UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

DETECÇÃO E CARACTERIZAÇÃO MOLECULAR DE AGENTES ANAPLASMATACEAE EM XENARTHRA NO BRASIL

Ana Cláudia Calchi
Médica Veterinária

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Coorientadora: Profa Dra. Rosangela Zacarias Machado

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TÍTULO DA DISSERTAÇÃO: DETECÇÃO E CARACTERIZAÇÃO DE AGENTES ANAPLASMATACEAE EM XENARTHRA NO BRASIL

AUTORA: ANA CLÁUDIA CALCHI
ORIENTADOR: MARCOS ROGÉRIO ANDRÉ
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Jaboticabal, 08 de julho de 2020

DADOS CURRICULARES DA AUTORA

Ana Cláudia Calchi – nascida na cidade de Pirassununga, São Paulo, em 13 de março de 1994. Filha de Ana Lúcia de Mattos Calchi e Aldemir Aparecido Calchi. Graduada em Medicina Veterinária pela Universidade Estadual "Júlio de Mesquita Filho" – Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, Jaboticabal, São Paulo, no ano de 2017. Durante a graduação, foi bolsista de Iniciação Científica da Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) de novembro de 2014 a agosto de 2015, atuando na área de Produção Animal, sob orientação da Profa. Dra. Silvana Martinez Baraldi Artoni, com o trabalho intitulado "Sulfato de Glucosamina e de Condroitina sobre o desempenho e qualidade do tecido ósseo e articular em frangos de corte, de 1 a 10 dias de idade, submetido às dietas deficientes de Cálcio e Fósforo" (Processo número 2014/15505-3). Foi bolsista pela mesma instituição de financiamento de dezembro de 2016 a julho de 2017, atuando na área de Parasitologia Veterinária, sob orientação do Prof. Dr. Marcos Rogério André, com o trabalho intitulado "Detecção Molecular de Agentes Anaplasmatacea em Canídeos Selvagens no Pantanal Sul Matogrossense" (Processo número 2015/22387-0). Foi monitora voluntária da disciplina de Parasitologia Veterinária II, ministrada pelo Prof. Dr. Marcos Rogério André, de agosto a dezembro de 2016. Em agosto de 2018, ingressou no curso de Mestrado do Programa de Pós-graduação em Medicina Veterinária (Medicina Veterinária Preventiva) na FCAV/UNESP, sob orientação do Prof. Dr. Marcos Rogério André, com bolsa de estudo da FAPESP (Processo número 2018/13838-6).

EPÍGRAFE

“Tenho a impressão de ter sido uma criança brincando à beira-mar, divertindo-me em descobrir uma pedrinha mais lisa ou uma concha mais bonita que as outras, enquanto o imenso oceano da verdade continua misterioso diante aos meus olhos.” (Isaac Newton)

DEDICATÓRIA

Aos meus pais, Ana Lúcia de Mattos Calchi e Aldemir Aparecido Calchi, à minha tia Nilza Maria de Mattos e ao meu avô Luiz de Mattos

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Detecção e caracterização molecular de agentes Anaplasmataceae em Xenarthra no Brasil"**, protocolo nº 014125/18, 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 10 de outubro de 2018.

Vigência do Projeto	06/11/2018 a 06/12/2020
Espécie / Linhagem	Superordem Xenarthra
Nº de animais	110
Peso / Idade	Variável
Sexo	Variável
Origem	Animais atropelados em rodovias do Mato Grosso do Sul e animais que chegarem para necropsia no Serviço de Patologia de Animais Selvagens da Faculdade de Ciências Agrárias e Veterinárias, UNESP, campus de Jaboticabal

Jaboticabal, 10 de outubro de 2018.

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DETECÇÃO E CARACTERIZAÇÃO MOLECULAR DE AGENTES ANAPLASMATACEAE EM XENARTHRA NO BRASIL

RESUMO - Os agentes Anaplasmataceae são α-proteobactérias Gram-negativos intracelulares obrigatórias que são transmitidos principalmente por vetores artrópodes. Embora os mamíferos da Superordem Xenarthra (preguiças, tamanduás e tatus) tenham sido implicados como reservatórios de vários agentes zoonóticos, apenas poucos estudos procuraram detectar agentes Anaplasmataceae neste grupo de mamíferos. Este estudo teve por objetivo investigar a ocorrência e diversidade genética de *Anaplasma* spp. e *Ehrlichia* spp. em amostras de sangue e baço de Xenarthra de vida livre de quatro estados no Brasil (São Paulo, Mato Grosso do Sul, Rondônia, e Pará). Foram efetuados ensaios de triagem por meio de nPCR e cPCR para detectar os genes *rrs* e *dsb* de *Anaplasma* spp. e *Ehrlichia* spp., respectivamente. Os ensaios foram positivos em 27,57% (91/330) para *Anaplasma* spp. e 24,54% (81/330) para *Ehrlichia* spp. Das 91 amostras positivas para *Anaplasma* spp., 56,04% foram positivas num ensaio de PCR convencional visando a região intergênica 23S-5S. Análises filogenéticas e de distância baseadas no gene *rrs* alocaram as sequências de *Anaplasma* de preguiças (*Bradypus variegatus*, *Bradypus* sp., *Choloepus* spp. e *C. didactylus*) capturadas nos estados de Rondônia e Pará em um único clado, que estava estreitamente relacionado com o clado de *A. marginale*, *A. ovis*, e *A. capra*. As sequências detectadas nos tamanduás-mirins (*Tamandua tetradactyla*) de São Paulo foram alocadas em um clado filogeneticamente relacionado com as sequências de *Anaplasma* spp. detectadas em *Nasua nasua*, *Leopardus pardalis*, e *Cerdocyon thous* no Brasil. Estas sequências foram posicionadas perto das sequências de *A. odocoilei*. A análise do genótipo corroborou as descobertas anteriores e demonstrou a circulação de dois genótipos distintos de *Anaplasma* em animais do norte e sudeste do Brasil. O primeiro genótipo era novo. O segundo foi previamente detectado em *N. nasua*, no estado de Mato Grosso do Sul. As análises da região intergênica também demonstraram dois genótipos distintos de *Anaplasma*. As sequências detectadas em Xenarthra dos estados do Pará e Rondônia estavam relacionadas com as de *A. marginale*, *A. ovis*, e *A. capra*. Enquanto que as sequências de *Anaplasma* spp. detectadas em Xenarthra de São Paulo foram alocadas em um clado irmão ao de *A. phagocytophilum*. As análises baseadas no gene *dsb* agruparam as sequências de *Ehrlichia* spp. de São Paulo, Mato Grosso do Sul, e Pará com sequências de *E. canis* e as de Rondônia e Pará com *E. minasensis*. Os dados indicam a ocorrência de *E. canis* e *E. minasensis* e duas possíveis novas espécies de *Anaplasma* spp. em mamíferos de vida livre da Superordem Xenarthra no Brasil.

Palavras-chave: *Anaplasma* spp., diversidade genética*, Ehrlichia* spp., preguiças, tamanduás, tatus

MOLECULAR DETECTION AND CHARACTERIZATION OF ANAPLASMATACEAE AGENTS IN XENARTHRA FROM BRAZIL

ABSTRACT – Anaplasmataceae agents are obligatory intracellular Gram-negative α-proteobacteria that are transmitted mostly by arthropod vectors. Although mammals of the Superorder Xenarthra (sloths, anteaters, and armadillos) have been implicated as reservoirs for several zoonotic agents, only few studies have sought to detect Anaplasmataceae agents in this group of mammals. This study aimed to investigate the occurrence and genetic diversity of *Anaplasma* spp. and *Ehrlichia* spp. in blood and spleen samples of free-living Xenarthra from four different states in Brazil (São Paulo, Mato Grosso do Sul, Rondônia, and Pará). Nested and conventional PCR screening assays were performed to detect the *rrs* and *dsb* genes of *Anaplasma* spp. and *Ehrlichia* spp., respectively. The assays were positive in 27.57% (91/330) of the *Anaplasma* spp. and 24.54% (81/330) of the *Ehrlichia* spp. Of the 91 positive *Anaplasma* spp. samples, 56.04% were positive in a conventional PCR assay targeting the 23S-5S intergenic region. Phylogenetic and distance analyses based on the *rrs* gene allocated *Anaplasma* sequences from sloths captured in Rondônia and Pará states in a single clade, which was closely related to the *A. marginale*, *A. ovis,* and *A. capra* clades. The sequences detected in southern anteaters from São Paulo were allocated in a clade closely related to sequences of *Anaplasma* spp. detected in *Nasua nasua*, *Leopardus pardalis,* and *Cerdocyon thous* in Brazil. These sequences were positioned close to *A. odocoilei* sequences. Genotype analysis corroborated previous findings and demonstrated the circulation of two distinct *Anaplasma* genotypes in animals from north and southeast Brazil. The first genotype was new. The second was previously detected in *N. nasua* in Mato Grosso do Sul state. The intergenic region analyses also demonstrated two distinct genotypes of *Anaplasma*. The sequences detected in Xenarthra from Pará and Rondônia states were closely related to those in *A. marginale*, *A. ovis,* and *A. capra*. *Anaplasma* spp. sequences detected in Xenarthra from São Paulo and were allocated close to those in *A. phagocytophilum*. The analyses based on the *dsb* gene grouped the *Ehrlichia* spp. sequences with sequences of *E. canis* (São Paulo, Mato Grosso do Sul, and Pará) and *E. minasensis* (Rondônia and Pará). The data indicate the occurrence of *E. canis* and *E. minasensis* and two possible new *Candidatus* species of *Anaplasma* spp. in free-living mammals of the Superorder Xenarthra in Brazil.

Keys words: *Anaplasma* spp., genetic diversity, *Ehrlichia* spp., sloths, anteaters, armadillos

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CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

Doenças transmitidas por vetores artrópodes representam 22,8% das doenças emergentes atuais (Zanella, 2016). As mudanças climáticas, ambientais e ecológicas, favorecem a ruptura de ciclos de microrganismos naturalmente estabelecidos e a consequente emergência de enfermidades, bem como a expansão da distribuição de vetores e maior aproximação da fauna selvagem com animais domésticos e seres humanos. Como consequência, verifica-se uma maior interação entre reservatórios, vetores e hospedeiros susceptíveis, acarretando em graves problemas à saúde pública e animal (Shaw et al., 2001; André, 2018).

A família Anaplasmataceae (gêneros *Ehrlichia*, *Anaplasma*, *Neorickettsia, Wolbachia* e *'Candidatus* Neoehrlichia') é composta por bactérias Gram-negativas intracelulares obrigatórias que formam agregados (mórulas) em vacúolos intracitoplasmáticos de neutrófilos, monócitos, macrófagos, plaquetas e células endoteliais de mamíferos, a depender da espécie. Tais agentes são transmitidos, em sua maioria, por artrópodes, principalmente carrapatos, e podem causar doenças em humanos e animais (Dumler et al., 2001; Inokuma et al., 2001; Aguiar et al., 2014).

Esses agentes são responsáveis por três zoonoses de grande importância, sendo: erliquiose monocítica humana causada por *E. chaffeensis*, erliquiose granulocítica por *E. ewingii* e anaplasmose granulocítica humana por *A. phagocytophilum* (Karbowiak et al., 2016). Ademais, outras espécies já foram detectadas em humanos com sintomatologia, a saber: *E. canis*, *E. ruminantium*, *E. muris*, *A. platys, 'Candidatus* Neoehrlichia mikurensis' (Anderson et al, 1991; Perez, 1996; Buller et al., 1999; Louw et al., 2005; Bouza-Mora et al., 2007; Wellinder-Olsson, et al., 2010; Yabsley, 2010; Pritt et al., 2011; Maggi et al., 2013; Alvarado et al., 2014; Lynn et al., 2017). Os principais sintomas descritos são febre, dores de cabeça, mialgia, erupções cutâneas, dor abdominal, vômito, diarreia, sinais neurológicos e falência de órgãos em casos mais graves. Os achados laboratoriais consistem em

trombocitopenia, leucopenia, neutrofilia e elevação nos níveis de transaminases hepáticas (Doudier et al., 2010; Ismail et al., 2010).

No Brasil, vários genótipos de *Anaplasma* spp. e *Ehrlichia* spp. vêm sendo detectados em diferentes espécies de animais selvagens como cervídeos (Machado et al., 2006; Picoloto et al., 2010; Sacchi et al, 2012; Silveira et al., 2012, 2013, 2014; Mongruel et al., 2016), canídeos (André et al., 2012; Almeida et al., 2013; de Sousa et al., 2017); felinos (André et al., 2010; 2012; Widmer et al., 2011), quatis (de Sousa et al., 2017); roedores (Wolf et al., 2016; Benevenute et al., 2017; de Sousa et al., 2017; Braga et al., 2018), caititus e queixadas (Soares et al., 2017), gambás (Lopes et al., 2018) e aves (Machado et al., 2012; Werther et al., 2017).

A Superordem Xenarthra compreende um grupo monofilético de mamíferos originários da América do Sul. É composta por duas ordens, Cingulata e Pilosa, cuja sinapomorfia compreende a presença de articulações adicionais entre as vértebras lombares (Glass, 1985). A ordem Cingulata, composta pela família Dasypodidae, representada pelos tatus, é caracterizada pela presença de osteodermos. A ordem Pilosa, por sua vez, engloba animais com pelagem densa e ausência ou pouco desenvolvimento de dentes, abrangendo quatro famílias: Bradypodidae (preguiças-de-três-dedos), Megalonychidae (preguiças-de-dois-dedos), Cyclopedidae (tamanduaí) e Myrmecophagidae (tamanduás) (Gardner, 2005). Tais mamíferos estão distribuídos do Centro sul da América do Norte até o sul da América do Sul, sendo encontrados em todos os biomas no Brasil (Reis, 2006).

É sabido que mamíferos da Superordem Xenarthra, principalmente tatus, são reservatórios de inúmeros agentes zoonóticos, tais como *Trypanosoma cruzi*, *Leishmania* spp., *Toxoplasma gondii* e *Mycobacterum leprae* (Kluyber, 2016). Porém, raros são os estudos que buscaram investigar a ocorrência de agentes transmitidos por carrapatos neste grupo de animais. Até o momento, *Anaplasma marginale* foi detectado em um tamanduá-bandeira (*Myrmecophaga tridactyla*) na Argentina (Guillemi et al., 2016) e um novo genótipo de *Ehrlichia* sp. relacionado à *E. ruminantium* foi encontrado em uma preguiça preguiça-de-três-dedos (*Bradypus tridactylus*) no estado do Pará (Soares et al., 2017). Em vista disso, o presente estudo teve como objetivo investigar a ocorrência e a diversidade genética de *Anaplasma*

spp. e *Ehrlichia* spp. em mamíferos da Superodem Xenarthra amostrados em quatro diferentes estados brasileiros.

2. OBJETIVOS

2.1. Geral

O presente estudo teve como objetivo investigar a ocorrência molecular e a diversidade genética de agentes dos gêneros *Ehrlichia* spp. e *Anaplasma* spp. em preguiças, tamanduás e tatus de vida livre capturados nos estados de Rondônia, Pará, Mato Grosso do Sul e São Paulo.

2.2. Específicos

- Investigar a presença e quantificar o DNA de bactérias da família Anaplasmataceae (*Ehrlichia* spp. e *Anaplasma* spp.) em amostras de sangue de preguiças e sangue/tecidos de tamanduás e tatus de vida livre nos estados de Rondônia, Pará, Mato Grosso do Sul e São Paulo;
- Investigar a presença de DNA das espécies *E. canis, E. chaffeensis e A. phagocytophilum,* por meio de ensaios de PCR em tempo real quantitativa específicos baseados nos genes *dsb, vlpt e msp-2*, respectivamente;
- Caracterizar molecularmente as amostras positivas com base em ensaios de PCR convencional baseados nos genes 16S rRNA, *groEL, gltA, sodB* e *omp-1* e da construção de árvores filogenéticas;
- Analisar o número e a diversidade de genótipos de *Ehrlichia* spp. e *Anaplasma* spp. detectados em amostras biológicas de mamíferos da Superordem Xenarthra.

3. REVISÃO DE LITERATURA

3.1. Superordem Xenarthra

A Superordem Xenarthra compreende espécies de preguiças, tamanduás e tatus, com origem no Hemisfério Sul (Gondwana) há cerca de 100 milhões de anos atrás (m.a.) (Delsuc et al., 2004; Murphy et al., 2007; Moraes-Barros e Arteaga, 2015). Apesar de fósseis já terem sido encontrados no Eoceno da Antártica (Vizcaíno e Scillato-Yané, 1995), Eoceno da Europa (Storch e Richter, 1992), Paleoceno da Ásia (Storch e Richter, 1992) e Paleoceno da Austrália (Simpson, 1980), esse grupo de animais se estabeleceu na América do Sul.

Durante o período Terciário, os mamíferos Xenarthra que habitavam a América do Sul passaram por um período considerável de radiação evolutiva (Simpson, 1980). Após a formação do Istmo do Panamá, um corredor terrestre que uniu a América do Norte com a do Sul por volta de 3 m.a., esses animais puderam se dispersar para o Norte do continente. Esse período ficou conhecido como Grande Intercâmbio Biótico Americano (GABI), que possibilitou a migração de mamíferos Xenarthra e Marsupilia para a América do Norte, assim como de animais das famílias Cervidae, Felidae, Tapiridae, Ursidae e Gomphotheriidae para a América do Sul (Webb, 1985; Tonni et al., 1992; Carlini et al., 2008).

Mamíferos Xenarthra conseguiram se estabelecer de forma eficaz na América do Norte. Autores sugerem que, devido ao fato de tais animais serem capazes de se alimentar de diferentes partes das plantas, incluindo partes de baixo valor nutricional, houve uma diminuição da competição por alimentos com herbívoros nativos. Adicionalmente, a grande diversidade do grupo evitou a concorrência ecológica entre os membros da própria Superordem. Porém, a taxa de radiação ou diversificação foi menor em relação a que ocorreu na América do Sul (McDonald et al., 2005).

Autores sugerem que a diversificação de mamíferos Xenarthra foi influenciada pela elevação andina e pela flutuação do nível do mar no Cenozóico. Para Delsuc et al., 2004, a diversificação dos tamanduás e tatus no Eoceno, a radiação das preguiças e Tolypeutinae, a diversificação dos Euphractine e a divisão dos *Dasypus* no Mioceno ocorreram devido à crise tectônica andina.

Até o Pleistoceno, foram reconhecidos 218 gêneros de Xenarthra (McKenna e Bell, 1997; Moraes-Barros et al., 2015). Porém, uma extinção em massa ocorreu no final desse período, ocasionando a queda da diversidade do grupo e o

desaparecimento de várias espécies, principalmente aqueles animais de grande porte (Koch e Barnosky, 2006).

Atualmente, a Superordem Xenarthra é dividida em duas ordens: Cingulata e Pilosa. A primeira é caracterizada pela presença de osteodermos e composta por uma única família (Dasypodidae), que compreende tatus. Já a segunda engloba animais com pelagem densa e ausência ou pouco desenvolvimento dos dentes. É composta por quatro famílias, a saber: Bradypodidae (preguiças-de-três-dedos), Megalonychidae (preguiças-de-dois-dedos), Cyclopedidae (tamanduaí) e Myrmecophagidae (tamanduás) (Gardner, 2005). No total, são reconhecidas 38 espécies, sendo: seis, dez e 22 de preguiças, tamanduás e tatus, respectivamente (ASASG, 2020), distribuídas do centro-sul da América do Norte ao Sul da América do Sul (Gardner, 2005). No Brasil, ocorrem 23 espécies distribuídas por todos os biomas do país.

Os mamíferos que abrangem esse grupo possuem diferentes hábitos locomotores, alimentares e ecológicos. Como consomem alimentos com baixo teor energético, apresentam baixo metabolismo e baixa temperatura corpórea, características estas que influenciam diretamente na área de vida e no padrão de atividade desses animais. As preguiças possuem hábitos solitários, com exceção no período de acasalamento e cuidados com os filhotes (Queiroz, 1995). São folívoras e arborícolas, passam a maior parte do tempo em repouso na copa das árvores, descendo para o solo apenas para defecar (intervalos de 8 dias para *B. variegatus*) e para deslocamento em áreas abertas, uma vez que em áreas florestais o deslocamento é realizado nas extremidades de galhos e lianas (Montgomery e Sunquest, 1975; Cassano, 2006). Membros da família Mymercophagidae, composta pelo tamanduá-bandeira (*M. trydactyla)* e tamanduá-mirim (*Tamandua tetradactyla)* possuem hábitos solitários e são mimercófagos. O tamanduá-bandeira, possui hábitos terrestres e apenas um período de atividade durante o dia, o qual juntamente com a escolha do habitat, variam de acordo com a temperatura do ambiente e estação. Em temperaturas de 17 a 27ºC há maior preferência por áreas de baixa cobertura vegetal (savanas e campos) e em frio ou calor extremo há preferência por áreas florestais. Ainda, em estações mais quentes a atividade é mais noturna e no inverno é diurna (Alves e Mourão, 2006; Braga, 2010; Bertassoni, 2017). Por sua vez, o tamanduá-

mirim, possui hábito diurno, crepuscular ou noturno a depender do local. São animais escansoriais, predominantemente arborícola, mas com descidas ao solo para alimentação e deslocamento. Em períodos de inatividade eles se abrigam em ocos de árvores ou tocas produzidas por outros animais (Rodrigues et al., 2001; Rodrigues e Marinho, 2003; Gardner, 2008; Catapani, 2014). Já os tamanduaís (Cyclopedidae) são exclusivamente arborícolas e noturnos (ASASG, 2020). Os tatus formam o grupo mais diverso dos Xenarthra, são solitários, com hábitos principalmente noturnos, com exceção de algumas espécies pertencentes ao gênero *Euphractus*, as quais são mais diurnas (Redford e Wetzel, 1985; Emmons, 1997). São animais escavadores, de grande importância para a estruturação e manutenção da comunidade local, uma vez que as tocas produzidas por eles podem servir de refúgio para outros animais (Kinlaw, 1999; Uribe, 2004). Possuem preferência por áreas abertas, com árvores e arbustos, bordas de floresta e florestas (Redford e Wetzel, 1985; Emmons e Feer, 1997; Trolle, 2003; Medri, 2008). Em relação aos hábitos alimentares, são classificados em quatro categorias: carnívoros/onívoros que se alimentam de matéria vegetal e animal (*Chaetophractus* spp., *Euphractus* spp., *Zaedys* spp.); insetívoros generalistas fossoriais e terrestres (*Chlamuphorus* spp. e *Dasypus* spp., respectivamente), os quais alimentam-se de insetos e matéria vegetal; e insetívoros especialistas, os quais se alimentam de cupins e formigas (*Priodontes* spp., *Cabassous* spp., *Tolypeutes* spp.) (Redford, 1985).

Há muitas incertezas acerca da filogenia dessa Superordem, devido à raridade de algumas espécies e falta de dados acerca das delimitações e distribuições de alguns desses animais (Superina et al., 2014). Adicionalmente, a posição exata desse grupo dentro da filogenia dos placentários é controversa. A hipótese de que se trata de um grupo monofilético é suportada por caracteres morfológicos e moleculares (Engelmann, 1985; Gaudin, 1999; Delsuc et al., 2001, 2004). As principais características morfológicas são a presença de articulações adicionais entre as vértebras lombares com fusão das vértebras sacrais, costelas esternais ossificadas, osso septomaxilar no nariz e dentes homodontes (Piveteau and Dechaseaux, 1958; Glass, 1985; Rose, 2006).

Análises filogenéticas baseadas no gene mitocondrial de 31 espécies de Xenarthra (**Figura 1**) mostraram que tatus são os animais mais antigos e mais

diversificados dentro dessa Superordem. A separação entre as duas ordens (Cingulata e Pilosa) ocorreu há aproximadamente 67,7 milhões de anos. Por outro lado, preguiças e tamanduás separaram-se há 58,5 m.a. Adicionalmente, demonstrou-se que a fase de maior especiação do grupo ocorreu há 10-15 m.a., durante um período de resfriamento intenso que, juntamente com a corrente circumpolar Antártica e a última fase da elevação andina, promoveu aridez na América do Sul e consequente formação dos biomas secos (Caatinga, Cerrado, Chaco e Pampas). Por fim, por meio de análises biogeográficas, verificaram que as regiões Pan-Amazônica e de Mata Atlântica foram o berço da evolução dos Xenarthra durante o Paleógeno (Gibbi et al., 2016).

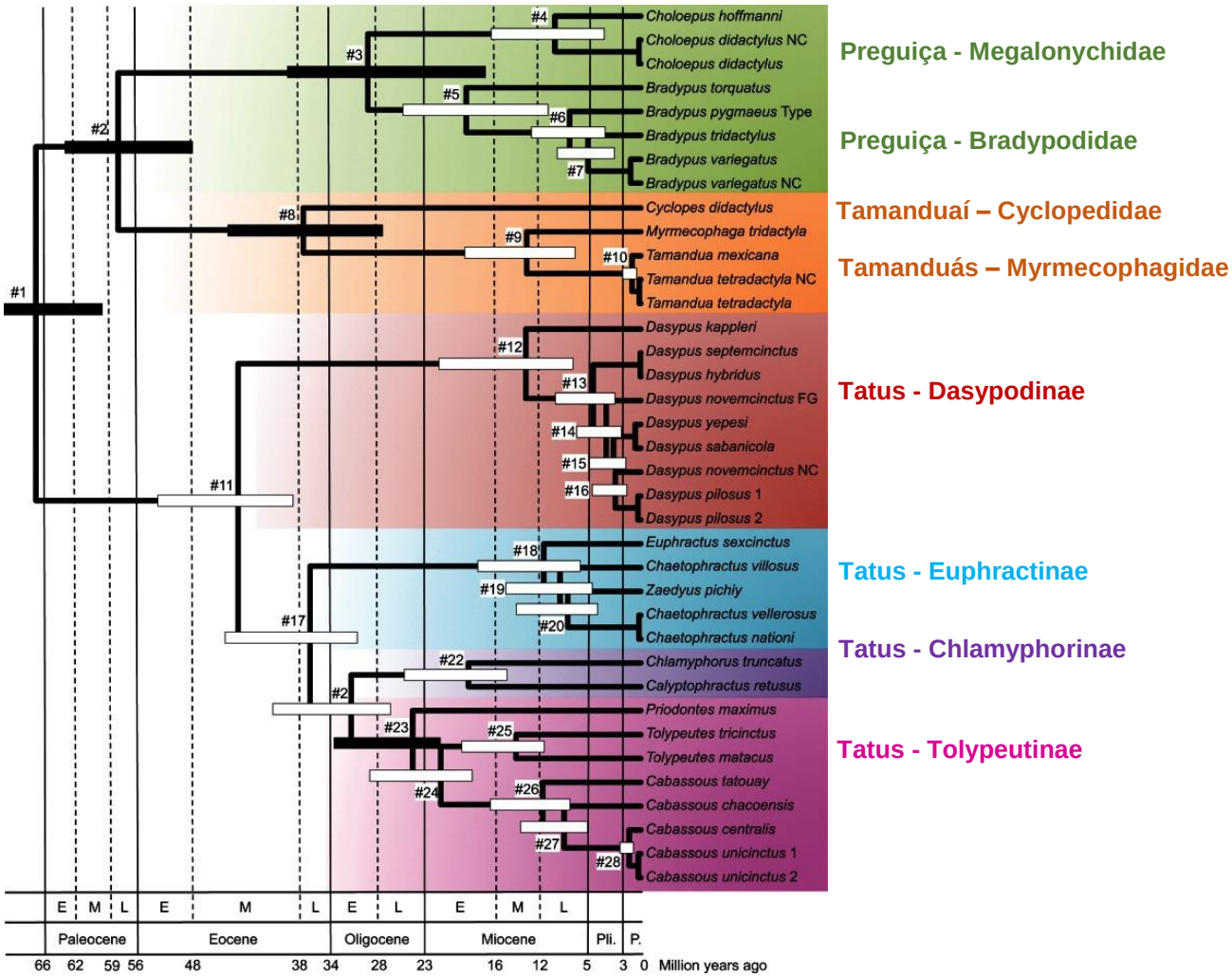

Figura 1. Escala de tempo molecular com análise Bayesiana para todas as espécies existentes de Xenarthra. Barras de nós brancas indicam incerteza em torno das estimativas de idade com 95% de intervalo de credibilidade. Barras de nós pretas indicam nós usados como restrições de calibração a priori. Linhas verticais delimitam os principais períodos geológicos do Cenozóico seguindo a Escala de Tempo Geológico de 2012 da Sociedade Geológica da América. E = early; M=middle; L = late; Pli. = Plioceno; P. = Pleistoceno. Fonte: adaptado de Gibb et al. (2016).

De acordo com a lista vermelha de espécies ameaçadas da IUCN (International Union for Conservation of Nature), as espécies *Cabassous chacoensis* (Tatu-de-rabo-mole-do-Chaco), *Dasypus hybridus* (Tatuíra), *Dasypus sabanicola* (Tatu-das-savanas), *Tolypeutes matacus* (Tatu-bola) e *Zaedyus pichiy* (Piche) são consideradas como quase ameaçadas à extinção. Por outro lado, *Bradypus torquatus* (Preguiça-de-Coleira), *Myrmecophaga tridactyla* (Tamanduá-bandeira), *Priodontes maximus* (Tatu-Canastra) e *Tolypeutes tricinctus* (Tatu-bola) estão classificadas como espécies vulneráveis. Por fim, *Bradypus pygmaeus* (Preguiça-anã) é considerada como espécie criticamente ameaçada (IUCN, 2020). O decréscimo na população deste grupo de animais ocorre devido à caça e constante perda e fragmentação do habitat pelo desmatamento.

3.1.1. Espécies de carrapatos parasitas de mamíferos Xenarthra no Brasil

Estudos realizados em diferentes estados brasileiros mostraram grande especificidade em relação às diferentes espécies de carrapatos que acometem Xenarthra.

Em todas as regiões do Brasil, preguiças são parasitadas principalmente pelas espécies *Amblyomma varium* e *A. geayi*, tamanduás-mirins e tamanduás-bandeiras por *A. nodosum*, *A. sculptum* e *A. calcaratum*, já tatus são frequentemente parasitados por *A. pseudoconcolor* e *A. auricularium* (Botelho et al., 1989; Labruna et al., 2002; Marques et al., 2002; Labruna et al., 2004; Arzua et al., 2005; Bechara et al., 2006; Dantas-Torres et al., 2009; Miranda et al., 2010; Garcia et al., 2013; Soares et al., 2014; Martins et al., 2015; Acosta et al., 2016; Coelho et al., 2016; Kluyber et al., 2016; Cançado et al., 2017; Gonzalez et al., 2017; Martins et al., 2017a; Martins et al., 2017b; Nascimento et al., 2017; Oliveira et al., 2017; Luz et al., 2018; Muñoz-García et al., 2019). A **Tabela 1** apresenta as espécies de carrapatos encontrados em Xenarthra em diferentes estados brasileiros.

