Revisiting the Hybridization Processes in the Triatoma brasiliensis Complex (Hemiptera, Triatominae): Reproductive Isolation between Triatoma petrocchiae and T. b. brasiliensis and T. lenti

Luiza Maria Grzyb Delgado 1,4, Jader de Oliveira 2,3,4*, Amanda Ravazi 1, Fernanda Fernandez Madeira 4, Yago Visinho dos Reis 1, Heloisa Pinotti 3, Ana Beatriz Bortolozo de Oliveira 4, Isabella da Silva Masarin 1, Maurício Lilioso 5, Elaine Folly-Ramos 6, Cleber Galvão 7,*, Maria Tercília Vilela de Azeredo-Oliveira 4, João Aristeu da Rosa 3 and Kaio Cesar Chaboli Alevi 1,2,3

Abstract: Triatoma petrocchiae is a species morphologically similar to T. b. brasiliensis (which resulted in a synonymization event); despite this similarity, genetic, morphological, and experimental crossbreeding studies confirmed the specific status of T. petrocchiae. Considering that both species have been reported living in sympathy and that, for a long time, most species of the T. brasiliensis complex were considered only chromatic variants of T. b. brasiliensis, we carried out experimental crosses between T. b. brasiliensis and T. petrocchiae (to confirm whether these species are reproductively isolated)
between *T. lenti* and *T. petrocchiae* (to assess whether *T. petrocchiae* also presents prezygotic isolation with the other species of the *T. brasiliensis* complex). Reciprocal experimental crosses were conducted, and weekly, the eggs were collected, counted, and separated in new containers to assess the hatch rate. Neither cross resulted in hybrids, demonstrating that there are pre-zygotic reproductive barriers installed between *T. petrocchiae* and the other species of the *T. brasiliensis* complex. On the basis of the results above, we demonstrated that *T. petrocchiae* is reproductively isolated from *T. b. brasiliensis* and *T. lenti*. Furthermore, we suggest that *T. petrocchiae* is the species most derived from the *T. brasiliensis* complex.

**Keywords:** triatomines; reproductive barriers; hybridization; speciation; Chagas disease vectors

### 1. Introduction

Triatomines (Hemiptera, Triatominae) are hematophagous insects of great importance to public health, as they are considered the main form of transmission of the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), the etiological agent of Chagas disease [1] (a neglected disease that affects about 8 million people and results in 10,000 deaths per year [1]). There are currently 157 species grouped into 18 genera and five tribes [2–5]. The Triatomini and Rhodniini tribes have the largest number of species (115 and 24, respectively) and are the most important from an epidemiological point of view (since *Panstrongylus megistus* (Burmeister, 1835), *Triatoma infestans* Klug, 1834, *T. brasiliensis brasiliensis* Neiva, 1911, *T. dimidiata* (Latreille, 1811), and *Rhodnius prolixus* Stål, 1859 are of worldwide importance in the transmission of the disease [6]).

The genus *Triatoma* Laporte, 1832 is the most representative (81 species) and the most morphologically diversified [6,7]. This genus is paraphyletic [8,9], and species are grouped into complexes and subcomplexes [9–12]. The *T. brasiliensis* complex is a grouping of endemic species from Brazil [13] composed of six species and two subspecies that share a common ancestry: *T. b. brasiliensis* Neiva, 1911, *T. b. macromelasoma* Galvão, 1965, *T. juazeirensis* Costa and Félix, 2007, *T. sherlocki* Papa et al., 2002, *T. petrocchiae* Pinto and Barreto, 1925, *T. lenti* Sherlock and Serafin, 1967, *T. bahiensis* Sherlock and Serafin, 1967, and *T. melania* Neiva and Lent, 1941 [14–18]. The last taxon grouped in this complex was *T. petrocchiae* [18], a species reported in the states of Bahia, Ceará, Pernambuco, Paraíba, and Rio Grande do Norte [13,19]; however, the potential distribution map published by Caranha et al. [20] suggests that this species could also be found in the states of Piauí, Alagoas, and Sergipe, where the species has not been recorded to date.

