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Genetic diversity and lack of molecular evidence for hemoplasma cross-species transmission between wild and synanthropic mammals from Central-Western Brazil

Luiz Ricardo Gonçalves[a,b], Heitor Miraglia Herrera[c], Wesley Arruda Gimenes Nantes[c], Filipe Martins Santos[c], Grasiela Edith de Oliveira Porfírio[d], Wanessa Teixeira Gomes Barreto[c], Gabriel Carvalho de Macedo[c], Willian de Oliveira Assis[c], João Bosco Vilela Campos[c], Thiago Merighi Vieira da Silva[b], Leticia Colovatti Mariano[b], Darci Moraes Barros-Battesti[b], Rosangela Zacarias Machado[b], Marcos Rogério André[b,*]

[a] Agricultural Microbiology Program at School of Agrarian and Veterinary Sciences (FCAV/UNESP), Jaboticabal, São Paulo, Brazil
[b] Department of Veterinary Pathology, School of Agrarian and Veterinary Sciences (FCAV/UNESP), Jaboticabal, São Paulo, Brazil
[c] Dom Bosco Catholic University, (UCDB), Campo Grande, Mato Grosso do Sul, Brazil
[d] Graduate Program of Natural Resources, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

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ABSTRACT

Globally, hemotropic mycoplasmas (hemoplasmas) comprise an emerging or remerging bacteria group that attaches to red blood cells of several mammal's species and in some cases, causing hemolytic anemia. Herein, we assessed the occurrence, genetic diversity, the factors coupled to mammals infection, and the phylogeographic distribution of hemoplasmas in sylvatic and synanthropic mammals and their associated ectoparasites from Brazil. We collected spleen and/or blood samples from synanthropic rodents (*Rattus rattus* [*N* = 39] and *Mus musculus* [*N* = 9]), sylvatic rodents (*Hydrochoerus hydrochaeris* [*N* = 14]) and opossums (*Didelphis albiventris* [*N* = 43]). In addition, ticks (*Amblyomma* spp. [*N* = 270] and lice (*Polyplax spinulosa* [*N* = 6]) specimens were also sampled. Using a PCR targeting the 16S rRNA region, out of 48 small rodents, 14 capybaras and 43 opossums DNA samples, hemoplasma DNA was found in 25%, 50%, and 32.5% animals, respectively. Besides, we reported hemoplasma DNA in *Amblyomma* sp. (22.2% [2/9]) and lice (100% [2/2]) pools samples from rats, and one female *A. sculptum* DNA sample (3% [1/33]) obtained from a capybara. Additionally, and in agreement with ML analysis, the network analyses showed a clear phylogenetic separation among the hemoplasmas genotypes found in the different host species sampled, thus, suggesting the absence of cross-species hemoplasmas transmission between the mammals trapped. Finally, using the NTC network analysis, we reported the same 16S rRNA *Mycoplasma* genotype circulating in *Rattus* sampled in Brazil, Hungary, and Japan.

1. Introduction

Interactions among sympatric vertebrate hosts, vectors and pathogens shape infectious diseases occurrence, infection load, the timing of outbreaks and invasiveness of pathogens (Hoberg and Brooks, 2015; Cohen et al., 2018). Recently, the occurrence of arthropod-borne diseases in humans and animals have increased due to distinct factors, such as climate change (higher temperatures increased pathogen propagation and disease incidence), affecting the species behavior and immunity; urbanization (causing biotic homogenization); deforestation and natural environment encroachment (predisposing a higher contact among wildlife, humans and domestic animals); and globalization (e.g. wildlife and livestock trades). As a consequence, these factors may affect the transmission dynamics of pathogens, the emergence and re-emergence of infectious diseases and the spillover of humans and domestic animals' pathogens to wildlife and vice-versa (Chomel et al., 2007; Dar and Reshi, 2014; Otranto et al., 2015; Price et al., 2019).

Rodentia and Didelphimorphia comprise two important mammal groups, playing an crucial role in the maintenance of the ecological balance. Whereas rodents (order Rodentia) are widely distributed in different habitats and represent the largest order of mammals (over 2200 species), the family Didelphidae (order Didelphimorphia) is found

* Corresponding author.
E-mail address: mr.andre@unesp.br (M.R. André).

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only throughout the nearctic and neotropical zones and comprises 98 species (Wilson and Reeder, 2005; IUCN – https://www.iucnredlist.org – Accessed on August 2019).

Among the different ecological roles, these animals are important reservoirs for many pathogens (e.g. *Trypanosoma* spp., *Leishmania* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*), and play a crucial role to immature tick stages development (Labruna, 2009; Stuen et al., 2013; Roque et al., 2014; Rodrigues et al., 2019).

