UNIVERSIDADE ESTADUAL PAULISTA “JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – CÂMPUS DE BOTUCATU (IBB/UNESP)
PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (ZOOLOGIA)

Roberto Dezan Vicente

Estudos morfológicos, citológicos, moleculares e epidemiológicos em híbridos de triatomíneos resultantes do cruzamento entre *Triatoma sordida* e *T. rosai* (Hemiptera, Triatominae)

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Zoologia), junto ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia) do Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu.

Orientador: Prof. Dr. Kaio Cesar Chaboli Alevi

Botucatu

2023

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Vicente, Roberto Dezan.
Estudos morfológicos, citológicos, moleculares e epidemiológicos em híbridos de triatomíneos resultantes do cruzamento entre Triatoma sordida e T. rosai (Hemiptera, Triatominae) / Roberto Dezan Vicente. - Botucatu, 2023

Dissertação (mestrado)Unesp Botucatu
Orientador: Prof. Dr. Kaio Cesar Chaboli Alevi
Capes: 20400004

Palavras-chave: doença de Chagas; vetores; biologia molecular; morfologia; citogenética.

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Botucatu

2023

DEDICATÓRIA

Dedico esta conquista aos professores que nunca deixaram de acreditar em mim, mesmo com as dificuldades ao longo do processo.

À minha família, que sempre me apoiou e esteve presente em cada conquista da minha vida.

Ao meu noivo, André Santana, que sempre esteve ao meu lado, vibrando e me incentivando em cada momento.

AGRADECIMENTOS

Agradeço primeiramente a Deus, por toda bondade e amor comigo, que sempre me proporcionou o necessário para o cumprimento dos seus sonhos em minha vida. Agradeço por tamanho carinho e fidelidade de suas promessas.

Sou grato à minha família pelo apoio que me deram durante toda minha jornada de estudos.

Ao meu amigo e orientador, Prof. Dr. Kaio Cesar Chaboli Alevi, por todas as oportunidades de crescimento pessoal e profissional, por toda a paciência e dedicação, mesmo em tempos difíceis. Agradeço a dedicação e o incentivo, o que me manteve motivado durante o projeto. Sou muito grato a você!

Agradeço à Seção de Pós-Graduação e corpo docente da UNESP, Câmpus Botucatu, pela transferência de conhecimento e contínuo suporte, bem como aos órgãos de fomento FAPESP, CAPES, CNPq e FAPERJ pelo financiamento do projeto de pesquisa e da taxa de publicação do artigo científico resultante da dissertação de Mestrado.

Meus sinceros agradecimentos a todos aqueles que participaram desta conquista!

"A alegria não chega apenas no encontro do achado,

mas faz parte do processo da busca..."

(Paulo Freire)

RESUMO

Triatoma sordida é uma espécie da América do Sul (distribuído no Brasil, Paraguai, Uruguai e na Bolívia) que já foi encontrada naturalmente infectada pelo *Trypanosoma cruzi* em regiões de peri e intradomicílio. A partir de populações alopátricas de *T. sordida* da Argentina, *T. rosai* foi recentemente descrita. Essa espécie ocupa diferentes ecótopos e também foi coletada infectada pelo *T. cruzi* em ambientes naturais. Em condições de laboratório, *T. sordida* e *T. rosai* são capazes de cruzar e produzir híbridos (embora a maioria da prole híbrida morra antes de atingir a fase adulta). Diante das alterações climáticas e ambientais que estão resultando em mudanças significativas no padrão de distribuição, na história natural e no comportamento de espécies (incluindo patógenos e vetores de doenças), o estudo de híbridos de triatomíneos apresentam implicações evolutivas e, sobretudo, epidemiológicas. Dessa forma, realizamos cruzamentos experimentais entre *T. sordida* e *T. rosai* e caracterizamos os parâmetros morfológicos, citológicos, moleculares e epidemiológicos dos híbridos. A caracterização morfológica da genitália feminina mostrou que as características de ambas as espécies parentais segregaram na prole híbrida. As análises citogenéticas mostraram metáfases regulares (sem erros de pareamento). Do ponto de vista filogenético, o marcador mitocondrial *Citocromo B* (*CitB*) relacionou os híbridos com *T. sordida* e o nuclear Espaçador Interno transcrito-1 (ITS-1) relacionou os híbridos com *T. rosai*. Por fim, tanto parentais, como híbridos defecaram durante a hematofagia. Assim, os híbridos resultantes do cruzamento entre *T. sordida* e *T. rosai* apresentaram segregação de caracteres fenotípicos de ambas as espécies parentais, 100% de homeologia entre os cromossomos homeólogos, relação filogenética com *T. sordida* e com *T. rosai* (com *CitB* e ITS-1, respectivamente) e, finalmente, padrões de alimentação e defecação semelhantes aos parentais.