The species *T. brasiliensis sensu stricto* is currently divided into two subspecies (*T. b. brasiliensis* and *T. b. macromelasoma*), which can be differentiated by morphological characters: *T. b. brasiliensis* presents a pronotum with 1 + 1 brownish-yellow areas extending from the posterior portion of the anterior lobe to the posterior lobe, femora with broad brownish-yellow rings, and membrane of hemelytra with a lumen of cells that are not darkened; *T. b. macromelasoma* presents a pronotum with 1 + 1 narrow brownish-yellow stripes on the submedian carinae, not attaining its apex, legs with an incomplete brownish-yellow ring on the apical half of the femora, and hemelytra with membrane cells that are darkened on the central portion [21]. In addition to the phenotypic divergences, these species have a different geographic distribution: while *T. b. brasiliensis* has been noted in the states of Ceará, Maranhão, Paraíba, Piauí, and Rio Grande do Norte, *T. b. macromelasoma* is endemic to Pernambuco [13,19].

*Triatoma petrocchiae* is a species morphologically similar to *T. b. brasiliensis*, which led Lucena [22] to propose the synonymization of species, considering *T. petrocchiae* only as a chromatic variant of *T. b. brasiliensis*. However, Espinola [23] carried out experimental crosses between *T. b. brasiliensis* and *T. petrocchiae* from Paulo Afonso, Bahia, Brazil, and observed that these species did not produce viable hybrids. On the basis of this, Lent and
Insects Wygodzinsky [24] revalidated the specific status of *T. petrocchiae* from morphological data (the status was corroborated with genetic analyses using allozyme electrophoresis [25]). The interspecific crosses performed by Espinola [23] were proposed because chromatic variations were observed in the populations of *T. b. brasiliensis* from Paulo Afonso, Bahia. The authors indicated that there are similarities in the coloration between *T. b. brasiliensis* and *T. petrocchiae* and, above all, these species share the same ecological niche. Considering that these species have been reported living in sympatry [26,27] and that, in 1971, most species of the *T. brasiliensis* complex were still considered only chromatic variants of *T. b. brasiliensis*, there is a need to confirm whether *T. b. brasiliensis* and *T. petrocchiae* are really reproductively isolated (mainly because all other species in this complex are capable of producing hybrids [15,28–32]). On the basis of the assumptions above, we carried out experimental crosses between *T. b. brasiliensis* and *T. petrocchiae* (to corroborate the results of Espinola [23]) and between *T. lenti* and *T. petrocchiae* (to assess whether *T. petrocchiae* also presents prezygotic isolation with the other species of the *T. brasiliensis* complex).

2. Materials and Methods

Reciprocal experimental crosses were conducted between *T. b. brasiliensis* (from Currais Novos (Pedra do Sino), Rio Grande do Norte, Brazil, collected in wild ecotopes (geographic coordinates: 6°17’06.8" S 36°29’51.9" W) and *T. petrocchiae* (from Caiçá, Rio Grande do Norte, Brazil, collected in wild ecotopes (geographic coordinates: 6°27’47.6" S 37°09’11.3" W)) and between *T. lenti* (from Macaubas, Bahia, Brazil, collected in peridomical ciliary ecotopes (geographic coordinates: 13°11’25.7" S 42°31’56.3" W)) and *T. petrocchiae* (Figure 1). The insects used in the experiment came from colonies kept in the Triatominae insectary of the School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, São Paulo, Brazil. The experimental crosses were conducted in the Triatominae insectary, according to the experiments of Mendonça et al. [30], Neves et al. [33], and Pinotti et al. [32]: the insects were sexed as 5th instar nymphs [34], and males and females were kept separately until they reached the adult stage to guarantee the virginity of the insects used in the crosses. For the experimental crosses, three couples from each set were placed in plastic jars (diameter 5 cm × height 10 cm) (each couple in a jar) and kept at room temperature (average of 24 °C [35]) and an average relative humidity of 63% [35]). Weekly, the couples were fed on duck blood, and the eggs were collected, counted, and separated into new containers to assess the hatch rate.

