



## Diversity and molecular characterization of novel hemoplasmas infecting wild rodents from different Brazilian biomes

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### ABSTRACT

Although hemoplasma infection in domestic animals has been well documented, little is known about the prevalence and genetic diversity of these bacteria in wild rodents. The present work aimed to investigate the occurrence of hemotropic mycoplasmas in wild rodents from five Brazilian biomes, assessing the 16S rRNA phylogenetic position of hemoplasma species by molecular approach. Spleen tissues were obtained from 500 rodents, comprising 52 different rodent species trapped between 2000 and 2011. DNA samples were submitted to previously described PCR protocols for amplifying *Mycoplasma* spp. based on 16S rRNA, followed by sequencing and phylogenetic inferences. Among 457 rodent spleen samples showing absence of inhibitors, 100 (21.9%) were PCR positive to *Mycoplasma* spp. The occurrence of hemotropic mycoplasmas among all sampled rodents was demonstrated in all five biomes and ranged from 9.3% (7/75) to 26.2% (38/145). The Blastn analysis showed that amplified sequences had a percentage of identity ranging from 86 to 99% with other murine hemoplasmas. The ML phylogenetic analysis of 16S rRNA gene of 24 positive randomly selected samples showed the presence of ten distinct groups, all clustering within the *Mycoplasma haemofelis*. The phylogenetic assessment suggests the circulation of novel hemoplasma species in rodents from different biomes in Brazil.

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### 1. Introduction

Hemotropic mycoplasmas are epicytellar erythrocytic bacteria attach to red blood cells of a wide variety of wild and domestic animals, including humans. Although hemoplasma infection in domestic animals has been well documented, little is known about the prevalence, genetic diversity, and routes of transmission, interactions among bacteria-host-vectors, and the impact of infection on wild animal's health [1–3]. Blood-sucking arthropods (ticks, fleas and lice) are responsible for the transmission of hemotropic mycoplasmas among animals [4,5].

Phylogenetic analyses have identified two major clusters of hemoplasmas, comprising *Mycoplasma suis* and *Mycoplasma haemofelis* groups. Several hemoplasma species infecting wild and domestic animals were included in these two clusters [6,7].

Phylogenetic approaches have been showing the circulation of a wide and diverse number of genotypes and/or *Candidatus* to new hemoplasma species in wild animals, including some with zoonotic potential [8,9].

Rodentia is the most diversified and widespread order of mammals. A wide number of species is found in different habitats, including semi-aquatic, terrestrial and semi-fossorial ones in Brazil [10]. Rodent species may act as reservoirs for diverse zoonotic pathogens [11–13]. However, the zoonotic potential of rodent hemoplasmas has not yet been established [14].

Recently, *Mycoplasma haemomuris*, one of two hemotropic species of the *Mycoplasma* genus currently recognized in rodents, has been separated in two subgroups genetically distinct: '*Candidatus M. haemomuris* subsp. musculi', detected in small Japanese field mouse (*Apodemus argenteus*) an endemic species from Japan [15] and '*Candidatus M. haemomuris* subsp. ratti', detected in *Rattus rattus* [16]. According to the authors, the separation of *M. haemomuris* in two species depends mostly on the host species where these strains have been detected rather than the geographical

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localization where positive animals have been sampled. Experimental and/or field studies have incriminated lice (*Polyplax serrata* and *P. spinulosa*) as vectors of *M. coccoides* and *M. haemomuris* among rodents [14,17].

Considering the large number of hemotropic mycoplasma species, the crescent number of *Candidatus* to new species recently described in different species of mammals and the wide spectrum of rodents distributed around the world, the diversity of hemoplasmas infecting rodents are presumably more complex than is currently supposed.

Regarding the occurrence of hemotropic mycoplasmas in rodents in Brazil, novel hemoplasmas have been reported in capybaras in the state of Paraná [18]. Besides, hemoplasmas have been detected in several other wild animals in Brazil, including wild carnivores [19], cervids [20] and non-human primates [21,22].