Tabela 1. Espécies de carrapatos já encontradas em mamíferos Xenarthra em diferentes estados brasileiros

Hospedeiro	Localidade	Espécies de carrapatos	Referências
Bradypus variegatus	São Paulo	*Amblyomma varium* , *A. aureolatum*	Martins et al., 2015; Gonzalez et al., 2017; Martins et al., 2017a; Martins et al., 2017b
	Alagoas	*A. varium*	Dantas-Torres et al., 2009
	Pará	*A. varium, A. geayi*	Nascimento et al., 2017
Bradypus torquatus	Rio de Janeiro	*A. varium, A. longirostre*	Luz et al., 2018
	Espírito Santo	*A. varium*	Acosta et al., 2016
***Bradypus* sp.**	Paraná	*A. geayi*	Arzua et al., 2005
Choloepus sp.	Rondônia	*A. varium*	Labruna et al., 2004
Choloepus didactylus	Pará	*A. geayi*	Nascimento et al., 2017
Tamandua tetradactyla	São Paulo	*A. nodosum*, *A. sculptum*, *A. calcaratum*, *A. brasiliensis*, *Rhipicephalus sanguineus*	Labruna et al., 2002; Martins et al., 2015; Gonzalez et al., 2017; Martins et al., 2017a; Martins et al., 2017b
	Rio de Janeiro	*A. nodosum*	Luz et al., 2018
	Mato Grosso	*A. nodosum*	Soares et al., 2014
	Mato Grosso do Sul	*A. nodosum, A. sculptum, A. parvum*	Bechara et al., 2006; Cançado et al., 2017
	Pernambuco	*A. nodosum, A. auricularium, R. sanguineus*	Oliveira et al., 2017
	Pará	*A. nodosum, A. goeldii, A. humerale, A. naponense, A. calcaratum*	Soares et al., 2014; Nascimento et al., 2017
	Rondônia	*A. nodosum, A. calcaratum*	Labruna et al., 2004
	Paraná	*A. nodosum, A. aureolatum, A. calcaratum*	Arzua et al., 2005
M. tridactyla	São Paulo	*A. nodosum*, *A. sculptum, A. triste, A. calcaratum, A. brasiliensis, R. microplus*	Labruna et al., 2002; Martins et al., 2015; Coelho et al., 2016
	Espírito Santo	*A. nodosum*	Acosta et al., 2016

	Minas Gerais	*A. calcaratum, A. maculatum, A. pseudoconcolor*	Botelho et al., 1989
	Mato Grosso do Sul	*A. nodosum, A. sculptum, A. scalpturatum, A. parvum, A. ovale, A. triste*	Bechara et al., 2006; Cançado et al., 2017
	Goiás	*A. pseudoconcolor, A. sculptum, A. nodosum*	Martins et al., 2011
	Pernambuco	*A. nodosum*	Dantas-Torres et al., 2009
	Rondônia	*A. calcaratum*	Labruna et al., 2004
Cabassous unicinctus	Mato Grosso do Sul	*A. sculptum*	Kluyber et al., 2016
Dasypodidae	Minas Gerais	*A. pseudoconcolor*	Botelho et al., 1989
	Goiás	*A. pseudoconcolor, A. sculptum, A. nodosum*	Martins et al., 2011
	Mato Grosso do Sul	*A. pseudoconcolor, A. sculptum*	Cançado et al., 2017
Dasypus septemcinctus	Rio de Janeiro	*A. auricularium*	Luz et al., 2018
Dasypus novencinctus	São Paulo	*A. sculptum*	Martins et al., 2017b
	Mato Grosso	*A. auricularium*	Soares et al., 2014
	Mato Grosso do Sul	*A. parvum; A. sculptum*	Kluyber et al., 2016
	Pará	*A. humerale*	Soares et al., 2014
Euphractus sexcinctus	Mato Grosso do Sul	*A. pseudoconcolor; A. auricularium; A. parvum; A. sculptum*	Bechara et al., 2006; Kluyber et al., 2016
Priodontes maximus	Mato Grosso	*A. auricularium, A. pseudoconcolor, A. brasiliensis, A. fuscum, A. parvum, A sculptum*	Kluyber et al., 2016; Miranda et al., 2010

3.2. Família Anaplasmataceae

A família Anaplasmataceae, pertencente à ordem Rickettsiales, é formada pelos gêneros *Anaplasma*, *Ehrlichia*, *Neorickettsia*, *Wolbachia* e '*Candidatus* Neoehrlichia'. Compreendem alfa-proteobactérias Gram-negativas intracelulares obrigatórias, transmitidas por carrapatos (*Anaplasma* spp., *Ehrlichia* spp. e '*Candidatus* Neoehrlichia'), helmintos (*Neorickettsia* spp.) ou compreendem endossimbiontes de invertebrados (*Wolbachia* spp.) (Dumler et al., 2001).

Anaplasma spp. e *Ehrlichia* spp., a depender da espécie, podem infectar neutrófilos, monócitos, macrófagos, plaquetas, eritrócitos e células endoteliais de mamíferos (Rikihisa, 1991; Dumler et al., 2001; Allsopp, 2010; Aguiar et al, 2014), onde formam microcolônias (mórulas) em vacúolos intracitoplasmáticos (Doudier et al., 2010). As bactérias possuem dois tipos morfológicos: uma forma densamente corada, a qual contém uma condensação central ou excêntrica relativamente densa de fios de cromatina, e um forma reticulada, a qual possui uma matriz solta homogênea de fios de cromatina, entre os quais os ribossomos também estão espalhados (Popov et al., 1998; Battilani et al., 2017).

A manutenção da infecção destes agentes dos gêneros *Ehrlichia* e *Anaplasma* ocorre pela interação de vetores artrópodes com os hospedeiros vertebrados no ecossistema. Carrapatos, ao se alimentarem de um hospedeiro infectado, ingerem as bactérias, as quais, por sua vez, penetram no epitélio do intestino médio do invertebrado e realizam a primeira replicação. Posteriormente, as mesmas migram até as glândulas salivares, invadem as células epiteliais e passam pelo segundo ciclo de replicação, penetram na secreção salivar e são transmitidas a outro hospedeiro durante o repasto sanguíneo do carrapato (Ueti et al., 2009; Rar e Golovljova, 2011). Hospedeiros vertebrados desenvolvem infecção persistente, atuando como reservatórios desses agentes, os quais são responsáveis por causar enfermidades em animais e humanos (Kocan et al., 1992; Dumler et al., 2001; Parola et al., 2003; Doudier et al., 2010; Villar et al., 2016).

Atualmente o gênero *Anaplasma* é composto pelas espécies *A. phagocytophilum, A. marginale*, *A. centrale*, *A. ovis*, *A. bovis, A. platys, A. odocoilei* e *A. capra.* Já o gênero *Ehrlichia* é composto por *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris*, *E. ruminantium* e *E. minassensis* (Dumler et al., 2001; Tate et al., 2013; Li et al., 2015; Cabezas-Cruz et al., 2016).

Embora ‘*Candidatus* Neoehrlichia’ spp. não tenha sido oficializado como novo gênero, devido a grande dificuldade de cultivo desse agente, o mesmo vem sendo encontrada em diversas espécies de carrapatos e mamíferos. Esse novo gênero forma um clado irmão ao de *Ehrlichia* spp. Até o momento, foram descritas pelo menos quatro possíveis espécies, a saber: ‘*Candidatus* Neoehrlichia mikurensis’, ‘*Candidatus* Neoehrlichia lotoris’, ‘*Candidatus* Neoehrlichia australis’, ‘*Candidatus* Neoehrlichia arcanae’ e ‘*Candidatus* Neoehrlichia chilensis’ (Kawahara, 2004; Yabsley et al., 2018; Gofton et al., 2016; Müller et al., 2017).

Devido à grande inespecificidade dos sinais clínicos, técnicas laboratoriais devem ser utilizadas para um diagnóstico preciso. A detecção direta por meio de esfregaços sanguíneos permite observar a presença de mórulas em células sanguíneas. Entretanto, essa técnica é de baixa sensibilidade, uma vez que depende do número de células infectadas, sendo este maior na fase aguda da doença, e da experiência do microscopista (Paddock e Child, 2003). Técnicas sorológicas, por sua vez, mostram-se mais sensíveis, porém reações cruzadas podem ocorrer, dificultando a obtenção de um diagnóstico preciso. Além disso, resultados falsos-negativos podem ser obtidos durante a fase inicial da doença, em que os anticorpos ainda estão em processo de produção (Paddock e Child, 2003). Por fim, o uso de ensaios moleculares estão cada vez mais se difundindo, devido à elevada sensibilidade e especificidade, embora em alguns países ainda sejam considerados técnicas caras, dificultando o seu uso.

3.2.1. Agentes etiológicos

3.2.1.1. Anaplasma phagocytophilum

Anaplasma phagocytophilum recebeu esse nome após a reclassificação realizada por Dumler et al. (2001), o qual, a partir de análises moleculares dos genes 16S RNA ribossomal (16S rRNA), “heat shock operon” (*groEL*), e genes de proteínas de superfície, verificou que as bactérias até então nomeadas de *Ehrlichia phagocytophila*, *Ehrlichia equi* e agente da erliquiose granulocítica humana tratavam-se da mesma espécie.

O protótipo de *A. phagocytophilum* foi descrito pela primeira vez em 1940 (Gordon, 1940), após ser identificado como o agente causador da febre transmitida pelo carrapato em ovinos, doença descrita inicialmente na Escócia em 1932 (Macleod, 1932).

Essa bactéria infecta neutrófilos de uma ampla variedade de vertebrados como carnívoros, ruminantes, roedores, aves, répteis e seres humanos (Stuen et al., 2013; Dugat et al, 2015; Kocan et al., 2015). Está distribuída principalmente nos Estados Unidos, Europa e Ásia, mas já foi descrita na América do Sul e África (Bakken e Dumler, 2000; Stuen et al., 2013).

Em humanos, é responsável por causar uma zoonose emergente, conhecida como anaplasmose granulocítica humana (HGA), descrita pela primeira vez nos Estados Unidos em 1994 (Chen et al., 1994). Essa doença é amplamente distribuída nos Estados Unidos, devido à presença do carrapato vetor. As principais manifestações clínicas e laboratoriais são febre, dor de cabeça, mialgia, mal-estar, leucopenia, trombocitopenia e injúria hepática. Já foi observado, com menor frequência, a presença de náusea, vômito, tosse, diarreia, artralgia e confusão mental (Dumler et al., 2005; Lotric-Furlan et al., 2006; Strle, 2004; Rar e Golovljova, 2011).

Em ruminantes, causa uma enfermidade conhecida por Febre da Pastagem ou Febre do Carrapato, caracterizada por febre, fraqueza, anorexia, abortamento e queda na produção de leite. Por ser um agente imunossupressor, favorece a ocorrência de infecções secundárias, principalmente por *Staphylococcus aureus* (Woldehiwet, 2006; Woldehiwet, 2010; Dugat et al., 2015). Em equinos, a doença é denominada anaplasmose granulocítica equina, cujos sinais são febre, anorexia, apatia, inapetência, icterícia, edema dos membros, ataxia e dificuldade de locomoção (Madigan e Gribble, 1987; Franzén et al., 2007; Battilani et al., 2017). A anaplasmose granulocítica canina, por sua vez, é caracterizada por febre, letargia, anorexia, claudicação, êmese, diarreia, poliúria, polidipsia, mucosas pálidas, epistaxe, espleno e linfoadenomegalia (Kohn et al., 2008; Nair et al., 2016; Battilani et al., 2017).

A transmissão se dá pela picada de diferentes espécies de carrapatos do gênero *Ixodes*. Enquanto que no centro-oeste e nordeste dos EUA a principal espécie vetora é *I. scapularis*, no oeste, tal agente é transmitido por *I. pacificus* e *I. spinipalpis* (Des Vignes e Fish, 1997; Burkot et al., 2001). Na Europa, o principal vetor é *I. ricinus*,

já na Rússia e Ásia é *I. persulcatus* (Woldehiwet et al., 2010). Ademais, DNA de *A. phagocytophilum* já foi detectado em *Dermacentor silvarium*, *D. reticulatus*, *D. variabilis*, *D. occidentalis*, *I. ovatus*, *I. trianguliceps*, *I. ventalloi*, *Haemaphysalis concinna*, *Amblyomma americanum* (Bown et al., 2003; Santos et al., 2004; Ohashi et al., 2005; Cao et al., 2006; Clark. 2012; Paulauskas et al., 2012; Tomanovic et al., 2013).

Nos EUA, os principais reservatórios são Cervo-da-cauda-branca (*Odocoileus virginianus*), camundongo-de-patas-brancas (*Peromyscus leucopus*) no leste e roedores selvagens (*Neotoma fuscipes*, *Tamias ochrogenys*) no oeste do país (Nicholson et al., 1999; Tate et al., 2005; Rar e Golovljova, 2011). Já na Europa, ovelhas, veado-europeu (*Capreolus capreolus*), veado-vermelho (*Cervus elaphus*) e camurça (*Rupicapra rupicapra*) são os principais reservatórios (Liz et al., 2002; Rar e Golovljova, 2011).

A diversidade genética de *A. phagocytophilum* é considerável, sendo que várias variantes já foram descritas, tanto patogênicas como não-patogênicas. Estudos já demonstraram que a Variante-1, comum em cervos-da-cauda-branca, pode infectar cabras, porém não infecta roedores (Massung et al., 2003; Reichard et al., 2009). Além disso, algumas amostras que ocorrem em humanos podem infectar roedores, mas não cervos-da-cauda-branca (Reichard et al., 2009). Amostras detectadas em esquilos e camundongo-de-patas-brancas podem acometer roedores, equinos, cães e humanos (Foley et al., 2009; Rar e Golovljova, 2011).

3.2.2.2.Anaplasma marginale

Anaplasma marginale foi detectado pela primeira vez na África do Sul por Sir Arnold Theiler, em 1910, o qual encontrou inclusões localizadas na superfície dos eritrócitos de bovinos doentes (Theiler, 1910).

Esse agente infecta eritrócitos de ruminantes domésticos e selvagens. É responsável por causar uma doença denominada anaplasmose bovina, cujos principais sinais clínicos são febre, perda de peso, letargia, icterícia, abortamento, queda na produção de leite e morte (Kocan et al., 2003). Essa enfermidade é cosmopolita, com maior incidência em regiões tropicais e subtropicas, ocasionando

grande impacto econômico na produção, devido sua alta morbidade e mortalidade (Kocan et al., 2010; Silaghi et al., 2017).

Pode ser transmitido pela picada de carrapatos dos gêneros *Dermacentor* em regiões temperadas, e *Rhipicephalus* em regiões tropicais, sendo que mais de 20 espécies foram descritas como vetores (Kocan et al., 2010; Rar e Golovljova, 2011; Silaghi et al., 2017). Ademais, a transmissão mecânica por meio de insetos hematófagos, tais como *Stomoxys calcitrans, Haematobia irritans* e dípteros tabanomorphos, e por meio de fômites contaminados com sangue também pode ocorrer, sendo a principal forma de transmissão em regiões onde não há a presença do vetor (Ewing, 1981; Kocan et al., 2010; da Silva et al., 2013; Battilani et al., 2017). Transmissão transplacentária já foi relata e gera bezerros saudáveis e infectados permanentemente (Kocan et al., 2015).

Além de bovinos, *A. marginale* já foi detectado em cervo-de-cauda-branca, búfalos-d'agua (*Bubalus bubalis*), bisão americano (*Bison bison*), cervo-de-cauda-negra (*Odocoileus hemionus columbianus*) (Kocan et al., 2010; Battilani et al., 2017), cervo-do-Pantanal (*Blastocerus dichotomus*) (Machado et al., 2006; Silveira et al., 2012), veado-campeiro (*Ozotocerus bezoarticus leucogaster*) (Picoloto et al., 2010); veado-catigueiro (*Mazama gouazoubira*) (Silveira et al., 2012) e búfalos africanos (*Syncerus caffer*) (Machado et al., 2016).

Grande diversidade genética é evidenciada em *A. marginale*, uma vez que vários estudos demonstraram que um mesmo bovino ou um mesmo rebanho ou região possuem diferentes genótipos circulantes do patógeno, o que dificulta a criação de vacinas para esse agente (Vidotto et al., 2004; Estrada-Peña et al., 2009; Poohl et al., 2013; Machado et al., 2015; Silva et al., 2015; Silva et al., 2016; de Souza Ramos et al., 2019).

3.2.2.3. Anaplasma centrale

Anaplasma centrale foi descrito em 1911 por Sir Arnold Theiler, caracterizado por formar inclusões na área central de eritrócitos de bovinos (Theiler, 1911; Potgieter e Van Rensburg, 1987).

Trata-se de uma bactéria intimamente relacionada com *A. marginale*, porém com menor ocorrência. Está distribuída principalmente nas regiões tropicais e

subtropicais e é responsável por causar sinais mais brandos de anaplasmose em ruminantes, sendo considerado um agente naturalmente atenuado e candidato à vacina viva contra *A. marginale* (Kocan, 2010; Rar and Golovljova, 2011; Kocan, 2015; Bensaid, 2018).

Pode ser transmitido de forma mecânica ou biológica. *Rhipicephalus simus* é considerado o principal vetor, porém DNA de *A. centrale* já foi detectado em *Amblyomma* sp. e *Haemaphysalis punctata*, mas a competência vetorial não foi demonstrada nessas espécies (Potgieter e Van Rensburg, 1987; Palomar et al., 2015; Teshale et al., 2015).

3.2.2.4. Anaplasma ovis

Anaplasma ovis foi descrito pela primeira vez em uma ovelha em 1912 (Bevan, 1912). Infecta eritrócitos de ovelhas, cabras e ruminantes selvagens como renas (*Rangifer tarandus*), veado-europeu (*Capreolus capreolus*) e camelos (*Camelus dromedarius*) (de la Fuente et al., 2008; Renneker et al., 2013; Ybañes et al., 2014; Li et al., 2015b; Selmi et al., 2020). Possui um possível potencial zoonótico, uma vez que foi detectado em um ser humano no Chipre (Chochlakis et al., 2010). Encontra-se distribuído na África, Ásia, Europa e EUA (Renneker et al., 2013).

Os sinais clínicos causados por este espécie de *Anaplasma* são brandos, mas em situação de estresse e imunossupressão pode causar febre, fraqueza, anorexia, mucosas pálidas, abortamento e queda na produção de leite (Battilani et al., 2017).

Os principais vetores biológicos para o referido agente são carrapatos das espécies *Rhipiceplahus bursa*, *R. turanicus*, *R. sanguineus*, *Dermacentor andersoni* e o díptero *Melophagus ovinus* (de la Fuente et al., 2008; Aktas et al., 2009; Belkahia et al., 2019; Cabezas-Cruz et al., 2019; Hosseini-Chegeni et al., 2020). O agente já foi molecularmente detectado em *R. microplus* (Matsimbe et al., 2017).

3.2.2.5. Anaplasma bovis

Anaplasma bovis foi descrito pela primeira vez em bovinos em 1936 (Donatien e Lestoquardm 1936). Infecta monócitos e macrófagos teciduais (Worthington and Bigalke, 2001; Liu, 2012) e ocorre principalmente na África, Ásia e América do Sul,

apesar de já ter sido descrito na Europa e EUA (Ybañez et al., 2014; Battilani et al., 2017).

É transmitido pelos carrapatos *Rhipicephalus appendiculatus*, *Amblyomma variegatum*, *Hyalomma* spp., *Rhipicephalus sanguineus* e *Haemaphysalis* spp. (Nair, 2013; Battilani, 2017). Possui como principais reservatórios bovinos (*Bos taurus*) e búfalo africanos (Ooshiro et al., 2008). Adicionalmente, tal agente já foi descrito em cervídeos (*Cervus elaphus*, *Cervus nippon*, *Mazama goazoubira*, *Blastocerus dichotomus*, *Hydropotes inermis*), cabras, guaxinins (*Procyon lotor*, *Nyctereutes procyonoides*), cães, gato-leopardo (*Prionailurus bengalensis*) e musaranho (*Elephantulus myurus*) (Sashika et al., 2011; Silveira et al., 2012; Atif, 2016; Kang et al., 2018; Lee et al., 2018).

Embora os bovinos acometidos sejam geralmente assintomáticos (Noaman e Shayan, 2010), podem apresentar febre, perda de peso, diarreia, edema cerebral e danos no sistema nervoso (Ybañez et al., 2014; Battilani et al., 2017).

3.2.2.6. Anaplasma platys

Anaplasma platys é um agente cosmopolita que infecta plaquetas de cães e foi descrito pela primeira vez em 1978 na Flórida (Harvey et al., 1978). Embora muitos cães infectados sejam assintomáticos, pode causar uma enfermidade conhecida como trombocitopenia cíclica canina, cujos sinais clínicos e laboratoriais são febre, perda de peso, apatia, mucosas pálidas, petéquias, linfoadenomegalia e trombocitopenia, (Bradfield et al., 1996; Bouzouraa et al., 2016).

Um novo genótipo, denominado *A. platys-like,* foi encontrado infectando neutrófilos de ruminantes na Itália. Tal variante apresenta baixa identidade nucleotídica com *A. platys* com base no gene *groEL* e posicionava-se filogeneticamente em um subclado dentro do clado de *A. platys* (Zobba et al., 2014).

Além de cães, esse agente já foi detectado em humanos (Maggi et al., 2013; Arraga-Alvarado et al., 2014; Breitschwerdt et al., 2014), gatos (Lima et al., 2010; Salakij et al., 2012; Zobba et al., 2015), raposas-vermelhas (*Vulpes vulpes*) (Cardoso et al., 2015), cervídeos (Sacchi et al., 2012; Silveira et al., 2012), ruminantes domésticos (bovinos, caprinos e ovinos), bubalinos e camelídeos (Chocohlakis et al., 2009; Djiba et al., 2013; Berggoetz et al., 2014; Tay et al., 2014; Dahmani et al., 2015;

Li et al., 2015b; Yang et al., 2015; Machado et al., 2016; Qiu et al., 2016; Ben Said et al., 2017; Gioia et al., 2018; Koh et al., 2018; Pradeep et al., 2018; Chien et al., 2019; Dahmani et al., 2019; Fernandes et al., 2019; Selmi et al. 2019; Zhou et al., 2019; Alanazi et al., 2020).

É transmitido principalmente pelo carrapato *R. sanguineus* sensu lato, mas já foi detectado em *Dermacentor auratus*, *Ixodes persulcatus*, *Haemaphysalis longicornise*, *H. bispinosa*, *R. turanicus*, *R. microplus*, *R. bursa*, *Hyalomma dromedari*, *Hyalomma anatolicum* (Simpson et al., 1991; Parola et al., 2003; Brown et al., 2005; Kim et al., 2006; Aktas et al., 2009; Harrus et al., 2010; Ybañez et al., 2012; Tay et al., 2014; Campos-Calderon et al., 2016; Koh, 2017; Yu et al., 2017; Chisu et al., 2018; Rehaman et al., 2018; Selmi et al., 2019). Recentemente, Snellgrove et al. (2020) comprovaram a transmissão transestadial e transovariana de *A. platys* por R. sanguienus sensu stricto nos EUA.

3.2.2.7. Anaplasma odocoilei

Anaplasma odocoilei foi descrito pela primeira vez em 2013, infectando plaquetas de cervídeos (Tate et al., 2013). Após infecção experimental em cervos-da-cauda-branca, notou-se que alguns animais apresentaram trombocitopenia transitória, mas sem manifestações clínicas (Tate et al., 2013). Adicionalmente, foi descrito também em cervos-da-cauda-branca (*O. virginianus yucatanensis*) no México (Ojeda-Chi et al., 2018).

Por tratar-se de um agente descoberto recentemente, são poucas as informações a respeito de transmissão, sinais clínicos e distribuição. Até o momento, DNA de *A. odocoilei* foi detectado em *Amblyomma americanum*, porém não houve comprovação vetorial (Trout et al., 2017).

3.2.2.8. Anaplasma capra

Uma nova espécie de *Anaplasma* spp. foi detectada em cabras na China (Zhou et al., 2010). Posteriormente, Li et al. (2015) detectaram a mesma espécie nos mesmos hospedeiros, nomeando-a então de *Anaplasma capra*. A partir daí, essa nova espécie foi descrita infectando cabras (Yang et al., 2017; Peng et al., 2018; Seo et al., 2019), ovelhas (Peng et al., 2018; Shi et al., 2020), bovinos (Seo et al., 2018),

ruminantes selvagens (Yang et al., 2018), cervídeos (Amer et al., 2019; Jouglin et al., 2019) e cães (Shi et al., 2019).

Possui caráter zoonótico, uma vez que foi detectada em seres humanos que apresentavam febre, dor de cabeça, tonturas, mialgias, erupções cutâneas, linfadenopatia e sintomas gastrointestinais na China (Li et al., 2015).

Não há informações acerca da distribuição e transmissão do agente, embora DNA do mesmo tenha sido detectado em carrapatos da espécie *Ixodes persulcatus*, *Haemaphysalis longicornis*, *Haemaphysalis qinghaiensis*, *R. microplus* (Li et al., 2015; Sun et al., 2015; Yang et al., 2016; Guo et al., 2019).

3.2.2.9. Ehrlichia canis

Ehrlichia canis foi descrita pela primeira vez na Argélia, em 1935, por Donatien e Lestoquard, que observaram inclusões intracitoplasmáticas em células mononucleares de cães infestados por carrapatos. Posteriormente, esse agente foi determinado como o responsável por causar a doença conhecida como erliquiose monocítica canina (CME) (Rikihisa, 1991).

Essa bactéria infecta monócitos de uma ampla variedade de animais selvagens e domésticos, com maior predominância em cães. Possui distribuição mundial, com maior prevalência em regiões tropicais e subtropicais, devido à presença do carrapato vetor. Duas espécies com capacidade vetorial comprovada são *R. sanguineus* sensu lato (linhagem tropical) e *Dermacentor variabilis* (Groves et al., 1975; Johnson et al., 1998; Moraes-Filho et al., 2015).

Em cães, a doença ocorre em três fases: aguda, subclínica e crônica. A aguda tem duração de 2 a 4 semanas, com início de 8 a 20 dias após a picada do carrapato. É caracterizada por febre, apatia, dispneia, anorexia, esplenomegalia, trombocitopenia, leucopenia, anemia e hiperglobulinemia. A fase subclínica pode durar meses ou anos, é geralmente assintomática e pode ser caracterizada por trombocitopenia e hiperglobulinemia moderada. Animais subclinicamente infectados atuam como reservatórios para o agente. Já a fase crônica assume características de doença auto-imune, e é caracterizada pelos mesmos sinais da fase aguda, como letargia anorexia e perda de peso. Adicionamente, podem ser verificados hemorragia, hematúria, epistaxe, sinais neurológicos (ataxia, disfunção neuromotora, disfunção

vestibular central ou periférica e hiperestesia localizada ou generalizada) e glomerulonefrite (Codner e Farrim-Smith, 1986; Gregory e Forrester, 1990; Cordner e Malin, 1992; Couto, 1998; Cohn, 2003; Skotarczak, 2003).

Tal agente já foi descrito em seres humanos, associados com sintomatologia clínica (Fishbein et al., 1987; Maeda et al., 1987; Dawson et al., 1991; Perez et al., 1996; Perez et al., 2006), bem como em pessoas assintomáticas doadoras de sangue (Bolsa-Mora et al., 2017). Segundo Perez et al. (2006), indivíduos que vivem em áreas endêmicas onde a prevalência de cães positivos pela PCR é alta, pode ser mais acometidas.

3.2.2.10. Ehrlichia chaffeensis

Ehrlichia chaffeensis infecta monócitos e é responsável por causar uma zoonose conhecida por erliquiose monocítica humana (HME). Embora o agente tenha sido descrito em 1991, o primeiro relato da doença foi reportado em 1986 nos Estados Unidos (Anderson et al., 1991; Dawson et al., 1991).

A HME é amplamente distribuída nos Estados Unidos, acometendo principalmente as regiões sudeste, centro-sul e meio-Atlântico do país, devido a presença do carrapato vetor (Walker et al., 1996; Dahlgren et al. 2011; Mansfield et al., 2017). Somente entre os anos de 2012 a 2016 foram relatados 6.786 novos casos em diferentes estados do país (Mogg et al., 2020). A doença já foi descrita também em Portugal (Morais et al., 1991), Venezuela (Martinez et al., 2008), Camarões (Ndip et al., 2009) e Sérvia (Arsic et al., 2014). Adicionalmente, evidências sorológicas de exposição à *E. chaffeensis* em humanos foram relatadas no Mali, Coréia do Sul, Sudeste Asiático, Peru e Brasil, mas não houve comprovação por métodos moleculares, uma vez que apenas resultados sorológicos podem apresentar reatividade cruzada com *A. phagocytophilum* (Calic et al. 2004; Costa et al., 2005, 2006; Thomas et al., 2009).

Há registros de infecção em outras espécies de animais, tais como cães, bovinos, cabras, raposas (*Vulpes vulpes*), coiotes (*Canis latrans*), cervos-sika-do-sul-do-Japão (*Cervus nippon*) e roedores (Wen et al., 2003; Kim et al., 2006; de Los Santos et al., 2007; André, 2018; Lee et al., 2009; Doudier et al., 2010).

Nos Estados Unidos, carrapatos da espécie *Amblyomma americanum* e veados-de-cauda-branca são considerados os principais vetores e reservatórios de *E. chaffeensis*, respectivamente (Rar e Golovljova, 2011). DNA do agente em questão já foi encontrado em *Dermacentor variabilis, I. pacificus*, *H. longicornis*, *I. persulcatus*, *R. sanguineus*, *A. testudinarum*, *Haemaphysalis yeni*, *A. parvum*, que podem estar relacionados com a transmissão em outras regiões onde não há a presença de *A. americanum* (Cao et al., 2000; Kim et al., 2003; Walker et al., 2004; Lee et al., 2005; Tomassone et al., 2008; Ndip et al., 2010; Rar e Golovljova, 2011).

Os sinais clínicos e laboratoriais mais comumente observados em humanos são febre, dores de cabeça, mialgia, náuseas, leucopenia, neutrofilia, trombocitopenia e elevação da atividade das transaminases hepáticas (Doudier et al., 2010; Rar e Golovljova, 2011).

3.2.2.11. Ehrlichia ewingii

Ehrlichia ewingii foi reconhecida como uma nova espécie em 1992, sendo anteriormente descrita como uma cepa de *E. canis* (Anderson et al., 1992). Diferentemente das demais espécies, infecta neutrófilos, sendo responsável por causar uma zoonose denominada Erliquiose Granulocítica Humana (HGE), (Buller et al., 1999; Pritt et al., 2011). Pode acometer cães, causando a Erliquiose Granulocítica Canina (Anderson et al., 1992).

HGE ocorre na América do Norte, no centro-oeste dos Estados Unidos. A transmissão ocorre via picada do carrapato *A. americanum* (Anziani et al., 1990; Madison-Antenucci et al., 2020). Ao menos 22 casos da doença são registrados por ano no país (Madison-Antenucci et al., 2020). Cervídeos, principalmente *O. virginianus*, e cães são considerados reservatórios para o agente, porém já houve detecção em cabras (Yabsley et al., 2002; Thomas et al., 2009; Biggs et al., 2016).

A doença causada tanto em humanos como em cães é considerada mais moderada quando comparada àquela causada por *E. chaffeensis* e *E. canis*, respectivamente (Thomas et al., 2009; Rar e Golovljova, 2011). Em humanos, pacientes imunossuprimidos, como por exemplo portadores de HIV ou recém transplantados, possuem sintomatologia mais severa (Paddock et al., 2001). Os principais sintomas e achados laboratoriais são febre, dor de cabeça, náuseas,

mialgia, trombocitopenia acompanhada ou não de leucopenia e aumento das enzimas hepáticas (Buller et al., 1999; Yabsley et al., 2002; Bigg et al., 2016).

Além dos Estados Unidos, esse agente já foi detectado em cães e carrapatos *R. sanguineus* em Camarões (Ndip et al., 2007), em roedores e carrapatos da espécie *H. longicornis* na Coréia (Kim et al., 2006; Matsumoto et al., 2011).

3.2.2.12. Ehrlichia muris

Ehrlichia muris infecta macrófagos e foi isolada pela primeira vez de um roedor selvagem (*Eothnomys kageus*) no Japão, em 1983. A princípio, foi considerada um agente que afetava apenas murinos, causando esplenomegalia moderada (Wen et al., 1995).

Em 2009, uma nova amostra com potencial zoonótico (*E. muris* –like, atualmente denominada *E. muris* subsp. *euaclairensis*) foi detectada em humanos nos EUA, associada à febre, dor de cabeça, mialgia, trombocitopenia e linfopenia. Porém, até o momento, os únicos registros de casos da doença foram registrados nos estados de Wiscosin e Minnesota nos EUA (Pritt et al., 2009; Wormser e Pritt, 2015; Pritt et al., 2017; Xu et al., 2018).

Acredita-se que as principais espécies de carrapatos vetores de *E. muris* sejam *Haemaphysalis flava* no Japão, *I. persulcatus* no leste Europeu e *I. ricinus* na Europa ocidental (Pritt et al., 2017). Diferentes espécies de roedores são incriminadas como possíveis reservatórios, sendo elas *Apodemos speciosus*, *A. argenteus, A. flavicollis* e *E. kageus* (Kawahara et al.,1996, 1999; Smetanová et al., 2007). Evidências sorológicas de exposição a este agente foram encontras em humanos, javalis, cães, cervídeos, ursos e macacos no Japão, mas reações cruzadas não podem ser descartadas (Kawahara et al., 1999; Pritt et al., 2017).

Nos EUA, *E. muris* subsp. *euaclairensis* possui como vetor e reservatório *I. scapularus* e camundongos de patas-brancas (*Peromycus leucopus*), respectivamente (Telford et al., 2011; Stromdahl et al., 2014; Wormser e Pritt, 2015; Saito e Walker, 2015; Lynn et al., 2015, 2017).

Adicionalmente, DNA de *E. muris* foi detectado em outras espécies de carrapatos, tais como *I. cookei* nos EUA (Xu et al., 2018) e *I. pavlovskyi* na Sibéria

(Rar et al., 2017). Além disso, um cão mostrou-se positivo em Minessota (Hegarty et al., 2012).

3.2.2.13. Ehrlichia ruminantium

Ehrlichia ruminantium infecta células endoteliais, neutrófilos e macrófagos, e descrita pela primeira vez em 1925 (Cowdry, 2005a, 2005b; Allsopp, 2010). É responsável por causar uma doença denominada *Heartwater* ou *Cowdriosis* em ruminantes, com ocorrência apenas na África Sub-Saariana e em ilhas do Caribe (Jongeian e Uilenberg, 2004).

É transmitida por dez espécies de carrapatos do gênero *Amblyomma*, sendo as principais *A. variegatum* e *A. hebraeum*. Sua distribuição está relacionada com a distribuição dos carrapatos vetores. DNA deste agente já foi detectado em *R. microplus*, mas não houve comprovação vetorial (Biguezoton et al., 2016). Adicionalmente, pode ocorrer transmissão vertical, caso o bezerro ingira leucócitos infectados no colostro (Deem et al., 1996). Ruminantes domésticos e selvagens são considerados os principais reservatórios do agente (Rar e Golovljova, 2011).