![Figure 1. Distribution map of species used in experimental crosses.](image-url)

### 3. Results and Discussion

The experimental crosses between *T. petrocchiae* and *T. b. brasiliensis* did not result in hybrids (Table 1), demonstrating that there are pre-zygotic reproductive barriers installed...
between these species (confirming the specific status of *T. petrocchiae* according to the biological species concept [36,37]). These results obtained for the cross between *T. b. brasiliensis* and *T. petrocchiae* from Rio Grande do Sul (the state where the species were also collected in the same rock outcrop spot [38]) agree with those obtained by Espínola [23] when they crossed specimens from Paulo Afonso, Bahia.

Table 1. Experimental crosses performed between *T. petrocchiae* x *T. b. brasiliensis* and *T. lenti*.

| Crossing Experiments | Number of Eggs | Total | Egg Fertility |
|----------------------|----------------|-------|---------------|
|                      | C1    | C2    | C2          |       |
| ♀ *T. b. brasiliensis* x *T. petrocchiae* ♂ | 48    | 40    | 56          | 144   | 0% |
| ♀ *T. petrocchiae* x *T. b. brasiliensis* ♂ | 38    | 45    | 37          | 120   | 0% |
| ♀ *T. lenti* x *T. petrocchiae* ♂ | 42    | 33    | 27          | 102   | 0% |
| ♀ *T. petrocchiae* x *T. lenti* ♂ | 36    | 28    | 22          | 86    | 0% |

Whereas in 1971, the current species *T. melanica*, *T. sherlocki*, and *T. juazeirensis* were considered only phenotypic variants and/or subspecies of *T. b. brasiliensis* [14,39–42], we conducted a survey of the literature on the triatomine already noted in Paulo Afonso, Bahia to confirm which species Espínola [22] had crossed with *T. petrocchiae*, and we observed that only *T. b. brasiliensis* and *T. petrocchiae* were the species of the *T. brasiliensis* complex notifi ed for the municipality [43]. Furthermore, to ensure that the *T. brasiliensis* complex triatomines collected in Paulo Afonso, Bahia, were correctly identified as *T. b. brasiliensis* and *T. petrocchiae*, we evaluated some specimens collected in this municipality that were deposited in the entomologic collections of the Faculty of Public Health of the University of Sao Paulo, Brazil (Figure 2). On the basis of this information, we confirmed that the specimens used in the experiments of Espínola [23] were *T. b. brasiliensis*.

Figure 2. Triatomines deposited in the entomologic collections of the Faculty of Public Health of the University of Sao Paulo, Brazil. (A, B) *T. b. brasiliensis* ♀ (Brazil, Paulo Afonso, BA. Ident. Sherlock/68); (C) *T. b. brasiliensis* ♂ (Brazil, Paulo Afonso, BA. Ident. Sherlock/68); (D, E) *T. petrocchiae* ♂ (Brazil, Paulo Afonso, BA. Ident. Sherlock/62).
Although Espinola [23] suggested that *T. petrocchiae* and *T. b. brasiliensis* share the same ecological niche, Liloso et al. [27] recently demonstrated that while *T. b. brasiliensis* is mainly associated with rodents, the food sources of *T. petrocchiae* were strongly associated with reptiles of the *Tropidurus* and *Hemidactylus* genera; this suggests that *T. petrocchiae* is the single member within this complex that is associated with reptiles, indicating a distinct niche occupation related to the trophic resources. These results point to the possible presence of a prezygotic reproductive barrier due to ecological isolation between *T. petrocchiae* and members of the *T. brasiliensis* complex. However, other possible prezygotic barriers cannot be ruled out, such as mechanical isolation, as the morphological analysis of the external female genitalia evidenced some unique characteristics for *T. petrocchiae* [44].

