

Ethics approval All experimental procedures were approved by the “Instituto Chico Mendes de Biodiversidade” (ICMBio) (SISBIO 57450-1) and by the Ethics Committee on Animal Use of the School of Agricultural and Veterinary Sciences, UNESP (CEUA FCAV/UNESP 010050/17).

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material Sequences were deposited in GenBank under submission numbers MT002345-MT002354 and MT002356.

Code availability Not applicable.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Priscila Ikeda; Jaire Marinho Torres; Taline Revollo Menezes, and Elizabete Captivo Lourenço. The first draft of the manuscript was written by Priscila Ikeda; Taline Revollo Menezes and Marcos Rogério André and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Supplementary material:

Supplementary table 1. PCR assay results for piroplasmid detection based on the 18S rRNA, *hsp-70*, *cox-1* and β -*tubulin* genes and ITS-2 intergenic region. The 18S rRNA-nested PCR protocol described by Jefferies et al. (2007) was used to screen samples for piroplasmid DNA.

#Spleen samples	Host species	Capture location	18S rRNA (~ 800 pb) (Jefferies et al., 2007)	18S rRNA (~ 600 pb) (Yabsley et al., 2006)	18S rRNA (~1500 pb) (Greay et al., 2018)	<i>hsp-70</i> (~ 740 pb) (Soares et al., 2011)	β - <i>tubulina</i> (~ 1500 pb) (Zamoto et al., 2004)	ITS-2 (~ 300 pb) (Shock et al., 2012)
3	<i>Artibeus planirostris</i>	CEA Polonês	+ NS	-	-	-	-	-
5	<i>Platyrrhinus lineatus</i>	CEA Polonês	+ NS	-	-	-	-	-
12	<i>Artibeus planirostris</i>	CEA Polonês	+ NS	-	-	-	-	-
13	<i>Artibeus planirostris</i>	CEA Polonês	+ NS	-	-	-	-	-
18	<i>Platyrrhinus lineatus</i>	CEA Polonês	+ NS	-	-	-	-	-
55*	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	-	-	-	-	-
62*	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	-	-	-	-	-
63*	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	-	-	-	-	-

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#Blood samples	Host species	Capture location	18S rRNA (~ 800 pb) (Jefferies et al., 2007)	18S rRNA (~600 pb) (Yabsley et al., 2006)	18S rRNA (~1500 pb) (Greay et al., 2018)	<i>hsp-70</i> (~ 740 pb) (Soares et al., 2011)	β - <i>tubulina</i> (~ 1500 pb) (Zamoto et al., 2004)	ITS-2 (~ 300 pb) (Shock et al., 2012)
40	<i>Artibeus planirostris</i>	CEA Florestinha	+S	-	-	-	-	-
55*	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	-	-	-	-	-
59	<i>Phyllostomus discolor</i>	CEA Florestinha	+ S	-	+S	-	-	-
61	<i>Phyllostomus discolor</i>	CEA Florestinha	+ NS	-	-	-	-	-
62*	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	+S	+S	-	-	-
63*	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	-	-	-	-	-
66	<i>Phyllostomus discolor</i>	CEA Florestinha	+ NS	+S	+S	-	-	-
72	<i>Phyllostomus discolor</i>	CEA Florestinha	+S	-	-	-	-	-
87	<i>Artibeus lituratus</i>	CEA Polonês	+S	-	-	-	-	-

Legend: positive and sequenced samples (+S); positive but not sequenced samples (+NS); negative samples for the tested genes (-); animals positive for both spleen and blood samples as determined by nPCR assay targeting the 18S rRNA gene (*)

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Supplementary table 2. nBLAST analysis results of 18S rRNA piroplasmid sequences detected in non-hematophagous bats from Central-Western Brazil.

# Animal	Sample type	Host Species	Capture location	PCR Protocol	Identity (%)	Query Coverage (%)	Organism (GenBank accession number)
40	Blood	<i>Artibeus planirostris</i>	CEA Florestinha	Jefferies et al. (2007)	99.87%	100%	<i>Babesia vogeli</i> (MH100719)
55	Spleen	<i>Phyllostomus discolor</i>	CEA Florestinha	Jefferies et al. (2007)	92.14%	100%	<i>Theileria bicornis</i> (MF536661)
	Blood				92.3%	99%	<i>Theileria bicornis</i> (MF536661)
59	Blood	<i>Phyllostomus discolor</i>	CEA Florestinha	Greay et al. (2018)	93.59%	100%	<i>Theileria bicornis</i> (MF536661)
62	Spleen	<i>Phyllostomus discolor</i>	CEA Florestinha	Jefferies et al. (2007)	93.03%	100%	<i>Theileria bicornis</i> (MF536661)
	Blood				92.96%	100%	<i>Theileria bicornis</i> (MF536661)
					Yabsley et al. (2006)	93.56%	100%
63	Spleen	<i>Phyllostomus discolor</i>	CEA Florestinha	Jefferies et al. (2007)	93.50%	100%	<i>Theileria bicornis</i> (MF536661)
	Blood				92.63%	99%	<i>Theileria bicornis</i> (MF536661)
66	Blood	<i>Phyllostomus discolor</i>	CEA Florestinha	Greay et al. (2018)	93.44%	99%	<i>Theileria bicornis</i> (MF536661)
72	Blood	<i>Phyllostomus discolor</i>	CEA Florestinha	Jefferies et al. (2007)	92.6%	98%	<i>Theileria bicornis</i> (MF536661)
87	Blood	<i>Artibeus lituratus</i>	CEA Polonês	Jefferies et al. (2007)	100%	100%	<i>Babesia</i> sp. (MK830995)

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Supplementary Table 3. Description and length of each sequence used in both phylogenetic analyses including the sequences obtained from non-hematophagous bats from Central-Western Brazil.

GenBank Accession Number	Organism	Length (pb)
KU958545	<i>Babesia crassa</i> - <i>Ixodes simplex</i> Hungary	410
KU958551	<i>Babesia canis</i> - <i>Ixodes simplex</i> Romania	420
KU958547	<i>Theileria capreoli</i> - <i>Ixodes simplex</i> Hungary	425
KP835549	<i>Babesia canis</i> - <i>Nyctalus noctula</i> Hungary	431
KP835550	<i>Babesia canis</i> - <i>Pipistrellus pygmaeus</i> Hungary	431
KU958549	<i>Theileria orientalis</i> - <i>Ixodes simplex</i> Hungary	432
KU958550	<i>Theileria</i> sp. - <i>Ixodes simplex</i> Hungary	432
KY657241	<i>Babesia vesperuginis</i> - <i>Argas vespertilionis</i> Hungary	448
KU958544	<i>Babesia vesperuginis</i> - <i>Ixodes ariadnae</i> Hungary	448
MG011454	<i>Babesia vesperuginis</i> - <i>Nyctalus noctula</i> Czech Republic	514
MT002353	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Spleen 62	577
MT002354	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Spleen 63	620
MT002352	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Spleen 55	623
AJ871610	<i>Babesia vesperuginis</i> - <i>Pipistrellus pipistrellus</i> United Kingdom	624
MT002351	Piroplasmid sp. <i>Artibeus</i> spp. - <i>Artibeus lituratus</i> Blood 87	708
MT002345	Piroplasmid sp., <i>Artibeus</i> spp. - <i>Artibeus planirostris</i> Blood 40	741
MT002349	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Blood 63	758
MT002350	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Blood 72	761
MT002346	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Blood 55	769
MG706129	<i>Babesia</i> sp. - <i>Pteropus rufus</i> Madagascar	970
KJ024367	<i>Theileria lestoquardi</i>	1036
MH790142	<i>Babesia</i> sp. - <i>Pteropus rufus</i> Madagascar	1089
MG685811	<i>Babesia</i> sp. - <i>Pteropus rufus</i> Madagascar	1110
KY867435	<i>Babesia ovis</i>	1354
KT937390	<i>Theileria ornithorhynchi</i>	1398

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MT002347	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Blood 59*	1455
MT002356	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Blood 66*	1467
MT002348	Piroplasmid n. sp., <i>P. discolor</i> - <i>Phyllostomus discolor</i> Blood 62*	1571
EU642511	<i>Theileria equi</i>	1573
HQ184406	<i>Theileria cervi</i>	1591
EU583386	<i>Babesia gibsoni</i>	1592
MF155937	<i>Plasmodium falciparum</i>	1628
GU194290	<i>Babesia major</i>	1629
AY485690	<i>Cytauxzoon manul</i>	1630
MF288026	<i>Babesia peircei</i>	1642
MF288029	<i>Babesia ugwidiensis</i>	1644
KY953227	<i>Theileria ornithorhynchi</i>	1646
KC790443	<i>Babesia lengau</i>	1648
AY150059	<i>Babesia bovis</i>	1649
LC005773	<i>Babesia venatorum</i>	1666
XR 003751973	<i>Babesia ovata</i>	1677
MK240324	<i>Babesia crassa</i>	1683
AY533147	<i>Babesia motasi</i>	1684
M87565	<i>Babesia rodhaini</i>	1684
HQ840960	<i>Babesia bigemina</i>	1689
AF244911	<i>Babesia leo</i>	1690
AF499604	<i>Theileria bicornis</i>	1693
Z15104	<i>Babesia caballi</i>	1694
AF244912	<i>Babesia felis</i>	1694
EU109716	<i>Babesia</i> sp.Coco	1698
AY596279	<i>Babesia orientalis</i>	1700
L19079	<i>Babesia rossi</i>	1711
AY072925	<i>Babesia vogeli</i>	1713
AY072926	<i>Babesia canis</i>	1714

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EF222255	<i>Babesia</i> sp. - <i>Hydrochaeris hydrochaeris</i> Brazil	1714
AF158702	<i>Babesia conradae</i>	1717
U16369	<i>Babesia odocoilei</i>	1723
FJ944827	<i>Babesia capreoli</i>	1724
Z48751	<i>Babesia divergens</i>	1726
MF536661	<i>Theileria bicornis</i>	1729
AF078815	<i>Theileria mutans</i>	1737
EU083802	<i>Theileria sergenti</i>	1740
L02366	<i>Theileria parva</i>	1742
M64243	<i>Theileria annulata</i>	1744
HM538266	<i>Theileria orientalis</i>	1748
U09833	<i>Babesia microti</i>	1763
AY260172	<i>Theileria ovis</i>	1766
HQ289870	<i>Babesia duncani</i>	1768
AY679105	<i>Cytauxzoon felis</i>	1774
L19080	<i>Cytauxzoon felis</i>	1774
EU052685	<i>Cardiosporidium cionae</i>	1919
JQ627152	<i>Plasmodium falciparum</i>	1921
DQ200887	<i>Babesia poeala</i>	2841

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CHAPTER 6 - Final Considerations

In the present work, we investigated the molecular occurrence and genetic diversity of *Anaplasmataceae*, *Bartonellaceae*, *Coxiellaceae*, *Mycoplasmataceae* and Piroplasmida agents in bats sampled in the periurban region of Campo Grande, Mato Grosso do Sul, Brazil.

