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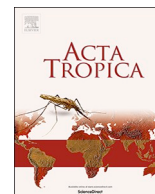
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# Molecular eco-epidemiology on the sympatric Chagas disease vectors *Triatoma brasiliensis* and *Triatoma petrocchia*: Ecotopes, genetic variation, natural infection prevalence by trypanosomatids and parasite genotyping

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## ABSTRACT

*Triatoma petrocchia* is the newly member of the *Triatoma brasiliensis* species complex. This species overlaps with *T. brasiliensis* in geographic and ecotypic occupation in the sylvatic habitat because both inhabit rocky outcrops in the semi-arid portion of Brazilian northeast. In this region *T. brasiliensis* is the most important Chagas disease vector because it constantly colonizes domiciles. In contrast, *T. petrocchia* is rarely found in peri or intradomestic habitats – reason why little is known about this species. Therefore, Here, we present information for the first time on the *T. petrocchia* ecotopes, genetic diversity, *Trypanosoma cruzi* prevalence/genotyping in comparison to *T. brasiliensis*. We found *T. brasiliensis* ( $N = 223$ ) and *T. petrocchia* ( $N = 69$ ) in co-habitation in rocky outcrops in three Districts of Paraíba and Rio Grande do Norte states. Forty-tree *T. petrocchia* insects of eleven sampling spots (composing three geographic populations) were genotyped for the mitochondrial Cyt B gene and little geographic structure was observed. Tajima's  $D$  test suggested that species is evolving toward a mutation-drift equilibrium in our collection range. Sylvatic *T. petrocchia* had 4% (3/68) of infected insects by *T. cruzi*, whereas *T. brasiliensis* had 26% (59/223). Fluorescent Fragment Length Barcoding demonstrated that all three *T. petrocchia* harbored TcI whereas *T. brasiliensis* had TcI, but also TcIII, TcII/TcVI and *T. rangeli* genotype A, sometimes under mixed infections. None of infected *T. petrocchia* were carrying mixed infections. However, this result should be confirmed using a larger pool of infected bugs. We here presented the first documentation of *T. rangeli* infecting *T. brasiliensis*. The finding of infected *T. petrocchia* calls for constant vector monitoring because the epidemiologic scenario is dynamic and sylvatic vectors are progressively found in adaptation to anthropic environments.

## 1. Introduction

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and is placed as one of the most important neglected disease in the world. It affects about 6–7 million people worldwide, mostly in Latin

America (WHO, 2016). Anthropogenic changes in natural ecosystems have influenced the distribution of vectors on geographic and ecotypic scales. In Brazil, this can be illustrated by the growing finding of home colonization by sylvatic Chagas disease vectors (Almeida et al., 2009, 2000), which increases the risk of human Chagasic infection due to the

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approximation between vectors and humans – even for accidental infections, as for oral contaminations.

Among Chagas disease vectors, *Triatoma brasiliensis* is the main concern in the semi-arid portion of Northeastern Brazil, where it coexists with *T. petrochiae* – a species rarely collected and poorly studied. They differ greatly in epidemiological terms: whereas *T. brasiliensis* occupies a variety of ecotopes – sylvatic, peridomestic and domestic (Costa et al., 1998, 2003) – *T. petrochiae* is rarely found in the peridomestic and domestic habitats, being considered exclusively sylvatic (Lent and Wygodzinsky, 1979). However, this species occasionally invades domiciles (Costa et al., 2003; Barbosa-Silva et al., 2019).