Palavras-chaves: doença de Chagas; vetores, biologia molecular; morfologia; citogenética

ABSTRACT

Triatoma sordida is a species from South America (distributed in Brazil, Paraguay, Uruguay and Bolivia) that has already been found naturally infected by *Trypanosoma cruzi* in peri and intradomicile. From allopatric populations of *T. sordida* from Argentina, *T. rosai* was recently described. This species occupies different ecotopes and was also collected infected by *T. cruzi* in natural environments. Under laboratory conditions, *T. sordida* and *T. rosai* are able to cross and producing hybrids (although most hybrid offspring die before reaching adulthood). Faced with climate and environmental changes that are resulting in significant changes in the distribution pattern, natural history and behavior of species (including pathogens and disease vectors), the study of triatomine hybrids has evolutionary and, above all, epidemiological implications. Thus, we carried out experimental crosses between *T. sordida* and *T. rosai* and characterized the morphological, cytological, molecular and epidemiological parameters of the hybrids. The morphological characterization of the female genitalia showed that the characteristics of both parental species segregated in the hybrid offspring. Cytogenetic analyzes showed regular metaphases (without pairing errors). From the phylogenetic point of view, the mitochondrial marker *Cytochrome B* (*CytB*) related the hybrids with *T. sordida* and the nuclear Spacer Internal Transcript-1 (ITS-1) related the hybrids with *T. rosai*. Finally, both parents and hybrids defecated during hematophagy. Thus, the hybrids resulting from the cross between *T. sordida* and *T. rosai* showed segregation of phenotypic characters of both parental species, 100% homeology between homologous chromosomes, phylogenetic relationship with *T. sordida* and *T. rosai* (with *CytB* and ITS -1, respectively) and, finally, feeding and defecation patterns similar to the parents.

Keywords: Chagas diseases; vectors, molecular biology; morphology; cytogenetics

SUMÁRIO

1. INTRODUÇÃO

Os triatomíneos são insetos hematófagos classificados na ordem Hemiptera, subordem Heteroptera, família Reduviidae e subfamília Triatominae que apresentam grande importância epidemiológica, pois são considerados como a principal forma de transmissão do protozoário *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), agente etiológico da doença de Chagas (DC) (WHO, 2022). Essa doença é negligenciada, não tem cura na fase crônica, atinge cerca de sete milhões de pessoas e coloca em risco de infecção, aproximadamente, outras 70 milhões em todo o mundo, sendo o tratamento com os fármacos Benznidazol e Nifurtimox efetivo apenas na fase aguda da doença (que, na maioria dos casos, é assintomática ou apresenta sintomas inespecíficos) (CDC, 2022; WHO, 2022).

Atualmente, existem 157 espécies descritas na subfamília Triatominae (sendo 154 espécies vivas e três espécies fósseis), agrupadas em 18 gêneros e cinco tribos (ALEVI et al., 2021; GIL-SANTANA et al., 2022; OLIVEIRA CORREIA et al., 2022). Embora existam espécies com maior ou menor grau de importância na transmissão vetorial da DC [com destaque para *Triatoma infestans* Klug, 1834, *Panstrongylus megistus* (Burmeister, 1835), *T. brasiliensis* Neiva, 1911, *T. pseudomaculata* Corrêa & Espínola, 1964 e *T. sordida* (Stål, 1859), que apresentam maiores competências vetoriais no Brasil (GALVÃO, 2014)], todos os triatomíneos, de ambos os sexos e em qualquer fase do desenvolvimento após a eclosão (ninfas e adultos), são considerados como potenciais vetores da DC.

