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Molecular detection and phylogenetic analysis of *Trypanosoma* spp. in Neotropical primates from Rio de Janeiro State, Brazil[1]

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ABSTRACT.- Guimarães A., Santos H.A., Balthazar D.A., Kierulff M.C.M., Baptista M.N.M., Oliveira A.F.X., Stocco N.V., Mureb E.N., Costa A.C., Raimundo J.M. & Baldani C.D. 2022. **Molecular detection and phylogenetic analysis of *Trypanosoma* spp. in Neotropical primates from Rio de Janeiro State, Brazil.** *Pesquisa Veterinária Brasileira 42:e07059, 2022.* Instituto Nacional da Mata Atlântica, Av. José Ruschi 4, Centro, Santa Teresa, ES 29650-000, Brazil. E-mail: andresaguimaraes02@yahoo.com.br

Trypanosoma spp. infection is a problem in many tropical countries, infecting several animal species, including humans. The aim of the present study was to identify the *Trypanosoma* species in Neotropical primates from Rio de Janeiro state and compare the results with other reports both phylogenetically and geographically. Molecular detection was based on the 18 SSU gene. The sequences obtained in the PCR were sequenced and compared with others previously deposited in GenBank. These sequences were used to perform phylogenetic analysis and make a distribution map of primate species infected by *Trypanosoma* species in Brazil. Among 34 monkeys, five capuchin monkeys (*Sapajus* spp.) and one marmoset (*Callithrix* spp.) showed *Trypanosoma* spp. sequences in the same clade of *Trypanosoma minasense* and three capuchin monkeys' sequences were in the same clade of *Trypanosoma cruzi*. The Atlantic Forest and the Brazilian Amazon are the regions with the highest frequency of studies about *Trypanosoma* spp. and variety of Neotropical primate hosts. These are areas that deserve attention regarding the conservation of biodiversity, but it also makes evident the lack of studies with Neotropical primates in other regions of the country, as well as multidisciplinary studies to better understand the host pathogen relationships.

INDEX TERMS: Molecular detection, phylogenetic, Neotropical primates, *Trypanosoma cruzi*, *Trypanosoma minasense*, *Sapajus*, *Callithrix*, Brazil.

RESUMO.- [**Detecção molecular e análise filogenética de *Trypanosoma* spp. em primatas neotropicais do estado do Rio de Janeiro, Brasil.**] A infecção por *Trypanosoma* spp. é um problema em muitos países tropicais, infectando várias espécies animais, incluindo humanos. O objetivo do presente estudo foi identificar as espécies de *Trypanosoma* em primatas neotropicais no estado Rio de Janeiro e comparar os resultados com outros relatos, tanto filogeneticamente quando geograficamente. A detecção molecular foi baseada no gene SSU 18. As sequências obtidas na PCR foram sequenciadas e comparadas com outras previamente depositadas no GenBank. Essas sequências foram utilizadas para análises filogenéticas e confeccionar um mapa de distribuição de espécies de primatas infectadas por espécies de *Trypanosoma* no Brasil. Entre 34 macacos, cinco macacos-prego (*Sapajus* spp.) e um sagui (*Callithrix* spp.) apresentaram sequências de *Trypanosoma* spp. no mesmo clado de *Trypanosoma minasense* e três sequências de macacos-prego estavam no mesmo clado de *Trypanosoma cruzi*. A Mata Atlântica e a

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Amazônia brasileira são as regiões com maior frequência de estudos sobre *Trypanosoma* spp. e variedade de primatas neotropicais hospedeiros. São áreas que merecem atenção no que se refere à conservação da biodiversidade, mas também evidencia a carência de estudos com PNH em outras regiões do país e de estudos multidisciplinares para melhor compreender as relações do patógeno hospedeiro.

TERMOS DE INDEXAÇÃO: Detecção molecular, filogenética, primatas neotropicais, *Trypanosoma cruzi*, *Trypanosoma minasense*, *Sapajus*, *Callithrix*, Brasil.