*Triatoma brasiliensis* and *T. petrochiae* overlap in geographic distribution in several states within the Caatinga eco-region, such as Paraíba, Pernambuco and Rio Grande do Norte (Costa et al., 2003; Caranha et al., 2011; Silva et al., 2012; Almeida et al., 2016; Galvão et al., 2003). *T. petrochiae* is also found in the state of Bahia (Sherlock and Guitton, 1967), where *T. juazeirensis* occurs – a member of *T. brasiliensis* species complex. Besides *T. juazeirensis* and *T. petrochiae*, this group is composed of seven more members: *T. bahiensis*, *T. lenti*, *T. melanica*, *T. petrochiae*, and *T. sherlocki*, and two subspecies: *T. brasiliensis* (here referred as *T. brasiliensis*) and *T. b. macromelasoma* (Oliveira et al., 2017). Even though *T. petrochiae* is the most genetically differentiated within this complex, it is morphologically similar to *T. brasiliensis*, so that they were in synonymy in the past (Lucena, 1970). It was raised later to the specific taxonomic level by Lent and Wygodzinsky (1979) based on consistent morphological differences and reproductive incapacity for cross-mating experiments (Espinola, 1971). However, the morphological similarities between both species raise problem for those involved in vector control because there is no objective way to recognize nymphs (Dale et al., 2018).

Besides the geographic distribution, *T. brasiliensis* and *T. petrochiae* also overlap in ecotope, being found sometimes over the same rocky complex in the sylvatic environment (Almeida et al., 2016), although the precise ecotope of this species is still unknown. In rocky outcrops, *T. brasiliensis* has been recorded to be associated mainly with rodents of Caviidae family – but it also feeds on a variety of animals in peridomestic, including birds, dogs, cats, sheep, lizards, frogs, cows, among others. (Costa et al., 1998; Valença-Barbosa et al., 2014, 2015; Almeida et al., 2016; Bezerra et al., 2018). However, the food sources of *T. petrochiae* remain unknown, although Lent and Wygodzinsky (1979) mention it is probably associated with *Kerodon rupestris* (Rodentia: Caviidae).

Specially in the state of Rio Grande do Norte, *T. brasiliensis* can be found with high prevalence of *T. cruzi* infection in the domestic and sylvatic habitats, often with mixed parasite lineages (Lilioso et al., 2017; Almeida et al., 2016). On the other hand, it is not known whether *T. petrochiae* can be participating in the wild transmission cycle of this parasite. Phylogenetic reconstruction allocated *T. petrochiae* at the basal position of *T. brasiliensis* species complex with high support (Oliveira et al., 2017), but there is no information on its intraspecific genetic variability. Since *T. brasiliensis* is a vector that participate actively in the Chagas disease epidemiology, this study aimed to present the first information on the *T. petrochiae* ecotopes, genetic diversity, *T. cruzi* prevalence/genotyping in comparison to *T. brasiliensis*.

