Triatoma vitticeps (Stal, 1859) (Hemiptera, Triatominae): A Chagas Disease Vector or a Complex of Vectors?

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Abstract. *Triatoma vitticeps* is a Chagas disease vector that was found infected with *Trypanosoma cruzi* in homes. As this species is endemic from Brazil (Bahia, Espírito Santo, Minas Gerais, and Rio de Janeiro) and no study comparing the specimens from different Brazilian states was conducted, we analyzed the genetic distance (16S rDNA, Cyt b, and COI mitochondrial genes) and the chromosomal characteristics for *T. vitticeps* from Minas Gerais, Rio de Janeiro, and Espírito Santo. All specimens showed the same cytogenetic characteristics. On the other hand, the different mitochondrial genes demonstrated high intraspecific variation between the genetic distances of *T. vitticeps* from different states ranging from 2.3% to 7.2%. Based on this, our results suggest that possibly what is characterized as *T. vitticeps* is a complex of cryptic species (or subspecies).

The Chagas disease is caused by the protozoan *Trypanosoma cruzi* (Chagas, 1909) and transmitted to humans mainly through contact with feces of insects from the Triatominae subfamily. This disease is among the main neglected diseases in Latin America and is estimated that approximately 6–7 million people are infected worldwide; in Brazil there are about 2–3 million people who are infected with *T. cruzi*.^{1,2}

The vector control is the main way to minimize the incidence of new cases of Chagas disease. In Brazil, after significant reduction of vectors, the number of cases with the acute form of Chagas disease has been reduced dramatically.^{3,4} Characterizing the different species of the triatomines and the structure of the populations of these vectors can assist in the activities of vector control programs because there are species with greater or lesser degrees of importance in the transmission of *T. cruzi*.^{4,5}

Triatoma vitticeps (Stål, 1859) is an endemic species from Brazil, distributed in the states of Bahia, Espírito Santo, Minas Gerais, and Rio de Janeiro.⁵ This triatomine was initially characterized as a sylvatic species,⁶ but adults of *T. vitticeps* often invade houses attracted by light and form small colonies in domiciliary regions.⁷ In addition, worrying rates of domiciliary capture occurred in Rio de Janeiro^{8,9} and high rates of *T. cruzi* infection were observed in *T. vitticeps* captured in homes of Espírito Santo,⁷ although it has been shown that this species does not have the habit of defecating during hematophagy.¹⁰

Souza et al.¹¹ analyzed the population dynamics of *T. vitticeps* in the state of Minas Gerais, by means of morphometry, isoenzymes, and random amplification of polymorphic DNA. The authors observed migration between domestic, peridomiciliary, and sylvatic habitats and gene flow between the sylvatic and domestic habitats. However, as no study comparing the specimens from different Brazilian states was conducted, we analyzed the genetic (mitochondrial

markers—16S rDNA, Cyt b, and COI genes) and cytogenetic aspects (karyotype and heterochromatin) of *T. vitticeps* from Minas Gerais, Rio de Janeiro, and Espírito Santo.

Ten adult males of each Brazilian state were used for cytogenetic analysis. The specimens were provided by the insectariums of FCFAR/UNESP, Araraquara, São Paulo, Brazil, and of the IOC/FIOCRUZ, Rio de Janeiro, Brazil. The seminiferous tubules were torn apart, crushed, and fixed on slides in liquid nitrogen. The cytogenetic technique of C-banding¹² was then applied for the characterization of chromosome number and heterochromatin pattern and the analysis was performed using a Jenaval light microscope (Zeiss) attached to a digital camera and an Axio Vision LE 4.8 image analyzer (Copyright 2006–2009 Carl Zeiss Imaging Solutions GmbH). For the analysis of the genetic distance between *T. vitticeps* coming from different states of the southeastern region of Brazil were used sequences deposited in the GenBank (Table 1), and the Tamura 3-parameter model was used to calculate genetic distances pairwise in the MEGA 6.0 software.¹³ The specimens used in cytogenetic studies were from the same populations of the material used for the molecular analysis.^{14–16}