Triatoma sordida (Figura 1C,D) é, atualmente, a espécie mais capturada em regiões de peridomicílio do Brasil (GALVÃO, 2014). Esse triatomíneo endêmico da América do Sul (distribuído no Brasil, Paraguai, Uruguai e na Bolívia) já foi encontrado naturalmente infectado pelo *T. cruzi* em regiões peri e intradomiciliares (GALVÃO, 2014; ALEVI et al., 2020a). Recentemente, Alevi et al. (2020a) realizaram uma revisão da literatura e evidenciaram a importância vetorial de *T. sordida*, o que ressalta a necessidade de atenção dos programas de controle de vetores para essa espécie.

A partir de populações alopátricas de *T. sordida* da Argentina, Alevi et al. (2020b) descreveram *T. rosai* Alevi et al., 2020 (Figura 1A,B). Essa espécie ocupa diferentes ecótopos e já foi coletada infectada pelo *T. cruzi* em ambientes naturais, o que levou Costa et al. (2021) a incluí-la na lista de espécies descritas a partir de 1979 que podem mudar o *status* epidemiológico atual da DC.

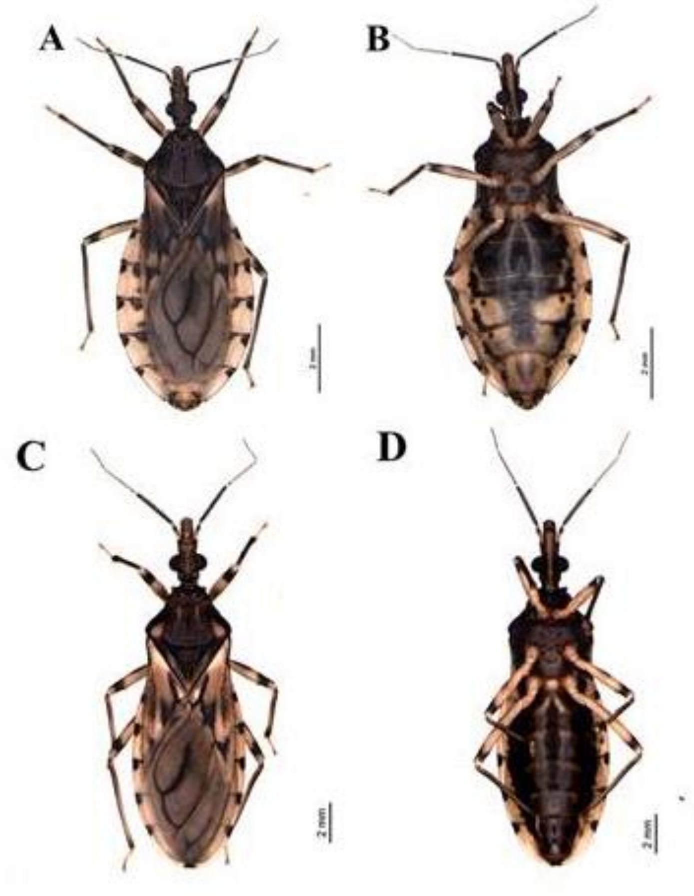

Figura 1. *Triatoma rosai* (A, B) e *Triatoma sordida* (C, D) adultos. Figura adaptada de Alevi et al. (2020b).

Em condições de laboratório, *T. sordida* e *T. rosai* são capazes de cruzar e produzir híbridos (embora a maioria da prole híbrida morra antes de atingir a fase adulta) (ALEVI et al., 2020b). O estudo da capacidade de hibridação é uma importante ferramenta taxonômica para Triatominae, pois permite caracterizar as barreiras reprodutivas pré e/ou pós-zigóticas, confirmando *o status* específico das espécies parentais, a partir do conceito biológico de espécie (ALEVI et al., 2018, 2020b; NASCIMENTO et al., 2019; RAVAZI et al., 2021).