In the current scenario, the hemotropic mycoplasmas (also known as hemoplasmas) emerge as pathogens that could impact on humans and animals' health (Maggi et al., 2013). The hemoplasmas are uncultivated cell wall-less bacteria that attach to red blood cells surface of a wide range of animals. Besides, the hemoplasmas are mainly transmitted via direct contact with blood - e.g., aggressiveness among animals -, and produce a primary acute infection that is followed by a persistent latent infection (Neimark et al., 2005; Cohen et al., 2018). Once the host is infected, hemoplasmas could induce acute hemolysis, and the disease is characterized by anorexia, lethargy, dehydration, weight loss, and occasionally death (Willi et al., 2007).

In Brazil, hemoplasmas DNA has been detected in domestic animals, such as cats (Miceli et al., 2013; André et al., 2014), dogs (Ramos et al., 2010; Valle et al., 2014), goats (Machado et al., 2017), cattle (Girotto et al., 2012; de Mello et al., 2019) and sheep (Souza et al., 2019). Concerning the wildlife, these agents have been molecularly detected in free-ranging and captive wild felids (Willi et al., 2007; André et al., al.,2011; de Souza et al., 2017; Furtado et al., 2019), wild and captive canids (André et al., al.,2011; de Souza et al., 2017), bats (Ikeda et al., 2017), non-human primates (Bonato et al., 2015; de Melo et al., 2019), wild and synanthropic rodents (Vieira et al., 2009; Gonçalves et al., 2015), procyonids (de Souza et al., 2017; Cubilla et al., 2017), deer (Grazziotin et al., 2011), wild boars (Dias et al., 2019) and opossums (Massini et al., 2019). On the other hand, hemoplasmas DNA has not been detected in ectoparasites in Brazil so far (de Souza et al., 2017).

Although extensive surveys have been conducted in Brazil, several biological aspects of hemoplasmas epidemiology remain poorly assessed. Thus, the elucidation of these bacterial cycles in nature, including the genetic diversity, identification of hosts, vectors and the species distribution in a particular ecotope shows great importance.

Thereby, the present study aimed to investigate the occurrence and the genetic diversity of hemoplasmas infecting marsupials, wild and synanthropic rodents and associated ectoparasites in urban areas and forest fragments in central-western Brazil.

2. Materials and methods

2.1. Study sites, mammal trapping and sample collection

Between May 2017 and August 2018, 105 mammals belonging to four species were sampled in different sites of Campo Grande municipality (-20^0 42′ 30″ S, -54^0 61′ 60″ W), state of Mato Grosso de Sul, Central-Western Brazil (Table 1). Forty-eight small rodents were trapped in urban areas (four sites) and urban forest fragments (four sites). Additionally, fourteen capybaras (*Hydrochoerus hydrochaeris*) – the largest rodent in South America – were trapped in three urban forest fragments. Lastly, 43 marsupials were sampled in six urban forest fragments. The small mammals were caught using Tomahawk and Sherman live traps baited with a mix of bananas, paçoca, oat flakes and tinned sardines. Once captured, the small rodents were chemically immobilized using a combination of ketamine hydrochloride (100 mg/mL) and acepromazine (10 mg/mL) (1:9) intramuscularly. After that, spleen fragments were collected under sterile conditions, placed into DNase and RNase-free microtubes containing etanol (100%), and maintained at −20 °C until DNA extraction. On the other hand, the marsupials (*Didelphis albiventris*) were anesthetized with a chemical association of Ketamine (20 mg/kg) and Xylazine (2 mg/kg) intramuscularly. Blood samples were collected from the marsupials' lateral caudal veins, placed to DNase and RNase-free anticoagulant ethylenediaminetetraacetic acid (EDTA)-contaning microtubes, and maintained at −20 °C until DNA extraction. Finally, the capybaras were initially immobilized using an anesthetic dart containing TELAZOL® 100 (4 mg/kg). Subsequently, approximately 2–5 mL of blood was collected from the femoral vein into EDTA-buffered vacutainer tubes. The samples were kept on ice until arrival in the laboratory and stored at −20 °C until DNA extraction. The following data were recorded from each animal: the presence of ectoparasites, gender weight, and sampling point.

All animals were checked for the presence of ectoparasites. Once collected, the ectoparasites were placed in microtubes containing absolute etanol (Merck) and maintained at −20 °C until morphological identification and DNA extraction. The morphological identification was performed using previously described taxonomic keys (Onofrio et al., 2005; Martins et al., 2010).