The present study aimed to investigate the occurrence and assessment of the 16S rRNA phylogenetic position of hemoplasma species infecting wild rodents from different Brazilian biomes through molecular approach.

## 2. Material and methods

### 2.1. Distribution and rodent species sampled

Between 2000 and 2009, different rodent species (52) were trapped in five Brazilian biomes (Fig. 1) (<http://www.informativoflorestal.com.br>). Animals were caught using Tomahawk and Shermann “live-traps” during previous studies performed by the Laboratories of Trypanosomatid Biology and of Biology and Parasitology of Wild Mammals Reservoirs Laboratories, Oswaldo Cruz Institute, Rio de Janeiro, Brazil [23–25]. Euthanasia of animals was performed for taxonomic identification

and/or diagnosis of parasites. Rodents were chemically immobilized using an association of ketamine hydrochloride (100 mg/mL) and acepromazine (10 mg/mL) intramuscularly. When the death of anesthetized animal did not occur after total blood collection, then euthanasia was performed through the application of Potassium Chloride 19.1% via intracardiac (2 mL/kg).

Spleen tissues from 500 rodents were collected and stored in DNase and RNase-free microtubes, containing ethanol and stored at  $-20^{\circ}\text{C}$  until DNA extraction. Sampling procedures were approved by Brazilian Institute for the Environment and the Natural Renewable Resources (IBAMA), (IBAMA/CGFAU/LIC 3665-1) and Oswaldo Cruz Foundation (FIOCRUZ) ethics committee (P0007-99; P0179-03; P0292/06; L0015-07).

### 2.2. PCR assays

DNA was extracted from 10 mg of each rodent spleen tissue using the DNeasy® Blood & Tissue Kit (Qiagen®, Valencia, California, USA), according to manufacturer’s instructions. DNA concentration and absorbance ratio (260/280) nm were measured using a spectrophotometer (Nanodrop, Thermo Scientific, USA). Microtubes containing ultra-pure sterile water were intercalated between each series of twenty rodent spleen samples and submitted to DNA extraction.

In order to discard the presence of PCR inhibitors, each sample of spleen extracted DNA was used as a template in an internal control PCR targeting the mammal IRBP gene (“Interphotoreceptor Retinoid Binding Protein”) as previously described [26]. Negative samples to IRBP gene-PCR were subsequently submitted to another internal control PCR targeting the GAPDH gene as previously described [27]. Positive samples to above described internal

