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Major Article

Characteristics of Triatomine infestation and natural *Trypanosoma cruzi* infection in the State of Rio Grande do Norte, Brazil

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ABSTRACT

Introduction: Natural and artificial ecotope infestation by the *kissing bug* triatomines and their colonization and infection by *Trypanosoma cruzi*, the Chagas disease agent, were evaluated in nine municipalities of the State of Rio Grande do Norte, Brazil. **Methods:** Following identification, triatomine intestinal contents were analyzed by direct microscopic examination, xenoculture, and polymerase chain reaction (PCR) for parasite detection. *Trypanosoma cruzi* isolates were genotyped using three different markers. **Results:** Of 842 triatomines captured, 65% were *Triatoma brasiliensis*, 17.8% *Triatoma pseudomaculata*, 12.5% *Panstrongylus lutzi*, and 4.7% *Rhodnius nasutus*. *Triatoma brasiliensis* and *P. lutzi* adults were found in the intradomicile. *T. brasiliensis*, *T. pseudomaculata*, and *R. nasutus* nymphs and adults were found in the peridomicile and wild environment. Intradomiciliary and peridomiciliary infestation indexes were 5.6% and 33.7%, respectively. In the peridomicile, chicken coops were the most infested ecotope. The *T. cruzi* triatomine infection rate was 30.2%, of which PCR detected 29%. *P. lutzi* (78.1%), *T. brasiliensis* (24.5%), and *T. pseudomaculata* (22.7%) were the most infected species. TcII and III genotypes were detected in *T. brasiliensis* and TcIII in *P. lutzi*. **Conclusions:** *T. brasiliensis* was found in all environments and most ecotopes with high *T. cruzi* infection rates. High infection rates were also detected in *T. pseudomaculata* and *P. lutzi*, suggesting their role in the interchange between the wild and peridomestic transmission cycles. The combination of PCR, microscopic examination, and xenoculture contributed to improving *T. cruzi* infection evaluation in triatomine bugs. The TcII and TcIII genotypes were predominant in the study area.

Keywords: *Trypanosoma cruzi*. Triatominae natural infection. PCR. Xenoculture. Direct microscopic examination.

INTRODUCTION

Trypanosoma cruzi is the etiological agent of Chagas disease, and its main vectors belong to the genera *Panstrongylus*, *Rhodnius*, and *Triatoma*. Among these blood-sucking reduviid bugs of the subfamily Triatominae, 70 of the over 148 Triatominae species described[1] are naturally infected by *T. cruzi*[2]. Infection is maintained primarily within three overlapping cycles: domiciliary, peridomiciliary, and sylvatic[3]. Vector transmission remains the most important route of parasite to human transmission owing to the natural distribution of *T. cruzi* in the triatomine species adapted to domestic or peridomestic environments; this adaptation to human dwellings strongly determines human infection rates[4].

The Brazilian Northeast, one of the poorest and most underdeveloped regions within Brazil, is considered the most important region therein for American trypanosomiasis, where native species like *Triatoma brasiliensis* Neiva, 1911, *Triatoma pseudomaculata* Corrêa and Espínola, 1964, *Panstrongylus lutzi* Neiva & Pinto, 1926, and *Rhodnius nasutus* Stal, 1859 are widespread[5] [6]. *Triatoma brasiliensis* is distributed in nine Northeast states, Tocantins, and Minas Gerais[7]. Considered the main vector of *T. cruzi* in the Northeastern semiarid regions, it colonizes both sylvatic and domestic environments but is more frequently captured in peridomestic areas[5] [8] [9] [10] [11]. Triatomine control remains problematic as this region is the center of dispersion and has higher *T. brasiliensis* concentrations[12] [13] [14]; it is further aggravated by local vector control activity discontinuation and wild and domestic environmental overlap[15] [16] [17]. *Triatoma pseudomaculata* also demonstrates high domiciliation capacity,

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thereby increasing its colonization rate in several states[5] [18]; such native triatomines that sporadically invade or reinvade human dwellings further complicate vector control consolidation[18].

Triatomines can be infected with several flagellates and *T. cruzi* infection level evaluation in wild, peridomiciliary, and domiciliary environments has relevance for control programs aimed at reducing human infections[19] [20]. *Trypanosoma cruzi* has been detected in the excreta or intestinal contents of triatomines by direct microscopic examination (DME)[19] [21], necessitating the ability to distinguish this protozoan from other trypanosomatids. Although DME is reliable and relatively inexpensive, disadvantages related to sensitivity, specificity[22] [23] [24], and poor performance on dead insects[25] exist. Xenoculture is used for quality control to confirm negative intestinal content results ascertained by DME, whereas positive cultures allow for *T. cruzi* strain isolation[26] pursuant to e.g. genetic studies. For detecting *T. cruzi* in reduviid bug feces or urine and mammalian blood samples, polymerase chain reaction (PCR) is generally superior [21] [22] [27] and can also genotype *T. cruzi* using different targets.