All animal captures were in accordance with the licenses obtained from the Instituto Chico Mendes de Conservação da Biodiversidade (license number 56,912–2), Imasul (license number 05/2017) and endorsed by the Ethics Committee of FCAV/UNESP University under the number: 01,952/18.

2.2. DNA extraction and molecular detection of hemoplasmas

DNA was extracted from 10 mg of each small rodent spleen tissue and 200 μL of blood samples from capybaras and marsupials, using the DNeasy® Blood & Tissue Kit (Qiagen®, Valencia, California, USA), according to manufacturer's instructions. Furthermore, the collected ectoparasites were submitted to DNA extraction individually and/or in pools (the tick nymphs were pooled up to 3 individuals and the larvae up to 7 individuals from the same host - the lice were pooled up to 2 specimens from the same host), using the commercial kit above mentioned.

In order to discard the presence of PCR inhibitors, all extracted mammal DNA samples were used as a template in an internal control PCR targeting the mammal *gapdh* gene as previously described (Birkenheuer et al., 2003). Likewise, all arthropod DNA samples were submitted to internal control targeting the 16S rRNA as previously described (Black and Piesman, 1994). Internal control-PCR positive samples were subsequently submitted to a broad-range hemoplasma PCR assay targeting the 16S rRNA gene.

Previously described PCR protocols were utilized to amplify *Mycoplasma* spp. 16S rRNA gene, using two sets of primers, namely HemMycop16S-41 s (5′-GYATGCMTAAYACATGCAAGTCGARCG-3′) and HemMyco16S-938as (5′-CTCCACCACTTGTTCAGGTCCCCGTC-3′) (fragment of 800 bp), and HemMycop16S- 322 s (5′-GCCCATATTCCT ACGGGAAGCAGCAGT-3′) and HemMycop16S-1420as (5′-GTTTGACG GGCGGTGTGTACAAGACC-3′) (fragment of 800 bp) as previously described (Maggi et al., 2013). Sequences derived from each amplicon obtained from each primer set (with an overlap of 600 bp) were used to build a larger consensus sequence (approximately 1200 bp). '*Candidatus* Mycoplasma haemobos' DNA (MF992084) obtained from a naturally infected buffalo (Gonçalves et al., 2018) and ultra-pure sterile water were used as positive and negative controls, respectively.

2.3. Phylogenetic analysis

The amplicons obtained from 16S rRNA-based PCR assays were purified using the EXOSAP-IT® (Applied Biosystems). Purified amplified DNA fragments were submitted to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyser – Applied Byosystem/ Perkin Elmer). Consensus sequences were obtained through the analysis of electropherograms using the Phred-Phrap program (Ewing et al., 1998). The Phred quality score (peaks around each base call) was established at ≥20 (99% in accuracy of the base call). Hemoplasma-16S rRNA sequences were identified by BLASTn using the

Table 1
Number and animal species postitive to hemoplasmas targeting the 16S rRNA.

Animal species	Sample type	N⁰ of sampled animals	Occurrence of hemoplasma% (N⁰)	Ectoparasite species	N⁰ of sampled ectoparasites	Occurrence of hemoplasma% (N⁰)
Mammals				Arthropds		
Rodentia				Ixodida/Phthiraptera*		
R. rattus	DNA from spleen tissues	39	30.7% (12/39)	*Amblyomma* sp.[a]	62	22.2% (2/9)[b]
				*P. spinulosa**	6	100% (2/2)[b]
M. musculus	DNA from spleen tissues	9	0% (0/9)	–	–	–
H. hydrochaeris	DNA from whole blood	14	50% (7/14)	*A. dubitatum*	42	0% (0/42)
				A. sculptum	36	3.3% (1/33) [c]
				Amblyomma sp.[a]	2	0% (0/2)
Didelphimorphia						
D. albiventris	DNA from whole blood	43	32.5% (14/43)	*A. dubitatum*	70	0% (0/28)[b]

[a] *Amblyomma* sp. refers to larvae sampled – In these specimens only the genus was reported.
[b] Ectoparasites-DNA pool samples.
[c] Three out of 36 *Amblyomma*-DNA samples were negative to the endogenous control (16S rRNA).

Megablast (following default parameters), aligned with sequences available in GenBank using Clustal/W (Thompson et al., 1994), and adjusted in Bioedit v. 7.0.5.3 (Hall, 1999). The phylogenetic analysis was performed using Maximum Likelihood (ML) method, inferred with RAxML-HPC BlackBox (7.6.3.) (Stamatakis et al., 2008) and performed in CIPRES Science Gateway (Miller et al., 2010). The Akaike Information Criterion (AIC) available on MEGA v. 5 software (Tamura et al., 2011) was applied to identify the most appropriate model of nucleotide substitution. GTR+ G + I model was chosen as the most appropriate for the phylogenetic analysis of the 16S rRNA alignment.