## 2. Methods

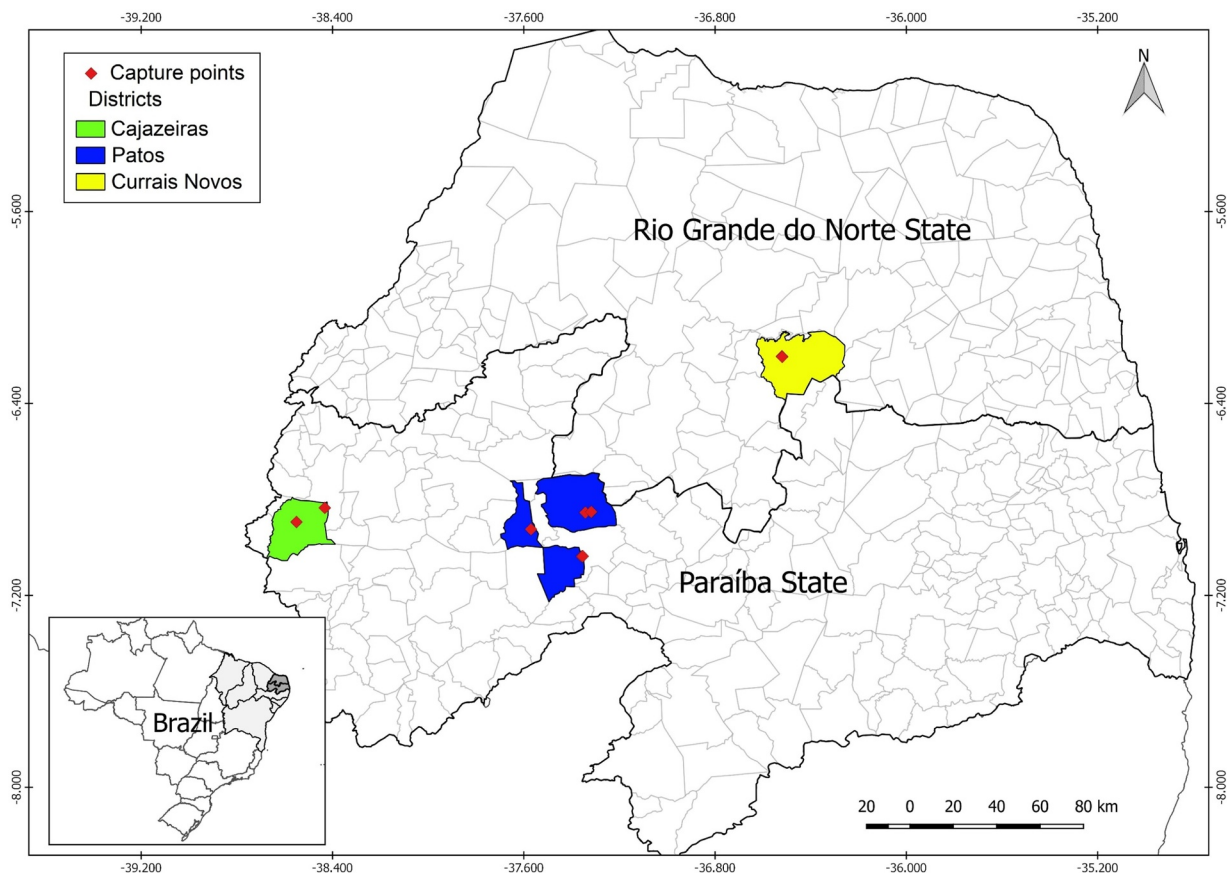
**Captures** – Searches for insects were conducted in the states of Paraíba (PB) and Rio Grande do Norte (RN), Brazil, across a range of 240 km (East-West) and 95 km (North-South) (–06°58'48,0" to –06°08'49,2" and –38°35'27,6" to –36°29'09,6"). The sampling was distributed in three districts: Cajazeiras (PB; two sampling spots), Patos (PB; seven sampling spots) and Currais Novos (RN; two sampling spots) which are here referred as geographic populations (Fig. 1). All sampled spots were within the biogeographic zone known as Caatinga, a mosaic of xerophytic, deciduous, semi-arid thorn scrubs, and forest (Leal et al.,

2003). The peridomestic ecotopes were considered within a radius of 50 m from the domiciles, where domesticated animals slept or were raised. In peridomestic, most of triatomines were captured in chicken coops and in goat and pig corrals. Sylvatic ecotopes were represented by rocky outcrops, although we searched *T. brasiliensis* and *T. petrochiae* in other kind of ecotopes, as cacti, bird nests, and palms where no samples of these two species were found. All captures were conducted manually with the aid of tweezers by three field workers. Peridomestic captures were performed during the day whereas sylvatic searches were nocturnal. We obtained permission from house owners/residents to search for insects in all properties and we visited four houses in each spot – staying for around one hour in each house. During the same period and with the same staff we performed captures in sylvatic environments, where triatomines could be found in rocky outcrops – starting at the sunset (~05:00 h PM) and the effort lasted for approximately five hours in each spot. Populations were delimited as the group of insects collected in the same district, but from varied sampling spots.

**Natural infection prevalence** – All collected insects were stored individually in an Eppendorf tube at –20 °C in absolute ethanol until processed. The gut of each insect was macerated in liquid nitrogen by using a sterile crusher. The DNeasy Blood & Tissue Kit (Qiagen) was used for DNA isolation, following the manufacturer's recommended protocol. The primers 121 and 122 (*T. cruzi* kDNA) we used to access *T. cruzi* natural infection prevalence by following the amplification protocol of Sturm et al. (1989) and Wincker et al. (1994). Products of PCR were applied to 2% (w/v) agarose gels and submitted to electrophoresis at 80 V for 40 min. The presence of bands indicates that the sample was positive for tripanosomatids. We used water as the negative control and DNA extracted from *T. cruzi* culture (TcI) as the positive one. The same insect DNA was used for an additional PCR to verify if the intestinal DNA did not have inhibitors for PCR. For this purpose we amplified the insect Cyt B gene as the target, by using the cybTprR (Oliveira et al., 2017) and 7432F primers (Monteiro et al., 2003), as previously described. These PCR products were also used to genotype the bugs.