Such methodology has increased the rate of positivity of infection in field studies, which is especially important in areas where there is scarce information on vector infection following control and surveillance programs. A recent seroepidemiological survey showed high seroprevalence for municipalities in the west and central mesoregions of the State of Rio Grande do Norte (RN)[28], and genotyping studies identified *T. cruzi* I (TcI) in *T. brasiliensis* and TcIII (formerly called TcIIc) in armadillos[29], TcI and TcII in humans, TcII and TcIII in *T. brasiliensis*, and TcIII in *P. lutzi*[15].

The purpose of this study was to evaluate the occurrence of triatomine infestation of natural and artificial ecotopes, and the colonization and *T. cruzi* infection in triatomines collected from different environments in the west and central mesoregions of the State RN, Brazil. Diagnostic method efficacy and reproducibility were evaluated and the *T. cruzi* populations isolated from positive triatomines were genotyped to establish their genetic groups.

METHODS

Study area

State of Rio Grande do Norte, located in northeastern Brazil, is divided into 167 municipalities distributed in four mesoregions: West, Central, Agreste, and East. About 90% of the territory represents arid and semiarid climates, where the predominant biome is the Caatinga, characterized as containing thorny shrubs, irregular structure, and partially uncovered soil. This study was conducted in the municipalities of Apodi, Caraúbas, Governador Dix-Sept Rosado, Lucrécia, Mossoró, Severiano Melo, São Miguel, Caicó, and *Serra Negra do Norte* in the West and Central mesoregions (**Figure 1**), which were selected in reference to the seroepidemiological survey conducted between 2007 and 2009[28].

Triatomine collection and identification

Triatomines were captured in rural areas of the nine municipalities in intradomicile, peridomicile, and wild environments from March 2009 to August 2012. The intradomiciliary environment was surveyed in 250 domiciliary units (DU) together with 187 artificial structures in the peridomicile such as chicken coops (n = 110), corrals (n = 25), pigsties (n = 2), piles of tiles (n = 44), old stone fence structures (n = 2), and dry carnauba palm tree (*Copernicia prunifera*) straw (n = 4). Entomological indicators were used to calculate the colonization and species peridomiciliary and domiciliary infestation indices[30].

Wild environment captures were performed in Apodi, Caraúbas, and *Serra Negra do Norte* in rock outcrops (n = 9), stone fences (n = 2), bird's nests (n = 5), and carnauba palm trees (n = 16), the latter as previously described[31].

In *Serra Negra do Norte*, wild triatomines capture was conducted at the Seridó Ecological Station (ESEC-Seridó)/ *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio), created by Decree 87222 of 05/31/1982, Law 6902 04.27.1982 as a Conservation Unit. Some adult insects were captured in station lodgings and were classified as an undefined ecotope. Insects were captured both day and night by the principal author with the assistance of the respective Municipal Health Secretariat technicians via manual searches using tweezers and a flashlight in all environments and without the use of insect dislodging substances, and were individually identified as described[32].

Natural infection of triatomine bugs

Direct microscopic examination. Collected triatomines were examined individually and their intestinal content was removed under aseptic conditions and placed in a well of a 24-well plate containing 500µL sterile saline solution. After homogenization, 5µL suspension was used to identify trypanosomatid forms via DME at 400× magnification[26] and smear stained by Giemsa (1,000×).

Xenoculture. Approximately 250µL intestinal content suspension was seeded in 15mL tubes containing liver infusion tryptose culture medium[33] plus McNeal Novy Niccole or blood agar and incubated at 28°C. Aliquots were examined after 15, 30, and 60 days by DME (400×)[26].

PCR with species-specific primers. Deoxyribonucleic acid (DNA) was extracted via phenol-chloroform[34] using 200µL diluted insect intestinal content solution (v/v) in 0.2M guanidine-HCl 6M/ethylenediaminetetraacetic acid (EDTA)[35] maintained for 5-7 days at room temperature and stored at 4ºC until DNA extraction. PCR amplifications were performed in duplicate, as described[34], using specific primers to identify *T. cruzi*[36]. PCR assays were performed in a DNA clean chamber to avoid contamination and positive and negative controls were used to monitor each step.

For *Trypanosoma cruzi* and *Trypanosoma rangeli* Tejera, 1920 differential diagnosis, multiplex PCR was performed