Experimental crosses between *T. petrocchiae* and *T. lenti* also did not result in hybrids (Table 1). Unlike *T. b. brasiliensis*, which cohabits rock outcrops with *T. petrocchiae* [27,38], there are no reports of *T. petrocchiae* and *T. lenti* living in sympatry (on the contrary, they inhabit municipalities in the state of Bahia that are at least 800 km away [43]). This result is in accordance with what was proposed by Oliveira et al. [18], which suggests that *T. petrocchiae* is the most distant species from the *T. brasiliensis* complex. The genomic incompatibility resulting in the inability to produce hybrids with *T. lenti* points to the hypothesis that *T. petrocchiae* was possibly the first species to be derived from the common ancestor of the *T. brasiliensis* complex (since all other species in the complex produce hybrids [15,28–32]).

The prezygotic isolation observed between *T. petrocchiae* and species of the *T. brasiliensis* complex was only observed when members of this complex (*T. b. brasiliensis*) were crossed with other subcomplexes, such as *T. sordida* [45], *T. infestans* [45], and *T. viticeps* subcomplexes [33] (which are species phylogenetically distant from *T. b. brasiliensis* [8,9]). The reproductive barrier that possibly prevents hybrids between other species of the *T. brasiliensis* complex is based on post-zygotic reproductive isolation due to hybrid collapse (as noted by Mendonça et al. [30] and Alevi et al. [31]). It was believed that *T. melanica* was the most differentiated form of the complex [37]; however, according to the results of experimental crosses and the high genetic distance observed between *T. petrocchiae* and members of the *T. brasiliensis* complex [46], the most differentiated species from a genetic point of view is *T. petrocchiae*.

Before concluding, it is worth mentioning that the low number of eggs produced by crosses can be a limiting factor for the research. Furthermore, it is important that new crosses between *T. petrocchiae* and all members of the *T. brasiliensis* complex be carried out to confirm that this species is indeed reproductively isolated from all other species in the complex or whether prezygotic isolation is restricted to *T. b. brasiliensis* and *T. lenti* (as noted by Espinola et al. [23] and in the present manuscript).

4. Conclusions

On the basis of the results above, we demonstrated that *T. petrocchiae* is reproductively isolated from *T. b. brasiliensis* and *T. lenti* (confirming the specific status of *T. petrocchiae*). Furthermore, we demonstrated that these species have prezygotic reproductive isolation and suggest that *T. petrocchiae* is the species most derived from the *T. brasiliensis* complex.

**Author Contributions:** Conceptualization, L.M.G.D., J.d.O., C.G., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; methodology, L.M.G.D., J.d.O., A.R., H.P., F.F.M., Y.V.d.R., A.B.B.d.O., I.d.S.M., M.L., E.F.-R. and K.C.C.A.; formal analysis, L.M.G.D., J.d.O., A.R., H.P., F.F.M., Y.V.d.R., A.B.B.d.O., I.d.S.M., M.L., E.F.-R. and K.C.C.A.; investigation, L.M.G.D., J.d.O., A.R., C.G., F.F.M., H.P., Y.V.d.R., A.B.B.d.O., I.d.S.M., M.L., E.F.-R., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; resources, L.M.G.D., C.G., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; writing—original draft preparation, L.M.G.D. and K.C.C.A.; writing—review and editing, L.M.G.D., J.d.O., A.R., C.G., F.F.M., Y.V.d.R., A.B.B.d.O., M.L., E.F.-R., M.T.V.d.A.-O., J.A.d.R. and K.C.C.A.; supervision, J.d.O. and K.C.C.A.; project administration, L.M.G.D. and K.C.C.A.; funding acquisition, L.M.G.D., C.G., M.T.V.d.A.-O., and K.C.C.A. All authors have read and agreed to the published version of the manuscript.
Funding: This research was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil)—Finance Code 001, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All relevant data are within the manuscript.

