Differential Pattern of Infection of Sylvatic Nymphs and Domiciliary Adults of *Triatoma infestans* with *Trypanosoma cruzi* Genotypes in Chile

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Abstract. In Chile, the main vector of Chagas disease, *Triatoma infestans*, is under control after insecticide spraying. However, it has been found colonizing wild habitats. This study evaluated *Trypanosoma cruzi* infection of sylvatic and domiciliary *T. infestans* and identified their parasite genotypes. The sample studied was composed mainly of *T. infestans* sylvatic nymphs and domiciliary adults from a semi-urban area with human dwellings under vector control surveillance. Results showed prevalences of 57.7% in nymphs and 68.6% in adults. Hybridization tests showed a major *T. cruzi* lineage (TcI) circulating in sylvatic (93.3%) and domiciliary (100%) *T. infestans*. TcII, TcV, and TcVI were also detected, mainly in nymphs, suggesting differential adaptation of *T. cruzi* lineages among instars. We also discuss the origin of domiciliary individuals of *T. infestans* and the risk of human infection by triatomines of sylvatic foci that invade houses despite vector control programs.

INTRODUCTION

Trypanosoma cruzi is the etiologic agent of Chagas disease, a vector borne illness with approximately 10 million persons infected in the Western Hemisphere.¹ This parasite is transmitted through contact with contaminated feces of hematophagous insects of the subfamily Triatominae. Approximately 70 species of triatomines and more than 73 mammalian genera are naturally infected with *T. cruzi*.^{2,3}

Trypanosoma cruzi has a unique mitochondrion, the kinetoplast, which has DNA (kDNA) in maxicircles, analogous to the mitochondrial genome of other eukaryotes (25–50 identical copies per cell), and in minicircles, which are small molecules of different sequence classes (10,000–20,000 copies/cell) that code for guide RNAs, participating in the mitochondrion RNA editing.⁴ The minicircles are organized into four 120-bp conserved regions called conserved sequence blocks (CSBs) separated by four variable regions of approximately 250-bp each.⁵ Minicircle DNA amplification yields a product from the variable region, a highly polymorphic sequence present in different minicircle classes, and useful for *T. cruzi* typing by means of hybridization tests with a panel of well-characterized variable regions as kDNA probes. This method of high discrimination capacity has already been validated with large numbers of *T. cruzi* clones and stocks conducted in different settings.^{6,7} Moreover, a recent study showed that genotyping a large number of *T. cruzi* stocks and clones by means of hybridization was concordant with other methods, as cytochrome b gene sequencing and polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP).⁸ Similarly, genotyping results obtained with hybridization tests of *T. cruzi* patient samples from Colombia were concordant with the markers minixon, 24α ribosomal RNA, and cytochrome oxidase subunit II genes.⁹

Trypanosoma cruzi has been classified into six genetic subdivisions or lineages: TcI–TcVI.¹⁰ Some studies have suggested that TcII, TcV, and TcVI are associated with domestic envi-

ronments and chronic chagasic patients, whereas TcIII and TcIV are mainly involved in the sylvatic cycles, and TcI participates in both.^{10,11} Previous studies based on PCR minicircle DNA-based detection and genotyping directly from chronic patient blood samples showed that TcI, TcII, and TcV, including mixed infections, were the most prevalent in hyperendemic areas of Chile located 100–400 km north of the present study site. The genotype TcVI was also detected but at low rate.^{12,13} However, in the sylvatic cycle, TcVI frequency is variable according to the mammal¹⁴ and vector species.¹⁵ These results are concordant with those reported for 99 *T. cruzi* stocks from Chile, which were found to be TcI, TcII, TcV, and TcVI, but not TcIII or TcIV.¹⁶ Using PCR–RFLP, Rozas and others reported TcI, TcIIb (currently TcII), TcIIa or TcIIc (currently TcIII and TcIV), and TcIIId or TcIIe (TcV and TcVI) in single and mixed infections of mammals, *Mepraia spinolai* and humans from the Coquimbo Region, the hyperendemic area of Chile.¹⁷ More recently, the presence of TcIII was documented in *Triatoma infestans* from Chile by using three microsatellite markers, with TcI, a hybrid (TcV + TcVI), and unknown lineages.¹⁸