Out of the 135 sampled bats, 43 (31.85%) had positive blood and/or spleen samples for only one studied agent; 26 (19.25%) showed to be negative for all studied agents; and seven (5.15%) had negative blood and spleen samples, but was parasitized by a positive ectoparasite. This latter group of animals were represented by animal #10, whose Streblidae fly *Megistopoda aranea* was positive for *Ehrlichia* spp.; animals #91, #93, #95, #106, #122, whose Macronyssidae mites (*Steatonyssus* sp.) were positive for *Ehrlichia* spp.; and the animal #120, whose Streblidae fly *M. aranea* was positive for *Bartonella* spp.

In the remaining 59 (43.7%) animals, co-positivity was observed in 31 individuals when considering only spleen and/or blood samples:

- 🦇 10 animals positive for *Mycoplasma* spp. + *Neorickettsia* spp.
- 🦇 04 positive animals for *Neorickettsia* spp. + *Bartonella* spp.
- 🦇 03 positive animals for *Mycoplasma* spp. + *Bartonella* spp.
- 🦇 03 positive animals for *Anaplasma* spp. + *Neorickettsia* spp.
- 🦇 03 positive animals for *Mycoplasma* spp. + *Neorickettsia* spp. + *Bartonella* spp.
- 🦇 Individual bats were co-positive for the following agents:
 - **Ehrlichia* spp. + *Neorickettsia* spp.
 - **Ehrlichia* spp. + Piroplasmida
 - **Mycoplasma* spp. + Piroplasmida
 - **Neorickettsia* spp. + Piroplasmida
 - **Mycoplasma* spp. + *Ehrlichia* spp.
 - **Mycoplasma* spp. + *Ehrlichia* spp. + *Neorickettsia* spp.
 - **Mycoplasma* spp. + *Neorickettsia* spp. + Piroplasmida
 - **Mycoplasma* spp. + *Anaplasma* spp. + *Neorickettsia* spp. + Piroplasmida

The remaining 27 animals had at least one sample (blood and/or spleen) positive for one or more agents together with positivity of one or more associated ectoparasites, also positive for one or more different agents:

- ✶ 12 animals that were positive for one agent in spleen and/or blood samples, and another agent detected in the associated ectoparasite;
- ✶ 09 animals positive for two or more agents in spleen and/or blood samples, and another agent in the associated ectoparasites;
- ✶ 3 animals positive for one agent in spleen and/or blood samples and two or more different agents detected in the associated ectoparasites;
- ✶ 3 animals positive for two or more agents in both spleen/blood and ectoparasites samples.

The combinations of positivity found among blood/spleen and/or ectoparasite samples collected from the same individual are shown in **Table 1** and more details of each sample can be found in **Table 2**. Complementary information can be found in **Appendices 1-3**.

Table 1. Combinations of co-positivity for *Anaplasmataceae*, *Bartonellaceae*, *Mycoplasmataceae*, and *Piroplasmida* agents molecularly detected in blood and/or spleen samples and ectoparasites samples collected from the same bat host captured in Campo Grande, MS, Brazil.

Number of animals showing positive samples for the combination	Combination of co-positivity in blood and/or spleen samples	Combination of co-positivity in ectoparasites samples (mite and/or bat fly and/or tick)
04	<i>Mycoplasma</i> spp.	<i>Ehrlichia</i> spp.
03	<i>Neorickettsia</i> spp.	<i>Ehrlichia</i> spp.
02	<i>Mycoplasma</i> spp.	<i>Bartonella</i> spp.
02	<i>Neorickettsia</i> spp.	<i>Bartonella</i> spp.
02	<i>Neorickettsia</i> spp.	<i>Ehrlichia</i> spp. + <i>Bartonella</i> spp.
02	<i>Mycoplasma</i> spp. + <i>Neorickettsia</i> spp.	<i>Ehrlichia</i> spp. + <i>Bartonella</i> spp.
01	<i>Piroplasmida</i>	<i>Ehrlichia</i> spp.
01	<i>Mycoplasma</i> spp.	<i>Ehrlichia</i> spp. + <i>Bartonella</i> spp.
01	<i>Mycoplasma</i> spp. + <i>Piroplasmida</i>	<i>Ehrlichia</i> spp.

01	<i>Mycoplasma</i> spp. + Piroplasmida	<i>Anaplasma</i> spp.
01	<i>Mycoplasma</i> spp. + <i>Bartonella</i>	<i>Bartonella</i> spp.
01	<i>Mycoplasma</i> spp. + <i>Neorickettsia</i> spp.	<i>Ehrlichia</i> spp.
01	<i>Neorickettsia</i> spp. + <i>Bartonella</i> spp.	<i>Bartonella</i> spp.
01	<i>Mycoplasma</i> spp. + Piroplasmida + <i>Bartonella</i> spp.	<i>Ehrlichia</i> spp.
01	<i>Mycoplasma</i> spp. + Piroplasmida + <i>Neorickettsia</i> spp.	<i>Bartonella</i> spp.
01	<i>Mycoplasma</i> spp. + <i>Ehrlichia</i> spp. + <i>Neorickettsia</i> spp.	<i>Ehrlichia</i> spp.
01	<i>Mycoplasma</i> spp. + <i>Anaplasma</i> spp. + <i>Neorickettsia</i> spp. + Piroplasmida	<i>Bartonella</i> spp.
01	<i>Mycoplasma</i> spp. + <i>Anaplasma</i> spp. + <i>Ehrlichia</i> spp. + Piroplasmida	<i>Ehrlichia</i> spp. + <i>Neorickettsia</i> spp.

Table 2. Description of sample type positive for each studied agent collected from the same bat individual that showed co-positivity. All collected Macronyssidae mites were identified as *Steatonyssus* sp. and all collected ticks were identified as *Ornithodoros hasei*. All animals were sampled in periurban regions of Campo Grande, Mato Grosso do Sul, Brazil.

Animal ID	Capture Site	Bat species	<i>Bartonella</i> spp.	Hemoplasmas	<i>Anaplasma</i> spp.	<i>Ehrlichia</i> spp.	<i>Neorickettsia</i> spp.	Piroplasmida
MCG 01	CEA Polonês	<i>Artibeus lituratus</i>				spleen	spleen	
MCG 02	CEA Polonês	<i>Artibeus lituratus</i>		spleen			blood	
MCG 03	CEA Polonês	<i>Artibeus planirostris</i>				blood		spleen
MCG 05	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood	blood		Macronyssidae mite		spleen
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>		spleen and blood			spleen and blood	
MCG 12	CEA Polonês	<i>Artibeus planirostris</i>				Bat fly (<i>M. aranea</i>)		spleen
MCG 13	CEA Polonês	<i>Artibeus planirostris</i>		spleen and blood				spleen
MCG 15	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood	blood			spleen	
MCG 16	CEA Polonês	<i>Artibeus planirostris</i>	blood				spleen	
MCG 20	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood				blood	
MCG 22	CEA Polonês	<i>Platyrrhinus lineatus</i>			spleen		spleen	
MCG 23	CEA Polonês	<i>Eptesicus furinalis</i>	bat fly (<i>T. dugesii</i> complex)	spleen		Macronyssidae mite		
MCG 25	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood	blood				
MCG 30	CEA Polonês	<i>Platyrrhinus lineatus</i>	Bat fly (<i>T. dugesii</i> complex)				blood	

MCG 32	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood			blood	
MCG 34	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood			spleen	
MCG 35	CEA Polonês	<i>Artibeus planirostris</i>		spleen and blood		blood	
MCG 38	CEA Florestinha	<i>Artibeus planirostris</i>		spleen and blood	bat fly (<i>M. aranea</i>)		
MCG 39	CEA Florestinha	<i>Artibeus planirostris</i>		spleen and blood		blood	
MCG 40	CEA Florestinha	<i>Artibeus planirostris</i>				spleen	blood
MCG 42	CEA Florestinha	<i>Carollia perspicillata</i>	blood and bat fly (2 <i>T. dugesii</i> complex)	spleen			
MCG 43	CEA Florestinha	<i>Artibeus planirostris</i>		blood		blood	
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>	blood	spleen and blood		spleen	
MCG 51	CEA Polonês	<i>Artibeus planirostris</i>	blood	blood		spleen	
MCG 54	CEA Florestinha	<i>Molossus molossus</i>		blood		blood	
MCG 55	CEA Florestinha	<i>Phyllostomus discolor</i>	bat fly (<i>T. costalimai</i>)	spleen and blood		blood	spleen and blood
MCG 56	CEA Florestinha	<i>Phyllostomus discolor</i>		blood	bat fly (<i>T. costalimai</i>), Spinturnicidae mite (1 <i>P. torrealbai</i> and 1 <i>P. acutisternus</i>), Macronyssidae mite and tick		
MCG 57	CEA Florestinha	<i>Phyllostomus discolor</i>	bat fly (<i>T. costalimai</i>)*	spleen and blood	bat fly (<i>T. costalimai</i>)*	spleen and blood	

MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>	bat fly (<i>T. costalimai</i>)	blood	blood		blood	blood
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>		spleen and blood	blood		blood	blood
MCG 62	CEA Florestinha	<i>Phyllostomus discolor</i>		spleen and blood	blood	blood and Spinturnicidae mite (2 <i>Periglischrus</i> sp.)	tick	spleen and blood
MCG 63	CEA Florestinha	<i>Phyllostomus discolor</i>		blood		Macronyssidae mite and tick (2)		spleen and blood
MCG 64	CEA Florestinha	<i>Phyllostomus discolor</i>		blood		Macronyssidae mite		
MCG 65	CEA Florestinha	<i>Phyllostomus discolor</i>	bat fly (<i>T. costalimai</i>)	blood				
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>		blood			blood	blood
MCG 68	CEA Florestinha	<i>Phyllostomus discolor</i>		blood			blood	
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>		spleen		spleen	spleen and blood	
MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>		spleen and blood		bat fly (2 <i>S. hertigi</i>) and Spinturnicidae mite (<i>Periglischrus</i> sp.)	blood	
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>		blood		blood and Macronyssidae mite	blood	
MCG 72	CEA Florestinha	<i>Phyllostomus discolor</i>		spleen	tick			blood
MCG 74	CEA Polonês	<i>Eptesicus furinalis</i>	Macronyssidae mite*			Macronyssidae mite*	blood	
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>	Macronyssidae mite*	spleen		Macronyssidae mite*	blood	