**Trypanosoma cruzi genotyping**–The fluorescence fragment length barcode (FFLB) method was used for the *T. cruzi* genotyping. It discriminates the different Discrete Typing Units (TcI–VI) by size polymorphisms in specific regions of the 18S and 28S ribosomal RNA genes (Hamilton et al., 2011). It is a method based on PCR with fluorescent primers of which amplified fragments with varied sizes are interpreted in the automated sequencer. The technique can distinguish *T. cruzi* from *T. rangeli* and their different subtypes – except for TcII to TcVI (hereafter referred as TcII/TcVI). All DNA samples were from DNA from the triatomine digestive tract without passing for cultures.

**Triatoma petrochiae genotyping and genetic diversity statistics** – Sequences were obtained from both strands. The consensus sequences were edited in Seqman software (Swindell and Plasterer, 1997). The alignment was obtained via Clustal W 2.0 software (Larkin et al., 2007). Haplotypes were deposited in GenBank under the accession numbers MN177967 to MN177974. A phylogram was built with the resulting Cyt B sequences via Bayesian inference. The matrix was run in BEAST 1.8.0 (Drummond et al., 2012) with 10 million generations and sampling every 1000 simulations. A strict molecular clock model (with default rate of 1.0) and the constant-size coalescent tree prior were applied for this analysis. Tracer 1.7 (Rambaut et al., 2018) was used to check parameter convergence with a stationary state, using minimum threshold for ESS (Effective Sample Size) value of 200. TreeAnnotator (available on BEAST package) was used to summarize all the generated trees in the highest credibility tree, which was visualized and edited using FigTree 1.4.4 (available at <http://tree.bio.ed.ac.uk/software/figtree/>). The evolutionary model was chosen according to the Akaike Information Criterion in MrModeltest (Nylander, 2004). The haplotype network was obtained using the median-joining algorithm (Bandelt et al., 1999) implemented in PopART (Leigh and Bryant, 2015).



**Fig. 1.** Points of capture of *T. petrocchia* and *T. brasiliensis* in the states of Paraíba and Rio Grande do Norte. Geographic districts are in yellow (Currais Novos), blue (Patos) and green (Cajazeiras).

Summary statistics of genetic diversity of *T. petrocchia* were calculated for insects collected in the same geographic spot or populations (insects from the same district). The null hypothesis of neutrality was tested using the Tajima's *D* statistic (Tajima, 1989) and Fu's *FS* (Fu, 1997). The following population genetic summary statistics were calculated: number of haplotypes (*H*), haplotype diversity ( $H_d$ ) and nucleotide diversity ( $\pi$ ). A hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed to assess population genetic structure; samples from each geographic spot (Table 2) were grouped according to the three districts (Cajazeiras, Patos and Currais Novos). The DnaSP v6 software (Rozas et al., 2017) was used to generate the files to run the analyses described above in Arlequin 3.5 software (Excoffier and Lischer, 2010). The significance of molecular statistics (Tajima's *D*, Fu's *FS*,  $F_{CT}$ ,  $F_{SC}$ ,  $F_{ST}$ ) were tested using 1000 permutations, being considered significant those at *p* values < 0.05.