Chagas disease is endemic to Chile. Three sylvatic triatomine species have been described: the kissing bugs *M. spinolai* Porter 1934, *M. gajardoi* Frias, Henry and Gonzalez 1998, and *M. parapatrica*.¹⁹ *Triatoma infestans* Klug 1834 is the only domiciliary vector, which has been subjected to control measures and prevention.²⁰ Recently, *T. infestans* was found colonizing wild areas in rural localities of central Chile inhabiting terrestrial bromeliads of the genus *Puya* spp.,²¹ and rock piles.²² The bromeliad *Puya* spp. provides shelter to numerous small mammals,²³ similar as in other countries, where species from the genera *Rhodnius* and *Triatoma* have also been found in bromeliads, among other ecotopes.²⁴ There are no other documented findings of this vector inhabiting sylvatic habitat in Chile before those reported.²¹

The sylvatic cycle of *T. cruzi* is present in these rural areas that harbor wild *T. infestans*. During the spring–summer season, winged adults enter to houses that are regularly sprayed, representing a risk factor for acquiring Chagas disease to persons that inhabit these dwellings.^{21,22}

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FIGURE 1. Location of the study site in Chile. Projection UTM WGS 84, 19 S. **A**, Location of the hills of Cerro Lonquén in the Metropolitan Region (above). **B**, Location of the Metropolitan Region of Chile (below, left). **C**, Location of Chile in South America (below, right).

In the context of a study that intended to determine the origin of *T. infestans* found within human dwellings, we addressed the question at the level of *T. cruzi* populations circulating in domiciliary findings and sylvatic *T. infestans* from the same area. To do so, we compared the abundances of different *T. cruzi* lineages found in these triatomines.

MATERIALS AND METHODS

Study area. Field studies were carried out in the Metropolitan Region (Figure 1B), 27–30 km south of Santiago, the capital of Chile, in South America (Figure 1C). The study area, Cerro Lonquén, is a mountain range located in Calera de Tango and Talagante Counties and maintains native flora and fauna (Figure 1A). People build their homes over slopes, usually with solid materials, such as cement or bricks. Crops and more dwellings are located on the mountainsides. During summer, persons usually sleep with their windows opened, unaware of the entrance of triatomines. Maps were generated using ArcGis 9.3 software (ESRI, Redlands, CA).

Specimens. The domiciliary *T. infestans* were obtained from the Regional Sanitary Authority, an institution that receives reports of triatomines found inside human dwellings. Authorized personnel performed manual collection of every triatomine and gathered those collected previously by the householders, registering the date, sampling site, and geo-references. The infested houses are included in the National Vector Control Program that involves, among other measures, spraying those dwellings for three years to prevent reinfestation, an action that can be extended if new kissing bugs are found. A total of 118 domiciliary insects were provided for the study, all dead adults collected during 2006–2008 in spring, summer, and autumn. Their specific site of collection is shown in Figure 1A.

Sylvatic *T. infestans* were captured in terrestrial bromeliads by using carbon dioxide-emitting traps baited with dry ice, yeast plus water and sugar, or laboratory mice (*Mus musculus*). Traps were placed overnight in several occasions during summer in 2003–2004 and 2007–2008. Sampling sites are shown in Figure 1A. The closest sylvatic triatomine capture site to a human dwelling under vector control program was located 117 meters away (determined by using ArcGis 9.3 software). Captured triatomines were taken to the laboratory, where they were placed in individual recipients in a climatic chamber with controlled temperature (27°C) and relative humidity (70%). Monthly, each *T. infestans* was individually fed on rabbits (*Oryctolagus cuniculus*) until processing to minimize the risk of interference with the infection status of the triatomines.²⁵ Because in their natural environment, these sylvatic *T. infestans* had rabbits as potential food sources, they were familiarized with these hosts. The protocol of maintenance and care of the experimental animals was approved by the Bioethics Committee of the Faculty of Veterinary Medicine from the University of Chile. All individuals were identified as *T. infestans* and classified by stage according to taxonomic keys.^{3,26}

DNA extraction and *T. cruzi* detection. After the insects were killed, the abdomens of each insect were cut and macerated with 200 μ L of 6 M guanidine-HCl, 200 mM EDTA and incubated overnight with 20 μ L of proteinase K (10 mg/mL); the DNA in resulting material was extracted by using the QIAamp Mini Kit (QIAGEN, Hilden, Germany). The eluted material was used as the DNA template. Amplification was performed by using PCR as described²¹ with oligonucleotides

121 and 122, which anneal to CSB2 and CSB3 of minicircles, respectively. Each experiment included a negative control that contained distilled water and a positive control that contained purified *T. cruzi* kDNA. Amplification products were subjected to electrophoresis on 2% agarose gels stained with ethidium bromide and visualized with ultraviolet light.