A doença pode ocorrer de quatro formas: hiperaguda, aguda, subaguda e subclínica. A hiperaguda é uma forma rara, caracterizada por febre, dispneia, convulsões, diarreia e morte. A forma mais comum é a aguda, em que o animal apresenta febre, inapetência, depressão, apatia, tosse úmida, mucosas cianóticas, dispneia e sinais neurológicos, sendo que a morte ocorre em até uma semana do início dos sinais. Na forma subaguda, há relatos de febre prolongada, tosse e incoordenação; os animais podem se recuperar ou vir a óbito em até duas semanas. Já a forma subclínica é mais branda e ocorre em animais com boa imunidade (Kasari et al., 2010; Saito e Walker, 2016).

Como o agente possui tropismo pelas células endoteliais, os danos provocados nos capilares causam aumento da permeabilidade levando à disfunção cardíaca e respiratória, que pode ocasionar hidropericárdio, hidrotórax e edema cerebral (Allsopp, 2015; Saito e Walker, 2016).

E. ruminantium já foi detectada em cães sintomáticos e em humanos que vieram a óbito por decorrência da doença, o que demonstra seu potencial zoonótico (Allsopp e Allsopp, 2001; Allsopp, 2015; Louw et al., 2005).

3.2.2.14. Ehrlichia minasensis

Ehrlichia minasensis foi descrita pela primeira vez em 2016, isolada a partir de carrapatos *R. microplus* encontrados em bovinos de Minas Gerais (Cabezas-Cruz et al., 2016). Possui alta homologia com *E. canis* (de Aguiar et al., 2019). Bovinos infectados podem apresentar febre, depressão, letargia e trombocitopenia (de Aguiar et al., 2014; Carvalho et al., 2016; de Aguiar et al., 2019).

Vetores e reservatórios ainda não estão ainda bem definidos. Essa bactéria já foi detectada em cervídeos (Gajadhar et al., 2010; Lobanov et al., 2012), bovinos (Gadjahar et al., 2010; de Aguiar et al., 2014; Hailemariam et al., 2017) Ainda, vem sendo detectada em diversas espécies de carrapatos: *R. microplus*, *R. sanguineus* (Cabezas-Cruz et al., 2012; Carvalho et al., 2016; Iweiebor et al., 2017; Cicculli et al., 2019), *Hyalomma* spp. (Cicculli et al., 2019; Rehman et al., 2019) e *Haemaphysalis hystricis* (Li et al., 2019). Até o momento, transmissão transestadial de *E. minasensis* foi comprovada somente em *R. microplus* (Carvalho et al., 2016).

3.2.2.15. '*Candidatus* Neoehrlichia'

'*Candidatus* Neoehrlichia mikurensis' foi detectada pela primeira vez em células endoteliais de baços de ratos (*Rattus novergicus)* e em seus ectoparasitas (*Ixodes ovatus*) no Japão (Kawahara, 2004). Em 2010, foi detectada em um humano com leucemia linfocítica crônia na Suécia, este apresentava febre, exantema e complicações tromboembólicas (Wellinder-Olsson, et al., 2010). Posteriormente, o mesmo agente foi detectado em humanos assintomáticos e sintomáticos apresentando alguma doença imunossupressora concomitante em outros países europeus e na China. Os principais sintomas da infecção por esse agente incluem febre, mialgia, exantema e vasculite inflamatória sistêmica culminando em trombose, tromboembolismo e aneurisma (Fehr et al., 2010; von Loewenich et al., 2010; Pekova et al., 2011; Li et al., 2012; Grankvist et al., 2014; Welc-Falęciak et al., 2014; Andréasson et al., 2015; Quarsten et al., 2017).

O referido agente possui como reservatório diversas espécies de roedores, com comprovação de transmissão transplacentária nesses animais (Burri et al., 2014; Obiegala et al., 2014). Os possíveis vetores são carrapatos das espécies *Ixodes*

ricinus e *I. persulcatus*, mas já hove detecção em *I. hexagonus, I. trianguliceps, I. frontalis* e *Dermacentor reticulatus* (Rar e Golovljova, 2011; Silaghi et al., 2016; Portilo et al., 2018).

Além de roedores, '*Candidatus* Neoehrlichia mikurensis' vem sendo detectada em ursos-europeus (*Ursus arctos arctos*), texugo-europeu (*Meles meles),* camurça (*Rupicapra rupicapra*), muflão (*European mouflons*), ouriços (*Erinaceus roumanicus*) e cães domésticos (Diniz et al., 2011; Beck et al., 2014; Földva´ri et al., 2014; Hofmann-Lehmann et al., 2016; Hornok et al., 2018).

Por outro lado, '*Candidatus* Neoehrlichia lotoris' foi detectada em guaxinins (*Procyon lotoris)* apenas nos EUA (Munderloh et al., 2007; Yabsley et al., 2008). Testes experimentais provaram que roedores e coelhos são refratários à infecção (Yabsley et al., 2008). Não há informações acerca de vetores e ocorrência em outros animais.

Adicionalmente, novos candidatos a espécies filogeneticamente relacionados com "*Candidatus* N. lotoris' vem sendo descritos em diferentes animais e localizaçãoes. 'Candidatus Neoehrlichia sp.'. (FU98) foi detectado em raposas (*Vulpes vulpes*), texugo-europeu e guaxinins na Europa (Hodži´c et al. 2015; Hornok et al., 2017; Hodži´c et al., 2017; Hodži´c et al., 2018). '*Candidatus* Neoehrlichia australis' e '*Candidatus* Neoehrlichia arcana' foram detectados em *Ixodes holocyclus* na Austrália (Gofton et al., 2016). Mais recentemente, '*Candidatus* Neoehrlichia chilensis' foi detectado em roedores selvagens (*Abrothrix* sp. e *Mus musculus*) e em *Ixodes* sp. *sigelos* no Chile (Müller et al., 2018; Muñoz-Leal et al., 2019).

3.2.3. Ocorrência de Agentes Anaplasmataceae em Animais Selvagens no Brasil

É crescente o números de estudos acerca da detecção de agentes Anaplasmataceae em animais selvagens no país. Tais trabalhos auxiliam na investigação de espécies já descritas e novos genótipos de agentes transmitidos por vetores que circulam no Brasil, bem como, permite um melhor entendimento da epidemiologia e na identificação de possíveis reservatórios e vetores.

3.2.3.1. Anaplasma spp.

Anaplasma spp. filogeneticamente próximo a *A. phagocytophilum* foi detectado com base no gene 16S rRNA em um carcará (50%[1/2]) (*Caracara plancus*) e urubus (40%[2/5]) (*Coragyps atratus*) no estado de São Paulo (Machado et al., 2012); em gatos-do-mato-pequeno (16% [4/25]) (*Leopardus tigrinus*) oriundos de zoológicos do estado de São Paulo (André et al., 2012); em caititus (100% [3/3]) (*Pecari tajacu*) e queixadas (100%[11/11]) (*Tayassu pecari*) nos estados de Mato Grosso e Pará (Soares et al., 2017); em gansos-do-Orinoco (4,84% [3/62]) (*Neochen jubata*) em Goiás (Werther et al., 2017) e em um jacuguaçu (50%[1/2]) (*Penelope obscura*) amostrado no Paraná (Mongruel et al., 2017). Adicionalmente, foi detectado com base no gene *groESL* em um veado-catingueiro (50%[1/2]) (*Mazama gouazoubira*) no estado do Paraná (Mongruel et al., 2016)

Anaplasma marginale foi detectado com base no gene *msp-5* em cervos-do-Pantanal (57,14% [4/7]) (*Blastocerus dichotomus*) na Usina Hidroelétrica Porto Primavera entre os estados de São Paulo e Mato Grosso do Sul (Machado et al., 2006) e em veados-campeiro (*Ozotocerus bezoarticus leucogaster*) no Pantanal Sul-Matogrossense (Picoloto et al., 2010). Com base nos genes *msp4* e *msp1α,* foi encontrado em cervos-do-Pantanal (75%[3/4]) e veados-catingueiro (80%[20/25]) em Minas Gerais (Silveira et al., 2012).

Sequências 16S rRNA de *Anaplasma* spp. filogeneticamente próximo a *Anaplasma platys* foram detectadas em cervos-do-Pantanal na Usina Hidroelétrica Porto Primavera (Sacchi et al., 2012).

No Pantanal sul-matogrossense, genótipos 16S rRNA de *Anaplasma* spp. filogeneticamente próximos a *A. bovis* e *A. phagocytophilum* foram detectados em quatis (22,58% [7/31]), um cachorro-do-mato (1,28%[1/78]) e em roedores (4,54%[5/110]) (*T. fosteri, C. laticeps, G. agilis, T. macrurus*) (de Sousa et al., 2017). Adicionalmente, genótipos 16S rRNA de *Anaplasma* spp. filogeneticamente próximos a *A. phagocytophilum*, *A. odocoilei* e a uma sequência detectada em um espécime de *Hylaeamys megacephalus* foram detectados em roedores (1,97%[9/458]) (*Rattus rattus, Akodon mostensis, Sphiggurus villosus, Calomys cerqueirae*) amostrados nos biomas Caatinga e Mata Atlântica (Benevenute et al., 2017).

Por fim, Soares et al. (2017) detectaram duas sequências 16S rRNA de *Anaplasma* sp. filogeneticamente relacionadas *A. phagocytophilum* e a *A. bovis* em veados mateiros (*Mazama americana*) amostrados em Mato Grosso (100% [1/1]) e Pará (50% [1/2]), respectivamente.

3.2.3.2. Ehrlichia spp.

Estudos baseados nos genes 16S rRNA, *groESL* e *dsb*, mostraram a presença de *Ehrlichia* sp. filogeneticamente próxima a *E. chaffeensis* em cervos-do-Pantanal (*Blastocerus dichotomus*), na proximidade da usina hidrelétrica Porto Primavera, no rio Paraná, com positividades de 42,85% (3/7) (Machado et al., 2006) e 42,65% (61/143) (Sacchi et al., 2012); e em veados-catingueiros (*Mazama gouazoubira*) (4%[1/25]) no estado de Minas Gerais (Silveira et al., 2012).

Sequências 16S rRNA de *Ehrlichia* spp. filogeneticamente próximas à *E. canis* foram detectadas em amostras de roedores (1,1%[5/428]) (*Trichomys laurentius*, *Holochilus fosteri*, *Clyomys laticeps* e *Calomys cerqueirai*) nos biomas Pantanal, Cerrado e Caatinga (Benevenute et al., 2017); em cachorros-do-mato (2,5%[2/78]) (*Cerdocyon thous*), quatis (3,2%[1/32]) (*Nasua nasua*) e roedores (13,64%[15/110]) (*Thrichomys fosteri, Oecomys mamorae, Gracilinamus agilis, Thylamys macrurus* e *Monodelphis domestica*) no Pantanal Sul-Matogrossense (de Sousa et al., 2017); em cutias (16,7%[4/24]) (*Dasyprocta azarae*) amostradas em São Luís, Maranhão (Braga et al., 2018); e em um gambá (2,7% [1/37]) (*Didelphis aurita*) no Rio de Janeiro (Guimarães et al., 2019).

Um novo genótipo de *Ehrlichia* spp. foi detectado em felídeos selvagens (7% [5/72]) (*Puma yagouaroudi*, *Leopardus tigrinus*, *Leopardus wiedii*) mantidos em cativeiro em zoológicos do estado de São Paulo e Brasília. Enquanto a filogenia pelo gene 16S rRNA mostrou proximidade dos genótipos encontrados com sequências de *E. canis*, a inferência filogenética pelo gene *omp-1* os posicionou em um clado distinto, entre sequências de *Ehrlichia* e *Anaplasma*, sugerindo a circulação de uma nova espécie de *Ehrlichia* sp. nesses animais (André et al., 2010).

André et al. (2012) detectaram a presença de sequências 16S rRNA de *Ehrlichia* spp. filogeneticamente relacionadas à *E. chafeensis* e *E. canis* em 15 e 16 carnívoros selvagens mantidos em cativeiro, respectivamente. Onze felídeos

(11,11%[11/99]) (*Leopardus tigrinus, Leopardus pardalis, Puma concolor, Panthera tigres, Puma yagouaroundi, Panthera leo*) e quatro canídeos (4%[4/100]) (*Cerdocyon thous, Canis lupus*) continham sequências 16S rRNA filogeneticamente próximas a *E. chaffeensis* em amostras sanguíneas. Ainda, 10 felídeos (10,1%) (*Puma concolor*, *Leopardus tigrinus, Panthera leo, Puma yagouaroundi, Leopardus pardalis)* e seis canídeos (6%) (*Speothos venaticus*, *Cerdocyon thous*) continham sequências 16S rRNA de *E. canis* em amostras sanguíneas.

Genótipos 16S rRNA de *Ehrlichia* spp. filogeneticamente próximos à *E. chaffeensis* e *E. canis* e um genótipo *omp-1* relacionado filogeneticamente com genótipos previamente detectados em felídeos selvagens no Brasil foram detectados em gansos-do-Orinoco (38,7% [24/62]) (*Neochen jubata*) amostrados no estado de Goiás (Werther et al., 2017) e em aves carnívoras (14,28[3/21]) (*Falcos sparverius*, *Coragyps atratus* e *Neochen jubata*) nos estados de Mato Grosso e São Paulo (Machado et al., 2012).

Com base em ensaios de PCR baseados nos genes 16S rRNA e *dsb*, uma provável nova espécie de *Ehrlichia* foi detectada em onças-pintadas (20%[2/10]) (*Panthera onca*) no Pantanal Sul-Matogrossense O novo genótipo encontrado alocou-se em um clado único, irmão ao clado de *E. ruminantium* (Widmer et al., 2011). Posteriormente, um estudo realizado por Almeida et al. (2013) detectou fragmentos de DNA de *Ehrlichia* spp, a partir dois genes supracitados em amostras de sangue de cachorros-do-mato (3,44%[2/58]) do Espírito Santo. Na filogenia, as sequências alocaram-se no mesmo clado da sequência previamente detectada em onças-pintadas. O mesmo resultado foi encontrado por Soares et al. (2017), em que sequências *dsb* detectadas em queixadas (27,27%[3/11]) (*Tayassu pecari*) nos estados de Mato Grosso e Pará, alocaram-se no mesmo clado descrito anteriormente.

Lopes et al. (2018) encontraram um novo genótipo *dsb* de *Ehrlichia* sp. a partir de uma amostra de baço de gambá (16,66%[1/6]) (*Didelphis albiventris*) amostrado no Rio Grande do Norte. Tal genótipo foi denominado *Ehrlichia* sp. strain Natal e alocou-se em um clado sozinho, irmão ao clado de *E. ewingii*.

3.2.4. Ocorrência de Anaplasmataceae em Xenarthra

Raros são os estudos acerca da ocorrência de agentes Anaplasmataceae em animais da Superordem Xenarthra. Guillemi et al. (2016) detectaram a presença de mórulas de *A. marginale* em esfregaço sanguíneo de um tamanduá-bandeira na Argentina. A identidade do agente foi confirmada por ensaios de PCR e sequenciamento baseados nos genes *msp-5* e *msp1-α*.

No Brasil, Soares et al. (2017) detectaram um novo genótipo *dsb* de *Ehrlichia* sp. em uma preguiças-de-três-dedos (*Bradypus tridactylus*) capturada no Pará. O referido genótipo foi alocado em um clado separado, irmão ao clado de *E. ruminantium* e próximo às sequências de *Ehrlichia* spp. detectadas em um equino no sul do Brasil (Vieira et al., 2016) e uma onça-pintada no Pantanal (Widmer et al, 2011). Essa mesma preguiça mostrou-se positiva em ensaio de PCR para agentes Anaplasmataceae baseado gene 16S rRNA. Embora a sequência tenha se alocado em clado próximo ao de *A. phagocytophilum*, os autores questionaram tratar-se de coinfecção ou algum erro de posicionamento filogenético, devido ao pequeno tamanho do fragmento gênico avaliado (300 pb).

4. REFERÊNCIAS

Acosta IC, Martins TF, Marcili A, Soares HS, Krawczak FS, Vieira FT, Labruna MB (2016) Ticks (Acari: Ixodidae, Argasidae) from humans, domestic and wild animals in the state of Espírito Santo, Brazil, with notes on rickettsial infection. **Veterinary Parasitology: Regional Studies and Reports** 3:66-69.

Aguiar DM et al. (2014). A novel *Ehrlichia* genotype strain distinguished by the TRP36 gene naturally infects cattle in Brazil and causes clinical manifestations associated with ehrlichiosis. **Ticks and Tick-borne Diseases** 5:537-544.

Aktas M, Altay K, Dumanli N, Kalkan A (2009). Molecular detection and identification of *Ehrlichia* and *Anaplasma* species in ixodid ticks. **Parasitology Research** 104:1243–1248.

Alanazi AD, Nguyen VL, Alyousif MS, Manoj RR, Alouffi AS, Donato R, Sazmand A, Mendonza-Roldan JA, Dantas-Torres F, Otranto D (2020) Ticks and associated pathogens in camels (*Camelus dromedarius*) from Riyadh Province, Saudi Arabia. **Parasites & Vectors** 13:1-9.

Almeida AP, Souza TD, Marcili A, Labruna MB (2013) Novel *Ehrlichia* and *Hepatozoon* agents infecting the Crab-Eating Fox (*Cerdocyon thous*) in Southeastern Brazil. **Journal of Medical Entomology** 50:640-646.

Allsopp MT, Allsopp BA (2001) Novel *Ehrlichia* genotype detected in dogs in South Africa. **Journal of Clinical Microbiology** 39:4204–4207.

Allsopp BA (2010) Natural history of *Ehrlichia ruminantium*. **Veterinary Parasitology** 167:123–135.

Allsopp BA (2015) Heartwater- *Ehrlichia ruminantium* infection. **Revue Scientifique et Technique (International Office of Epizootics)** 34:557-568.

Alves CSP, Mourão G (2006). Responses of a specialized insectivorous mammal (*Myrmecophaga tridactyla*) to variation in ambient temperature. **Biotropica** 38:52-56.

Amer S, Kim S, Yun Y, Na KJ (2019) Novel variants of the newly emerged *Anaplasma capra* from Korean water deer (*Hydropotes inermis argyropus*) in South Korea. **Parasites & vectors** 12:365.

Anderson BE, Dawson JE, Jones DC, Wilson KH (1991) *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. **Journal of Clinical Microbiology** 29:2838–2842.

Anderson BE, Greene CE, Jones DC, Dawson JE (1992) *Ehrlichia ewingii*. sp. Nov., the etiologic agent of canine granulocytic ehrlichiosis. **International Journal of Systematic Bacteriology** 42:299–302.

Andréasson K, Jönsson G, Lindell P, Gülfe A, Ingvarsson R, Lindqvist E, Marsal J (2015). Recurrent fever caused by *Candidatus* Neoehrlichia mikurensis in a rheumatoid arthritis patient treated with rituximab. **Rheumatology (Oxford, England)** 54:369.

André MR, Adania CH, Machado RZ, Allegreti SM, Felippe PAN, Silva KF, Nakaghi ACH (2010) Molecular detection of *Ehrlichia* spp. in endangered Brazilian wild captive felids. **Journal of Wildlife Diseases** 46:1017-1023.

André MR, Dumler JS, Scorpio DG, Teixeira RHF, Allegretti SM, Machado RZ (2012) Molecular detection of tick-borne bacterial agents in Brazilian and exotic captive carnivores. **Ticks and Tick-Borne Diseases** 3: 247-253.

André MR (2018) Diversity of Anaplasma and *Ehrlichia*/*Neoehrlichia* agents in terrestrial wild carnivores worldwide: implications for human and domestic animal health and wildlife conservation. **Frontiers in Veterinary Science** 5:293.7

Anziani OS, Ewing SA, Barker RW (1990) Experimental transmission of a granulocytic form of the tribe Ehrlichieae by *Dermacentor variabilis* and *Amblyomma americanum* to dogs. **American Journal of Veterinary Research** 51:929–931.

Arraga-Alvarado CM, Qurollo BA, Parra OC, Berrueta MA, Hegarty BC, Breitschwerdt EB (2014) Case report: molecular evidence of *Anaplasma platys* infection in two women from Venezuela. **The American Journal of Tropical Medicine and Hygiene** 91:1161–1165.

Arsić B, Gligić A, Ristanović E, Lako B, Potkonjak A, Peruničić M, Pavlović M (2014) A case of human monocytic ehrlichiosis in Serbia. **Srpski arhiv za celokupno lekarstvo** 142:79-82.

Arzua M, Onofrio VC, Barros-Battesti DM (2005) Catalogue of the tick collection (Acari, Ixodida) of the Museu de História Natural Capão da Imbuia, Curitiba, Paraná, Brazil. **Revista Brasileira de Zoologia** 22:623-632.

ASASG (2020) Os xenartros. Disponível em: <https://www.xenarthrans.org/pt/especies-2/> Acesso em:03 abr. 2020.

Atif FA (2016) *Alpha proteobacteria* of genus *Anaplasma* (Rickettsiales: Anaplasmataceae): epidemiology and characteristics of *Anaplasma* species related to veterinary and public health importance. **Parasitology** 143:659-685.

Bakken JS, Dumler JS (2000) Human granulocytic ehrlichiosis. **Clinical Infectious Diseases** 31:554–560.

Battilani M, de Arcangeli S, Balboni A, Dondi F (2017). Genetic diversity and molecular epidemiology of *Anaplasma*. **Infection, Genetics and Evolution** 49:195-211.

Bechara GH, Szabó MPJ, Duarte JMB, Matushima ER, Pereira MC, Rechav Y, Keirans JE, Fielden LJ (2000) Ticks associated with wild animals in the Nhecolândia Pantanal, Brazil. **Annals of the New York Academy of Sciences** 916:289-297.

Beck R, C ˇ ubric´ C ˇ urik V, Racˇic´ I, S ˇ prem N, Vujnovic´ A (2014) Identification of *Candidatus* Neoehrlichia mikurensis and *Anaplasma* species in wildlife from Croatia. Proceedings of the 1st Conference on Neglected Vectors and Vector-Borne Diseases (EurNegVec) Cluj-Napoca **Parasites & Vectors**, 7(Suppl 1): O28.

Belkahia H, Said MB, Ghribi R, Selmi R, Asker AB, Yahiaoui M, Bousrih M, Daaloul-Jedidi, Messadi L. (2019). Molecular detection, genotyping and phylogeny of *Anaplasma* spp. in *Rhipicephalus* ticks from Tunisia. **Acta tropica** 191:38-49.

Benevenute JL et al. (2017) Assessment of a quantitative 5'nuclease real-time polymerase chain reaction using *groEL* gene for *Ehrlichia* and *Anaplasma* species in rodents in Brazil. **Ticks and Tick-Borne Diseases** 8:646-656.

Ben Said M, Belkahia H, El Mabrouk N, Saidani M, Alberti A, Zobba R, Cherif A, Mahjoub T, Bouattour A, Messadi L (2017) *Anaplasma platys*-like strains in ruminants lifrom Tunisia. **Infection, Genetics and Evolution** 49:226–233.

Ben Said M, Belkahia H, Messadi L (2018) *Anaplasma* spp. in North Africa: a review on molecular epidemiology, associated risk factors and genetic characteristics. **Ticks and Tick Borne Disease** 9:543-555.

Berggoetz M, Schmid M, Ston D, Wyss V, Chevillon C, Pretorius AM, Gern L (2014) Tick-borne pathogens in the blood of wild and domestic ungulates in South Africa: interplay of game and livestock. **Ticks and Tick Borne Diseases** 5:166–175.

Bertassoni A (2017). **Ecologia espacial e populacional de tamanduá-bandeira (*Myrmecophaga tridactyla*) em estação ecológica de domínio cerrado, São Paulo, Brasil.** 127 f. Tese (Doutorado em Biologia Animal) – UNESP, São José do Rio Preto.

Bevan LEW (1912) Anaplasmosis of sheep. **The Veterinary Journal** 68:400–401.

Biggs HM et al. (2016) Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, ehrlichioses, and anaplasmosis—United States. **MMWR Recommendations and Reports** 65:1– 44.

Biguezoton A et al. (2016) *Ehrlichia ruminantium* infects *Rhipicephalus microplus* in West Africa. **Parasites & Vectors** 9:354

Botelho JR, Linardi PM, Encarnação CD (1989) Interrelações entre Acari Ixodidae e hospedeiros Edentata da Serra da Canastra, Minas Gerais, Brasil. **Memórias do Instituto Oswaldo Cruz** 84:61-64.

Bouza-Mora L, Dolz G, Solórzano-Morales A, Romero-Zuñiga JJ, Salazar-Sánchez L, Labruna MB, Aguiar DM (2017) Novel genotype of *Ehrlichia canis* detected in samples of human blood bank donors in Costa Rica. **Ticks and tick-borne diseases** 8:36-40.

Bouzouraa T, René-Martellet M, Chêne J, Attipa C, Lebert I, Chalvet-Monfray K, Cadoré JL, Halos L, Chabanne L (2016) Clinical and laboratory features of canine *Anaplasma platys* infection in 32 naturally infected dogs in the Mediterranean basin. **Ticks and Tick Borne Diseases** 7:1256-1264.

Bown KJ, Begon M, Bennett M, Woldehiwet Z, Ogden NH (2003) Seasonal dynamics of *Anaplasma phagocytophila* in a rodent-tick (*Ixodes trianguliceps*) system, United Kingdom. **Emerging Infecious Diseases** 9:63–70.

Bradfield JF, Vore SJ, Pryor WH Jr (1996) *Ehrlichia platys* infection in dogs. **Laboratory Animal Science** 46:565–568.

Braga FG (2010). **Ecologia e comportamento de tamanduá-bandeira *Myrmecophaga tridactyla* Linnaeus, 1758 no município de Jaguariaíva, Paraná**. 104f. Tese (Doutorado em Ciências Florestais) – Universidade Federal do Paraná, Curitiba.

Braga MSCO, Pereira JG, Fernandes SJ, Marques ICL, Jesus RP, Ferreira GS, Xavier DR, Benevenute JL, Machado RZ, André MR (2018) Molecular detection of Anaplasmataceae agents in *Dasyprocta azarae* in northeastern in Brasil. **Revista Brasileira de Parasitologia Veterinária** 27:99-105.

Breitschwerdt EB, Hegarty BC, Qurollo BA, Saito TB, Maggi RG, Blanton LS, Bouyer DH (2014) Intravascular persistence of *Anaplasma platys*, *Ehrlichia chaffeensis*, and

Ehrlichia ewingii DNA in the blood of a dog and two family members. **Parasites & Vectors** 7:298.

Brown GK, Martin AR, Roberts TK, Dunstan RH (2005) Molecular detection of *Anaplasma platys* in lice collected from dogs in Australia. **Australian Veterinary Journal** 83:101-102.

Buller RS et al. (1999) *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. **The New England Journal of Medicine** 15:148–155.

Burkot TR, Maupin GO, Schneider BS, Denatale C, Happ CM, Rutherford JS, Zeidner NS (2001) Use of a sentinel host system to study the questing behavior of *Ixodes spinipalpis* and its role in the transmission of *Borrelia bissettii*, human granulocytic ehrlichiosis, and *Babesia microti*. **The American Journal of Tropical Medicine and Hygiene** 65:293– 299.

Burri C, Schumann O, Schumann C, Gern L (2014). Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi*, *Candidatus* Neoehrlichia mikurensis, *Rickettsia helvetica*, *R. monacensis* and *Anaplasma phagocytophilum*?. **Ticks and tick-borne diseases** 5:245-251.

Cabezas-Cruz A, Zweygarth E, Ribeiro MFB, da Silveira JAG, de la Fuente J, Grubhoffer L, Valdés JJ, Passos LMF (2012) New species of *Ehrlichia* isolated from *Rhipicephalus (Boophilus) microplus* shows an ortholog of the *E. canis* major immunogenic glycoprotein gp36 with a new sequence of tandem repeats. **Parasites & Vectors** 5:291.

Cabezas-Cruz A, Zweygarth E, Vancova M, Broniszewska M, Grubhoffer L, Passos LMF, Ribeiro MFB, Alberdi P, de la Fuente J (2016) *Ehrlichia minasensis* sp. nov., isolated from the tick *Rhipicephalus microplus*. **International Journal of Systematic and Evolutionary Microbiology** 66:1426–1430.

Cabezas-Cruz A et al. (2019). Epidemiology and genetic diversity of *Anaplasma ovis* in goats in Corsica, France. **Parasites & vectors** 12:3.

Calic SB, Galvão MA, Bacellar F, Rocha CM, Mafra CL, Leite RC, Walker DH (2004) Human ehrlichioses in Brazil: first suspect cases. **Brazilian Journal of Infectious Diseases** 8:259-262.

Campos-Calderon L, Abrego-Sanchez L, Solorzano-Morales A, Alberti A, Tore G, Zobba R, Jimenez-Rocha AE, Dolz G (2016) Molecular detection and identification of Rickettsiales pathogens in dog ticks from Costa Rica. **Ticks and Tick Borne Diseases** 7:1198–1202.

Cançado PHD, Faccini JLH, Mourão GDM, Piranda EM, Onofrio VC, Barros-Battesti DM (2017) Current status of ticks and tick-host relationship in domestic and wild animals from Pantanal wetlands in the state of Mato Grosso do Sul, Brazil. **Iheringia. Série Zoologia** 107:1-8

Cao WC, Gao YM, Zhang PH, Zhang XT, Dai QH, Dumler JS, Fang LQ, Yang H (2000) Identification of *Ehrlichia chaffeensis* by nested PCR in ticks from Southern China. **Journal of Clinical Microbiology** 38:2778–2780.

Cao WC, Zhan L, He J, Foley JE, De Vlas SJ, Wu XM, Yang H, Richardus JH, Habbema JD (2006) Natural *Anaplasma phagocytophilum* infection of ticks and rodents from a forest area of Jilin Province, China. **The American Journal of Tropical Medicine and Hygiene** 75:664–668.

Cardoso L, Gilad M, Cortes HCE, Nachum-Biala Y, Lopes AP, Vila-Viçosa MJ, Simões M, Rodrigues PA, Baneth G (2015) First report of *Anaplasma platys* infection in red foxes (*Vulpes vulpes*) and molecular detection of *Ehrlichia canis* and *Leishmania infantum* in foxes from Portugal. **Parasites & Vectors** 8:144.

Carvalho IT et al. (2016) Minimum infection rate of *Ehrlichia minasensis* in *Rhipicephalus microplus* and *Amblyomma sculptum* ticks in Brazil. **Ticks and Tick Borne Diseases** 7:849-852.

Carlini AA, Zurita AE, Aguilera OA (2008). North American Glyptodontines (Xenarthra, Mammalia) in the Upper Pleistocene of northern South America. **Paläontologische Zeitschrift** 82:125.

Cassano CR (2006). **Ecologia e conservação da preguiça-de-coleira (*Bradypus torquatus* Illiger, 1811) no sul da Bahia**. 106f. Dissertação (Mestrado em Zoologia) - Universidade Federal de Santa Cruz, Ilhéus.

Catapani, M. L. (2014). **Comportamento de tamanduá-mirim, Tamandua tetradactyla (Linnaeus, 1758)(Pilosa, Myrmecophagidae) em condições de cativeiro: implicações ao bem-estar**. 53f. Dissertação (Mestrado em Ecologia e Recursos Naturais – Universidade Federal de São Carlos, São Carlos.

Chen SM, Dumler JS, Bakken JS, Walker DH (1994) Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. **Journal of Clinical Microbiology** 32:589–595.

Chien NTH, Nguyen TL, Bui KL, Nguyen TV, Le TH (2019) *Anaplasma marginale* and *A. platys* characterized from dairy and indigenous cattle and dogs in Northern Vietnam. **Korean Journal of Parasitology** 57:43-47.

Chisu V, Zobba R, Lecis R, Sotgiu F, Masala G, Foxi C, Pisu D, Alberti A (2018) *GroEL* typing and phylogeny of *Anaplasma* species in ticks from domestic and wild vertebrates. **Ticks and Tick Borne Diseases** 9:31–36.

Chochlakis D, Ioannou I, Sharif L, Kokkini S, Hristophi N, Dimitriou T, Tselentis Y, Psaroulaki A (2009) Prevalence of *Anaplasma* sp. in goats and sheep in Cyprus. **Vector Borne Zoonotic Diseases** 9:457–463.

Cicculli V, Masse S, Capai L, de Lamballerie X, Charrel R, Falchi A (2019) First detection of *Ehrlichia minasensis* in *Hyalomma marginatum* ticks collected from cattle in Corsica, France. **The Journal of Veterinary Medical Science** 5:243-248.

Clark KL (2012) *Anaplasma phagocytophilum* in small mammals and ticks in northeast Florida. **Journal of Vector Ecology** 37:262–268.

Codner EC, Farris-Smith LL (1986) Characterization of the subclinical phase of ehrlichiosis in dogs. **Journal of the American Veterinry Medical Association** 189*:47*-50.

Codner EC, Maslin W (1992) Investigation of renal protein loss in dogs with acute experimentally induced *Ehrlichia canis* infection. **American Journal of Veterinary Research** 53:264-9.

Coelho WMD (2016) Prevalência de Ixodídeos em animais silvestres atropelados nas rodovias do interior paulista. **Revista Conexão Eletrônica** 13:1163-1166.

Cohn LA (2003) Ehrlichiosis and related infections. **Veterinay Clinics of North America: Small Animal Practice** 33:863–884.