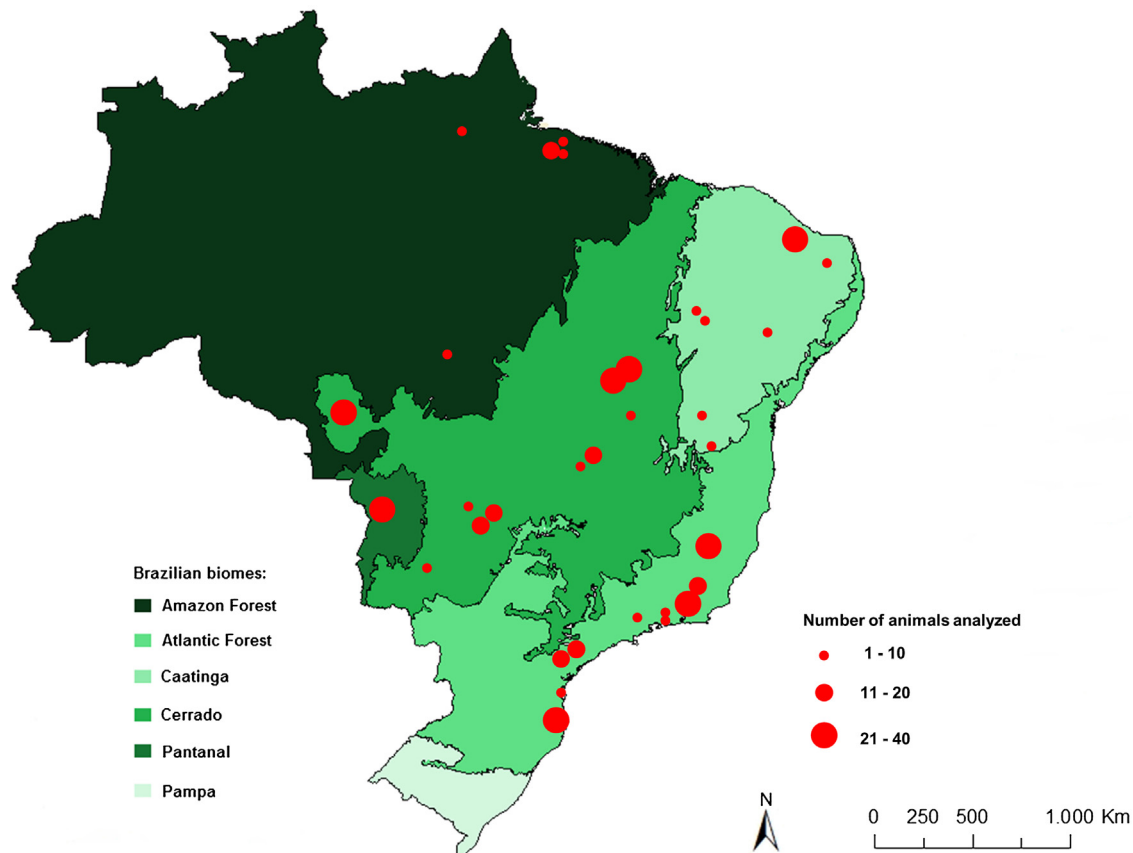


Fig. 1. Distribution of rodents sampled in different Brazilian biomes.

control PCR protocols were subsequently submitted to PCR assays targeting 16S rRNA-*Mycoplasma* spp.

Previously described PCR protocols based on 16S rRNA were utilized to amplify *Mycoplasma* spp. DNA, using two sets of primers, namely HemMycop16S-41s (5'-GYATGCMATAAYACATGCAAGTCGARGC-3') and HemMyco16S-938as (5'-CTCCACCAC-TTGTTCAGGTCCCGTC-3') (fragment of ~800 bp), and HemMycop16S-322s (5'-GCCCATATTCCTACGGGAAGCAGCAGT-3') and HemMycop16S-1420as (5'-GTTTGACGGGCGGTGTGTACAAGACC-3') (fragment of ~800 bp) as previously described [8]. *M. haemofelis* DNA obtained from a naturally infected cat [28] was used as positive control. Ultra-pure sterile water was used as negative control in all PCR assays described above. The 16S rRNA *Mycoplasma* spp.-positive samples were additionally submitted to RNaseP gene-*Mycoplasma* sp. (165 bp) PCR assay using the oligonucleotides HemoMyco RNaseP30s (5'-GATKGTGYGAGYATATAAAAAATAAARCTCRAC-3') and HemoMyco RNaseP200as (5'-GMGGRGTTTACCGCGTTTCAC-3') as previously described [8].

### 2.3. Statistical analysis

The Pearson's Chi-squared test was used to evaluate the association between biomes and the number of rodents positive for hemoplasmas using the SAS program (Statistical Analysis System version 9.2). Significance was defined as  $p < 0.05$ .

### 2.4. Phylogenetic analysis

PCR products of the expected size for each assay were purified from the gel using Silica Bead DNA Gel Extraction Kit (Fermentas, São Paulo, SP, Brazil). Amplicons were sequenced in both directions using the same PCR primers (forward and reverse) by Sanger sequencing at (ABI Prism 310 Genetic Analyser–Applied Biosystem/Perkin Elmer). Consensus sequences were obtained through the analysis of the sense and antisense sequences using the CAP3 program (<http://mobyle.pasteur.fr/cgi-bin/MobylePortal/portal.py>). Comparisons with sequences deposited in GenBank were done using the basic local alignment search tool (BLASTn) [29]. The sequences were aligned with sequences published in GenBank using Clustal/W [30] and manually adjusted in Bioedit v. 7.0.5.3 [31]. Phylogenetic inference based on maximum likelihood criterion (ML), was inferred with RAxML-HPC BlackBox 7.6.3 [32] through the CIPRES Science Gateway [33]. Akaike information criterion was used in Mega 5.05 to identify the most appropriate model of nucleotide substitution. GTRGAMMA+I model was chosen as the most appropriate for the Maximum Likelihood analysis of the 16S rDNA alignment.

