Hybrid Collapse Confirms the Specific Status of *Triatoma bahiensis* Sherlock and Serafim, 1967 (Hemiptera, Triatominae), an Endemic Species in Brazil

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Abstract. Six to seven million people worldwide are estimated to be infected with *Trypanosoma cruzi*, the etiologic agent of Chagas disease transmitted mainly by triatomines. *Triatoma bahiensis* was recently collected in the Brazilian state of Bahia and revalidated using different approaches. The main criterion used to define a “good” biological species is reproductive isolation, so we evaluated the cytogenetics of first-generation (F1) hybrids resulting from the experimental cross between *T. bahiensis* females and *Triatoma lenti* males to possibly characterize the postzygotic isolation associated with the hybrid breakdown. All cells analyzed presented a karyotype of $2n = 22$ and a pair of univalent autosomes. This chromosome behavior represents hybrid collapse and underscores the specific status of *T. bahiensis*. Thus, we have characterized the presence of the hybrid collapse phenomenon in an experimental cross, and we confirm the specific status of *T. bahiensis*, thus contributing to the Triatominae taxonomy.

Six million to seven million people worldwide are estimated to be infected with *Trypanosoma cruzi* (Chagas, 1909), the parasite which causes Chagas disease.¹ In Latin America, *T. cruzi* parasites are mainly transmitted by contact with feces or urine of infected blood-sucking triatomine bugs, and vector control is considered the most useful method for preventing Chagas disease.¹

In Brazil, there are currently 68 triatomine species distributed throughout the country's 27 states.^{2–6} In the state of Bahia, there are 25 species distributed among the genera *Cavernicola* (one species), *Eratyrus* (one species), *Panstrongylus* (five species), *Psammolestes* (one species), *Rhodnius* (three species), and *Triatoma* (14 species).^{2–4}

Triatoma bahiensis Sherlock and Serafim, 1967 was recently collected in the south-central region of the Brazilian state of Bahia in an area known to be endemic for *Triatoma lenti*.⁷ For more than three decades, *T. bahiensis* was considered to be synonymous with *T. lenti* Sherlock and Serafim, 1967.⁸ Mendonça et al.³ used morphological, morphometric, molecular, cytogenetic, and experimental crosses to revalidate *T. bahiensis*. Although neither cytogenetic analysis (same characteristics) nor molecular analysis (relatively low genetic distance) generated enough support for the differentiation between *T. bahiensis* and *T. lenti*, the low viability of experimental crosses combined with morphological and morphometric data do, in fact, allow for this differentiation.³ These tools have corroborated the phylogenetic relationship between the *Triatoma brasiliensis* complex and *T. bahiensis*.³

The main criterion that defines a “good” biological species is reproductive isolation; however, this type of insulation must be considered under natural conditions.⁹ Thus, although experimental crosses under laboratory conditions are not conclusive, they provide important knowledge on the evolutionary

phenomena involved in the conceptualization of biological species (reproductive isolation).

In the subfamily Triatominae, several events involving prezygotic isolation (for example, mechanical isolation)¹⁰ have been observed, as have events involving reproductive postzygotic isolation—most commonly as sterility¹¹ and hybrid collapse.¹² Experimental crosses have proven to be important in the taxonomy of the *T. brasiliensis* complex: the specific status of *Triatoma petrocchiae* Pinto and Barreto, 1925 was confirmed by reproductive isolation when crossed with *T. brasiliensis* Neiva, 1911 (the eggs did not hatch);¹³ genetic incompatibility was found between melanica populations of *T. brasiliensis* and *T. brasiliensis*,¹⁴ which provided important evidence for the revalidation of *Triatoma melanica* Costa, Argolo and Felix, 2006 as species;¹⁵ and, more recently, the occurrence of hybrid collapse has corroborated the specific status of *T. lenti* and *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira and Barata, 2002.¹²

Because the experimental cross between *T. bahiensis* and *T. lenti* produced hybrids (which makes prezygotic isolation barriers unfeasible),³ we evaluated the cytogenetics of the first-generation (F1) hybrids to characterize the possible postzygotic isolation factors involved in the hybrid breakdown observed by Mendonça et al.³

Three adult male hybrids resulting from the experimental cross between *T. bahiensis* females and *T. lenti* males were analyzed cytogenetically. It is important to note that the reciprocal crosses between *T. lenti* females and *T. bahiensis* males resulted in only two adult females, and ovary analysis is not feasible in cytogenetic studies because meiotic stages are not usually observed in their ovaries;¹⁶ for more details on the conditions of experimental crosses, see Mendonça et al.³ The hybrids were dissected, and the testicles were removed and fixed in a methanol and acetic acid solution (3:1). Seminiferous tubules were shredded and smashed, and the microscope slides were set in liquid nitrogen. The coverslips were removed after the sample was frozen in liquid nitrogen and the biological material was fixed on the slides. The samples were then stained using the lacto-acetic orcein cytogenetic

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FIGURE 1. Diakinesis (A) and metaphase I (B) of hybrids resulting from the experimental cross between *T. bahiensis* female $\times$ *T. lenti* male. Note the univalent autosomes (arrow). Y = Y sex chromosome; X = X sex chromosome. Bar: 10 μ m.

technique,^{17,18} and the genetic affinity between the parental species was evaluated through the pairings between the homeologous chromosomes of the hybrids during prophase (diakinesis) and metaphase I and II. At least 50 cells in different stages were observed, as per the classification provided by Campos-Soto et al.¹⁶

All of the cells analyzed presented a karyotype of $2n = 22$ (20A + XY) and a pair of univalent autosomes (Figure 1A, B, arrow). This chromosome behavior shows that the cross between a *T. bahiensis* female and a *T. lenti* male is unviable because of the hybrid collapse. This finding underscores the specific status of *T. bahiensis* because two species have distinct genomes when their chromosomes differ in structure and gene content, and, as a result, no pairing occurs between one or more pairs of homeologous chromosomes during the meiosis of hybrids.¹⁹

These findings show that, although *T. bahiensis* and *T. lenti* are considered morphologically related (differentiated initially by only two orange-red spots on each side of the corium),²⁰ these species underwent genomic reorganization during speciation, as suggested by the speciation of *Rhodnius colombiensis* Mejia, Galvão and Jurberg, 1999 by means of the experimental crossing with *Rhodnius pallescens* Barber, 1932.²¹ The presence of univalent chromosomes results in unviable gametes, as has been described previously for other hybrids.^{11,22}

Sherlock and Guitton²³ collected *T. lenti* specimens in Bahia, and all were positive for *T. cruzi*. Recently, *T. cruzi* was again isolated from *T. lenti* from Bahia.²⁴ Although there are no reports in the literature of *T. bahiensis* being infected by *T. cruzi*, we would like to call attention to the benefit of future entoepidemiological studies on the possible presence of *T. bahiensis* among the triatomines collected in this state to definitively determine the epidemiological importance of this species as a vector.

Thus, we characterize the presence of the hybrid collapse phenomenon in an experimental cross between *T. bahiensis* females and *T. lenti* males, and we confirm the specific status of *T. bahiensis*, thus contributing to the Triatominae taxonomy.

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