First record of *Triatoma vitticeps* (Hemiptera, Reduviidae) colonising a brick house in an urban area of a devastated Atlantic Forest fragment in Rio de Janeiro State, Brazil

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BACKGROUND *Triatoma vitticeps* (Stål, 1859), a primarily sylvatic vector of *Trypanosoma cruzi* (Chagas, 1909), has been reported invading dwellings and peridomiciliary ecotopes in several regions of Brazil.

OBJECTIVES and METHODS Here, we report for the first time, the colonisation of a brick house by *T. vitticeps* in the municipality of São José do Vale do Rio Preto, Rio de Janeiro State.

FINDINGS Inside the house, indicators of colonisation were recorded, including 15 eggs, 11 nymphs, two exuviae, and 19 adult specimens. *T. cruzi* infection was detected in 13 specimens and the blood meal source identification, through amplification of DNA fragments indicated the presence of human blood as one of the feeding sources in seven specimens.

MAIN CONCLUSIONS The infested dwelling was treated with insecticide to control the infestation, and residents were appropriately instructed to remain vigilant for the presence of triatomines and to contact health authorities if new specimens were detected.

Key words: Triatominae - vectors - intradomiciliary infestation - colonisation - Chagas disease

Currently, 159 Triatominae species are recognised as potential vectors of *Trypanosoma cruzi* (Chagas, 1909), the parasite that causes Chagas disease (CD).[1,2] The great majority of the vectors are sylvatic, distributed across the Americas and Brazil harbours the highest diversity of triatomine species.[1,3,4]

In the State of Rio de Janeiro, *T. cruzi* vectors are primarily sylvatic species associated with the Atlantic Forest. Among these, *Triatoma vitticeps* (Stål, 1859) is a predominantly wild triatomine distributed in the Brazilian states of Bahia, Espírito Santo, Minas Gerais and Rio de Janeiro.[1,5,6] Although primarily associated with forest environments, adults of *T. vitticeps* frequently invade rural dwellings, attracted by artificial light or in search of blood meals.[7] This synanthropic behaviour links sylvatic transmission cycles to human exposure, particularly in peridomiciliary settings near woodpiles and animal shelters, conferring significant epidemiological relevance to *T. vitticeps* in areas where *T. cruzi* circulates.

The ecology and biology of *T. vitticeps* have been extensively investigated, encompassing aspects of its life cycle, feeding behaviour, defecation patterns, and blood meal sources.[8,9] These studies indicate that the relatively long interval between the end of feeding and defecation is one of the main factors limiting the vectorial efficiency of this species.[9] Nevertheless, blood meal analyses have revealed frequent ingestion of human blood by adults captured indoors, demonstrating direct contact with human populations.[8,10]

The invasion and the establishment of *T. vittice*ps in anthropogenic environments has been reported by several authors. Peridomiciliary colonies of this species infected with *T. cruzi* were found in rural areas of Espírito Santo State, Brazil.[11] The population dynamics of that species in Minas Gerais, Brazil showed the infested domiciles were located closer to open fields rather than to the sylvatic environment.[12] More recently, the ecological and spatial dynamics of this species were modelled and the results pointed out that CD transmission in Espírito Santo is driven by wind-facilitated *T. vitticeps* invasion, with higher mammal biodiversity, predicting increased *T. cruzi* infection rates.[13]

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Furthermore, previous records show *T. vitticeps* colonising domicile in the Afredo Chaves municipality, Espírito Santo State, suspected of transmitting CD.[14,15] In Rio de Janeiro State, the species was recorded colonising wattle and daub houses, in rural areas, in Miguel Pereira and São Fidelis municipalities.[16,17] The present paper reports, for the first time, colonisation by *T. vitticeps* in a brick house located in an urban area adjacent to a severely degraded fragment of the Atlantic Forest in Rio de Janeiro State, Brazil.

MATERIALS AND METHODS

Study area and specimen collection - Following a request from the Municipal Health Secretary, the inspected dwelling was in an urbanised area within a heavily degraded fragment of the Atlantic Forest in the municipality of São José do Vale do Rio Preto (SJVRP), Rio de Janeiro State, Brazil, at an altitude of 615 m (22.1600933º S, 42.9361117º W) (Fig. 1). The well finished brick house is situated near several other dwellings constructed with similar materials, including tile roofs, and is surrounded by a peridomiciliary area containing domestic animals such as birds, dogs, and cats. According to residents, opossums are frequently observed in the surroundings, including peridomiciliary areas. Active searches for triatomines in the infested dwelling were systematically conducted in each of its six rooms (one toy room, one bedroom, one living room, one bathroom, a kitchen, and a laundry).

The presence of triatomines was investigated in all potential shelters within the house and in the peridomiciliary areas. The exhaustive sampling effort took 5 h to be completed and was carried out in clockwise direction, in each of the house compartments, plus the roof. All specimens were manually collected using tweezers, placed in plastic containers and subsequently transported to the Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos (LNIRTT), Instituto Oswaldo Cruz. In the laboratory, specimens were identified using the taxonomic keys,[5,6] and recorded according to developmental stage, including the number of eggs, exuviae, and male and female adults. One male voucher specimen was deposited under number 20627 in the Triatomine Collection of Instituto Oswaldo Cruz (CTIOC), Rio de Janeiro, Brazil. After the taxonomic identification 16 specimens were selected for analysis.

Due to the relevant risk of the finding for public health, this report was based on a single inspection, regarding the status of colonisation of the captured species in the urban brick residence. Once the searches were carried out, an official technical report was elaborated by LINRTT and sent to the Ministry of Health author-