Acknowledgments: We thank Maria Anice Mureb Sallum for loaning the specimens of T. b. brasilien-
sis and T. petrocchiae deposited in “Coleção Entomológica de Referência” of the Faculdade de Saúde Pública (FSP/USP), Brazil. We also thank Vagner José Mendonça for his support in collecting T. lentii.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. Chagas Disease (American Trypanosomiasis). Available online: http://www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis) (accessed on 7 October 2021).

2. Alevi, K.C.C.; De Oliveira, J.; Garcia, A.C.C.; Cristal, D.C.; Delgado, L.M.G.; Bittinelli, I.D.F.; Dos Reis, Y.V.; Ravazi, A.; De Oliveira, A.B.B.; Galvão, C.; et al. Triatoma rosai sp. nov. (Hemiptera, Triatominae): A new species of Argentinian Chagas disease vector described based on integrative taxonomy. *Insects* **2020**, *11*, 830. [CrossRef]

3. Zhao, Y.; Galvão, C.; Cai, W. *Rhodnius micki*, a new species of Triatomaíneae (Hemiptera, Reduviidae) from Bolivia. *ZooKeys* **2021**, *1012*, 71–93. [CrossRef]

4. Costa, J.; Dale, C.; Galvão, C.; Almeida, C.E.; Dujardin, J.P. Do the new triatomine species pose new challenges or strategies for monitoring Chagas disease? An overview from 1979–2021. *Mem. Inst. Oswaldo Cruz* **2021**, *116*, e210015. [CrossRef]

5. Dale, C.; Justi, S.A.; Galvão, C. *Belminus santosmalletae* (Hemiptera: Heteroptera: Reduviidae): New Species from Panama, with an Updated Key for Belminus Stål, 1859 Species. *Insects* **2021**, *12*, 686. [CrossRef]

6. Galvão, C. *Vetores da Doença de Chagas No Brasil*, 1st ed.; Sociedade Brasileira de Zoologia: Curitiba, Brazil, 2014.

7. Galvão, C. Taxonomia dos vetores da doença de Chagas da forma à molécula, quase três séculos de história. In *Atualidades em Medicina Tropical No Brasil: Vetores*; Oliveira, J., Alevi, K.C.C., Camargo, L.M.A., Menegueti, D.U.O., Eds.; Strictu Sensu Editora: Porto Alegre, Brazil, 2020; pp. 9–37.

8. Justi, S.A.; Russo, C.A.M.; Mallet, J.R.D.S.; Obara, M.T.; Galvão, C. Molecular phylogeny of Triatomini (Hemiptera: Reduviidae: Triatominae). *Parasites Vectors* **2014**, *7*, 149. [CrossRef]

9. Justi, S.A.; Galvão, C.; Schrago, C.G. Geological changes of the Americas and their influence on the diversification of the Neotropical kissing bugs (Hemiptera: Reduviidae: Triatominae). *PLoS Negl. Trop. Dis.* **2016**, *10*, 4. [CrossRef]

10. Schofield, C.J.; Galvão, C. Classification, evolution, and species groups within the Triatominae. *Acta Trop.* **2009**, *110*, 88–100. [CrossRef]

11. Pita, S.; Lorite, P.; Nattero, J.; Galvão, C.; Alevi, K.; Teves, S.C.; Azeredo-Oliveira, M.T.; Panzera, F. New arrangements on several species subcomplexes of *Triatoma* genus based on the chromosomal position of ribosomal genes (Hemiptera -Triatominae). *Infect. Genet. Evol.* **2016**, *43*, 225–231. [CrossRef][PubMed]

12. Alevi, K.C.C.; Oliveira, J.; Azeredo-Oliveira, M.T.V.; Rosa, J.A. *Triatoma vitticeps* subcomplex (Hemiptera, Reduviidae, Triatominae): A new grouping of Chagas disease vectors from South America. *Parasites Vectors* **2017**, *10*, 180. [CrossRef]

13. Costa, J.; Dornak, L.L.; Almeida, C.E.; Peterson, A.T. Distributional potential of the *Triatoma brasiliensis* species complex at present and under scenarios of future climate conditions. *Parasites Vectors* **2014**, *7*, 238. [CrossRef]