Diante das alterações climáticas e ambientais, mudanças significativas no padrão de distribuição, na história natural e no comportamento de espécies (incluindo patógenos e vetores de doenças) estão ocorrendo (PARMESAN; YOHE, 2003), o que pode resultar em eventos de hibridação provenientes da quebra barreiras reprodutivas físicas, temporais e comportamentais (CHUNCO, 2014). Chunco (2014) ressalta que essas questões ambientais, provavelmente influenciarão drasticamente no aumento da probabilidade de hibridação para as comunidades futuras, resultando potencialmente em eventos de introgressão, especiação ou, até mesmo, extinção.

Como já demonstrdo, o estudo de híbridos de triatomíneos apresentam implicações taxonômicas, evolutivas e, sobretudo, epidemiológicas. Do ponto de vista medico-sanitário, menor tempo de defecação (MARTÍNEZ-IBARRA et al., 2016a) e maior aptidão (MARTÍNEZ-IBARRA et al., 2016b, 2017) foram observados nos híbridos resultantes de cruzamentos entre *Triatoma* spp., demonstrando que esses organismos podem desempenhar um papel importante na transmissão do agente etiológico da DC (MARTÍNEZ-IBARRA et al., 2016a, 2016b, 2017, 2021; MERAZ-MEDINA et al., 2019).

2. OBJETIVOS

2.1 Objetivo geral

Realizar cruzamentos experimentais interespecíficos entre *T. sordida* e *T. rosai* e caracterizar os parâmetros morfológicos, citológicos, moleculares e epidemiológicos dos híbridos.

2.2 Objetivo específico

a. Avaliar a dinâmica dos cruzamentos experimentais, por meio da análise da cópula interespecífica, da oviposição, da taxa de eclosão dos ovos e da viabilidade dos híbridos;

b. Analisar a viabilidade reprodutiva dos híbridos, por meio de análises citogenéticas do padrão de pareamento entre os cromossomos homeólogos;

c. Analisar o padrão de segregação das características fenotípicas dos parentais nos híbridos, por meio da caracterização das genitálias feminina por microscopia de varredura;

d. Caracterizar a relação filogenética dos híbridos com *T. rosai* e *T. sordida*, por meio de marcadores moleculares (marcador nuclear ITS-1 e mitocondrial *Cyt b*);

e. Avaliar a competência vetorial dos híbridos, por meio da análise do tempo de repastos e tempo de defecações durante a hematofagia.

3. RESULTADOS (apresentados na forma de artigo científico)

3.1 Artigo científico publicado na revista internacional *Pathogens* (FI 4,53)

VICENTE RD, MADEIRA FF, BORSATTO KC, GARCIA ACC, CESARETTO DC, DELGADO LMG, BITTINELLI IF, MELLO DV, REIS YV, RAVAZI A, GALVÃO C, AZEREDO-OLIVEIRA MTV, ROSA JA, OLIVEIRA J, ALEVI KCC. Morphological, Cytological and Molecular Studies and Feeding and Defecation Pattern of Hybrids from Experimental Crosses between *Triatoma sordida* and *T. rosai* (Hemiptera, Triatominae). Pathogens, v. 11, p. 1302. DOI: https://doi.org/10.3390/pathogens11111302

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Morphological, Cytological and Molecular Studies and Feeding and Defecation Pattern of Hybrids from Experimental Crosses between *Triatoma sordida* and *T. rosai* (Hemiptera, Triatominae)

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Citation: Vicente, R.D.; Madeira, F.F.; Borsatto, K.C.; Garcia, A.C.C.; Cristal, D.C.; Delgado, L.M.G.; Bittinelli, I.d.F.; De Mello, D.V.; Dos Reis, Y.V.; Ravazi, A.; et al. Morphological, Cytological and Molecular Studies and Feeding and Defecation Pattern of Hybrids from Experimental Crosses between *Triatoma sordida* and *T. rosai* (Hemiptera, Triatominae). *Pathogens* **2022**, *11*, 1302. https://doi.org/10.3390/pathogens11111302