INTRODUCTION

Wild primate parasites are relevant for conservation biology and human health because of their high potential to infect humans (Maia da Silva et al. 2008). Chagas disease, a zoonotic disease also called American trypanosomiasis, is of great importance in public health in Brazil. Recent studies show that most cases/outbreaks of trypanosomiasis currently occur in different regional settings due to ingestion of its insect vector and are related to the local interaction of humans with their surroundings, as well as to local ecological peculiarities in general (Jansen et al. 2018, 2015). Neotropical primates, especially in the Amazon region, are commonly infected by *Trypanosoma cruzi* and *Trypanosoma rangeli*, parasites that also infect humans and several other mammals (Maia da Silva et al. 2008). The capuchin monkey, *Sapajus libidinosus,* and the golden lion tamarin, *Leontopithecus rosalia*, are examples of species that showed high rates of positive hemocultures for *T. cruzi* infection, suggesting they may be important wild reservoir hosts in Brazil (Jansen et al. 2018). Other primate species may be of great importance as wild reservoirs of *Trypanosoma* spp. in different biomes and, therefore, a continuous study on the involvement of Neotropical primates in the spread of *Trypanosoma* spp., as well as the maintenance of its cycle, is necessary.

Trypanosoma minasense is a widely distributed parasite detected in 32 species or subspecies of non-human primates (Ziccardi et al. 1996). Although widespread, it is still unclear whether it causes disease in primates, and further studies are needed to understand the animal-parasite relationship in non-human primates. Unlike *T. cruzi* and *T. rangeli*, *Trypanosoma minasense* is not a zoonosis. There are no other *Trypanosoma* species frequently found only in Brazilian Neotropical primates, except those reported to cause opportunistic infection in captive animals, such as *Trypanosoma lewisi* (Maia da Silva et al. 2010).

In addition to the possibility of being infected, Neotropical primates suffer from natural hybridization between species, especially marmosets. Moreover, invasive species resulting from animal trafficking or incorrect fauna management can be released outside their original distribution and hybridize with native species. Consequently, hybrids compete with natives for habitat and food, reduce local biodiversity, and can transmit pathogens to animals in non-endemic regions where they become sick more easily (Malukiewicz et al. 2015).

Trypanosome detection can be done by different methodologies, such as microscopy, serological tests, isolation in culture and xenodiagnoses. Currently, the main diagnostic methods fot *Trypanosoma* spp. infections are through molecular biology tests, such as the polymerase chain reaction (PCR) and its variations. Therefore, establishing a standardized barcode protocol for the detection and identification of trypanosomes is a priority (Hutchinson & Stevens 2018). Extremely sensitive, PCR-based methods have been used to amplify small target regions in both the 18 small subunit ribosomal RNA (rRNA) and the 28S large subunit rRNA (Hamilton & Stevens 2011, Hutchinson & Stevens 2018).

New technologies provide opportunities for new and enhanced approaches to disease control through increased surveillance, data analysis, easy communication, and fast information sharing (WHO 2015). Phylogenetic studies allow the identification of *Trypanosoma* species and strains in a given area, as well as their proximity to others previously identified in different locations. Knowledge about the diversity of mammalian reservoirs and invertebrate vectors of *Trypanosoma* spp. in wild habitats is essential to better understand the dynamics of transmission cycles (Santos et al. 2019).

The present study aims to identify Neotropical primates infected by *Trypanosoma* spp., through the phylogenetic study of 18 SSU gene fragments derived from Neotropical primates' blood samples, and to characterize the species that are circulating in this population from the state of Rio de Janeiro.

MATERIALS AND METHODS

Study area and sampling efforts. The study was conducted with capuchin monkeys (*Sapajus* spp.) and marmosets (*Callithrix* spp.) rescued from 2016 to 2018 by the "Centros de Triagem de Animais Silvestres" (Wild Animal Triage Center - CETAS) of the state of Rio de Janeiro. Rescues occurred mainly due to electric shocks, irregular captivity, attacks by other animals, or accidental entry into homes. Unfortunately, it was not possible to retrieve the original geographic coordinates of the collection sites. Authors state that the experiments were conducted in accordance with national guidelines and regulations for the care and use of animals. Blood collection and clinical examination of the animals were performed during their periodic examination, an action provided for in CETAS "Protocols of clinical care for wildlife animals". The project was authorized by the Brazilian Institute for Biodiversity Conservation (SISBIO/ICMBio Permission No. 62830-1). All primates were classified only by genus (*Sapajus* or *Callithrix*) due to the presence of hybrid animals. In addition to the lack of details about the origin, the appearance and pelage of some animals were not compatible with those of the endemic species originally found in the region.