### 3. Results

**Parasite prevalence and trypanosomatid genotyping** – We collected 292 insects, and *T. brasiliensis* (Tb, *N* = 223) was most abundant compared to *T. petrocchia* (Tp, *N* = 69) (Table 1). Most of insects were collected in the sylvatic environment: the exception is regarding one *T. brasiliensis* population collected in Currais Novos, of which 26 insects were collected in the peridomestic environment and seven in the sylvatic environment. Because it was the unique population exhibiting insects collected in both environments, it is discussed later with the genotypic distribution of trypanosomatids (Fig. 2). Regarding natural infection prevalence, both species were found infected: whereas *T. brasiliensis* exhibited 26% of overall natural infection by trypanosomatids, *T. petrocchia* exhibited only 4%. In Patos district only *T. brasiliensis* population was infected (8%), whereas both were infected in Cajazeiras

(Tb = 4%, Tp = 8%) and Currais Novos (Tp = 8%, Tb = 68%) populations.

We genotyped the trypanosomatids of 41 bugs via FFLB; *T. cruzi* I (TcI) was the prevailing genotype because it was present in 34 of the 41 insects, also in mixed infections. It was the unique genotype observed for Cajazeiras (*N* = 3) and Patos populations (*N* = 3), as well as for all *T. petrocchia* (*N* = 3). Single TcI infections were present in 30 insects (73%) (Table 1).

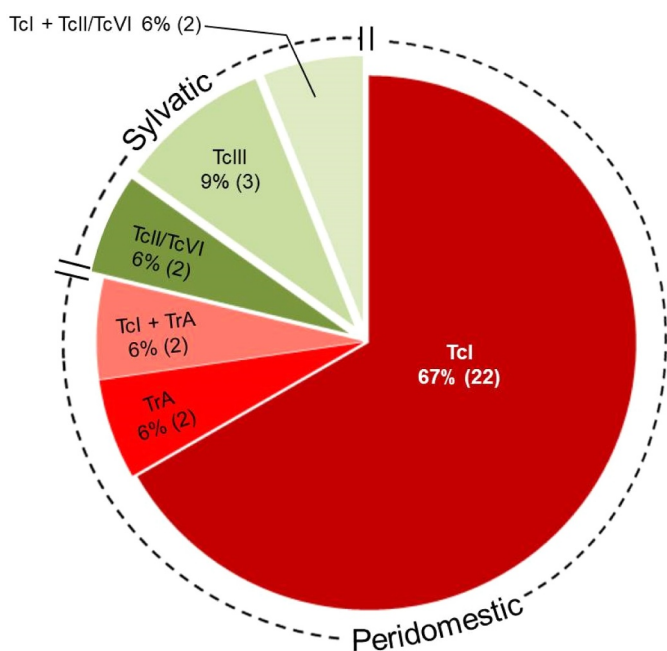
Insects from Currais Novos district were the only ones with an appropriate sample size (*N* = 33) of parasitic genotyping for inferring on ecotypic circulation by trypanosomatids in *T. brasiliensis*. Regarding peridomestic *T. brasiliensis* insects, 22 (67%) were infected by TcI, two (6%) had *T. rangeli* genotype A (TrA) and two (6%) had TcI mixed with TrA. Concerning sylvatic insects, two (6%) had TcII/TcVI, three (9%) had TcIII and two (6%) had TcI mixed with TcII/TcVI (Table 1, Fig. 2).

**Genetic diversity and structure, haplotype network and demographic signals** – We obtained 43 individual sequences of *T. petrocchia* with 509 bp for Cyt B. The alignment exhibited eight haplotypes and 15 polymorphic sites. Haplotype ( $H_d$ ) and nucleotide diversity ( $\pi$ ) levels for the whole dataset were 0.65 and 0.0078, respectively (Table 2). These values were not uniform for population level, ranging from 0.66 to 1 for  $H_d$  and from 0.0075 to 0.0137 for  $\pi$ . Samples from CZ27, ST289 and SJ290 spots did not present polymorphic sites, resulting in null diversity. In the population analysis, Currais Novos presented the highest diversity levels and Cajazeiras the lowest (Table 2). The haplotype network (Fig. 3) showed little geographic structure. The most frequent haplotype was shared by individuals from almost every sampling spot, which is separated from the others by at least six mutational steps, forming two major groups. These groups are also corroborated in the phylogenetic reconstruction through Bayesian inference with high posterior probability (Supplementary File 1). Only individuals from the

**Table 1**  
Collected insects in sylvatic and peridomestic environments, prevalence of natural infection by *Trypanosoma cruzi* and parasite genotyping.