Costa PSGD, Brigatte ME, Greco DB (2005) Antibodies to *Rickettsia rickettsii, Rickettsia typhi, Coxiella burnetii, Bartonella henselae, Bartonella quintana*, and *Ehrlichia chaffeensis* among healthy population in Minas Gerais, Brazil. **Memórias do Instituto Oswaldo Cruz** 100:853-859.

Costa PSG, Valle LMDC, Brigatte ME, Greco DB (2006) More about human monocytotropic ehrlichiosis in Brazil: serological evidence of nine new cases. **Brazilian Journal of Infectious Diseases** 10:7-10.

Couto CG (1998) Doenças Rickettsiais In: Birchard, Sherdig. **Manual Saunders: Clínica de pequenos animais**. São Paulo:Roca, p.139-142.

Cowdry EV (1925a) Studies on the etiology of heartwater. 1. Observation of a rickettsia, *Rickettsia ruminantium* (n. sp.), in the tissues of infected animals. **Journal of Experimental Medicine** 42:231–252.

Cowdry EV (1925b) Studies on the etiology of heartwater. 2. *Rickettsia ruminantium*(n. sp.) in the tissues of ticks transmitting the disease. **Journal of Experimental Medicine** 42:253–274.

Dahmani, M., Davoust, B., Benterki, M.S., Fenollar, F., Raoult, D., Mediannikov, O., 2015. Development of a new PCR-based assay to detect Anaplasmataceae and the first report of *Anaplasma phagocytophilum* and *Anaplasma platys* in cattle from Algeria. Comp. Immunol. Microbiol. Infect. Dis. 39, 9–45.

Dahmani M, Davoust B, Sambou M, Bassene H, Scandola P, Ameur T, Raoult D, Fenollar F, Mediannikov O (2019) Molecular investigation and phylogeny of species of the Anaplasmataceae infecting animals and ticks in Senegal. **Parasites & Vectors** 12:1-15.

Dahlgren FS, Mandel EJ, Krebs JW, Massung RF, McQuiston JH (2011) Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000–2007. **The American Journal of Tropical Medicine and Hygiene** 85:124–131.

Dantas-Torres F, Ferreira DR, de Melo LM, Lima PAC, Siqueira DB, Rameh-de-Albuquerque L C, de Melo AV, Ramos JA (2010) Ticks on captive and free-living wild animals in northeastern Brazil. **Experimental and Applied Acarology** 50:181.

da Silva AS, Lopes LS, Diaz JD, Tonin AA, Stefani LM, Araújo DN (2013) Lice outbreak in buffaloes: evidence of *Anaplasma marginale* transmission by sucking lice *Haematopinus tuberculatus*. **Journal of Parasitology** 99:546-547.

Dawson JE, Anderson BE, Fishbein DB, Sanchez JL, Goldsmith CS, Wilson KH, Duntley CW (1991) Isolation and characterization of an *Ehrlichia* sp. from a patient diagnosed with human ehrlichiosis. **Journal of Clinical Microbiology** 29:2741-2745.

de Aguiar DM, Araújo Junior JP, Nakazato L, Bard E, Aguilar-Bultet L, Vorimore F, Popov VL, Colodel EM, Cabezas-Cruz A (2019) Isolation and Characterization of a Novel Pathogenic Strain of *Ehrlichia minasensis*. **Microorganisms** 7:528.

Deem SL, Norval RA, Donachie PL, Mahan SM (1996) Demonstration of vertical transmission of *Cowdria ruminantium*, the causative agent of heartwater, from cows to their calves. **Veteriary Parasitology** 61:119–132.

de la Fuente J, Ruiz-Fons F, Naranjo V, Torina A, Rodríguez O, Gortázar C, (2008) Evidence of *Anaplasma* infections in European roe deer (*Capreolus capreolus*) from southern Spain. **Research in Veterinary Science** 84:382–386.

de Los Santos JR et al. (2007) Experimental infection of dairy calves with *Ehrlichia chaffeensis*. **Journal of Medical Microbiology** 56:1660-1668.

Delsuc F, Catzeflis FM, StanhopeMJ, Douzery EJP (2001) The evolution of armadillos, anteaters, and sloths depicted by nuclear and mitochondrial phylogenies: implications for the status of the enigmatic fóssil Eurotamandua. **Proceedings of the Royal Society of London** 268:1605–1615.

Delsuc F, Vizcaíno SF, Douzery EJP (2004) Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. **BMC Evolutionary Biology** 4:1–13.

de Sousa KCM, Calchi AC, Herrera HM, Dumler JS, Barros-Battesti DM, Machado RZ, André MR (2017) Anaplasmataceae agentes among wild mammals and ectoparasites in Brazil. **Epidemiology & Infection** 145:3424-3437.

de Souza Ramos IA et al. (2019). Genetic diversity of *Anaplasma marginale* in beef cattle in the Brazilian Pantanal. **Ticks and Tick Borne Diseases** 10:805-814.

Des Vignes F, Fish D (1997) Transmission of the agent of human granulocytic ehrlichiosis by host-seeking *Ixodus scapularis* (Acari:Ixodidae) in southern New York state. **Journal of Medical Entomology** 34:379–382.

Diniz PP, Schulz BS, Hartmann K, Breitschwerdt EB (2011) '*Candidatus* Neoehrlichia mikurensis' infection in a dog from Germany. **Journal of clinical microbiology** 49:2059–2062.

Djiba ML, Mediannikov O, Mbengue M, Thiongane Y, Molez JF, Seck MT, Fenollar F, Raoult D, Ndiaye M (2013) Survey of *Anaplasmataceae* bacteria in sheep from Senegal. **Tropical Animal Health and Production** 45:1557–1561.

Donatien A, Lestoquard F (1936) *Rickettsia bovis*, nouvelle espèce pathogène pour le beouf. Bulletin de la Societe de Pathologie Exotique 29:1057-1061.

Doudier B, Olano J, Parola P, Brouqui P (2010) Factors contributing to emergence of *Ehrlichia* and *Anaplasma* spp. as human pathogens. **Veterinary Parasitology** 167:149-154.

Dugat T, Lagrée AC, Maillard R, Boulouis HJ, Haddad N (2015) Opening the black box of *Anaplasma phagocytophilum* diversity: current situation and future perspectives. **Frontiers in Cellular and Infection Microbiology** 5: 61:1-18.

Dumler JS, Barbet AF, Bekker CPJ, Dasch GA, Palmer GH, Stuart CR, Rikihisa Y, Rurangirwa FR (2001) Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales*: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six news species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. **International Journal of Systematic and Evolutionary Microbiology** 51:2145-2165.

Dumler JS, Choi KS, Garcia-Garcia JC, Barat NS, Scorpio DG, Garyu JW, Grab DJ, Bakken JS (2005) Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. **Emerging Infectious Diseases** 11:1828–1834.

Emmons L, Feer F (1997). **Neotropical rainforest mammals: a field guide**. Chicago:The University the Chicago Press, 307p.

Engelmann GF (1985) The phylogeny of the Xenarthra. In: Montgomery GG (Ed.) **The Evolution and Ecology of Armadillos, Sloths and Vermilinguas** Washington and London: Smithsonian Institution Press. p. 51-64.

Estrada-Peña A, Naranjo V, Acevedo-Whitehouse K, Mangold AJ, Kocan KM, de la Fuente J (2009) Phylogeographic analysis reveals association of tick-borne pathogen, *Anaplasma marginale*, *msp1α* sequences with ecological traits affecting tick vector performance. **BMC Biology** 57:1-13.

Ewing SA (1981) Transmission of *Anaplasma marginale* by arthropods. In: PROCEEDINGS OF THE SEVENTH NATIONAL ANAPLASMOSIS CONFERENCE Starkville:MS, p.425–434.

Fehr JS, Bloemberg GV, Ritter C, Hombach M, Lüscher TF, Weber R, Keller PM (2010). Septicemia caused by tick-borne bacterial pathogen *Candidatus* Neoehrlichia mikurensis. **Emerging infectious diseases** 16:1127.

Fernandes SJ, Matos CA, Freschi CR, de Souza Ramos IA, Machado RZ, André MR (2019) Diversity of *Anaplasma* species in cattle in Mozambique. **Ticks and Tick Borne Diseases** 10:651-664.

Fishbein DB, Sawyer LA, Holland CJ, Hayes EB, Okoroanyanwa W, Williams D, Sikes RK, Ristic M, McDade JE (1987) Unex·plained febrile illness after exposure to ticks. **JAMA** 257*:3100*-3104.

Földva´ri G, Jahfari S, Rigo´ K, Jablonszky M, Szekeres S, Majoros G, To´th M, Molna´r V, Coipan EC, Sprong H (2014) *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum* in urban hedgehogs. **Emerging Infectious Diseases** 20:496–498.

Foley JE, Nieto NC, Massung R, Barbet A, Madigan J, Brown RN (2009) Distinct ecologically relevant strains of *Anaplasma phagocytophilum*. **Emerging Infectious Diseases** 15:842–843.

Frazén P, Berg AL, Aspan A, Gunnarsson A, Pringle J (2007) Death of a horse infected experimentally with *Anaplasma phagocytophilum*. **The Veterinary Record** 160:122-125.

Gajadhar AA, Lobanov V, Scandrett WB, Campbell J, Al-Adhami B (2010) A novel *Ehrlichia* genotype detected in naturally infected cattle in North America. **Veterinary Parasitology** 173:324–329.

Garcia MV, Silva DCD, Almeida RFCD, Cunha RC, Matias J, Barros JC., Andreotti R, Szabó MPJ (2013). Environmentally associated ticks (Acari: Ixodidae) in campo grande, mato grosso do sul, Brazil. **Revista Brasileira de Parasitologia Veterinária** 22:124-128.

Gardner AL (2005) Order Pilosa. In: Wilson DE, Reeder DM (Ed) **Mammal Species of the World**. Baltimore: Johns Hopkins University Press p.94-99.

Gardner AL (Ed.) (2008). **Mammals of South America, volume 1: marsupials, xenarthrans, shrews, and bats** (Vol. 2). University of Chicago Press.

Gaudin TJ, Branham DG (1998) The phylogeny of the Myrmecophagidae (Mammalia, Xenarthra, Vermilingua) and the relationship of Eurotamandua to the Vermilingua. **Journal Mammal of Evolution** 5:237-265.

Gibb GC, Condamine FL, Kuch M, Enk J, Moraes-Barros N, Superina M, Poinar HN, Delsuc F. (2016). Shotgun mitogenomics provides a reference phylogenetic framework and timescale for living xenarthrans. **Molecular Biology and evolution** 33:621-642.

Gioia GV et al. (2018) Bovine anaplasmosis and tick-borne pathogens in cattle of the Galapagos Islands. **Transboundary and Emerging Diseases** 65:1262-1271.

Glass BP (1985) **History of classification and nomenclature in Xenarthra (Edentata). The Evolution and ecology of armadillos, sloths, and vermilinguas**. Washington and London:Smithsonian University Press p.51-64.

Gofton AW, Doggett S, Ratchford A, Ryan U, Irwin P (2016). Phylogenetic characterisation of two novel Anaplasmataceae from Australian *Ixodes holocyclus* ticks:'*Candidatus* Neoehrlichia australis' and '*Candidatus* Neoehrlichia arcana'. **International journal of systematic and evolutionary microbiology** 66:4256-4261.

Gonzalez IHL, Labruna MB, Chagas CRF, Salgado PAB, Monticelli C, Morais LH, Antunes TC, Ramos PL, Martins TF (2017) Ticks infesting captive and free-roaming wild animal species at the São Paulo Zoo, São Paulo, Brazil. **Revista Brasileira de Parasitologia Veterinária** 26:496-499.

Gordon WS, Brownlee A, Wilson DR (1940) Studies in louping-ill, tick-borne fever and scrapie In:3RD INTERNATIONAL CONGRESS OF MICROBIOLOGY. **Report of Proceedings** NewYork, p.362–363.

Grankvist A et al. (2014) Infections with the tick-borne bacterium '*Candidatus* Neoehrlichia mikurensis' mimic noninfectious conditions in patients with B cell malignancies or autoimmune diseases. **Clinical Infectious Diseases** 58:1716–1722.

Gregory C, Forrester SO (1990) *Ehrlichia canis, E.equi, E. risticci* infections. In: Greene CE **Infectious diseases of the dog and cat**. Philadelphia: W. B. Saunders, p.404-414.

Groves MG, Dennis GL, Amyx HL, Huxsoll DL (1975) Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). **American Journal of Veterinary Research** 36:937-940.

Guillemi EC et al. (2016). Molecular identification of *Anaplasma marginale* in two autochthonous South American wild species revealed an identical new genotype and its phylogenetic relationship with those of bovines. **Parasites & Vectors** 9:305.

Guimarães A, Raimundo JM, Silva ATD, Carpintero FM, Pires JR, Benevenute JL, Machado RZ, André MR, Baldani CD (2019) Detection of a putative novel genotype of *Ehrlichia* sp. from opossums (*Didelphis aurita*) from Brazil. **Revista Brasileira de Parasitologia Veterinária** 28:140-144.

Guo WP, Zhang B, Wang YH, Xu G, Wang X, Ni X, Zhou EM (2019) Molecular identification and characterization of *Anaplasma capra* and *Anaplasma platys*-like in *Rhipicephalus microplus* in Ankang, Northwest China. **BMC Infectious Diseases** 19:434.

Hailemariam Z, Krücken J, Baumann M, Ahmed JS, Clausen PH, Nijhof AM (2017) Molecular detection of tick-borne pathogens in cattle from southwestern Ethiopia. **PLoS One** 12:e0188248.

Harrus S, Perlman-Avrahami A, Mumcuoglu KY, Morick D, Eyal O, Baneth G (2010) Molecular detection of *Ehrlichia canis*, *Anaplasma bovis*, *Anaplasma platys*, *Candidatus* Midichloria mitochondrii and *Babesia canis vogeli* in ticks from Israel. **Clinical Microbiology and Infection** 17:459-463.

Harvey JW, Simpson CF, Gaskin JM (1978) Cyclic thrombocytopenia induced by a Rickettsia-like agent in dogs. **The Journal of Infectious Diseases** 137:182-188.

Hegarty BC, Maggi RG, Koskinen P, Beall MJ, Eberts M, Chandrashekar R, Breitschwerdt EB (2012) *Ehrlichia muris* infection in a dog from Minnesota. **Journal of Veterinary Internal Medicine** 26:1217-1220.

Hodžić A, Cézanne R, Duscher GG, Harl J, Glawischnig W, Fuehrer HP (2015). *Candidatus* Neoehrlichia sp. in an Austrian fox is distinct from *Candidatus* Neoehrlichia mikurensis, but closer related to *Candidatus* Neoehrlichia lotoris. **Parasites & vectors** 8:1-4.

Hodžić A, Mitkovà B, Modrý D, Juránková J, Frgelecová L, Forejtek P, Duscher GG (2017). A new case of the enigmatic *Candidatus* Neoehrlichia sp.(FU98) in a fox from the Czech Republic. **Molecular and Cellular Probes** 31:59-60.

Hodzic A, Mrowietz N, Cezanne R, Bruckschwaiger P, Punz S, Habler VE, Fuehrer HP (2018). Occurrence and diversity of arthropod-transmitted pathogens in red foxes (*Vulpes vulpes*) in western Austria, and possible vertical (transplacental) transmission of *Hepatozoon canis*. **Parasitology** 145:335-344.

Hofmann-Lehmann R, Wagmann N, Meli ML, Riond B, Novacco M, Joekel D, Carrapiço T. (2016). Detection of' *Candidatus* Neoehrlichia mikurensis' and other

Anaplasmataceae and Rickettsiaceae in Canidae in Switzerland and Mediterranean countries. **Schweizer Archiv für Tierheilkunde** 158:691-700.

Hornok S, Trauttwein K, Takács N, Hodžić A, Duscher GG, Kontschán J (2017). Molecular analysis of *Ixodes rugicollis*, *Candidatus* Neoehrlichia sp.(FU98) and a novel *Babesia* genotype from a European badger (*Meles meles*). **Ticks and tick-borne diseases** 8:41-44.

Hornok S, Horváth G, Takács N, Farkas R, Szőke K, Kontschán J (2018). Molecular evidence of a badger-associated *Ehrlichia* sp., a *Candidatus* Neoehrlichia lotoris-like genotype and *Anaplasma marginale* in dogs. **Ticks and tick-borne diseases** 9:1302-1309.

Hosseini-Chegeni A, Tavakoli M, Goudarzi G, Telmadarraiy Z, Sharifdini M, Faghihi F, Ghanbari M K (2020). Molecular Detection of *Anaplasma marginale* and *Anaplasma ovis* (Rickettsiales: Anaplasmataceae) in Ixodid Tick Species in Iran. **Archives of Razi Institute** 75:39-46.

Ismail N, Bloch KC, Mcbride JW (2010) Human ehrlichiosis and anaplasmosis. **Clinics in Laboratory Medicine** 30:261-292.

Inokuma H, Brouqui P, Drancourt M, Raolt D (2001) Citrate synthase gene sequence: a new tool for phylogenetic analysis and identification of *Ehrlichia*. **Journal of Clinical Microbiology** 39:3031-3039.

IUCN (2020) The red list. Disponível em:<https://www.iucnredlist.org/species/>. Acesso em:03 abr. 2020.

Iweriebor BC, Mmbaga EJ, Adegborioye A, Igwaran A, Obi LC, Okoh AI (2017) Genetic profiling for *Anaplasma* and *Ehrlichia* species in ticks collected in the Eastern Cape Province of South Africa. **BMC Microbiology** 17:45.

Johnson EM, Ewing SA, Barker RW, Fox JC, Crow DW, Kocan KM (1998) Experimental transmission of *Ehrlichia canis* (Rickettsiales: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). **Veterinary parasitology** 74:277-288.

Jongejan F, Uilenberg G (2004) The global importance of ticks. **Parasitology** 129:3–14.

Jouglin M, Blanc B, de la Cotte N, Bastian S, Ortiz K, Malandrin L (2019) First detection and molecular identification of the zoonotic *Anaplasma capra* in deer in France. **PloS One** 14: e0219184

Kang JG et al. (2018). Molecular detection of *Anaplasma*, *Bartonella*, and *Borrelia theileri* in raccoon dogs (*Nyctereutes procyonoides*) in Korea. **The American Journal of Tropical Medicine and Hygiene** 98:1061-1068.

Karbowiak G, Biernat B, Stanczak J, Werszko J, Wróblewski P, Szerwczyk T, Sytykiewicz H (2016). The role of particular ticks developmental stages in the circulation of tick-borne pathogens in Cental Europe. **Annals of Parasitology** 62:267-284.

Kasari TR, Miller RS, James AM, Freier JE (2010) Recognition of the threat of *Ehrlichia ruminantium* infection in domestic and wild ruminants in the continental United States. **Journal of the American Veterinry Medical Association** 237:520–530.

Kawahara M, Suto C, Shibata S, Futohashi M, Rikihisa Y (1996) Impaired antigen specific responses and enhanced polyclonal stimulation in mice infected with *Ehrlichia muris*. **Microbiology and Immunology** 40: 575–581.

Kawahara M, Ito T, Suto C, Shibata S, Rikihisa Y, Hara K, Hirai K (1999) Comparison of *Ehrlichia muris* strains isolated from wild mice and ticks and serologic evidence of humans and animals with *E. Muris* as antigen. **Journal of Clinical Microbiology** 37:1123–1129.

Kawahara M, Rikihisa Y, Isogai E, Takahashi M, Misumi H, Suto C, Tsuji M (2004). Ultrastructure and phylogenetic analysis of '*Candidatus* Neoehrlichia mikurensis' in the family Anaplasmataceae, isolated from wild rats and found in Ixodes ovatus ticks. **International journal of systematic and evolutionary microbiology** 54:1837-1843.

Kim CM, Kim MS, Park MS, Park JH, Chae JS (2003) Identification of *Ehrlichia chaffeensis, Anaplasma phagocytophilum*, and *A. bovis* in *Haemaphysalis longicornis* and *Ixodes persulcatus* ticks from Korea. **Vector Borne and Zoonotic Diseases** 3:17–26.

Kim CM et al. (2006) Tick-borne rickettsial pathogens in ticks and small mammals in Korea. **Applied and Environmental Microbiology** 72:5766-5776.

Kinlaw AL (1999). A review of burrowing by semi-fossorial vertebrates in arid environments. **Journal of Arid Environments** 41:127-145.

Kluyber D (2016) **Avaliação da prevalência de patógenos zoonóticos de importância para a saúde pública em tatus de vida livre – Mato Grosso do Sul.** 142p. Dissertação (Mestrado em Doenças Tropicais e Saúde Internacional) – USP, São Paulo.

Kluyber D, Martins TF, Desbiez AJ, Amorim M, Massocato G, Labruna MB (2016) Ticks (Acari: Ixodidae) infesting armadillos (Cingulata: Dasypodidae) in the Pantanal wetland, Mato Grosso do Sul, Brazil. **Systematic and applied acarology** 21:1087-1092.

Kocan KM, Goff WL, Stiller D, Claypool PL, Edwards W, Ewing SA, Hair JA, Barron SJ (1992) Persistence of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) in male *Dermacentor andersoni* (Acari: Ixodidae) transferred successively from infected to susceptible cattle. Journal of Medical Entomology 29:657–668.

Kocan KM, de la Fuente J (2003) Co-feeding studies of ticks infected with *Anaplasma marginale*. **Veterinary Parasitology** 112:295–305.

Kocan KM, de la Fuente F, Blouin EF, Coetzee JF, Ewing AS (2010) The natural history of *Anaplasma marginale*. **Veterinary Parasitology** 167:95-107.

Kocan KM, de la Fuente J, Cabezas-Cruz A (2015) The genus *Anaplasma*: new challenges after reclassification. **Revue Scientifique et Technique (International Office of Epizootics)** 34:577-586.

Koch PL, Barnosky AD (2006) Late quartenary extinctions: State of the debate. **Annual Review of Ecology, Evolution and Systematics** 37:215-250.

Koh FX, Kho KL, Kisomi MG, Wong LP, Bulgiba A, Tan PE, Lim YAL, Nizam QNH, Panchadcharam C, Tay ST (2017) *Ehrlichia* and *Anaplasma* infections: Serological evidence and tick surveillance in peninsular Malaysia. **Journal of Medical Entomology** 55:269-276.

Koh FX, Panchadcharam C, Sitam FT, Tay ST (2018) Molecular investigation of *Anaplasma* spp. in domestic and wildlife animals in Peninsular Malaysia. **Veterinary Parasitology: Regional Studies and Reports** 13:141-147.

Kohn B, Galke D, Beelitz P, Pfister K (2008) Clinical features of canine granulocytic anaplasmosis in 18 naturally infected dogs. **Journal of Veterinary Internal Medicine** 22:1289-1295.

Labruna MB, de Paula CD, Lima TF, Sana DA (2002) Ticks (Acari: Ixodidae) on wild animals from the Porto-Primavera hydroelectric power station area, Brazil. **Memórias do Instituto Oswaldo Cruz** 97:1133-1136.

Labruna MB, Camargo LMA, Terrassini FA, Ferreira F, Schumaker TT, Camargo EP (2005) Ticks (Acari: Ixodidae) from the state of Rondônia, western Amazon, Brazil. **Systematic and Applied Acarology** 10:17-32.

Lee SO, Na DK, Kim CM, Li YH, Cho YH, Park JH, Lee JH, Eo SK, Klein TA, Chae JS (2005) Identification and prevalence of *Ehrlichia chaffeensis* infection in *Haemaphysalis longicornis* ticks from Korea by PCR, sequencing and phylogenetic analysis based on 16S rRNA gene. **Journal of Veterinary Science** 6:151–155.

Lee M, Yu D, Yoon J, Li Y, Lee J, Park J (2009) Natural co-infection of *Ehrlichia chaffeensis* and *Anaplasma bovis* in a deer in South Korea. **Journal of Veterinary Medical Science** 71:101–103.

Lee M et al. (2018). Molecular detection and phylogenetic analysis of tick-borne pathogens in wild Korean water deer and farmed elk in Gyeongbuk and Gangwon Provinces of Korea. **Journal of Veterinary Medical Science** 80:1473-1478.

Li H et al. (2012) Human infection with *Candidatus* Neoehrlichia mikurensis, China. **Emerging infectious diseases** 18:1636–1639.

Li H et al. (2015) Human infection with a novel tick-borne Anaplasma species in China: a surveillance study. **The Lancet Infectious Disease** 15:663-670.

Li, Y et al. (2015b) Anaplasma infection of Bactrian camels (*Camelus bactrianus*) and ticks in Xinjiang, China. **Parasites & Vectors** 8:313.

Li J, Liu X, Mu J, Yu X, Fei Y, Chang J, Bi Y, Zhou Y, Ding Z, Yin R (2019) Emergence of a Novel *Ehrlichia minasensis* Strain, Harboring the Major Immunogenic Glycoprotein trp36 with Unique Tandem Repeat and C-Terminal Region Sequences, in *Haemaphysalis hystricis* Ticks Removed from Free-Ranging Sheep in Hainan Province, China. **Microorganisms** 7:369.

Lima ML, Soares PT, Ramos CA, Araújo FR, Ramos RA, Souza II, Faustino MA, Alves LC (2010) Molecular detection of *Anaplasma platys* in a naturally-infected cat in Brazil. Braz. **Journal of Microbiology** 4:381–385.

Liu Z, Ma M, Wang Z, Wang J, Peng Y, Li Y, Guan G, Luo J, Yin H (2012) Molecular survey and genetic identification of *Anaplasma* species in goats from central and southern China. **Applied and Environmental Microbiology** 78:464–470.

Liz JS, Sumner JW, Pfister K, Brossard M (2002) PCR detection and serological evidence of granulocytic ehrlichial infection in roe deer (*Capreolus capreolus*) and chamois (*Rupicapra rupicapra*). **Journal of Clinical Microbiology** 40:892–897.

Lobanov VA, Gajadhar AA, Al-Adhami B, Schwantje HM (2012) Molecular study of free-ranging mule deer and white-tailed deer from British Columbia, Canada, for evidence of *Anaplasma* spp. and *Ehrlichia* spp. **Transboundary and Emerging Diseases** 59:233–243.

Lopes MG, Muñoz-Leal S, de Lima JTR, Fournier GFDSR, Acosta ICL, Martins T F, Ramirez DG, Gennari SM, Labruna MB (2018) Ticks, rickettsial and erlichial infection in small mammals from Atlantic forest remnants in northeastern Brazil. **International Journal for Parasitology: Parasites and Wildlife** 7:380-385.

Lotric-Furlan S, Rojko T, Petrovec M, Avsic-Zupanc T, Strle F (2006) Epidemiological, clinical and laboratory characteristics of patients with human granulocytic anaplasmosis in Slovenia. **Wiener Klinische Wochenschrift** 118:708–713.

Louw M, Allsopp MT, Meyer EC (2005) *Ehrlichia ruminantium*, an emerging human pathogen - a further report. **South Africa Medical Journal** 95:948.

Luz HR, Costa Neto SFD, Weksler M, Gentile R, Faccini JLH (2018) Ticks parasitizing wild mammals in Atlantic Forest areas in the state of Rio de Janeiro, Brazil. **Revista Brasileira de Parasitologia Veterinária** 27:409-414.

Lynn GE, Oliver JD, Nelson CM, Felsheim RF, Kurtti TJ, Munderloh UG (2015) Tissue distribution of the *Ehrlichia muris*-like agent in a tick vector. **PLoS One** 10:e0122007.

Lynn GE, Oliver JD, Cornax I, O'Sullivan MG, Munderloh UG (2017) Experimental evaluation of *Peromyscus leucopus* as a reservoir host of the *Ehrlichia muris*-like agent. **Parasites & vectors** 10:48.

Machado RZ, Duarte JMB, Dadnone AS, Szabó MPJ (2006) Detection of *Ehrlichia chaffeensis* in Brazilian marsh deer (*Blastocerus dichotomus*). **Veterinary Parasitology** 139:262–266.

Machado RZ, André MR, Werther K, de Sousa E, Gavioli FA, Alves Junior JRF (2012) Migratory and carnivorous birds in Brazil: reservoirs for *Anaplasma* and *Ehrlichia* species?. **Vector-Borne and Zoonotic Diseases** 12:705-708.

Machado RZ, Silva JB, André MR, Gonçalves LR, Matos CA, Obregón D (2015) Outbreak of anaplasmosis associated with the presence of different *Anaplasma marginale* strains in dairy cattle in the states of São Paulo and Goiás, Brazil. **Revista Brasileira de Parasitologia Veterinária** 24:483-46.

Machado RZ, Teixeira MMG, Rodrigues AC, André MR, Gonçalves LR, da Silva JB, Pereira CL (2016) Molecular diagnosis and genetic diversity of tick-borne Anaplasmataceae agentes infecting the African buffalo *Syncerus caffer* from Marromeu Reserve in Mozambique. **Parasites & Vectors** 9:454.

MacLeod J, Gordon WS (1932) Studies in tick-borne fever of sheep. I.Transmission by the tick, *Ixodes ricinus*, with a description of the disease produced. **Parasitolology** 25:273–283.

Madigan JE, Gribble D (1987) Equine ehrlichiosis in northern California: 49 cases (1968–1981). **Journal of the American Veterinary Medical Association** 190, 445–448.

Madison-Antenucci S, Kramer LD, Gebhardt LL, Kauffman E (2020) Emerging Tick-Borne Diseases. **Clinical Microbiology Reviews** 33: e00083-18.

Maeda K, Markowitz N, Hawley RC, Ristic M, Cox D, McDade JE (1987) Human infection with *Ehrlichia canis*, a leukocytic rickettsia. **New England Journal of Medicine** 316:853-856.

Maggi RG, Mascarelli PE, Havenga LN, Naidoo V, Breitschwerdt EB (2013) Coinfection with *Anaplasma platys*, *Bartonella henselae* and *Candidatus* Mycoplasma haematoparvum in a veterinarian. **Parasites & Vectors** 6:103.

Mansfield KL, Cook C, Ellis RJ, Bell-Sakyi L, Johnson N, Alberdi P, de la Fuente J, Fooks AR (2017) Tick-borne pathogens induce differential expression of genes promoting cell survival and host resistance in Ixodes ricinus cells. **Parasites & Vectors** 10:81.

Marques S, Barros-Battesti DM, Faccini JLH, Onofrio VC (2002) Brazilian distribution of *Amblyomma varium* Koch, 1844 (Acari: Ixodidae), a common parasite of sloths (Mammalia: Xenarthra). **Memórias do Instituto Oswaldo Cruz** 97:1141-1146.

Martinez MC, Gutierrez CN, Monger F, Ruiz J, Watts A, Mijares VM, Rojas MG, Triana-Alonso FJ (2008) *Ehrlichia chaffeensis* in child, Venezuela. **Emerging and Infectious Diseases** 14:519–520.

Martins TF, Furtado MM, Anah TDA, Silveira L, Sollmann R, Tôorres NM, Labruna MB (2011) Ticks on free-living wild mammals in Emas National Park, Goiás State, central Brazil. **Systematic and Applied Acarology** 16:201-206.

Martins TF, Teixeira RHF, Labruna MB (2015) Ocorrência de carrapatos em animais silvestres recebidos e atendidos pelo Parque Zoológico Municipal Quinzinho de Barros, Sorocaba, São Paulo, Brasil. **Brazilian Journal of Veterinary Research and Animal Science** 52:319-324.

Martins TF et al. (2017). Diversidade de carrapatos (Acari: Ixodidae) em animais silvestres recebidos pelo zoológico municipal de Guarulhos. **Ars Veterinaria** 33:20-25.

Martins TF, Milanelo L, Krawczak FDS, Furuya HR, Fitorra LS, Dores FTD, Pedro VS, Hippolito AG, Labruna MB (2017) Diversity of ticks in the wildlife screening center of São Paulo city, Brazil. **Ciência Rural** 47:1-6.

Massung RF, Priestley RA, Miller NJ, Mather TN, Levin ML (2003) Inability of a variant strain of *Anaplasma phagocytophilum* to infect mice. **Journal of Infectious Diseases** 188:1757–1763.

Matsimbe AM, Magaia V, Sanches GS, Neves L, Noormahomed E, Antunes S, Domingos A (2017) Molecular detection of pathogens in ticks infesting cattle in Nampula province, Mozambique. **Experimental and Applied Acarology** 73:91-102.

Matsumoto K, Takeuchi T, Yokoyama N, Katagiri Y, Ooshiro M, Zakimi S, Gaowa, Kawamori F, Ohashi N, Inokuma H (2011) Detection of the new *Ehrlichia* species closely related to *Ehrlichia ewingii* from *Haemaphysalis longicornis* in Yonaguni Island, Okinawa, Japan. **Journal of Veterinary Medical Science** 73:1485-1488.

McDonald HG (2005) Paleoecology of extinct xenarthrans and the Great American Biotic Interchange. **Bulletin of the Florida Museum of Natural History** 45:313-333.

McKenna MC, Bell SK (1997) **Classification of Mammals above the Species Level**. New York:Columbia University Press 646p.

Medri ÍM (2008). **Ecologia e história natural do tatu-peba, *Euphractus sexcinctus* (Linnaeus, 1758), no Pantanal da Nhecolândia, Mato Grosso do Sul.** 167f. Tese (Doutorado em Ecologia) – Universidade de Brasília, Brasília.