Anesthesia was performed by immobilizing the animals with ketamine hydrochloride (10mg/kg) and valium (5mg), injected intramuscularly. Blood samples were then collected from each animal in proportion to its body weight (1% of body weight at maximum) and transferred to tubes with an anticoagulant. Blood smear slides were prepared from the samples for all subjects. Blood samples were stored frozen at -20°C until DNA extraction. We did not find insects in the cages or ectoparasites in the animals during blood collection. After recovery from anesthesia, the animals were returned to their enclosures and fed.

Blood smear slides were prepared from each animal's blood samples. The smears were fixed with methanol, stained with Diff-Quick, and each one was analyzed twice under a NIKON™ optical microscope at 1000x magnification by the same observer. Each slide was observed under the microscope for at least 30 minutes.

Molecular detection of trypanosomatid infections. DNA was extracted from 200µL of each EDTA-whole blood sample using the ReliaPrep™ Blood gDNA Miniprep System (Promega™) according

to the manufacturer's instructions. Ultra-pure sterile water was used as negative controls in each batch of samples to assess DNA contamination during the extraction of total DNA.

Nested PCR targeting a portion of the variable region of the small subunit ribosomal gene (18 SSU) was performed in two rounds. For the first round, each 25µL PCR reaction mixture contained 3µL of DNA, polymerization buffer 1x, 0.2mM dNTPs, 100mM MgCl2, 1.0U of Taq DNA polymerase (Promega™), and 10pmol of the following external primers: TRY927F (5'-GAAACAAGAAACACGGGAG-3') and TRY927R (5'-CTACTGGGCAGCTTGGA-3') (Smith et al. 2008). Thermal cycling was conducted in a Veriti Thermal Cycler (Applied Biosystems™) for 3 min at 94˚C, 30 cycles at 94˚C for 30 s, 55˚C for 60 s, and 72˚C for 90 s and 72˚C for 10 minutes. For the second round of PCR, 1µL of products from the first amplification was used as a template with the following internal primers: SSU561F (5'-TGGGATAACAAAGGAGCA-3') and SSU561R (5'-CTGAGACTGTAACCTCAAAGC-3'), using the same PCR reaction mixture and cycle conditions described above (Smith et al. 2008). We considered positive all samples that produced a band of approximately 600 bp on the second round.

Purification and sequencing of positive samples for *Trypanosoma* sp. PCR positive samples were selected for purification and subjected to sequencing. The amplification products were purified with Wizard SV Gel and PCR Clean-Up System (Promega™) kit according to the manufacturer's recommendations.

DNA sequencing was performed by the Sanger method (Sanger et al. 1977), using ABI 3730 DNA analyzer (Applied Biosystems™). The same primers for the PCR reaction were used for sequencing. Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit. Runs were performed in 36cm capillaries using POP7 polymer. Sequences were analyzed by Sequencing Analysis 5.3.1 software using Base Caller KB.

Phylogenetic analysis. Consensus sequences were obtained by analyzing sense and antisense sequences using Bioedit v. 7.0.5.3 (Hall 1999). We used the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) to assess the similarity of the 18-rDNA sequences to other nucleotide sequences of *Trypanosoma* spp. available from GenBank. A minimum identity of 97%, coverage of 98% and e-value of 1e -100 was considered to define the *Trypanosoma* species.

We built a database from 18-rDNA sequences from *Trypanosoma* spp. that infect neotropical primates and contains the H7-H8 variable regions. A total of 57 18-rDNA sequences were retrieved from GenBank. Our sequences were aligned with those available on GenBank using Clustal/W (Thompson et al. 1994) and fitted using Bioedit v. 7.0.5.3 (Hall 1999). The phylogenetic reconstruction was inferred using the Maximum Likelihood method. Nucleotide substitution models were selected based on the Akaike information criterion (AIC) in Mega X software (Kumar et al. 2018). The 2-parameter Kimura model was used to calculate evolutionary distances. The combination of phylogenetic clusters was assessed using a bootstrap test with 1000 replicates to test different phylogenetic reconstructions. The phylogenetic valuation was performed in Mega X software (Kumar et al. 2018).