Populations	<i>Triatoma petrocchia</i>			<i>Triatoma brasiliensis</i>		
	Collected	Infected(%)	Genotypes	Collected	Infected	Genotypes
Cajazeiras	11	2(18%)	TcI(N = 2)	49	2(4%)	TcI(N = 2)
Patos	49	0(0)	–	101	7(8%)	TcI(N = 3)
Currais Novos	9	1(11%)	TcI(N = 1)	73	50(68%)	TcI(N = 22), TcII/TcVI(N = 2), TcIII (N = 3), TrA(N = 2)
Total	69	4%		223	26%	

Mixed infections were recorded for *T. brasiliensis* from Currais Novos district: two for TcI + TcII/VI and two for TcI + TrA. Supplementary File 1 illustrates the ecotypic distribution of *T. cruzi* genotypes in *T. brasiliensis* from Currais Novos.



**Fig. 2.** Ecotypic distribution for all combination of trypanosomatid genotypes observed in thirty-three *Triatoma brasiliensis* collected in Currais Novos. Peridomestic genotypes are in reddish and sylvatic are in greenish.

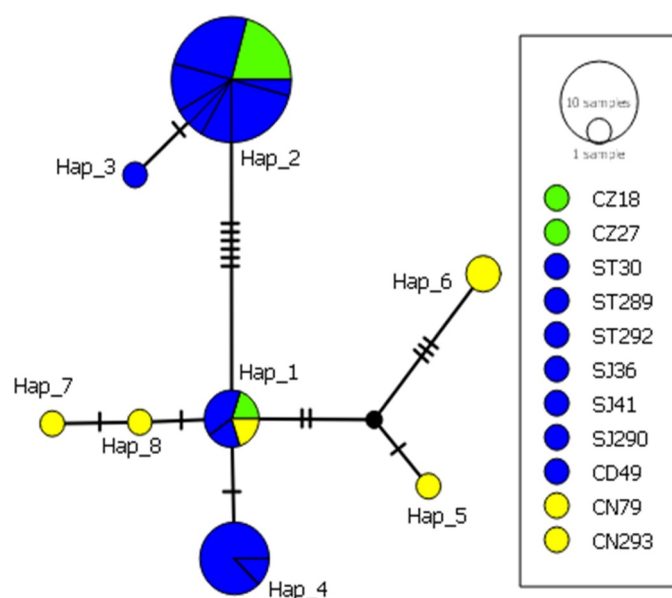
CZ18 and Currais Novos spots (CN79 and CN293) did not share this most frequent haplotype. Individuals from Currais Novos population exhibited four of the five exclusive haplotypes detected.

The neutrality tests did not show any signals of demographic events

**Table 2**  
Summary statistics of genetic diversity of *Triatoma petrocchia* calculated with a matrix of 43 gene fragments of 509 bp for the mitochondrial Cyt B gene.

Populations	N	S	H	Hd (s.d.)	$\pi$ (s.d.)	FS (P)	D (P)	Coordinates	
								South	West
Sampling spots									
Cajazeiras population	6	6	2	0.33 (0.21)	0.0039 (0.0025)	2.99 (0.90)	-1.36 (0.052)		
CZ18	1	-	1	-	-	-	-	-6.835261	-38.432389
CZ27	5	0	1	0	0	-	-	-6.894055	-38.550555
Patos population	31	8	4	0.56 (0.07)	0.0064 (0.0007)	4.76 (0.96)	1.95 (0.98)		
ST30	16	8	4	0.69 (0.07)	0.0075 (0.0006)	3.53 (0.94)	2.07 (0.99)	-7.037938	-37.355797
ST289	3	0	1	0	0	-	-	-7.037502	-37.35664
ST292	1	-	1	-	-	-	-	-7.036844	-37.353282
SJ36	1	-	1	-	-	-	-	-6.854806	-37.3426
SJ41	3	6	2	0.66 (0.31)	0.0078 (0.003)	2.63 (0.83)	-	-6.851956	-37.317123
SJ290	5	0	1	0	0	-	-	-6.851782	-37.3177
CD49	2	7	2	1.00 (0.50)	0.0137 (0.007)	1.94 (0.54)	-	-6.924627	-37.569704
C. Novos population	6	8	5	0.93 (0.12)	0.0078 (0.0013)	-0.74 (0.24)	0.83 (0.79)		
CN79	5	8	4	0.90 (0.16)	0.008 (0.0016)	0.21 (0.45)	0.66 (0.73)	-6.203250	-36.520194
CN293	1	-	1	-	-	-	-	-6.205600	-36.519002
Total	43	15	8	0.65 (0.07)	0.0078 (0.0007)	1.91 (0.80)	0.44 (0.73)		