MCG 79	CEA Polonês	<i>Platyrrhinus lineatus</i>	blood and bat fly (<i>T. costalimai</i>)		spleen
MCG 82	CEA Polonês	<i>Eptesicus furinalis</i>	Macronyssidae mite*	Macronyssidae mite*	blood
MCG 83	CEA Polonês	<i>Platyrrhinus lineatus</i>	bat fly (<i>T. dugesii</i> complex)		blood
MCG 88	CEA Polonês	<i>Platyrrhinus lineatus</i>		spleen	blood
MCG 89	CEA Polonês	<i>Platyrrhinus lineatus</i>		spleen	blood
MCG 90	CEA Polonês	<i>Artibeus lituratus</i>		Macronyssidae mite	blood
MCG 92	CEA Florestinha	<i>Eumops perotis</i>		Macronyssidae mite	blood
MCG 94	CEA Florestinha	<i>Molossus molossus</i>		Macronyssidae mite	blood
MCG 99	CEA Polonês	<i>Chiroderma villosus</i>		spleen	blood
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>		sblood	spleen
MCG 101	CEA Polonês	<i>Platyrrhinus lineatus</i>		spleen	spleen
MCG 108	CEA Polonês	<i>Artibeus planirostris</i>		blood	spleen
MCG 123	CEA Polonês	<i>Eptesicus furinalis</i>		Macronyssidae mite	spleen
MCG 125	CEA Polonês	<i>Artibeus lituratus</i>	blood	spleen and blood	
MCG 126	CEA Polonês	<i>Artibeus lituratus</i>	blood	blood	
MCG 132	CEA Polonês	<i>Eptesicus furinalis</i>	Macronyssidae mite	blood	

* The positive DNA sample was obtained from the same individual ectoparasite.

Despite the attempts of isolation by both pre-enrichment liquid medium and chocolate agar plates, we were not able to successfully isolate *Bartonella* spp. Nonetheless, we found a high genotypic diversity that was verified not only among vertebrate hosts, but also within the same biological sample. Such diversity showed to be higher in vertebrate hosts than in arthropods. Indeed, both vertebrate and invertebrate hosts seem to play a role in the maintenance of diversity of this agent. Similarly, hemoplasmas seem to be genetically diverse among bats around the world.

We also showed the detection of *Anaplasma*, *Ehrlichia* and *Neorickettsia* in bats sampled in Brazil, which leads to a new epidemiological scenario, mainly regarding the role of bats in the occurrence of the Potomac Horse Fever. In the present study, only one tick (*Ornithodoros hasei*) was positive for *Neorickettsia* spp., while the remaining positive samples were represented by bats' blood and/or spleen samples. Unfortunately, it was not possible to search for trematodes in these animals. Therefore, additional studies should be performed in order to investigate the bat-associated trematodes involved in the epidemiology of *Neorickettsia* spp. in Brazil. Additionally, epidemiological studies should be performed in an attempt to establish a possible correlation between the presence of bats and the occurrence or neorickettsiosis in a certain region.

Interestingly, the ectoparasites collected from bats in the current study showed a higher positivity for *Bartonella* spp. and *Ehrlichia* spp. than for the other studied agents (**Table 3**). Regarding bat flies, 11/64 (17.18%) were positive for *Bartonella* spp., 7/64 (10.93%) were positive for *Ehrlichia* spp., whereas only 1/64 (1.56%) was positive for hemoplasmas and none was positive for *Anaplasma* spp. and *Neorickettsia* spp. When it comes to mites, 6/46 (13.04%) of Spinturnicidae mites and 19/21 (90.47%) of Macronyssidae mites showed positivity for *Ehrlichia* spp. When considering the other studied agents, only 4/21 (19.04%) Macronyssidae mites were positive for *Bartonella* spp. Regarding the *O. hasei* ticks, 3/19 (15.78%) were positive for *Ehrlichia* spp. and 1/19 (5.26%) was positive for *Anaplasma* spp. and *Neorickettsia* spp. Based on these results, it seems that mites may play a role in the transmission and maintenance of these agents among bats. Future studies, with a larger number of bat-associated arthropods, should investigate the role of these ectoparasites in the maintenance and transmission especially of *Anaplasmataceae* agents in Brazil.

Table 3. Positivity of the ectoparasites collected from bats sampled in periurban region of Campo Grande, Mato Grosso do Sul, Brazil for *Anaplasmataceae*, *Bartonellaceae* and *Mycoplasmataceae*.

Specie	Ectoparasite Host	Sample	Agent positivity
<i>Trichobius dugesii</i> complex	<i>Eptesicus furinalis</i>	Bat fly #23	<i>Bartonella</i> spp.
<i>Trichobius dugesii</i> complex	<i>Platyrrhinus lineatus</i>	Bat fly #30	<i>Bartonella</i> spp.
<i>Trichobius dugesii</i> complex	<i>Carollia perspicillata</i>	Bat fly #42 M1	<i>Bartonella</i> spp.
<i>Trichobius dugesii</i> complex	<i>Carollia perspicillata</i>	Bat fly #42 M2	<i>Bartonella</i> spp.
<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Bat fly #55	<i>Bartonella</i> spp.
<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Bat fly #57 M	<i>Bartonella</i> spp.
<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Bat fly #59	<i>Bartonella</i> spp.
<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Bat fly #65 F1	<i>Bartonella</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #74	<i>Bartonella</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #75	<i>Bartonella</i> spp.
<i>Trichobius dugesii</i> complex	<i>Platyrrhinus lineatus</i>	Bat fly #79	<i>Bartonella</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #82	<i>Bartonella</i> spp.
<i>Trichobius dugesii</i> complex	<i>Platyrrhinus lineatus</i>	Bat fly #83	<i>Bartonella</i> spp.
<i>Megistopoda aranae</i>	<i>Artibeus planirostris</i>	Bat fly #120	<i>Bartonella</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus</i> cf. <i>furinalis</i>	Bat mite #132	<i>Bartonella</i> spp.
<i>Steatonyssus</i> sp.	<i>Myotis nigricans</i>	Bat mite #04	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Platyrrhinus lineatus</i>	Bat mite #05	<i>Ehrlichia</i> spp.
<i>Megistopoda aranea</i>	<i>Artibeus lituratus</i>	Bat fly #10	<i>Ehrlichia</i> spp.
<i>Megistopoda aranea</i>	<i>Artibeus planirostris</i>	Bat fly #12	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #23	<i>Ehrlichia</i> spp.
<i>Megistopoda aranea</i>	<i>Artibeus planirostris</i>	Bat fly #38	<i>Ehrlichia</i> spp.
<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Bat fly #56.5	<i>Ehrlichia</i> spp.
<i>Periglischrus torrealbai</i>	<i>Phyllostomus discolor</i>	Bat mite #56.1	<i>Ehrlichia</i> spp.
<i>Periglischrus acutisternus</i>	<i>Phyllostomus discolor</i>	Bat mite #56.2	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #56	<i>Ehrlichia</i> spp.
<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae #56.2	<i>Ehrlichia</i> spp.
<i>Trichobius costalimai</i>	<i>Phyllostomus discolor</i>	Bat fly #57.5	<i>Ehrlichia</i> spp.
<i>Periglischrus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #62.1	<i>Ehrlichia</i> spp.
<i>Periglischrus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #62.2	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #63	<i>Ehrlichia</i> spp.
<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae #63.1	<i>Ehrlichia</i> spp.
<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae #63.4	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #64	<i>Ehrlichia</i> spp.
<i>Periglischrus torrealbai</i>	<i>Phyllostomus discolor</i>	Bat mite #67	<i>Ehrlichia</i> spp.
<i>Strebla hertigi</i>	<i>Phyllostomus discolor</i>	Bat fly #70.1	<i>Ehrlichia</i> spp.
<i>Strebla hertigi</i>	<i>Phyllostomus discolor</i>	Bat fly #70.2	<i>Ehrlichia</i> spp.
<i>Periglischrus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #70.1	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Phyllostomus discolor</i>	Bat mite #71	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #74	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #75	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #82	<i>Ehrlichia</i> spp.

<i>Steatonyssus</i> sp.	<i>Artibeus lituratus</i>	Bat mite #90	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eumops perotis</i>	Bat mite #91	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eumops perotis</i>	Bat mite #92	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eumops perotis</i>	Bat mite #93	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Molossus molossus</i>	Bat mite #94	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eumops perotis</i>	Bat mite #95	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #106	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Artibeus planirostris</i>	Bat mite #122	<i>Ehrlichia</i> spp.
<i>Steatonyssus</i> sp.	<i>Eptesicus furinalis</i>	Bat mite #123	<i>Ehrlichia</i> spp.
<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae #62.2	<i>Neorickettsia</i> spp.
<i>Ornithodoros hasei</i>	<i>Phyllostomus discolor</i>	Tick larvae #72	<i>Anaplasma</i> spp.
<i>Megistopoda aranea</i>	<i>Artibeus planirostris</i>	Bat fly #27	<i>Mycoplasma</i> spp.

Although *Coxiella burnetii* was not detected in the sampled bats, the impossibility of testing the ectoparasites precluded any assumption on the occurrence of this agent in the system vertebrate host-ectoparasite, as assessed by *Bartonellaceae*, *Anaplasmataceae* and *Mycoplasmataceae* in the present work.

Noteworthy, possible new species of Piroplasmida were detected in the sampled bats. There is a need for additional characterization of the detected agents, such as isolation, morphology description, and sequencing of additional molecular markers, mitochondrial and whole genomes. The role of bat-associated ticks in the transmission of these new piroplasmids should be further assessed.

Little is known about the influence of these agents on bat health and future studies may include this type of assessment. Although possible zoonotic agents have been detected in bats around the world (for instance, hemoplasmas closely related to '*Candidatus* *Mycoplasma haemohominis*', '*Candidatus* *Bartonella mayotimonensis*', '*Candidatus* *Bartonella rousetii*', *C. burnetii*, *Anaplasma phagocytophilum*, and *Babesia microti*), very little is effectively known about the role of these animals in the transmission of these pathogens to humans.