## 3. Results

### 3.1. Detection of hemoplasmas

Out of 500 rodent spleen samples analyzed, 457 were positive to internal control (IRBP gene) PCR. All negative samples to IRBP gene-PCR were also negative to GAPDH gene PCR and were excluded from subsequent analysis. Among 457 rodent spleen samples analyzed, 100 (21.9%) were PCR positive to *Mycoplasma* spp. (Table 1). The occurrence of hemotropic mycoplasmas among sampled rodents in each one of the five different biomes was of 9.3% (7/75) in the Caatinga, 12.5% (4/32) in the Pantanal, 24.7% (43/174) in the Cerrado, 25.8% (8/31) in the Amazon Forest and 26.2% (38/145) in Atlantic Forest biome. The statistical analysis showed that occurrence of hemoplasma is influenced by different biomes ( $\chi^2 = 11.24$ ,  $p = 0.024$ ). Statistical difference was observed

**Table 1**  
Number and distribution of rodent species positive to *Mycoplasma* spp.

Biome/state	Species	No. of positive rodents to internal control (IRBP gene)	No. of positive rodents to <i>Mycoplasma</i> spp. (16S rRNA)
<b>Atlantic forest</b>			
Rio de Janeiro	<i>Akodon</i> sp.	27	5
	<i>Necomys lasiurus</i>	4	3
	<i>Brucepattersonius</i> sp.	1	–
	<i>Delomys dorsalis</i>	9	5
	<i>Euryoryzomys russatus</i>	2	–
	<i>Oligoryzomys nigripes</i>	4	–
	<i>Oligoryzomys</i> sp.	1	1
	<i>Oxymycterus dasythricus</i>	3	–
	<i>Oxymycterus</i> sp.	3	2
	<i>Trinomys dimidiatus</i>	1	–
	<i>Trinomys iheringi</i>	3	–
	<i>Sciurus</i> sp.	1	–
	<i>Sphiggurus</i> sp.	1	–
	<i>Sphiggurus villosus</i>	1	–
	<i>Nectomys squamipes</i>	4	–
	<i>Thrichomys apereoides</i>	10	1
	Minas Gerais	<i>Oligoryzomys nigripes</i>	7
<i>Calomys cerqueirae</i>		3	2
<i>Oligoryzomys</i> sp.		4	2
<i>Oligoryzomys nigripes</i>		11	3
<i>Oligoryzomys flavescens</i>		3	1
São Paulo	<i>Akodon montensis</i>	11	5
	<i>Calomys tener</i>	10	3
	<i>Mus musculus</i>	2	–
	<i>Akodon montensis</i>	5	1
	<i>Mus musculus</i>	1	–
Santa Catarina	<i>Euryoryzomys russatus</i>	10	–
	<i>Sooretamys angouya</i>	2	–
	<i>Mus musculus</i>	1	–
	<i>Mus musculus</i>	1	–
<b>Amazon forest</b>			
Pará	<i>Rattus rattus</i>	8	6
	<i>Mus musculus</i>	1	–
	<i>Proechimys goeldii</i>	7	–
	<i>Proechimys roberti</i>	2	–
	<i>Proechimys gr.cuvieri</i>	2	–
	<i>Proechimys</i> sp.	3	–
	<i>Zygodontomys</i> sp.	2	–
	<i>Rattus rattus</i>	2	2
Mato Grosso	<i>Proechimys</i> sp.	3	–
	<i>Necomys lasiurus</i>	1	–
	<i>Necomys lasiurus</i>	1	–
Bahia	<i>Necomys lasiurus</i>	1	–
	<i>Mus musculus</i>	1	–
	<i>Thrichomys inermis</i>	7	–
	<i>Thrichomys laurentius</i>	2	–
	<i>Thrichomys</i> sp.	3	2
	<i>Galea spixii</i>	8	–
	<i>Galea spixii</i>	12	–
Ceará	<i>Mus musculus</i>	7	–
	<i>Rattus rattus</i>	12	2
	<i>Thrichomys laurentius</i>	8	–
	<i>Rhipidomys macrurus</i>	3	1
Piauí	<i>Thrichomys laurentius</i>	7	2
	<i>Cavia</i> sp.	1	–
Rio Grande do Norte	<i>Thrichomys</i> sp.	3	–
	<i>Thrichomys</i> sp.	3	–
<b>Cerrado</b>			
Mato Grosso	<i>Rattus rattus</i>	3	3
	<i>Necomys lasiurus</i>	15	3
	<i>Calomys</i> sp.	16	–
Mato Grosso do Sul	<i>Calomys expulsus</i>	1	–
	<i>Necomys lasiurus</i>	6	3
	<i>Nectomys rattus</i>	3	1
	<i>Nectomys</i> sp.	3	–
	<i>Nectomys squamipes</i>	2	2
Goiás	<i>Necomys lasiurus</i>	9	2
	<i>Necomys lasiurus</i>	9	2
	<i>Cerradomys</i> sp.	3	–

