

## Major Article

# Synanthropic triatomines (Hemiptera: Reduviidae): infestation, colonization, and natural infection by trypanosomatids in the State of Rio Grande do Norte, Brazil

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### Abstract

**Introduction:** The ecoepidemiological situation in the State of Rio Grande do Norte, Brazil is characterized by frequent invasion and colonization of domiciliary units (DUs) by several triatomine species, with high rates of natural infection by *Trypanosoma cruzi*. **Methods:** We evaluated the possibility of vector transmission of *T. cruzi* based on records of the occurrence of domiciled triatomines collected by the Secretariat of State for Public Health from 2005 to 2015. During this period, 67.7% (113/167) of municipalities conducted at least one active search and 110 recorded the presence of insects in DUs. These activities were more frequent in municipalities considered to have a high and medium-level risk of *T. cruzi* transmission. **Results:** Of 51,569 captured triatomines, the most common species were *Triatoma brasiliensis* (47.2%) and *T. pseudomaculata* (40.2%). Colonies of *T. brasiliensis*, *T. pseudomaculata*, *T. petrocchiae*, *Panstrongylus lutzi*, and *Rhodnius nasutus* were also recorded in the intradomicile and peridomicile. Natural infection by trypanosomatids was detected in 1,153 specimens; the highest rate was found in *R. nasutus* (3.5%), followed by *T. brasiliensis* (2.5%) and *T. pseudomaculata* (2.4%). There have been high levels of colonization over the years; however, not all infested DUs have been sprayed. **Conclusions:** This is the first report of intradomicile and peridomicile colonization by *P. lutzi*. These results demonstrate the risk of new cases of infection by *T. cruzi* and reinforce the need for continuous entomological surveillance in the State of Rio Grande do Norte.

**Keywords:** Triatominae. Trypanosomatids. Natural infection. Entomological surveillance.

### INTRODUCTION

More than a century after the discovery of human infection by *Trypanosoma cruzi* (Chagas, 1909), Chagas disease remains a serious public health problem. It is endemic to 21 countries of Latin America, and the number of infected individuals is estimated to be around 6–7 million<sup>1</sup>, among whom 12,000 die annually<sup>2</sup>; in addition, more than 70 million people are at risk of infection<sup>1</sup>.

Among the transmission mechanisms of *T. cruzi*, classic vector transmission remains the most important parasite transmission route to humans because of the natural distribution of *T. cruzi* in triatomine species adapted to domestic or peridomestic environments. Of the 155 species of triatomine described (Hemiptera: Reduviidae), including two fossil species, more than 60 are autochthonous in Brazil<sup>3-5</sup>. However, vector competence which includes the ability to adapt and colonize the domiciliary environment and the consequent infection of humans and domestic animals is limited to only some species<sup>6</sup>. The Chagas Disease Control Program was set up in the 1970s to prevent human-vector contact through the use of residual-action insecticides in infested DUs, resulting in the interruption of domestic transmission by *Triatoma infestans* Klug, 1834, an

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allochthonous species and the most important vector in Brazil from an epidemiological standpoint<sup>7</sup>. However, it is noteworthy that there are no records of the presence of this vector in the State of Rio Grande do Norte<sup>8</sup>.

The autochthonous species *Triatoma brasiliensis* Neiva, 1911, *Triatoma pseudomaculata* Corrêa & Espínola, 1964, *Triatoma petrocchiae* Pinto & Barreto, 1925, *Triatoma melanocephala* Neiva & Pinto, 1923, *Panstrongylus diasi* Pinto & Lent, 1946, *Panstrongylus lutzi* Neiva & Pinto, 1923, *Panstrongylus megistus* Burmeister, 1835, *Psammolestes tertius* Lent & Jurberg, 1965, and *Rhodnius nasutus* Stål, 1859 have been found in artificial and wild environments<sup>9-12</sup>.