***Trypanosoma cruzi* genotyping.** To genotype *T. cruzi*, DNA blot analyses were performed with variable volumes of each PCR product (30–150 ng of DNA). Samples subjected to electrophoresis were transferred onto Hybond N⁺ nylon membranes (Amersham, Little Chalfont, United Kingdom) and cross-linked with ultraviolet light to fix the DNA. The membranes were pre-hybridized for at least two hours at 55°C and hybridized under high stringency conditions as described.¹³ Different *T. cruzi* clones (TcI: sp104 cl1, TcII: CBB cl3, TcV: NR cl3 and TcVI: V195 cl1) were used as DNA template to generate probes that were used to determine by hybridization the parasite(s) genotype(s) infecting each *T. infestans*. The same clones were used as controls (Figure 2). Construction of genotype specific probes was performed as described.⁷ After hybridization with ³²P-labeled DNA probes, each membrane was washed three times for 30 minutes with 2 $\times$ SSC (0.3 M NaCl, 0.03 M sodium citrate), 0.1% sodium dodecyl sulfate at 55°C, and analyzed with the Molecular Imager FX (Bio-Rad Laboratories, Hercules, CA).

FIGURE 2. Representative results of *Trypanosoma cruzi* genotypes by means of hybridization tests of sylvatic and domiciliary *Triatoma infestans* samples and *T. cruzi* controls. **A**, Agarose gel with electrophoresed PCR products. **B**, Hybridization with TcI probe. **C**, Hybridization with TcII probe. **D**, Hybridization with TcV probe. **E**, Hybridization with TcVI probe. Lane 1, 100-basepair (bp) DNA ladder; lanes 2–5, control samples, from left to right: TcI (clone 20 sp.104 cl1), TcII (clone 33 CBB cl3), TcV (clone 39 NR cl3), and TcVI (clone 43 v195 cl1); lane 6, negative control; lanes 7–11, samples from sylvatic *T. infestans*; lanes 12–14, samples from domiciliary *T. infestans*.

Statistical analyses. We compared the overall genotype composition of sylvatic and domiciliary findings of *T. infestans* by using the G test for goodness of fit.²⁷ The presence of single and mixed infections between sylvatic and domiciliary individuals was compared by using the chi-square test. This test was also used to compare the proportion of infection with the most prevalent genotype and secondary genotypes. We calculated the mean and SD of the Shannon-Weiner diversity index by using 1,000 simulations for each group (sylvatic and domiciliary) in EstimateS version 8.02 software (Colwell RK, 2006). Results were compared by using a two-sample Student *t*-test in Stata version 9.1 (StataCorp LP, College Station, TX). The confidence level for all tests was 95%.

RESULTS

A total of 118 domiciliary and 104 sylvatic *T. infestans* were analyzed by PCR. The distribution of developmental instars of the analyzed insects and their infection with *T. cruzi* are shown in Table 1. The overall prevalence of *T. cruzi* was 68.6% in *T. infestans* found inside dwellings and 57.7% in insects from wild ecotopes. The prevalence between domiciliary and sylvatic triatomines was not significantly different ($\chi^2 = 2.86$, degrees of freedom [df] = 1, $P = 0.091$). Infection in younger nymphs (I and II instars) was 46.7%, but was not significantly different from the prevalence of 62.3% detected in III, IV, and V instars ($\chi^2 = 2.39$, df = 1, $P = 0.122$).

The hybridization results for the 141 infected specimens are summarized in Table 2. There were samples positive with one, two, and three probes. Controls hybridized only with their complementary counterpart probes. Representative results of *T. cruzi* genotypes by means of hybridization tests are shown in Figure 2.