***Trypanosoma* distribution in Neotropical primates in Brazil.** We searched the National Center for Biotechnology Information (NCBI) using combinations of the terms "*Trypanosoma*", "neotropical primate", "Brazil", and each genus of Neotropical primate found in the country as keywords. Our goal was to describe the distribution of *Trypanosoma* species, identified by molecular diagnosis, infecting neotropical primates in Brazil. We selected a copy of the deposited sequence for each genus of *Trypanosoma* and primate by location. The GenBank accession number of each sequence selected is listed in Table 1. For studies with multiple deposited sequences, only one is represented on the map, along with the sequences of our study. There was no distinction between genes or targets used for the molecular detection of the *Trypanosoma* stretch. We did not include sequences of *Trypanosoma* spp. deposited without host identification.

RESULTS

The study was conducted with a total of 34 neotropical primates, of which 26 were capuchin monkeys (*Sapajus* spp.) and eight were marmosets (*Callithrix* spp.), all rescued from 2016 to 2018 by the CETAS of the state of Rio de Janeiro. All marmosets appeared to be hybrids. Most of the capuchin monkeys sampled in this study had the appearance of *Sapajus nigritus*, the species native to this region. However, some capuchin monkeys had lighter fur and were probably hybrids with species introduced or brought in by animal trafficking. The animals were clinically healthy, active, free of ectoparasites, and without noticeable lesions. They have been held captive in CETAS for months or years.

Eight *Sapajus* sp. (30.8%) and one *Callithrix* sp. (12.5%) had positive nested PCR. All samples were purified and sequenced (GenBank MW332110-MW332118). Samples of five *Sapajus* sp. (19.2%) and one *Callithrix* sp. (12.5%) exhibited sequences of *Trypanosoma* clustered within the same clade as *Trypanosoma minasense* in the phylogenetic tree (Fig.1), with BLAST analysis showing 99% identity between the sequences and the species. Three sequences from *Sapajus* spp. (11.5%) were in the same clade as *Trypanosoma cruzi*, with 99% to 100% identity in BLAST analysis. We did not observe any parasitic form of *Trypanosoma* in the microscopic analysis.

The Amazon and the Atlantic Forest are the two most biodiverse biomes in Brazil and, therefore, where most studies are concentrated. Thus, in our study, these biomes had the highest frequencies of T*rypanosoma* species, as well as a wide variety of primate hosts (Fig.2).

Neotropical primates of the Cebidae family were the most observed hosts of *Trypanosoma* spp., followed by primates of the Callitrichidae family. Regarding GenBank records, most originate in the north of the country (61.5%) in the Amazon Forest biome, followed by the Southeast (33.3%) in the Atlantic Forest, and by the Midwest (5.1%) in the Cerrado biome. Among the studies with Neotropical primate hosts, *T. cruzi* was detected in 43.6% of the records, followed by *T. rangeli* (33.3%), *T. lewisi* (15.4%), and *T. minasense* (7.7%). In the Amazon Forest, there was a higher detection of *T. rangeli* (45.8%), followed by *T. cruzi* (41.7%), while in the Atlantic Forest, there was a higher detection of *T. cruzi* (38.5%) than the other *Trypanosoma* species.

DISCUSSION

Primates of distinct taxa may act as important reservoirs of *Trypanosoma* species. In the present study, no parasitic form was found in the microscopic analysis. In contrast, nine positive samples were detected by molecular methods. Indeed, the probability of finding infectious forms on light microscopy may be influenced by the low parasitemia in infections by *Trypanosoma* spp. (Ziccardi & Lourenço-de-Oliveira 1999, Tenório et al. 2014, Coimbra et al. 2019), while PCR is considered a more accurate diagnostic methodology (Tenório et al. 2014).