The first seroepidemiological survey conducted in Rio Grande do Norte indicated a prevalence of 15.5% of individuals infected<sup>13</sup>. A recent seroepidemiological survey performed among dwellers in municipalities of the West and Central mesoregions showed an estimated seroprevalence of 6.5% and 3.3%, respectively<sup>14</sup>. Despite the decline in prevalence rates and incidence of infection, the possibility of vector transmission cannot be ruled out. This hypothesis is supported by the diagnosis of *T. cruzi* infection in a child living in a rural area of Várzea municipality (Agreste Mesoregion) whose mother had negative serologic test results<sup>15</sup>. In addition, the high potential for recolonization of the artificial environment by *T. brasiliensis* after chemical treatment results in the population remaining continuously exposed to risk of infection<sup>16,17</sup>. In this context, knowledge of the diversity, geographic distribution, and natural

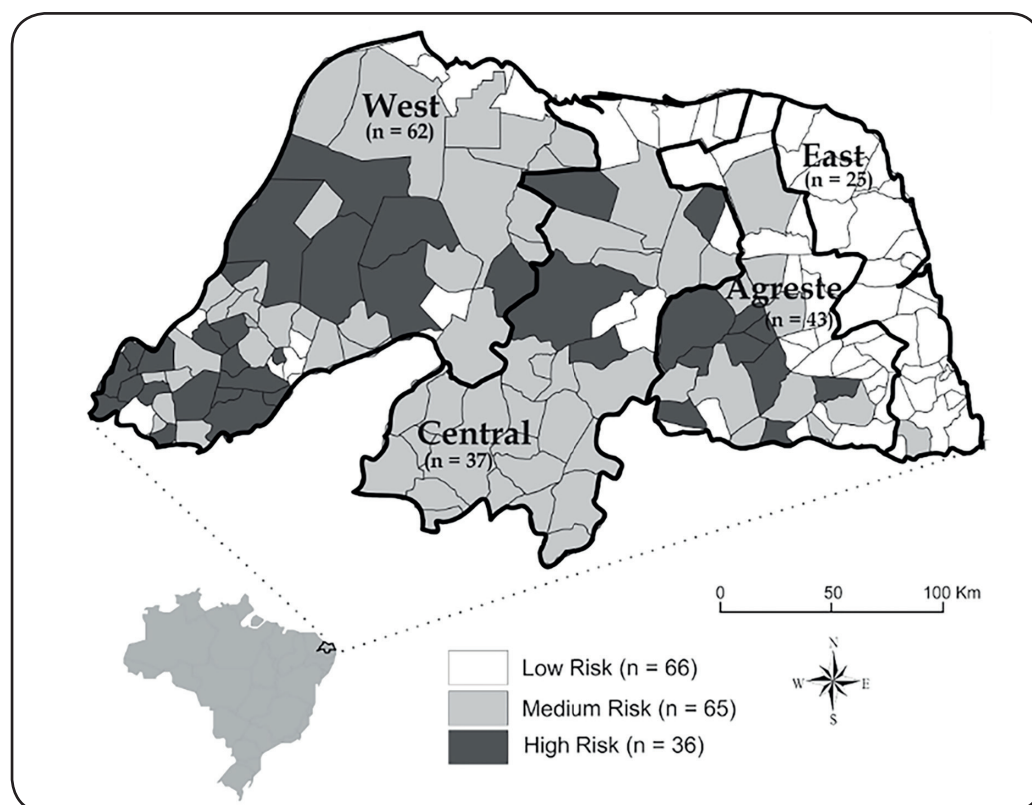
infection rate of autochthonous triatomine species is essential to support control and surveillance activities<sup>18,19</sup>. However, this information remains scarce for the State of Rio Grande do Norte. Therefore, the aim of this study was to evaluate the occurrence and territorial distribution of domiciled triatomines, natural infection by trypanosomatids, and entomological surveillance activities using the entomological data set collected by the Secretariat of State for Public Health from 2005 to 2015.

## METHODS

### Study area

The State of Rio Grande do Norte, located in the Northeastern Region of Brazil, has an area of 52,811.1 km<sup>2</sup>, which corresponds to 0.62% of the Brazilian territory, and an estimated population of 3.5 million. The state is divided into 167 municipalities distributed throughout four mesoregions: West, Central, Agreste, and East<sup>20</sup>. According to data from the Ministry of Health provided by the Secretariat of State for Public Health, 36 (21.6%) of the 167 municipalities are classified as having a high risk of *T. cruzi* transmission, 65 (38.9%) have a medium risk, and 66 (39.5%) have a low risk of transmission (**Figure 1**).

