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Bat trypanosomes from Tapajós-Arapiuns Extractive Reserve in Brazilian Amazon

Tripanossomas de morcegos da Reserva Extrativista Tapajós-Arapiuns na Amazônia Brasileira

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Abstract

Trypanosoma comprises flagellates able to infect several mammalian species and is transmitted by several groups of invertebrates. The order Chiroptera can be infected by the subgenera *Herpetosoma*, *Schizotrypanum*, *Megatrypanum* and *Trypanozoon.* In this study, we described the diversity of bat trypanosomes and inferred phylogenetic relationships among the trypanosomes from bats caught in Tapajós-Arapiuns Extractive Reserve (Resex) in Pará state. Trypanosomes from bats were isolated by means of hemoculture, and the molecular phylogeny was based on the trypanosome barcode (SSUrDNA V7V8 variable region). A total of 111 bats were caught in the area, belonging to three families (Emballonuridae, Molossidae and Phyllostomidae) and 12 species. The bat trypanosome prevalence, as evaluated through hemoculture, was 9% all positive cultures were cryopreserve (100% of isolation success). Phylogenetic trees grouped nine isolates in *T. cruzi marinkellei* branch and only one in *T. dionisii* branch. Studies on bat trypanosome diversity are important for identifying pathogenic species and for generating support for control measures, especially in such areas where humans inhabit the forest with close contact with bat species. In addition, this is the first study in Resex Tapajós-Arapiuns extractive reserve and further studies should be conducted to elucidate the role of these parasites as environmental degradation biomarkers.

Keywords: *Trypanosoma*, Chiroptera, Amazon, phylogeny, taxonomy.

Resumo

Trypanosoma compreende flagelados capazes de infectar diversas espécies de mamíferos e são transmitidos por diferentes grupos de invertebrados. A ordem Chiroptera pode ser parasitada pelos subgêneros *Herpetosoma*, *Schizotrypanum*, *Megatrypanum* e *Trypanozoon*. Neste estudo é descrita a diversidade de tripanossomas de morcegos capturados na Reserva Extrativista Tapajós-Arapiuns, no Estado do Pará. Os tripanossomas de morcegos foram isolados através de hemocultura e os estudos filogenéticos baseados na região de barcode de tripanossomas (SSUrDNA V7V8 região variável). Foram capturados 111 morcegos pertencentes a três famílias (Emballonuridae, Molossidae e Phyllostomidae) e 12 espécies. A prevalência dos tripanossomas de morcegos, avaliada por hemocultura, foi de 9% para as culturas positivas e todas foram criopreservadas (100% de eficiência no isolamento). As árvores filogenéticas agruparam nove isolados no ramo de *T. cruzi marinkellei* e um único isolado de *T. dionisii*. Estudos sobre a diversidade de tripanossomas de morcegos são importantes para identificar espécies patogênicas e gerar suporte para medidas de controle, principalmente em áreas silvestres com contato entre as populações humanas e de morcegos. Além disso, este foi o primeiro estudo realizado na Resex Tapajós-Arapiuns e novos estudos devem ser conduzidos para elucidar o papel destes parasitas como marcadores de degradação ambiental.

Palavras-chave: *Trypanosoma*, Chiroptera, Amazônia, filogenia, taxonomia.

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Introduction

The genus *Trypanosoma* comprises protozoan flagellates that are able to infect several vertebrate species and are transmitted by several groups of invertebrates. More than 30 trypanosome species belonging to the subgenera *Herpetosoma*, *Schizotrypanum*, *Megatrypanum* and *Trypanozoon* have been described in bats (order Chiroptera) (HOARE, 1972; MARINKELLE, 1976).

The Most descriptions of the species of trypanosomes of bats are based on host of origin and morphological characters. Phylogenetic studies on trypanosomes of bats are scarce and only *T. dionisii*, *T. vespertilionis, Trypanosoma* sp. bat (isolated in Africa) and *T. cruzi marinkellei* were included in phylogenetic trees (STEVENS & GIBSON, 1999; STEVENS & RAMBAUT, 2001; HAMILTON et al., 2007). Recently, *T. erneyi* and *T. livingstonei* from African bats and *T. teixeirae* from flying fox in Australia were described and positioned in phylogenetic trees (LIMA et al., 2012, 2013; BARBOSA et al., 2016).