Trypanosoma minasense was isolated for the first time in axenic blood culture from a naturally infected marmoset, *Callithrix penicillata*, from Brazil (Ziccardi et al. 1996). It does not infect triatomine bugs and nothing is known about its vectors in nature (Ziccardi et al. 1996). In wild individuals of *Callithrix* sp. from Jardim Botânico, Rio de Janeiro, hemoparasites identified as *T. minasense* were found in 33% of the animals through morphometric data and in 20% of them based on DNA sequence similarity, also exhibiting size polymorphism (Coimbra et al. 2019). The infection rate was similar to that found in the present study (19.2% for *Sapajus* sp. and 12.5% for *Callithrix* sp.), indicating that *T. minasense* is present in the state of Rio de Janeiro. However, the high specificity of this parasite with Neotropical primate hosts suggests that it does not pose a risk to public health (Coimbra et al. 2019).

Trypanosoma spp. sequences from three *Sapajus* spp. (11.5%) were in the same clade of *Trypanosoma cruzi*, with BLAST analysis showing 99% to 100% of identity. *T. cruzi* strains detected in *Sapajus* spp. in the studied region were not classified as discrete typing units (DTU), as recommended by Zingales et al. (2009). Future studies will be carried out to establish the *T. cruzi* DTUs that occurs in *Sapajus* spp. Neotropical primates infected with *T. cruzi* may develop cardiac manifestations such as right atrial enlargement and systolic and diastolic abnormalities of both ventricles (Zabalgoitia et al. 2003). However, the animals in our study did not show

Table 1. Non-human primates from Brazil detected with *Trypanosoma* species in GenBank

Identification* (id_host)	NHP species (host_sp)	*Trypanosoma* species (tryp_sp)	Accession number GenBank (Idd)	Locality	Latitude (Lat_y)	Longitude (Long_x)
1	*Alouatta fusca*	*Trypanosoma lewisi*	GU252216.1	Mogi Mirim/SP	-23.0432	-45.9292
2	*Alouatta fusca*	*Trypanosoma lewisi*	GU252209.1	Mogi Mirim/SP	-23.5432	-46.6292
3	*Alouatta stramineus*	*Trypanosoma rangeli*	AY491760	Monte Negro/RO	-10.2568	-63.3165
4	*Aotus* sp.	*Trypanosoma lewisi*	MF403115.1	Amazonas	-2.01684	-66.0561
5	*Aotus* sp.	*Trypanosoma rangeli*	AY491757	Amazonas	-3.21684	-66.0561
6	*Aotus* sp.	*Trypanosoma cruzi*	EU856376.1	Belem/PA	-1.941	-49.0905
7	*Aotus* sp.	*Trypanosoma lewisi*	GU252219.1	Belem/PA	-1.341	-48.5905
8	*Aotus* sp.	*Trypanosoma lewisi*	GU252212.1	Belem/PA	-2.041	-48.0905
9	*Callicebus lugens*	*Trypanosoma rangeli*	AY491751	Rio Negro/AM	-2.05883	-60.4111
10	*Callicebus moloch clipeus*	*Trypanosoma rangeli*	AY491750	P. de Castro/AC	-10.281	-67.7727
11	*Callithrix jacchus*	*Trypanosoma lewisi*	GU252221.1	Mogi Mirim/SP	-23.0432	-46.6292
12	*Callithrix* sp.	*Trypanosoma minasense*	MN066342.1	Rio de Janeiro/RJ	-22.395	-43.9601
13	*Cebuella pygmaea*	*Trypanosoma rangeli*	AY491752	RioBranco/AC	-9.9863	-67.9727
14	*Cebus albifrons*	*Trypanosoma cruzi*	EU856371.1	RioNegro/AM	-3.41684	-60.8561
15	*Sapajus apella*	*Trypanosoma cruzi*	EU856370.1	RioBranco/AC	-9.9863	-68.9712
16	*Leontopithecus chrysomelas*	*Trypanosoma cruzi*	KU145427.1	Rio de Janeiro (collection)	-22.9068	-43.2729
17	*Leontopithecus chrysomelas*	*Trypanosoma cruzi*	KT390213.1	Rio de Janeiro (collection)	-22.7068	-43.5729
18	*Leontopithecus rosalia*	*Trypanosoma cruzi*	KT390197.1	Rio de Janeiro (collection)	-22.5068	-43.0729
19	*Leontopithecus rosalia*	*Trypanosoma cruzi*	KU145431.1	Rio de Janeiro (collection)	-22.6068	-42.2
20	*Mico chrysoleucus*	*Trypanosoma cruzi*	KU298449.1	Brasília (zoo)	-15.8267	-47.9218
21	*Saguinus bicolor*	*Trypanosoma rangeli*	FJ997562.1	Amazonas	-3.61684	-64.4561
22	*Saguinus bicolor*	*Trypanosoma cruzi*	EU755241.1	Manaus/AM	-3.04431	-60.1072
23	*Saguinus fuscicollis*	*Trypanosoma cruzi*	JN040972.1	Acre	-8.7238	-71.812
24	*Saguinus fuscicollis*	*Trypanosoma cruzi*	JF421307.1	Acre	-9.1238	-70.0001
25	*Saguinus fuscicollis weddelli*	*Trypanosoma rangeli*	AY491755	Acre	-9.0238	-70.812
26	*Saguinus labiatus*	*Trypanosoma rangeli*	AY491756	Acre	-9.6789	-70
27	*Saguinus labiatus*	*Trypanosoma cruzi*	EU856378.1	Placido de Castro/AC	-10.281	-68.1727
28	*Saguinus labiatus*	*Trypanosoma rangeli*	KP001266.1	São Paulo	-22.5432	-46.9292
29	*Saguinus midas*	*Trypanosoma cruzi*	EU856369.1	Manaus/AM	-3.61684	-59.7072
30	*Saguinus niger*	*Trypanosoma cruzi*	KU298450.1	Brasília (zoo)	-15.5267	-47.5218
31	*Saimiri sciureus*	*Trypanosoma rangeli*	FJ997561.1	Amazonas	-3.81684	-65.2561
32	*Saimiri sciureus*	*Trypanosoma rangeli*	AY491768.1	Manaus/AM	-3.21684	-59.6072
33	*Saimiri sciureus*	*Trypanosoma rangeli*	AY491747	Marajo Island/PA	-0.95192	-50.7103
34	*Saimiri sciureus*	*Trypanosoma cruzi*	EU755219.1	Marajo Island/PA	-1.99813	-50.9306
35	*Saimiri* sp.	*Trypanosoma rangeli*	EF071550.1	São Paulo	-21.9432	-47.6292
36	*Saimiri ustus*	*Trypanosoma cruzi*	EU755251.1	Manaus/AM	-3.61684	-60.2072
37	*Callithrix* sp.	*Trypanosoma minasense*	MW332118.1	Rio de Janeiro	-2.067807	-42.79417
38	*Sapajus* sp.	*Trypanosoma minasense*	MW332110.1	Rio de Janeiro	-21.7	-42.79417
39	*Sapajus* sp.	*Trypanosoma cruzi*	MW332112.1	Rio de Janeiro	-2.166397	-41.852909