N = number of samples, S = number of segregating sites, H = number of haplotypes, Hd = Haplotype diversity,  $\pi$  = nucleotide diversity, FS = FU's FS and D = Tajima's D, p = p-value.



**Fig. 3.** Median-joining network showing the relationship among the eight *Triatoma petrocchia* haplotypes based on a 509 bp fragment of cytochrome b mitochondrial gene. Samples were coded according to the districts: Currais Novos in yellow, Patos in blue and Cajazeiras in green (as in Fig. 1). Haplotypes 1 to 8 were deposited in GenBank under the accession codes MN177967 to MN177974.

(Table 2). AMOVA revealed that most of the variation was found within populations (approximately 60%) which reinforces the pattern of little geographic structure for *T. petrocchia* (Table 3).

**Table 3**  
AMOVA based on Cytochrome b gene variation for *Triatoma petrocchiae* (d.f. = degrees of freedom, statistically significant values are in bold).

Source of variation	d.f.	Variation (%)	Statistics	p
Among districts	2	26.68	$F_{CT} = 0.26$	0.07
Among populations within districts	8	15.33	$F_{SC} = 0.20$	0.06
Within populations	32	<b>57.99</b>	$F_{ST} = 0.42$	0.001

#### 4. Discussion

Based on the variation on multiple mitochondrial genes (Cyt B, COI, 12S and 16S) and morphometric information, *T. petrocchiae* was the last member to be included in the *T. brasiliensis* species complex (Oliveira et al., 2017). This species overlaps with *T. brasiliensis* in geographic and ecotypic distribution in the sylvatic habitat because both inhabit rocky outcrops in semi-arid region of Brazilian northeast (Almeida et al., 2016; Costa et al., 2003). Unlike *T. brasiliensis*, *T. petrocchiae* is considered exclusively sylvatic, and here we present the first study on *T. petrocchiae* ecotope choice, genetic diversity, *T. cruzi* prevalence/genotyping in comparison to its associated species – *T. brasiliensis*.

The high prevalence of natural infection in *T. brasiliensis* populations has been shown by several authors (Alencar 1987; Costa et al., 1998, 2003; Bezerra et al., 2018), which seems to be much higher for the state of Rio Grande do Norte (Almeida et al., 2016; Lilio et al., 2017; Barbosa-Silva et al., 2019). Our results support the idea that TcI is the predominant genotype in anthropic environments whereas TcII/TcIV and TcIII are preferentially sylvatic. However, none of *T. cruzi* genotypes were ecotypically exclusive, as already observed by other authors in Rio Grande do Norte (Almeida et al., 2016; Barbosa-Silva et al., 2016) and Ceará (Bezerra et al., 2018). The flow of *T. cruzi* genotypes may be explained by the pronounced gene flow of *T. brasiliensis* between ecotopes – probably by flying, as observed by Almeida et al. (2016). It is important to mention that we present here the first finding of *T. rangeli* in *T. brasiliensis*, appearing also in mixed infection. Our dataset does not allow robust conclusion whether TcI is a genotype predominant in *T. petrocchiae* because we detected only three sylvatic insects infected. Isolating and cultivating *T. cruzi* may result in loss of diversity, and we here used a technique (FFLB) that allows to genotype the trypanosomatids by using the DNA from the insect gut contents.