The subgenus *Schizotrypanum* comprises the species *Trypanosoma erneyi* and *T. dionisii*, which only infect bats, and *T. cruzi marinkellei*, which has the ability to infect many orders of mammals (MARINKELLE, 1976; LIMA et al., 2012).

T. dionisii is transmitted by bugs of the family Cimicidae, which are widely distributed, while *T. cruzi marinkellei* is transmitted by bugs of the family Reduviidae (*Cavernicola pilosa*) and only occurs in the Americas. In Brazil, the studies conducted have found *T. cruzi* (TCI and TcBat group), *T. cruzi marinkellei* and *T. dionisii* through hemoculture evaluations (MARCILI et al., 2009a; CAVAZZANA et al., 2010).

Trypanosoma dionisii has been detected in the bat families Phyllostomidae, Molossidae, Vespertilionidae and Noctilionidae in all biomes analyzed, from northern to southern Brazil, while *T. cruzi marinkellei* has been found in phyllostomid and vespertilionid bats in northern, central and southeastern areas and *T. cruzi* in the bat families Phyllostomidae, Vespertilionidae, Noctilionidae and Thyropteridae in the Amazon, Pantanal and Atlantic rainforest biomes (COSTA et al., 2016, 2015; ACOSTA et al., 2014; MARCILI et al., 2009a; CAVAZZANA et al., 2010). Additionally, *Trypanosoma erneyi* has been described in bats in Africa (LIMA et al., 2012).

There are few studies on *Trypanosoma* in wild mammals or vectors in the state of Pará. *Trypanosoma cruzi* lineages have been described in marsupials, rodents, carnivores, armadillos and triatomines (MARCILI et al., 2009a, b). New species of *Trypanosoma* were described recently in the bat genus *Pteronotus* in the state of Rondônia and in blood from bats in Pará (LIMA et al., 2015). In Altamira, *T. cruzi*, *T. cruzi marinkellei* and *T. wauwau* were isolated (COSTA et al., 2016). The Tapajós-Arapiuns Resex is a protected area designated for sustainable use of natural resources with difficult access with humans inhabit small villages inside the Amazon rainforest, with close contact with bats and limited information regarding trypanosome infection.

In the present study, we inferred phylogenetic relationships among the trypanosomes from bats caught in the Tapajós-Arapiuns Resex area, based on SSU rDNA sequences. Two species were described: *T. cruzi marinkellei* and *T. dionisii* from phyllostomid bats.

Materials and Methods

Study areas and bats caught

The bats were caught in the Tapajós-Arapiuns extractive reserve (Resex), mostly located in municipality of Santarem, in the Pará state (02º20’-03º40’ S; 55º00’-56º00’ W) on the left bank of the Tapajós river (Figure 1). The entire area of the Tapajós-Arapiuns Resex (677,513.24 ha) is included in the Amazon biome (annual average temperature 27 °C; annual precipitation greater than 2400 mm). More than 3000 families inhabit the area of the Tapajós-Arapiuns Resex and conduct extractive activities consisting of fishing and hunting for their own consumption (ICMbio, 2014).

Bat-catching was undertaken in each area for five nights in April 2015. Six mist nets (7 × 3 m) were set up from 18:00 to 23:00 h and were checked every 30 min for the presence of bats. Artificial shelters on the roofs of schools were also set up and checked. The bats caught were anesthetized and blood samples were collected by means of heart puncture.

The animals were caught and manipulated in accordance with the recommendations of the Brazilian Institute for the Environment and Renewable Natural Resources and Chico Mendes Institute for Biodiversity Conservation (IBAMA-ICMBio) and the procedures used were approved by the Animal Research Committee of the University of Santo Amaro.