Finally, the present work showed, for the first time, the occurrence of coinfection by several arthropod and trematode-borne agents in non-hematophagous bats from Brazil and respective ectoparasites, which leads to a complex epidemiological scenario, with challenges mainly when it comes to diagnosis and the establishment of preventive measures taking into account both Animal and Public Health.

Appendices

Appendix 1. Identification and details of each bat specimen sampled in periurban region of Campo Grande, Mato Grosso do Sul, Brazil

Animal ID	Capture site	Specie	Ectoparasite presence	Forearm measure	Gender	Age	Reproductive status
MCG 01	CEA Polonês	<i>Artibeus lituratus</i>	Yes	73,6	Male	Adult	Active
MCG 02	CEA Polonês	<i>Artibeus lituratus</i>	Yes	73,8	Female	Adult	Inactive
MCG 03	CEA Polonês	<i>Artibeus planirostris</i>	No	60,7	Female	Adult	Inactive
MCG 04	CEA Polonês	<i>Myotis nigricans</i>	Yes	37,8	Male	Adult	Active
MCG 05	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	49,8	Female	Adult	Inactive
MCG 06	CEA Polonês	<i>Artibeus planirostris</i>	No	67,2	Male	Adult	Active
MCG 07	CEA Polonês	<i>Artibeus planirostris</i>	Yes	59,6	Female	Juvenile	Inactive
MCG 08	CEA Polonês	<i>Artibeus lituratus</i>	Yes	73,7	Male	Adult	Active
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>	Yes	36,7	Male	Adult	Active
MCG 10	CEA Polonês	<i>Artibeus lituratus</i>	Yes	73,4	Male	Adult	Active
MCG 11	CEA Polonês	<i>Artibeus planirostris</i>	Yes	57,8	Male	Adult	Active
MCG 12	CEA Polonês	<i>Artibeus planirostris</i>	Yes	62,4	Female	Juvenile	Inactive
MCG 13	CEA Polonês	<i>Artibeus planirostris</i>	No	63	Male	Juvenile	Inactive
MCG 14	CEA Polonês	<i>Artibeus planirostris</i>	Yes	65,6	Male	Juvenile	Inactive
MCG 15	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	50	Female	Juvenile	Inactive
MCG 16	CEA Polonês	<i>Artibeus planirostris</i>	Yes	64,1	Female	Juvenile	Inactive
MCG 17	CEA Polonês	<i>Artibeus planirostris</i>	Yes	62,3	Female	Adult	Post-lactating
MCG 18	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	49,1	Female	Juvenile	Inactive
MCG 19	CEA Polonês	<i>Artibeus planirostris</i>	Yes	61,6	Female	Juvenile	Inactive
MCG 20	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	48,9	Male	Juvenile	Inactive
MCG 21	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	49,9	Female	Juvenile	Inactive
MCG 22	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	47,4	Male	Juvenile	Inactive
MCG 23	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	39,9	Female	Juvenile	Inactive

MCG 24	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	48,7	Female	Juvenile	Inactive
MCG 25	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	50,9	Female	Juvenile	Inactive
MCG 26	CEA Polonês	<i>Artibeus planirostris</i>	Yes	64,3	Female	Juvenile	Inactive
MCG 27	CEA Polonês	<i>Artibeus planirostris</i>	Yes	60,7	Female	Juvenile	Inactive
MCG 28	CEA Polonês	<i>Artibeus lituratus</i>	Yes	69,5	Female	Adult	Inactive
MCG 29	CEA Polonês	<i>Artibeus planirostris</i>	No	63,4	Male	Juvenile	Inactive
MCG 30	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	50,7	Female	Juvenile	Inactive
MCG 31	CEA Polonês	<i>Artibeus lituratus</i>	No	78,4	Male	Adult	Inactive
MCG 32	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	50,3	Female	Juvenile	Inactive
MCG 33	CEA Polonês	<i>Artibeus planirostris</i>	Yes	64,9	Female	Juvenile	Inactive
MCG 34	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	50,4	Female	Juvenile	Inactive
MCG 35	CEA Polonês	<i>Artibeus planirostris</i>	Yes	62,4	Female	Adult	Inactive
MCG 36	CEA Florestinha	<i>Artibeus planirostris</i>	No	63,7	Female	Adult	Inactive
MCG 37	CEA Florestinha	<i>Carollia perspicillata</i>	Yes	45,4	Male	Adult	Active
MCG 38	CEA Florestinha	<i>Artibeus planirostris</i>	Yes	61,9	Female	Juvenile	Inactive
MCG 39	CEA Florestinha	<i>Artibeus planirostris</i>	Yes	62,8	Female	Juvenile	Inactive
MCG 40	CEA Florestinha	<i>Artibeus planirostris</i>	Yes	63,4	Female	Adult	Inactive
MCG 41	CEA Florestinha	<i>Platyrrhinus lineatus</i>	Yes	51,4	Male	Juvenile	Inactive
MCG 42	CEA Florestinha	<i>Carollia perspicillata</i>	Yes	44,3	Male	Juvenile	Inactive
MCG 43	CEA Florestinha	<i>Artibeus planirostris</i>	Yes	62	Male	Juvenile	Inactive
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>	Yes	66,3	Male	Adult	Active
MCG 45	CEA Polonês	<i>Myotis nigricans</i>	Yes	38,5	Female	Juvenile	Inactive
MCG 46	CEA Polonês	<i>Artibeus lituratus</i>	Yes	72,4	Male	Adult	Active
MCG 47	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	46,4	Male	Adult	Active
MCG 48	CEA Polonês	<i>Artibeus planirostris</i>	No	60,5	Male	Juvenile	Inactive
MCG 49	CEA Polonês	<i>Artibeus lituratus</i>	No	73,4	Male	Adult	Active
MCG 50	CEA Polonês	<i>Artibeus lituratus</i>	No	76,1	Male	Adult	Active
MCG 51	CEA Polonês	<i>Artibeus planirostris</i>	No	64,1	Male	Adult	Active
MCG 52	CEA Polonês	<i>Molossus molossus</i>	No	36,4	Female	Juvenile	Inactive

MCG 53	CEA Polonês	<i>Artibeus lituratus</i>	No	73,1	Male	Adult	Active
MCG 54	CEA Florestinha	<i>Molossus molossus</i>	No	40,4	Male	Juvenile	Inactive
MCG 55	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	63,6	Male	Adult	Active
MCG 56	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	63,7	Male	Adult	Active
MCG 57	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	60,8	Male	Adult	Active
MCG 58	CEA Florestinha	<i>Artibeus lituratus</i>	Yes	74,6	Male	Adult	Active
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	61,2	Male	Adult	Inactive
MCG 60	CEA Florestinha	<i>Molossops temminckii</i>	No	30	Male	Adult	Active
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	68,7	Male	Adult	Active
MCG 62	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	62,3	Male	Adult	Active
MCG 63	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	64,7	Male	Adult	Active
MCG 64	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	63,7	Male	Adult	Active
MCG 65	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	60,4	Male	Adult	Active
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	63,2	Male	Adult	Active
MCG 67	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	63,5	Male	Adult	Active
MCG 68	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	62,8	Male	Adult	Active
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>	Yes	45	Female	Juvenile	Inactive
MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	64,3	Male	Adult	Active
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	59,3	Male	Juvenile	Inactive
MCG 72	CEA Florestinha	<i>Phyllostomus discolor</i>	Yes	64,7	Male	Juvenile	Inactive
MCG 73	CEA Florestinha	<i>Molossus molossus</i>	No	41,8	Male	Adult	Active
MCG 74	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	36,8	Male	Juvenile	Inactive
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	39,7	Female	Juvenile	Inactive
MCG 76	CEA Polonês	<i>Artibeus lituratus</i>	No	73	Male	Adult	Active
MCG 77	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	47	Male	Juvenile	Inactive
MCG 78	CEA Polonês	<i>Artibeus lituratus</i>	Yes	77,8	Male	Adult	Active
MCG 79	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	50,2	Female	Juvenile	Inactive
MCG 80	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	46,8	Female	Juvenile	Inactive
MCG 81	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	46,9	Female	Juvenile	Inactive

MCG 82	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	36,5	Male	Juvenile	Inactive
MCG 83	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	49,8	Female	Juvenile	Inactive
MCG 84	CEA Polonês	<i>Artibeus lituratus</i>	Yes	66,9	Female	Juvenile	Inactive
MCG 85	CEA Polonês	<i>Artibeus lituratus</i>	No	71	Female	Adult	Post-lactating
MCG 86	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	49,1	Male	Adult	Inactive
MCG 87	CEA Polonês	<i>Artibeus lituratus</i>	Yes	73	Male	Juvenile	Inactive
MCG 88	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	46,8	Female	Juvenile	Inactive
MCG 89	CEA Polonês	<i>Platyrrhinus lineatus</i>	Yes	46,2	Male	Juvenile	Inactive
MCG 90	CEA Polonês	<i>Artibeus lituratus</i>	Yes	69,3	Male	Juvenile	Inactive
MCG 91	CEA Florestinha	<i>Eumops perotis</i>	Yes	80,3	Female	Adult	Post-lactating
MCG 92	CEA Florestinha	<i>Eumops perotis</i>	Yes	82,5	Female	Adult	Post-lactating
MCG 93	CEA Florestinha	<i>Eumops perotis</i>	Yes	82,9	Male	Adult	Inactive
MCG 94	CEA Florestinha	<i>Molossus molossus</i>	Yes	40	Male	Juvenile	Inactive
MCG 95	CEA Florestinha	<i>Eumops perotis</i>	Yes	81,9	Male	Adult	Active
MCG 96	CEA Polonês	<i>Artibeus lituratus</i>	No	73,7	Male	Adult	Active
MCG 97	CEA Polonês	<i>Artibeus lituratus</i>	Yes	77,5	Male	Adult	Active
MCG 98	CEA Polonês	<i>Artibeus lituratus</i>	No	72,9	Male	Adult	Active
MCG 99	CEA Polonês	<i>Chiroderma villosus</i>	No	52,2	Female	Adult	Active
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>	No	72,5	Male	Adult	Active
MCG 101	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	48,1	Male	Juvenile	Inactive
MCG 102	CEA Polonês	<i>Artibeus lituratus</i>	Yes	72,8	Male	Juvenile	Inactive
MCG 103	CEA Polonês	<i>Artibeus planirostris</i>	No	58,1	Male	Juvenile	Inactive
MCG 104	CEA Polonês	<i>Artibeus lituratus</i>	Yes	71,1	Male	Juvenile	Inactive
MCG 105	CEA Polonês	<i>Artibeus lituratus</i>	No	70	Male	Adult	Active
MCG 106	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	38,4	Female	Juvenile	Inactive
MCG 107	CEA Polonês	<i>Artibeus planirostris</i>	No	65	Female	Adult	Post-lactating
MCG 108	CEA Polonês	<i>Artibeus planirostris</i>	No	66,5	Female	Adult	Inactive
MCG 109	CEA Polonês	<i>Artibeus planirostris</i>	No	61,7	Female	Juvenile	Inactive
MCG 110	CEA Polonês	<i>Artibeus planirostris</i>	No	64,6	Female	Juvenile	Inactive