Academic Editor: Hans-Peter Fuehrer

Received: 16 September 2022
Accepted: 4 November 2022
Published: 6 November 2022

Abstract: Under laboratory conditions, *Triatoma rosai* and *T. sordida* are able to cross and produce hybrids. In the face of climate and environmental changes, the study of hybrids of triatomines has evolutionary and epidemiological implications. Therefore, we performed morphological, cytological and molecular studies and characterized the feeding and defecation pattern of hybrids from crosses between *T. sordida* and *T. rosai*. The morphological characterization of the female genitalia of the hybrids showed that characteristics of both parental species segregated in the hybrids. Cytogenetic analyzes of hybrids showed regular metaphases. According to molecular studies, the mitochondrial marker Cytochrome B (*CytB*) related the hybrids with *T. sordida* and the nuclear marker Internal Transcribed Spacer 1 (ITS-1) related the hybrids with *T. rosai*. Both parents and hybrids defecated during the blood meal. Thus, the hybrids resulting from the cross between *T. sordida* and *T. rosai* presented segregation of phenotypic characters of both parental species, 100% homeology between homeologous chromosomes, phylogenetic relationship with *T sordida* and with *T. rosai* (with *CytB* and ITS-1, respectively), and, finally, feeding and defecation patterns similar to the parents.

Keywords: Chagas disease vectors; hybridization; molecular biology; morphology; cytogenetics

1. Introduction

Chagas disease is a neglected disease caused by the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) [1,2] that affects about seven million people worldwide [1,2]. This disease is mostly transmitted when humans come into contact with faeces and/or urine of triatomines (Hemiptera, Triatominae) infected by *T. cruzi* (vector-borne transmission) [1,2]. As Chagas disease has no cure in the chronic phase and the acute phase is usually asymptomatic [1,2], the World Health Organization points out that vector control is considered as the main measure to reduce the incidence of new infections [1,2].

There are currently 157 species (154 extant species and three fossils) grouped into 18 genera and five tribes that are potential vectors of *T. cruzi* [3–5]. In the last ten years (2012–2022), 13 species of triatomines were described [3,5]. However, among them, only two show clues of house invasion or domiciliation: *Triatoma huehuetenanguensis* Lima-Cordón et al., 2019 (Hemiptera, Triatominae) and *T. rosai* Alevi et al., 2020 [6].

Triatoma rosai is a related species of *T. sordida* (Stål, 1859), and was recently described using integrative taxonomy [7]. Although phylogenetically related, these species show differences in morphological, morphometric, genetic, and cytogenetic aspects, as well as in electrophoresis and cuticular hydrocarbons pattern [7]. Under laboratory conditions, these species are able to cross and produce hybrids (although the vast majority of hybrid offspring die before reaching adulthood) [7].

The study of hybridization capacity is an important taxonomic tool for Triatominae [7–10], because the characterization of pre- and/or post-zygotic reproductive barriers allows confirming the specific status of parental species from the biological species concept [11–13]. Furthermore, in the face of anthropogenic climate and environmental changes that are producing significant changes in the distribution pattern, natural history and behavior of species (including pathogens and disease vectors) [14,15], the study of hybrids of these insect vectors has evolutionary and, above all, epidemiological implications.

Shorter defecation time [16] and greater fitness [17,18] has been observed in the hybrids resulting from crosses between *Triatoma* spp., demonstrating that that triatomine hybrids can play an important role in the transmission of Chagas disease [16–20]. Both *T. rosai* and *T. sordida* are species that have already been collected naturally infected by *T. cruzi* [21–25] and that have vector importance for the epidemiology of Chagas disease.

Based on the above, we performed morphological, cytological and molecular studies and we characterized the feeding and defecation pattern of hybrids from experimental crosses between *T. sordida* and *T. rosai*.

2. Materials and Methods

2.1. Sampling

We examined specimens of *T. rosai* from Department San Miguel, Province of Corrientes, Argentina, specimens of *T. sordida* from Seabra, Bahia, Brazil and adult hybrids resulting from the cross between *T. rosai* ♀and *T. sordida* ♂and between *T. rosai* ♂and *T. sordida* ♀. The analyzed species came from live colonies kept in the Triatominae Insectarium of the São Paulo State University "Julio de Mesquita Filho", School of Pharmaceutical Sciences, Araraquara, São Paulo, Brazil. In addition, interspecific crosses were also carried out in the Insectarium to obtain hybrids in both gender combinations (as detailed by Alevi et al. [7]).