Regarding genetic variation in *T. petrocchiae*, the most pronounced differentiation is six mutational steps exhibited between two haplotypes within groups well supported phylogenetically, in which populations from Currais Novos population (CN) are the only ones restricted to one of these groups. The group that includes CN population is likely the derived one, because the other has a haplotype (H2) with the most pronounced proportion of individuals, being represented by several geographic spots – a characteristic of the ancestral haplotypes (Almeida et al., 2008; 2016; Monteiro et al., 2004). The differentiation between groups from sides separated by six mutations is at the border line for what is observed between *T. brasiliensis* subspecies, which exhibit nine mutational steps of differentiation for the same marker and fragment length (Monteiro et al., 2004).

Analysis of molecular variance (AMOVA) did not evidence geographic explanation for the distribution of genetic variation. Tajima's *D* test was used to access signatures of recent historical demographic events, suggesting the species is evolving in mutation-drift equilibrium in our collection range because neither overall taxa nor most of populations exhibited significant Tajima's *D* values (Tajima, 1989). This pattern was in accordance to what is observed for *T. brasiliensis* (Almeida et al., 2008, 2016), for which most of populations also fit the neutral theory model at equilibrium between mutation and genetic drift.

We present the first consistent information on *T. petrocchiae* ecotope since we found almost all bugs in rocky outcrops. Although Lent and Wygodzinsky (1979) mentioned this insect occupies burrows of *Kerodon rupestris* (Rodentia, Caviidae), we believe it only shares the habitat with *T. brasiliensis* in rocky outcrops. It seems that the participation of *T. petrocchiae* in the parasite cycle does not seem to be as relevant as *T. brasiliensis* since this last species exhibits higher prevalence of natural infection by *T. cruzi*. The finding of single infection by *T. cruzi* in only three infected bugs needs to be validated using a larger dataset. Recently, Barbosa-Silva et al. (2019) also found infected *T. petrocchiae*, with a parasite prevalence of 1.3% via classic methods by searching parasites by optical microscopy. The higher *T. cruzi* prevalence observed here (4%) may be explained by the higher resolution of molecular techniques.

A recent Chagas disease outbreak was observed in the state of Rio Grande do Norte (Lilio et al., 2017; Vargas et al., 2018). Evidence suggests that *T. brasiliensis* was the vector involved in this outbreak; however the participation of other vectors must not be ruled out since several other triatomine species were already recorded in this state (Gurgel-Goncalves et al., 2012). Strategies for monitoring the risk of Chagas disease infection cover essentially the study of spatial distribution and diversity of vectors, reservoirs and its etiologic agent *T. cruzi* (Costa and Lorenzo, 2009). We present the first molecular eco-epidemiological study on *T. petrocchiae*. The finding of infected *T. petrocchiae* calls for further studies on its food sources. Recently Barbosa-Silva et al. (2019) found *T. petrocchiae* colonizing domiciles, representing 0.47% of all bugs collected. As mentioned by Waleckx et al. (2015), the ecotypic vector distribution may be dynamic, and therefore it requires continuous monitoring, especially in face of the finding of *T. petrocchiae* infected by *T. cruzi*.

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#### CRediT authorship contribution statement

**Tarcianne M. Lima-Oliveira:** Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Fernanda von Hertwig M. Fontes:** Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Maurício Lilio:** Investigation, Methodology, Writing - original draft, Writing - review & editing. **Dayane Pires-Silva:** Methodology, Writing - review & editing. **Marta Maria Geraldete Teixeira:** Methodology, Writing - review & editing. **Jose Gabriel Vergara Meza:** Methodology, Writing - review & editing. **Myriam Harry:** Writing - review & editing. **Jonathan Fileé:** Writing - review & editing. **Jane Costa:** Writing - review & editing. **Carolina Valença-Barbosa:** Investigation, Methodology. **Elaine Folly-Ramos:** Investigation, Writing - review & editing. **Carlos Eduardo Almeida:** Conceptualization, Funding acquisition, Writing - original draft, Writing - review & editing.

#### Declaration of Competing Interest

None.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.actatropica.2019.105188](https://doi.org/10.1016/j.actatropica.2019.105188).

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