Risk stratification associated with vector transmission has been proposed, to guide and sustain control actions in the states and municipalities of Brazil. Entomological, environmental and demographic indicators have been used to classify the degree of risk in each area<sup>21,22</sup>.



**FIGURE 1:** Stratification of risk areas for *T. cruzi* transmission in different mesoregions of the State of Rio Grande do Norte, Brazil, according to entomological data of the Secretariat of State for Public Health from 2005 to 2015.

## Entomological data

The database for this study, containing annual records of triatomines captured in domiciliary units (DUs) from 2005 to 2015, was provided by the Secretariat of State for Public Health, which is responsible for managing the information collected by those municipalities that have carried out epidemiological surveillance activities. Information on the occurrence of triatomine species and the developmental stage, capture site (intradomicile or peridomicile), and trypanosomatid infection rate were used in this study. Manual captures were exhaustively carried out in DUs by endemic disease control agents, without the use of insect dislodging substances. Capture time was approximately 1 hour/two agents/DU. The identification of triatomines was based on the identification key proposed by Lent and Wygodzinsky<sup>23</sup>. Parasitological examination of triatomines was conducted by abdominal compression and examination of fecal material with optical microscopy by technicians of Regional Units of Public Health of the State.

According to the Secretariat of State for Public Health, active searches in high risk and medium risk municipalities are conducted in all positive localities, in which the presence of triatomines has been registered during the previous year, as well as neighboring localities up to 1 km away. Other locations are randomly selected and included, to assess infestation. In these cases, it is recommended that all positive houses be sprayed with insecticide. In low risk municipalities, entomological surveillance is performed passively, followed by detailed entomological surveillance if triatomines are found in the DUs.

Data regarding the number of surveyed DUs, positive DUs, and sprayed DUs were also analyzed. This information was used to calculate several annual indicators recommended by the World Health Organization<sup>24</sup> and Pan American Health Organization<sup>25</sup>, e.g., (i) infestation index (number of DUs with a presence of triatomines  $\times$  100 / number of DUs surveyed); (ii) peridomestic infestation index (number of DUs with triatomines in the peridomicile  $\times$  100 / number of peridomiciliary units examined); (iii) intradomiciliary infestation rate (number of DUs with triatomines in the intradomicile  $\times$  100 / number of intradomiciliary units examined); (iv) colonization index (number of DUs with nymphs  $\times$  100 / number of DUs with triatomines); and (v) natural infection index by trypanosomatids (number of infected triatomines  $\times$  100 / number of insects examined). According to their ecological definitions, the intradomicile corresponds to the interior of the dwelling where inhabitants sleep and the peridomicile refers to the area surrounding the dwelling that is affected by human activity<sup>26</sup>.

## Statistical analysis

Categorical variables were expressed as absolute and relative frequencies and were estimated using Stata version 15.1 (StataCorp LLC, College Station, TX, USA). We evaluated triatomine infestation in the DUs according to risk stratification by municipality, using the Pearson chi-squared test. The significance level was set at  $p < 0.05$ . The coefficient of determination ( $R^2$ ) was calculated using linear regression analysis to evaluate variation in the entomological indices (infestation, peridomiciliary infestation, intradomiciliary infestation, and intradomiciliary colonization) over the study period (years). Values of  $R^2 > 0.6$  were considered to indicate significant correlation.

## RESULTS

Active searches for triatomines in DUs were performed at least once in 113 (67.7%) of the 167 municipalities in Rio Grande do Norte, among which 110 (65.9%) had records of infestation. During a 7- to 10-year period, active searches were performed in 27/36 (75%) and 42/65 (64.6%) of municipalities with high and medium risk for transmission of *T. cruzi*, respectively. However, active searches were conducted in only two (3.0%) low risk municipalities, which was much lower than that observed in municipalities with medium and high transmission risk ( $p < 0.001$ ) (Table 1).