The overall genotype composition of *T. cruzi* lineages between domiciliary and sylvatic *T. infestans* was significantly different ($\chi^2 = 18.87$, df = 3, $P < 0.001$). Some samples showed a complex hybridization pattern with more than one probe, suggesting that those cases represent mixed infections with different *T. cruzi* lineages (Table 2). The comparison between sylvatic and domiciliary specimens regarding the presence of single or mixed infections was significantly different ($\chi^2 = 9.06$, df = 1, $P = 0.003$), and there were higher single infections in domiciliary insects. Comparison of single and mixed infections between sylvatic instars I–II and domiciliary insects showed significant differences ($\chi^2 = 5.29$, df = 1, $P = 0.021$). Comparison of sylvatic instars III–IV–V and domiciliary specimens also showed significant differences ($\chi^2 = 6.87$, df = 1, $P = 0.009$).

TABLE 1

Stage distribution of collected insects and positivity to *Trypanosoma cruzi* in sylvatic and domiciliary *Triatoma infestans* determined by polymerase chain reaction, Chile

Collection site	Instar	No. <i>T. infestans</i> collected	No. (%) positive <i>T. infestans</i>
Sylvatic	I	19	7 (36.8)
	II	26	14 (53.9)
	III	20	12 (60.0)
	IV	14	9 (64.3)
	V	19	12 (63.2)
	Adult	6	6 (100.0)
	Subtotal	104	60 (57.7)
Domiciliary	Adult	118	81 (68.6)
Total	All combined	222	141 (63.5)

TABLE 2

Single and mixed hybridization patterns in wild and domiciliary positive *Triatoma infestans* specimens, Chile*

Collection site	Instar	Single	Mixed	Total
Sylvatic	I	5 (71.4)	2 (28.6)	7
	II	9 (64.3)	5 (35.7)	14
	III	10 (83.3)	2 (16.7)	12
	IV	7 (77.8)	2 (22.2)	9
	V	5 (41.7)	7 (58.3)	12
	Adult	4 (66.7)	2 (33.3)	6
	Subtotal	40 (66.7)	20 (33.3)	60
Domiciliary	Adult	71 (87.7)	10 (12.4)	81
Total	All combined	111 (78.7)	30 (21.3)	141

*Values are no. (%) of insects from each instar by collection site.

TcI was the predominant genotype in domiciliary (100%) and sylvatic (93.3%) triatomines and was detected as single and mixed infections with the other *T. cruzi* lineages. Domiciliary adults were almost exclusively infected with TcI, and different secondary lineages were frequent among sylvatic nymphs (Table 3). Within sylvatic specimens, 20 of the 60 positive samples showed mixed infections of two or three lineages, and TcI was always present. The patterns detected in sylvatic specimens were TcI + TcII, TcI + TcV, TcI + TcVI, TcI + TcII + TcV, TcI + TcII + TcVI, and TcI + TcV + TcVI. Only ten of 81 positive insects found inside dwellings showed mixed infections, either with TcI + TcV or TcI + TcVI. Thirty-six of 60 infected sylvatic kissing bugs had only TcI, and there were also four single infections with TcV. Single infections in 71 positive domiciliary *T. infestans* all had the TcI lineage. Comparison between sylvatic and domiciliary insects regarding the proportion of TcI in positive insects was significantly different ($\chi^2 = 5.12$, df = 1, $P = 0.024$), as was the presence of the other genotypes (TcII or TcV or TcVI) tested as a group ($\chi^2 = 9.59$, df = 1, $P = 0.002$). The diversity index (H') was 0.463 in the sylvatic population and 0.183 in the domiciliary population. The mean H' index obtained with EstimateS (1,000 random runs with replacement) with $n = 89$ was 0.78 (SD = 0.32) for the sylvatic sample and with $n = 91$ was 0.74 (SD = 0.22) for the domiciliary sample, which were not statistically different ($t = -0.9752$, df = 178; $P = 0.3308$).

DISCUSSION

Triatoma infestans has adapted to the stable environments provided by human dwellings.²⁸ However, it has been found occasionally in sylvatic habitats.^{29–32} In Bolivia, sylvatic populations are extensively documented, and nymphs and adults have been found in several ecotopes.^{33–39} In Chile, sylvatic foci of *T. infestans* with all nymphal and adult instars were documented inhabiting terrestrial bromeliads.²¹ Reinfestation of human dwellings may follow active dispersal of winged adults from the wild foci to houses, attracted by light during hot weather.^{40,41} There are studies reporting 200 meters,²⁰ 500 meters,^{42,43} 1,500 meters,⁴⁴ and $\leq 2,000$ meters⁴⁵ of dispersal distance, depending on factors as temperature and nutrition. *Triatoma infestans* shows nocturnal activity and sporadically observed flights, which may bias dispersal estimations.⁴¹ More recently, it has been suggested that wild *T. infestans* from highlands of Bolivia gradually disperses over small distances by walking within patches that might be characterized as a continuous land cover,⁴⁶ the same locomotion pattern presented by nymphs from Argentina.⁴⁵