**Table 1** (Continued)

Biome/state	Species	No. of positive rodents to internal control (IRBP gene)	No. of positive rodents to <i>Mycoplasma</i> spp. (16S rRNA)
Tocantins	<i>Hylaeamys megacephalus</i>	7	–
	<i>Necomys lasiurus</i>	6	1
	<i>Nectomys rattus</i>	5	2
	<i>Nectomys</i> sp.	2	1
	<i>Oecomys</i> gr. <i>bicolor</i>	4	–
	<i>Oryzomys megacephalus</i>	2	–
	<i>Rattus rattus</i>	4	1
	<i>Rhipidomys</i> sp.	10	5
	<i>Oligoryzomys nigripes</i>	2	–
	<i>Oligoryzomys fornesi</i>	2	1
	<i>Calomys</i> sp.	27	4
	<i>Hylaeamys</i> sp.	9	3
	<i>Necomys lasiurus</i>	9	6
	<i>Rhipidomys</i> sp.	5	2
	<i>Mus musculus</i>	1	1
	<i>Juliomys</i> sp.	1	–
	<i>Oligoryzomys</i> sp.	6	–
	<i>Cerradomys</i> sp.	3	–
<i>Oecomys</i> sp.	6	2	
<i>Oryzomys</i> sp.	2	–	
<b>Pantanal</b>			
Mato Grosso do Sul	<i>Clyomys laticeps</i>	8	–
	<i>Holochilus</i> sp.	6	–
	<i>Thrichomy fosteri</i>	18	4
<b>Total</b>		<b>457</b>	<b>100 (21.88%)</b>

only in Caatinga vs Cerrado ( $p=0.005$ ), Caatinga vs Amazon Forest ( $p=0.027$ ) and Caatinga vs Atlantic Forest ( $p=0.003$ ).

Unfortunately, all positive samples to 16S rRNA gene that were submitted to RNaseP gene PCR showed weak bands intensity which precluded sequencing these fragments.

### 3.2. Phylogenetic analyses

The Blastn analysis showed that amplified sequences shared percentage of identity ranging from 86 to 99% with other murine hemoplasmas ('*Candidatus M. haemomuris* subsp. musculi', '*Candidatus M. haemomuris* subsp. ratti' [formerly *M. haemomuris*], *M. coccoides* and with other sequences detected in rodents from Brazil and Hungary deposited in Genbank (Table 2).

The ML phylogenetic analysis based on a fragment of 1500 pb (after the alignment by Bioedit) of 16S rRNA gene of 24 positive randomly selected samples showed the presence of ten distinct

clusters. All clusters were positioned within *M. haemofelis* group and showed a strong separation supported by high bootstrap values (ranging from 97 to 100). The 16S rRNA hemoplasma sequences amplified from sylvatic rodents spleen samples clustered in eight different groups (clusters 1, 2, 3, 4, 5, 6, 8 and 10) (Fig. 2).

