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Occurrence of *Mycoplasma* spp. in the golden-headed lion tamarin (*Leontopithecus chrysomelas*) in Southern Bahia, Brazil

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Abstract: The presence and zoonotic potential of *Mycoplasma* spp. have already been identified in primates from different regions of the world. Information on the consequences of these parasites for the health of non-human primates, especially those threatened with extinction, such as the golden-headed lion tamarin (*Leontopithecus chrysomelas*), is scarce in the literature. The issue requires greater attention, as it can contribute to species conservation actions. Thus, the main objective of the study was to investigate the presence of *Mycoplasma* spp. in blood samples subjected to PCR for the 16S rRNA gene of 21 golden-headed lion tamarins living in anthropically altered environments in southern Bahia, Brazil. Additionally, the hematological parameters of infected animals were evaluated. Of the assessed animals (n=21), 38% were positive for *Mycoplasma* spp., showing an identity between 88.0 and 99.0% for this bacterium. There was no difference between positive and negative animals for *Mycoplasma* spp. concerning the hematological parameters evaluated. The data show that *Mycoplasma* spp. is present in the golden-headed lion tamarin in southern Bahia, which may affect animal and public health.

Keywords: Conservation; hemoparasites; hemoplasmas; primates; South America.

1. Introduction

The golden-headed lion tamarin (*Leontopithecus chrysomelas*) is a primate endemic to the Atlantic Forest in southern Bahia, northeastern Brazil (Teixeira, 2022), and it is an endangered species (criterion A3c) (Oliveira et al., 2021). Most local golden-headed lion tamarin populations live in areas under anthropogenic influence, such as degraded secondary forest and agricultural systems (e.g., agroforestry systems of cocoa (*Theobroma cacao*) plantations, popularly known as *cabrucas* (Bernal-Valle et al., 2025) generally surrounded by agricultural areas, pastures, and human settlements, which increases the risk of disease transmission among wild and domestic animals, and humans (Tomori and Oluwayelu, 2023). Thus, a One Health approach is required to assess the health status of these individuals to understand the epidemiology of *Mycoplasma* sp. in this threatened species (Leendertz et al., 2017), particularly in an environment sustaining greater risk of zoonotic disease emergence (Han et al., 2016), and contribute to the development and implementation of ongoing and future conservation strategies to protect these golden-headed lion tamarin populations.

Bacteria of the genus *Mycoplasma* spp. are essential to public and veterinary health. These bacteria adhere to the surface of erythrocytes from various wild and domestic mammals, including humans (Paul et al., 2020; Strandberg et al., 2023; Lignon et al., 2025). It is believed that hematophagous arthropods such as fleas, lice, ticks, and mosquitoes can transmit these bacteria (Paul et al., 2020). Most *Mycoplasma* spp. are species-specific, however, there are reports of infection by *Mycoplasma* spp. in species that are not the primary hosts (Obara et al., 2011). Human infection has been reported in pregnant women, immunocompromised patients and people exposed to animals and arthropods (Steer et al., 2011; Paul et al., 2020).

Infections by *Mycoplasma* spp. have been reported worldwide in some species of non-human primates such as *Macaca fascicularis* (Sricharern et al., 2021), *Macaca fuscata* (Sashida et al., 2014) and *Macaca assamensis* (Rucksaken et al., 2024). In Brazil, infections by *Mycoplasma* spp. have been reported in *Alouatta* spp. (Cubilla et al., 2017; Melo et al. 2019), *Sapajus apella*, *Saimiri sciureus* and *Saguinus midas niger* (Bonato et al., 2015), *Sapajus nigritus* (Cubilla et al., 2017), *Sapajus flavius* (Ramalho et al., 2017), and *Macaca mulatta* maintained in laboratory (Mongruel et al., 2022). Mycoplasma species found in primates were '*Candidatus* Mycoplasma kahanei' (Neimark et al., 2002), '*Ca.* M. aoti' (Barker et al., 2011) and '*Ca.* M. haemomacaque' (Mongruel et al., 2022; Narapakdeesakul et al., 2024; Rucksaken et al., 2024). In addition to these species, there are suspicions of others that have not yet been identified in non-human primates (Bonato et al., 2015; Ramalho et al., 2017). Some studies report that primates infected by *Mycoplasma* spp. may present hemolytic anemia (Contamin and Michel, 1999; Santos et al., 2013); however, this change is not always observed (Cubilla et al., 2017; Ramalho et al., 2017; Mongruel et al., 2022).