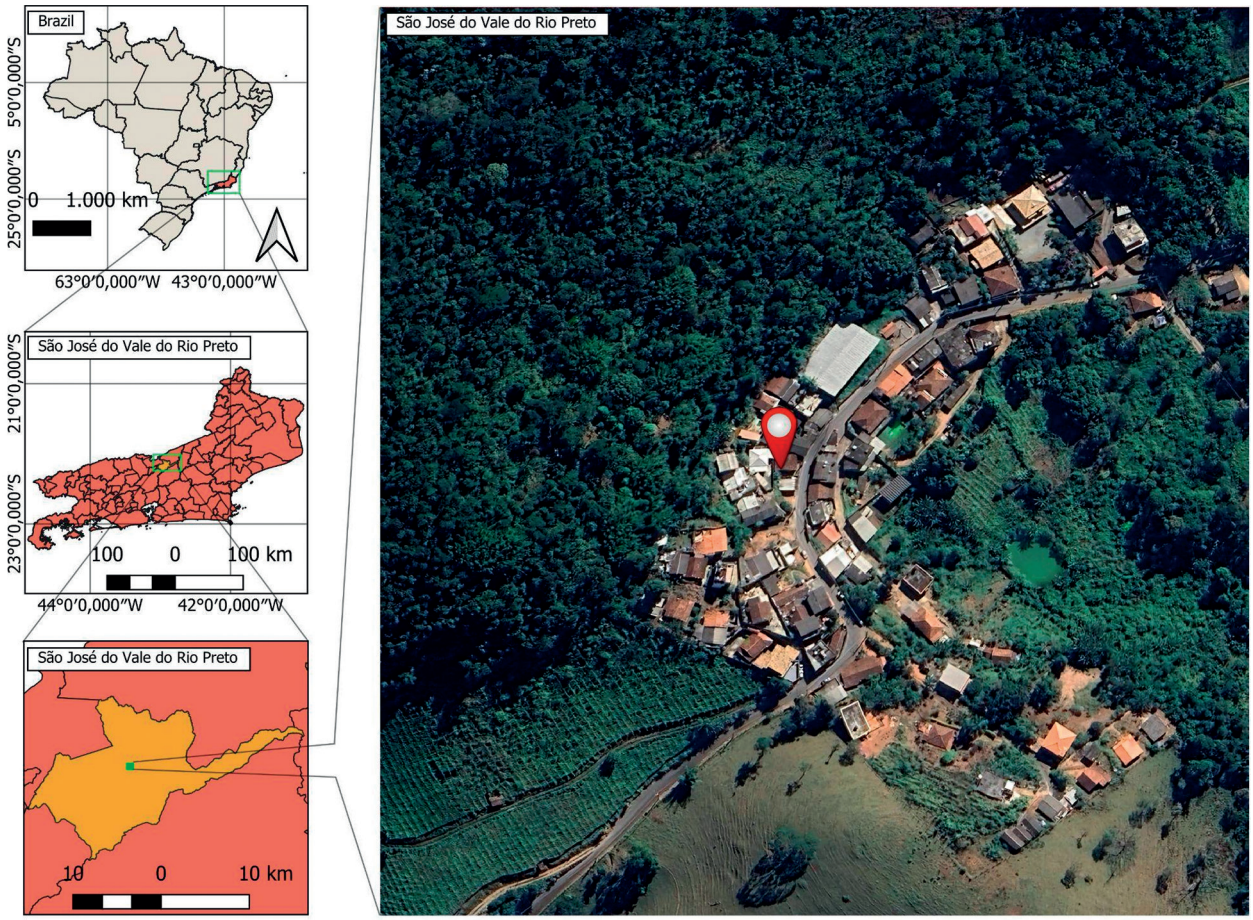

Fig. 1: map showing the location of the residence infested by *Triatoma vitticeps*, in São José do Vale do Rio Preto, Rio de Janeiro State, Brazil.

ities reporting the results and stressing the importance of implementing vector monitoring activities in SJVRP (Supplementary data). Furthermore, at the end of the exhaustive sampling efforts the residents were properly instructed and requested to leave the house while the spraying treatment was applied for eliminating the infestation.

DNA extraction - The DNeasy Blood & Tissue Kit protocol (Qiagen) was used for DNA extraction from the intestinal contents of the specimens. Due to the preservation condition of the specimens received by the laboratory, dried specimens were excluded from molecular analysis, and only engorged insects were selected to ensure greater quantity and quality of DNA in the intestinal content, allowing higher reliability in the identification of the blood meal source. Each specimen was dissected in the terminal region of the abdomen for removal of the intestinal content. The intestinal content was macerated using autoclaved plastic pestles and stored in sterile microcentrifuge tubes at -20ºC until the beginning of molecular analyses.

Trypanosoma cruzi infection and blood meal source analyses - To detect natural infection by *T. cruzi* in the captured specimens, the specific primers TcH2AF and TcH2AR were employed. The primers TcH2AF and TcH2AR amplify the histone H2A region. Polymerase chain reaction (PCR) reactions were performed in final volume of 25 µL, using: 12.5 µL Green Taq Polymerase Master Mix (Celco), 0.5 µL of each primer (forward and reverse), 2 µL of DNA, and nuclease-free water to complete the final volume. A fixed volume of 2 µL of intestinal content DNA was used in all PCR reactions. To prevent contamination with human DNA, DNA extraction and PCR procedures were performed in a biosafety cabinet using sterile materials. PCR cycling for the detection of *T. cruzi* was performed under the conditions previously described,[18] with an initial denaturation at 95ºC for 5 min, followed by 15 cycles of denaturation at 95ºC for 30 s and, extension at 72ºC for 1 min, followed by 30 cycles of denaturation at 95ºC for 30 s, annealing at 60ºC for 30 s, and extension at 72ºC for 30 s, with a final extension at 72ºC for 5 min. To ensure the quality of the DNA present in the samples, primers already described[19] were used to amplify a fragment of the insect mitochondrial Cytochrome oxidase subunit I (COI).

For blood meal source identification, specific primers[20] were used for the detection of vertebrate DNA from commonly found animals in domestic environments (birds, dogs, and cats) and humans. The second PCR was carried out for the specific primers with an initial denaturation at 94ºC for 6 min, followed by 40 cycles of 30 s at 94ºC, annealing at 60ºC for 30 s, and extension at 72ºC for 1 min, followed by a final extension at 72ºC for 10 min. The reactions were performed in a gradient thermal cycler and maintained at 12ºC (PR-96E, MIULAB) until collection. PCR products were confirmed by electrophoresis on 1 - 1.5% agarose gels stained with GelRed 20× (Biotium) were run in TBE 1X buffer for 35 min at 90 V. A 100 bp DNA ladder (Ludwig) was used as a molecular weight marker, and gels were visualised under UV light using a transilluminator (Loccus).

Positive controls for *T. cruzi* and vertebrate-specific primers (*Homo sapiens*, *Gallus gallus*, *Felis catus* and *Canis familiaris*) were obtained from samples provided by the Institute of Biology of the State University of Campinas (UNICAMP), and negative controls (distilled water replaced in DNA sample) were included and run on the same gel. Amplicons of 234 bp were interpreted as positive for *T. cruzi*, while amplicons of 470 bp, 476 bp, 1179 bp, and 197 pb were considered positive for human, dog, cat, and bird DNA, respectively (Table I).

TABLE I

List of primer sequences for detection of *Trypanosoma cruzi* infection and vertebrate-specific blood meal source used in the analyses of *Triatoma vitticeps* specimens, collected in the intradomiciles in São José do Vale do Rio Preto, Rio de Janeiro, Brazil

Primer	Sequence	Target	Amplicon size (bp)	Reference
TcH2AF	5'-GAGAGTGATCGTGGGAGAGC-3'	*T. cruzi*	234	Paiva et al.[18]
TcH2AR	5'-AGTGGCAGACTTTGG GGTC-3'			
COI (LCO1490)	5'-GGTCAACAAATCATAAAGATATTGG-3'	Triatominae	710	Folmer et al.[19]
COI (HCO2198)	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'			
Human-F	5'-GTAGTACATAAAAACCCAATCCACATC-3'	*Homo sapiens*	470	Ribeiro Jr et al.[20]
Human-R	5'-GTCGGATACAGTTCACTTTAGCTACC-3'			
Avian-F	5'-ATAGAATGGCCTGGGTTGAAAAG-3'	Class avian	197	Ribeiro Jr et al.[20]
Avian-R	5'-AAGTTTTTCACACAGAGGGTGGT-3'			
Cat -F	5'-ACAGGATCAGAAACCTTTATCTGACTA-3'	*Felis catus*	1179	Ribeiro Jr et al.[20]
Cat-R	5'-TTTGACTTAAAATTTATGGTTTGGTTT-3'			
Dog-F	5'-GTCAATGGTTTCAGGACATATAGTTTT-3'	*Canis Familiaris*	476	Ribeiro Jr et al.[20]
Dog-R	5'-TATTGTATGCACTTAGTCCTGTTTTTG-3'			

RESULTS

During a single systematic and exhaustive search covering all rooms, the roof and the peridomiciliary areas of the house, a total of 47 specimens of *T. vitticeps* were found (Table II, Fig. 2).