The cluster 1 consisted of five sequences that were obtained from three different rodent species (*Akodon* sp. [two sequences], *Delomys dorsalis* [one sequence], and *Necomys lasiurus* [one sequence] sampled in Atlantic Forest, and *N. lasiurus* [one sequence] sampled in Cerrado biome). Two sequences, amplified from *Thrichomys laurentius* and *Rhipidomys macrurus* sampled in Caatinga biome, were positioned in the cluster 2. The three sequences comprising the cluster 3 were amplified from rodents belonging to *Oligoryzomys nigripes* species captured in Atlantic Forest. The cluster 4 consisted of only one sequence amplified from *Rhipidomys* sp. captured in the Cerrado. Three sequences were grouped in cluster 5 (one detected in *Necomys rattus*, one in *Necomys* sp. and another in *N. lasiurus* species, which were sampled in Cerrado). The cluster 6 consisted on only one sequence, which was detected in *Thrichomys fosteri* in Pantanal biome. The cluster 8 consisted on two sequences obtained from *Akodon* sp. and *N. lasiurus*, which were captured in the Atlantic Forest. The cluster 10 consisted of only one sequence detected in *Akodon* sp. captured in the Atlantic Forest, closely related to *Mycoplasma* sp. detected in capybaras from Brazil. The percentage of identities (Blastn) between sequences obtained in the present study and other previously described is showed in Table 2.

Among the six synanthropic rodents (*R. rattus* captured in Amazon Forest and Cerrado) sequences, one (Cluster 7) was closely related to '*Candidatus M. haemomuris* subsp. ratti' detected in *R. rattus* from Japan (AB758435), showing 99% identity by Blast analysis. The five other sequences (Cluster 9) were closely related to *Mycoplasma* sp. detected in *R. norvegicus* from Japan (AB752303) and Hungary (KJ739312), both showing 99% identity by Blast analysis.

## 4. Discussion

Several studies have suggested that hemotropic mycoplasmas have co-evolved with many animal species, including humans. The development of molecular assays primarily targeting the 16S rRNA gene has resulted in an increased number of recognitions of these pathogens in both animals and humans [14,34–36].

Herein we related an occurrence of 21.9% for hemoplasmas infecting sylvatic rodents from five different Brazilian biomes, as well as the phylogenetic assessment of hemoplasma genotypes circulating in wild rodents from Brazil. In Japan, 11.1% (1/9) of synanthropic rodents sampled were positive to *Mycoplasma*