Miranda FR, Teixeira RHF, Gazêta GS, Serra-Freire NM, Amorim M (2010) Presence of *Amblyomma cajennense* in wild giant armadillos (*Priodontes maximus*) of the Pantanal Matogrossense, Brazil. **Edentata** 11:73-75.

Mogg M, Wang HH, Baker A, Derouen Z, Borski J, Grant WE (2020) Increased Incidence of *Ehrlichia chaffeensis* Infections in the United States, 2012 Through 2016. **Vector-Borne and Zoonotic Diseases** Epub ahead of print.

Mongruel ACB, Benevenute JL, Ikeda P, André MR, Machado RZ, Carrasco ADOT, Seki MC (2017) Detection of *Anaplasma* sp. phylogenetically related to *A. phagocytophilum* in a free-living bird in Brazil. **Revista Brasileira de Parasitologia Veterinária** 26:505-510.

Mongruel AC, Benevenute JL, André MR, Carrasco AO, Machado RZ, Seki MC (2017) Molecular characterization of *Anaplasma* sp. in free-living gray brockets (*Mazama gouazoubira*). **Vector Borne Zoonotic Diseases** 17:165-171.

Montgomery GG, Sunquist ME (1975) Impact of sloths on Neotropical forest energy flow and nutrient cycling. In: Golley FB, Medina E. **Tropical Ecology Systems**: Trends in Terrestrial and Aquatic Research. Berlin: Springer-Verlog, p.69-98.

Moraes-Barros N, Arteaga MC (2015) Genetic diversity in Xenarthra and its relevance to patterns of neotropical biodiversity. **Journal of Mammalogy** 96:690-702.

Moraes-Filho J, Krawczak FS, Costa FB, Soares JF, Labruna MB (2015) Comparative evaluation of the vector competence of four South American populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. **PLoS One** 10:e0139386.

Morais JD, Dawson JE, Greene C, Filipe AR, Galhardas LC, Bacellar F (1991) First European case of ehrlichiosis. **Lancet** 338:633–634.

Müller A, Monti G, Otth C, Sepúlveda P, Bittencourt P, Nachum-Biala Y, Gutierrez R. Harrus S (2018). "*Candidatus* Neoehrlichia chilensis" sp. nov.: Molecular detection and characterization of a novel Anaplasmataceae in wild rodents from Valdivia, southern Chile. **Transboundary and emerging diseases 65**:357-362.

Munderloh UG, Yabsley MJ, Murphy SM, Luttrell MP, Howerth EW (2007). Isolation and establishment of the raccoon *Ehrlichia*-like agent in tick cell culture. **Vector-Borne and Zoonotic Diseases** 7:418-425.

Muñoz-García CI, Guzmán-Cornejo C, Rendón-Franco E, Villanueva-García C, Sánchez-Montes S, Acosta-Gutierrez R, Romero-Callejas E, Díaz-Lopez H, Martinéz-Carrasco C, Berriatua E (2019) Epidemiological study of ticks collected from the northern tamandua (*Tamandua mexicana*) and a literature review of ticks of Myrmecophagidae anteaters. **Ticks and tick-borne diseases** 10:1146-1156.

Muñoz-Leal S, Lopes MG, Marcili A, Martins TF, González-Acuña D, Labruna MB (2019). Anaplasmataceae, *Borrelia* and *Hepatozoon* agents in ticks (Acari: Argasidae, Ixodidae) from Chile. **Acta tropica** 182:91-103.

Murphy WJ, Pringle TH, Crider TA, Springr MS, Miller W (2007) Using genomic data to unravel the root of the placental mammal phylogeny. **Genome Research** 17:413–421.

Nair AS, Ravindran R, Lakshmanan B, Sreekumar C, Kumar SS, Raju R, Tresamol PV, Vimalkumar MB, Saseendranath MR (2013) Bovine carriers of *Anaplasma marginale* and *Anaplasma bovis* in South India. **Tropical Biomedicine** 30:105–112.

Nair ADS, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, Ganta RR (2016) Comparative experimental infection study in dogs with *Ehrlichia canis*, *E. chaffeensis*, *Anaplasma platys* and *A.phagocytophilum*. **PLoS ONE** 11:e0148239.

Nascimento KKGD, Veríssimo SMM, Raia VDA, Guimarães RCS, Seade GCC, Azevedo ACP, Matos SP, de Oliveira JM, Bezerra IA, Martins TF (2017) Tick fauna of wild animals received and attended at the Santarém Zoological Park, western Pará State, Brazil. **Ciência Rural** 47:1-5.

Ndip LM, Labruna M, Ndip RN, Walker DH, McBride JW (2009) Molecular and clinical evidence of *Ehrlichia chaffeensis* infection in Cameroonian patients with undifferentiated febrile illness. **Annals of Tropical Medicine and Parasitology** 10:719–725.

Ndip LM, Ndip RN, Esemu SN, Walker DH, McBride JW (2010) Predominance of *Ehrlichia chaffeensis* in *Rhipicephalus sanguineus* ticks from kennel-confined dogs in Limbe, Cameroon. **Experimental and Applied Acarology** 50:163–168.

Ndip LM, Ndip RN, Ndive VE, Awuh JA, Walker DH, McBride JW (2007) *Ehrlichia* species in *Rhipicephalus sanguineus* ticks in Cameroon. **Vector Borne and Zoonotic Diseases** 7:221–227.

Nicholson WL, Castro MB, Kramer VL, Sumner JW, Childs JE (1999) Dusky-footed wood rats (*Neotoma fuscipes*) as reservoirs of granulocytic Ehrlichiae (Rickettsiales:Ehrlichieae) in Northern California. **Journal of Clinical Microbiology** 37:3323–3327.

Noaman V, Shayan P (2010) Molecular detection of *Anaplasma bovis* in cattle from central part of Iran. **Veterinary Research Forum** 1:117–122.

Obiegala A, Pfeffer M, Pfister K, Tiedemann T, Thiel C, Balling A, Silaghi C (2014). *Candidatus* Neoehrlichia mikurensis and *Anaplasma phagocytophilum*: prevalences

and investigations on a new transmission path in small mammals and ixodid ticks. **Parasites & Vectors** 7:563.

Ohashi N, Inayoshi M, Kitamura K, Kawamori F, Kawaguchi D, Nishimura Y, Naitou H, Hiroi M, Masuzawa T (2005) *Anaplasma phagocytophilum*-infected ticks, Japan. **Emerging Infectious Diseases** 11:1780–1783.

Ojeda-Chi MM, Rodriguez-Vivas RI, Esteve-Gasent MD, de León AP, Modarelli JJ, Villegas-Perez S (2019) Molecular detection of rickettsial tick-borne agents in white-tailed deer (*Odocoileus virginianus yucatanensis*), mazama deer (*Mazama temama*), and the ticks they host in Yucatan, Mexico. **Ticks and Tick-borne Diseases** 10:365-370.

Oliveira GMB, Martins TF, Pereira LCM, Nicola PA, Horta MC (2017) Ocorrência de carrapatos em *Tamandua tetradactyla* (Linnaeus, 1758) na Caatinga, Nordeste do Brasil. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia** 69:865-869.

Ooshiro M, Zakimi S, Matsukawa Y, Katagiri Y, Inokuma H (2008) Detection of *Anaplasma bovis* and *Anaplasma phagocytophilum* from cattle on Yonaguni Island, Okinawa, Japan. **Veterinary Parasitology** 154:360-364.

Paddock CD (2001) Infections with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in persons coinfected with human immunodeficiency virus. **Clinical Infectious Diseases** 33:1586–1594.

Paddock CD, Childs JE (2003) *Ehrlichia chaffeensis*: a Prototypical Emerging Pathogen. **Clinical of Microbiology Reviews** 16:37–64.

Paglia AP et al. (2012). Lista Anotada dos Mamíferos do Brasil 2ª Edição/Annotated Checklist of Brazilian Mammals. **Occasional papers in conservation biology** 6:1-82.

Palomar AM, Portillo A, Santibáñez P, Mazuelas D, Roncero L, García-Álvarez L, Santibáñez S, Gutiérrez Ó, Oteo JÁ (2015) Detection of tick-borne *Anaplasma bovis*, *Anaplasma phagocytophilum* and *Anaplasma centrale* in Spain. **Medical and Veterinary Entomology** 29:349-353.

Parola P, Cornet JP, Sanogo YO, Miller RS, Thien HV, Gonzalez JP, Raoult D, Telford III SR, Wongsrichanalai C (2003) Detection of *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., and other eubacteria in ticks from the Thai-Myanmar border and Vietnam. **Journal of Clinical Microbiology** 41:1600–1608.

Paulauskas A, Radzijevskaja J, Rosef O (2012) Molecular detection and characterization of *Anaplasma phagocytophilum* strains. **Comparative Immunology, Microbiology & Infectious Diseases** 35:187–195.

Pekova S, Vydra J, Kabickova H, Frankova S, Haugvicova R, Mazal O, Kozak T (2011). *Candidatus* Neoehrlichia mikurensis infection identified in 2 hematooncologic patients: benefit of molecular techniques for rare pathogen detection. **Diagnostic microbiology and infectious disease** 69:266-270.

Peng Y, Wang K, Zhao S, Yan Y, Wang H, Jing J, Jian F, Wang R, Zhang L, Ning C (2018) Detection and phylogenetic characterization of *Anaplasma capra*: an emerging pathogen in sheep and goats in China. **Frontiers in Cellular and Infection Microbiology** 8:283.

Perez M, Rikihisa Y, Wen B (1996) *Ehrlichia canis*-like agent isolated from a man in Venezuela: antigenic and genetic characterization. **Journal of Clinical Microbiology** 34:2133-2139.

Perez M, Bodor M, Zhang C, Xiong Q, Rikihisa Y (2006) Human infection with *Ehrlichia canis* accompanied by clinical signs in Venezuela. **Annals of the New York Academy of Sciences** 1078:110-117.

Picoloto G, Lima RF, Olegario LAO, Carvalho CME, Lacerda ACR, Tomas WM (2010) Real time polymerase chain reaction to diagnose *Anaplasma marginale* in cattle and deer (*Ozotoceros bezoarticus leucogaster*) of the Brazilian Pantanal. **Revista Brasileira de Parasitologia Veterinária** 19:186-188.

Piveteau J, Dechaseaux C. (1958) **Traité de Paléontologie**. Paris:Editeurs 604p.

Poohl AE, Cabezas-Cruz A, Ribeiro MFB, Silveira JAG, Silaghi C, Pfister K, Passos LMF (2013) Detection of genetic diversity of *Anaplasma marginale* isolates in Minas Gerais, Brazil. **Revista Brasileira de Parasitologia Veterinária** 22:129-135

Popov VL, Han VC, Chen SM, Dumler JS, Feng HM, Andreadis TG, Tesh RB, Walker DH (1998) Ultrastructural differentiation of the genogroups in the genus *Ehrlichia*. **Journal of Medical Microbiology** 47:235-251.

Portillo A, Santibáñez P, Palomar AM, Santibáñez S, Oteo JA (2018). '*Candidatus* Neoehrlichia mikurensis' in Europe. **New Microbes and New Infections** 22:30-36.

Potgieter FT, van Rensburg L (1987) Tick transmission of *Anaplasma centrale*. Onderstepoort. **Journal of Veterinary Research** 54:5-7.

Pradeep R.K et al. (2019) Molecular characterization of South Indian field isolates of bovine *Babesia* spp. and *Anaplasma* spp. **Parasitology Research** 118:617-630.

Pritt BS et al. (2011) Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. **The New England Journal of Medicine** 365:422– 429.

Pritt BS et al. (2017) Proposal to reclassify *Ehrlichia muris* as *Ehrlichia muris* subsp. *muris* subsp. nov. and description of *Ehrlichia muris* subsp. *eauclairensis* subsp. nov., a newly recognized tick-borne pathogen of humans. **Intenational Journal of Systematic and Evolutionary Microbiology** 67:2121–6.

Qiu H, Kelly PJ, Zhang J, Luo Q, Yang Y, Mao Y, Yang Z, Li J, Wu H, Wang C (2016) Molecular detection of *Anaplasma* spp. and *Ehrlichia* spp. in ruminants from twelve provinces of China. **Canadian Journal of Infectious Diseases and Medical Microbiology** 2016:1-9.

Quarsten H, Grankvist A, Høyvoll L, Myre IB, Skarpaas T, Kjelland V, Noraas S(2017). *Candidatus* Neoehrlichia mikurensis and *Borrelia burgdorferi* sensu lato detected in the blood of Norwegian patients with erythema migrans. **Ticks and tick-borne diseases** 8;715-720.

Queiroz HL (1995) **Preguiças e guaribas: os mamíferos folívoros arborícolas do Mamirauá**. Conselho Nacional de Desenvolvimento Científico e Tecnológico, Diretoria de Unidades de Pesquisa, 160p.

Rar V, Golovljova I (2011) *Anaplasma*, *Ehrlichia*, and "*Candidatus* Neoehrlichia" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. **Infection, Genetics and Evolution** 11:842-1861.

Rar V et al. (2017). Detection and genetic characterization of a wide range of infectious agents in *Ixodes pavlovskyi* ticks in Western Siberia, Russia. **Parasites & vectors** 10:258.

Redford KH, Wetzel RM (1985). *Euphractus sexcinctus*. **Mammalian Species** 252:1-4.

Rehman A, Conraths FJ, Sauter-Louis C, Krücken J, Nijhof AM (2019) Epidemiology of tick-borne pathogens in the semi-arid and the arid agro-ecological zones of Punjab province, Pakistan. **Transboundary and Emerging Diseases** 66:526-536.

Reichard MV, Roman RM, Kocan KM, Blouin EF, de la Fuente J, Snider TA, Heinz RE, West MD, Little SE, Massung RF (2009) Inoculation of whitetailed deer (*Odocoileus*

virginianus) with Ap-V1 or NY-18 strains of *Anaplasma phagocytophilum* and microscopic demonstration of Ap-V1 in *Ixodes scapularis* adults that acquired infection from deer as nymphs. **Vector Borne and Zoonotic Diseases** 9:565–568.

Reis NR, Peracchi AL, Pedro WA, Lima IP (2006) **Mamíferos do Brasil***.* Londrina: Eds 437p.

Renneker S et al. (2013) Can *Anaplasma ovis* in small ruminants be neglected any longer? **Transboundary and Emerging Disease** 60:105-112.

Rikihisa Y (1991) The tribe Ehrlichieae and ehrlichial diseases. **Clinical Microbiology Reviews** 4:286-308.

Rodrigues FH, Marinho-Filho J, Dos Santos HG (2001). Home ranges of translocated lesser anteaters *Tamandua tetradactyla* in the cerrado of Brazil. **Oryx** 35:166-169.

Rodrigues FHG, Marinho-Filho JS (2003). Diurnal rest sites of translocated lesser anteaters (*Tamandua tetradactyla*) in the cerrado of Brazil. **Edentata** 5:44-46.

Rose KD (2006) **The beginning of the Age of Mammals**. Baltimore:The Johns Hopkins University Press 428p.

Sacchi ABV, Duarte JMB, André MR, Machado RZ (2012) Prevalence and molecular characterization of Anaplasmataceae agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*). **Comparative Immunology, Microbiology and Infectious Diseases** 35:325-334.

Saito TB, Walker DH (2015) A Tick vector transmission model of monocytotropic ehrlichiosis. **Journal of Infectous Diseases** 212:968–77.

Saito TB, Walker DH (2016). Ehrlichioses: An important one health opportunity. **Veterinary sciences** *3*:20.

Salakij C, Lertwatcharasarakul P, Salakij J, Nunklang K, Rattanakunuprakarn J (2012) Molecular characterization of *Anaplasma platys* in a domestic cat from Thailand. **Comparative Clinical Pathology** 21:345–348.

Santos AS, Santos-Silva MM, Almeida VC, Bacellar F, Dumler JS (2004) Detection of *Anaplasma phagocytophilum* DNA in Ixodes ticks (*Acari: Ixodidae*) from Madeira island and Setubal district, mainland Portugal. **Emerging Infectious Diseases** 10:1643–1648.

Sashika M, Abe G, Matsumoto K, Inokuma H (2011) Molecular survey of *Anaplasma* and *Ehrlichia* infections of feral raccoons (*Procyon lotor*) in Hokkaido, Japan. **Vector Borne and Zoonotic Diseases** 11:349-354.

Selmi R, Said MB, Dhibi M, Yahia HB, Messadi L (2019) Improving specific detection and updating phylogenetic data related to *Anaplasma platys*-like strains infecting camels (*Camelus dromedarius*) and their ticks. **Ticks and Tick Borne Disases** 10:101260.

Selmi R, Said M B, Dhibi M, Yahia HB, Abdelaali H, Messadi L (2020) Genetic diversity of *groEL* and *msp4* sequences of *Anaplasma ovis* infecting camels from Tunisia. **Parasitology international** 74:101980.

Seo MG, Ouh IO, Lee H, Geraldino PJL, Rhee MH, Kwon OD, Kwak D (2018) Differential identification of *Anaplasma* in cattle and potential of cattle to serve as reservoirs of *Anaplasma capra*, an emerging tick-borne zoonotic pathogen. **Veterinary Microbiology** 226:15-22.

Seo HJ, Jin BC, Kim KH, Yoo MS, Seong KW, Jeong SJ, Bang-Hun H, Cho YS (2019) Molecular Detection and Phylogenetic Analysis of *Anaplasma* spp. in Korean Native Goats from Ulsan Metropolitan City, Korea. **Vector-Borne and Zoonotic Diseases** 19:773-776.

Shaw SE, Birtles RJ, Day MJ (2001) Review: Arthropod-transmitted infectious diseases of cats. **Journal of Feline Medicine and Surgery** 3:193-209.

Shi K et al. (2019) Dogs as new hosts for the emerging zoonotic pathogen *Anaplasma capra* in China. **Frontiers in Cellular and Infection Microbiology** 9:394.

Shi Y, Yang J, Guan G, Liu Z, Luo J, Song M (2020) Molecular investigation of *Anaplasma* species in sheep from Heilongjiang Province, northeast China identified four *Anaplasma* species and a novel genotype of *Anaplasma capra*. **Parasitology International** 76:102072.

Silaghi C, Beck R, Oteo JA, Pfeffer M, Sprong H (2016). Neoehrlichiosis: an emerging tick-borne zoonosis caused by *Candidatus* Neoehrlichia mikurensis. **Experimental and applied acarology** 68:279-297.

Silaghi C et al. (2017) Guidelines for the direct detection of *Anaplasma* spp. in diagnosis and epidemiological studies. **Vector Borne and Zoonotic Diseases** 17:12–22.

Silva JB, Goncalves LR, de Mello Varani A, André MR, Machado RZ (2015) Genetic diversity and molecular phylogeny of *Anaplasma marginale* studied longitudinally under natural transmission conditions in Rio de Janeiro, Brazil. **Ticks and Tick Borne Disease** 6:499–507.

Silva JB, André MR, Machado RZ (2016) Low genetic diversity of *Anaplasma marginale* in calves in an endemic area for bovine anaplasmosis in the state of São Paulo, Brazil. **Ticks and Tick-borne Diseases** 7:20-25.

Silveira JA, Rabelo EM, Ribeiro MF (2012) Molecular detection of tickborne pathogens of the family Anaplasmataceae in Brazilian Brown Brocket Deer (*Mazama*

gouazoubira, Fischer, 1814) and Marsh Deer (*Blastocerus dichotomus*, Illiger, 1815). **Transboundary and Emerging Diseases** 59:353-360.

Silveira JAG, Rabelo EML, Lacerda ACR, Borges PAL, Tomás WM, Pellegrin AO, Tomich RGP, Ribeiro MFB (2013) Molecular detection and identification of hemoparasites in pampas deer (*Ozotocerus bezoarticus* 1019 Linnaeus, 1758) from the Pantanal Brazil. **Ticks and Tick-Borne Diseases** 4:341–345.

Silveira JAG, Rabelo EML, Lima PCS, Chaves BN, Ribeiro MFB (2014) Post-mortem hemoparasites detection in free-living Brazilian brown brocket deer (*Mazama gouazoubira*, Fischer 1814). **Revista Brasileira de Parasitologia Veterinária** 23:206–215.

Simpson GG (1980). **Splendid Isolation: The Curious History of South American Mammals.** New Haven:Yale University Press p.275.

Simpson RM, Gaunt SD, Hair JA, Kocan KM, Henk WG, Casey HW (1991) Evaluation of *Rhipicephalus sanguineus* as a potential biologic vector of *Ehrlichia platys*. **American Journal of Veterinary Research** 52:1537-1541.

Skotarczak B (2003) Canine ehrlichiosis. **Annals of Agricultural and Environmental Medicine** 10:137–141.

Smetanová K, Boldis V, Kocianová E, Spitalska E (2007) Detection of *Ehrlichia muris* in a yellow-necked mouse (*Apodemus flavicollis*) in Central Slovakia. **Acta virologica** 51:69.

Snellgrove AN, Krapiunaya I, Ford SL, Stanley HM, Wickson AG, Hartzer KL, Levin ML (2020). Vector competence of *Rhipicephalus sanguineus* sensu stricto for *Anaplasma platys*. **Ticks and Tick-borne Diseases,** 101517.

Soares HS, Barbieri AR, Martins TF, Minervino AH, de Lima JT, Marcili A, Gennari SM, Labruna MB (2015) Ticks and rickettsial infection in the wildlife of two regions of the Brazilian Amazon. **Experimental and Applied Acarology** 65:125-140.

Soares HS, Marcili A, Barbieri ARM, Minervino AHH, Malheiros AF, Gennari SM, Labruna MB (2017) Novel *Anaplasma* and *Ehrlichia* organisms infecting the wildlife of two regions of the Brazilian Amazon. **Acta Tropica** 174:82-87.

Storch G, Richter G (1992). The ant-eater *Eurotamandua*: a South American in Europe. In.: Schaal S., Ziegler W (Eds.) **Messel: An Insight into the History of Life and of the Earth**. Oxford: Clarendon Press p.209–215.

Strle F (2004) Human granulocytic ehrlichiosis in Europe. **International Journal of Medical Microbiology** 293:27–35.

Stromdahl E, Hamer S, Jenkins S, Sloan L, Williamson P, Foster E, Nadolny R, Elkins C, Vince M, Pritt B (2014) Comparison of phenology and pathogen prevalence, including infection with the *Ehrlichia muris*–like (EML) agent, of *Ixodes scapularis* removed from soldiers in the midwestern and the northeastern United States over a 15 year period (1997–2012). **Parasites & Vectors** 7:553.

Stuen S, Granquist EG, Silaghi C (2013) *Anaplasma phagocytophilum* a widespread multi-host pathogen with highly adaptive strategies. **Frontiers in Cellular and Infection Microbiology** 22:3-31.

Sun XF, Zhao L, Wen HL, Luo LM, Yu XJ (2015) *Anaplasma* species in China. **The Lancet Infectious Diseases** 15:1263-1264.

Superina M, Pagnutti N, Abba AM (2014) What do we know about armadillos? An analysis of four centuries of knowledge about a group of South American mammals, with emphasis on their conservation. **Mammal Review** 44:69–80.

Tate CM, Mead DG, Luttrell MP, Howerth EW, Dugan VG, Munderloh UG, Davidson WR (2005) Experimental infection of white-tailed deer with *Anaplasma phagocytophilum*, etiologic agent of human granulocytic anaplasmosis. **Journal of Clinical Microbiology** 43:3595–3601.

Tate CM, Howeth EW, Mead DG, Dugan VG, Luttrell MP, Sahora AI, Munderloh UG, Davidson WR, Yabsley MJ (2013) *Anaplasma odocoilei* sp. nov. (family Anaplasmataceae) from white-tailed deer (*Odocoileus virginianus*). **Ticks and Tick-borne Diseases** 4:110-119.

Tay ST, Koh FX, Kho KL, Ong BL (2014) Molecular survey and sequence analysis of *Anaplasma* spp. In cattle and ticks in a Malaysian farm. **Tropical Biomedicine** 31:769–776.

Telford III SR, Goethert HK, Cunningham JÁ (2011). Prevalence of *Ehrlichia muris* in Wisconsin deer ticks collected during the mid 1990s. **The Open Microbiology Journal** 5:18–20.

Teshale S, Geysen D, Ameni G, Asfaw Y, Berkvens D (2015) Improved molecular detection of *Ehrlichia* and *Anaplasma* species applied to *Amblyomma* ticks collected from cattle and sheep in Ethiopia. **Ticks and Tick Borne Diseases** 6:1-7.

Theiler A (1910) *Anaplasma marginale* (gen. and spec. nov). The marginal points in the blood of cattle suffering from a specific disease In: Theiler A (Ed.) **Report of the Government on Veterinary Bacteriology in Transvaal. Department of Agriculture, South Africa, 1908–1909** p. 7–64.

Theiler A (1911) Further investigations into anaplasmosis of South African cattle. In: **First Report of the Director of Veterinary Research**, Union of South Africa, pp. 7–46.

Thomas RJ, Dumler JS, Carlyon JA (2009) Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *Ehrlichia ewingii* ehrlichiosis. **Expert Review of Anti-infective Therapy** 7:709–722.

Tomanović S, Chochlakis D, Radulovic Z, Milutinovic M, Cakic S, Mihaljica D, Tselentis Y, Psaroulaki A (2013) Analysis of pathogen co occurrence in host-seeking adult hard ticks from Serbia. **Experimental and Applied Acarology** 59:367–376.

Tomassone L, Nunez P, Gurtler RE, Ceballos LA, Orozco MM, Kitron UD, Farber M (2008) Molecular detection of *Ehrlichia chaffeensis* in *Amblyomma parvum* ticks, Argentina. **Emerging and Infectious Diseases** 14:1953–1955.

Tonni EP, Alberdi MT, Prado JL, Bargo S, Cione LA (1992) Changes of mammal assemblage in the pampean region (Argentina) and their relation with the Plio-Pleistocene boundary. **Palaeocology, Palaeogeography, Palaeoclimatology** 95:179-194.

Trolle M (2003). Mammal survey in the southeastern Pantanal, Brazil. **Biodiversity & Conservation** 12:823-836.

Trout RTF, Hendricks BM, Pompo K, Mays SE, Paulsen DJ, Operario DJ, Houston AE (2017) Investigating the Adult Ixodid Tick Populations and Their Associated *Anaplasma*, *Ehrlichia*, and *Rickettsia* Bacteria at a Rocky Mountain Spotted Fever Hotspot in Western Tennessee. **Vector Borne and Zoonotic Diseases** 17:527-538.

Ueti MW, Knowles DP, Davitt CM, Scoles GA, Baszler TV, Palmer GH (2009) Quantitative differences in salivary pathogen load during tick transmission underlie strainspecific variation in transmission efficiency of *Anaplasma marginale*. **Infection and Immunity** 77:70–75.

Uribe MCA (2004). **Efeito da estrutura do ambiente e da fragmentação florestal no uso do habitat por tatus (Xenarthra: Dasypodidae) na Amazônia Central.** 52f. Dissertação (Mestrado em Ciências Biológicas) – INPA, UFAM, Manaus.

Vidotto MC, Kano FS, Gregori F (2004) Phylogenetic analysis of *Anaplasma marginale* strains from Paraná State Brazil, using the *msp1α* and *msp4* genes. **Journal of Veterinary Medicine Series B – Infectious** 53:404-411.

Villar M, López V, Ayllón N, Cabezas-Cruz A, López JA, Vázquez J, Alberdi P, de la Fuente J (2016) The intracellular bacterium *Anaplasma phagocytophilum* selectively manipulates the levels of vertebrate host proteins in the tick vector *Ixodes scapularis*. **Parasitic & Vectors** 9:467.

Vizcaíno SF, Scillato-Yané GJ (1995) An Eocene Tardigrade (Mammalia, Xenarthra) from Seymour Island, West Antarctica. **Antarctic Science** 7:407–408.

von Loewenich FD, Geißdörfer W, Disqué C, Matten J, Schett G, Sakka SG, Bogdan C. (2010). Detection of "*Candidatus* Neoehrlichia mikurensis" in two patients with severe febrile illnesses: evidence for a European sequence variant. **Journal of clinical microbiology** 48:2630-2635.

Walker DH, Dumler JS (1996) Emergence of the ehrlichioses as human health problems. **Emerging Infectious Diseases** 2:18 –29.

Walker DH, Ismail N, Olano JP, McBride JW, Yu XJ, Feng HM (2004) *Ehrlichia chaffeensis*: a prevalent, life-threatening, emerging pathogen. **Transactions of the American Clinical and Climatological Association** 115:375.

Webb SD (1985) Late Cenozoic mammal dispersals between the Americas. In.: Stehli FG e Webb SD (Eds.) **The Great American Biotic Interchange**. New York:Plenum Press 108p.

Welc-Falęciak R, Siński E, Kowalec M, Zajkowska J, Pancewicz SA (2014). Asymptomatic "*Candidatus* Neoehrlichia mikurensis" infections in immunocompetent humans. **Journal of clinical microbiology** 52:3072-3074.

Welinder-Olsson C, Kjellin E, Vaht K, Jacobsson S, Wennerås C (2010). First case of human "*Candidatus* Neoehrlichia mikurensis" infection in a febrile patient with chronic lymphocytic leukemia. **Journal of clinical microbiology** 48:1956-1959.

Wen B, Rikihisa Y, Mott J, Fuerst PA, Kawahara M, Suto C (1995) *Ehrlichia muris* sp. nov., identified on the basis of 16S rRNA base sequences and serological, morphological, and biological characteristics. **International Journal of Systematic and Bacteriology** 45:250–254.

Wen B, Cao W, Pan H (2003) Ehrlichiae and ehrlichial diseases in China. **Annals of the New York Academy of Sciences** 990:45–53.

Werther K, Luzzi MC, Gonçalves LR, de Oliveira JP, Junior JRFA, Machado RZ, André MR (2017) Arthropod-borne agents in wild Orinoco geese (*Neochen jubata*) in Brazil. **Comparative immunology, microbiology and infectious diseases** 55:30-41.

Widmer CE, Azevedo FC, Almeida AP, Ferreira F, Labruna MB (2011). Tick-borne bacteria in free-living jaguars (*Panthera onca*) in Pantanal, Brazil. **Vector-Borne and Zoonotic Diseases** 11:1001-1005.

Woldehiwet Z (2006) *Anaplasma phagocytophilum* in ruminants in Europe. **Annals of the New York Academy of Sciences** 1078:446-460.

Woldehiwet Z (2010) The natural history of *Anaplasma phagocytophilum*. **Veterinary Parasitology** 167:108-22.

Wolf RW et al. (2016) Novel *Babesia* and *Hepatozoon* agents infecting non-volant small mammals in the Brazilian Pantanal, with the first record of the tick *Ornithodoros guaporensis* in Brazil. **Ticks Tick-Borne Diseases** 7:449-456.

Wormser GP, Pritt B (2015) Update and commentary on four emerging tick-borne infections*: Ehrlichia muris*-like agent, *Borrelia miyamotoi*, deer tick virus, Heartland virus, and whether ticks play a role in transmission of *Bartonella henselae*. **Infectious Disease Clinics of North America** 29:371–81.

Worthington RW, Bigalke RD (2001). A review of the infectious diseases of African wild ruminants, Onderstepoort. **Journal of Veterinary Research** 68:291–323.

Xu G, Pearson P, Rich SM (2018) *Ehrlichia muris* in *Ixodes cookei* ticks, northeastern United States, 2016–2017. **Emerging Infectious Diseases** 24:1143.

Yabsley MJ, Varela AS, Tate CM, Dugan VG, Stallknecht DE, Little SE, Davidson WR (2002) *Ehrlichia ewingii* infection in white-tailed deer (*Odocoileus virginianus*). **Emerging and Infectious Diseases** 8:668–671.

Yabsley MJ, Murphy SM, Luttrell MP, Wilcox BR, Howerth EW, Munderloh UG (2008). Characterization of '*Candidatus* Neoehrlichia lotoris'(family Anaplasmataceae) from raccoons (*Procyon lotor*). **International journal of systematic and evolutionary microbiology** 58:2794.

Yabsley MJ (2010) Natural history of *Ehrlichia chaffeensis*: vertebrate hosts and tick vectors from the United States and evidence for endemic transmission in other countries. **Veterinary Parasitology** 167:136-148.

Yang J, Li Y, Liu Z, Liu J, Niu Q, Ren Q, Chen Z, Guan G, Luo J, Yin H (2015) Molecular detection and characterization of *Anaplasma* spp. in sheep and cattle from Xinjiang, northwest China. **Parasites & Vectors** 8:108.

Yang J, Liu Z, Niu Q, Liu J, Han R, Liu G, Shi Y, Luo J, Yin H (2016) Molecular survey and characterization of a novel *Anaplasma* species closely related to *Anaplasma capra* in ticks, northwestern China. **Parasites & Vectors** 9:603.

Yang J, Liu Z, Niu Q, Mukhtar MU, Guan G, Liu G, Luo J, Yin H (2018) A novel genotype of "*Anaplasma capra*" in wildlife and its phylogenetic relationship with the human genotypes. **Emerging Microbes & Infections** 7:1-4.

Ybañez AP, Matsumoto K, Kishimoto T, Inokuma H (2012) Molecular analyses of a potentially novel *Anaplasma* species closely related to *Anaplasma phagocytophilum* detected in sika deer (*Cervus nippon yesoensis*) in Japan. **Veterinary Microbiology** 157:232–236.