A total of 51,569 triatomines were captured, belonging to the species *T. brasiliensis*, *T. pseudomaculata*, *T. petrocchia*, *P. lutzi*, *P. megistus*, and *R. nasutus*. Of these, 40,386 (78.3%) insects were captured in the peridomicile and 11,183 (21.7%) in the intradomicile. *Triatoma brasiliensis* (47.2%) and *T. pseudomaculata* (40.2%) were the most frequent species, followed by *R. nasutus* (7.2%). In the intradomicile, *T. brasiliensis* corresponded to 63.31% of the specimens captured, which together with *T. pseudomaculata* represented 85.4% of captures in the peridomicile. Nymphs and adults of *T. brasiliensis*, *T. pseudomaculata*, *T. petrocchia*, *P. lutzi*, and *R. nasutus* were captured in the intradomicile and peridomicile; this is indicative of the colonization of these environments by these species. Only adult *P. megistus* specimens were captured in DUs. The natural infection rate by flagellates that are morphologically similar to *T. cruzi* was 2.5%. *Rhodnius nasutus* had the highest infection index (3.5%), followed by *T. brasiliensis* (2.5%), *T. pseudomaculata* (2.4%), *P. lutzi* (1.4%), and *T. petrocchia* (1.3%) (Table 2).

**TABLE 1:** Evaluation of triatomine infestation (active search) in domiciliary units of different municipalities according to risk stratification during period 2005–2015.

Number of years evaluated in period	Municipalities, n (%)			p
	High risk	Medium risk	Low risk	
7 to 11	27 (75.0)	42 (64.6)	2 (3.0)	< 0.001
0 to 6	9 (25.0)	23 (35.4)	64 (97.0)	
<b>Total</b>	<b>36 (100.0)</b>	<b>65 (100.0)</b>	<b>66 (100.0)</b>	

n (%), absolute and relative frequency.

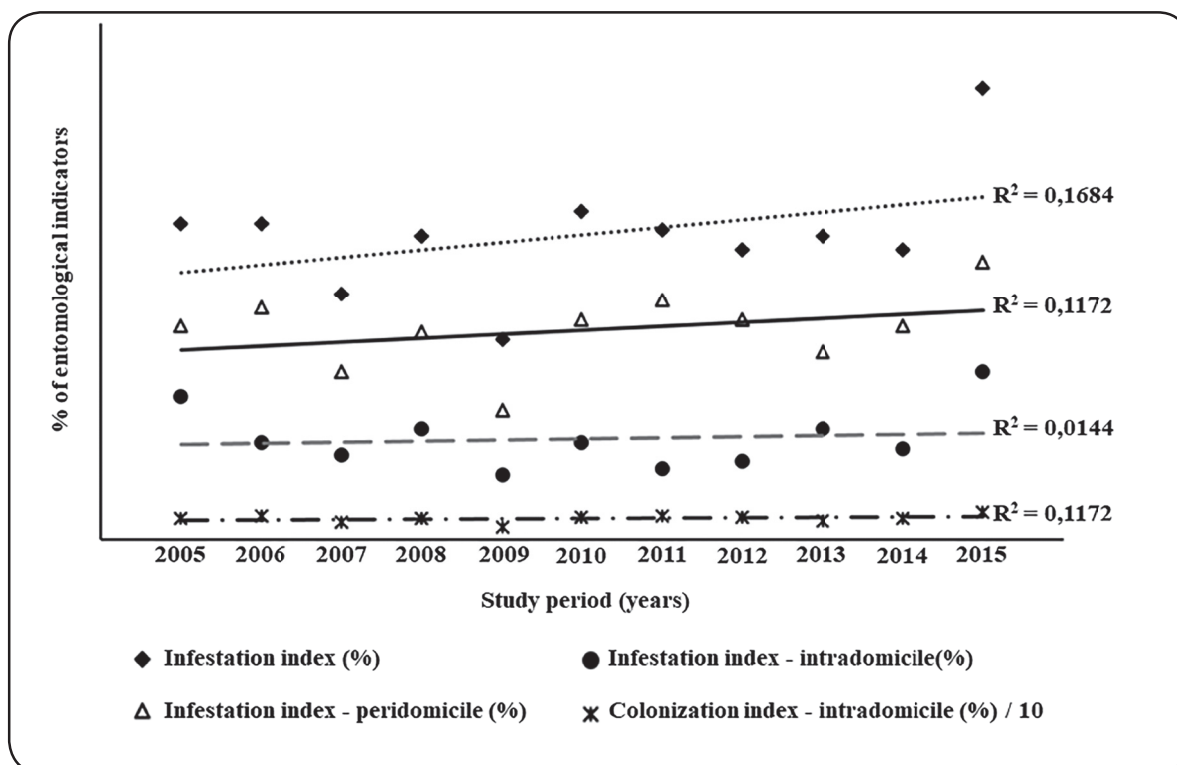
**TABLE 2:** Distribution of triatomines by species, developmental stage, and infection index by trypanosomatids, in the intradomicile and peridomicile during 2005–2015.