All specimens analyzed showed the same cytogenetic characteristics: karyotype $2n = 24$ ($20A + X_1X_2X_3Y$) and heterochromatin restricted to only one of the X sex chromosomes and in the Y sex chromosome (Figure 1). These results demonstrate that *T. vitticeps* does not present intraspecific chromosome variation as already observed for other triatomines.¹⁷

On the other hand, the different mitochondrial genes analyzed demonstrated high intraspecific variation between the genetic distances of *T. vitticeps* from different states ranging from 2.3% to 7.5% (Tables 2–4). Pereira et al.¹⁸ consider that

TABLE 1

Sequences of *Triatoma vitticeps* coming from different Brazilian states deposited in the GenBank

<i>T. vitticeps</i>	COI	Cyt b	16S
Espírito Santo	AF021219	KF826896	EU827202
Rio de Janeiro	KC249397	KC249304	KC249088
Minas Gerais	KC249395	KC249301	KC249085

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FIGURE 1. Metaphase I of *Triatoma vitticeps* stained by C-banding. Note the karyotype $2n = 24$ ($20A + X_1X_2X_3Y$) and heterochromatin restricted to only one of the X sex chromosomes and in the Y sex chromosome. X = X sex chromosome; Y = Y sex chromosome. Bar: 10 μ m. This figure appears in color at www.ajtmh.org.

taxa with a genetic distance greater than 2% represent new species that are possibly cryptic. For Triatominae, a genetic distance of 2.48%, associated with other tools, already supports specific status (as recently observed for *Triatoma bahiensis* Sherlock & Serafim, 1967).¹⁹

Based on this, different authors evaluated the specific status of triatomines by means of genetic distance using mitochondrial genes: Alevi et al.²⁰ through the 16S gene corroborates the specific status of *Triatoma pintodiasi* Jurberg et al. (2013); Alevi et al.²¹ through the 16S gene also have proposed the synonymization between *Nesotriatoma bruneri* Usinger (1944) and *Nesotriatoma flavida* (Neiva, 1911); Ceretti-Junior et al.¹⁴ based on the 16S gene reported low intraspecific variation between genetic distances of triatomines (0–0.6%), except for relationships between *Triatoma brasiliensis* Neiva (1911) specimens (1.3%) and *Triatoma sordida* (Stål, 1859) specimens (1%) from different geographic populations (specimens that possibly suffered cryptic speciation)^{22,23}; Panzera et al.²³ using the COI gene observed a genetic distance of 5.3% between *T. sordida* from Brazil and from Argentina and suggest that

TABLE 2

Genetic distances for different populations of *Triatoma vitticeps* with the mitochondrial genes: COI

<i>T. vitticeps</i>	(1)	(2)	(3)
(1) Espírito Santo	0.000	–	–
(2) Rio de Janeiro	0.068	0.000	–
(3) Minas Gerais	0.072	0.075	0.000

TABLE 3

Genetic distances for different populations of *Triatoma vitticeps* with the mitochondrial genes: Cyt b

<i>T. vitticeps</i>	(1)	(2)	(3)
(1) Espírito Santo	0.000	–	–
(2) Rio de Janeiro	0.050	0.000	–
(3) Minas Gerais	0.067	0.063	0.000

T. sordida from Argentina is possibly a new cryptic species of *T. sordida*; Abad-Franch et al.²⁴ used the genetic distance of cyt b (8.7%) between *Rhodnius robustus* Larrousse (1927) and *Rhodnius barretti* to characterize that *R. barretti* represents a distinct entity within the “robustus lineage”; and Rua et al.²⁵ suggested that populations of *Triatoma sanguisuga* (Leconte, 1855) are divided into two subspecies because they have genetic distances close to five for cyt b.

Recently, *T. vitticeps* and *Triatoma melanocephala* were grouped in the monophyletic subcomplex *T. vitticeps* because they presented karyotypic differences when compared with the other triatomines in South America.²⁶ As observed for *T. vitticeps*, other populations of triatomines (e.g., *T. brasiliensis*) also did not present intraspecific chromosomal variations²⁷ and presented genetic distances²² that supported the description of new species. Alevi et al.²⁸ highlighted that gene evolution and chromosomal evolution are events mediated by different factors, being that the events that may lead to variations in DNA sequences are more common than those events that lead to chromosomal changes.