14. Monteiro, F.A.; Donnelly, M.J.; Beard, C.B.; Costa, J. Nested clade and phylogeographic analyses of the Chagas disease vector *Triatoma brasiliensis* in Northeast Brazil. *Mol. Phylogenet. Evol.* **2004**, *32*, 46–56. [CrossRef]

15. Mendonça, V.J.; Alevi, K.C.C.; Pinotti, H.; Gurgel-Goçalves, R.; Pita, S.; Guerra, A.L.; Panzera, F.; de Araújo, R.F.; de Azeredo-Oliveira, M.T.V.; da Rosa, J.A. Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the *T. brasiliensis* species complex. *Zootaxa* **2016**, *4107*, 239–254.

16. Mendonça, V.J.; Silva, M.T.A.; Araújo, R.F.; Martins Júnior, J.; Bacci Júnior, M.; Almeida, C.E.; Costa, J.; Graminha, M.; Cicarelli, R.M.B.; Rosa, J.A. Phylogeny of *Triatoma sherlocki* (Hemiptera: Reduviidae: Triatominae) inferred from two mitochondrial genes suggests its location within the *Triatoma brasiliensis* complex. *Am. J. Trop. Med. Hyg.* **2009**, *81*, 856–864. [CrossRef]

17. Gardim, S.; Almeida, C.E.; Takiya, D.; Oliveira, J.; Araújo, R.F.; Cicarelli, R.M.; da Rosa, J.A. Multiple mitochondrial genes of some sylvatic Brazilian *Triatoma*: Non-monophyly of the *T. brasiliensis* subcomplex and the need for a generic revision in the Triatomini. *Infect. Genet. Evol.* **2014**, *23*, 74–79. [CrossRef]

18. Oliveira, J.; Marcat, P.L.; Takiya, D.M.; Mendonça, V.J.; Belintani, T.; Bargues, M.D.; Mateo, L.; Chagas, V.; Folly-Ramos, E.; Cordeiro-Estréla, P.; et al. Combined phylogenetic and morphometric information to delimitand unify the *Triatoma brasiliensis* species complex and the Brasiliensis subcomplex. *Acta Trop.* **2017**, *170*, 140–148. [CrossRef]