The adult specimens were found in a single room (the toy room). According to the homeowner, specimens were also frequently observed in the living room and in the bedroom near the bed. Because spots like triatomine faeces were observed on the wall, near the ceiling, in the toy room (where most of the adult specimens were collected), additional searches on the roof were conducted (Fig. 3). Tiles were removed above the area marked with the faecal spots observed indoors. This inspection revealed several dry leaves, bird feathers, fur, sticks and rodent faeces. Among this material, nymphs, exuviae, and eggs of *T. vitticeps* were found (Fig. 4A-D). Other areas of the roof were equally inspected however, no additional signs of triatomine colonisation were found.

TABLE II

Number of *Triatoma vitticeps* specimens collected in intradomiciliary environment of a brick house in urban area of São José do Vale do Rio Preto municipality, Rio de Janeiro, Brazil

Specimens	Number	Place
Females	9	Toy room
Males	10	Toy room
Nymphs (5th instar)	1	Roof, under the tiles
Nymphs (2th instar)	2	Roof, under the tiles
Nymphs (1th instar)	8	Roof, under the tiles
Eggs	15	Roof, under the tiles
Exuviae	2	Roof, under the tiles
Total	47	

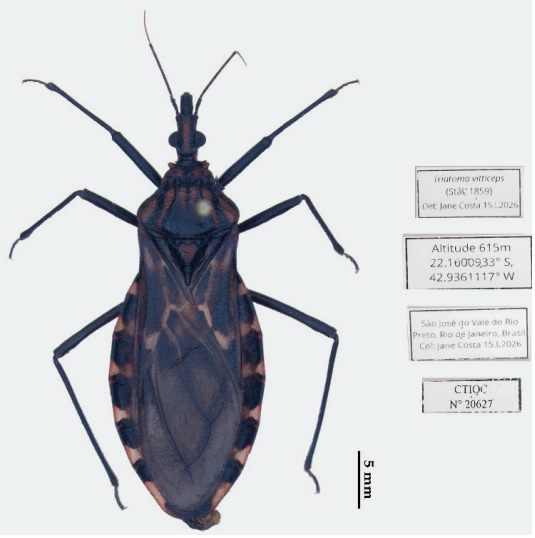

Fig. 2: *Triatoma vitticeps* male specimen collected in the intradomicile, in São José do Vale do Rio Preto, Rio de Janeiro State, Brazil, and deposited in the Triatominae Collection of Instituto Oswaldo Cruz.

From the sixteen specimens tested, thirteen were positive for *T. cruzi* infection. Only three specimens tested negative for *T. cruzi* infection. The expected amplification of the COI gene fragment was observed in fifteen specimens. Blood meal source identification, based on the amplification of DNA fragments, indicated the presence of human blood as one of the feeding sources, with seven triatomine specimens testing positive. None of the specimens tested positive for dog or cat DNA. One specimen presented a mixed blood meal, with human and bird DNA detected; this specimen was also positive for *T. cruzi* infection.

DISCUSSION

Over recent decades, Latin America has observed a notable increase in new records of triatomine species occurrences. Those belonging to the genera *Triatoma* and *Panstrongylus* are the most frequently detected in human dwellings and exhibit high rates of *T. cruzi* infection. Infected triatomine species have been recorded across a broad geographic range, from Argentina to the United States. Certain species have become increasingly intrusive, repeatedly invading dwellings and, in some instances, establishing stable colonies in urban environments, leading to a rise in reported encounters.[4,21,22,23] This trend is exemplified by the recent first records of *Panstrongylus geniculatus* (Latreille 1811) and *Panstrongylus megistus* (Burmeister 1835) on Ilha Grande, an Atlantic Forest island, illustrating the intrusive behaviour of native triatomines into anthropic environments.[24,25] These findings highlight the ongoing geographic expansion and adaptive potential of these vectors, underscoring the need for enhanced urban and peri-urban surveillance to mitigate CD transmission risk amid changing ecological and anthropogenic landscapes.[26,27,28]

Endemic from Brazil and considered primarily invasive, *T. vitticeps* has been recorded invading houses attracted by light from nearby forest remnants in the Atlantic Forest biome.[5,6] As previously mentioned, records of *T. vitticeps* colonising domiciles and peridomiciles in rural areas were reported in literature.[11,12,16,17] The colonisation of anthropic ecotopes by *T. vitticeps* in Alfredo Chaves was reported and the authors emphasised there are no indications of change in the sylvatic habits of that species, suggesting the colonies found were associated with opossum nests, a result of an expansion of the sylvatic environment into the peridomicile.[11]

Additional studies have confirmed the presence of both nymphs, indicating active breeding, and adults inside houses and associated outbuildings, particularly in areas of Rio de Janeiro, Minas Gerais and Espirito Santo, where the species is frequently associated with opossum nests (*Didelphis aurita*).[7,9,16,29] Despite the fact *T. vitticeps* presents small domiciliary colonies, these records suggest a broader ecological plasticity than previously recognised for this vector species, raising concerns in terms of public health.

The findings here reported confirm the colonisation capacity of *T. vitticeps* since eggs, nymphs, adults and exuviae were found in the intradomiciliary area. The previously published studies on *T. vitticeps* show-

Fig. 3: entomological surveillance activities carried out in an urban residential area, in São José do Vale do Rio Preto, Rio de Janeiro State, Brazil. (A) Technical visit to the residence for inspection and guidance to residents. (B) Removal of triatomine specimens and organic materials (feathers, dry leaves, sticks, fur, rodent faeces) accumulated in the shelters on the roof of the dwelling. (C) Manual capture of an adult *Triatoma vitticeps* specimen using entomological tweezer. (D) Inspection of internal structures of the dwelling and observation of spots like triatomine faeces.

ing the species colonising wattle and daub houses in rural areas, and infection by *T. cruzi* in anthropogenic rural settings demonstrate our results are not unprecedented. However, the present report reveals the inedited finding of *T. vitticeps* colonising a brick house of an urban area, in SJVRP. It is also worth mentioning that according to the local health authorities, there are no previous records of *T. vitticeps* occurring in the referred municipality.