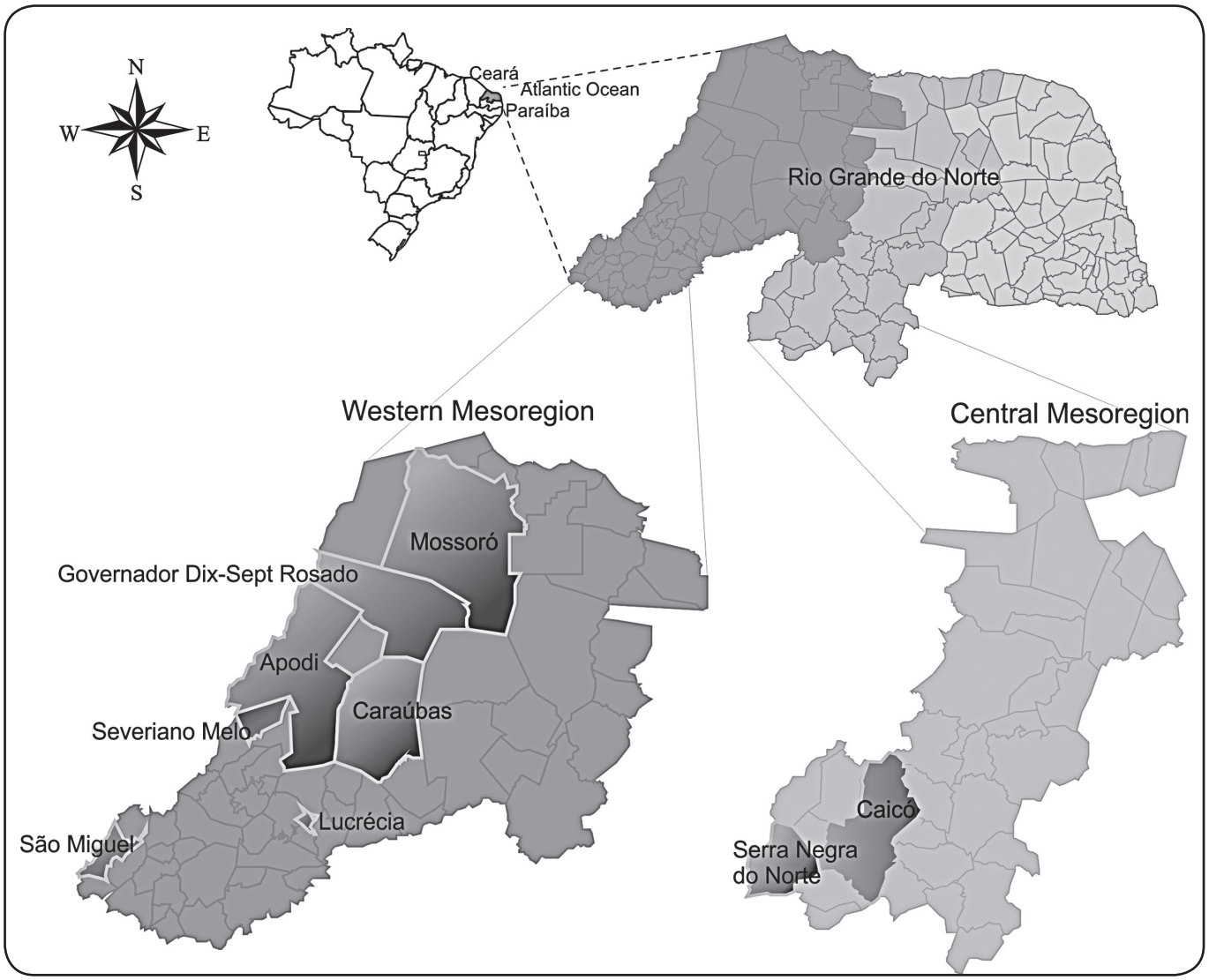

FIGURE 1 - Map of the State of Rio Grande do Norte highlighting the West and Central mesoregions. The study area showing the surveyed municipalities is shown in dark grey.

as described[37]. As positive controls, *Trypanosoma cruzi* cell cultures of the Colombian strains (*T. cruzi* I), JG (*T. cruzi* II), and *T. rangeli* were used.

Trypanosoma cruzi isolates for genotyping

Cultured parasite genomic DNA obtained using phenol-chloroform was used as PCR assay templates[38]. *Trypanosoma cruzi* isolates were typed using three different parasite genomic sequences as reported[39], with the 24Sα ribosomal (rRNA) gene D7 domain[40], mitochondrial cytochrome oxidase subunit 2 gene (COII)[41], and the spliced leader genes intergenic region[42] as markers for six discrete typing units (DTUs)[43], using *T. cruzi* reference strains[15] [44] [45] and clones[46] [47] as DTU controls.

All PCR products were analyzed by 6% polyacrylamide gel electrophoresis and visualized by silver staining[48].

Statistical analysis

To quantify the concordance between the results of different methods, generalized Kappa (*hat k*) coefficients were estimated and an approximate 95% confidence interval ($CI_{95\%}$) for *k* [lower confidence limit (LCL) and upper confidence limit (UCL)][49] was calculated and classified accordingly[50].

RESULTS

Table 1 shows that were captured 842 triatomines including *T. brasiliensis* (65%), *T. pseudomaculata* (17.8%), *P. lutzi* (12.5%), and *R. nasutus* (4.7%). *T. brasiliensis* was predominant in intradomicile (n = 12), peridomicile (n = 323), and wild environments (n = 212). **Table 1** shows specimen numbers per species, environment, and municipality.