In this study, ~~we~~ it was investigated the occurrence of *Mycoplasma* spp. through molecular analysis in populations of the golden-headed lion tamarin in southern Bahia, Brazil. There were also investigated hematological changes associated with the occurrence of *Mycoplasma* spp. on animals.

2. Material and Methods

2.1. Ethical Statement

This work was approved by the Animal Research Ethics Committee of the Universidade Estadual de Santa Cruz under protocol # 018/2015. The Brazilian Environmental Agency (ICMBio/SISBIO) approved permission to capture and collect biological materials under protocols # 234576 and # 471783.

2.2. Studied animals and blood sample collection

From October 2015 to March 2017, blood samples were collected from 21 adult individuals, 12 males and nine females (Table 1), belonging to five groups of free-ranging golden-headed lion tamarins. Three of these groups (RIB, MRO and OZA) lived in fragments of degraded forest inserted in an agricultural matrix (called DFAM) of pastures, rubber trees (*Hevea brasiliensis*), cupuaçu (*Theobroma grandiflorum*), cocoa (*Theobroma cacao*) plantations and ephemeral crops in the rural area of the municipality of Una, Bahia, Brazil (15°15'52" S, 39°8'46" W). The other two groups (ALM and BOM) lived in areas of cocoa agroforestry systems (called *cabruca*) belonging to private farms in the rural area near the city of Ilhéus, Bahia, Brazil (14°39'S, 39°11'W). These environments (DFAM and *cabruca*) are approximately 107 km apart and there are no connections between tamarin populations studied in these two locations. *L. chrysomelas* groups that live in these areas have been habituated and monitored for ecology, behavior and health studies since 2013 (De Vleeschouwer and Oliveira, 2017; Costa et al., 2020; Bernal-Valle et al., 2025). Both of these environments (DFAM and *cabruca*) present specific challenges to groups of tamarins, such as higher hunting levels and human contact compared with groups living in more preserved areas (Costa et al., 2020).

To collect blood samples, tamarins were captured in tomahawk traps (0.48 m length × 0.15 m width × 0.15 m height), using bananas as bait, following the procedures described by Catenacci et al. (2022). After capture, animals were taken to a field laboratory and sedated (10 mg/kg Ketamine and 0.3 mg/kg Midazolam, intramuscularly), following Catenacci et al. (2016). After sedation, animals were evaluated for general health status, and blood samples were collected by puncture of the femoral vein, in the arteriovenous plexus of the inguinal region, with a maximum collection volume of 3 mL (Catenacci et al., 2022). Tamarins 6 and 113 had their blood collected at two different times. Tamarin 6 in June/2016 and March/2017 and Tamarin 113 in March/2016 and March/2017. The other animals had their blood collected only once (Table 1).

After collection, blood samples were stored in 4ml sterile tubes containing EDTA K3 anticoagulant (Vacuplast, Osasco, Brazil) and cooled for hematological analysis, which took place the day after collection. The tamarins were kept in the laboratory overnight to ensure full recovery from anesthesia and after feeding them banana, they were released ~~them~~ early in the morning of the next day at the site where they were captured. This procedure has been used and adapted to ensure animal safety, resulting in a very safe process with no detrimental effects on the animals if performed adequately (Costa et al., 2020; Catenacci et al., 2022).