2.4. Identification and genetic relationship of identified hemoplasmas genotypes

The 16S rRNA aligned sequences amplified in the present study were utilized to identify the genotypes using the DnaSP v5.10 (Librado and Rozas, 2009). To investigate the genetic relationship among hemoplasmas genotypes detected in the present study and those previously detected in rodents and marsupials retrieved from GenBank, a Neighbor-Net network was constructed, using the pairwise genetic distances with SplitsTree v4.14.6 (Huson and Bryant, 2006). Additionally, the different genotypes identified were submitted to TCS network inferred using the Population Analysis with Reticulate Trees (popART v. 1.7) (Leigh and Bryant,2015).

3. Results

3.1. Ectoparasites and hemoplasma occurrence and BLASTn analysis

Ectoparasites were found in 10.4% (5/48) of small rodents. Also, 71.4% (10/14) of trapped capybaras were infested by ticks. Lastly, ticks were observed in 32.5% (14/43) of the sampled opossums. All ectoparasites species sampled are shown in (Table 1).

Except for three tick-DNA samples obtained from capybaras, all arthropod and mammal DNA samples were positive to 16S rRNA and *gapdh* endogenous control PCR assays, respectively. The tick samples negative in arthropod-16S rRNA PCR assay were excluded from subsequent analyses.

Out of 48 small rodents, 14 capybaras and 43 opossums DNA samples, hemoplasma DNA was found in 25%, 50% and 32.5% animals, respectively. Besides, 16S rRNA hemoplasma was detected in 22.2% (2/9) of the *Amblyomma* sp. larvae and 100% (2/2) of the *Polyplax spinulosa* DNA samples collected from *R. rattus*. Additionally, one female *Amblyomma sculptum* DNA sample (3% [1/33]) obtained from one capybara was positive to hemoplasma. Nonetheless, all 28 *Amblyomma dubitatum* nymphs DNA samples were negative to *Mycoplasma* spp. (Table 1).

The positive samples obtained in hemoplasmas-PCR showing high intensity amplicons in agarose gel electrophoresis were selected and submitted to sequencing. The BLASTn analysis showed that hemoplasmas-16S rRNA sequences detected in rodents and their associated ectoparasites (N = 5) shared percentage of identity ranging from 99.5% to 100% with other murine hemoplasmas detected in Brazil (KT215635, KT215639, KT215642) and Japan (AB758439). In addition, the sequences detected in capybaras (N = 3) were identical (100% of identity) to a *Mycoplasma* sp. sequence (FJ667773) previously reported in a capybara from Brazil. Finally, the sequences identified in opossums (N = 5) were identical to *Mycoplasma* sp. sequence (MH158514) detected in an opossum (*D. albiventris*) from Brazil and shared 98.8% of identity with '*Candidatus* Mycoplasma haemodidelphis' (AF178676) detected in a marsupial (*Didelphis virginiana*) in the USA. All sequences amplified in the present study showed query coverage of 100%. The amplified 16S rRNA sequences were deposited in Genbank under accession numbers: MN423253-MN423265.

3.2. Phylogenetic and genotype analyses

In accordance with BLAST analysis, one sequence detected in *R. rattus* (MN423261) and another sequence amplified in *P. spinulosa* (MN423265) clustered together with '*Candidatus* M. haemomuris' sequences (KM258432, KT215635, AB758435) previously detected in synanthropic rodents (*R. novergicus* and *R. rattus*) from Brazil and Japan. Additionally, other two sequences detected in *R. rattus* (MN423262 and MN423263) and another one identified in *Amblyomma* sp. larvae (MN423264) collected from *R. rattus* were positioned together with other sequences detected in synanthropic rodents from Brazil (KT215639), Japan (AB752303) and Hungary (KJ739312) (Fig. 1).

Regarding the sequences identified in capybaras (MN423253-MN423255), three sequences grouped with hemoplasma sequences (FJ667773 and FJ667774) previously detected in capybaras in southern Brazil. Besides, these sequences closely positioned to a hemoplasma sequence (KY002651) detected in *Nasua nasua* trapped in central-western Brazil, albeit remaining in a separate branch (Fig. 1).

Regarding the hemoplasma sequences identified in opossums, five sequences (MN423256-MN423260) were phylogenetically related to *Mycoplasma* sp. sequences (MH158514 and MH158515) previously detected in *D. albiventris* in southern Brazil, and to '*Candidatus* M. haemodidelphis' (AF178676), previously detected in *D. virginiana* from the USA. Interestingly, this cluster was closely related to *Mycoplasma* sp.