The presence of eggs, nymphs, exuviae and adults of *T. vitticeps* in intradomiciliary environment provide clear evidence the species was colonising an urban brick house. Nevertheless, the finding was based on one single sampling event. Due to the public health risk it represents, the infested house was sprayed with insecticide the day after the exhaustive triatomine capture was carried out. In order to get a broad understanding of a possible adaptation process of *T. vitticeps* to anthropic ecotopes further studies must be conducted in the mentioned municipality.

Triatoma vitticeps specimens captured inside dwellings have consistently shown high rates of natural infection with *T. cruzi*.[9] The analysed specimens in this report also confirm *T. vitticeps* as a highly infected vector, since from 16 tested specimens, 13 were positive for *T. cruzi*, and despite these high infection rates with the etiologic agent of CD and other trypanosomes, *T. vitticeps* has traditionally been classified as a secondary vector, mainly due to its low tendency to colonise houses and the long interval between hematophagy and defecation, which reduces the efficiency of classical vectorial transmission.[9] However, this species has been shown to harbour single and mixed infections involving multiple *T. cruzi* discrete typing units (TcI-TcIV), as well as *T. cruzi marinkellei* and *T. dionisii*. Importantly, fatal cases of acute CD associated with oral transmission following the manipulation of infected *T. vitticeps* specimens have been documented.[30]

Because the recognition of an anthropophilic triatomine is also key to define a relevant CD vector in the

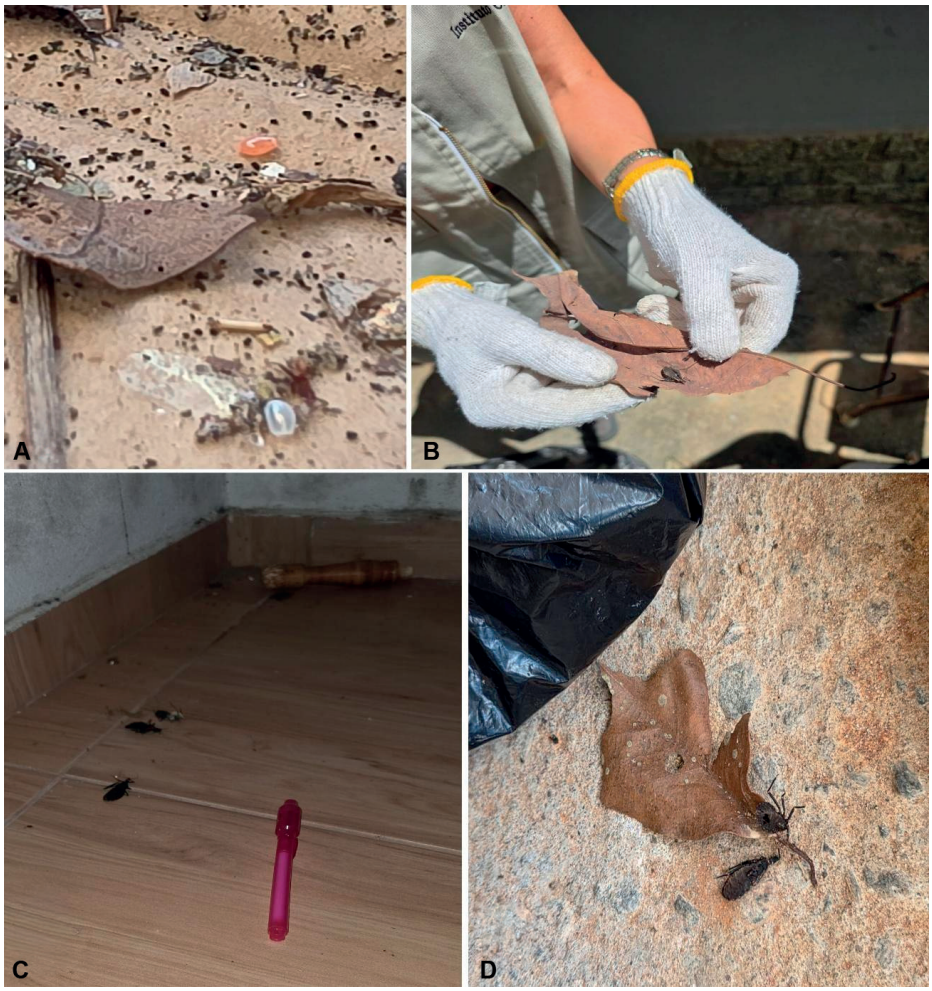

Fig. 4: evidences of *Triatoma vitticeps* colonising an urban brick house, in São José do Vale do Rio Preto, Rio de Janeiro State, Brazil. The presence and capture of triatomine bugs in the domiciliary environment: (A) *T. vitticeps* eggs found among the organic material accumulated under the tiles. (B-C) Manual inspection of dry leaves and organic material found under the tiles, serving as triatomine bug shelter, where exuviae were found. (D) Intradomiciliary environment, showing the toy room, where most of the captured triatomine bugs were found.

classical *T. cruzi* transmission, this characteristic has been evaluated for several species.[31] For *T. vitticeps*, results were obtained through the precipitin test[8,10,16] demonstrating the studied species presents a significant feeding eclecticism and marked anthropophily. In the present study, anthropophily was evaluated using specific primers for vertebrate DNA detection of commonly found animals in domestic environments like birds, dogs, cats, and humans.[20] From the 13 tested specimens, seven were positive for human blood, stressing the importance to constantly monitor the area studied for triatomine infestation in the domiciliary ecotopes.

The present report highlights the importance of advising residents to carefully collect and deliver suspected "kissing bugs" (*barbeiros*) to local health services for identification and testing, rather than crushing them, as was recommended to inhabitants of the neighbourhood where the infestation was detected. In addition, evaluating environmental changes that may be facilitating triatomine invasions in the study area is essential for implementing proactive measures to prevent new infestations.[13,27,32,33]

The presence of *T. vitticeps* (eggs, adults and nymphs) in the domiciliary environment of a brick house reinforces the need for sustained entomological surveillance. Emerging evidence also suggests that what is currently identified as *T. vitticeps* may represent a complex of cryptic species, which could help explain observed variations in behaviour, infection rates, and patterns of domiciliary invasion.[34] Also, a detailed study on the *T. vitticeps* phenotype, encompassing specimens from a broad range of its geographic distribution, is recommended since distinct phenotypes may present distinct epidemiological importance requiring distinct control strategies.[35] Furthermore, detailed studies of the *T. cruzi* lineages and the food sources of the mentioned vector are necessary.