Ybañez AP, Sashika M, Inokuma H (2014) The phylogenetic position of *Anaplasma bovis* and inferences on the phylogeny of the genus *Anaplasma*. **The Journal of Veterinary Medical Science** 76:307–312.

Yu P, Liu Z, Niu Q, Yang J, Abdallah MO, Chen Z, Liu G, Luo J, Yin H (2017) Molecular evidence of tick-borne pathogens in *Hyalomma anatolicum* ticks infesting cattle in Xinjiang Uygur Autonomous Region, Northwestern China. **Experimental and Applied Acarology** 73:269-281.

Zanella JRC (2016) Zoonoses emergentes e reemergentes e sua importância para saúde e produção animal. **Pesquisa Agropecuária Brasileira** 51:510-519.

Zhou Z, Nie K, Tang C, Wang Z, Zhou R, Hu S, Zhang Z (2010) Phylogenetic analysis of the genus *Anaplasma* in Southwestern China based on 16S rRNA sequence. **Research in Veterinary Science** 89:262-265.

Zhou Z et al. (2019) Molecular epidemiology and risk factors of *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. infection in cattle in Chongqing, China. **PloS One** 14:e0221359.

Zobba R, Anfossi AG, Pinna Parpaglia ML, Dore GM, Chessa B, Spezzigu A, Rocca S, Visco S, Pittau M, Alberti A (2014) Molecular investigation and phylogeny of *Anaplasma* spp. In Mediterranea ruminants reveal the presence of neutrophil-tropic

strains closely related to *A. platys*. **Applied and Environmental Microbiology** 80:271–80.

Zobba R, Anfossi AG, Visco S, Sotgiu F, Dedola C, Pinna Parpaglia ML, Battilani M, Pittau M, Alberti A (2015) Cell tropism and molecular epidemiology of *Anaplasma platys*-like strains in cats. **Ticks and Tick Borne Diseases** 6:272–280.

CAPÍTULO 2 – *Ehrlichia* spp. and *Anaplasma* spp. in Xenarthra mammals from Brazil, with evidence of novel *Candidatus* Anaplasma spp.[1]

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Abstract

Anaplasmataceae agents are obligatory intracellular Gram-negative α-proteobacteria that are transmitted mostly by arthropod vectors. Although mammals of the Superorder Xenarthra (sloths, anteaters, and armadillos) have been implicated as reservoirs for several zoonotic agents, only few studies have sought to detect Anaplasmataceae agents in this group of mammals. This study aimed to investigate the occurrence and genetic diversity of *Anaplasma* spp. and *Ehrlichia* spp. in blood and spleen samples of free-living Xenarthra from four different states in Brazil (São Paulo, Mato Grosso do Sul, Rondônia, and Pará). Nested and conventional PCR screening assays were performed to detect the *rrs* and *dsb* genes of *Anaplasma* spp. and *Ehrlichia* spp.,

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respectively. The assays were positive in 27.57% (91/330) of the *Anaplasma* spp. and 24.54% (81/330) of the *Ehrlichia* spp. Of the 91 positive *Anaplasma* spp. samples, 56.04% were positive in a conventional PCR assay targeting the 23S-5S intergenic region. Phylogenetic and distance analyses based on the *rrs* gene allocated *Anaplasma* sequences from sloths captured in Rondônia and Pará states in a single clade, which was closely related to the *A. marginale*, *A. ovis,* and *A. capra* clades. The sequences detected in southern anteaters from São Paulo were allocated in a clade closely related to sequences of *Anaplasma* spp. detected in *Nasua nasua*, *Leopardus pardalis,* and *Cerdocyon thous* in Brazil. These sequences were positioned close to *A. odocoilei* sequences. Genotype analysis corroborated previous findings and demonstrated the circulation of two distinct *Anaplasma* genotypes in animals from north and southeast Brazil. The first genotype was new. The second was previously detected in *N. nasua* in Mato Grosso do Sul state. The intergenic region analyses also demonstrated two distinct genotypes of *Anaplasma*. The sequences detected in Xenarthra from Pará and Rondônia states were closely related to those in *A. marginale*, *A. ovis,* and *A. capra*. *Anaplasma* spp. sequences detected in Xenarthra from São Paulo and were allocated close to those in *A. phagocytophilum*. The analyses based on the *dsb* gene grouped the *Ehrlichia* spp. sequences with sequences of *E. canis* (São Paulo, Mato Grosso do Sul, and Pará) and *E. minasensis* (Rondônia and Pará). The data indicate the occurrence of *E. canis* and *E. minasensis* and two possible new *Candidatus* species of *Anaplasma* spp. in free-living mammals of the Superorder Xenarthra in Brazil.

Keys words: Anaplasmataceae, sloths, armadillos, anteaters, genetic diversity

1. Introduction

The Anaplasmataceae family (order Rickettsiales) is formed by the genera *Anaplasma*, *Ehrlichia*, *Neorickettsia,* and *Wolbachia* [1]. They are comprised of obligatory intracellular Gram-negative bacteria, that transmit mostly through arthropod vectors, mainly ticks. These agents can infect different mammalian cells depending on the infecting species. *A. marginale*, *A. centrale,* and *A. ovis* infect erythrocytes; *A. platys* infects platelets and neutrophils; *A. odocoilei* infects platelets; *A.*

phagocytophilum and *E. ewingii* infect granulocytes; *A. bovis*, *E. chafeensis*, *E. canis*, *E. muris,* and *Neorickettsia* spp. infect monocytes and macrophages; *E. ruminantium* infects endothelial cells, neutrophils, and macrophages; and *Wolbachia* spp. is an invertebrate endosymbiont [1, 2]. They form microcolonies (morulas) in intracytoplasmic vacuoles [3]. Domestic and wild animals are considered reservoirs, and favor the spread of these agents that can cause diseases in animals and humans [1, 3, 4].

In Brazil, several genotypes of *A. phagocytophilum, A. marginale, A. bovis, A. platys, E. canis, E. chafeensis*, and potential new species, that are not yet named, have been detected in different species of wild animals, including deer [5, 6, 7, 8, 9, 10, 11], wild canids [12, 13, 14], wild felids [12, 15, 16], coatis [14], rodents [14, 17, 18, 19], caititus and peccaries [20], opossums [21, 22], and birds [23, 24].

The Xenarthra superorder is composed of two orders: Cingulata and Pilosa. Cingulata is characterized by the presence of a bone carapace. A representative animal is the armadillo. Pilosa features a dense coat and the absence or underdevelopment of teeth. Representative animals are sloths and anteaters [25, 26]. These animals are distributed from the central southern region of North America to southern of South America [26]. They are considered reservoirs of several zoonotic agents [27].

Studies on Anaplasmataceae agents in Xenarthra are scarce. *Anaplasma marginale* was detected in a giant anteater (*Myrmecophaga tridactyla*) in Argentina by blood smears and molecular confirmation by PCR assays based on the *msp-5* and *msp1-α* genes [28]. Genotypes of *Ehrlichia* spp., based on the *dsb* gene, and *Anaplasma* spp., based on the 16S rRNA gene, were detected in a three-toed sloth (*Bradypus tridactylus*) in the state of Pará in northern Brazil [30].

The present study aimed to investigate the occurrence and genetic diversity of *Anaplasma* spp. and *Ehrlichia* spp. in Xenarthra mammals sampled in four different Brazilian states.

2. Material and Methods

2.1. Ethical Statement

Animal procedures and management protocols were approved by the Institute Chico Mendes for Conservation of Biodiversity (SISBIO N º 53798-5) and by the Ethics Committee on Animal Use of the Biomedical Sciences Institute – University of São Paulo (protocol number 98) and the School of Agricultural and Veterinarian Sciences (FCAV/UNESP; protocol number 11.794). All methods and experimental protocols were performed in accordance with the relevant FCAV/UNESP Ethics Committee guidelines and regulations.

2.2. Sampling and sampled species

The biological samples of Xenarthra mammals originated from four Brazilian states: Mato Grosso do Sul (MS), São Paulo (SP), Pará (PA), and Rondônia (RO). The total of 330 animals included 188 brown-throated sloths (*Bradypus variegatus*), three *Bradypus* spp., five two-toed sloths (*Choloepus didactylus*), 31 *Choloepus* spp., 31 southern anteaters (*Tamandua tetradactyla*), 52 Giant Anteaters (*Myrmecophaga tridactyla*), three southern naked-tailed armadillos (*Cabassous unicinctus*), eight nine-banded armadillos (*Dasypus novemcinctus*), eight six-banded armadillo (*Euphractus sexcinctus*), and one giant armadillo (*Priodontes maximus*) (**Figure 1**).

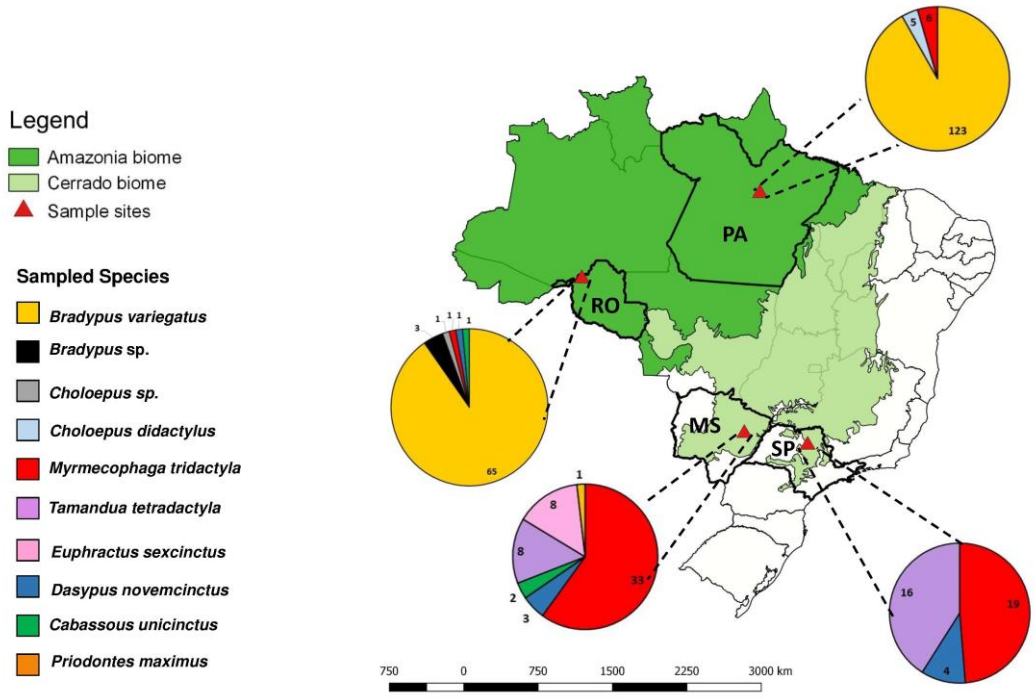

Figure 1. Number and origin of the mammals from the Superorder Xenarthra sampled in Brazil.

Spleen samples were collected by the Bandeiras e Rodovias Project team and by the Wildlife Pathology Service of FCAV/UNESP during necropsies of road-killed animals in the Cerrado biome in Mato Grosso do Sul (20°26'48.3"S 52°54'11.6"W) and São Paulo (21°17'33.2"S 48°19'53.4"W). In the state of Mato Grosso do Sul, 55 spleen samples (33 giant anteaters, eight southern anteaters, eight six-banded armadillos, three southern nine-banded armadillos, one giant armadillo, and two southern naked-tailed armadillos) were collected during 2017 and 2018. In São Paulo state, 39 spleen samples (19 giant anteaters, 16 southern anteaters, and four southern nine-banded armadillos) were obtained from 2011 to 2018. In addition, 236 blood samples were collected by cephalic vein venipuncture from sloths, armadillo and anteaters during the filling process of a hydroelectric power plant in Amazonia biome in the states of Rondônia (3°07'20.2"S 51°46'31.5"W) (n=102, comprising 65 brown-throated sloths, 31 *Choloepus* spp., three Bradypus spp., one giant anteater, one nine-banded armadillo, and one southern naked-tailed armadillo) and Pará (9°16'21.1"S 64°37'59.2"W) (n=134, comprising 123 brown-throated sloths, five two-toed sloths, and six giant anteaters).

Spleen fragments and blood samples were stored in KASVI® RNAse and DNAse-free microtubes at –70°C until DNA extraction.

2.3. Molecular analysis

DNA was extracted from 10 mg of each spleen tissue and 200 µL of each blood sample using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA), according to the manufacturer' s instructions. The presence of amplifiable DNA was verified by a conventional PCR (cPCR) assay targeting the mammalian endogenous glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) gene [29]. The positive samples were subjected to specific PCR assays for *Anaplasma* spp. and *Ehrlichia* spp. (**Figure 2**).

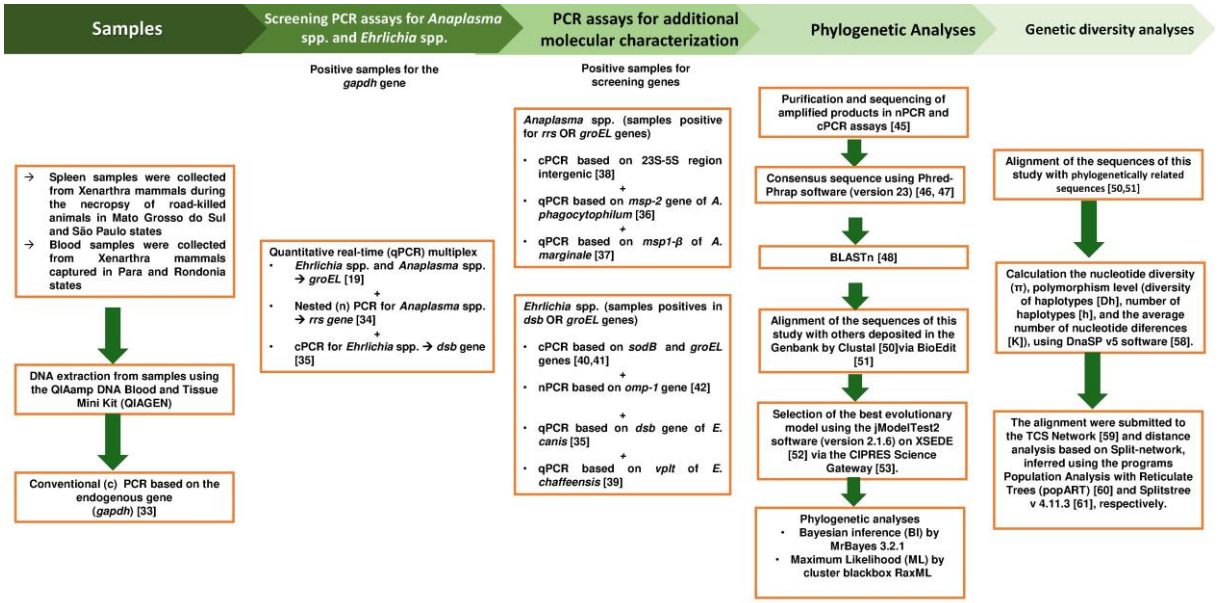

Figure 2. Workflow with the steps performed in the methodology of this study.

2.3.1. *Anaplasma* spp. detection

Screening for *Anaplasma* spp. based on the *groEL* and *rrs* genes was performed using multiplex qPCR (quantitative real-time PCR) [18] and nested PCR (nPCR) [30] protocols, respectively (**Tables 1 and 2**). The positive samples, for at least one of the two protocols, were tested by qPCR assays based on the *msp-2* [31] and *msp1-β* [32] genes for *A. phagocytophilum* and *A. marginale*, respectively (**Table 1**). cPCR assays for *Anaplasma* spp. based on the 23S-5S intergenic region were also performed [33] **(Table 2)**.

Table 1. Description of primers, hydrolysis probes and thermal sequences used in qPCR assays for *Ehrlichia* spp. and *Anaplasma* spp.

Agents (target-genes)	Primers	Hydrolysis probe (TaqMan)	Thermal sequences	Reference
Ehrlichia spp. (*groEL* gene)*	5'-GCGAGCATAATTACTCAGAG-3' 5'-CAGTATGGAGCATGTAGTAG-3'	TET- 5'-CATTGGCTCTTGCTATTGCTAAT-3'[BHQ2a-Q]3'	- 95°C for 3 minutes; 40 cycles: 95°C for 10 minutes and 52,8°C for 30 seconds	[18]
Anaplasma spp. (*groEL* gene)*	5'-TTATCGTTACATTGAGAAGC-3' 5'GATATAAAGTTATTAAAAGTATAAAGC-3'	Cy-5- 5'-CCACCTTATCATTACACTGAGACG-3'[BHQ2a-Q]3'	- 95°C for 3 minutes; 40 cycles: 95°C for 10 minutes and 52,8°C for 30 seconds	[18]
E. canis (*dsb* gene)	5' – TTGCAAAATGATGTCTGAAGATATGAAACA – 3' 5' – GCTGCTCCACCAATAAATGTATCYCCTA – 3'	5' FAM AGCTAGTGCTGCTTGGGCAACTTTGAGTGAA-[BHQ – 1-3']	95ºC for 5 minutes, 40 cycles: 95ºC for 15 seconds and 60ºC for 1 minute	[34]
E. chaffeensis (*vlpt* gene)	5'-CTAATTCTGATTTACACGAGTCTTC-3' 5'-GCATCATCTTCGAATTGAACTTC-3'	5'[TAMRA] (TTGAGTGTCC[BHQ2a-Q]3'	95°C for 3 minutes, 40 cycles: 95°C for 10 minutes e 55°C for 30 seconds	[35]
A.phagocytophilum (*msp-2* gene)	5`-AGTTTGACTGGAACACACCTGATC-3 5`-CTCGTAACCAATCTCAAGCTCAAC-3`	5`[FAM] (939p-TTAAGGACAACATGCTTGTAGCTATGGAAG-GCA-[TAMRA])	50°C for 2 minutes, 95°C for 10 minutes, 40 cycles: 95°C for 15 seconds and 60°C for 1 minute	[31]
A. marginale (*msp1-β* gene)	5'-TTGGCAAGGCAGCAGCTT-3' 5'-TTCCGCGAGCATGTTGCAT-3'	5'[FAM] (TCGGTCTAACATCTCCAGGCTTTCAT-3'-BHQ1	95ºC for 10 minutes, 40 cycles: 95ºC for 15 seconds and 60ºC for 1 minute	[32]

*qPCR multiplex

Table 2. Description of primers, amplicon sizes and thermal sequences used in conventional and nested PCR assays for *Ehrlichia* spp. and *Anaplasma* spp.

Agents	Primers sequences	Size (bp)	Thermal sequences	Reference
Anaplasma spp. (*rrs* gene) - **Screening** External primers - gE3a - gE10R Internal primers - gE2 - gE9f	5´-CACATGCAAGTCGAACGGATTATTC-3´ 5´-TTCCGTTAAGAAGGATCTAATCTCC´-3´ 5´-GGCAGTATTAAAAGCAGCTCCAGG-3´ 5´-AACGGATTATTCTTTATAGCTTGCT-3´	932 546	94°C for 5 minutes 40 cycles: 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minutr 72°C for 5 minutes	[30]
Anaplasma spp. (ITS – 23S-5S) *-ITS2F* -ITS2R	5'-AGGATCTGACTCTAGTAACGAG-3' 5'-CTCCCATGTCTTAAGACAAAG-3'	300	94ºC for 2 minutes, 35 cycles; 94ºC for 30 seconds, 58ºC for 30 seconds, 72ºC for 1 minute 72ºC for 5 minutes.	[33]
Ehrlichia spp. (*dsb* gene) - **Screening** -dsb-330 (F) -dsb-728 (R)	5'-GATGATGTCTGAAGATATGAAACAAAT-3' 5'-CTGCTCGTCTATTTTACTTCTTAAAGT-3'	409	95°C for 2 minutes; 50 cycles: 95°C for 15 seconds, 58°C for 30 seconds and 72°C por 30 segundos 72°C for 5 minutes	[34]
Ehrlichia spp. (*groEL* gene*)* -groEL124-F1 -groEL808-R1	5'-ATTAAGCCAGAAGAACCATTAGC-3' 5'-TACTGCAATATCACCAAGCATATC-3'	680	95ºc for 5 minutes, 40 cycles: 95ºC for 30 seconds, 54ºC for 30 seconds and 72ºC for 30 seconds 72ºC for 5 minutes	[36]
Ehrlichia spp. *(sodB* gene) *-sodbEhr1600-F* *-sodbEhrl600-R*	5'-ATGTTTACTTTACCTGAACTTCCATATC-3' 5'-ATCTTTGAGCTGCAAAATCCCAATT-3'	600	94ºC for 3 minutes; 55 cycles: 94ºC for 10 seconds; 58ºC for 10 seconds; 72ºC por 15 seconds 72ºC for 30 seconds 72ºC for 5 minutes	[37]
Ehrlichia spp. (*omp-1* gene) External primers *-conP28-F1* *-conP28-R1* Internal primers *-conP28-F2* *-conP29-R2*	5'AT(C/T)AGT(G/C)AAA(A/G)TA(T/C)(A/G)T(G/A)CCAA-3' 5'TTA(G/A)AA(A/G)G(C/T)AAA(C/T)CT(T/G)CCTCC-3' 5'CAATGG(A/G)(T/A)GG(T/C)CC(A/C)AGA(AG)TAG-3' 5'TTCC(T/C)TG(A/G)TA(A/G)G(A/C)AA(T/G)TTTAGG-3'	300	94°C for 3 minutes, 35 cycles: 94°C for 1 minute, 50°C for 1 minute and 72°C for 2 minutes 72ºC for 5 minutes	[38]

2.3.2. *Ehrlichia* spp. detection

Screening for *Ehrlichia* spp. based on *groEL* and *dsb* genes was performed using multiplex qPCR (quantitative real-time PCR) [18] and cPCR [34] protocols, respectively (**Tables 1 and 2**). The positive samples, for at least one of the two protocols, were tested by qPCR assays based on *dsb* [34] and *vlpt* [35] genes for *E. canis* and *E. chaffeensis*, respectively (**Table 1**). Additionally, cPCR assays for *Ehrlichia* spp. were based on the *groEL* [36] and *sodB* genes [37]. In addition, nPCR assays for *Ehrlichia* spp. based on the *omp*-1 gene [38], were performed **(Table 2)**.

2.3.3. Reaction conditions

All qPCR assays were performed with a final volume of 10 µL containing 1 µL of DNA sample (concentration mean: 162.5 and 50.55 ng/µL for spleen and blood samples, respectively), 0.2 µM of each primer and hydrolysis probe, 5 µL GoTaq Probe qPCR Master Mix (Promega Corporation, Madison WI, USA), and sterilized ultrapure water (Nuclease-Free Water; Promega Corporation) q.s. 9 µL. PCR amplifications were performed in low-profile multiplate unskirted PCR plates (Bio-Rad, Hercules, CA USA) using a CFX96 Thermal Cycler (Bio-Rad). Quantification of the number of copies of target DNA/µL was performed using IDT psmart plasmids (Integrated DNA Technologies, Coralville, IA, USA) containing the target sequences. Serial dilutions were performed to construct standard curves with different plasmid DNA concentrations (2.0×10^7 to 2.0×10^0 copies/µL). The number of plasmid copies/µL of the amount (g/µL) of DNA/plasmid (bp) was determined by multiplying by 6.022×10^{23}. Each qPCR assay was performed in duplicate for each DNA sample. All duplicates showing cycle quantification (Cq) values differing by >0.5 were re-tested. Amplification efficiency (E) was calculated from the slope of the standard curve in each run ($E= 10^{-1/slope}$). The reactions followed the standards established by the Minimum Information for Publication of Quantitative real-time PCR experiments [39].

All the cPCR assays were performed using 5 µL of the DNA samples (concentration mean: 162.5 and 50.55 ng/µL for spleen and blood samples, respectively) in a mixture containing 1.25 U Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, California, USA), PCR buffer (PCR buffer 10 X – 100 nM Tris-HCl, pH 9.0, 500 mM KCl), 0.2 mM deoxynucleotides (dATP, dTTP, dCTP, and dGTP) (Invitrogen,

Carlsbad, California, United States), 1.5 mM of magnesium chloride (Invitrogen, Carlsbad, CA, United States), 0.5 µM of each primer (Invitrogen), and sterile ultrapure water (Invitrogen) q.s.25 µL. In nPCR assays, 1 µL of the amplified product from the first PCR reaction was used as the target DNA in the second reaction. DNA samples from *A. phagocytophilum,* kindly provided by Prof. John Stephen Dumler (Uniformed Services University of the Health Sciences, Bethesda, MD, USA), and *E. canis*, obtained from DH82 cells infected with the Jaboticabal strain of *E. canis* [40], were used as positive controls. Sterile ultrapure water (Nuclease-Free Water, Promega Corporation) was used as a negative control. The products were separated by 1% agarose gel electrophoresis at an electric current of 100 V/150 mA for 50 min. The gel was stained with 1% ethidium bromide (Life Technologies, Carlsbad, CA, USA) and examined under ultraviolet light illumination using the ChemiDoc MP Imaging System (Bio-Rad) and photographed using Image Lab Software version 4.1.

The cPCR and nPCR amplified products were purified using the ExoSAP-IT PCR Product Cleanup Reagent (Applied Biosystems, Foster City, CA, USA) and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Waltham, MA, USA) and the ABI PRISM 310 DNA Analyzer (Applied Biosystems) [41].

2.4. Phylogenetic analyses

The sequences obtained were submitted to a quality-screening test using Phred-Phrap software (version 23) [42, 43] to evaluate the quality of the electropherograms and to obtain the consensus sequences from the alignment of the sense and antisense sequences. The BLASTn program [44] was used to compare the obtained nucleotide sequences with previously deposited sequences in the GenBank database [45].

The consensus sequences obtained in this study and those retrieved from GenBank were aligned using ClustalW software version 7 [46] using Bioedit v. 7.0.5.3 [47]. The best evolutionary model was chosen using the jModelTest2 software (version 2.1.6) on XSEDE [48] via the CIPRES Science Gateway [49]. The phylogenetic analyses were based on Bayesian inference (BI) and maximum likelihood (ML) methods. The BI analyses were performed using MrBayes 3.1.2 [50] via the CIPRES

Science Gateway. Markov chain Monte Carlo simulations were run for 10^6 generations with a sampling frequency of every 100 generations and a 25% burn-in. ML analyses were performed using the Blackbox RaxML cluster [51] using 1000 bootstrapping replicates [52]. The phylogenetic trees were edited using TreeGraph 2.0.56-381 beta software [53].

2.5. Genetic diversity and genealogies

The genetic diversity analyses for the *rrs* gene and 23S-5S intergenic region of *Anaplasma* spp. and for the *dsb* gene of *Ehrlichia* spp. were performed with the sequences obtained in this study aligned to phylogenetically closer sequences of *A. phagocytophilum*, *A. marginale*, *A. ovis*, *A. odocoilei*, *Anaplasma* spp., *E. canis*, *E. minasensis*, and *Ehrlichia* spp. retrieved from GenBank. Clustal/W software [46] via Bioedit v. 7.0.5.3 [47] was used for the alignment. The sequences used were at least 420 bp, 310 bp, and 310 bp for the *rrs* gene, 23S-5S intergenic region, and *dsb* gene, respectively. Sequences that were smaller in size were excluded from the phylogenetic analysis. These alignments were used to calculate the nucleotide diversity (π), polymorphism level (diversity of haplotypes [Dh], number of haplotypes [h], and the average number of nucleotide differences [K]), using DnaSP v5 software [54]. The sequences were submitted to the TCS Network [55] and distance analysis based on the split-network was inferred using the programs Population Analysis with Reticulate Trees (popART) [56] and Splitstree v 4.14.6 [57], respectively.

3. Results

3.1. Occurrence of Anaplasmataceae agents in Xenarthra mammals

The mean concentration of the extracted DNA was 162.5 ± 37.8 and 50.55 ± 12.1 ng/µL for spleen and blood samples, respectively. All 330 DNA samples were positive in the cPCR assay based on the endogenous *gapdh* gene. A total of 147 (44.54%) animals were positive for at least one agent.

Financial restraints limited the selection of samples for sequencing to only a few among the large number of positive samples. The selection was based on two steps. First, we selected samples that presented high intensity amplicons in agarose gel electrophoresis. These samples were then separated according to animal species and

region of origin. A random selection was performed to obtain at least one representative of each positive species and of each location.

3.2. *Anaplasma* spp.

Of the 330 samples analyzed, 91 (27.57%) were positive for *Anaplasma* spp. based on the *rrs* gene (**Table 3**). No sample was positive in the qPCR for *Anaplasma* spp. based on the *groEL* gene. Of the 91 samples positive in the screening assays for *Anaplasma* spp., 51 (56.04%) samples were positive for the 23S-5S intergenic region of *Anaplasma* spp., and 7/91 (7.7%) samples were positive in the qPCR assay for *A. phagocytophilum* based on the *msp-2* gene. The latter positive samples were not quantified because of the low amount of DNA of the agent in the tested samples (Monte Carlo effect) [42] (**Table 3**). All samples were negative in the qPCR assays for *A. marginale* (*msp1-β* gene) (**Table 1-Supplementary Material)**.

Table 3. Molecular detection of *Anaplasma* spp. and *Ehrlichia* spp. in biological samples (blood or spleen) of Xenarthra mammals.

Xenarthra species (nº sampled)	**PCR assays for *Anaplasma* spp. - nº positive (%)/nº positive by state**			**PCR assays for *Ehrlichia* spp. - nºpositive (%)/nº positive by state**
	nPCR screening (*rrs* gene)	**cPCR (23S-5S intergenic region)**	**qPCR (*msp-2* gene)**	**cPCR screening (*dsb* gene)**
Bradypus variegatus (188)	65 (34.57%) / 34 PA, 31 RO	39 (20.74%) / 18 PA, 19 RO	2 (3.07%) / 2 PA	56 (29.78%) / 42PA, 14RO
Bradypus sp. (3)	2 (66.66%) / 2 RO	2 (66.6%) / 2 RO	0	2 (66.66%) / 2 RO
Choloepus didactylus (5)	2 (40%) / 2 PA	1 (20%) / 1 PA	0	1 (20%) / 1PA
Choloepus sp (31)	13 (41.93%)/ 13 RO	6 (19.35%) / 6 RO	0	5 (16.12%) / 5 RO
Tamandua tetradactyla (31)	5 (16.12%) / 1 PA, 4 SP	3 (9.677%) / 3 SP	5 (16.12%) / 1PA, 4 SP	10 (32.25%) / 1 PA, 4 MS, 5 SP
Myrmecophaga tridactyla (52)	0	NT	NT	7 (13.46%) / 3 SP, 4 MS
Cabassous unicinctus (3)	2 (66.66%) / 2 MS	0	0	0
Dasypus novemcinctus (8)	1 (12.5%) / 1 MS	0	0	0
Euphractus sexcinctus (8)	1 (12.5%) / 1 MS	0	0	0
Priodontes maximus (1)	0	NT	NT	0
Total = 330	91	51	7	81

PA=Para; RO=Rondônia; SP=São Paulo; MS=Mato Grosso do Sul; NT: samples not tested due to negative results in the screening PCR assays.

Table Supplementary Material 1. Results of efficiency, R^2, slope and Y-intercept of qPCR assays for *Ehrlichia* spp. and *Anaplasma* spp.

Gene (Agent)	Efficiency Variation (mean)	R^2 Variation (mean)	Slope Variation (mean)	Y-intercept Variation (mean)
groEL (*Ehrlichia* spp.)	84.7 - 97% (90.70%)	0.971 - 0.991 (0.9835)	-3.753 - -3.397 (-3.5715)	38.762 - 42.30 (40.04)
groEL (*Anaplasma* spp.)	100.3 -104.3% (102.6%)	0.993 - 0.991 (0.991)	-3.314 - -3.224 (-3.261)	37.461 - 38.61 (38.15)
msp-2 (*A. phagocytophilum)*	100.1 - 103.5% (101.02%)	0.991 - 0.998 (0.995)	-3.319 - -3.24 (-3.297)	38.118 - 39.195 (38.549)
msp1-β (*A. marginale)*	97.2 - 104.1% (100.65%)	0.994 - 0.988 (0.991)	-3.392 - -3.228 (-3.31)	44.775 - 45.229 (45.002)
dsb (E. canis)	99.2 -103.8% (101.5%)	0.987 - 0.993 (0.99)	-3.341 - -3.235 (-3.288)	41.645 - 42.256 (41.9505)
vlpt (*E. chaffeensis)*	101.5 - 104.6% (103.05%)	0.908 - 0.975 (0.942)	-3.286 - -3.217 (-3.251)	42.209 - 43.204 (42.706)

3.2.1. *rrs* gene

Of the 91 samples, 25 (27.47%) positive samples for *Anaplasma* spp., based on the *rrs* gene, were sequenced. The sequences obtained were deposited in GenBank under accession numbers MT199810 to MT199833.