TABLE 1 - Number of triatomine bugs captured in different environments in the west and central mesoregions in nine municipalities in the State of Rio Grande do Norte, Brazil (2009-2012).

Municipalities	Triatomine species								Total	
	Triatoma brasiliensis		*Triatoma pseudomaculata*		*Panstrongylus lutzi*		*Rhodnius nasutus*			
	n	%	n	%	n	%	n	%	n	%
Central mesoregion										
Caicó	23	4.3	0	0.0	0	0.0	1	2.5	24	2.9
Serra Negra do Norte	127	23.2	38	25.3	102	97.1	0	0.0	267	31.8
West mesoregion										
Apodi	35	6.4	20	13.3	0	0.0	21	52.5	76	9.0
Caraúbas	303	55.4	65	43.4	3	2.9	18	45.0	389	46.2
Governador Dix-Sept	50	9.2	0	0.0	0	0.0	0	0.0	50	5.9
Rosado										
Lucrécia	0	0.0	7	4.7	0	0.0	0	0.0	7	0.8
Mossoró	4	0.7	0	0.0	0	0.0	0	0.0	4	0.5
São Miguel	1	0.1	12	8.0	0	0.0	0	0.0	13	1.5
Severiano Melo	4	0.7	8	5.3	0	0.0	0	0.0	12	1.4
Total	**547**	**65.0**	**150**	**17.8**	**105**	**12.5**	**40**	**4.7**	**842**	**100.0**
Environments Wild										
nymphs	89	42.0	16	27.5	0	0.0	6	33.3	111	28.5
adults	123	58.0	42	72.5	102	100.0	12	66.7	279	71.5
Peridomicile										
nymphs	200	61.9	54	58.6	0	0.0	9	40.9	263	60.2
adults	123	30.1	38	41.4	0	0.0	13	59.1	174	39.8
Intradomicile										
nymphs	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
adults	12	100.0	0	0.0	3	100.0	0	0.0	15	100.0

In the wild, 48.5% (16/33) of the ecotopes were infested by triatomines. High infestation was observed in stone fences (100%) and rock outcrops (75%) in *Serra Negra do Norte* in comparison with other ecotopes. In the peridomicile, the infestation index was 33.7% (63/187) with *T. brasiliensis* and *T. pseudomaculata* at indices at 26.2% (49/187) and 5.9% (11/187), respectively, primarily in chicken coops. The intradomicile infestation index was 5.6% (14/250). **Table 2** shows the colonization index of each ecotope by triatomine species.

All the insects were examined; 30.2% exhibited *T. cruzi* infection, with *P. lutzi* demonstrating the highest rates (78.1%). The highest infection rate was detected by PCR (29%), detecting *T. cruzi* infection in most *P. lutzi* (69.5%) and *T. brasiliensis* (24.3%) specimens (**Figure 2A**). Kappa evinced no agreement between *T. cruzi* detection methods for *T. brasiliensis* [*hat k* = −0.0112; $CI_{95\%}$ = (−0.0596; 0.0372)], *T. pseudomaculata* [*hat k* = 0.0036; $CI_{95\%}$ = (−0.0888; 0.0960)], *P. lutzi* [*hat k* = 0.0690; $CI_{95\%}$ = (−0.0414; 0.1794)], and *R. nasutus* (*hat k* = 0.1652; $CI_{95\%}$ = (−0.0137; 0.3441)]. The global infection index was 0.12 [$CI_{95\%}$ = (0.0773; 0.1553)] indicating slight methodological agreement, but significantly different from zero as excluded by the CI.

Figure 2B shows that the highest infected specimen rate (53.3%) occurred in the intradomicile [primarily *P. lutzi* (66.7%)], followed by the wild environment (40.5%) and peridomicile (20.1%) [primarily *P. lutzi* and *T. pseudomaculata* (78.4% and 27.2%, respectively)].

In the wild, *T. brasiliensis* presented high infection indices in *Serra Negra do Norte* (31.4%) and Caraúbas (26.1%) rock outcrops. *T. cruzi* infection was also detected in *R. nasutus* in carnauba palm trees. In the peridomicile, *T. brasiliensis* presented high infection indexes in piles of tiles (38.1%) and corrals (37.5%) in Governador Dix Sept Rosado and Caraúbas, respectively, and *T. pseudomaculata* in chicken coops (30.8%) in Caraúbas (**Table 2**).

Intestinal contents of all infected triatomines and 100 uninfected specimens were submitted to multiplex PCR using primers for *T. rangeli* and produced no overlapping data, thus confirming that *T. cruzi*-specific amplification.

Trypanosoma cruzi DTUs were identified in 15 samples from three municipalities. We isolated 3 TcII and 3 TcIII stocks from *T. brasiliensis* captured in Caicó and *Serra Negra do Norte*, respectively,and 9 TcIII *T. cruzi* were isolated from *P. lutzi* in Caraúbas (**Table 3**).

TABLE 2 - Entomological indicators observed in the ecotopes of different environments in the municipalities of the State of Rio Grande do Norte.