Animal	Sex	Group	Environment	Collection period
Tamarin 92	F	RIB	DFAM	Mar/2016
Tamarin 126	M	RIB	DFAM	Mar/2017
Tamarin 115	M	MRO	DFAM	Mar/2016
Tamarin 102	M	MRO	DFAM	Oct/2015
Tamarin 116	F	MRO	DFAM	Oct/2015
Tamarin 117	M	MRO	DFAM	Oct/2015
Tamarin 95	F	OZA	DFAM	Mar/2016
Tamarin 109	F	OZA	DFAM	Mar/2017
Tamarin 113	F	OZA	DFAM	Mar/2016 and Mar/2017
Tamarin 123	M	OZA	DFAM	Mar/2017
Tamarin 14	M	ALM	CAB	Jun/2016
Tamarin 15	F	ALM	CAB	Jun/2016
Tamarin 17	M	ALM	CAB	Mar/2017
Tamarin 19	F	ALM	CAB	Mar/2017
Tamarin 23	M	ALM	CAB	Mar/2017
Tamarin 5	M	BOM	CAB	Jun/2016
Tamarin 6	F	BOM	CAB	Jun/2016 and Mar/2017
Tamarin 7	F	BOM	CAB	Mar/2017
Tamarin 10	M	BOM	CAB	Jun/2016
Tamarin 11	M	BOM	CAB	Jun/2016
Tamarin 12	M	BOM	CAB	Jun/2016

Table 1 – Characteristics of *Leontopithecus chrysomelas* from degraded areas in Una (DFAM), Bahia, Brazil, and *cabruca* areas (CAB), in Ilhéus, Bahia, Brazil, and blood collection period.

2.3. Hematological analyses

A complete blood cell count (CBC; i.e., hemogram, leucogram, and platelets) was performed using an automatic counter cell control (ABX Vetcounter, Horiba, Montpellier, France). Differential leukocyte count, aiming to determine the percentage of basophils, eosinophils, rod neutrophils, segmented neutrophils, lymphocytes, and monocytes, was obtained from examination of blood smears with rapid panoptic staining (Laborclin, Pinhais, Brazil). Values for total plasma protein were obtained using a manual refractometer. Reference values used to evaluate hematological parameters were based on the ranges established for *Leontopithecus* sp. (Verona and Pissinati, 2014). After the blood count was performed, whole blood samples were stored in sterile plastic cryotubes, free of DNase and RNase, and at -20°C for subsequent molecular analysis (Mongruel et al., 2022).

2.4. Molecular Diagnostics

Molecular protocols were used according to Criado-Fornelio et al. (2003) for. DNA was extracted from 200 µL of each tamarin blood sample, using a DNeasy Blood & Tissue extraction kit (Qiagen, Valencia, USA), according to the manufacturer's instructions. DNA concentration was measured using a spectrophotometer (Nanodrop, Thermo Scientific, Waltham, MA, USA). Microtubes containing sterile ultrapure water were used as a negative control. A feline sample positive for *Mycoplasma haemofelis* was used as the positive control. Samples were subjected to conventional Polymerase Chain Reaction (PCR) for 16S rRNA gene, whose primer oligonucleotides (HBT-F) – 5'- ATACGGCCCATATTCCTACG – 3', HBT-R – 5'- TGCTCCACCACTTGTTCA-3') were used to amplify a 600bp fragment (Criado-Fornelio et al., 2003). The amplification reaction was performed using a 25 µL total volume reaction, containing 5 µL of DNA, 0.2 mM of each deoxynucleotide, 0.5 µM of each oligonucleotide primer, 1.0 mM MgCl2, 10X PCR buffer, 1.5 IU Taq DNA Polymerase, and completing the remaining volume with ultrapure distilled water. Samples were subjected in the thermocycler (Biocycler MJ96G, Waltham, USA) to an initial denaturation at 95ºC for 2 minutes, 40 cycles consisting of denaturation at 94ºC for 1 minute, annealing at 60ºC for 45 seconds, and extension at 72ºC for 45 seconds, followed by a final extension at 72ºC for 5 minutes.

2.5. Sequencing and phylogenetic analysis

Products resulting from PCR were purified using PurelinkTM Quick gel extraction & PCR Purification combo Kit (Invitrogen, Carlsbad, USA). Purified DNA fragments amplified from positive samples were subjected to the capillary electrophoresis method (Sanger et al., 1977) on a 3500xl genetic analyzer (Applied Biosystems, Waltham, USA). Electropherograms generated after sequencing were evaluated by observing the quality of the peaks corresponding to each sequenced base, using the FinchTV program (Geospiza research team). Furthermore, BioEdit v.7.0.5.3 (Hall et al., 2011) and BLAST (Altschul et al., 1997) programs were used to compare the percentage identity of sequences obtained with others previously deposited in GenBank.