* Identification numbers are the same as the map in Figure 2.

symptoms or clinical changes during veterinary examinations, and we did not perform more specific cardiographic tests.

The most common way of transmission of *Trypanosoma* spp. is through an invertebrate vector (Zabalgoitia et al. 2003, Sathler-Avelar et al. 2017, Jansen et al. 2018, Drozino et al. 2019). Capuchin monkeys and marmosets have a habit of sleeping in tangles of vines, bromeliads, and in the base of palm leaves, places where the vector may be present. Oral transmission in Neotropical species seems relevant (Zabalgoitia et al. 2003, Sathler-Avelar et al. 2017), since their insect-eating habits May predispose these animals to ingest infected triatomines or other bugs (Zabalgoitia et al. 2003, Sathler-Avelar et al. 2017).

Chagas disease is a neglected disease that constitutes a public health problem worldwide (WHO 2015). Capuchin monkeys should be investigated as reservoir hosts for *T. cruzi*. A 20-year data collection and analyses study demonstrated that primates from different Brazilian biomes can be infected by *T. cruzi*, especially species of *Sapajus*, *Leontopithecus, Alouatta,* and *Callithrix* (Jansen et al. 2018). In addition, *T. cruzi* is transmitted and remains in the wild, even though most individuals of the infected species have low parasitemia (WHO 2015). Thus, primates from the Atlantic Forest may be a source of infection for other animals and humans. It is important to note that a single examination of the animal only shows a snapshot of the infection. For a better perspective, constant studies with Neotropical primates and arthropod vectors are recommended.