Ecotopes	Infested ecotopes %	*Triatoma brasiliensis* %			*Triatoma pseudomaculata* %			*Panstrongylus lutzi* %			*Rhodnius nasutus* %		
		II	CI	NII	II	CI	NII	II	CI	NII	II	CI	NII
Wild environment													
Apodi													
bird nests	20.0	0.0	0.0	0.0	20.0	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0
carnauba palm trees	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caraúbas													
carnauba palm trees	42.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	42.8	33.3	16.7
rock outcrops	60.0	60.0	33.3	26.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Serra Negra do Norte													
rock outcrops	75.0	75.0	100.0	31.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
stone fences	100.0	100.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ecological station lodging	100.0	100.0	0.0	68.7	100.0	0.0	23.7	100.0	0.0	78.4	0.0	0.0	0.0
Total	**48.5**	**27.2**	**31.2**	**31.1**	**6.0**	**6.2**	**15.5**	**3.0**	**0.0**	**78.4**	**18.2**	**21.4**	**16.7**
Peridomicile													
Apodi													
corrals	22.2	22.2	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
carnauba palm tree straw	66.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	100.0	4.8
chicken coops	13.3	13.3	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
piles of tiles	20.0	20.0	100	22.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caicó													
stone fences	100.0	100.0	100	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
chicken coops	10.0	10.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
carnauba palm tree straw	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Caraúbas													
corrals	31.2	31.2	80.0	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
chicken coops	67.5	52.5	100.0	12.1	15.0	100.0	30.8	0.0	0.0	0.0	0.0	0.0	0.0
pigsties	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
piles of tiles	38.9	38.9	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Governador Dix-Sept Rosado													
chicken coops	33.3	33.3	100.0	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
piles of tiles	60.0	60.0	100.0	38.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lucrécia													
chicken coops	20.0	0.0	0.0	0.0	20.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mossoró													
chicken coops	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
São Miguel													
chicken coops	40.0	20.0	0.0	0.0	20.0	100.0	41.7	0.0	0.0	0.0	0.0	0.0	0.0
Serra Negra do Norte													
chicken coops	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
piles of tiles	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Severiano Melo													
chicken coops	57.1	42.8	0.0	0.0	14.3	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	**33.7**	**26.2**	**73.0**	**19.2**	**5.9**	**15.9**	**27.1**	**0.0**	**0.0**	**0.0**	**0.5**	**1.6**	**4.5**

Continue...

TABLE 2 - Continuation.

Ecotopes	Infested ecotopes %	*Triatoma brasiliensis* %			*Triatoma pseudomaculata* %			*Panstrongylus lutzi* %			*Rhodnius nasutus* %		
		II	CI	NII	II	CI	NII	II	CI	NII	II	CI	NII
Intradomicile													
Apodi	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caicó	10.7	10.7	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caraúbas	8.0	2.0	0.0	0.0	0.0	0.0	0.0	6.0	0.0	66.7	0.0	0.0	0.0
Governador Dix-Sept Rosado	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lucrécia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mossoró	12.0	12.0	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
São Miguel	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Serra Negra do Norte	18.7	18.7	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Severiano Melo	5.9	5.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	**5.6**	**4.4**	**0.0**	**50.0**	**0.0**	**0.0**	**0.0**	**1.2**	**0.0**	**66.7**	**0.0**	**0.0**	**0.0**

II: infestation index; **CI:** colonization index; **NII:** natural infection index.