Isolation of bat trypanosomes

The blood samples were collected by means of heart puncture and were inoculated into vacutainer tubes containing a biphasic medium (15% sheep red blood cells with 4% blood agar base) and liquid LIT medium supplemented with 20% FBS (COSTA et al., 2016). The culture was incubated at 28 °C, and the isolates were cryopreserved in liquid nitrogen in the Brazilian Trypanosomatid Collection (Coleção Brasileira de Tripanossomatídeos, CBT), in the Department of Preventive Veterinary Medicine and Animal Health, School of Veterinary Medicine, University of São Paulo, Brazil. No primary samples (blood samples) were obtained to molecular diagnosis. The most caught bats were released and the amount of blood collected was prioritized for isolation.

Molecular and phylogenetic analysis

Samples of DNA from the trypanosome cultures were extracted using the phenol-chloroform method. The V7V8 barcode region of the SSU rDNA gene was amplified by means of a conventional polymerase chain reaction (PCR), as previously described (FERREIRA et al., 2008; VIOLA et al., 2008). PCR products of the expected size were purified by means of Exosap-IT (Affymetrix) and were sequenced in an automated sequencer (Applied Biosystems/PerkinElmer, model ABI Prism 3500 Genetic, Foster City, California, USA), in accordance with the manufacturer’s recommendations.

The sequences obtained were aligned with sequences that had previously been determined for other *Schizotrypanum* species available in GenBank, using ClustalX (THOMPSON et al., 1997), and were adjusted manually using GeneDoc (NICHOLAS et al., 1997).

The phylogenetic tree was constructed using maximum parsimony (MP) and Bayesian analysis (B). MP was implemented in PAUP version 4.0b10 (SWOFFORD, 2002) with 500 bootstrap replicates, random stepwise addition starting trees (with random addition sequences) and TBR branch swapping. Bayesian analysis was performed through MrBayes v3.1.2 (HUELSENBECK & RONQUIST, 2001) with 1,000,000 MCMC generations. The first 25% of the trees represented burn-in, and the remaining trees were used to calculate Bayesian posterior probability.

Results

A total of 111 bats were caught in the area, belonging to three families (Emballonuridae, Molossidae and Phyllostomidae) and 12 species (Table 1). The bat trypanosome prevalence, as evaluated through hemoculture, was 9%. Ten positive hemocultures were