Species	Intradomicile				Peridomicile				Total collected		Infection index (%)
	A	N	T	%	A	N	T	%	n	%	
<i>Triatoma brasiliensis</i>	4,219	2,861	7,080	63.31	11,215	6,048	17,263	42.75	24,343	47.20	2.5 (559/22,507)
<i>Triatoma pseudomaculata</i>	2,455	1,061	3,516	31.44	10,832	6,394	17,226	42.65	20,742	40.22	2.4 (462/(19,277))
<i>Triatoma petrocchiai</i>	50	2	52	0.46	125	65	190	0.47	242	0.47	1.3 (2/150)
<i>Panstrongylus lutzi</i>	174	40	214	1.91	1,831	486	2,317	5.74	2,531	4.91	1.4 (28/1,937)
<i>Panstrongylus megistus</i>	10	0	10	0.09	7	0	7	0.02	17	0.03	0 (0/17)
<i>Rhodnius nasutus</i>	106	205	311	2.78	2,375	1,008	3,383	8.38	3,694	7.17	3.5 (102/2,915)
<b>Total</b>	<b>7,014</b>	<b>4,169</b>	<b>11,183</b>	<b>21.7</b>	<b>26,385</b>	<b>14,001</b>	<b>40,386</b>	<b>78.3</b>	<b>51,569</b>	<b>100</b>	<b>2.5 (1,153/46,803)</b>

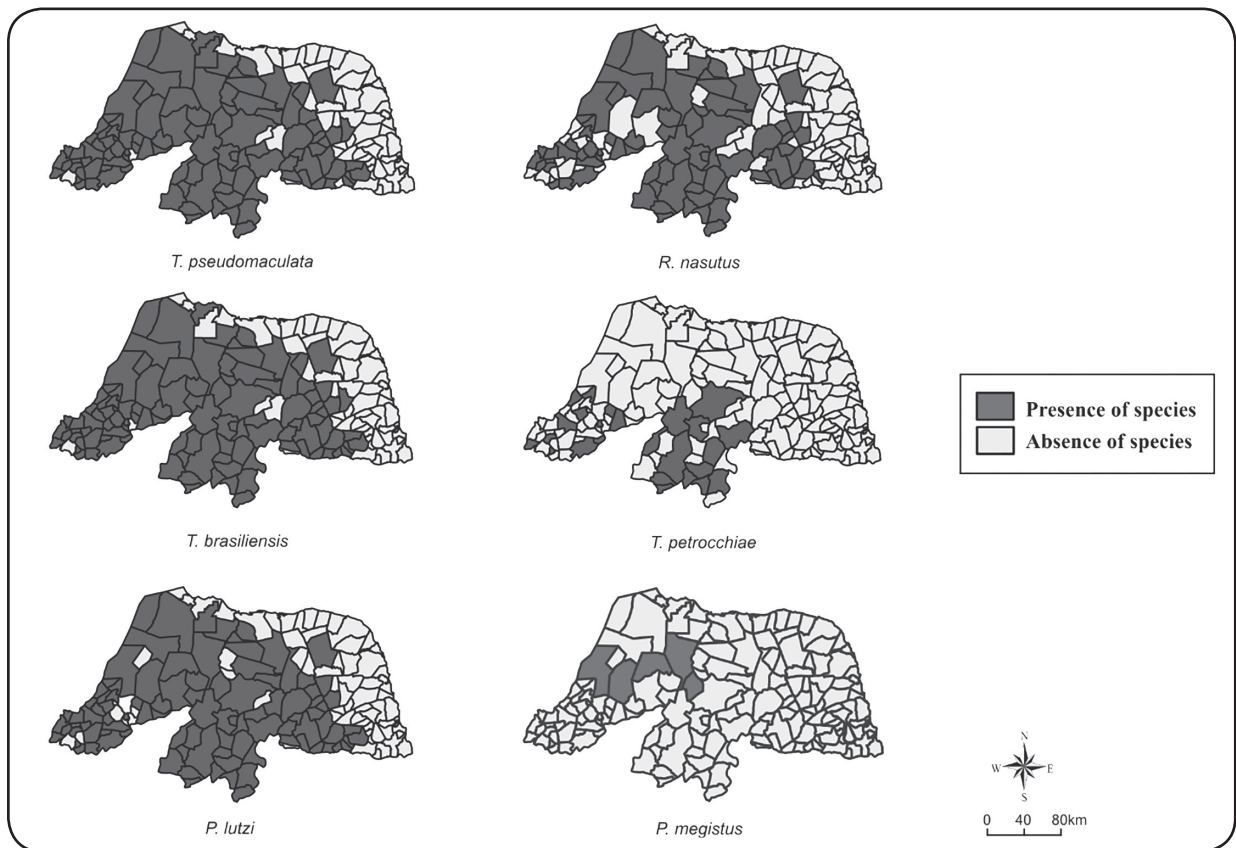
**A:** adult; **N:** nymph; **T:** total; **%:** percentage of total infected triatomines adult and nymphs at the intradomicile, peridomicile and total collected; Infection index (**%**), number of infected specimens / number of specimens examined.