TABLE 3

Triatoma infestans infected with each genotype of *Trypanosoma cruzi* from sylvatic habitat and dwellings determined by DNA blotting, Chile*

Collection site	Instar	TcI	TcII	TcV	TcVI	Total†
Sylvatic	I	5 (71.4)	1 (14.3)	4 (57.1)	1 (14.3)	7
	II	13 (92.9)	2 (14.3)	4 (28.6)	3 (21.4)	14
	III	12 (100.0)	1 (8.3)	0 (0.0)	1 (8.3)	12
	IV	8 (88.9)	1 (11.1)	3 (33.3)	1 (11.1)	9
	V	12 (100.0)	3 (25.0)	1 (8.3)	4 (33.3)	12
	Adult	6 (100.0)	2 (33.3)	0 (0.0)	1 (16.7)	6
Domiciliary	Subtotal	56 (93.3)	10 (16.7)	12 (20.0)	11 (18.3)	60
	Adult	81 (100.0)	0 (0.0)	6 (7.4)	4 (4.9)	81
Total	All combined	137 (97.2)	10 (7.1)	18 (12.8)	15 (10.6)	141

* Values are no. (%) specimens from each instar.

† Mixed infections. Thus, sum of percentages of infection may be > 100%.

We propose that *T. infestans* adults may reach human dwellings by flying from sylvatic foci, although dispersal from undetected foci is possible. Recolonization is prevented by the National Control Program, which sprays these houses with long-lasting insecticides (lambda-cyhalothrin). However, it has been reported elsewhere that *T. infestans* can rapidly infest buildings after a spraying campaign.⁴⁷ There were no findings of nymphal instars inside dwellings that had winged adults, strengthening the idea that there are no residual colonies within those houses.

Recent phylogeographic studies of *T. infestans* from Chile by using mitochondrial cytochrome c oxidase subunit I sequences, including samples obtained from the same insects used in this study, showed that sylvatic and domiciliary insects are not segregated in the phylogeographic network.⁴⁸ These findings suggest an absence of or low population structure, thus enabling gene flow between sylvatic and domiciliary insects.

In this study, we determined the prevalence of infection and *T. cruzi* lineages from insects of different developmental instars collected in the sylvatic foci and from adults present in human dwellings. Infection in earlier nymphal instars was rather high, indicating that insects became infected early during their lives, as described for *T. infestans*⁴⁹ and other triatomine species,⁵⁰ suggesting that they feed on vertebrate hosts infected with two or more *T. cruzi* lineages, which is frequent in nature.^{14,23,51} We cannot discard the route of direct transmission between triatomines by coprophagy or cannibalism,^{25,52–54} given that all triatomines must obtain their symbionts at least once in their lifetime, probably by coprophagy.^{25,55} Most of the time, field insects are starving, which enhances their cannibalistic behavior towards other triatomines that were able to feed, probably acquiring *T. cruzi* in the process, in case that the engorged insect was infected.⁵³

We report high infection rates with *T. cruzi* in adults found in sylvatic and domiciliary habitats, and lower levels of infection among the nymphal instars found in the sylvatic foci. The *T. cruzi* genotypes found among insects from houses and sylvatic foci were similar, suggesting the sylvatic origin of the domiciliary *T. infestans* adults.