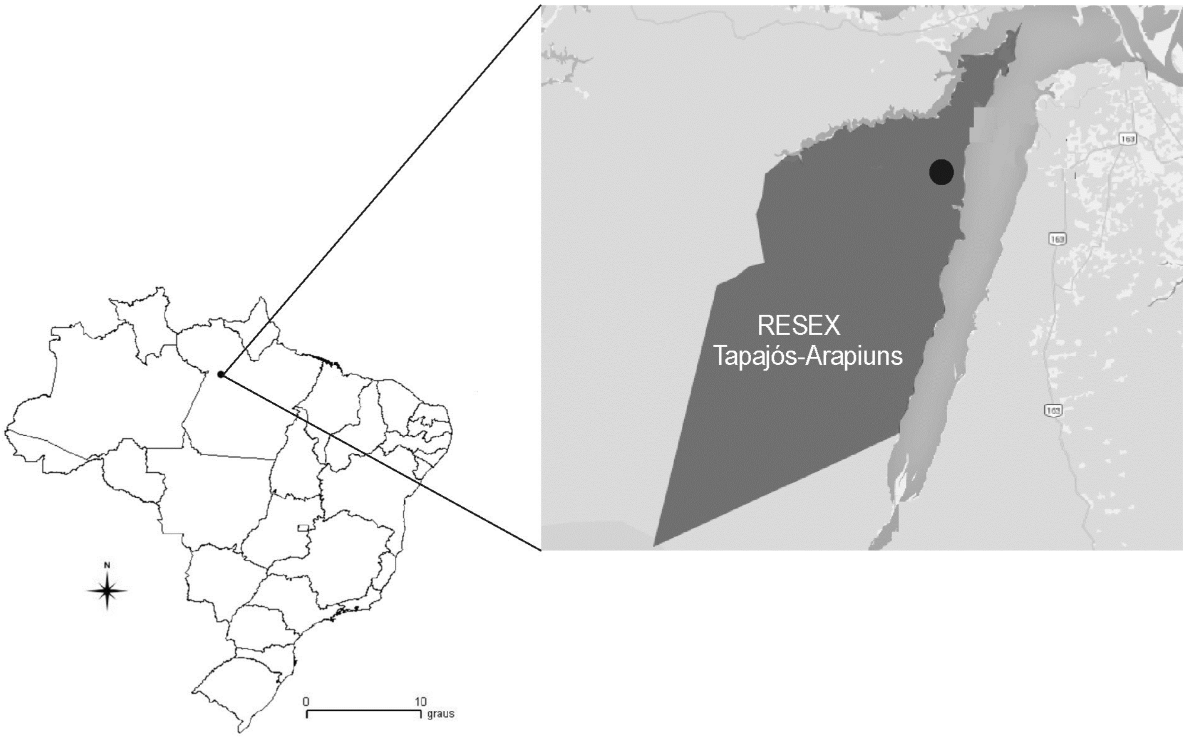

Figure 1. Map of Resex Tapajós-Arapiuns. Geographical origin of bats caught in the Resex Tapajós-Arapiuns in Alter do Chão, Pará state, Brazil.

Table 1. Hosts species and haemoculture positivity of bats examined in this study.

Order	Host			No. of individuals	
	Family	**Genus**	**Species**	**Examined/Positive**[a]	**Total**
Chiroptera	Phyllostomidae	*Artibeus*	*obscurus*	1/0	0
		Carollia	*perspicillata*	51/8	8
		Glossophaga	*soricina*	2/1	1
		Lichonycteris	*degener*	1/0	0
		Mimon	*crenulatum*	1/1	1
		Sturnira	*lilium*	4/0	0
		Vampyressa	*pusilla*	7/0	0
		Platyrrhinus	*lineatus*	1/0	0
	Molossidae	*Molossus*	*ater*	1/0	0
			molossus	40/0	0
	Emballonuridae	*Rhynchonycteris*	*naso*	1/0	0
		Saccopteryx	*gymnura*	1/0	0
Total	**3**	**11**	**12**	**111/10**	**10**

[a] Haemoculture.

obtained, only from phyllostomid bats, and all of the cultures were established and cryopreserved in CBT (Table 2).

The morphology of the cultured forms and the biological behavior in culture media were similar to those of the subgenus *Schizotrypanum* in all isolates from phyllostomid bats.

Phylogenetic relationships based on the trypanosome barcode were inferred by means of maximum parsimony and Bayesian analysis, and congruent topologies were generated for *Schizotrypanum* trypanosomes (Figure 2). The subgenus *Schizotrypanum* is a monophyletic group (100% of similarity of sequences, 100%

Table 2. Trypanosome isolates, host and geographic origin and sequences of SSU rDNA used for phylogenetic analysis.