MCG 111	CEA Polonês	<i>Carollia perspicillata</i>	No	44,5	Female	Juvenile	Inactive
MCG 112	CEA Polonês	<i>Artibeus planirostris</i>	No	66	Male	Juvenile	Inactive
MCG 113	CEA Polonês	<i>Artibeus planirostris</i>	No	66,1	Female	Juvenile	Inactive
MCG 114	CEA Polonês	<i>Platyrrhinus lineatus</i>	No	48	Female	Juvenile	Inactive
MCG 115	CEA Florestinha	<i>Artibeus planirostris</i>	No	64,7	Female	Adult	Post-lactating
MCG 116	CEA Florestinha	<i>Artibeus lituratus</i>	No	77,3	Female	Adult	Post-lactating
MCG 117	CEA Florestinha	<i>Artibeus lituratus</i>	No	70,9	Male	Adult	Active
MCG 118	CEA Florestinha	<i>Artibeus lituratus</i>	No	72,6	Male	Adult	Active
MCG 119	CEA Florestinha	<i>Artibeus lituratus</i>	No	77,7	Male	Adult	Active
MCG 120	CEA Florestinha	<i>Artibeus planirostris</i>	Yes	64,9	Female	Adult	Post-lactating
MCG 121	CEA Florestinha	<i>Artibeus lituratus</i>	No	78,1	Female	Adult	Post-lactating
MCG 122	CEA Polonês	<i>Artibeus planirostris</i>	No	60,7	Male	Juvenile	Inactive
MCG 123	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	36,8	Male	Juvenile	Inactive
MCG 124	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	39	Female	Juvenile	Inactive
MCG 125	CEA Polonês	<i>Artibeus lituratus</i>	Yes	73,3	Male	Adult	Inactive
MCG 126	CEA Polonês	<i>Artibeus lituratus</i>	Yes	77,5	Female	Adult	Post-lactating
MCG 127	CEA Polonês	<i>Carollia perspicillata</i>	No	45,8	Male	Juvenile	Inactive
MCG 128	CEA Florestinha	<i>Artibeus lituratus</i>	Yes	74,9	Female	Adult	Inactive
MCG 129	CEA Florestinha	<i>Artibeus lituratus</i>	No	73,5	Male	Adult	Active
MCG 130	CEA Florestinha	<i>Artibeus lituratus</i>	No	67,2	Male	Adult	Active
MCG 131	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	36,3	Female	Juvenile	Inactive
MCG 132	CEA Polonês	<i>Eptesicus furinalis</i>	Yes	40,2	Female	Adult	Inactive
MCG 133	CEA Florestinha	<i>Artibeus lituratus</i>	Yes	73,8	Male	Juvenile	Inactive
MCG 134	CEA Florestinha	<i>Artibeus lituratus</i>	Yes	72,6	Male	Adult	Active
MCG 135	CEA Florestinha	<i>Artibeus lituratus</i>	No	74,3	Male	Adult	Active

Appendix 2. Results of optical spectrophotometry measurement (by Nanodrop device) in spleen and blood DNA samples collected from each bat specimen sampled in periurban region of Campo Grande, Mato Grosso do Sul, Brazil

Animal ID	Capture Site	Specie	Spleen DNA Samples			Blood DNA Samples		
			Concentration (ng/μL)	260/280 ratio	260/230 ratio	Concentration (ng/μL)	260/280 ratio	260/230 ratio
MCG 01	CEA Polonês	<i>Artibeus lituratus</i>	103,4	1,87	2,23	6,2	3,75	0,29
MCG 02	CEA Polonês	<i>Artibeus lituratus</i>	110,9	1,83	2,07	10,2	2,31	0,44
MCG 03	CEA Polonês	<i>Artibeus planirostris</i>	105,4	1,84	2,14	7,4	2,32	0,37
MCG 04	CEA Polonês	<i>Myotis nigricans</i>	32,8	1,81	2,2	N/A		
MCG 05	CEA Polonês	<i>Platyrrhinus lineatus</i>	226	1,87	2,42	8,2	1,95	1,46
MCG 06	CEA Polonês	<i>Artibeus planirostris</i>	74,1	1,85	2,2	14,8	2,06	0,54
MCG 07	CEA Polonês	<i>Artibeus planirostris</i>	45,3	1,87	2,03	13,9	1,91	0,65
MCG 08	CEA Polonês	<i>Artibeus lituratus</i>	99,2	1,87	2,31	5,1	2,76	0,23
MCG 09	CEA Polonês	<i>Glossophaga soricina</i>	106,8	1,86	2,34	3,8	4,95	0,18
MCG 10	CEA Polonês	<i>Artibeus lituratus</i>	100,2	1,8	1,95	9,7	2,29	0,41
MCG 11	CEA Polonês	<i>Artibeus planirostris</i>	51,4	1,88	2,21	11	2,3	0,51
MCG 12	CEA Polonês	<i>Artibeus planirostris</i>	70,4	1,83	2,01	6,3	1,95	0,22
MCG 13	CEA Polonês	<i>Artibeus planirostris</i>	146,6	1,86	2,28	9,9	2,3	0,37
MCG 14	CEA Polonês	<i>Artibeus planirostris</i>	80,5	1,87	2,39	7,3	2,48	0,34
MCG 15	CEA Polonês	<i>Platyrrhinus lineatus</i>	160,6	1,87	2,38	2,7	6,07	0,15
MCG 16	CEA Polonês	<i>Artibeus planirostris</i>	76,4	1,88	2,3	10,9	1,96	0,52
MCG 17	CEA Polonês	<i>Artibeus planirostris</i>	122	1,84	2,09	9,8	2,22	0,44
MCG 18	CEA Polonês	<i>Platyrrhinus lineatus</i>	53,8	1,86	2,04	8,3	2,25	0,33
MCG 19	CEA Polonês	<i>Artibeus planirostris</i>	55,2	1,88	2,07	7,7	2,11	0,33
MCG 20	CEA Polonês	<i>Platyrrhinus lineatus</i>	98,9	1,84	2,17	4,8	2,55	0,25
MCG 21	CEA Polonês	<i>Platyrrhinus lineatus</i>	75	1,88	2,5	9,9	2,27	0,43
MCG 22	CEA Polonês	<i>Platyrrhinus lineatus</i>	63	1,9	2,42	4,5	1,5	0,36
MCG 23	CEA Polonês	<i>Eptesicus furinalis</i>	20,3	2,01	1,59	6,2	2,5	0,29

MCG 24	CEA Polonês	<i>Platyrrhinus lineatus</i>	153,3	1,87	2,37	3,5	3,89	0,17
MCG 25	CEA Polonês	<i>Platyrrhinus lineatus</i>	99,7	1,95	2,16	9,9	2,13	0,47
MCG 26	CEA Polonês	<i>Artibeus planirostris</i>	51,7	1,95	1,88	15,1	1,82	0,5
MCG 27	CEA Polonês	<i>Artibeus planirostris</i>	171,9	1,85	2,15	6,4	1,91	0,3
MCG 28	CEA Polonês	<i>Artibeus lituratus</i>	155	1,75	1,78	13,8	1,98	0,59
MCG 29	CEA Polonês	<i>Artibeus planirostris</i>	117,2	1,93	2,31	11,2	2,04	0,48
MCG 30	CEA Polonês	<i>Platyrrhinus lineatus</i>	78,5	2	2,18	2,6	3,6	0,13
MCG 31	CEA Polonês	<i>Artibeus lituratus</i>	87,6	1,92	1,97	17	1,95	0,68
MCG 32	CEA Polonês	<i>Platyrrhinus lineatus</i>	82,2	1,89	2,35	3,9	2,54	-2
MCG 33	CEA Polonês	<i>Artibeus planirostris</i>	61,8	1,96	2,04	12,2	1,94	0,49
MCG 34	CEA Polonês	<i>Platyrrhinus lineatus</i>	51,8	1,93	2,02	8	1,93	0,34
MCG 35	CEA Polonês	<i>Artibeus planirostris</i>	99,7	1,94	2,25	23,3	1,89	0,87
MCG 36	CEA Florestinha	<i>Artibeus planirostris</i>	65,4	1,93	2,12	10	1,98	0,43
MCG 37	CEA Florestinha	<i>Carollia perspicillata</i>	75,3	1,93	2,17	6,9	2,43	31,04
MCG 38	CEA Florestinha	<i>Artibeus planirostris</i>	120,1	1,92	2,2	4	1,55	0,67
MCG 39	CEA Florestinha	<i>Artibeus planirostris</i>	44,3	1,92	1,79	7,1	2,21	0,32
MCG 40	CEA Florestinha	<i>Artibeus planirostris</i>	66	1,95	2,08	2,1	2,28	-4,9
MCG 41	CEA Florestinha	<i>Platyrrhinus lineatus</i>	33,6	1,85	1,24	3,6	1,98	0,5
MCG 42	CEA Florestinha	<i>Carollia perspicillata</i>	76,7	1,92	2,22	11,5	1,76	1,12
MCG 43	CEA Florestinha	<i>Artibeus planirostris</i>	80,6	1,9	1,94	12,1	2,17	0,53
MCG 44	CEA Polonês	<i>Artibeus planirostris</i>	104,1	1,9	2,06	8,9	1,96	0,38
MCG 45	CEA Polonês	<i>Myotis nigricans</i>	160,9	1,88	2,24	13,3	2,04	0,55
MCG 46	CEA Polonês	<i>Artibeus lituratus</i>	31	1,9	1,78	3,6	4,56	0,19
MCG 47	CEA Polonês	<i>Platyrrhinus lineatus</i>	112,9	1,91	2,31	2,5	4,75	0,14
MCG 48	CEA Polonês	<i>Artibeus planirostris</i>	163,5	1,9	2,26	10	2,05	0,44
MCG 49	CEA Polonês	<i>Artibeus lituratus</i>	51,2	1,89	1,92	3,8	2,62	0,19
MCG 50	CEA Polonês	<i>Artibeus lituratus</i>	67,8	1,89	2,1	7,8	1,97	0,34
MCG 51	CEA Polonês	<i>Artibeus planirostris</i>	63,3	1,89	2,18	11,2	1,85	1,38
MCG 52	CEA Polonês	<i>Molossus molossus</i>	7,2	2,69	1,19	4,4	2,05	0,2