After the entomological survey, local inhabitants were instructed to promptly contact health authorities whenever suspected insects are found in their homes or surrounding areas. In this context, digital surveillance and citizen science initiatives such as *WhatsBarb* tool,[36] and the CD Portal (https://chagas.fiocruz.br/), launched by the Oswaldo Cruz Foundation (Fiocruz), Brazil, are

crucial to disseminate knowledge about CD. These initiatives facilitate early detection, rapid communication with surveillance services, and proper taxonomic identification of triatomines, strengthening entomological surveillance in areas where new infestations are detected.[1,4]

In order to check in details and evaluate the results of the insecticide spraying in the infested brick house here reported and, in the neighbourhood, future searches are programmed in collaboration with the technicians of the local Health Secretary. A research project is going to be implemented to provide a comprehensive and integrative analysis allowing the understanding of the *T. cruzi* cycles occurring in the infested area, as carried out by our team for other vector species.[31,37]

In conclusion - Here we report, for the first time, a triatomine colonisation in SJVRP, by *T. vitticeps*, a species primarily considered a sylvatic vector. Specimens were found infesting a brick house, representing a new and unexpected event recorded in a highly degraded fragment of the Atlantic Forest, in an urban area. Laboratory analyses showed *T. vitticeps* specimens were infected by *T. cruzi* and reacted positively for human blood as a food source, confirming public health risks.

Integrative studies, including all aspects of the *T. cruzi* transmission cycle are required to understand the eco-epidemiological dynamics of CD in SJVRP. The mentioned endeavours are imperative to support proactive measures aimed at preventing human infection. In addition, continuous educational campaigns are crucial to raise awareness among the locals.

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AUTHORS' CONTRIBUTION

JC and CG were the main contributors to the conceptualisation and design of the study; JC, GG and CJS carried out field work; JC, JPSOC, FCOF, DSR and CG conducted the writing, literature review and manuscript input; JC, GG, FCOF, JPSOC, EOCJ, CJS, DRS and CG data acquisition, analysis, and interpretation; JPSOC and FCOF prepared figures and tables. None of the authors have any conflicts of interest to declare.

DATA AVAILABILITY

The contents underlying the research text are included in the manuscript.

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OPEN PEER REVIEW

Memórias do IOC thanks the anonymous reviewers for their contribution to the peer review of this work.

FIRST REVIEW ROUND

REVIEWERS' COMMENTS

REVIEWER #1

The manuscript presents a relevant and potentially publishable record, particularly by documenting the intradomiciliary colonization of Triatoma vitticeps in an urban environment, which carries important epidemiological implications. However, despite its merits, the study exhibits conceptual, methodological, and, most notably, interpretative weaknesses that should be addressed to enhance its scientific robustness and to avoid extrapolations that may be questioned during peer review.

Overall, the manuscript is well structured and clearly written. Nevertheless, there is a recurring tendency to overemphasize the findings without sufficient analytical support. The title is strong and appropriate; however, the claim of a "first record" requires more rigorous substantiation. It should be clearly specified whether this refers to the first record for the municipality, for a particular type of urban construction, or for a fully documented domiciliary colonization including all developmental stages. This distinction is essential, as previous records of domiciliary colonization by T. vitticeps exist in southeastern Brazil, and the originality of the study may be questioned if its scope is not precisely defined.

In this context, it is crucial to incorporate existing literature demonstrating colonization or establishment of this species in anthropogenic environments. Relevant studies include: Peridomiciliary colonies of Triatoma vitticeps (Stål, 1859) (Hemiptera, Reduviidae, Triatominae) infected with Trypanosoma cruzi in rural areas of the state of Espírito Santo, Brazil (Biral dos Santos et al., Memórias do Instituto Oswaldo Cruz 100(5): 471–473, 2005); Population dynamics of Triatoma vitticeps (Stål, 1859) in Itanhomi, Minas Gerais, Brazil (de Souza et al., Memórias do Instituto Oswaldo Cruz 103(1): 14–20, 2008); and Unveiling the ecology and spatial dynamics of Trypanosoma cruzi, its DTUs and Triatoma vitticeps in the Atlantic Forest of south-eastern Espírito Santo State, Brazil (Testai et al., PLoS Neglected Tropical Diseases 20(3): e0014111, 2026). These studies demonstrate that colonization and infection in anthropogenic settings are not entirely unprecedented, even if the specific context presented here may be.

The abstract is clear but would benefit from greater quantitative precision. The expression "several indicators of colonization" should be replaced with objective data, such as the number of nymphs, eggs, and adults observed. Additionally, the statement regarding Trypanosoma cruzi infection should avoid vague wording such as "presented and discussed" and instead include the key findings, even in summarized form, to strengthen its impact.

The introduction is generally well contextualized but contains some redundancies and overly broad generalizations. While the discussion of synanthropic behavior and epidemiological relevance is appropriate, it should be more directly aligned with the specific knowledge gap addressed by the study. The central objective remains somewhat unclear whether the study aims to demonstrate colonization, assess epidemiological risk, or discuss urban adaptation. At present, these aspects are addressed simultaneously, which dilutes the focus. Incorporating overlooked studies directly addressing the ecology and domiciliation of T. vitticeps including those cited above would significantly improve the contextual framework.

The methodology presents several critical issues. Although the collection procedures are adequately described, there is no standardization of sampling effort. The study is based on a single inspection, which substantially limits inferences regarding stable colonization, and this limitation should be explicitly acknowledged. The selection of only 16 adults for molecular analysis also requires clearer justification, particularly given that 47 specimens were collected. Furthermore, restricting the analysis to engorged insects reduces representativeness and may introduce bias in the identification of blood meal sources.

The molecular section is functional but lacks detail expected by current standards. There is no information regarding the use of positive and negative controls for each primer in the blood meal analyses, nor is there discussion of potential cross-amplification. The statement that results were "adopted due to the rapidity and high quality of the analyses" is not appropriate for a scientific manuscript and should be removed or reformulated based on technical criteria.

In the results section, a major concern is the classification of 13 out of 16 specimens as "possibly positive" for T. cruzi. In PCR-based analyses, results should be clearly defined as positive or negative according to objective criteria. The use of "possibly" undermines data reliability and raises methodological concerns. This issue should be clarified, whether in terms of band intensity, reproducibility, or predefined thresholds for positivity. Without such clarification, the validity of the results may be questioned.

Additionally, the results could be presented in a more quantitative and structured manner. For example, the spatial distribution of insects within the household could be better organized in a table or described more objectively. Table 1 also requires revision to correct typographical errors and ensure consistent formatting.

The discussion represents the most sensitive section of the manuscript. Although it provides useful context, it relies on excessive extrapolation from a single sampling event. The text suggests broader changes in epidemiological dynamics and urban adaptation; however, the data are limited to a single household and a single collection event. The tone should therefore be moderated, clearly framing the findings as a punctual yet relevant record. In this regard, incorporating additional literature is essential to support and calibrate the interpretations. The recent study by Testai et al. (2026) is particularly relevant, as it offers an integrated perspective on vector and parasite ecology in the Atlantic Forest and could strengthen the discussion of infection patterns, parasite diversity, and spatial dynamics.

The discussion of oral transmission, although conceptually valid, appears somewhat disconnected from the data presented and may be perceived as overstating the study's implications. It would be more appropriate to maintain focus on domiciliary colonization and vector–human contact, as supported by the blood meal analysis.