The 16S rRNA sequences from sequenced samples were used for phylogenetic analysis to corroborate similarity search data and classify the sequences at the species level. A database with 70 sequences from the 16S region of *Mycoplasma* spp. was created and used as the reference for constructing the phylogeny. Sequences were aligned with sequences generated in a study using MAFFT v.7.0 software (Katoh and Standley, 2003). The best evolutionary model was estimated as TIM3 by the ModelTest-NG v.0.1.7 program (Darriba et al., 2020), based on the information value of the Akaike information criterion (AIC). An aligned database was used to construct the phylogenetic analysis using the maximum likelihood method, with 1000 bootstrap replicates in the IQ-Tree1 program (Nguyen et al., 2015). The final image was visualized and constructed using the R v.4.1.0 program (R Core Team, 2018).

2.6. Data analysis and statistics

Initially, the Lilliefors test was applied to assess whether hematological parameters had a normal distribution. Subsequently, we used the t-student test to evaluate whether there is a difference between positive and negative animals for *Mycoplasma* spp., concerning hematological parameters: erythrocyte count, hematocrit, hemoglobin, leukocytes, segmented neutrophil, rod neutrophil, lymphocyte, eosinophil, monocyte, platelets, total plasma proteins (PPT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (HCM) and mean corpuscular hemoglobin count (MCHC), considering a significance level of $p<0.05$.

2.7. Results

Out of the 21 studied individuals, 38% (8/21) were Mycoplasma-PCR-positive: two lived in cabruca (Tamarin 19 [ALM group] and Tamarin 6 [BOM group]) and six in DFAM (Tamarin 92 [RIB group], Tamarins 102 and 115 [MRO group], and three [Tamarins 95, 109 and 113 [OZA group]).

Six positive animals from both areas had their samples sequenced (Tamarins 92 [RIB group], Tamarins 95, 109, and 113 [OZA group], Tamarin 6 [BOM group], and Tamarin 19 [ALM group]). These samples had between 88% and 99% identity for *Mycoplasma* spp. (Table 2).

Animal	Group	Environment	Percentage of identity with *Mycoplasma* sp.
Tamarin 92	RIB	DFAM	93%
Tamarin 95	OZA	DFAM	99%
Tamarin 109	OZA	DFAM	88%
Tamarin 113	OZA	DFAM	93%
Tamarin 6	BOM	CAB	97%
Tamarin 19	ALM	CAB	97%

Table 2 – Percentage of identity with *Mycoplasma* spp. of DNA samples sequenced for 16S rRNA gene of *Leontopithecus chrysomelas* from degraded areas in Una (DFAM), Bahia, Brazil, and *cabruca* areas (CAB), in Ilhéus, Bahia, Brazil, during October/2015 to March/2017.

Hematological parameters of studied tamarins were within the reference value for the genus *Leontopithecus* (Table 3). There was no difference between *Mycoplasma* spp. positive and negative hematological parameters evaluated (Table 3).

Parameters	*Leontopithecus* sp. (free living)*	*Leontopithecus* sp. (captivity)*	*Mycoplasma* spp. (negative; n=13)	*Mycoplasma* spp. (positive; n=8)	*t-test*	*p*
Erythrocytes (x10^6/µl)	9.26 ± 2.65	5.7 ± 0.6	5.4 ± 0.43	5.63 ± 0.38	-1.63	0.12
Hematocrit (%)	36.67 ± 5.78	45.5 ± 3.5	35.25 ± 4.75	36.95 ± 2.5	-1.60	0.12
Hemoglobin (g/dl)	-	15.4 ± 1.6	11.28 ± 1.56	12.16 ± 1.67	-1.20	0.24
Leukocytes (x10^3/ µl)	8.85 ± 3.92	7.13 ± 2.84	8.0 ± 3.28	9.37 ± 3.40	-0.64	0.52
Segmented neutrophil (%)	61.1 ± 10.24	44.2 ± 2.51	67.7 ± 13.10	72.7 ± 6.0	-0.96	0.34
Rod neutrophil (%)	-	0.1 ± 0.05	0.5 ± 0.60	0.3 ± 0.50	0	1
Lymphocyte (%)	30.5 ± 9.96	21.7 ± 1.15	28.6 ± 12.4	18.8 ± 8.8	0.84	0.39
Eosinophil (%)	0.7 ± 1.25	3.0 ± 0.40	0.25 ± 0.40	2.87 ± 1.57	-0.40	0.69
Monocyte (%)	6.65 ± 4.18	1.6 ± 0.18	1.58 ± 1.44	4.87 ± 6.31	-2.04	0.08
Basophil (%)	0 ± 0	0.01 a 0.06	0.0 ± 0.0	0.0 ± 0.0	-	-
Platelets (x10^3/ µl)	-	-	436.3± 182.58	527.12± 158.30	-1.14	0.26
PPT (g/dl)	6.27 ± 0.98	7.2 ± 1.5	6.93 ± 0.60	7.04 ± 0.57	-0.41	0.68
VCM (µm³)	-	-	64.75 ± 7.44	69.50 ±3.74	-1.66	0.11
HCM (pg)	-	-	21.18 ± 2.41	22.92 ± 1.46	-1.82	0.08
CHCM (g/dl)	-	-	33.02 ± 0.42	33.09 ± 0.88	-0.26	0.79