Brazil is considered one of the most megadiverse countries in terms of species in the world (Scarano 2007). Despite this, research on zoonoses in these animals is restricted to some areas of the territory. In the case of *Trypanosoma cruzi*, an important vector-borne zoonosis, studies on NPH hosts are not widespread among the states (Fig.2). Likewise, although the primates in the present study are widely distributed in

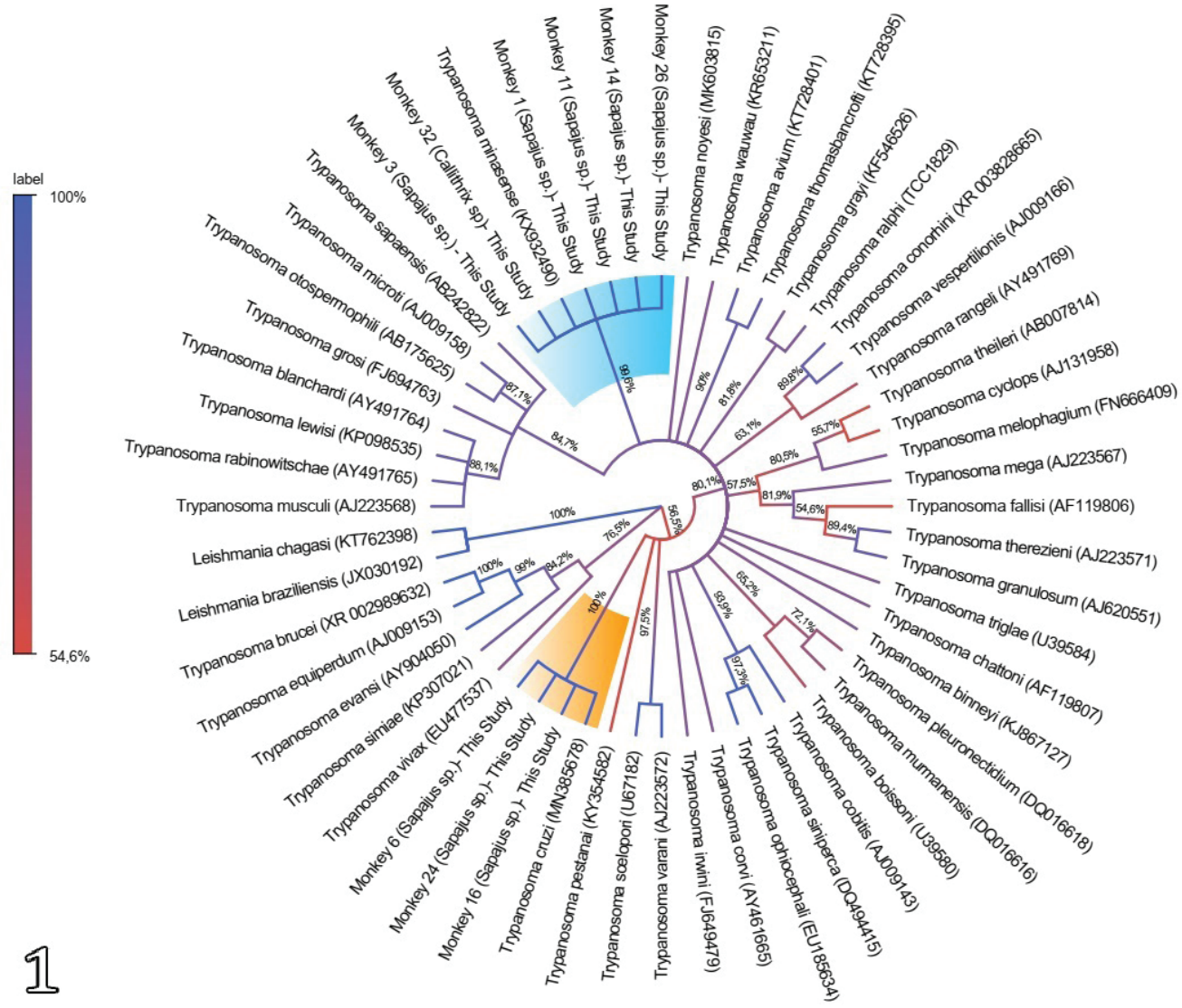

Fig.1. Phylogenetic analysis was inferred by using the Maximum Likelihood method and Kimura 2-parameter model based in 18 SSU *Trypanosoma* sequences from primates *Sapajus* spp. and *Callithrix* spp. from the state of Rio de Janeiro and related species (GenBank accession numbers are indicated before species name).