Thus, our results suggest that possibly what is characterized as *T. vitticeps* is a complex of cryptic species (or subspecies). We suggest that an extensive phylogeographic study (possibly with tools that characterize the presence of gene flow) be performed in *T. vitticeps* and be associated with taxonomic tools, such as experimental crosses between different populations of *T. vitticeps*, to evaluate the specific status of this vector of Chagas’ disease endemic to Brazil.

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TABLE 4

Genetic distances for different populations of *Triatoma vitticeps* with the mitochondrial genes: 16S rDNA

<i>T. vitticeps</i>	(1)	(2)	(3)
(1) Espírito Santo	0.000	–	–
(2) Rio de Janeiro	0.031	0.000	–
(3) Minas Gerais	0.023	0.023	0.000

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REFERENCES

- Martins-Melo FR, Ramos AN, Alencar CH, Heukelbach J, 2014. Prevalence of Chagas disease in Brazil: a systematic review and meta-analysis. *Acta Trop* 130: 167–174.
- World Health Organization, 2015. Chagas disease (American trypanosomiasis). *Wkly Epidemiol Rec* 90: 33–44.
- Silveira AC, Dias JC, 2011. The control of vectorial transmission. *Rev Soc Bras Med Trop* 44: 52–63.
- Costa J, Lorenzo M, 2009. Biology, diversity and strategies for the monitoring and control of triatomines-Chagas disease vectors. *Mem Inst Oswaldo Cruz* 104: 46–51.
- Galvão C, 2014. *Vetores da Doença de Chagas no Brasil*. Curitiba, Brazil: Sociedade Brasileira de Zoologia, 289.
- Gonçalves TCM, Rocha DS, Cunha RA, 2000. Feeding patterns of *Triatoma vitticeps* in the state of Rio de Janeiro, Brazil. *Rev Saude Publica* 34: 348–352.
- Santos CB, Ferreira AL, Leite GR, Ferreira GEM, Rodrigues AAF, Falqueto A, 2005. Peridomiliary colonies of *Triatoma vitticeps* (Stal, 1859) (Hemiptera, Reduviidae, Triatominae) infected with *Trypanosoma cruzi* in rural areas of the state of Espírito Santo, Brazil. *Mem Inst Oswaldo Cruz* 100: 471–473.
- Lorosa ES, Valente MVMP, Cunha V, Lent H, Jurberg J, 2003. Foco de doença de Chagas em Arcádia, estado do Rio de Janeiro, Brasil. *Mem Inst Oswaldo Cruz* 98: 885–887.
- Gonçalves TCM, Oliveira E, Dias LS, Almeida MD, Nogueira WO, Pires FDA, 1998. An investigation on the ecology of *Triatoma vitticeps* (Stål, 1859) and its possible role in the transmission of *Trypanosoma cruzi* in the locality of Triunfo, Santa Maria Madalena municipal district, state of Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 93: 711–717.
- Gonçalves TCM, Victório VMN, Jurberg J, Cunha V, 1988. Biologia do *Triatoma vitticeps* (Stal, 1859) em condições de laboratório (Hemiptera: Reduviidae: Triatominae). I Ciclo evolutivo. *Mem Inst Oswaldo Cruz* 83: 519–523.
- Souza RCM, Barbosa SE, Sonoda IV, Azeredo BMV, Romanha AJ, Diotaiuti L, 2008. Population dynamics of *Triatoma vitticeps* (Stal, 1859) in Itanhomi, Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz* 103: 14–20.
- Sumner AT, 1972. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 75: 305–306.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30: 2725–2729.