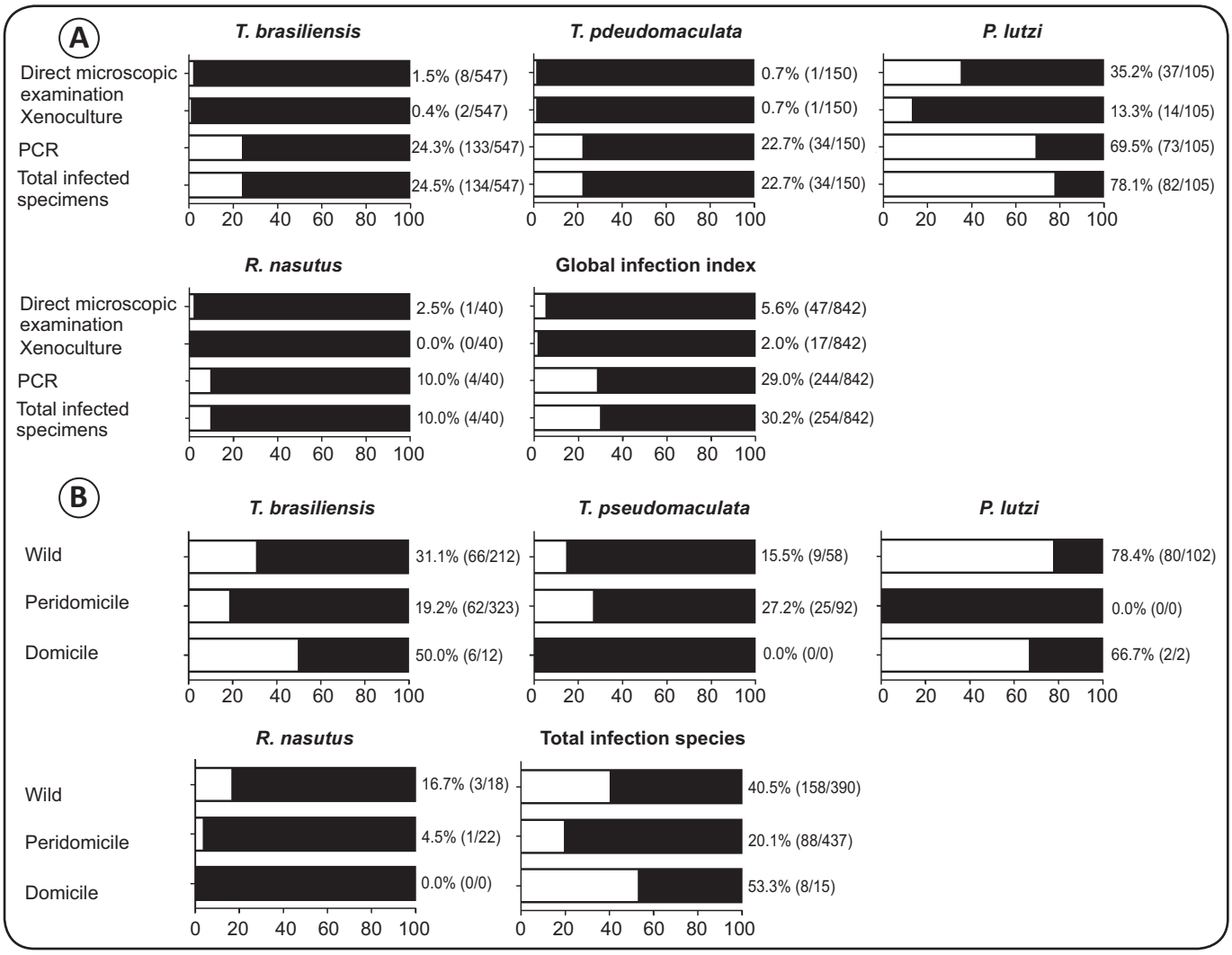

FIGURE 2 - Rate of natural infection of triatomine species by *Trypanosoma cruzi* as determined using different methods and in three distinct environments. A) Infection rate of triatomine species by detection method and global infection index. B) Infection rate of triatomines in different capture environments and total number of infected specimens. *T. brasiliensis:* *Triatoma brasiliensis*; ***T. pseudomaculata:*** *Triatoma pseudomaculata;* ***P. lutzi:*** *Panstrongylus lutzi;* ***R. nasutus:*** *Rhodnius nasutus;* **PCR:** polymerase chain reaction.

TABLE 3 - Geographical origin, development stage, environment, and genetic typing of *Trypanosoma cruzi* stocks from triatomine species.

Trypanosoma cruzi stocks	Triatomine species	Stage	Environment	DTU	Municipality
RN01	*Triatoma brasiliensis*	nymph	peridomiciliary	TcII	Caicó
RN02	*Triatoma brasiliensis*	adult	wild	TcIII	*Serra Negra do Norte*
RN03	*Triatoma brasiliensis*	adult	wild	TcII	*Serra Negra do Norte*
RN04	*Triatoma brasiliensis*	nymph	wild	TcIII	*Serra Negra do Norte*
RN05	*Triatoma brasiliensis*	nymph	wild	TcIII	*Serra Negra do Norte*
RN06	*Triatoma brasiliensis*	nymph	wild	TcII	*Serra Negra do Norte*
RN07	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN08	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN09	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN10	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN11	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN12	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN18	*Panstrongylus lutzi*	adult	intradomiciliary	TcIII	Caraúbas
RN19	*Panstrongylus lutzi*	adult	intradomiciliary	TcIII	Caraúbas
RN213	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
RN812	*Panstrongylus lutzi*	adult	wild	TcIII	*Serra Negra do Norte*
Col1.7G2*[47]	human	-	-	TcI	-
JG*[44]	human	-	-	TcII	-
RN19*[15]	human	-	-	TcIII	-
AM64*[45]	human	-	-	TcIV	-
3253*	human	-	-	TcV	-
CL Brener*[46]	*Triatoma infestans*	-	-	TcVI	-

DTU: discrete typing units: TcI, TcII, TcIII, TcIV, TcV and TcVI**.** **T. cruzi* strains and clones used as reference; **3253:** Lages-Silva et al. (unpublished data).