The section addressing anthropophily and blood meal sources is interesting but should also be interpreted with caution. The detection of human blood in seven individuals within a small sample size does not support robust conclusions about population-level feeding behavior. This finding should be presented as indicative rather than conclusive, ideally in comparison with previous studies demonstrating the species' eclectic feeding habits.

A notable strength of the study is the identification of a colony under the roof, including eggs, nymphs, and exuviae, which provides clear evidence of colonization. This represents the core contribution of the manuscript and should be emphasized further, including direct comparisons with similar records reported in peridomiciliary environments in southeastern Brazil.

It is also strongly recommended that the authors include a high-quality dorsal photograph of at least one collected specimen. This would allow proper documentation of the morphotype, support taxonomic identification, and enhance the overall robustness and transparency of the study.

The final portion of the discussion combines recommendations, future perspectives, and outreach aspects in a somewhat diffuse manner. The mention of tools such as WhatsBarb is interesting but appears tangential to the main scientific argument and could be presented more concisely.

The conclusions are coherent but partially repetitive and employ somewhat strong language given the level of evidence. They should be made more objective and strictly aligned with the data presented.

From a technical standpoint, there are several minor issues throughout the manuscript, including inconsistencies in species nomenclature, errors in figure legends, and problems in references and formatting. Although these do not compromise the scientific content, they should be carefully revised, as they affect the overall perception of quality.

In summary, the manuscript has clear merit and publication potential, particularly due to the record of intradomiciliary colonization in an urban setting. However, it requires substantial revisions, including more rigorous data interpretation, clearer methodological descriptions (especially regarding PCR procedures), reduction of unsupported extrapolations, improved data presentation, inclusion of a dorsal image documenting the specimen, and incorporation of relevant overlooked literature. Addressing these points will significantly strengthen the manuscript and reduce the likelihood of predictable reviewer criticisms.

REVIEWER #2

Thank you for presenting this detailed assessment. I have some comments to help with clarity but overall, excellent presentation.

- Please comment on exactly why the Municipal Health Secretary was requested to the specific home? Was this because a resident at the home reconginzed a triatomine? Where they being bitten by teh bug? Chagas disease concerns? Please be more specific and include in "Study area and specimen collection" section.

- How long was the investigation? Was it for one day? 8 hours? Did they return for multiple entomological assessments? Please put in the details of the investigation timeline.

- Figure 2 (D) shows fecal drop on the walls but how was this confirmed? Some insects will defecate on walls and it looks like triatomine feces. I suggest saying, "Inspection of internal structures of the dwelling and observation of suspected triatomine feces on the wall."

- Did you test for DTU I? Why not?

- Please provide more details of the blood meal analysis. This is very briefly mentioned. Human blood was detected but documenting the other sources aids in understanding the extent to the other blood meal sources and will also help with the ecology of domestic invasion of a triatomine like this.

- I like the pictures of the triatomines and where they were found. This is impactful. I found myself wanting to see at least one adult picture which shows clear morphological features of the bug. This would also be impactful for readers.

- Map is excellent. Fantastic!
- Please add in the Discussion the known prevalence of Chagas in this locality and in the Rio de Janeiro State.
- I would also add some sentences on the impact of a sylvatic vector invading into the peridomestic domain and now domicilatting a home. Why did this occur at this residence? As you mentioned this is a fragmented forest region and deforestation is being seen in many regions of Brasil and other regions where Chagas disease is endemic. Sylvatic and peridomestic vectors are now being seen more and more in these regions. I would make some comments about this and possible site some article displaying this:
- Cantillo-Barraza O, Gual-González L, Velásquez-Ortiz N, Medina Camargo MA, González P, Cruz-Saavedra L, Castillo A, Zuluaga S, Herrera G, Cowan H, Velez-Mira A, Patiño LH, Ramírez JD, Triana O, Nolan MS. Triatoma venosa and Panstrongylus geniculatus challenge the certification of interruption of vectorial Trypanosoma cruzi transmission by Rhodnius prolixus in eastern Colombia. PLoS Negl Trop Dis. 2025 Jan 27;19(1):e0012822.
- Meraz-Medina T, Vences-Blanco MO, Ramírez-Cervantes AG, Nogueda-Torres B, Martínez-Ibarra JA. First record and vital statistics of Triatoma longipennis (Heteroptera, Reduviidae, Triatominae) from Southern Mexico. Acta Trop. 2025 Nov;271:107873.
- Beatty NL, Bhosale CR, White ZS, Torhorst CW, Wilson KN, Dorleans R, Stenn TMS, Killets KC, Curtis-Robles R, Burkett-Cadena N, Nováková E, Hamer GL, Hamer SA, Wisely SM. Field evidence of Trypanosoma cruzi infection, diverse host use and invasion of human dwellings by the Chagas disease vector in Florida, USA. PLoS Negl Trop Dis. 2025 Jul 7;19(7):e0012920.

Other examples exist. I recommend discussing the importance a bit more as it is a great example of the changing dynamics of Chagas disease within the Americas.

Thank you for your work!

AUTHORS' RESPONSE TO THE REVIEWERS

Dear Editors,

We have forwarded the document with the responses to the reviewers.

Thank you for your contributions, which improved the manuscript. All changes have been considered and highlighted.

Sincerely,

The authors

Reviewer: 1

The manuscript presents a relevant and potentially publishable record, particularly by documenting the intradomiciliary colonization of Triatoma vitticeps in an urban environment, which carries important epidemiological implications. However, despite its merits, the study exhibits conceptual, methodological, and, most notably, interpretative weaknesses that should be addressed to enhance its scientific robustness and to avoid extrapolations that may be questioned during peer review.

A: Dear Reviewer, we thank the valuable comments, suggestions and corrections enhancing the scientific robustness of our manuscript. Bellow we answer each mentioned point one by one.

R1: Overall, the manuscript is well structured and clearly written. Nevertheless, there is a recurring tendency to overemphasize the findings without sufficient analytical support. The title is strong and appropriate; however, the claim of a "first record" requires more rigorous substantiation. It should be clearly specified whether this refers to the first record for the municipality, for a particular type of urban construction, or for a fully documented domiciliary colonization including all developmental stages. This distinction is essential, as previous records of domiciliary colonization by T. vitticeps exist in southeastern Brazil, and the originality of the study may be questioned if its scope is not precisely defined.

A: Thanks for bringing more clarity for this point. A few lines stressing the first record refers to the encounter of triatomines in a brick house were added. However, previous colonization process of T. vitticeps had already been found in a wattle and daub house in another near municipality. This point was also added in the manuscript. Besides that we stressed in the discussion, that according to the Municipal Health Secretary, no previous records of triatomines invading or colonizing houses were found either in the files of the Secretary of Heath of SJV Rio Preto or in the literature.