BLASTn analysis showed that nine *rrs Anaplasma* sequences obtained from animals sampled in Rondônia and Pará states showed identity ranging from 97.39 to 98.48% with sequences of *A. phagocytophilum* detected in *Haemaphysalis longicornis* (GU064895) and in Chinese water deer (*Hydropotes inermis*; KR611598) sampled in Korea. The 16 remaining sequences of animals from the same states showed identities ranging from 97.71 to 99.38% with *Anaplasma* spp. genotypes detected in *Rattus rattus* from Brazil (KY391803) and in a goat from Saudi Arabia (LC467273). Two sequences detected in Xenarthra sampled from São Paulo state showed identities of 99.04 and 99.50% with *Anaplasma* spp. detected in a Brazilian Brown Brocket Deer (*Mazama gouazoubira*; KF020580) and in a coati (*N. nasua*; KY499186) from Brazil, respectively. One sequence from São Paulo showed 98.69% identity with *A. phagocytophilum* detected in a dromedary from Tunisia (KC455363) (**Table 4**).

Table 4. Percentage of identity assessed by BLASTn of *Anaplasma* and *Ehrlichia* sequences detected in Xenarthra mammals .

Species – ID / Localization	Target gene	Query Coverage (%)	Identity (%)	GenBank accession numbers
B. variegatus – BM6 / PA	*Rrs*	99	99.35	*Anaplasma* sp. - rodent from Brazil (KY391803)
B. variegatus – BM27 / PA		100	99.34	
B. variegatus – BM31 / PA		100	99.35	
B. variegatus – BM37 / PA		98	99.35	
C. didactylus – BM85 / PA		100	99.11	
B. variegatus – BM117 / PA		99	99.33	
B. variegatus – BM175 / PA		100	99.10	
B. variegatus – BM353 / PA		98	99.36	
B. variegatus – BM415 / PA		97	99.36	
B. variegatus – PV709 / RO		100	98.58	
Bradypus sp. – PV1080 / RO		100	99.34	
B. variegatus – PV1190 / RO		99	99.38	
Choloepus sp.– PV1225 / RO		95	99.36	
B. variegatus – BM32 / PA	*Rrs*	100	98.37	*A. phagocytophilum* - *Haemaphysalis longicornis* from South Korea (GU064895)
B. variegatus – BM111 / PA		99	98.48	
B. variegatus – BM129 / PA		99	98.48	
Choloepus sp. – PV49 / RO		100	98.37	
B. variegatus – PV428 / RO		100	98.37	
B. variegatus – PV442 / RO		100	98.29	
Choloepus sp.– PV1102 / RO		100	98.44	
B. variegatus – BM80 / PA	*Rrs*	99	97.71	*Anaplasma* sp. - goat from Saudi Arabia (LC467273)
B. variegatus – BM128 / PA	*Rrs*	100	97.39	*A. phagocytophilum* - water-deer from Korea (KR611598)
T. tetradactyla – 60/SP	*Rrs*	100	98.69	*A. phagocytophilum* - dromedary from Tunisia (KC455363)
T. tetradactyla – 90/SP	*Rrs*	100	99.04%	*Anaplasma* sp. of Brazilian Brown Brocket (KF02058)
T. tetradactyla – 94/SP	*Rrs*	98	99.50	*Anaplasma* sp. – coati (*Nasua nasua*) from Brazil (KY499186)
B. variegatus – BM12 / PA	Intergenic region (23S-5S)	99	90.20	*A. marginale* – cattle from Brazil (CP023731)
B. variegatus – BM31 / PA		99	90.97	
B. variegatus – BM167 / PA		100	90.87	
B. variegatus – BM181 / PA		100	90.85	
B. variegatus – PV14 / PA		100	90.89	
B. variegatus – PV16/PA		100	90.89	
B. variegatus – PV399 / RO		100	90.91	
B. variegatus – PV737 / RO		100	90.89	
B. variegatus – PV415/ RO		100	90	
Bradypus sp. – PV1080 / RO		100	90.89	
Choloepus sp. – PV1177 / RO		100	90.89	
Choloepus sp. – PV1206 / RO		100	90.89	
T. tetradactyla – 58 / SP	Intergenic region (23S-5S)	100	90.74	*A. phagocytophilum* - sheep from Norway (CP01376)
T. tetradactyla – 66 / SP		99	90.58	
T. tetradactyla – NEC17 / MS	*dsb*	100	100	*E. canis* - dog from Colombia (MK783026)
T. tetradactyla – NEC19 / MS		100	100	
M. tridactyla – NEC34 / MS		100	100	
M. tridactyla – NEC63 / MS		100	100	
M. tridactyla – 75 / SP		100	100	
M. tridactyla – 83 / SP		100	100	
D. novemcinctus – 89 / SP		100	100	
B. variegatus – BM24 / PA		100	100	
B. variegatus – BM51 / PA		100	100	
B. variegatus – BM61 / PA		100	100	
T. tetradactyla – BM177 / PA	*dsb*	99	100	

B. variegatus – BM16 / PA	*dsb*	100	99.38	*E. minasensis* - cattle from Australia (MH500007)
B. variegatus – BM180 / PA		100	99.70	
B. variegatus – PV35 / RO		96	99.71	
B. variegatus – PV41 / RO		87	99.43	
B. variegatus – PV14 / RO		99	100	*E. minasensis - Rhipicephalus microplus* from Brazil (JX629808)
B. variegatus – PV726 / RO		99	99.47	
B. variegatus – PV737 / RO		93	98.96	
Choloepus sp. – PV1165 / RO		99	99.47	

The phylogenetic analysis inferred by the BI method (**Figure 3a**) positioned all the sequences obtained in Rondônia and Pará states in a single clade that was phylogenetically closer to *Anaplasma* spp. genotypes detected in rodents in Brazil (KP757841 and KY391803), with 89% branch support. Despite forming a single clade, the obtained sequences showed greater proximity to the clade of *Anaplasma* spp. found in ruminants (*A. capra*, *A. ovis,* and *A. marginale*). On the other hand, the sequences obtained from anteaters of São Paulo state were allocated to a clade closer to the sequences of *Anaplasma* spp. detected in ocelots (*Leopardus pardalis*), coatis (*Nasua nasua*), and crab-eating foxes (*Cerdocyon thous*) from the Pantanal natural region in southern Brazil, with 87% branch support. This clade was sister to the clade of *A. odocoilei*, with 90% branch support. Results of the ML analysis (**Figure 3b**) concurred (or agreed) partially with the BI analysis. While the sequences obtained in Rondônia and Pará states formed a single clade close to the clade of *Anaplasma* spp. found in ruminants, the *Anaplasma* sequences obtained from Xenarthra of São Paulo were subdivided into different clades, allocated close to the clade of *A. odocoilei.*

Additionally, four (BM31, BM32, BM128, and BM129) of the seven positive samples for *A. phagocytophilum,* based on the *msp2* gene, were sequenced for the *rrs* gene. Interestingly, three sequences had a high identity with *A. phagocytophilum* by the BLASTn analysis. However, in the phylogenetic analysis, they were positioned together with *rrs* sequences of other *Anaplasma* spp. obtained in Rondônia and Pará states. BLASTn directly presented a greater identity of one sequence (BM31) with *Anaplasma* sp. of *Rattus rattus*

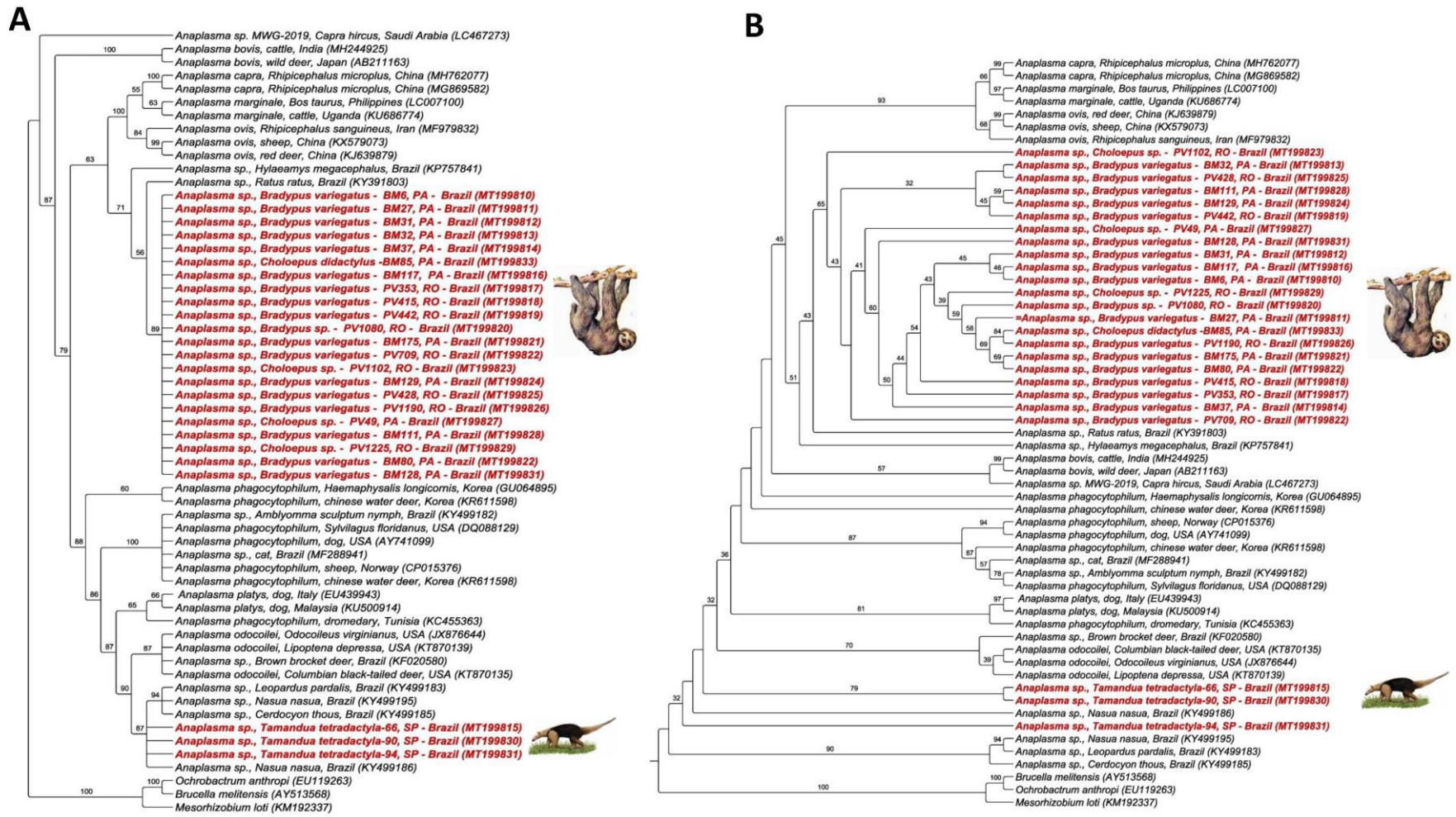

Figure 3. Phylogenetic analysis of *Anaplasma rrs* sequences based on the topology generated by Bayesian model (**A**) and Maximum Likelihood (**B**), with TVM+I+G as evolutionary model. *Ochrobactrum anthropi, Brucella melitensis* and *Mesorhzobium Ioti* were used as an external group.

Genotype analysis based on 38 *rrs Anaplasma* sequences, including 18 sequences obtained in this study and sequences of *A. marginale, A. ovis, A. phagocytophilum, A. odocoilei,* and *Anaplasma* spp., indicated the presence of 12 genotypes, with π = 0.01719, hd = 0.808, and K = 7.16643 (**Table 5** and **Figure 4a**). Eight genotypes comprised more than one sequence. Genotype #3 comprised two sequences of *A. phagocytophilum* detected in South Korea. Genotype #4 comprised 16 sequences detected in sloths from Rondônia and Pará (this study). Genotype #5 comprised three sequences of *A. phagocytophilum* detected in the United States and Norway. Genotype #6 grouped two sequences of *A. marginale* detected in cattle from the Philippines and Uganda. Genotype #7 grouped two sequences of *A. ovis* detected in sheep and deer in China. Genotype #8 grouped the two sequences detected in anteaters from São Paulo state (this study) and one sequence detected in a coati (*Nasua nasua*) from MS. Genotype #11 comprised three sequences of *A. odocoilei* sequences detected in cervids and a fly (*Lipoptena depressa*) in the United States. Finally, genotype #12 grouped sequences of *Anaplasma* sp. detected in ocelot (*Leopardus pardalis*), crab-eating fox (*Cerdocyon thous*), and coati from Brazil.

Regarding the genotype network (**Figure 4a**), it could be inferred that genotype #4, which comprises the *rrs* sequences of *Anaplasma* detected in sloths from Rondônia and Pará states, was derived from genotype #1, which comprises a sequence found in *Rattus* in Brazil (KY391803), upon a mutational event. Both genotypes originated from a median vector (genotype not contemplated in the presented tree). On the other hand, genotype #8, which covers the two sequences obtained in this study in anteaters from São Paulo state and a coati sequence obtained in the state of MS, originated from a median vector upon a mutational event. Moreover, the sequences of *A. odocoilei* also originated from the same median vector. Additionally, genotype #8 gave rise to genotype #12, composed of sequences obtained from different wild animals sampled in the state of MS.

Split-network analysis based on the *rrs* gene (**Figure 4b**) corroborated the genotype network, since the Rondônia and Pará sequences were all positioned together and closer to the *Anaplasma* spp. sequence previously detected in *R. rattus*. The phylogenetic analysis revealed that they were positioned closer to the *A. ovis*

sequences. The sequences detected in São Paulo were closely positioned to the sequences of *A. odocoilei*.

Table 5. Genetic diversity and polymorphisms of *Anaplasma rrs* and 23S-5S intergenic region and *Ehrlichia dsb* sequences.

Gene/agent	bp	N	VS	GC%	h	dh (mean±SD)	π (mean±SD)	K
rrs/Anaplasma spp.	417	38	21	0,519	12	0,808±0,059	0,01719±0,00118	7,16643
Intergenic region (23S-5S)/*Anaplasma* spp.	302	23	66	0,472	8	0,719±0,094	0,08454±0,01005	24,09486
dsb/Ehrlichia spp.	260	29	19	0,329	4	0,569±0,057	0,0357±0,00221	9,28079

N= number of sequences analyzed, VS=number of variable sites, GC%=C+G content, h=number of genotypes, dh=diversity of genotypes, SD=standard deviation, π=nucleotide diversity (per site), K=nucleotide difference number

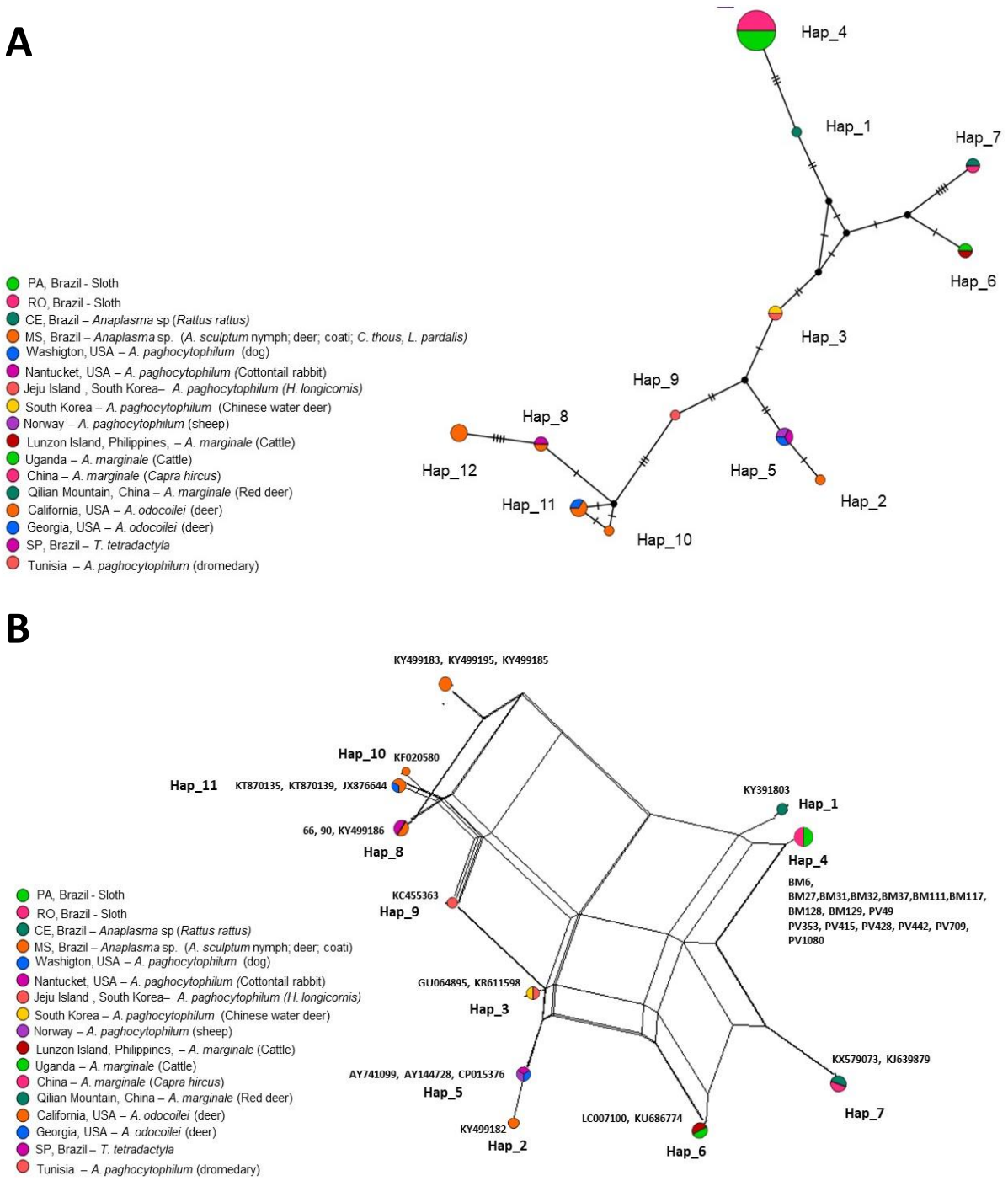

Figure 4. TCS network (**A**) and Split-network based on distance (**B**) of *rrs* sequences from *A. marginale*, *A. phagocytophilum*, *A. ovis* and those obtained in the present study.

3.2.2. 23S-5S region intergenic

Fourteen (27.45%) of 51 positive samples for *Anaplasma* spp. based on the 23S-5S intergenic region were sequenced. The sequences obtained were deposited in GenBank under access numbers MT267341 to MT267354.

BLASTn analysis showed that 12 sequences obtained in Rondônia and Pará states showed identity ranging from 90 to 90.91% with *A. marginale* detected in a bovine animal from Brazil (CP023731), and two sequences from São Paulo showed 90.58 and 90.74% identity with *A. phagocytophilum* detected in a sheep from Norway (CP015376) (**Table 4)**.

Phylogenetic analyses based on the 23S-5S intergenic region of *Anaplasma* spp. positioned the sequences detected in Xenarthra in two distinct clades, composed only of sequences found in this study. The first clade was composed of the sequences detected in sloths from the Rondônia and Pará states, which was in close proximity to the clade of *A. marginale* and *A. ovis*. The second clade was composed of sequences obtained from anteaters from SP, which were in close proximity to the clade of *A. phagocytophilum*. Both the BI (**Figure 5a**) and ML analyses (**Figure 5b**) presented the same topology, although ML presented a better definition of the clades. The index of clade support was 100 and 99% for Rondônia and Pará clade 1 and 89 and 100% for SP clade 2, in the BI and ML analyses, respectively. Of the seven (7.7%) positive samples in the qPCR for the *msp2* gene of *A. phagocytophilum*, only one (14.28%) was positive in the PCR based on the 23S-5S region. However, it was closer to *A. marginale* in both the BLASTn and phylogenetic analyses.

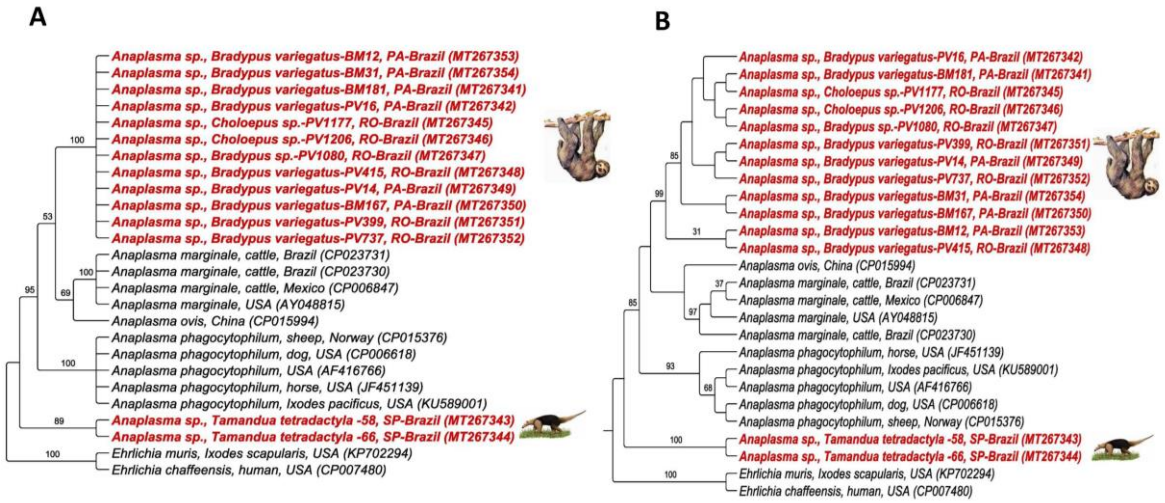

Figure 5. Phylogenetic analysis of *Anaplasma* 23S-5S sequences based on the topology generated by Bayesian (**A**) and Maximum Likelihood (**B**) models, with HKY+G as evolutionary model. *Ehrlichia muris* and *Ehrlichia chaffeensis* were used as an external group.

Genotype analysis based on 23 *Anaplasma* 23S-5S sequences, which included 14 sequences obtained from the Xenarthra sampled in this study as well as *A. marginale*, *A. ovis,* and *A. phagocytophilum* sequences, obtained eight different genotypes, with π=0.08454, hd=0.719, and K=24.09486 (**Table 5** and **Figure 6a**). Four genotypes comprised more than one sequence. Genotype #1 comprised all 12 *Anaplasma* 23S-5S sequences from sloths sampled in Rondônia and Pará states. Genotype #8 comprised two sequences detected in giant anteaters from São Paulo state. Genotype #2 comprised three sequences of *A. marginale* previously detected in Brazil and Mexico. Genotype #5 comprised two sequences of *A. phagocytophilum* detected in the United States and Norway. Based on the network of genotypes (**Figure 6a**), genotype #1 originated from a median vector and several mutational events. The same median vector also originated from genotypes #2 and #4, corresponding to sequences of *A. marginale*. Genotype #8 also originated from a median vector, which in turn originated from genotype #7, and gave rise to genotypes #5 and 6, comprised of *A. phagocytophilum* sequences.

Corroborating the genotype network findings, the split-network analysis, based on the 23S-5S intergenic region (**Figure 6b**), showed a clear separation between the sequences detected in Rondônia and Pará states, and those detected in São Paulo

state. Additionally, the Xenarthra sequences from Rondônia and Pará states were closer to the *A. marginale* and *A. ovis* sequences. The Xenarthra sequences from São Paulo were allocated close to *A. phagocytophilum*, corroborating the findings obtained in the other analyses.

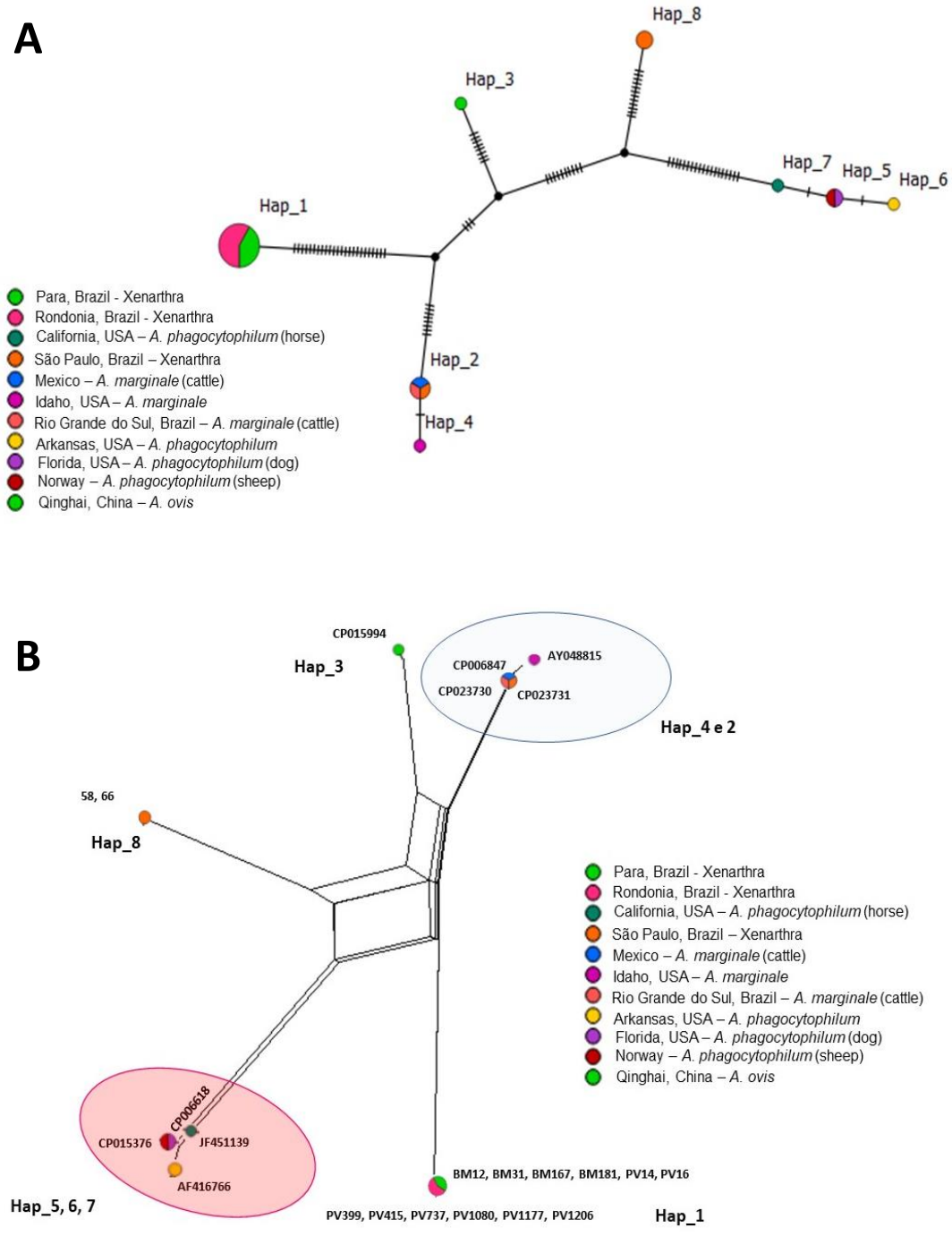

Figure 6. TCS network (**A**) and Split-network based on distance (**B**) of 23S-5S sequences from *A. marginale*, *A. phagocytophilum*, *A. ovis* and those obtained in this study.

3.3. *Ehrlichia* spp.

Of the 330 samples screened for *Ehrlichia* spp., 81 (24.54%) were positive in cPCR assays based on the *dsb* gene (**Table 3)**. No sample was positive in qPCR for *Ehrlichia* spp. based on the *groEL* gene. No samples were positive in the cPCR (*groEL* and *sodB* genes), in the nested PCR (*omp-1* gene) assays for *Ehrlichia* spp., and in the qPCR assays for *E. canis* (*dsb* gene) and *E. chaffeensis* (*vlpt* gene) (**Table 6-Supplementary Material)**.

Nineteen (23.45%) of 81 samples positive for *Ehrlichia* spp. PCR based on the *dsb* gene were sequenced. The sequences obtained were deposited in GenBank under access numbers MT212405 to MT212423.

BLASTn analysis showed that 10 sequences were identical to *E. canis* detected in a dog from Colombia (MK783026). The remaining nine sequences showed identities ranging from 98.96 to 100% with *E. minasensis* detected in *Rhipicephalus microplus* from Brazil (JX629808) and in bovine from Australia (MH500007) (**Table 4**).

Phylogenetic analyses based on the *dsb* gene of *Ehrlichia* spp. positioned the sequences obtained in this study in the *E. canis* and *E. minasensis* clades. The sequences obtained from São Paulo and Mato Grosso do Sul, and three from Pará were grouped in the clade of *E. canis*, and four sequences from Pará and all from Rondônia were grouped in the clade of *E. minasensis*. The topologies of phylogenetic trees obtained by both BI (**Figure 7a**) and ML (**Figure 7b**) methods corroborated the findings. Additionally, ML analysis inferred a subdivision within the *E. canis* clade. Five sequences found in Xenarthra mammals formed a minor clade close to a sequence detected in a dog from Colombia. Four sequences formed a second clade with the other sequences of *E. canis* analyzed. Finally, a sequence obtained from a *M. tridactyla* (83) from São Paulo was positioned separately from the others, with 98% branch support.

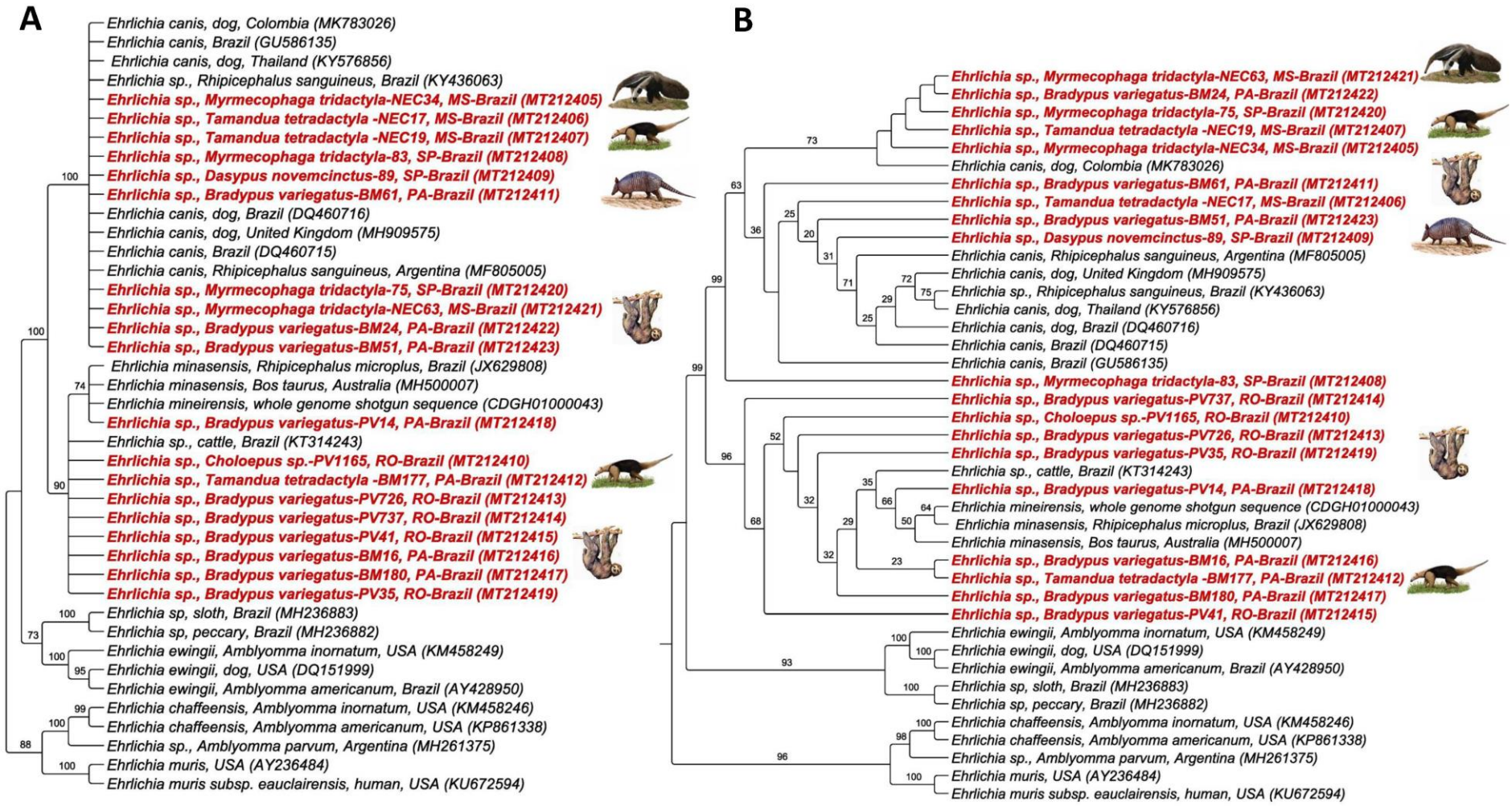

Figure 7. Phylogenetic analysis of *Ehrlichia dsb* sequences based on the topology generated by Bayesian model (**A**) and Maximum Likelihood (**B**), with TrN+I+G as evolutionary model. *Ehrlichia muris* and *Ehrlichia chaffeensis* were used as an external group.