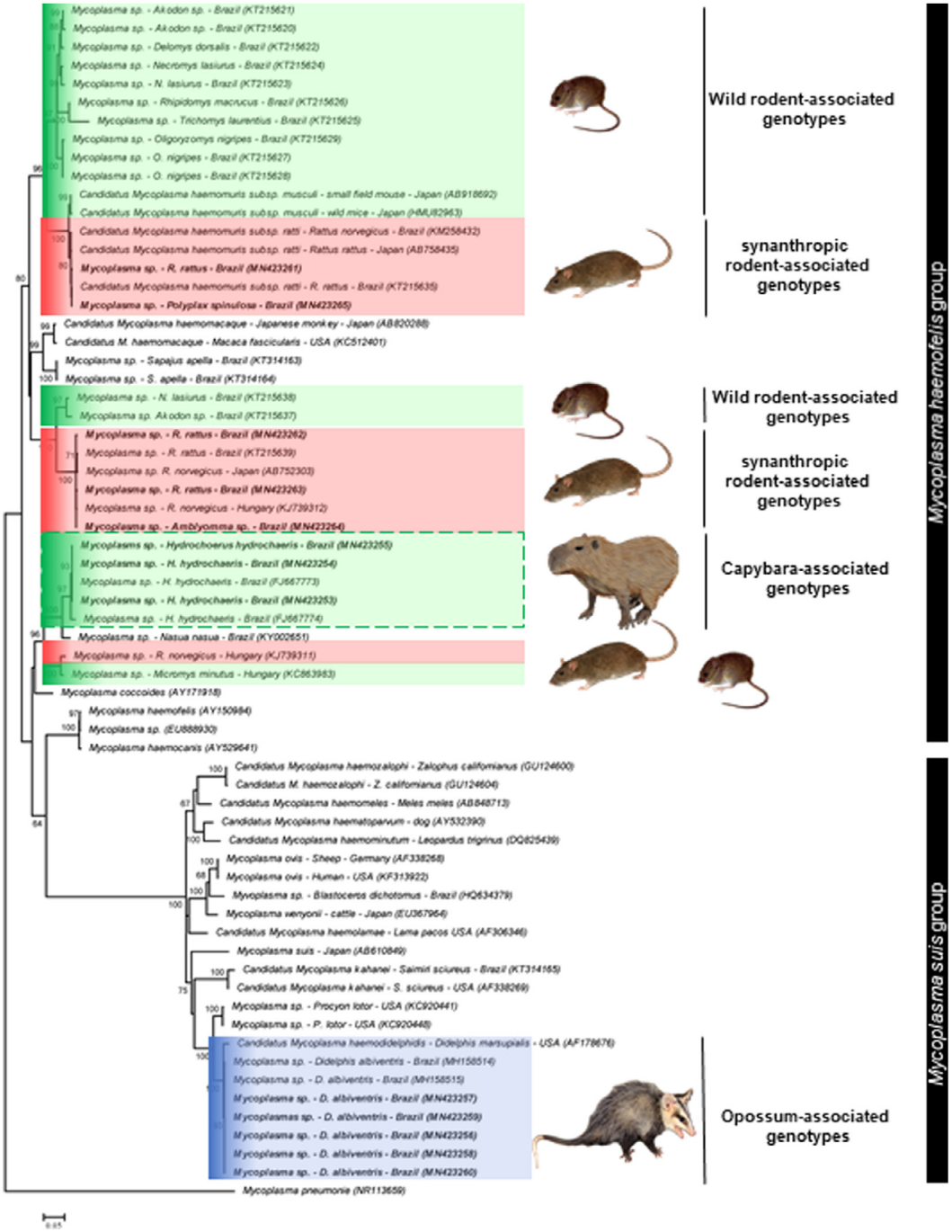

Fig. 1. Phylogenetic relationships within the *Mycoplasma* genus based on a fragment of 1500 bp of the 16S rRNA gene. The phylogenetic tree was inferred by using the maximum likelihood method. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to boostrap values higher than 60% accessed with 1000 replicates. *Mycoplasma pneumoniae* was used as outgroup.

sequences (KC920441 and KC920448) detected in *Procyon lotor* from the USA (Fig. 1). All clusters reported in the current study were supported by high bootstrap values ranging from 80% to 100%.