MCG 53	CEA Polonês	<i>Artibeus lituratus</i>	57,8	1,91	2,07	8,2	2,25	0,37
MCG 54	CEA Florestinha	<i>Molossus molossus</i>	2,5	3,33	0,94	9,5	1,66	0,31
MCG 55	CEA Florestinha	<i>Phyllostomus discolor</i>	76,4	1,89	2,16	13,9	1,86	0,52
MCG 56	CEA Florestinha	<i>Phyllostomus discolor</i>	46,4	1,82	1,54	6,8	1,66	0,27
MCG 57	CEA Florestinha	<i>Phyllostomus discolor</i>	99,3	1,86	2,23	6,7	1,77	0,26
MCG 58	CEA Florestinha	<i>Artibeus lituratus</i>	103,9	1,84	2,03	2,4	2,89	0,14
MCG 59	CEA Florestinha	<i>Phyllostomus discolor</i>	77,2	1,86	2,02	11,3	2,23	0,49
MCG 60	CEA Florestinha	<i>Molossops temminckii</i>	11,7	2,15	1,6	6,8	1,88	0,3
MCG 61	CEA Florestinha	<i>Phyllostomus discolor</i>	70,2	1,85	1,69	13,4	2,07	0,56
MCG 62	CEA Florestinha	<i>Phyllostomus discolor</i>	69,3	1,82	2,03	8,3	1,8	0,35
MCG 63	CEA Florestinha	<i>Phyllostomus discolor</i>	101,4	1,86	2,16	11,6	1,82	0,42
MCG 64	CEA Florestinha	<i>Phyllostomus discolor</i>	51	1,88	1,87	15,8	1,91	0,64
MCG 65	CEA Florestinha	<i>Phyllostomus discolor</i>	82,5	1,83	2,07	9	2,11	0,43
MCG 66	CEA Florestinha	<i>Phyllostomus discolor</i>	61,1	1,85	2,02	14,7	1,83	0,58
MCG 67	CEA Florestinha	<i>Phyllostomus discolor</i>	53,7	1,85	2,09	10,8	2,04	0,47
MCG 68	CEA Florestinha	<i>Phyllostomus discolor</i>	63,6	1,85	2,09	22,2	1,9	0,85
MCG 69	CEA Florestinha	<i>Carollia perspicillata</i>	103,4	1,84	2,33	12,9	1,95	0,45
MCG 70	CEA Florestinha	<i>Phyllostomus discolor</i>	70,5	1,87	2,24	9,9	2,03	0,46
MCG 71	CEA Florestinha	<i>Phyllostomus discolor</i>	87,5	1,85	2,1	22,8	1,91	0,81
MCG 72	CEA Florestinha	<i>Phyllostomus discolor</i>	68,5	1,85	1,92	6,7	1,53	0,21
MCG 73	CEA Florestinha	<i>Molossus molossus</i>	2,8	29,43	0,48	4,5	1,99	0,19
MCG 74	CEA Polonês	<i>Eptesicus furinalis</i>	15,3	2,15	2,06	4,7	2,05	0,22
MCG 75	CEA Polonês	<i>Eptesicus furinalis</i>	6,2	2,47	1,58	3	2,42	0,14
MCG 76	CEA Polonês	<i>Artibeus lituratus</i>	90	1,83	1,84	21,1	2,03	0,78
MCG 77	CEA Polonês	<i>Platyrrhinus lineatus</i>	65,7	1,92	2,13	6,4	1,86	0,31
MCG 78	CEA Polonês	<i>Artibeus lituratus</i>	123,1	1,89	2,24	5,6	2,04	0,26
MCG 79	CEA Polonês	<i>Platyrrhinus lineatus</i>	49,6	1,92	1,68	11,4	1,94	0,49
MCG 80	CEA Polonês	<i>Platyrrhinus lineatus</i>	60,2	1,92	2,03	13,8	2,01	0,61
MCG 81	CEA Polonês	<i>Platyrrhinus lineatus</i>	68,2	1,98	1,96	6,5	1,92	0,3

MCG 82	CEA Polonês	<i>Eptesicus furinalis</i>	32,7	1,93	1,52	7,1	1,81	0,29
MCG 83	CEA Polonês	<i>Platyrrhinus lineatus</i>	82,4	1,94	2,05	15,7	1,85	0,59
MCG 84	CEA Polonês	<i>Artibeus lituratus</i>	44,4	1,93	1,52	8,8	1,99	0,42
MCG 85	CEA Polonês	<i>Artibeus lituratus</i>	52,8	1,94	1,98	6,7	2,04	0,3
MCG 86	CEA Polonês	<i>Platyrrhinus lineatus</i>	57,9	1,94	1,95	10,6	2,01	0,45
MCG 87	CEA Polonês	<i>Artibeus lituratus</i>	66,9	1,96	1,99	8	1,96	0,36
MCG 88	CEA Polonês	<i>Platyrrhinus lineatus</i>	64,6	1,93	1,79	15,9	1,99	0,64
MCG 89	CEA Polonês	<i>Platyrrhinus lineatus</i>	69,2	1,98	2,09	5,7	1,87	0,23
MCG 90	CEA Polonês	<i>Artibeus lituratus</i>	93,7	1,92	2,24	18,3	1,91	0,68
MCG 91	CEA Florestinha	<i>Eumops perotis</i>	3,6	2,47	1,39	4,3	2,58	0,21
MCG 92	CEA Florestinha	<i>Eumops perotis</i>	10,4	2,1	1,58	9,5	1,97	1,41
MCG 93	CEA Florestinha	<i>Eumops perotis</i>	18,3	2,1	2	13,9	2,16	0,57
MCG 94	CEA Florestinha	<i>Molossus molossus</i>	19,5	2,04	1,93	15,4	1,78	0,58
MCG 95	CEA Florestinha	<i>Eumops perotis</i>	1,4	3,69	2	6,4	1,78	0,58
MCG 96	CEA Polonês	<i>Artibeus lituratus</i>	87,9	1,9	2,31	5,1	1,55	0,17
MCG 97	CEA Polonês	<i>Artibeus lituratus</i>	67,4	1,89	2,29	N/A		
MCG 98	CEA Polonês	<i>Artibeus lituratus</i>	104	1,9	2,31	6,4	2,18	0,28
MCG 99	CEA Polonês	<i>Chiroderma villosus</i>	81	1,88	2,26	1,9	2,83	-1,13
MCG 100	CEA Polonês	<i>Artibeus lituratus</i>	125,9	1,89	2,33	17,9	1,85	0,74
MCG 101	CEA Polonês	<i>Platyrrhinus lineatus</i>	50,4	1,93	2,1	11,8	2,35	0,57
MCG 102	CEA Polonês	<i>Artibeus lituratus</i>	78,8	1,88	2,13	6,8	2,53	0,33
MCG 103	CEA Polonês	<i>Artibeus planirostris</i>	63,9	1,89	2,05	2,2	2,46	-2,97
MCG 104	CEA Polonês	<i>Artibeus lituratus</i>	123,8	1,87	2,11	2,9	2,71	0,15
MCG 105	CEA Polonês	<i>Artibeus lituratus</i>	119,7	1,88	2,35	2,2	8,57	0,11
MCG 106	CEA Polonês	<i>Eptesicus furinalis</i>	3,7	2,71	0,49	2,1	4,23	0,1
MCG 107	CEA Polonês	<i>Artibeus planirostris</i>	50,5	1,84	1,82	13	2,2	0,56
MCG 108	CEA Polonês	<i>Artibeus planirostris</i>	46,6	1,88	2,02	14,8	1,78	0,58
MCG 109	CEA Polonês	<i>Artibeus planirostris</i>	38,3	1,94	1,41	10	2,06	0,44
MCG 110	CEA Polonês	<i>Artibeus planirostris</i>	22,7	1,99	1,54	10,5	1,9	0,48

MCG 111	CEA Polonês	<i>Carollia perspicillata</i>	33,3	1,95	2,19	17,2	2,07	0,65
MCG 112	CEA Polonês	<i>Artibeus planirostris</i>	8,7	2,01	3,58	14,2	2,01	0,6
MCG 113	CEA Polonês	<i>Artibeus planirostris</i>	17,3	1,83	1,3	22,2	1,98	0,82
MCG 114	CEA Polonês	<i>Platyrrhinus lineatus</i>	26,2	1,92	2,06	18,8	2,04	0,76
MCG 115	CEA Florestinha	<i>Artibeus planirostris</i>	23,4	1,92	2,36	10,7	1,89	0,51
MCG 116	CEA Florestinha	<i>Artibeus lituratus</i>	27,6	1,85	2,33	7,8	2,48	0,37
MCG 117	CEA Florestinha	<i>Artibeus lituratus</i>	19,3	1,94	3,35	11,2	2,19	0,48
MCG 118	CEA Florestinha	<i>Artibeus lituratus</i>	24,3	1,88	1,84	14,2	2,19	0,59
MCG 119	CEA Florestinha	<i>Artibeus lituratus</i>	37,4	1,9	2,22	17,7	2,03	0,67
MCG 120	CEA Florestinha	<i>Artibeus planirostris</i>	27,7	1,93	2,44	7	2,25	0,28
MCG 121	CEA Florestinha	<i>Artibeus lituratus</i>	28,9	1,86	2,61	8,4	2,26	0,38
MCG 122	CEA Polonês	<i>Artibeus planirostris</i>	13	1,89	2,39	2,1	11,16	0,13
MCG 123	CEA Polonês	<i>Eptesicus furinalis</i>	4,4	1,73	4,07	1,6	-3,16	0,08
MCG 124	CEA Polonês	<i>Eptesicus furinalis</i>	10,6	2,36	1,6	1,2	-12,14	0,07
MCG 125	CEA Polonês	<i>Artibeus lituratus</i>	23,3	2,13	1,83	5	2,56	0,29
MCG 126	CEA Polonês	<i>Artibeus lituratus</i>	22,6	1,88	1,54	12,2	2,1	0,65
MCG 127	CEA Polonês	<i>Carollia perspicillata</i>	19,6	1,96	1,62	20,6	2,1	0,65
MCG 128	CEA Florestinha	<i>Artibeus lituratus</i>	41,7	1,95	2,12	17,3	2,12	0,71
MCG 129	CEA Florestinha	<i>Artibeus lituratus</i>	27,4	2	1,79	6,9	2,32	0,3
MCG 130	CEA Florestinha	<i>Artibeus lituratus</i>	28,8	1,92	2,56	9,4	2,32	0,43
MCG 131	CEA Polonês	<i>Eptesicus furinalis</i>	4,7	1,55	1,6	10,3	2,18	0,44
MCG 132	CEA Polonês	<i>Eptesicus furinalis</i>	7,9	2,15	1,98	11,3	2,28	0,48
MCG 133	CEA Florestinha	<i>Artibeus lituratus</i>	28,1	1,99	2,73	7	2,06	0,34
MCG 134	CEA Florestinha	<i>Artibeus lituratus</i>	27,9	1,96	2,48	10,5	2,15	0,47
MCG 135	CEA Florestinha	<i>Artibeus lituratus</i>	26,6	1,98	1,38	13,8	1,99	0,54