R1: In this context, it is crucial to incorporate existing literature demonstrating colonization or establishment of this species in anthropogenic environments. Relevant studies include: Peridomiciliary colonies of Triatoma vitticeps (Stål, 1859) (Hemiptera, Reduviidae, Triatominae) infected with Trypanosoma cruzi in rural areas of the state of Espírito Santo, Brazil (Biral dos Santos et al., Memórias do Instituto Oswaldo Cruz 100(5): 471–473, 2005); Population dynamics of Triatoma vitticeps (Stål, 1859) in Itanhomi, Minas Gerais, Brazil (de Souza et al.,

Memórias do Instituto Oswaldo Cruz 103(1): 14–20, 2008); and Unveiling the ecology and spatial dynamics of Trypanosoma cruzi, its DTUs and Triatoma vitticeps in the Atlantic Forest of south-eastern Espírito Santo State, Brazil (Testai et al., PLoS Neglected Tropical Diseases 20(3): e0014111, 2026). These studies demonstrate that colonization and infection in anthropogenic settings are not entirely unprecedented, even if the specific context presented here may be.

A: We included all suggested references and a few lines mentioning the previous records of T. vitticeps colonizing in anthropic environments in rural areas of Espirito Santo and Minas Gerais. The species had already been recorded colonizing wattle and daub houses, in rural areas, in Miguel Pereira and São Fidelis municipalities, in Rio de Janeiro State, (Lorosa et al 2003 e 2008, respectively). All these points were emphasized in the introduction.

R1: The abstract is clear but would benefit from greater quantitative precision. The expression “several indicators of colonization” should be replaced with objective data, such as the number of nymphs, eggs, and adults observed. Additionally, the statement regarding Trypanosoma cruzi infection should avoid vague wording such as “presented and discussed” and instead include the key findings, even in summarized form, to strengthen its impact.

A: Precise information was included in the abstract and vague wording was changed by objective data.

R1: The introduction is generally well contextualized but contains some redundancies and overly broad generalizations. While the discussion of synanthropic behavior and epidemiological relevance is appropriate, it should be more directly aligned with the specific knowledge gap addressed by the study. The central objective remains somewhat unclear whether the study aims to demonstrate colonization, assess epidemiological risk, or discuss urban adaptation. At present, these aspects are addressed simultaneously, which dilutes the focus. Incorporating overlooked studies directly addressing the ecology and domiciliation of T. vitticeps including those cited above would significantly improve the contextual framework.

A: The introduction was modified stressing the demonstration of T. vitticeps collonization in rural areas in ES and MG, mentioning the previous records of colonization in wattle and daub houses in two other municipalities of RJ. In a near future, we plan to bring more details on ecology and epidemiological risks in the studied area. As recommended, we also added additional references in the discussion and mentioned separately the colonization and urban adaptation.

R1: The methodology presents several critical issues. Although the collection procedures are adequately described, there is no standardization of sampling effort. The study is based on a single inspection, which substantially limits inferences regarding stable colonization, and this limitation should be explicitly acknowledged. The selection of only 16 adults for molecular analysis also requires clearer justification, particularly given that 47 specimens were collected. Furthermore, restricting the analysis to engorged insects reduces representativeness and may introduce bias in the identification of blood meal sources.

A: Due to the relevance of the finding and the need to carry out control activities in the infested domicile we decided to focus in the protection of the residents rather than to extend the sampling efforts. That’s why just one search for insects was carried out. All the mentioned points were included the M&M and the official technical report elaborated by LINIRTT was included in the M&M as supplementary material.

Besides that, the results here presented are going to be crucial to support further studies, which are going to apply refined molecular methods to define the feeding sources of T. vitticeps in the area.

R1: The molecular section is functional but lacks detail expected by current standards. There is no information regarding the use of positive and negative controls for each primer in the blood meal analyses, nor is there discussion of potential cross-amplification. The statement that results were “adopted due to the rapidity and high quality of the analyses” is not appropriate for a scientific manuscript and should be removed or reformulated based on technical criteria.

A: More detailed and appropriate information on the molecular data was included in the manuscript.

R1: In the results section, a major concern is the classification of 13 out of 16 specimens as “possibly positive” for T. cruzi. In PCR-based analyses, results should be clearly defined as positive or negative according to objective criteria. The use of “possibly” undermines data reliability and raises methodological concerns. This issue should be clarified, whether in terms of band intensity, reproducibility, or predefined thresholds for positivity. Without such clarification, the validity of the results may be questioned.

A: The text was modified and the points mentioned were clarified and precisely presented in the results section.

R1: Additionally, the results could be presented in a more quantitative and structured manner. For example, the spatial distribution of insects within the household could be better organized in a table or described more objectively. Table 1 also requires revision to correct typographical errors and ensure consistent formatting.

A: Detailed results on spatial distribution of the insects in the house were added in the manuscript and the Table 1 was revised.

R1: The discussion represents the most sensitive section of the manuscript. Although it provides useful context, it relies on excessive extrapolation from a single sampling event. The text suggests broader changes in epidemiological dynamics and urban adaptation; however, the data are limited to a single household and a single collection event. The tone should therefore be moderated, clearly framing the findings as a punctual yet relevant record. In this regard, incorporating additional literature is essential to support and calibrate the interpretations. The recent study by Testai et al. (2026) is particularly relevant, as it offers an integrated perspective on vector and parasite ecology in the Atlantic Forest and could strengthen the discussion of infection patterns, parasite diversity, and spatial dynamics.

A: The tone about the infestation was moderated in the discussion, clearly framing the findings as a punctual yet relevant record. We also incorporated additional literature supporting and calibrating the interpretations.

R1: The discussion of oral transmission, although conceptually valid, appears somewhat disconnected from the data presented and may be perceived as overstating the study's implications. It would be more appropriate to maintain focus on domiciliary colonization and vector–human contact, as supported by the blood meal analysis.

A: We removed oral transmission paragraph.

R1: The section addressing anthropophily and blood meal sources is interesting but should also be interpreted with caution. The detection of human blood in seven individuals within a small sample size does not support robust conclusions about population-level feeding behavior. This finding should be presented as indicative rather than conclusive, ideally in comparison with previous studies demonstrating the species' eclectic feeding habits.

A: We agree with the considerations and the detection of human blood was presented as indicative rather than conclusive, and comparisons with previous studies demonstrating the species' eclectic feeding habits were included.

R1: A notable strength of the study is the identification of a colony under the roof, including eggs, nymphs, and exuviae, which provides clear evidence of colonization. This represents the core contribution of the manuscript and should be emphasized further, including direct comparisons with similar records reported in peridomiciliary environments in southeastern Brazil.

A: We agree and the colony found under the roof was emphasized and compared with similar records reported in peridomiciliary environments.

R1: It is also strongly recommended that the authors include a high-quality dorsal photograph of at least one collected specimen. This would allow proper documentation of the morphotype, support taxonomic identification, and enhance the overall robustness and transparency of the study.

A: A high-quality dorsal photograph was included in the manuscript.

R1: The final portion of the discussion combines recommendations, future perspectives, and outreach aspects in a somewhat diffuse manner. The mention of tools such as WhatsBarb is interesting but appears tangential to the main scientific argument and could be presented more concisely.

A: The final portion of the manuscript was reduced and the WhatsBarb was presented concisely.