Table 3 – Mean ± SD (standard deviation) and comparation of hematological parameters of *Leontopithecus chrysomelas* from dregraded areas in Una (DFAM), Bahia, Brazil, and *cabruca* areas (CAB), in Ilhéus, Bahia, Brazil, positive and negative for *Mycoplasma* spp., during October/2015 to March/2017**.** Symbol codes: - Data unavailable; *Based on Verona and Pissinati, 2014.

The nucleotide sequence data reported in this paper are available in GenBank under the accession numbers **PP621507**, **PP621508**, **PP621509**, **PP621510**, and **PP621511**. Three sequences, Tamarin95, Tamarin113_2017, and Tamarin109, were not accepted by NCBI due to their length. Despite their small length, it was still possible to use them in phylogenetic analysis.

From sequencing and phylogenetic analysis, it was possible to infer the position of *Mycoplasma* spp. in these golden-headed lion tamarins (Figure 1). All samples belonging to tamarins 92, 95, 109, 113, 6 and 19 grouped with clades of unidentified species of *Mycoplasma* spp.. Tamarin 6, whose blood samples were collected in two different years (June/2016 and March/2017), were grouped on the same branch, indicating that the same species of *Mycoplasma* spp. had been parasitizing the animal over the years (Figure 1). Samples from tamarin 6, which lived in the *cabruca* environment, were grouped in the same branch as tamarins 92, 109 and 113, which lived in the DFAM environment, indicating that they belong to the same species (Figure 1). Additionally, it was observed that the same animal (Tamarin 113), whose blood was collected in different years (March/2016 and March 2017), presented a different species of *Mycoplasma* spp. in each consecutive year of collection (Figure 1).