- Ceretti-Junior W, Vendrami DP, Gil JM, Barata JMS, Marrelli MT, 2008. Análise das relações taxonômicas e sistemáticas entre espécies de triatomíneos (Hemiptera, Reduviidae) de colônias mantidas pelo Serviço Especial de Saúde de Araraquara, inferida de seqüências do 16S rDNA mitocondrial. *Rev Bras Entomol* 52: 455–462.
- Gardim S, Almeida CE, Takiya DM, Oliveira J, Araújo RF, Cicarelli RMB, Rosa JA, 2014. 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* 23: 74–79.
- Justi SA, Russo CAM, Mallet JRS, Obara MT, Galvão C, 2014. Molecular phylogeny of Triatomini (Hemiptera: Reduviidae: Triatominae). *Parasit Vectors* 7: 149.
- Imperador CHL, Alevi KCC, Oliveira J, Rosa JA, Azeredo-Oliveira MTV, 2016. Cytogenetic analysis of *Triatoma pseudomaculata* Corrêa and Espínola, 1964 (Hemiptera, Triatominae) from different Brazilian states. *Genet Mol Res* 15, doi: 10.4238/gmr.15016937.
- Pereira LHG, Hanner R, Foresti F, Oliveira C, 2013. Can DNA barcoding accurately discriminate megadiverse neotropical freshwater fish fauna? *BMC Genet* 14: 20.
- Mendonça VJ, Alevi KCC, Pinotti H, Gurgel-Gongalves R, Pita S, Guerra AL, Panzera F, Araújo RF, Azeredo-Oliveira MTV, Rosa JA, 2016. Revalidation of *Triatoma bahiensis* Sherlock & Serafim, 1967 (Hemiptera: Reduviidae) and phylogeny of the *T. brasiliensis* species complex. *Zootaxa* 4107: 239–254.
- Alevi KCC, Guerra AL, Imperador CHL, Jurberg J, Moreira FF, Azeredo-Oliveira MTV, 2017. Mitochondrial gene confirms the specific status of *Triatoma pintodiasii* Jurberg, Cunha, and Rocha, 2013 (Hemiptera, Triatominae), an endemic species in Brazil. *Am J Trop Med Hyg* 96: 200–201.
- Alevi KCC, Reis YV, Guerra AL, Imperador CHL, Banho CA, Moreira FFF, Azeredo-Oliveira MTV, 2016. Would *Nesotriatoma bruneri* Usinger, 1944 be a valid species? *Zootaxa* 4103: 396–400.
- Monteiro FA, Donnelly MJ, Beard CB, Costa J, 2004. Nested clade and phylogeographic analyses of the Chagas disease vector *Triatoma brasiliensis* in northeast Brazil. *Mol Phylogenet Evol* 32: 46–56.
- Panzera F, Pita S, Nattero J, Panzera Y, Galvão C, Chavez T, Arias AR, Téllez LC, Noireau F, 2015. Cryptic speciation in the *Triatoma sordida* subcomplex (Hemiptera, Reduviidae) revealed by chromosomal markers. *Parasit Vectors* 8: 495.
- Abad-Franch F, Pavan MG, Jaramillo N, Palomeque FS, Dale C, Chaverra D, Monteiro FA, 2013. *Rhodnius barretti*, a new species of *Triatominae* (Hemiptera: Reduviidae) from western Amazonia. *Mem Inst Oswaldo Cruz* 108: 92–99.
- Rua N, Stevens L, Dorn PL, 2011. High genetic diversity in a single population of *Triatoma sanguisuga* (LeConte, 1855) inferred from two mitochondrial markers: cytochrome b and 16 S ribosomal DNA. *Infect Genet Evol* 11: 671–677.
- Alevi KCC, Oliveira J, Azeredo-Oliveira MTV, Rosa JA, 2017. *Triatoma vitticeps* subcomplex (Hemiptera, Triatominae): a new grouping of Chagas disease vectors from South American. *Parasit Vectors* 10: 180.
- Panzera F, Pérez R, Nicolini P, Hornos S, Costa J, Borges E, Diotaiuti L, Schofield CJ, 2000. Chromosome homogeneity in populations of *Triatoma brasiliensis* Neiva 1911 (Hemiptera-Reduviidae-Triatominae). *Cad Saude Publica* 16: 83–88.
- Alevi KCC, Nunes GM, Rosa JA, Azeredo-Oliveira MTV, 2015. Homogeneidade cromossômica em diferentes populações de *Panstrongylus megistus* do Brasil. *Rev Cienc Farm Basica Apl* 36: 307–310.