In agreement with ML analysis, the Neighbor-Net network analysis showed a clear phylogenetic separation among the hemoplasmas found in the different host species sampled in the present study and highlighted the richness of *Mycoplasma* species infecting rodents. In addition, we did not find rodent-associated – including those reported in capybaras – genotypes circulating in opossums and vice-versa (Fig. 2). Likewise, the TCS network analysis, performed using the sequences amplified in the current study and others previously detected in rodents and opossums retrieved from GenBank data base, showed the presence of 21 different genotypes (Fig. 3). The capybaras sequences detected in the present study together with a sequence previously detected in a capybara (FJ667773) from Brazil were classified as genotype #1. Also, the opossum sequences were grouped in the genotype #2, including the sequences previously detected in an opossum (MH158514 and MH158515) from Brazil. In addition, the '*Candidatus* M. haemodidelphis' sequence was herein classified as genotype #19. Lastly, the synanthropic rodent-related sequences amplified in the current study were grouped as genotypes #3 and #4. The genotype #3 consisted of five sequences, one detected in *R. rattus* and another detected in *Amblyomma* sp. larvae. Other three sequences were previously reported in *Rattus* spp. from Brazil and Japan. Among the eight sequences

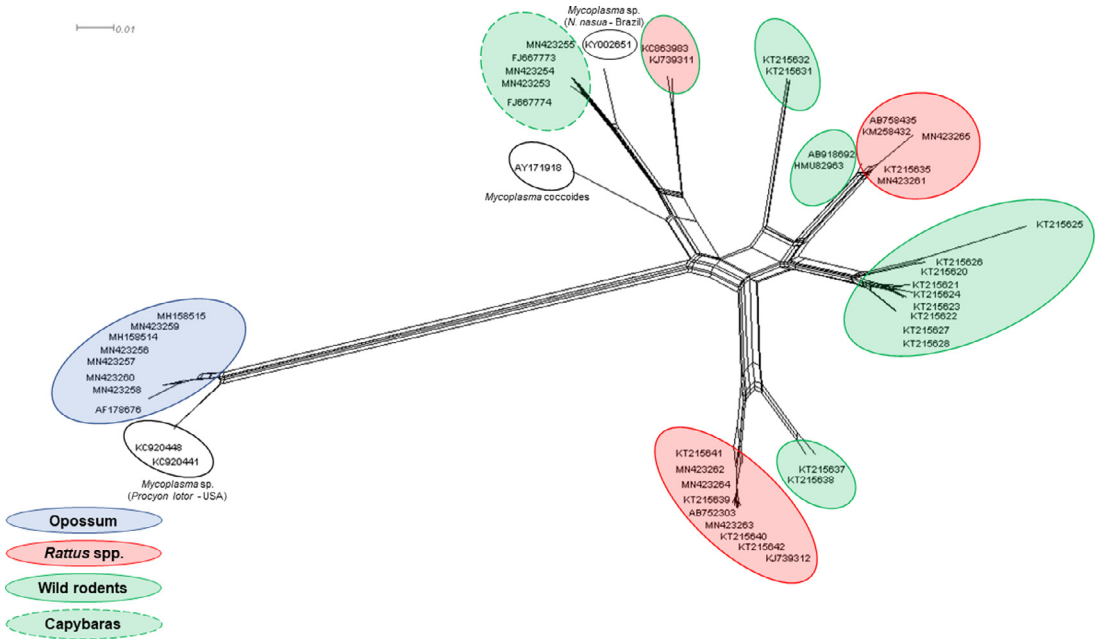

Fig. 2. Neighbor-Net analysis of 16S rRNA sequences obtained from wild and synanthropic rodents, opossum sampled in the present study and compared to related hemoplasmas sequences previously deposited in GenBank.