The number of municipalities conducting entomological control activities has varied over the years; the highest number was in 2007 ( $n = 95$ ). In 2015, there was a reduction in the number of municipalities that had performed vector control activities (data not shown). **Figure 2** shows that annual averages of the entomological indicators did not present significant variations in the studied period ( $R^2 < 0.6$ ).

*Triatoma brasiliensis*, *T. pseudomaculata*, *P. lutzi*, and *R. nasutus* were the most widely dispersed species in the state, recorded in 106, 107, 96, and 79 municipalities, respectively. *Triatoma petrocchiai* was restricted to 25 municipalities located in the West and Central mesoregions. *P. megistus* had the lowest distribution, restricted to five municipalities of the West Mesoregion (Apodi, Assú, Caraúbas, São Rafael, and Upanema) (**Figure 3**).



**FIGURE 2:** Evaluation of entomological indicators from 2005 to 2015 and coefficients of determination ( $R^2$ ).  $R^2 > 0.6$  was considered to indicate significant correlation.



**FIGURE 3:** Distribution of the six species of triatomines captured in domiciliary units of different municipalities in the State of Rio Grande do Norte, Brazil from 2005 to 2015.

## DISCUSSION

This study focused on the entomological surveillance in of the State of Rio Grande do Norte, Brazil, with an emphasis on certain aspects of the ecoepidemiology of Chagas disease in different municipalities of the state. We evaluated the presence of domiciled vectors and the index of natural infection by trypanosomatids, using the entomological data set collected by the Secretariat of State for Public Health from 2005 to 2015. Triatomines were recorded in most municipalities that performed entomological surveillance through active searches during the evaluation period. In Rio Grande do Norte and several other Brazilian states, control activities are recommended and supported according to risk stratification of domiciliary transmission of *T. cruzi*. Thus, municipalities are classified as high, medium, and low risk, based on an ecoepidemiological scenario characterized by endemic areas with autochthonous domiciled species<sup>19,22</sup>.

Entomological surveillance was more frequent in municipalities with high and medium risk of transmission whereas in most low risk municipalities, there were fewer control activities, resulting in fewer records of the Secretariat of State for Public Health. This suggests that transmission does not occur in these municipalities. However, this discontinuity of entomological surveillance, together with the presence of native species such as *T. brasiliensis*, *T. pseudomaculata*, and *P. lutzi*, which have a high index of natural infection by *T. cruzi* in DUs

as reported by Barbosa-Silva et al.<sup>10</sup> and Vargas et al.<sup>27</sup>, suggest risk of transmission of the parasite to the rural population in Rio Grande do Norte; this risk is evidenced in a recent outbreak of oral transmission in the municipality of Marcelino Vieira<sup>27</sup>.

In this scenario, *T. brasiliensis* and *T. pseudomaculata* were the most frequently captured in DUs. *Triatoma brasiliensis* is the most frequently captured species in the intradomicile<sup>9,10,22,28</sup>. Studies in the states of Rio Grande do Norte<sup>10</sup>, Ceará<sup>29-31</sup> and Pernambuco<sup>32,33</sup> have demonstrated the predominance of these species in the peridomicile, thus corroborating our data. The peridomicile is the site of interchange between wild and domestic populations of *T. brasiliensis*, which poses a great challenge to control interventions<sup>34</sup>. In this sense, the proximity of DUs in semi-arid regions to the wild environment favors infestation and reinfestation processes by triatomines<sup>16</sup>.