19. Dale, C.; Almeida, C.E. An updated and illustrated dichotomous key for the Chagas disease vectors of *Triatoma brasiliensis* species complex and their epidemiologic importance. *ZooKeys* **2018**, *805*, 33. [CrossRef][PubMed]
20. Caranha, L.; Gurgel-Goçalves, R.; Ramalho, R.D.; Galvão, C. New records and geographic distribution map of Triatoma petrochii Pinto and Barreto, 1925 (Hemiptera: Reduviidae: Triatominae). Check List. 2011, 7, 508–509. [CrossRef]
21. Costa, J.; Correia, N.C.; Neiva, V.L.; Cristina, T.; Goçalves, M.; Felix, M. Revalidation and redescription of Triatoma brasiliensis macromelasoma Galvão, 1956 and an identification key for the Triatoma brasiliensis complex (Hemiptera: Reduviidae: Triatominae). Mem. Inst. Oswaldo Cruz. 2013, 108, 785–789. [CrossRef] [PubMed]
22. Lucena, D. Estudos sobre a doença de Chagas no Brasil. Rev. Soc. Bras. Med. Trop. 1970, 22, 3–173.
23. Espínola, H. Reproductive isolation between Triatoma brasiliensis Neiva, 1911 and Triatoma petrophilii Pinto & Barreto, 1925 (Hemiptera Reduviidae). Rev. Bras. Biol. 1971, 31, 277–281. [PubMed]
24. Lent, H.; Wygodzinsky, P. Revision of the Triatominae (Hemiptera: Reduviidae) and their significance as vectors of Chagas disease. Bull. Am. Mus. Nat. Hist. 1979, 163, 123–520.
25. Monteiro, F.A.; Costa, J.; Sole-Cava, A.M. Genetic confirmation of the specific status of Triatoma petrophilii (Hemiptera: Reduviidae: Triatominae). Ann. Trop. Med. Parasitol. 1998, 92, 897–900. [CrossRef] [PubMed]
26. Lima-Oliveira, T.M.; Fontes, F.V.H.M.; Lilloso, M.; Pires-Silva, D.; Teixeira, M.M.G.; Meza, J.G.V.; Harry, M.; Fileé, J.; Costa, J.; Valencia-Barbosa, C.; et al. Molecular eco-epidemiology on the sympatric Chagas disease vectors Triatoma brasiliensis and Triatoma petrochii. Ecotopes, genetic variation, natural infection prevalence by trypanosomatids and parasite genotyping. Acta Trop. 2020, 201, 105188. [CrossRef]
27. Lilloso, M.; Pires-Silva, D.; Fontes, F.V.H.M.; Oliveira, J.; Da Rosa, J.A.; Vilela, R.; Folly-Ramos, E.; Almeida, C.E. Triatoma brasiliensis (Hemiptera: Reduviidae, Triatominae): A Chagas disease vector of T. brasiliensis species complex associated to reptiles. Infect. Genet. Evol. 2020, 82, 104307. [CrossRef]
28. Costa, J.; Almeida, C.E.; Dujardin, J.P.; Beard, C.B. Crossing experiments detect genetic incompatibility among populations of Triatoma brasiliensis Neiva, 1911 (Hemiptera, Reduviidae, Triatominae). Mem. Inst. Oswaldo Cruz. 2003, 98, 637–639. [CrossRef] [PubMed]
29. Correia, N.; Almeida, C.E.; Lima-Neiva, V.; Gumiel, M.; Lima, M.M.; Medeiros, L.M.O.; Rosa, J.A.; Costa, J. Crossing experiments confirm Triatoma sherlocki as a member of the Triatoma brasiliensis species complex. Acta Trop. 2013, 128, 162–167. [CrossRef] [PubMed]
30. Mendonça, V.J.; Alevi, K.C.C.; de Oliveira Medeiros, L.M.; Nascimento, J.D.; Azeredo-Oliveira, M.T.V.; Rosa, J.A. Cytogenetic and morphologic approaches of hybrids from experimental crosses between Triatoma lenti Sherlock & Serafim, 1967 and T. sherlocki Papa et al., 2002 (Hemiptera: Reduviidae). Infect. Genet. Evol. 2014, 26, 123–131. [PubMed]
31. Alvei, K.C.C.; Pinotti, H.; Araújo, R.F.; Azeredo-Oliveira, M.T.V.; Rosa, J.A.; Mendonça, V.J. Hybrid collapse confirm the specific status of Triatoma bahiensis Sherlock & Serafim, 1967 (Hemiptera, Triatominae). Am. J. Trop. Med. Hyg. 2018, 98, 475–477. [CrossRef]