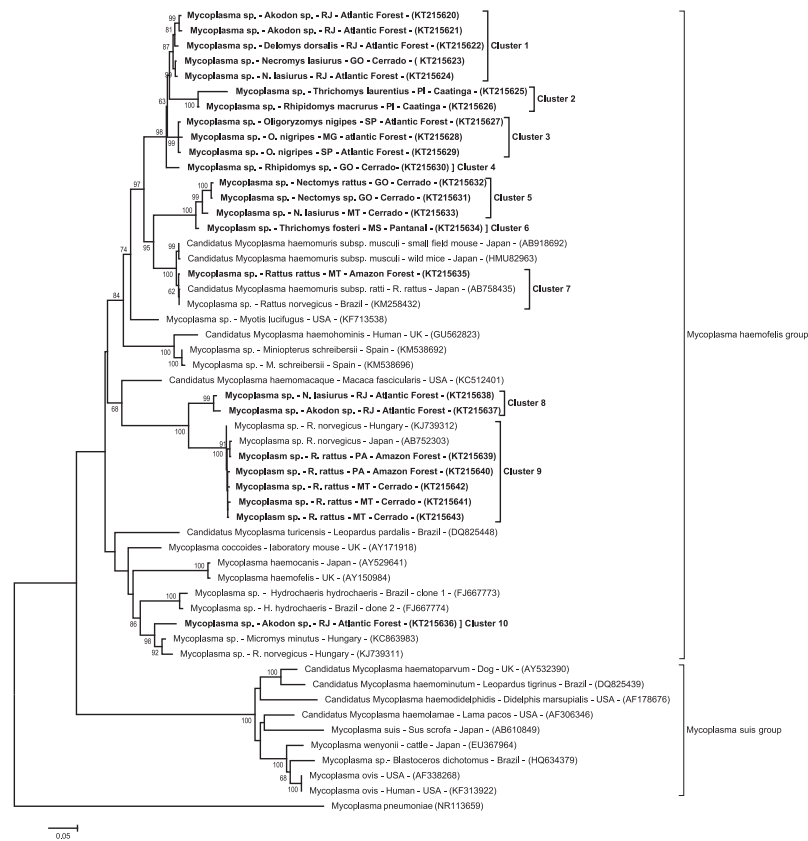
**Table 2**

Closest identity percentage (Blastn) between wild rodents hemoplasma 16S RNA sequences detected in Brazil and other murine hemoplasma sequences previously submitted to GenBank from others regions of the world.

	' <i>C. M. haemomuris</i> subsp. ratti' (AB758435)	' <i>C. M. haemomuris</i> subsp. musculi' (AB918692)	<i>Mycoplasma coccoides</i> (AY171918)	<i>Mycoplasma</i> sp. ( <i>Micromys min</i> ) (KC863983)	<i>Mycoplasma</i> sp. ( <i>R. norvegicus</i> ) (AB752303)	<i>Mycoplasma</i> sp. ( <i>Hydrochaeris hydrochaeris</i> ) (FJ667773)	<i>Mycoplasma</i> sp. ( <i>H. hydrochaeris</i> ) (FJ667774)
<b>Cluster 1</b>	93	93	91	90	90	89	89
<b>KT215625*</b>	89	89	86	86	85	86	86
<b>KT215626*</b>	93	93	90	90	89	89	89
<b>Cluster 3</b>	93	93	90	90	90	88	89
<b>Cluster 4</b>	93	93	89	90	89	88	89
<b>Cluster 5</b>	92	92	89	89	90	87	87
<b>Cluster 6</b>	93	93	89	89	89	88	88
<b>Cluster 7</b>	99	99	89	89	90	88	88
<b>Cluster 8</b>	90	89	90	89	93	88	89
<b>Cluster 9</b>	90	89	90	89	99	87	87
<b>Cluster 10</b>	91	89	93	96	89	92	92

\* The sequences highlighted belong to cluster 2.





**Fig. 2.** Phylogenetic relationships within the *Mycoplasma* genus based on a fragment of 1500 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 bootstrap values higher than 60% accessed with 1000 replicates. *Mycoplasma pneumoniae* was used as outgroup.

spp., although the low number of animals analyzed might have influenced the overall prevalence of hemoplasmas [37]. Recently, *Mycoplasma* spp. were detected in 67.3% (35/52) of rodents sampled in Hungary [14]. The smaller area where rodents were sampled in Hungary [14] – highlighting the aggregated distribution of parasitic diseases – compared to our study where rodents were sampled in five different biomes could explain the difference observed in the occurrence of hemoplasmas infecting wild rodents. Interesting, we observed statistical difference in the occurrence of *Mycoplasma* spp. between different biomes. Since hemoplasmas are vector-transmitted pathogens, we speculate that the distribution and abundance of arthropods may influence the occurrence of hemotropic mycoplasmas. Environmental factors, such as temperature, relative humidity, and seasonality may also have influence the *Mycoplasma* spp. occurrence. The Caatinga biome has a semi-arid climate, characterized by very low humidity, low rainfall and high temperatures with long dry seasons, when compared with other Brazilian biomes [38]. These factors may affect the development of arthropods stages and decrease the diversity and the abundance of vectors, which could explain the lower occurrence of *Mycoplasma* spp. in rodents from this biome. However, new studies are needed to access the gaps in biology of hemoplasmas. In Brazil, little is known about the occurrence and genetic diversity of hemoplasmas infecting wild animals. The first report of hemoplasma infecting rodents (*Hydrochaeris hydrochaeris*) in Brazil showed a higher occurrence (64%; 20/31) than that found in present study. The 16S rRNA sequences phylogenetic analyzes indicated divergence to other hemoplasma species previously detected in rodents [18].