Appendix 3. Identification and result of optical spectrophotometry measurement (by Nanodrop device) in ectoparasites samples collected from bats sampled in periurban region of Campo Grande, Mato Grosso do Sul, Brazil

Bat Host ID	Bat Host Specie	Capture Site	Ectoparasite sample ID*	Ectoparasite Specie	Concentration (ng/μL)	260/280 ratio	260/230 ratio
MCG 01	<i>Artibeus lituratus</i>	CEA Polonês	SM 01	<i>Periglischrus iheringi</i>	4,1	2,17	2,68
MCG 02	<i>Artibeus lituratus</i>	CEA Polonês	BF 02	<i>Paratrichobius longicrus</i>	5,5	3,23	-1,98
MCG 02	<i>Artibeus lituratus</i>	CEA Polonês	SM 02	<i>Periglischrus iheringi</i>	3,2	-42,47	0,81
MCG 04	<i>Myotis nigricans</i>	CEA Polonês	MM 04	<i>Steatonyssus</i> spp.	0,3	-1,44	0,27
MCG 05	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 05	<i>Periglischrus iheringi</i>	1,7	2,25	0,64
MCG 05	<i>Platyrrhinus lineatus</i>	CEA Polonês	MM 05	<i>Steatonyssus</i> spp.	0,6	-2,65	0,64
MCG 07	<i>Artibeus planirostris</i>	CEA Polonês	BF 07F	<i>Megistopoda aranae</i>	1,2	-9,31	-0,3
MCG 07	<i>Artibeus planirostris</i>	CEA Polonês	BF 07M	<i>Megistopoda aranae</i>	0,5	-1,38	-0,11
MCG 08	<i>Artibeus lituratus</i>	CEA Polonês	BF 08	<i>Megistopoda aranae</i>	0,8	-3,72	-0,19
MCG 08	<i>Artibeus lituratus</i>	CEA Polonês	SM 08	<i>Periglischrus iheringi</i>	6,1	1,95	3,07
MCG 09	<i>Glossophaga soricina</i>	CEA Polonês	BF 09	<i>Trichobius parasiticus</i> (complex)	0,5	-0,87	-0,15
MCG 10	<i>Artibeus lituratus</i>	CEA Polonês	BF 10	<i>Megistopoda aranae</i>	0,3	3,06	-0,07
MCG 11	<i>Artibeus planirostris</i>	CEA Polonês	BF 11	<i>Megistopoda aranae</i>	1,8	11,69	-0,39
MCG 11	<i>Artibeus planirostris</i>	CEA Polonês	SM 11	<i>Periglischrus iheringi</i>	0,7	-1,25	0,71
MCG 12	<i>Artibeus planirostris</i>	CEA Polonês	BF 12	<i>Megistopoda aranae</i>	1	-27,5	-0,18
MCG 14	<i>Artibeus planirostris</i>	CEA Polonês	SM 14	<i>Periglischrus iheringi</i>	0,5	-0,42	-0,29
MCG 16	<i>Artibeus planirostris</i>	CEA Polonês	BF 16	<i>Megistopoda aranae</i>	0,5	-0,78	-0,1
MCG 17	<i>Artibeus planirostris</i>	CEA Polonês	BF 17	<i>Megistopoda aranae</i>	1,8	-12,66	-0,4
MCG 18	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 18	<i>Periglischrus iheringi</i>	1,1	0,84	-16,67
MCG 19	<i>Artibeus planirostris</i>	CEA Polonês	BF 19F	<i>Megistopoda aranae</i>	1,6	7,58	-0,34
MCG 19	<i>Artibeus planirostris</i>	CEA Polonês	BF 19M	<i>Megistopoda aranae</i>	1	7,73	-0,2
MCG 20	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 20	<i>Trichobius dugesii</i> (complex)	2,8	2,19	-0,8
MCG 21	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 21	<i>Periglischrus iheringi</i>	2,2	8,19	-14,69
MCG 23	<i>Eptesicus furinalis</i>	CEA Polonês	BF 23	<i>Trichobius dugesii</i> (complex)	2,7	2,18	-0,56

MCG 23	<i>Eptesicus furinalis</i>	CEA Polonês	MM 23	<i>Steatonyssus</i> spp.	0,5	1,46	1,08
MCG 25	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 25	<i>Trichobius dugesii</i> (complex)	3,4	3,68	-0,8
MCG 25	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 25	<i>Periglischrus iheringi</i>	1,2	1,98	-3,73
MCG 26	<i>Artibeus planirostris</i>	CEA Polonês	SM 26	<i>Periglischrus iheringi</i>	0,6	-0,81	-1,27
MCG 27	<i>Artibeus planirostris</i>	CEA Polonês	BF 27	<i>Megistopoda aranae</i>	2,7	3,36	-1,35
MCG 27	<i>Artibeus planirostris</i>	CEA Polonês	SM 27	<i>Periglischrus iheringi</i>	1,7	2,4	3,42
MCG 28	<i>Artibeus lituratus</i>	CEA Polonês	SM 28	<i>Periglischrus iheringi</i>	0,9	-1,98	90,81
MCG 30	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 30	<i>Trichobius dugesii</i> (complex)	5,9	3,68	-2,67
MCG 32	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 32	<i>Periglischrus iheringi</i>	3,1	3,21	2,2
MCG 33	<i>Artibeus planirostris</i>	CEA Polonês	SM 33	<i>Periglischrus iheringi</i>	1,3	2,71	3,46
MCG 34	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 34	<i>Trichobius parasiticus</i> (complex)	1,3	-7,8	-0,27
MCG 34	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 34	<i>Periglischrus iheringi</i>	6,5	2,55	3,26
MCG 35	<i>Artibeus planirostris</i>	CEA Polonês	SM 35	<i>Periglischrus iheringi</i>	12,8	2,13	0,22
MCG 37	<i>Carollia perspicillata</i>	CEA Florestinha	BF 37	<i>Trichobius joblingi</i>	0,1	-0,17	-0,02
MCG 38	<i>Artibeus planirostris</i>	CEA Florestinha	BF 38	<i>Megistopoda aranae</i>	2,8	7,32	-7,1
MCG 39	<i>Artibeus planirostris</i>	CEA Florestinha	BF 39	<i>Megistopoda aranae</i>	1,5	13,8	-0,32
MCG 40	<i>Artibeus planirostris</i>	CEA Florestinha	BF 40	<i>Megistopoda aranae</i>	1	-33,72	-0,19
MCG 41	<i>Platyrrhinus lineatus</i>	CEA Florestinha	SM 41	<i>Periglischrus iheringi</i>	9,5	2,12	2,53
MCG 42	<i>Carollia perspicillata</i>	CEA Florestinha	BF 42 M1	<i>Trichobius dugesii</i> (complex)	-0,3	0,28	0,05
MCG 42	<i>Carollia perspicillata</i>	CEA Florestinha	BF 42 M2	<i>Trichobius dugesii</i> (complex)	-0,8	0,65	0,13
MCG 43	<i>Artibeus planirostris</i>	CEA Florestinha	BF 43M	<i>Megistopoda aranae</i>	1	-6,14	-0,21
MCG 43	<i>Artibeus planirostris</i>	CEA Florestinha	BF 43F	<i>Megistopoda aranae</i>	1,6	-7,49	-0,35
MCG 44	<i>Artibeus planirostris</i>	CEA Polonês	BF 44	<i>Megistopoda aranae</i>	0,4	-1,11	-0,07
MCG 55	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 55	<i>Trichobius costalimai</i>	-0,9	0,62	0,14
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 56F (s)	<i>Strebla hertigi</i>	0,2	-0,24	-0,03
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 56F1 (t)	<i>Trichobius costalimai</i>	-0,5	0,47	0,09
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 56F2 (t)	<i>Trichobius costalimai</i>	-0,8	0,53	0,11
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 56M1 (t)	<i>Trichobius costalimai</i>	0,8	0,71	0,15
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 56M2 (t)	<i>Trichobius costalimai</i>	-0,7	0,65	0,16

MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 56.1	<i>Periglischrus torrealbai</i>	1,9	2,51	0,09
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 56.2	<i>Periglischrus acutisternus</i>	2,4	3,33	-0,58
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	MM 56	<i>Steatonyssus</i> spp.	0,4	-1,81	0,12
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	T 56.1	<i>Ornithodoros hasei</i>	5,3	2,97	1,94
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	T 56.2	<i>Ornithodoros hasei</i>	2,8	7,96	2,53
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	T 56.3	<i>Ornithodoros hasei</i>	1,8	-8,82	0,06
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	T 56.4	<i>Ornithodoros hasei</i>	6,5	1,73	0,39
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	T 56.5	<i>Ornithodoros hasei</i>	-0,5	0,33	0,78
MCG 56	<i>Phyllostomus discolor</i>	CEA Florestinha	T 56.6	<i>Ornithodoros hasei</i>	-0,2	0,14	-0,18
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 57F1	<i>Trichobius costalimai</i>	0,3	-0,41	-0,07
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 57F2	<i>Trichobius costalimai</i>	1,6	5,71	-0,14
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 57F3	<i>Trichobius costalimai</i>	0,2	-0,23	-0,02
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 57F4	<i>Trichobius costalimai</i>	0,1	-0,06	-0,01
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 57M	<i>Trichobius costalimai</i>	-0,5	0,33	0,06
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 57	<i>Periglischrus torrealbai</i>	-0,4	0,78	0,2
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	T 57.1	<i>Ornithodoros hasei</i>	1,5	-2,81	-11,19
MCG 57	<i>Phyllostomus discolor</i>	CEA Florestinha	T 57.2	<i>Ornithodoros hasei</i>	0,7	-0,78	-1,59
MCG 58	<i>Artibeus lituratus</i>	CEA Florestinha	SM 58	<i>Periglischrus iheringi</i>	2,7	3,04	1,42
MCG 59	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 59	<i>Trichobius costalimai</i>	-0,3	0,37	0,05
MCG 59	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 59	<i>Periglischrus</i> spp.	1,4	-34,85	8,26
MCG 61	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 61	<i>Periglischrus torrealbai</i>	3,1	2,91	1,88
MCG 61	<i>Phyllostomus discolor</i>	CEA Florestinha	T 61	<i>Ornithodoros hasei</i>	1	-1,94	-0,57
MCG 62	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 62	<i>Trichobius costalimai</i>	0,3	-0,3	-0,04
MCG 62	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 62.1	<i>Periglischrus</i> spp.	1,5	7,25	-0,27
MCG 62	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 62.2	<i>Periglischrus</i> spp.	-0,6	0,55	0,07
MCG 62	<i>Phyllostomus discolor</i>	CEA Florestinha	T 62.1	<i>Ornithodoros hasei</i>	0,6	-0,71	-0,3
MCG 62	<i>Phyllostomus discolor</i>	CEA Florestinha	T 62.2	<i>Ornithodoros hasei</i>	-0,4	0,28	0,41
MCG 60	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 63M1	<i>Trichobius costalimai</i>	-0,3	0,23	0,05
MCG 61	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 63M2	<i>Trichobius costalimai</i>	-0,9	0,63	0,13