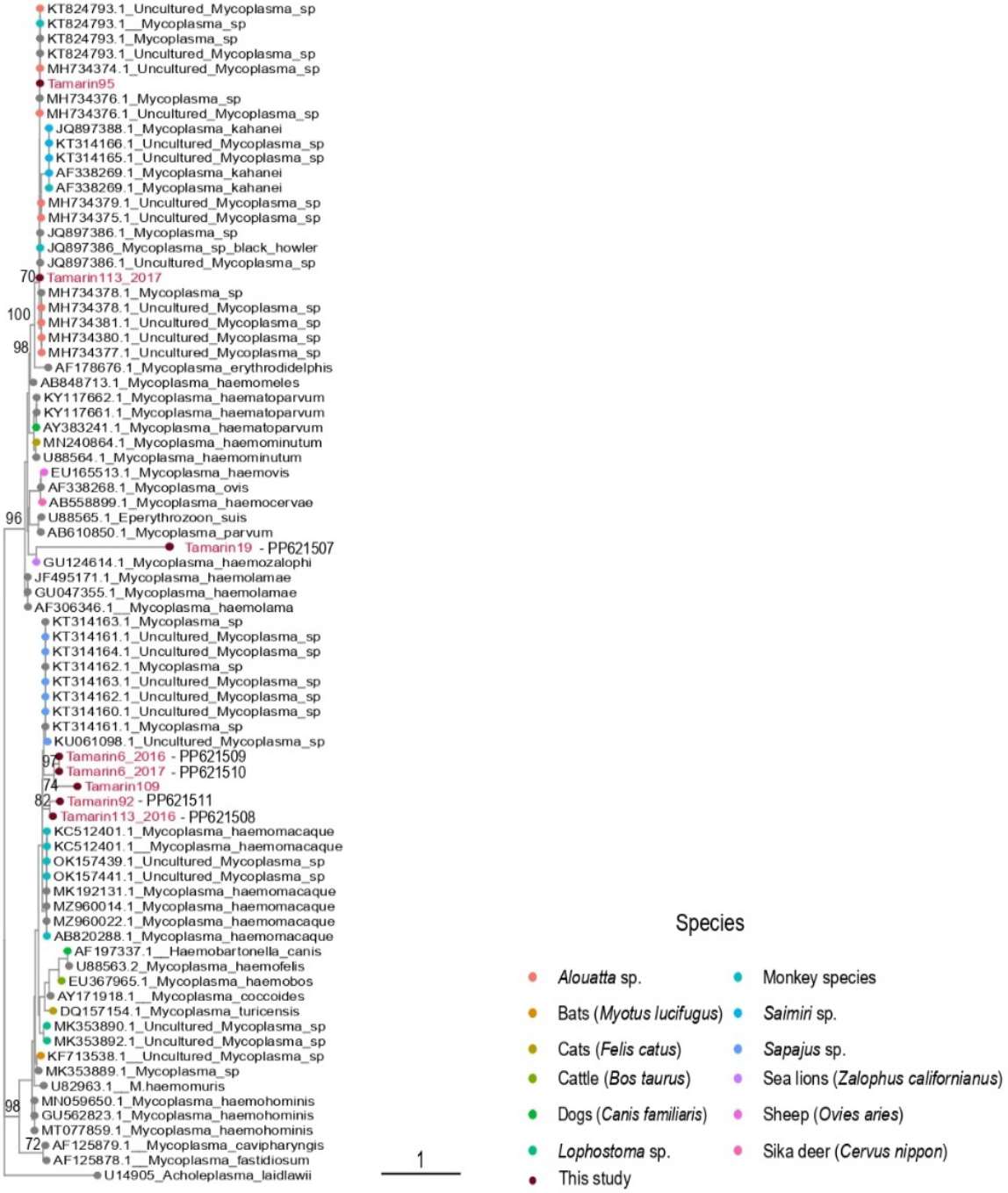

Figure 1 – Phylogenetic tree showing positions of different species of *Mycoplasma* spp. isolated from other hosts. Each host has a different color in the circle. Sequences generated in the work are coloured red. Analysis was based on 16S ribosomal rRNA of *Leontopithecus chrysomelas* from degraded areas in Una (DFAM), Bahia, Brazil, and *cabruca* areas (CAB), in Ilhéus, Bahia, Brazil, during October/2015 to March/2017. Nucleotide sequence data are available under the accession codes **PP621507**, **PP621508**, **PP621509**, **PP621510**, and **PP621511** in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

3. Discussion

Golden-headed lion tamarins from all analyzed groups presented positive results for *Mycoplasma* spp. in the present study. Considering the geographical separation between studied populations, this suggests that these parasites are widely distributed in the region where tamarins' populations live. In addition to compromising the health status of the animals, these infections can represent a risk to public health, due to the zoonotic potential of these parasites (Lignon et al., 2025) and human proximity in these environments. However, there are no reports of human infections caused by *Mycoplasma* spp. originating from non-human primates, the zoonotic transmission should not be underestimated (Narapakdeesakul et al., 2024), since there are already reports of

Mycoplasma haemofelis from felines to humans (Santos et al., 2008), *Mycoplasma suis* from pigs to humans (Yuan et al., 2009), as well as of *Candidatus* Mycoplasma haematoparvum, probably originating from contact with dogs (Maggi et al., 2013b).