Genotypes based on 29 *Ehrlichia* sequences were analyzed. These included 17 sequences obtained in this study as well as *E. canis* and *E. minasensis* sequences detected in different countries. A total of four genotypes were found, with π = 0.03570, hd = 0.569, and K = 9.28079 (**Table 5**). Genotype #1 comprised all sequences of *E. canis* retrieved from GenBank as well as the sequences detected in Xenarthra sampled in this study in São Paulo and Mato Grosso do Sul, and three sequences from Pará (BM24, BM51, and BM61). Genotype #2 comprised all *E. minasensis* sequences retrieved from GenBank, three Xenarthra sequences sampled in Rondônia, and four from Pará (BM16, BM177, BM180, PV14). Genotypes #3 and #4, on the other hand, comprised unique sequences detected in specimens of *B. variegatus* from Rondônia, PV337, and PV41, respectively. Based on genotype network analysis (**Figure 8a**), genotype #1 (*E. canis*) originated from genotype #3 through several mutational events. The latter seems to have originated from genotype #2 (*E. minasensis*), which, in turn, originated from genotype #4, both from a mutational event. The split-network analysis corroborated the main findings described by the genotype network analysis (**Figure 8b**).

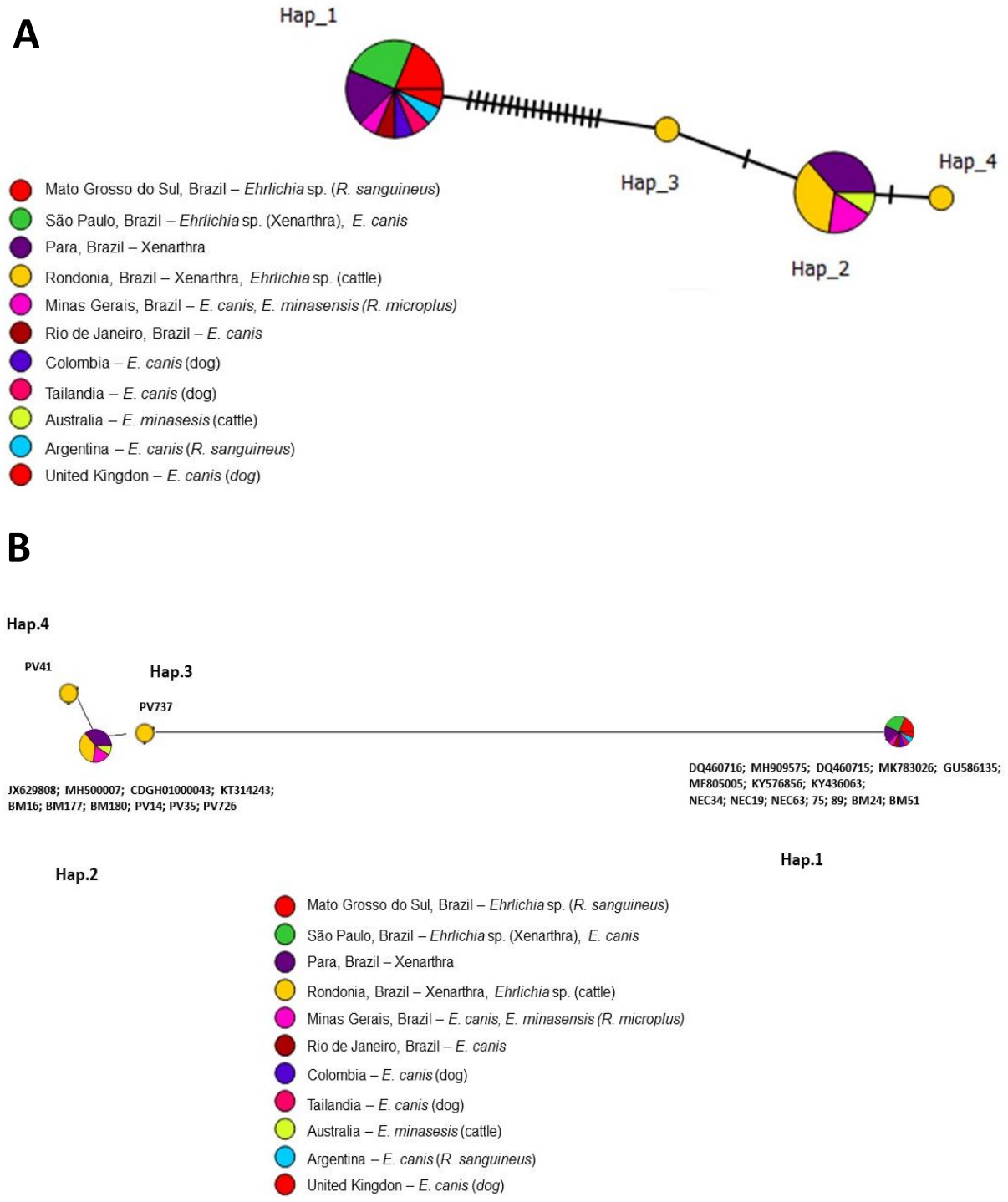

Figure 8. TCS network (**A**) and Split-network based on distance (**B**) of dsb sequences of E. canis, E. minasensis and those obtained in the present study.

3.4. Co-positivity for *Anaplasma* spp. and *Ehrlichia* spp.

Of the 147 positive animals, 25 (17%) were co-positive for *Anaplasma* spp. and *Ehrlichia* spp., among 21 sloths (18 *B. variegatus* [12 from Pará and six from

Rondônia], one *C. didactylus* from Pará, one *Bradypus* spp. and one *Choloepus* spp. from Rondônia), and four anteaters (three *T. tetradactyla* and one *M. tridactyla* from São Paulo).

4. Discussion

The present study revealed a high rate of positivity for Anaplasma spp. and Ehrlichia spp. in Xenarthra mammals sampled in four different Brazilian states. Of the 330 animals, 147 (44.54%) were positive for at least one of the agents. Of these, 25 (17%) were positive for both *Ehrlichia* spp. and *Anaplasma* spp. Until this study, molecular data concerning Anaplasmataceae agents in biological samples of the Superorder Xenarthra have been scant. Guillemi et al. [28] detected the presence of *A. marginale* morulae in blood smears from a giant anteater in Argentina, which was confirmed by PCR assays based on *msp-5* and *msp1-α* genes. In Brazil, Soares et al. [20] detected a new *dsb* genotype of *Ehrlichia* sp. in a three-toed sloth (*Bradypus tridactylus*) obtained in the state of Pará. The sequence was allocated a separate clade that was sister to the clade of *E. ruminantium,* and close to the sequences of *Ehrlichia* spp. detected in a horse and a fox in Brazil. The same animal was positive for the *rrs* gene, whose sequence was allocated in a clade close to *A. phagocytophilum*.

The present study reports the occurrence of two possible species of *Anaplasma* spp. in mammals of the Superorder Xenarthra from Brazil, since the two genes analyzed showed low identity values obtained by BLASTn and the phylogenetic findings positioned the sequences obtained in this study in single clades that were separate from the others.

BLAST analyses performed for *Anaplasma* spp. showed that all *rrs* sequences detected in sloths from the states of Rondônia and Pará showed identity values lower <99% (not exceeding 98.5%) with sequences of *A. ovis*, *A. marginale,* and *A. centrale*. Additionally, the sequences detected in anteaters from São Paulo also showed an identity <99% with sequences of *A. phagocytophilum* and *A. odocoilei*. However, these same sequences showed an identity >99% with an *Anaplasma* sequence previously detected in a coati from MS (KY4999186). Previous studies have defined *rrs* sequences as having at least 95% identity to be identified at the genus level and 99% to be identified at the species level [58, 59, 60]. In view of this, we have proposed two

new species circulating in these animals, and the species detected in São Paulo's anteaters is probably the same as that found in the Mato Grosso do Sul coatis. The analyses performed in the 23S-5S region intergenic sequences corroborated with the *rrs* gene. The identities obtained were quite low, not exceeding 90%, strengthening the hypothesis of two new species.

The phylogenetic analyses corroborated the BLASTn results. The analysis of the *rrs* sequences of *Anaplasma* spp. obtained from sloths of Rondônia and Pará were allocated close to two *Anaplasma* sequences previously detected in rodents (*R. rattus* and *H. megacephalus*) from Brazil. In addition, the clades formed by these two sequences and those found in the present study had a sister clade formed by *A. marginale*, *A. ovis,* and *A. capra*. The *Anaplasma* sequences detected in anteaters from São Paulo were allocated in a clade close to a new genotype of *Anaplasma* spp. previously detected in wild mammals from the Pantanal Sul-matogrossense.

A similar topology was observed in the phylogeny based on the 23S-5S intergenic region, in which the clade formed by the *Anaplasma* sequences from Xenarthra sampled in Rondônia and Pará was a sister clade that was formed by *Anaplasma* species detected in ruminants. The two *Anaplasma* sequences obtained from anteaters in the state of São Paulo were in a clade completely separated from the other sequences, although they were closer to the *A. phagocytophilum* clade, raising questions about the possible influence of the geographical location or host species on the occurrence of *Anaplasma* species that affect these animals.

Out of the seven samples positive in the qPCR for the *msp2* gene of *A. phagocytophilum*, only one (14.2%) was positive in the PCR based on the 23S-5S region. Despite this, it was phylogenetically related to the clade formed by *A. marginale* and *A. ovis*. Similarly, four samples that were also positive for the *msp2* gene were positioned close to the clade of *A. marginale*, *A. ovis,* and *A. capra* in the phylogenetic analysis based on the *rrs* gene. This potentially indicates the possibility of the aforementioned qPCR protocol to amplify *msp-2* gene fragments from *Anaplasma* species phylogenetically related to *A. phagocytophilum*. Alternatively, the animals may have been co-infected with *A. phagocytophilum* and the new Candidatus species. MSP2, an external membrane protein present in all *Anaplasma* species, is encoded by

several polymorphic genes in *A. marginale*, *A. centrale,* and *A. ovis,* and by only one gene in *A. phagocytophilum* [61].

To better understand the results, genetic diversity analysis and distance genealogies were performed. The results of both corroborated the previously presented phylogenetic positioning. For both the *rrs* gene and the 23S-5S intergenic region, the genotype analyses showed that the *Anaplasma* sequences obtained in Rondônia and Pará states formed new genotypes (#4 and #1, for *rrs* and intergenic regions, respectively), whereas the sequences obtained from anteaters in the SP state comprised one genotype (#8 for *rrs* and intergenic region).

In addition, the genotype network based on the *rrs* gene suggests that the genotype circulating in sloths in Rondônia and Pará states might have originated through three mutational events from genotype #1, which was detected in a *R. rattus* from Brazil, explaining the proximity of both in phylogenetic analysis. The genotype circulating in São Paulo anteaters is the same genotype previously found in a coati in Mato Grosso do Sul, which might have originated through a mutational event from a median vector. Additionally, the genotype network based on the intergenic region suggests that both genotypes (#1 and #8) found in this study might have originated from different median vectors through numerous mutational events. The distance analysis for both genes showed that *Anaplasma* sequences obtained from Xenarthra sampled in the northern region of the country were positioned apart from the others, but were closer to *A. marginale* and *A. ovis*. On the other hand, the sequences obtained from Xenarthra sampled in São Paulo were positioned apart, albeit closer to *A. odocoilei* and *A. phagocytophilum*, based on the *rrs* gene and intergenic region, respectively.

All analyses corroborated and provided strong evidence of the circulation of two new species of *Anaplasma* in Xenarhtra in Brazil, related to the region inhabited by these animals. We propose naming the species circulating in Rondônia and Pará states as *Candidatus Anaplasma amazonensis* and the species detected in São Paulo as *Candidatus Anaplasma brasiliensis*. Further studies are needed to validate these species as well as to determine the vectors responsible for their transmission.

Regarding the findings for *Ehrlichia* spp., all analyses including BLASTn, corroborated and grouped the sequences as *E. canis* or *E. minasensis*. In addition,

two sequences detected in sloths phylogenetically close to *E. minasensis* formed two new and distinct genotypes (#3 and #4).

The *rrs genotypes* phylogenetically related to *E. canis* have been increasingly described in wild animals from Brazil, and have already been detected in wild canids [12, 14], wild felids [15], rodents [14, 18, 19], coatis [14], and geese (*Neochen jubata*) [24]. *E. minasensis*, a recently described species that is genetically similar to *E. canis* [62, 63], can infect and cause clinical signs in cattle in central-western Brazil [64]. It has already been detected in cattle in North America [65], Ethiopia [66], Brazil [64], in cervids in Canada [67], dogs in Israel [68], and in several species of ticks [68, 69, 70, 71, 72, 73, 74].

Interestingly, the *dsb* sequences of *E. canis* obtained from Xenarthra mammals comprised animals sampled in the states of São Paulo, Mato Grosso do Sul, and portions of Pará. The *dsb* sequences of *E. minasensis* were obtained from animals from Rondônia and Pará states. Similar to the analysis of *rrs* and 23S-5S *Anaplasma* sequences, these findings again raise questions about the possible regionalization of the *Ehrlichia* and *Anaplasma* species found in Xenarthra from Brazil.

Tick vectors of *E. canis* include *R. sanguineus* sensu lato (tropical lineage) and *D. variabilis* [75, 76, 77]. Although DNA from *E. minasensis* has already been detected in tick species that include *Rhipicephalus* (*R. microplus* and *R. sanguineus*) [68, 69, 72, 73], *Hyalomma* spp. [71, 72], *Haemaphysalis hystricis* [74], and *Amblyomma sculptum* [72], the vectorial competence and capability have not been assessed thus far.

The clear dichotomy between the *Ehrlichia* and *Anaplasma* species that infect Xenarthra from different regions of the country may be related to the distribution and abundance of tick species that function as Anaplasmataceae vectors [78]. However, previous studies performed in different Brazilian states have shown that the different tick species that parasitize Xenarthra mammals are more correlated to the host species than to the geographic region. For instance, sloths are mainly parasitized by *Amblyomma varium* and *A. geayi*, with the latter found more in the state of Pará. While giant anteaters and southern anteaters are usually parasitized by A*. nodosum*, *A. sculptum,* and *A. calcaratum*, armadillos are frequently parasitized by *A. pseudoconcolor*, *A. auricularium,* and *A. sculptum* [79, 80, 81, 82, 83]. These studies

show the high parasitic specificity of some species of ticks that infest these animals. For instance, the ticks collected from anteaters from the state of São Paulo in the present study were all identified as *A. nodosum*, which was the most frequently found species in this group of animals (data not shown).

The vectorial capacity of the tick species frequently found in Xenarthra mammals for Anaplasmataceae agents is still unknown. Although the majority of ticks that parasitize this group of mammals belong to the genus *Amblyomma* spp., the main vectors of *Ehrlichia* spp. in Brazil belong to the genus *Rhipicephalus* spp. Further studies should be conducted to assess the vectorial capacity of these ticks and to better understand the genetic diversity of Anaplasmataceae agents that infect mammals of the Xenarhra superorder in Latin America as well as the possible role of these animals in the epidemiological cycle of these agents.

5. Conclusion

The present study showed the high occurrence of *Anaplasma* spp. (27.57%) and *Ehrlichia* spp. (24.54%) in free-living Xenarthra mammals sampled from four states in Brazil. In addition, the study provides the first description of the occurrence of *E. canis* and *E. minasensis* in this group of mammals. The analysis of two genetic regions of *Anaplasma* spp., one conserved (*rrs*), and another one more diverse (intergenic region 23S-5S) revealed similar results of the low identity in BLASTn analysis, phylogenetic positioning in two different clades that were separate from the others containing known species, and formation of two different genotypes from those comprising known *Anaplasma* species by the diversity analysis. Based on these findings, we propose two new Candidatus species—*Candidatus* Anaplasma amazonensis and *Candidatus* Anaplasma brasiliensis— in Xenarthra from Brazil.

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Competing interests

The authors declare no competing interests.

Data availability

The data tha support the findings of this study are openly available in National Center for Biotechnology Information at https://www.ncbi.nlm.nih.gov/, reference number *rrs:* MT199810-MT199833; *dsb*: MT212405-MT212423; 23S-4S intergenic region: MT267341-MT267354.

References

[1] Dumler, J.S. *et al*. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six news species combinations and designation of *Ehrlichia equi* and 'HGE agent'

as subjective synonyms of *Ehrlichia phagocytophila*. *Int. J. Syst. Evol. Microbiol.* **51**, 2145-2165 (2001).

[2] Rar, V. & Golovljova, I. *Anaplasma*, *Ehrlichia*, and "*Candidatus* Neoehrlichia" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infect. Genet. Evol.* **11**, 842-1861 (2011).

[3] Doudier, B., Olano, J., Parola, P., & Brouqui, P. Factors contributing to emergence of *Ehrlichia* and *Anaplasma* spp. as human pathogens. *Vet. Parasitol.***167**,149-154 (2010).

[4] Parola, P., Davoust, B., & Raoult, D. Tick and flea-born rickettsial emerging zoonoses. *Vet. Res.* **36**, 469–492 (2005).

[5] Machado, R. Z., Duarte, J.M.B., Dagnone, A.S., & Szabó, M.P.J. Detection of *Ehrlichia chaffeensis* in Brazilian marsh deer (*Blastocerus dichotomus*). *Vet. Parasitol.* **139**, 262–266 (2006).

[6]Picoloto, G. *et al.* Real time polymerase chain reaction to diagnose *Anaplasma marginale* in cattle and deer (*Ozotoceros bezoarticus leucogaster*) of the Brazilian Pantanal. *Rev. Bras. Parasitol. Vet.* **19**,186-188.

[7] Sacchi, A.B.V., Duarte, J.M.B., André, M.R., & Machado, R.Z. Prevalence and molecular characterization of Anaplasmataceae agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*). *Comp. Immunol. Microbiol. Infect. Dis.* **32**, 325-334 (2012).

[8] Silveira, J.A., Rabelo, E.M., & Ribeiro, M.F. Molecular detection of tickborne pathogens of the family Anaplasmataceae in Brazilian Brown Brocket Deer (*Mazama gouazoubira,* Fischer, 1814) and Marsh Deer (*Blastocerus dichotomus*, Illiger, 1815). *Transbound. Emerg. Dis.* **59**, 353–360 (2012).

[9] Silveira, J.A.G. *et al.* Molecular detection and identification of hemoparasites in pampas deer (*Ozotocerus bezoarticus* Linnaeus, 1758) from the Pantanal Brazil. *Ticks Tick Borne Dis.* **4**, 341–345.

[10] Silveira, J.A.G., Rabelo, E.M.L., Lima, P.C.S., Chaves, B.N., & Ribeiro, M.,F.,B. Post-mortem hemoparasites detection in free-living Brazilian brown brocket deer (*Mazama gouazoubira*, Fischer 1814). *Rev. Bras. Parasitol. Vet.* **23**, 206–215 (2014).

[11] Mongruel, A. C. *et al.* Molecular characterization of *Anaplasma* sp. in free-living gray brockets (*Mazama gouazoubira*). *Vector Borne Zoonotic Dis.* **17**, 165-171 (2016).

[12] André, M.R. *et al*. Molecular detection of tick-borne bacterial agents in Brazilian and exotic captive carnivores. *Ticks Tick Borne Dis.* **3**, 247-253 (2012).

[13] Almeida, A.P., Souza, T.D., Marcili, A., & Labruna, M.B. Novel *Ehrlichia* and *Hepatozoon* agents infecting the Crab-Eating Fox (*Cerdocyon thous*) in Southeastern Brazil. *J. Med. Entomol.* **50**, 640-646 (2013).

[14] de Sousa, K. C. M. *et al*. Anaplasmataceae agents among wild mammals and ectoparasites in Brazil. *Epidemiol. Infect.* **145**, 3424-3437 (2017).

[15] André, M.R. *et al.* Molecular and detection of *Ehrlichia* spp. in endangered Brazilian wild captive felids. *J. Wildl. Dis.* **46**, 1017 – 1023 (2010).

[16] Widmer, C.E., Azevedo, F.C., Almeida, A.P., Ferreira, F., & Labruna, M.B. Tick-borne bacteria in free-living jaguars (*Panthera onca*) in Pantanal, Brazil. *Vector Borne Zoonotic Dis.* **11**, 1001-1005 (2011).

[17] Wolf, R.W. *et al.* Novel *Babesia* and *Hepatozoon* agents infecting non-volant small mammals in the Brazilian Pantanal, with the first record of the tick *Ornithodoros guaporensis* in Brazil. *Ticks Tick Borne Dis.* **7**, 449-456 (2016).

[18] Benevenute, J.L. *et al.* Assessment of a quantitative 5’ nuclease real-time polymerase chain reaction using *groEL* gene for *Ehrlichia* and *Anaplasma* species in rodents in Brazil. *Ticks Tick Borne Dis.* **8**, 646-656 (2017).

[19] Braga, M.S.C.O. *et al.* Molecular detection of Anaplasmatacea agents in *Dasyprocta azarae* in northeastern in Brasil. *Rev. Bras. Parasitol. Vet.*, *27*, 99-105 (2018).

[20] Soares, H. S. *et al.* Ticks and rickettsial infection in the wildlife of two regions of the Brazilian Amazon. *Exp. Appl. Acarol.* **65**, 125-140 (2017).

[21] Lopes, M. G. *et al*. Ticks, rickettsial and erlichial infection in small mammals from Atlantic forest remnants in northeastern Brazil. *Int. J. Parasit. Parasites Wildl.* **7**, 380-385 (2018).

[22] Guimarães, A. *et al*. Detection of a putative novel genotype of *Ehrlichia* sp. from opossums (*Didelphis aurita*) from Brazil. *Rev. Bras. Parasitol. Vet.* **28**, 140-144 (2019).

[23] Machado, R. Z. *et al*. Migratory and carnivorous birds in Brazil: reservoirs for *Anaplasma* and *Ehrlichia* species?. *Vector Borne Zoonotic Dis.* **12**, 705-708 (2012).

[24] Werther, K. *et al*. Arthropod-borne agents in wild Orinoco geese (*Neochen jubata*) in Brazil. *Comp. Immunol. Microbiol. Infect. Dis.* **55**, 30-41 (2017).

[25] Gardner, A. L. Order Pilosa in *Mammal Species of the World* (Johns Hopkins University Press, 2005).

[26] Reis, N. R., Peracchi, A.L., Pedro, W. A., Lima, I. P. *Mamíferos do Brasil* (Eds, 2006).

[27] Kluyber, D. *et al.* Ticks (Acari: Ixodidae) infesting armadillos (Cingulata: Dasypodidae) in the Pantanal wetland, Mato Grosso do Sul, Brazil. *Syst. Appl. Acarol.* **21**, 1087-1092 (2016).

[28] Guillemi, E. C. *et al.* Molecular identification of *Anaplasma marginale* in two autochthonous South American wild species revealed an identical new genotype and its phylogenetic relationship with those of bovines. *Parasit. Vectors* **9**, 305 (2016).

[29] Birkenheuer, A. J., Levy, M. G., & Breitschwerdt, E. B. Development and evaluation of a seminested PCR for detection and differentiation of *Babesia gibsoni* (Asian genotype) and *B. canis* DNA in canine blood samples. *J. Clin. Microbiol.* **41**, 4172-4177 (2003).

[30] Massung, R. F. *et al.* Nested PCR assay for detection of granulocytic ehrlichiae. *J. Clin. Microbiol.* **36**, 1090-1095 (1998).

[31] Drazenovich, N., Foley, J., & Brown, R. N. Use of real-time quantitative PCR targeting the msp2 protein gene to identify cryptic *Anaplasma phagocytophilum* infections in wildlife and domestic animals. *Vector Borne Zoonotic Dis.* **6**, 83-90 (2006).

[32] Carelli, G. *et al.* Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. *Vet. Microbiol*. **124**, 107-14 (2007).

[33] Rejmanek, D., Bradburd, G., & Foley, J. Molecular characterization reveals distinct genospecies of *Anaplasma phagocytophilum* from diverse North American hosts. *J. Med. Microbiol.* **61**, 204 (2012).

[34] Doyle, C. K. *et al.* Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. *J. Mol. Diag.* **7**, 504-510 (2005).

[35] Reller, M., & Dumler, J. Development and Clinical Validation of a Multiplex Real-Time Quantitative PCR Assay for Human Infection by *Anaplasma phagocytophilum* and *Ehrlichia chaffeensis*. *Trop. Med. Infect. Dis.* **3**, 14 (2018).

[36] Müller, A. *et al.* "*Candidatus* Neoehrlichia chilensis" sp. nov.: Molecular detection and characterization of a novel Anaplasmataceae in wild rodents from Valdivia, southern Chile. *Transbound. Emerg. Dis.* **65**, 357-362 (2018).

[37] Nion, V. L. *et al.* Potentially novel *Ehrlichia* species in horses, Nicaragua. *Emerg. Infect. Dis.* **21**, 335 (2015).

[38] Inayoshi, M., Naitou, H., Kawamori, F., Masuzawa, T., & Ohashi, N. Characterization of *Ehrlichia* species from *Ixodes ovatus* ticks at the foot of Mt. Fuji, Japan. *Microbiol. Immunol.* **48**, 737-745 (2004).

[39] Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**, 611-622 (2009).

[40] Aguiar, D. M., Saito, T. B., Hagiwara, M. K., Machado, R. Z., & Labruna, M. B. Serological diagnosis of canine monocytic ehrlichiosis with Brazilian antigen of *Ehrlichia canis*. *Cien. Rural* **37**, 796-802 (2007).

[41] Sanger, F., Nicklen, S., & Coulson, A. R. DNA sequencing with chain-terminating inhibitors. *Proc. Nati. Acad. Sci.* **74**, 5463-5467 (1977).

[42] Ewing, B., Hillier, L., Wendl, M. C., & Green, P. Base-calling of automated sequencer traces usingPhred. I. Accuracy assessment. *Genome Res.* **8**, 175-185 (1998).

[43] Ewing, B., & Green, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* **8**, 186-194 (1998).

[44] Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410 (1990).

[45] Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. GenBank. *Nucleic Acids Res.* **41**, D36-D42 (2012).

[46] Thompson, J. D., Higgins, D. G., & Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673-4680 (1994).

[47] Hall, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* (Vol. 41, No. 41, pp. 95-98). London: Information Retrieval Ltd., c1979-c2000 (1999).

[48] Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772 (2012).

[49] Miller, M. A., Pfeiffer, W., & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop* (GCE), 01-08 (2010).

[50] Ronquist, F., & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574 (2003).

[51] Stamatakis, A., Hoover, P., & Rougemont, J. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **57**, 758-771 (2008).

[52] Felsenstein, J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783-791 (1985).

[53] Stöver, B. C., & Müller, K. F. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinform.* **11**, 7 (2010).

[54] Librado, P., & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451 -1452 (2009).

[55] Clement, M., Posada, D., & Crandall, K. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**, 1657-1660 (2000).

[56] Huson, D. H., & Bryant, D. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* **23**, 254-267 (2005).

[57] Leigh, J. W., & Bryant, D. Popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110-1116 (2015).

[58] Fry, N. K., Warwick, S., Saunders, N. A., & Embley, T. M. The use of 16S ribosomal RNA analyses to investigate the phylogeny of the family Legionellaceae. *Microbiol.* **137**, 1215-1222 (1991).

[59] Clarridge, J. E. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Microbiol. Rev.* **17**, 840-862 (2004).

[60] Koh, F. X., Kho, K. L., Panchadcharam, C., Sitam, F. T., & Tay, S. T. Molecular detection of *Anaplasma* spp. in pangolins (*Manis javanica*) and wild boars (*Sus scrofa*) in Peninsular Malaysia. *Vet. Parasitol.* **227**, 73-76 (2016).

[61] Battilani, M., De Arcangeli, S., Balboni, A., & Dondi, F. Genetic diversity and molecular epidemiology of *Anaplasma*. *Infec. Genet. Evol.* **49**, 195-211 (2017).

[62] Cabezas-Cruz, A. *et al. Ehrlichia minasensis* sp. nov., isolated from the tick *Rhipicephalus microplus*. *Int. J. Syst. Evol. Microbiol.* **66**, 1426-1430 (2016).

[63] Aguiar, D. M., Araujo, J. P., Nakazato, L., Bard, E., & Cabezas-Cruz, A. Complete Genome Sequence of an *Ehrlichia minasensis* Strain Isolated from Cattle. *Microbiol Resour Announc*, **8**, e00161-19 (2019).

[64] Aguiar, D. M. *et al.* A novel *Ehrlichia* genotype strain distinguished by the TRP36 gene naturally infects cattle in Brazil and causes clinical manifestations associated with ehrlichiosis. *Ticks Tick Borne Dis.* **5**, 537-544 (2014).

[65] Gajadhar, A. A., Lobanov, V., Scandrett, W. B., Campbell, J., & Al-Adhami, B. (2010). A novel *Ehrlichia* genotype detected in naturally infected cattle in North America. *Vet. Parasitol.* **173**, 324-329.

[66] Hailemariam, Z. *et al*. Molecular detection of tick-borne pathogens in cattle from Southwestern Ethiopia. *PloS One* **12**, e0188248 (2017).

[67] Lobanov, V. A., Gajadhar, A. A., Al-Adhami, B., & Schwantje, H. M. Molecular Study of Free-ranging Mule Deer and White-tailed Deer from British Columbia, Canada, for Evidence of Anaplasma spp. and Ehrlichia spp. *Transbound. Emerg. Dis.* **59**, 233-243 (2012).

[68] Cabezas-Cruz, A. *et al*. New species of *Ehrlichia* isolated from *Rhipicephalus (Boophilus) microplus* shows an ortholog of the *E. canis* major immunogenic glycoprotein gp36 with a new sequence of tandem repeats. *Parasites Vectors* **5**, 291 (2012).

[69] Iweriebor, B. C. *et al.* Genetic profiling for *Anaplasma* and *Ehrlichia* species in ticks collected in the Eastern Cape Province of South Africa. *BMC Microbiol.* **17**, 45 (2017).

[70] Thomson, K. *et al.* A new TaqMan method for the reliable diagnosis of *Ehrlichia* spp. in canine whole blood. *Parasites Vectors* **11**, 350 (2018).

[71] Rehman, A., Conraths, F. J., Sauter-Louis, C., Krücken, J., & Nijhof, A. M. Epidemiology of tick-borne pathogens in the semi-arid and the arid agro-ecological zones of Punjab province, Pakistan. *Transbound. Emerg. Dis.* **66**, 526-536 (2019).

[72] Carvalho, I. T. *et al.* Minimum infection rate of *Ehrlichia minasensis* in *Rhipicephalus microplus* and *Amblyomma sculptum* ticks in Brazil. *Ticks Tick Borne Dis.* **7**, 849-852 (2016).

[73] Cicculli, V. *et al*. First detection of *Ehrlichia minasensis* in *Hyalomma marginatum* ticks collected from cattle in Corsica, France. *Vet. Med. Sci.* **5**, 243-248 (2019).

[74] Li, J. *et al.* Emergence of a Novel *Ehrlichia minasensis* Strain, Harboring the Major Immunogenic Glycoprotein trp36 with Unique Tandem Repeat and C-Terminal Region Sequences, in *Haemaphysalis hystricis* Ticks Removed from Free-Ranging Sheep in Hainan Province, China. *Microorganisms*, *7*, 369 (2019).

[75] Groves, M. G., Dennis, G. L., Amyx, H. L., & Huxsoll, D. L. Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). *Am. J. Vet. Res.* **36**, 937-940 (1975).

[76] Moraes-Filho, J., Krawczak, F. S., Costa, F. B., Soares, J. F., & Labruna, M. B. Comparative evaluation of the vector competence of four South American populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. *PLoS One* **10**, e0139386 (2015).

[77] Johnson, E. M. *et al.* Experimental transmission of *Ehrlichia canis* (Rickettsiales: Ehrlichieae) by *Dermacentor variabilis* (Acari: Ixodidae). *Vet. Parasitol.* **74**, 277-288 (1998).

[78] Kocan, K. M., De La Fuente, J., & Cabezas-Cruz, A. The genus *Anaplasma*: new challenges after reclassification. *Rev. Sci. Tech.* **34**, 577-86 (2015).

[79] Marques, S., Barros-Battesti, D. M., Faccini, J. L. H., & Onofrio, V. C. Brazilian distribution of *Amblyomma varium* Koch, 1844 (Acari: Ixodidae), a common parasite of sloths (Mammalia: Xenarthra). *Mem. Inst. Oswaldo Cruz* **97**, 1141-1146 (2002).

[80] Soares, H. S. *et al.* Ticks and rickettsial infection in the wildlife of two regions of the Brazilian Amazon. *Exp. Appl. Acarol.* **65**, 125-140 (2015).

[81] Kluyber, D. *et al.* Ticks (Acari: Ixodidae) infesting armadillos (Cingulata: Dasypodidae) in the Pantanal wetland, Mato Grosso do Sul, Brazil. *Syst. Appl. Acarol.* **21**, 1087-1092 (2016).

[82] Martins, T. F. *et al.* Diversity of ticks in the wildlife screening center of São Paulo city, Brazil. *Cien. Rural* **47** (2017).

[83] Muñoz-García, C. I. *et al.* Epidemiological study of ticks collected from the northern tamandua (*Tamandua mexicana*) and a literature review of ticks of Myrmecophagidae anteaters. *Ticks Tick Borne Dis*. **10**, 1146-1156 (2019).