MCG 62	<i>Phyllostomus discolor</i>	CEA Florestinha	MM 63	<i>Steatonyssus</i> spp.	0,2	-0,49	0,17
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.1	<i>Ornithodoros hasei</i>	0,4	-0,44	-0,14
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.2	<i>Ornithodoros hasei</i>	0,6	-0,72	-0,75
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.3	<i>Ornithodoros hasei</i>	2,8	3,82	-2,56
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.4	<i>Ornithodoros hasei</i>	-1,4	0,67	0,3
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.5	<i>Ornithodoros hasei</i>	0,7	-0,92	-0,28
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.6	<i>Ornithodoros hasei</i>	1,2	-2,46	-1,01
MCG 63	<i>Phyllostomus discolor</i>	CEA Florestinha	T 63.7	<i>Ornithodoros hasei</i>	0,3	-0,25	-0,15
MCG 64	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 64 F1	<i>Trichobius costalimai</i>	-0,2	0,15	0,03
MCG 64	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 64 F2	<i>Trichobius costalimai</i>	-0,7	0,5	0,12
MCG 64	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 64 F3	<i>Trichobius costalimai</i>	-1,4	0,9	0,2
MCG 64	<i>Phyllostomus discolor</i>	CEA Florestinha	MM 64	<i>Steatonyssus</i> sp.	-0,2	0,23	-0,35
MCG 65	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 65F1	<i>Trichobius costalimai</i>	-0,1	0,1	0,01
MCG 65	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 65F2	<i>Trichobius costalimai</i>	0	0,02	0
MCG 65	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 65F3	<i>Trichobius costalimai</i>	0,1	-0,16	-0,02
MCG 65	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 65F4	<i>Trichobius costalimai</i>	0,2	-0,28	-0,04
MCG 65	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 65	<i>Periglischrus acutisternus</i>	4,2	7	1,44
MCG 66	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 66M1	<i>Trichobius costalimai</i>	-0,4	0,46	0,06
MCG 66	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 66M2	<i>Trichobius costalimai</i>	-1	0,58	0,2
MCG 66	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 66	<i>Periglischrus acutisternus</i>	3,6	3,44	11,69
MCG 67	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 67	<i>Periglischrus torrealbai</i>	1,1	-10,27	1,8
MCG 68	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 68M	<i>Trichobius costalimai</i>	-1	0,45	0,23
MCG 68	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 68F1	<i>Trichobius costalimai</i>	1,3	-329	-0,22
MCG 68	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 68F2	<i>Trichobius costalimai</i>	-1,2	0,66	0,18
MCG 68	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 68	<i>Periglischrus torrealbai</i>	1,4	-8,77	-14,89
MCG 69	<i>Carollia perspicillata</i>	CEA Florestinha	BF 69	<i>Trichobius joblingi</i>	3,4	2,47	0,48
MCG 70	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 70 F1	<i>Strebla hertigi</i>	1,9	4,33	-0,46
MCG 70	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 70 F2	<i>Strebla hertigi</i>	1,1	6,11	-0,14
MCG 70	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 70n	<i>Periglischrus</i> spp.	-0,9	0,64	0,11

MCG 70	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 70f1	<i>Periglischrus</i> spp.	0,2	-0,25	-0,04
MCG 70	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 70f2	<i>Periglischrus</i> spp.	1,5	56,52	-1,44
MCG 71	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 71	<i>Periglischrus</i> spp.	1,4	-15,41	0,89
MCG 71	<i>Phyllostomus discolor</i>	CEA Florestinha	MM 71	<i>Steatonyssus</i> spp.	1,2	-16,9	0,96
MCG 72	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 72F	<i>Trichobius costalimai</i>	-1,2	0,66	0,2
MCG 72	<i>Phyllostomus discolor</i>	CEA Florestinha	BF 72M	<i>Trichobius costalimai</i>	-1,2	0,76	0,15
MCG 72	<i>Phyllostomus discolor</i>	CEA Florestinha	SM 72	<i>Periglischrus acutisternus</i>	5,3	2,38	3,17
MCG 72	<i>Phyllostomus discolor</i>	CEA Florestinha	T 72	<i>Ornithodoros hasei</i>	-2	0,97	0,46
MCG 74	<i>Eptesicus furinalis</i>	CEA Polonês	MM 74	<i>Steatonyssus</i> spp.	2,1	1,72	0,56
MCG 75	<i>Eptesicus furinalis</i>	CEA Polonês	MM75	<i>Steatonyssus</i> spp.	1,2	4,52	0,69
MCG 78	<i>Artibeus lituratus</i>	CEA Polonês	SM 78	<i>Periglischrus iheringi</i>	2	2,12	1,92
MCG 79	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 79	<i>Trichobius dugesii</i> (complex)	0,6	11,07	-0,09
MCG 82	<i>Eptesicus furinalis</i>	CEA Polonês	MM 82	<i>Steatonyssus</i> spp.	0,5	-2,94	0,33
MCG 83	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 83	<i>Trichobius dugesii</i> (complex)	0,6	-1,06	-0,1
MCG 83	<i>Platyrrhinus lineatus</i>	CEA Polonês	SM 83	<i>Periglischrus iheringi</i>	1,2	1,02	1,03
MCG 84	<i>Artibeus lituratus</i>	CEA Polonês	SM 84	<i>Periglischrus iheringi</i>	2,5	5,51	-8,58
MCG 87	<i>Artibeus lituratus</i>	CEA Polonês	SM 87	<i>Periglischrus iheringi</i>	5,2	2,26	5,43
MCG 89	<i>Platyrrhinus lineatus</i>	CEA Polonês	BF 89	<i>Trichobius dugesii</i> (complex)	-0,1	0,05	0,01
MCG 90	<i>Artibeus lituratus</i>	CEA Polonês	SM 90	<i>Periglischrus iheringi</i>	2,9	4,36	2,94
MCG 90	<i>Artibeus lituratus</i>	CEA Polonês	MM 90	<i>Steatonyssus</i> spp.	0,8	1,59	0,75
MCG 91	<i>Eumops perotis</i>	CEA Florestinha	MM 91	<i>Steatonyssus</i> spp.	0,1	-0,31	-2,15
MCG 92	<i>Eumops perotis</i>	CEA Florestinha	MM 92	<i>Steatonyssus</i> spp.	1,3	8,76	1,67
MCG 93	<i>Eumops perotis</i>	CEA Florestinha	MM 93	<i>Steatonyssus</i> spp.	5	1,93	1,47
MCG 94	<i>Molossus molossus</i>	CEA Florestinha	MM 94	<i>Steatonyssus</i> spp.	1	1,95	
MCG 95	<i>Eumops perotis</i>	CEA Florestinha	MM 95	<i>Steatonyssus</i> spp.	17	1,98	2,48
MCG 97	<i>Artibeus lituratus</i>	CEA Polonês	SM 97	<i>Periglischrus iheringi</i>	1,6	-4,55	-4,7
MCG 102	<i>Artibeus lituratus</i>	CEA Polonês	SM 102	<i>Periglischrus iheringi</i>	11,3	2,24	3,14
MCG 104	<i>Artibeus lituratus</i>	CEA Polonês	SM 104	<i>Periglischrus iheringi</i>	19	2,14	2,77
MCG 106	<i>Eptesicus furinalis</i>	CEA Polonês	MM 106	<i>Steatonyssus</i> spp.	0	-0,05	-0,01

MCG 120	<i>Artibeus planirostris</i>	CEA Florestinha	BF 120 (m)	<i>Megistopoda aranae</i>	-0,8	0,71	0,15
MCG 122	<i>Artibeus planirostris</i>	CEA Polonês	MM 122	<i>Steatonyssus</i> spp.	-0,7	0,9	0,14
MCG 123	<i>Eptesicus furinalis</i>	CEA Polonês	MM 123	<i>Steatonyssus</i> spp.	-0,3	0,4	0,07
MCG 124	<i>Eptesicus furinalis</i>	CEA Polonês	SM 124	<i>Periglischrus</i> spp.	2,3	3,59	-3,99
MCG 126	<i>Artibeus lituratus</i>	CEA Polonês	BF 126	<i>Trichobius dugesii</i> (complex)	-0,4	0,4	0,07
MCG 128	<i>Artibeus lituratus</i>	CEA Florestinha	SM 128	<i>Periglischrus iheringi</i>	8,5	2,59	4,31
MCG 131	<i>Eptesicus furinalis</i>	CEA Polonês	MM 131	<i>Steatonyssus</i> spp.	1	8,27	-0,36
MCG 132	<i>Eptesicus furinalis</i>	CEA Polonês	MM 132	<i>Steatonyssus</i> spp.	-0,3	0,36	0,05
MCG 133	<i>Artibeus lituratus</i>	CEA Florestinha	SM 133	<i>Periglischrus iheringi</i>	27,7	1,95	2,58
MCG 134	<i>Artibeus lituratus</i>	CEA Florestinha	SM 134	<i>Periglischrus iheringi</i>	25,4	1,91	2,37

* SM = Spinturnicidae mite; MM = Macronyssidae mite; BF = Bat fly; T = Tick.