Brazil, studies involving Neotropical primates and *Trypanosoma* infections are still geographically limited, with a concentration of cases closer to study centers, as shown by the data available in GenBank (Fig.2). This factor limits the understanding of the epidemiology of *Trypanosoma* infections in Neotropical primates, making further studies in different regions necessaary to increase the knowledge of this ecological relationship.

Destruction, fragmentation, and decline of natural habitats followed by resource restriction, or, in some cases, species extinction, drive wild mammal populations to areas close to contact with humans and domestic animals (Roque & Jansen 2008). The same can happen as a result of animal trafficking, the introduction of exotic species and the rescue of threatened animals by environmental agencies, as those in the present study. Closer contact between humans and wild and domestic animals facilitates the emergence of zoonotic agents (WHO 2015, SCBD 2020).

Small numbers of individuals isolated in forest fragments may suffer genetic erosion. It results in the selection of individuals receptive to new pathogens or without resistance to pathogens already present. It also creates patches of high infection prevalence and risk of spillover to neighboring regions, highlitghting the need to align conservation and health goals, protect and maintain connectivity between natural areas, and reduce anthropogenic interference that causes biodiversity loss and emergence of new diseases (WHO 2015).

Regarding health concerns and the spread of diseases from animals to humans, the report calls for a "One Health" transition, in which agriculture, the urban environment, and wildlife are managed in a way that promotes healthy ecosystems and people (SCBD 2020). Within this concept, the study on *Trypanosoma* species, their arthropod vectors, their different hosts, and their territorial distribution should be approached together, bringing information to the population and allowing prevention strategies.

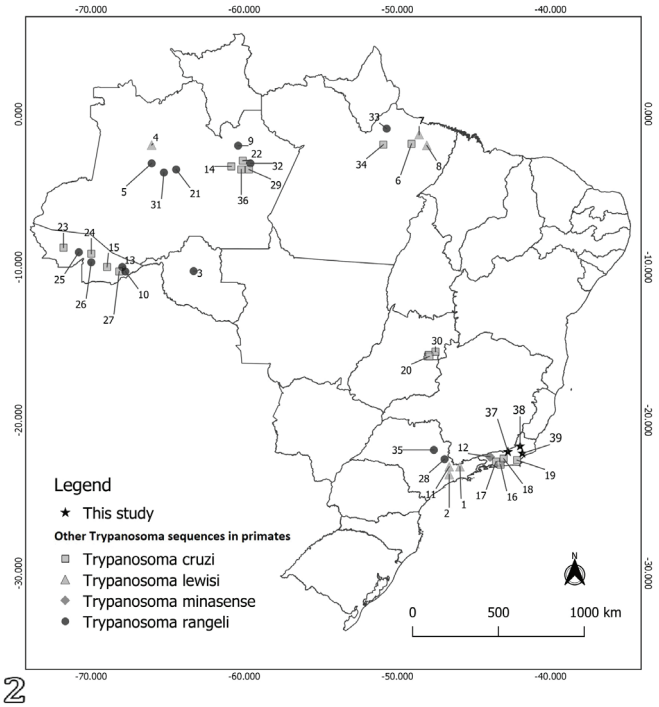

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Fig.2. Geographical distribution of *Trypanosoma* species infection in primates host from Brazil. Numbers on map are related with identification sample in the table.

CONCLUSION

The present study reports the detection of *Trypanosoma minasense* and *Trypanosoma cruzi* in *Callithrix* spp. and *Sapajus* spp. in the state of Rio de Janeiro. Further studies are needed to assess the role of *Sapajus* spp. in the epidemiological cycle of *T. cruzi* in humans and to classify the discrete typing units of *T. cruzi* circulating in these animals.

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