***Trypanosoma* species**	**Isolate code**	**Host**	**Geographic oringin SSUrDNA**		**Acession number[a]**
Trypanosoma rangeli	TCC643	*Platyrrhinus lineatus*	Miranda	MS	EU867803
T. dionisii	CBT58	*Sturnira lillium*	Pinheiros	ES	KF557744
	PJ	*Pipistrellus pipistrellus*	Belgium		AJ009152
	P3	*Pipistrellus pipistrellus*	England		AJ009151
	TCC289	*Eptesicus brasiliensis*	São Paulo	SP	FJ001651
	TCC633	*Sturnira lillium*	Miranda	MS	EU867812
	CBT214	*Glossophaga soricina*	Alter do Chão-Resex	PA	**KY454457**
T. erneyi	TCC1293	*Tadarida* sp.	Mozambique		JN040987
	TCC1294	*Tadarida* sp.	Mozambique		JN040988
	TCC1932	*Mops condylurus*	Mozambique		JN040990
	TCC1934	*Mops condylurus*	Mozambique		JN040991
T. cruzi marinkellei	B7	*Phyllostomus discolor*	São Felipe	BA	AJ009150
	CBT213	*Carollia perspicillata*	Alter do Chão-Resex	PA	**KY454456**
	CBT215	*Mimon crenulatum*	Alter do Chão-Resex	PA	**KY454458**
	CBT216	*Carollia perspicillata*	Alter do Chão-Resex	PA	**KY454459**
	CBT217	*Carollia perspicillata*	Alter do Chão-Resex	PA	**KY454460**
	CBT218	*Carollia perspicillata*	Alter do Chão-Resex	PA	**KY454461**
	CBT220	*Carollia perspicillata*	Alter do Chão-Resex	PA	**KY454462**
	CBT222	Caroliia perspicillata	Alter do Chão-Resex	PA	**KY454463**
	CBT223	*Carollia perspicillata*	Alter do Chão-Resex	PA	**KY454464**
	CBT224	Carollia perspicillata	Alter do Chão-Resex	PA	**KY454465**
T. cruzi	Y	*Homo sapiens*	São Paulo	SP	AF301912
	CLBR	*Triatoma infestans*	São Paulo	SP	AF245383
	Peru	*Homo sapiens*	Peru		X53917
	JJ	*Homo sapiens*	Barcelos	AM	AY491761
	TCC463	*Cebus albifrons*	Barcelos	AM	EU755224
	NRcl3	*Homo sapiens*	Chile		AF228685
	TCC186	*Homo sapiens*	Bolivia		FJ001630
	TCC862	*Euphractus sexcinctus*		RN	FJ183397
	TCC863	*Euphractus sexcinctus*		RN	FJ183376
	MT3663	*Panstrongylus geniculatus*		AM	AF288660
	TCC129	*Proechimys iheringi*	São Paulo	SP	FJ555652
	TCC793	*Myotis levis*	São Paulo	SP	FJ001634
	TCC480	*Noctilio albiventris*	Miranda	MS	EU867804
	TCC947	*Myotis nigricans*	São Paulo	SP	FJ001626
	TCC949	*Myotis nigricans*	São Paulo	SP	FJ001627
	G	*Didelphis marsupialis*		AM	AF239981
	TCC417	*Thyroptera tricolor*	Barcelos	AM	FJ001631
	TCC711	*Didelphis marsupialis*	Barcelos	AM	EU755229
	TCC1334	*Didelphis marsupialis*	Parauapebas	PA	EU755242
	TCC1456	*Monodelphis brevicaudata*	Pará	PA	FJ555623
	CBT100	*Artibeus lituratus*	Chapadinha	MA	KP197161
	CBT146	*Philander opossum*	Açailandia	MA	KP197162
	CBT147	*Philander opossum*	Açailandia	MA	KP197163
	CBT148	*Didelphis albiventris*	Açailandia	MA	KP197164
	CBT149	*Philander opossum*	Açailandia	MA	KP197165
	CBT150	*Didelphis marsupialis*	Açailandia	MA	KP197166
	CBT152	*Gracilinanus* sp.	Açailandia	MA	KP197167

[a] Sequences determined in this study and deposited in GenBank are underlined and bold; Brazilian states: Espirito Santo (ES); São Paulo (SP); Bahia (BA); Mato Grosso do Sul (MS); Amazonas (AM); Rio Grande do Norte (RN); Pará (PA); Maranhão (MA).