The predominance of TcI in sylvatic and domiciliary insects is consistent with data reported for efficiency of transmission of *T. cruzi* through *T. infestans*, which showed higher transmissibility for TcI and lower transmissibility for TcV, and being the lowest for TcII.⁵⁶ Moreover, in an experimental infection study *in vitro* and *in vivo*, in which a TcI genotype was tested individually and mixed with a TcII clonal genotype, the TcI genotype showed positive selection in co-cultivation.⁵⁷ A previous study performed in the same disease-endemic area

as this study showed that the synanthropic reservoir *Rattus rattus* was predominantly infected with TcI, and the wild rodent *Octodon degus* was mainly infected with TcV and TcII; TcI was the most prevalent lineage in the infected mammals in that area and TcVI the least prevalent lineage.²³ Some of these discrete typing units are better adapted to *T. infestans* than to vertebrate hosts; the most extreme case was shown with TcVI, which was absent in patient blood samples but was detected by *T. infestans* xenodiagnosis.¹² Recent studies using three microsatellites for *T. cruzi* genotyping reported that from 12 *T. infestans* found in houses in the Metropolitan Region that were tested, five had TcI, five had TcIII, and two had hybrid lineages.¹⁸ In this study, using the four probes described, we found no unknown lineages. Thus, it is unlikely that there is a genotype that has not been detected. However, it could be present as a mixed infection along with the other genotypes reported.

A deviation in *T. cruzi* genotypes distribution among sylvatic nymphs compared with domiciliary adults was observed in this study, in which TcII, TcV, and TcVI are frequent in the sylvatic nymphs. Assuming that the origin of the domiciliary *T. infestans* adults are sylvatic foci, this observation would indicate a differential adaptation of *T. cruzi* lineages among instars, with more TcII, TcV, and TcVI in nymphs compared with adults, including mixed infections with TcI. This observation would have great epidemiologic significance because the genotype vectorial capacity of *T. cruzi* would depend on the insect vector stage, among many other factors. Frequent elimination of some *T. cruzi* genotypes from mixed bi-clonal experimental infections of fully fed *T. infestans* was reported,⁵⁸ suggesting a tendency of *T. cruzi* to select a lineage from mixed infections.

Interactions as competition or stimulation between *T. cruzi* lineages likely exist, as observed in vertebrate^{14,59} and invertebrate hosts.^{58,60} Moreover, under long periods of starvation, 99.5% of *T. cruzi* in the rectum of an infected triatomine can be killed, and *T. cruzi* population density in the small intestine and rectum is reduced.⁶¹ Thus, adults probably undergo this process several times during their lifetime, which filters many genotypes and retains and proliferates those genotypes that are more resistant to adverse situations, both from the vector, such as trypanolytic compounds, digestive enzymes, and lectins,⁶² and environmental ones, such as resident bacteria in the gut, temperature variation,⁶² and resource shortage.⁵⁶ Another explanation might be that adults would have time to feed from hosts belonging to the domestic cycle of *T. cruzi*, acquiring genotypes present in higher frequency, and those new genotypes ingested would replace previous genotypes,

eliminating infections that were present in juvenile stage. The physiology of *T. infestans* also might vary according to their stage, affecting some *T. cruzi* genotypes more than others, which would enhance or decrease their populations inside the digestive tract according to their stage. Experimental studies comparing *T. cruzi* genotypes of nymphs and adults fed on the same infected hosts are needed to test the hypothesis of differential stage adaptation.

Our results indicate a preferential infection of adults of *T. infestans* with TcI, suggesting that TcI is the main lineage that circulates between the sylvatic cycle and the domestic cycle in adult vectors and synanthropic mammals in this area of Chile, creating an enhanced risk of human infections with this genotype. This finding is disturbing because TcI has showed to be resistant to benznidazole, usual chemotherapy for Chagas disease.⁶³

Another alternative to explain these results is that residual domiciliary populations of *T. infestans* might be playing a role in house reinfestation after the spraying campaign. However, in our case, insects found inside dwellings do not represent a recolonization because only adults have been found, although, that event might occur, given that adults of both sexes and/or fertilized females invade domestic habitats, as occurred with *T. infestans* in Bolivia⁶⁴ and with *Rhodnius prolixus* from different habitats in Venezuela after control campaigns.⁶⁵

In this new epidemiologic scenario, the risk of human dwelling re-infestation depends on the presence of sylvatic foci of *T. infestans* within their natural habitat, in this case, the bromeliad *Puya* sp., which provides refuge to mammals and to *T. infestans* colonies. There are probably more wild foci of *T. infestans* in the endemic area of Chile, not necessarily within bromeliads, as shown recently.²² Thus, it is important to continue the search for this vector, particularly in areas where there are unexplained sources of winged adults that invade houses. It is critical to study the surrounding area when these triatomines are found infected with *T. cruzi*, given the higher risk of transmission of Chagas disease to their inhabitants.

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