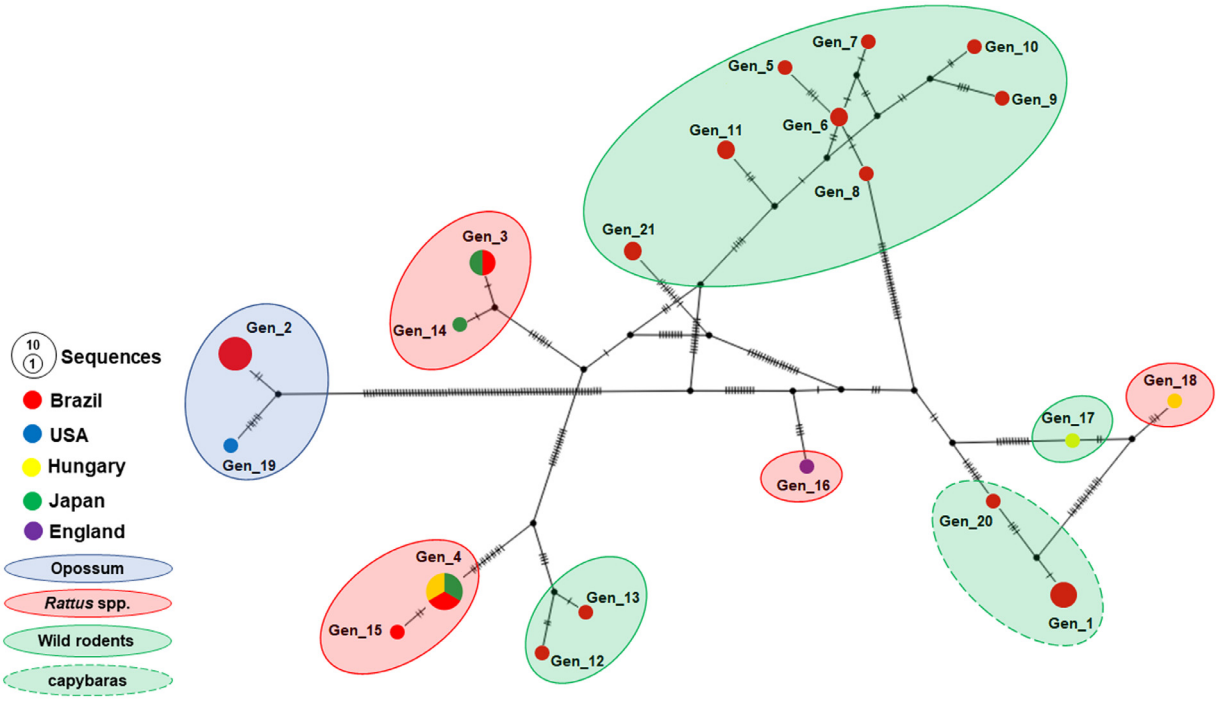

Fig. 3. NTC network analysis of 16S rRNA genotypes detected in wild and synanthropic rodents, opossum and related ectoparasites.

comprising the genotype #4, five were previously reported in *Rattus* spp. trapped in Brazil, Japan and Hungary. Other three sequences were detected in *R. rattus* and *P. spinulosa* in the present study (Fig. 3).

4. Discussion

In the present study, we reported the molecular occurrence and genetic diversity of hemoplasmas in rodents and marsupials and associated ectoparasites in central-western Brazil. Additionally, using a phylogeographic approach, the specificity of hemoplasma genotypes among different groups of mammals was assessed.

The prevalence of hemotropic mycoplasmas among different mammal species sampled around the world is widely variable, ranging from 0% to 78.8% (Willi et al., 2009; Vieira et al., 2015; de Souza et al., 2017; Volokhov et al., 2017; Millán et al., 2018; Souza et al., 2019). Herein, the occurrence of hemoplasmas was 25%, 50% and 32.5% among the small rodents, capybaras and opossuns samples, respectively. The difference observed in the occurrence of hemoplasmas has been assigned to distinct biological factors, such as group of analyzed animals (e,g., free-ranging vs captive animals) (Vieira et al., 2009), habitat types (e.g. undisturbed sites vs disturbed sites) (Volokhov et al., 2017), gregarious behavior of the hosts (de Souza et al., 2017), and age

(Persichetti et al., 2018). Interestingly, we found highest hemoplasma occurrence in rodents trapped in forest fragments. These results are congruent to those previously reported in raccoons from Georgia, USA (Volokhov et al., 2017). The highest occurrence of hemoplasma infection in rodents trapped in forest fragments could be partially attributed to aggressive interactions among these animals due to lower food availability when compared to urban areas – since the main route of rodent-associated hemoplasma transmission seems to be direct (Cohen et al., 2018). On the other hand, a higher availability of food in urban areas may have led to higher tolerance by rodents, reducing the aggressive interaction and hence, the hemoplasma transmission through infected saliva or blood. Also, the higher availability of food might improve the immune response against hemoplasma infection (Becker et al., 2014). However, the low number of animals sampled in the present study prevented an accurate statistical analysis and, therefore any speculation about it should be treated with caution. Additionally, considering that the hemoplasma occurrence could be attributed to distinct biological factors, further studies aiming at determining the biological parameters (e.g., host interactions and density, presence and richness of vectors species) that may play a role in hemoplasmas prevalence among different mammals are needed.

To the best authors' knowledge, this was the first molecular detection of hemoplasmas in ticks and lice from Brazil. Although the vector competence of *P. spinulosa* and *P. serrata* to *M. coccoides* has been determined (Berkenkamp and Wescott, 1988), a recent study (Cohen et al., 2018) suggested that *M. haemomuris*-like is mainly transmitted by rodent-rodent contact. Additionally, the authors did not find evidence of horizontal transmission of hemoplasmas by fleas (*Synosternus cleopatrae*). Therefore, while the real role of ticks in the hemoplasma transmission cycles remains poorly assessed, the PCR positive results found in ectoparasites should be analyzed carefully, since it may represent reminiscent DNA from mammal blood samples. Indeed, the tick vector competence for hemoplasmas should be conducted in future studies.