R1: The conclusions are coherent but partially repetitive and employ somewhat strong language given the level of evidence. They should be made more objective and strictly aligned with the data presented.

A: We changed the conclusion making it more objective and strictly aligned with the results.

R1: From a technical standpoint, there are several minor issues throughout the manuscript, including inconsistencies in species nomenclature, errors in figure legends, and problems in references and formatting. Although these do not compromise the scientific content, they should be carefully revised, as they affect the overall perception of quality.

A: We have corrected the inconsistencies in species nomenclature, legends and references formatting.

R1: In summary, the manuscript has clear merit and publication potential, particularly due to the record of intradomiciliary colonization in an urban setting. However, it requires substantial revisions, including more rigorous data interpretation, clearer methodological descriptions (especially regarding PCR procedures), reduction of unsupported extrapolations, improved data presentation, inclusion of a dorsal image documenting the specimen, and incorporation of relevant overlooked literature. Addressing these points will significantly strengthen the manuscript and reduce the likelihood of predictable reviewer criticisms.

A: Thanks, all mentioned points were acknowledged, corrected and precisely reviewed.

Reviewer: 2

R2: Thank you for presenting this detailed assessment. I have some comments to help with clarity but overall, excellent presentation.

R2: Please comment on exactly why the Municipal Health Secretary was requested to the specific home? Was this because a resident at the home reconginzed a triatomine? Where they being bitten by teh bug? Chagas disease concerns? Please be more specific and include in "Study area and specimen collection" section.

A: Thanks for the relevant issues. The resident of the infested house had already got in touch with the Municipal Health Secretary and also sent us a photo of an insect. By the photo it was possible to identify the insect as a triatomine. Once the insect has been identified we got in touch immediately with the local Municipal Health Secretary together with the Environmental Secretary and we visited residence and proceeded with the searches for triatomines in the residence. Several details were included in the manuscript.

R2: How long was the investigation? Was it for one day? 8 hours? Did they return for multiple entomological assessments? Please put in the details of the investigation timeline.

A: We completed the exhaustive searches for triatomines in five hours, including all rooms and the peridomiciliary area. A report was presented to the Municipal Health Secretary together and the Environmental Municipal Secretary. After this, a control spraying was carried out in all compartments of the house. During the treatment and for five days the house was closed, and the residents were asked to moved to another residence. After a week, a second search was carried out to confirm the treatment against the triatomine infestation was effective. Twenty days later a contact with the residents of the infested house confirmed that no more insects were found in the house. All details were included in the M & M including the official technical report sent to the health authorities.

-R2: Figure 2 (D) shows fecal drop on the walls but how was this confirmed? Some insects will defecate on walls and it looks like triatomine feces. I suggest saying, "Inspection of internal structures of the dwelling and observation of suspected triatomine feces on the wall."

A: The fecal drops were found in just one of the rooms and it was concentrated in a specific area of the room near the celling. From the out side, the roof was inspected, the tiles were remove on the area were the fecal drops were found. In this inspected area of the roof, nynphs, eggs, exuviae and adults were found. Eventually, triatomines were also found in other rooms of the house as mentioned by the owner. It was evident that in one of the rooms, the triatomines were more frequently found. This point is, now properly addressed in the manuscript.

R2: Did you test for DTU I? Why not?

A: At this moment, no DTU was tested. We plan to extend the study in the area and carry out a more detailed search for triatomines and characterization of the T. cruzi DTUs; due to the epidemiologic importance of this finding, we decided the turn public the very first results as soon as possible. The perspective of continuation of the scientific research in the area is now presented in the manuscript.

R2: Please provide more details of the blood meal analysis. This is very briefly mentioned. Human blood was detected but documenting the other sources aids in understanding the extent to the other blood meal sources and will also help with the ecology of domestic invasion of a triatomine like this.

A: More detailed information was added in the manuscript. We thank the suggestion and we do plan to continue detailed studies in the area.

R2: I like the pictures of the triatomines and where they were found. This is impactful. I found myself wanting to see at least one adult picture which shows clear morphological features of the bug. This would also be impactful for readers.

A: We included detailed picture of the T. vitticeps that was infesting the house

R2: Please add in the Discussion the known prevalence of Chagas in this locality and in the Rio de Janeiro State.

A: Thanks for the suggestions We included additional information on this matter

R2: I would also add some sentences on the impact of a sylvatic vector invading into the peridomestic domain and now domicilatting a home. Why did this occur at this residence? As you mentioned this is a fragmented forest region and deforestation is being seen in many regions of Brasil and other regions where Chagas disease is endemic. Sylvatic and peridomestic vectors are now being seen more and more in these regions. I would make some comments about this and possible site some article displaying this:

A: A few lines discussing the domiciliary colonization was added as well as its significance. However, regarding the references below, we'd rather keeping the focus on the T. vitticeps findings to avoid a very long discussion session. Also the list of references in the manuscript giving support to the discussion arguments are already encompassing the issues presented in the references below somehow.

R2: Cantillo-Barraza O, Gual-González L, Velásquez-Ortiz N, Medina Camargo MA, González P, Cruz-Saavedra L, Castillo A, Zuluaga S, Herrera G, Cowan H, Velez-Mira A, Patiño LH, Ramírez JD, Triana O,

Nolan MS. Triatoma venosa and Panstrongylus geniculatus challenge the certification of interruption of vectorial Trypanosoma cruzi transmission by Rhodnius prolixus in eastern Colombia. PLoS Negl Trop Dis. 2025 Jan 27;19(1):e0012822.

R2: Meraz-Medina T, Vences-Blanco MO, Ramírez-Cervantes AG, Nogueda-Torres B, Martínez-Ibarra JA. First record and vital statistics of Triatoma longipennis (Heteroptera, Reduviidae, Triatominae) from Southern Mexico. Acta Trop. 2025 Nov;271:107873.

R2: Beatty NL, Bhosale CR, White ZS, Torhorst CW, Wilson KN, Dorleans R, Stenn TMS, Killets KC, Curtis-Robles R, Burkett-Cadena N, Nováková E, Hamer GL, Hamer SA, Wisely SM. Field evidence of Trypanosoma cruzi infection, diverse host use and invasion of human dwellings by the Chagas disease vector in Florida, USA. PLoS Negl Trop Dis. 2025 Jul 7;19(7):e0012920.

R2: Other examples exist. I recommend discussing the importance a bit more as it is a great example of the changing dynamics of Chagas disease within the Americas.

A: Thanks. A few more lines on this matter, mentioning the importance of the reported finding was added in the discussion.

Thank you for your work!

A: Thanks very much for the nice suggestions; all of them were acknowledged and properly answered.

SECOND REVIEW ROUND

REVIEWERS' COMMENTS

REVIEWER #1

No other comments.

REVIEWER #2

Authors have provided a much improved manuscript with the recommended additional content from the Reviewers. I have no additional suggestions.

DECISION: ACCEPT SUBMISSION | RECEIVED 06 FEBRUARY 2026 | ACCEPTED 16 JUNE 2026