DISCUSSION

This general assessment of triatomine occurrence demonstrated that the species *T. brasiliensis*, *T. pseudomaculata*, *P. lutzi*, and *R. nasutus* continue to exist in artificial environments in RN State municipalities, where they have been registered since the 1950s[7] [8] [14] [15] [51] [52] and where high seroprevalence of human *T. cruzi* infection has been estimated[28]. The presence of other triatomine species has also been reported[5] [7] [8] [52], but these were not found in this study.

Triatoma brasiliensis and *Triatoma pseudomaculata* were found in most municipalities as expected, being the main species caught in the semiarid RN region. *R. nasutus* was captured only in Apodi, Caraúbas, and Caicó municipalities and *P. lutzi* was found only in *Serra Negra do Norte* and in Caraúbas, as described[51]. In agreement with our studies, *T. brasiliensis* was the most frequently identified species, followed by *T. pseudomaculata* and *R. nasutus*, as observed in several studies[9] [53].

We identified *T. brasiliensis* in the wild environment, peridomicile, and intradomicile with high *T. cruzi* infection rates. This species has been reported as semi-domestic, since it is an autochthonous species capable of colonizing domiciles and the peridomicile[12]. It is highly dispersed and frequent owing to its eurytopic characteristics, is not hygrophilous, is able to withstand very high temperatures, and thus is acclimatized to the vast expanse of the Northeastern region[13].

Despite the absence of intradomicile colonies, frequent adult *T. brasiliensis* invasions occur because of the proximity of the wild environment with DUs. Residential lighting attracts these insects[54], potentially explaining their localized capture. Peridomicile colonization was observed by *T. brasiliensis* and *T. pseudomaculata* nymphs, most frequently in chicken coops where they develop dense colonies, facilitating intradomiciliary invasion. *T. brasiliensis* was also found in piles of tiles, corrals, and associated with domestic and synanthropic animals, according to municipality. This species strongly associates with native rodents, especially *Galea* spp., which thrive

around rural dwellings in this state[55]. The epidemiological profile similarities among the municipalities studied suggest the need for interventions to prevent parasite transmission in this environment to domestic animals and humans.

Degradation of the natural wild vector habitat allows the bugs to move close to human habitations; such coexistence increases the probability of human infection[56]. In the wild, *T. brasiliensis* was mainly found colonizing rocky outcrops and stone fences. Its occurrence in Northeastern Brazil is associated with rock formation distribution[6]. However, this species can also be found in shrubby cacti co-occupied by native rodents in Ceará[57].

Triatoma pseudomaculata is widely distributed in the wild and is considered to be difficult to control[5]. *T. pseudomaculata* is primarily associated with Caatinga and areas of the Cerrado in Brazil[58], and is an arboricolous species[59]. Here, the natural habitat of *T. pseudomaculata* was identified as bird's nests, corroborating previous findings[32].

In the study area, the natural *R. nasutus* habitat was the Carnauba palm, considered the major ecotope of this species[9][60]. Colonization foci were observed in the peridomicile in dry carnauba straw used by the rural population to manufacture household items in Apodi. This can be explained by the proximity of palm trees with DUs that attract insects as previously described or by passive insect transport by residents living in carnauba extraction areas, where storing dry leaves in the peridomicile is common. In the State of Ceará, *R. nasutus* has been frequently found colonizing the peridomicile owing to the use of carnauba straw for chicken coop roof construction and household goods manufacture[9][61].

Panstrongylus lutzi was the most infected species in the intradomicile and wild environment. The former is worrying because of the *T. cruzi* transmission risk to domestic animals and humans and potential introduction of a new parasite genetic group into the transmission cycles, since this area is currently exclusively infected with DTU III[15]. *P. lutzi* has restricted distribution in areas of the semiarid northeast although it has wide geographical distribution and a high *T. cruzi* infection rate in the State of Pernambuco. Its domiciliation has occurred in the States of Ceará and Pernambuco[62][63]. *P. lutzi* is found in hollow *Auxemma oncocalyx* trunks in Ceará[64] and in armadillo burrows in the semiarid Caatinga in Bahia[59]. However, the *P. lutzi* natural habitat was not identified despite extensive searching in the wild environment of the Seridó Ecological Station where most specimens were captured; thus, further studies are required[15][51].

Currently, vector transmission is considered residual by a few native and peridomestic species such as *T. brasiliensis* and *T. pseudomaculata*. There is also a risk of progressive domiciliation of certain species previously considered sylvatic such as *P. lutzi* and the possibility of human infection directly related to the parasite enzootic cycle[62]. *P. lutzi* holds relevance toward maintaining the peridomestic and domestic *T. cruzi* transmission cycles, the risk of invasion and eventual colonization therein, and the consequent parasite transmission to domestic animals and humans. These results highlight the increasing epidemiological importance of *P. lutzi* and indicate the continuing necessity of maintaining epidemiological surveillance against *T. cruzi* transmission in the study area.