32. Pinotti, H.; Oliveira, J.; Ravazi, A.; Madeira, F.F.; Reis, Y.V.; Oliveira, A.B.B.; Azeredo-Oliveira, M.T.V.; Rosa, J.A.; Alevi, K.C.C. Revisiting the hybridization processes in the Triatoma brasiliensis complex (Hemiptera, Triatominae): Interspecific genomic compatibility point to a possible recent diversification of the species grouped in this monophyletic complex. PLoS ONE 2021, 16, e0257992. [CrossRef] [PubMed]
33. Neves, S.J.M.; Sousa, P.S.; Oliveira, J.; Ravazi, A.; Madeira, F.F.; Reis, Y.V.; de Oliveira, A.B.B.; Pinotti, H.; de Azeredo-Oliveira, M.T.V.; da Rosa, J.A.; et al. Prezygotic isolation confirms the exclusion of Triatoma melanocephala, T. vitticeps and T. tibiamaculata of the T. brasiliensis subcomplex (Hemiptera, Triatominae). Infect. Genet. Evol. 2020, 79, 104149. [CrossRef]
34. Rosa, J.A.; Barata, J.M.S.; Barelli, N.; Santos, J.L.F.; Belda Neto, F.M. Sexual distinction between 5th instar nymphs of six species (Hemiptera: Reduviidae). Mem. Inst. Oswaldo Cruz. 1992, 87, 257–264. [CrossRef]
35. Olaia, N.; Alevi, K.C.C.; de Oliveira, J.; Cacini, G.L.; Souza, E.D.S.; Pinotti, H.; da Silva, L.A.; da Rosa, J.A. Biology of Chagas disease vectors: Biological cycle and emergence rates of Rhodnius marabanhaensis Souza et al., 2016 (Hemiptera, Reduviidae, Triatominae) under laboratory conditions. Parasitol. Res. 2021, 120, 2939–2945. [CrossRef] [PubMed]
36. Mayr, E. Populações, Espécies e Evolução, 1st ed.; Editora Nacional: São Paulo, Brazil, 1963.
37. Mayr, E. Populations, Species, and Evolution; Harvard University Press: Cambridge, MA, USA, 1970.
38. Almeida, C.E.; Faucher, L.; Lavina, M.; Costa, J.; Harry, M. Molecular individual-based approach on Triatoma brasiliensis: Inferences on Triatoma foci, Trypanosoma cruzi natural infection prevalence, parasite diversity and feeding sources. PLoS Negl. Trop. Dis. 2016, 10, e004447. [CrossRef] [PubMed]
39. Cerqueira, R.L. Estudos sobre populações de triatomíneos silvestres encontrado em Santo Inácio—Bahia. Tese de Doutorado, Universidade de São Paulo, São Paulo, Brazil, 1982.
40. Costa, J.; Argolo, A.M.; Felix, M. Redescription of Triatoma melancora Neiva & Lent, 1941, New Status (Hemiptera: Reduviidae: Triatominae). Zootaxa 2006, 385, 47–52.
41. Costa, J.; Felix, M. Triatoma juazeirensis sp. nov. from the state of Bahia, Northeastern Brazil (Hemiptera: Reduviidae: Triatominae). Mem. Inst. Oswaldo Cruz. 2007, 102, 87–90. [CrossRef] [PubMed]
42. Papa, A.R.; Jurberg, J.; Carvalhalho, R.U.; Cerqueira, R.L.; Barata, J.M.S. Triatoma sherlocki sp. n. coletada na Bahia, Brasil (Hemiptera, Reduviidae, Triatominae). Entomol. Vect. 2002, 9, 133–146.
43. Souza, O.M.F.; Santos, C.G.S.; Santos, R.F.; Fonseca, E.O.L.; Lima, A.G.D. Triatomíneos da Bahia: Manual de Identificação e Orientações Para o Serviço; Editora Oxente: Paulo Afonso, Brazil, 2020.
44. Oliveira, J.; Almeida, C.E.; Mendonça, V.J.; Alevi, K.C.C.; Costa, J.; Rosa, J.A. Triatoma brasiliensis Species Complex: Characterization of the external female genitalia. J. Vector Ecol. 2020, 45, 57–68. [CrossRef]
45. Perlowagora-Szumlewics, A.; Correia, M.V. Induction of male sterility manipulation of genetic mechanisms present in vector species of Chagas disease (remarks on integrating sterile-male release with insecticidal control measures against vectors of Chagas disease). *Rev. Inst. Med. Trop. São Paulo* **1972**, *14*, 560–371.

46. Guerra, A.L.; Borsatto, K.C.C.; Pagliusi, N.D.; Madeira, F.F.; Oliveira, J.; Rosa, J.A.; Azeredo-Oliveira, M.T.V.; Alevi, K.C.C. Revisiting the Homoploid Hybrid Speciation Process of the *Triatoma brasiliensis macromelasoma* Galvão, 1956 (Hemiptera, Triatominae) Using Cytogenetic and Molecular Markers. *Am. J. Trop. Med. Hyg.* **2019**, *100*, 911–913. [CrossRef]