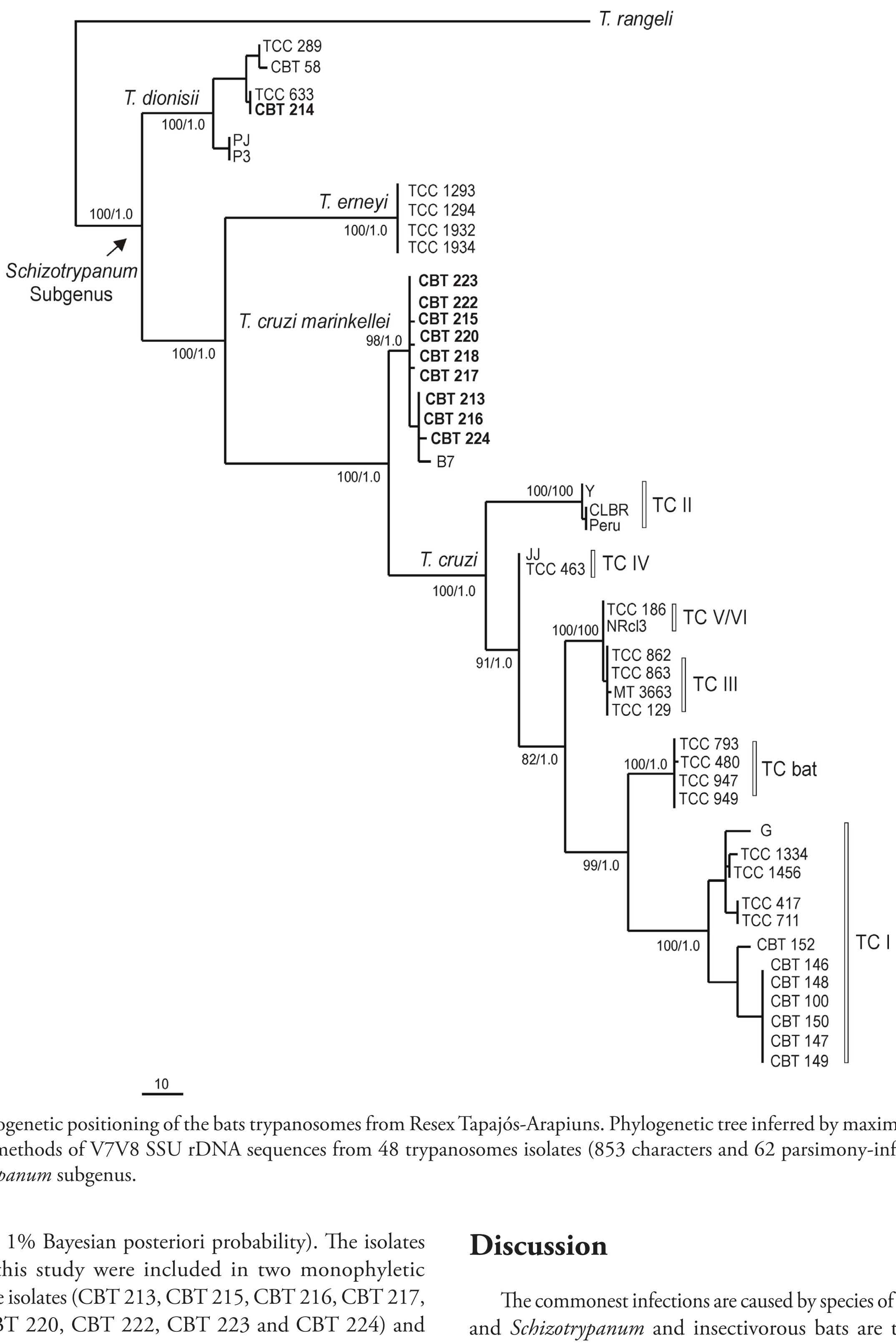

Figure 2. Phylogenetic positioning of the bats trypanosomes from Resex Tapajós-Arapiuns. Phylogenetic tree inferred by maximum parsimony and Bayesian methods of V7V8 SSU rDNA sequences from 48 trypanosomes isolates (853 characters and 62 parsimony-informative sites) from *Schizotrypanum* subgenus.

bootstrap and 1% Bayesian posteriori probability). The isolates obtained in this study were included in two monophyletic branches. Nine isolates (CBT 213, CBT 215, CBT 216, CBT 217, CBT 218, CBT 220, CBT 222, CBT 223 and CBT 224) and grouped with a reference strain (B7) of *T. cruzi marinkellei* (99% of similarity of sequences, 100% bootstrap and 1.0% *Bayesian* posteriori probability) (Figure 2). One isolate, CBT214 from *Glossophaga soricina*, was identified as on the *T. dionisii* branch with other sequences from Brazilian *T. dionisii* isolates (98% of similarity of sequences, 100% bootstrap and 1.0% *Bayesian* posterior probability) (Figure 2).