The absence of a huge identity (85–96%) between the sequences amplified from sylvatic rodent spleen samples in the present study

and those retrieved from Genbank database (Table 2) highlights the existence of a high degree of genetic diversity among hemotropic mycoplasmas infecting wild rodents in Brazil. Overall, the phylogenetic analysis based on 16S rRNA sequences obtained in the present study showed the presence of ten distinct clusters, suggesting the possibility of novel hemoplasma infecting rodents in Brazil.

Although a recent study have suggested that the two distinct subgroups of *M. haemomuris* depended on species differences between the natural hosts of these strains rather than the geographic location of strain collections [15], we noticed that the majority of clusters obtained in the present study consisted on hemoplasma sequences amplified from rodents sampled in a specific biome, except for clusters 1 and 9. Besides, it is important to emphasize that there is a huge overlap of distribution areas of most of the rodent species found infected in the present study [10] and, consequently, a given genotype may circulate in different rodent species coexisting in a certain biome.

Interestingly, hemotropic mycoplasma detected in sylvatic rodents in the present study were phylogenetically distant from others murine hemoplasmas (*M. coccoides*, ‘*Candidatus M. haemomuris* subsp. musculi’ and ‘*Candidatus M. haemomuris* subsp. rattii’). Considering the fact that synanthropic rodents (*R. rattus*, *R. norvegicus* and *M. musculus*) have an Asiatic origin and were introduced in Brazil only about 500yrs ago, we raise a hypothesis that the above mentioned hemotropic mycoplasma species were introduced together with synanthropic rodents during the European colonization. Additionally, our preliminary results suggest that these hemoplasmas species are restrict to synanthropic rodents or, at least, show a low occurrence among sylvatic rodents in Brazil. This hypothesis may have supported on the biology of synanthropic rodents in the environment. Although *R. rattus* and

*M. musculus* have been reported in all Brazilian states, they are usually found closely to human habitation [10]. On the other hand, the natural environment in which sylvatic rodents are found comprises woods, forest, gallery forests, veredas and other less disturbed areas [10]. This fact might have reduced the contact and exchange of pathogens through arthropod vectors between synanthropic and sylvatic rodents, which consequently generate a low occurrence of these three hemoplasma species among sylvatic rodents. In addition, all DNA sequences detected in *R. rattus* in the present study were grouped with other *Mycoplasma* spp. previously detected in synanthropic rodents (clusters 7 and 9). However, future studies sampling a large number of synanthropic rodents are much needed to corroborate to this hypothesis.

Although studies have suggested that hemotropic mycoplasmas co-evolved with several animal species [14,34–36], little is known about the origin, evolution and dispersion of hemoplasmas. According to our results, the *Mycoplasma* species detected in this present study already existed in Brazil circulating among the sylvatic rodents before introduction of synanthropic rodents and their respective hemoplasmas during the colonization. However, future studies using a phylogeographic approach, for example, are much needed to corroborate to this hypothesis.

Although the vector competence of *P. serrata* and *P. spinulosa* in transmitting murine hemoplasma (*M. coccoides*) has been confirmed [17,39], the wide and complex diversity of hemotropic mycoplasmas infecting different rodent species revealed by our study associated to the fact that DNA of *Mycoplasma* spp. has been detected in fleas (*Synosternus cleopatrae*) collected from rodents (*Gerbillus andersoni*) [40], highlights the need of future studies to assess the role of other arthropod vectors (fleas, ticks, mites, etc.) in transmission cycles of these pathogens among rodents and other animals.

## 5. Conclusion

The phylogenetic assessment based on 16S rRNA sequences supported by a relative low percentage of identity found by Blast analysis suggest the circulation of novel hemoplasma species in rodents from different biomes in Brazil.

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