The ML analysis corroborated the results obtained by BLASTn, since the *Rattus*-associated hemoplasma sequences grouped with other synanthropic rodents-associated *Mycoplasma* sequences previously detected (Gonçalves et al., 2015; Harasawa et al., 2015). Previously, studies proposed the split of *M. haemomuris* into two phylogenetic closely related subspecies based on different targets, such as 16S rRNA, ITS and *rnpB* (Sashida et al., 2013; Harasawa et al., 2015). Additionally, and in agreement with previous studies (Gonçalves et al., 2015; Sashida et al., 2013; Hornok et al., 2015), the ML analysis showed the occurrence of another hemoplasma species, which was strongly supported (bootstrap of 100%).

The phylogenetic analysis also showed that the capybaras sequences grouped with other sequences previously reported in this same rodent species and supported by high bootstrap value (97%) Finally, the opossum sequences that clustered with other sequences detected in an opossum from southern Brazil (Massini et al., 2019) were positioned slightly separated from '*Candidatus* Mycoplasma haemodidelphis' reported in *D. virginiana* in the USA (Messick et al., 2012). Therefore, future studies sampling a higher number of animals and targeting additional genes (23S rRNA, *rpoB, gyrB*) should be performed in order to assess the phylogenetic positioning of these species.

Interestingly, the genotypes reported in the present study infecting the trapped animals showed to be host-specific, and thus, suggesting the absence of cross-species transmission of hemoplasmas among small rodents, capybaras and opossums – even sharing some tick species. Future experimental studies should confirm this hypothesis. Similar results were described between urban raccoons and sympatric feral cats in Georgia, USA (Volokhov et al., 2017). On the other hand, Millán et al., 2018 reported that some genotypes were shared among different wild carnivore species (among different mustelids specimens, as well as between Mustelidae and Viverridae species) sampled in northern Spain. The spillover phenomenon – cross-species pathogens transmission – is promoted by successive processes that provide chances of an animal pathogen to establish the infection in another one (Plowright et al., 2017). The infection establishment is driven by synergism of distinct factors, such as host's distribution and behavior, pathogen prevalence, route of transmission, and genetic, physiological and immunological features of recipient hosts (Plowright et al., 2017). In this way, several biological features – not verified herein – can allow or not the cross-species transmission. Curiously, the cats and dogs-related hemoplasmas species/genotypes have been extensively reported in wild felids and canids, respectively (Willi et al., 2009; André et al., 2011; Harasawa et al., 2014; de Souza et al., 2017; Ghazisaeedi et al., 2017; Millán et al., 2018). Surely, these hemoplasmas species/genotypes carry specific factors that enable these pathogens to overcome every barrier and settle in another host. However, these factors seem to be absent among rodents. These findings are in accordance to a previous study (Gonçalves et al., 2015), who in a wide survey in Brazil sampling wild rodent and synanthropic rodent species concluded that the hemoplasmas reported in wild rodents are restrict to these animals and seem not to infect *R. rattus* or *M. musculus*. However, further studies aiming at identifying the factors that are crucial to cross-species transmission are needed.

The species belonging to the *Rattus* genus have been shown interesting models for ecological studies, mainly due to their dispersion around the globe (Aplin et al., 2011; Kosoy and Bai, 2019). Herein, using the Neighbor-Net and NTC network approaches, we demonstrated that the same genotypes circulate in synanthropic rodents sampled in Brazil, Japan and Hungary. Although only one partial gene was herein analyzed, these results agree with the hypothesis previously raised that *Rattus*-related hemoplasma genotypes detected in Brazil may have been introduced in Brazilian territories during the European colonization period (Gonçalves et al., 2015). However, further studies targeting other genes as well as the genomes are needed to solve it. Despite the wide distribution of *Rattus* spp. in Brazil, coupled with the unknown zoonotic potential of these genotypes, future studies aiming to assess the impact of these hemoplasmas in human and animal health should be carried out.

5. Conclusion

The present study reported a high occurrence of hemoplasma in synanthropic rodents, capybaras, opossums and associated ectoparasites sampled in central-western Brazil. Also, the assessment of the network based on 16S rRNA disclosed the hemoplasmas genetic richness occurring in rodents and highlighted the presence of the same genotype infecting synanthropic rodents from different countries. Finally, we did not find molecular evidence suggesting hemoplasmas cross-species transmission among the wild and synanthropic rodents, capybaras and opossums.

Declaration of competing interest

None.

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