Discussion

The commonest infections are caused by species of *Megatrypanum* and *Schizotrypanum* and insectivorous bats are the type most frequently infected (HOARE, 1972; MARINKELLE, 1976). The trypanosome species that can infect bats and have been included in phylogenetic studies are *T. dionisii*, *T. vespertilionis*, *Trypanosoma* sp. bat (isolated in Africa), *T. cruzi marinkellei* (STEVENS & RAMBAUT, 2001; STEVENS & GIBSON, 1999; HAMILTON et al., 2007), *T. desterrensis* (GRISARD et al., 2003), *T. wauwau* (LIMA et al., 2015) in Brazil, *T. erneyi* (LIMA et al., 2015), *T. livingstonei* (LIMA et al., 2013) in Africa and *T. teixeirae* in Austrália (BARBOSA et al., 2016).

The biomes with the highest prevalences of bat trypanosomes in Brazil are the Amazon biome with 45.2% according to microhematocrit and hemoculture (CAVAZZANA et al., 2010) and the Amazon/Cerrado biome with 15.5% according to hemoculture (MARCILI et al., 2013). The prevalence found in the Tapajós-Arapiuns Resex in the present study (9%) was low in comparison with other areas and studies.

However, in the present study, all the positive samples were isolated and cryopreserved (100% isolation success), while in other studies 20 a 35% of positive cultures did not generated isolates in several Brazilian biomes (CAVAZZANA et al., 2010; MARCILI et al., 2013; ACOSTA et al., 2014; COSTA et al., 2015, 2016).

The most isolates from Resex were positioned with *T. cruzi marinkellei* and only one isolate with *T. dionisii* isolates. In different studies of bat trypanosomes conducted in Brazil, *T. cruzi marinkellei* predominated, followed by *T. dionisii* and *T. cruzi*. Furthermore, *T. dionisii* and *T. cruzi* were isolated in all areas studied; meanwhile, *T. cruzi marinkellei* was not detected in southern or southeastern Brazil, except in Espirito Santo state (CAVAZZANA et al., 2010; ACOSTA et al., 2014).

The absence of *T. cruzi* isolates from the bats trypanosomes isolated from this study does not exclude their occurrence in the region. The diversity of bats species and trypanosomes associated of bats can be increase in new studies with more days, different area in Resex and sazonal variation.

Studies on diversity and associated parasites may provide information about the biology of hosts and host-parasite-environment relationships (GALLI et al., 2001; MADI & UETA, 2012). Neotropical bats are excellent quality indicators for different ecosystems (MEDELLÍN et al., 2000; WILLIG et al., 2007).

Parasites generally respond to environmental changes faster than their hosts. This provides a means of assessing environmental stress levels and creating a density regulator for the populations of their hosts. This has a great impact on the environment and on animal communities (SILVA-SOUZA et al., 2006).

Upward or downward variations in the amount of parasitism may be an indicator of human actions within the environment that can positively or negatively influence parasite development (LAFFERTY & KURIS, 1999). In Panamá, habitat fragmentation has increased the prevalence of *Trypanosoma* in the bat *Artibeus jamaicensis* caught in forest fragments, compared with those caught in areas of continuous forest (COTTONTAIL et al., 2009).

Studies aiming towards isolation and molecular characterization of bat trypanosomes in areas that have never previously been studied are extremely important for understanding the diversity of parasites in bats, as well as for identifying pathogenic species and generating support for control measures. In addition, studies should be conducted to confirm the role of these parasites as environmental degradation biomarkers.

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