All tamarin samples are grouped with clades of *Mycoplasma* spp. that infect tamarins and monkeys, suggesting that these species are ubiquitous in the environments studied. The phylogenetic tree shows that different Mycoplasma species may infect tamarins in different areas studied (DFAM and *cabruca*). This result differs from what was found by Sricharern et al. (2021), who detected the same species of *Candidatus* Mycoplasma haemomacaque infecting *Macaca fascicularis* in three different areas adjacent to human communities in Thailand. Tamarin samples grouped with clades of unidentified species of *Mycoplasma* spp., suggesting they are species not yet described in the literature. This result shows an open field for new research to be conducted in the region to investigate and identify species of *Mycoplasma* spp. that are parasitizing tamarins, in addition to evaluating the possible zoonotic potential of these parasites.

Samples from tamarins that lived in different environments, DFAM (Tamarins 92, 109 and 113) and *cabruca* (Tamarin 6), were grouped in the same branch of the phylogenetic tree. This result shows that although these tamarins are geographically separated, the same species of *Mycoplasma* spp. is circulating in different regions and parasitizing animals. Tamarin 113, in turn, presented different species of *Mycoplasma* spp. in each consecutive year of collection (2016 and 2017). This shows that PCR assays could detect the DNA of other parasite species in different collection years. Despite the positive results for infection by *Mycoplasma* spp., in hematological examination, both DFAM and *cabruca* animals presented mean values of hematological parameters within the normal range for the *Leontopithecus* genus, except for the hemoglobin parameter (12.16 ± 1 .67), which was below the normal range suggested by Verona and Pissinati (2014): (15.4 ± 1.6) for lion tamarins that were studied in captivity. However, Monteiro et al. (2010) found a mean hemoglobin value of 11.0 (SD± 1.6) for free-living golden-headed lion tamarins, indicating that values found in the present study were within usual standards for species. Reference values for hematological parameters may vary between authors due to blood collection conditions, the environment in which the animals live, and the characteristics of sampled animals, such as age, sex, and reproductive status, among others (Verona and Pissinati, 2014). Monteiro et al. (2010) carried out studies on golden-headed lion tamarins that lived freely in the Una-Bahia Biological Reserve; therefore, values obtained by this author are more reliable for comparison purposes with animals in the present study.

Although there is a report of hemolytic anemia in primates parasitized by *Mycoplasma* spp. (Santos et al., 2013), in the present study, no differences were observed in hematological parameters between animals testing positive and negative for this parasite. Similar results regarding the absence of changes in hematological parameters were also observed in other studies with primates (*Macaca fascicularis*: Maggi et al., 2013a; *Sapajus flavius*: Ramalho et al., 2017; *Macaca mulatta*: Mongruel et al., 2022). Cubilla et al. (2017) observed a decreased red blood cell (RBC) in infected black-horned capuchins (*Sapajus nigritus*). Still, it was unclear whether this was related to the hemoplasma infection since no statistical difference existed between the animals that tested positive and negative for this parameter. Furthermore, these animals had normal or high hematocrit and hemoglobin values. Therefore, future long-term studies, monitoring animals' health, may provide a more accurate prognosis about these infected animals' actual health and well-being.

Free-ranging primates may have increased risk of hemoplasma infection compared to captive primates due to increased exposure to arthropod vectors (Cubilla et al., 2017). Unfortunately, we did not evaluate the arthropod ectoparasites of golden-headed lion tamarins in the present study. The arthropod vectors and transmission routes of hemoplasmas in non-human primates remain unknown. Some studies have detected hemoplasma DNAs in ectoparasites, such as '*Candidatus* Mycoplasma haematohydrochoerus' in *Amblyomma dubitatum* tick (Vieira et al., 2021), and *Mycoplasma wenyonii* in *Stomoxys calcitrans* and Tabanus megalops flies (Thongmeesee et al., 2022). Therefore, further studies should be conducted about the potential *Mycoplasma* spp vectors infesting primates.

4. Conclusion

The present study showed that golden-headed lion tamarin populations in *cabruca* and DFAM areas are infected by unidentified species of *Mycoplasma* spp. We also identified infection by more than one *Mycoplasma* species in a single individual, which implies a novel epidemiological characteristic. Hematological abnormalities were not observed in infected animals. These results indicate that golden-headed lion tamarins in several locations in Southern Bahia, Brazil, may be infected and serve as reservoirs for this parasite.

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