The wide distribution of *P. lutzi* and *R. nasutus* draws attention to their recorded frequency among DUs in recent years, including their tendency to form intradomiciliary and peridomiciliary colonies, as shown in our study as well as that by Silveira and Martins<sup>35</sup>. The dispersion of *R. nasutus* in areas near carnaúba palm trees (*Copernicia prunifera*) and DUs should be considered when it comes to the domiciliation process, as these insects are attracted by light or food and can invade the intradomicile<sup>29</sup>.

The registration and distribution of *T. petrocchiai* were limited to municipalities of the West and Central mesoregions,

with a small number of specimens that were mainly captured in the intradomicile<sup>9</sup>. This species is sylvatic and its current distribution includes the states of Bahia, Ceará, Paraíba, and Pernambuco<sup>28,36,37</sup>.

In recent years, the species *P. megistus* has been captured less frequently in DUs of Rio Grande do Norte state, and its occurrence has been restricted to five municipalities in the West Mesoregion. Among these, Apodi and Caraúbas are included in the microregion of the Chapada do Apodi area, where there are remnants of the Atlantic Forest. This species is widely distributed in the Atlantic Forest biome<sup>38</sup>, in the humid Cerrado and Caatinga. *Panstrongylus megistus* is important from an epidemiological standpoint because of its potential to invade and colonize domiciles and its high rates of *T. cruzi* infection<sup>39</sup>.

The natural infection of triatomines by trypanosomatids showed a low index among the different species. Detection of *T. cruzi* infection in triatomines is traditionally done via abdominal compression and examination of fecal material with optical microscopy<sup>40</sup>. This technique is inexpensive but has some limitations such as low sensitivity and low reproducibility. Moreover, it is difficult to examine the developmental stage of triatomines using this method as the insects are not alive<sup>41</sup>, and staining quality may affect the specificity of the assay, thus hindering the morphological distinction between *T. cruzi* and other trypanosomatids, such as *Trypanosoma rangeli*<sup>42</sup>. Recent data have shown that a combination of methods (e.g., direct examination, xenoculture, and polymerase chain reaction) is best for detection of *T. cruzi*<sup>10,43</sup>.

Nymphs of *T. brasiliensis*, *T. pseudomaculata*, *P. lutzi*, *R. nasutus*, and *T. petrocchiae* in DUs indicate colonization of these environments. In this study, we recorded the first occurrence of colonization by the species *P. lutzi* in the intradomicile and peridomicile of Rio Grande do Norte state. It is possible that this species is undergoing a domiciliation process in the state. The proximity of *P. lutzi* to artificial ecotopes is of concern as it is a wild species, with high reported rates of natural infection by *T. cruzi* in the intradomicile<sup>10</sup>.

Taken together, the data demonstrate the need to reinforce political and administrative policies to strengthen entomological surveillance. In the evaluation of chemical control measures, we found that the number of sprayed DUs was lower than the number of infested DUs. Although this practice is inadequate, it has been recurrent over the years. This factor may have led to new cases of vector transmission by *T. cruzi*, especially when the main vectors (*T. brasiliensis* and *T. pseudomaculata*) are species of recognized epidemiological importance that are easily able to colonize and recolonize the artificial environment. Continuous and permanent control activities must be reinforced in endemic areas, to prevent the reestablishment of new foci of infestation in DUs. These efforts should include educational activities aimed at physical improvement and management of peridomestic ecotopes, especially those that commonly serve as shelters for these species<sup>16</sup>.

The presence of infected triatomines, especially *T. brasiliensis* and *T. pseudomaculata* that can colonize the intradomicile, serve as a warning regarding the risk of parasite

transmission. Our results also point to invasive species that have been acquiring epidemiological importance in the state, such as *P. lutzi* and *R. nasutus*. Therefore, to avoid contact with these vectors by animals and humans, entomological surveillance activities must be continuously carried out in high and medium risk municipalities and should be conducted more frequently in municipalities considered to have low risk. Moreover, vector control must be systematic to prevent domiciliary transmission of *T. cruzi*.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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