Here, we reported a slight agreement among *T. cruzi* detection methods used to evaluate infection in triatomines, and the importance of its association to field studies. *T. cruzi* infection varied among triatomine species according to method with PCR showing high positivity whereas detection by DME in intestinal contents was low. However, higher infection rates were reported with flagellates morphologically similar to *T. cruzi* in Pernambuco[63], and similar or higher triatomine infection rates have been reported between methods[19][23][24][25]. High PCR positivity in relation to DME has been previously observed, indicating its utility for epidemiological studies[19][23][24][65]. PCR can also directly detect *T. cruzi* vector infection thus improving triatomine evaluation and should be used to assess infection rates in dead insects[25] owing to its higher relative degree of precision. Thus, PCR represents the best tool for parasite detection, confirming the majority of infections ascertained by DME and xenoculture. However, these remain useful as the combination of methods can contribute to monitoring *T. cruzi* in triatomines and enhance confidence regarding triatomine positivity.

The *Trypanosoma cruzi* DTUs identified in 15 isolates from triatomine bugs were TcII and TcIII in *T. brasiliensis* and TcIII in *P. lutzi*. Previous findings showed these DTUs circulating in these species in the semiarid zone of RN[15][17] and highlight the need to understand *T. cruzi* population distribution in this area. Naturally heterogeneous *T. cruzi* populations involving TcI, TcII, and TcIII circulate among humans and triatomines in three different municipalities without domicile colonization[17], and high genetic similarities exist among *T. cruzi* populations circulating in different hosts, localities, and environments[16]. TcII was detected in the peridomicile in Caicó and in the wild in *Serra Negra do Norte*, indicating its participation in sylvatic and peridomestic cycles. The original primary host of TcII appears to be primates. Whereas its ecological niche has yet to be determined, isolates have been described from opossums in the Atlantic forest and from sylvatic primates, suggesting that such primates might be the primary original mammalian host[66]. TcII has also been isolated from the armadillo *Euphractus sexcintus* in the Paraguayan Chaco[67]. This DTU has been shown as the primary cause of severe acute and chronic Chagas disease in the Atlantic Forest and central region of Brazil and represents the etiological disease agent in São Felipe in the State of Bahia, where the domestic vector is *P. megistus*[67].

TcIII was isolated from *T. brasiliensis* in the wild environment and from *P. lutzi* captured in the domiciliary and wild environments of two different localities. TcIII is a poorly understood *T. cruzi* genetic group predominantly identified among wild cycles of parasite transmission infecting terrestrial mammals and triatomine vectors, but is also a potentially important emergent human disease agent[68]. A few triatomine species have been described in sylvatic TcIII transmission such as *Panstrongylus geniculatus* Latreille, 1811, and *Triatoma rubrovaria* Blanchard, 1843, both mainly sylvatic vectors frequently associated with terrestrial ecotopes[69]. TcIII is found

in a broad range of terrestrial mammals and its transmission may occur inside triatomine-infested burrows by both vectorial and oral routes[29] [70]. Overlapping geographic areas of TcIII and TcI isolates occur across South America, with shared wild mammals and vectors in terrestrial ecotopes[29]. TcIII in domestic transmission cycles, while intermittent[68], implies a role as a human disease agent. Furthermore, TcIII may be under-reported in both domestic and sylvatic transmission cycles because some typing methodologies fail to distinguish between TcIV and TcIII[71]. The TcII and TcIII identified in this study corroborate previous findings and reinforce the need for constant epidemiological surveillance of *T. brasiliensis* and *P. lutzi* to prevent TcIII spread to the domestic cycle[15].

TcI was not detected in triatomines in the studied area. However, most studies conducted in northeastern Brazil have isolated this DTU from *T. brasiliensis* and *T. pseudomaculata*, with most naturally infected *T. brasiliensis* isolates being TcI[72]. TcI has also been identified in *T. pseudomaculata* and *R. nasutus* in the peridomicile and natural environments of five State of Ceará peri-urban and urban localities[73]. Therein, high TcI and TcII infection rates were detected in peridomestic *T. brasiliensis* and *T. pseudomaculata*, respectively[74]. TcI has been most frequently identified in triatomines of the State of Mato Grosso do Sul[75]. The majority of isolates from humans, reservoirs, and vectors from Amazonia correspond to TcI[29] and it has been detected in humans from State of RN[15] [16], with recent isolates from patients with various clinical forms of Chagas disease[17]. *T. cruzi* genotypes isolated from triatomines can vary according to vector-specific physiology and ecological habitat[43], highlighting the necessary to accurately identify TcI in triatomines.

Our results suggest that combining methodologies contributed to increased parasite detection and to identifying infection foci to precisely determine triatomine distribution. Despite low parasite detection via xenoculture and DME, these methods allow the isolation of *T. cruzi* for further study. Owing to high peridomestic ecotope infestation and high *T. brasiliensis* and *T. pseudomaculata T. cruzi* infection, we recommend efficient entomological surveillance programs to detect possible colonization. This study contributes to our knowledge of *T. cruzi* diagnosis and identification in field-collected triatomines, further demonstrating that *T. cruzi* II and III predominate in the study area and, with future research, are essential for vector control and human infection prevention.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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