2.2. Morphological Studies in Scanning Electron Microscopy

For morphological characterization of the triatomines in Scanning Electron Microscope (SEM) (Topcon, Hasunuma-cho, Itabashi-Ku, Tokyo, Japan) (according to Rosa et al. [26]), four individuals of *T. rosai*, *T. sordida* and hybrids from both directions of crosses were used, emphasizing the study of the female external genitalia. For this study, the insects were cleaned in ultrasonic devices, dehydrated in graded series of alcohol, oven-dried at 45 °C for 20 min, and then fixed in small aluminum cylinders with colorless enamel. Afterward, they were metalized by sputtering for two minutes with 10 mA of power. After the metallization process, the samples were analyzed and photographed on the Topcon SM-300 SEM (Digital, Hasunuma-cho, Tokyo, Japan).

2.3. Cytogenetic Analysis

Four adult male hybrids from each gender combination were dissected and their testes removed and stored in a methanol:acetic acid solution (3:1). Slides were prepared by the cell-crushing technique (as described by Alevi et al. [27]), and cytogenetic analyses were performed to characterize spermatogenesis, with emphasis on the degree of pairing between the homeologous chromosomes, using the lacto-acetic orcein technique [27,28]. The slides were examined under a light microscope (Jenamed; Carl Zeiss, Jena, Germany) that was coupled with a digital camera with a 1000-fold magnification; AxioVision LE version 4.8 imaging software (Carl Zeiss) was used for analysis.

2.4. Molecular Analysis

Sequences of two molecular markers [Cytochrome B (*CytB*) and Internal Transcribed Spacer 1 (ITS-1)] obtained from *T. sordida* (n = 4), *T. rosai* (n = 4) and their hybrids (n = 4) as well as from *T. infestans* (Klug, 1834) (placed as outgroup) (Table 1) were submitted to the MEGA X program [29] and aligned by the Muscle method [30]. The alignments were concatenated by name using the Seaview4 program [31] and converted with the Mesquite program [32] for analysis in MrBayes 3.2 [33]. The data of each marker was also converted individually for analysis.

Table 1. Species and molecular markers used in the phylogenetic studies.

Species	*Cyt B*	ITS-1
T. sordida	MH054940	*
T. rosai	*	*
Hybrid [1]	*	*
Hybrid [2]	*	*

* Sequences obtained in this study [1] resulting from the cross between *T. sordida* ♂and *T. rosai* ♀; [2] resulting from the cross between *T. sordida* ♀and *T. rosai* ♂.

The best nucleotide substitution model (lowest Akaike Information Criterion value) for each marker was determined using the jModelTest 2 program [30], being HKY +G for *CytB* and GTR for ITS-1.

The phylogenetic reconstruction by Bayesian approach was performed in MrBayes 3.2 [34] for each marker, with a total of 100 million generations. Trees were sampled every 1000 generations in two independent runs, with burn-in set to 25%. The Tracer v. 1.7 program [35] was used to verify the stabilization (ESS values above 200) of the sampled trees and the generated phylogenetic tree of each analysis was viewed and edited in the FigTree v.1.4.4 [35] program, being rooted at the midpoint.

2.5. Feeding and Defecation Behavior

The feeding and defecation dynamics of *T. rosai*, *T. sordida*, and experimental hybrids were evaluated based on Diotaiuti et al. [36] with modifications: 20 adults of each species/hybrid were fed with mice and the mean period of time for feeding and mean period of time after beginning of feeding until defecation were monitored individually for one hour. The determination of the period of feeding time started with the beginning of the feeding process (when the insect inserted the mouthparts into the mouse) and ended when the insect stopped performing blood ingestion (when removing the mouthparts out of the mouse). The determination of the period of time until defecation started with the beginning of the feeding process and ended with the first release of excreta (feces/urine) by the insect (Figure 1). The period of feeding time and of the period of time until defecation were compared between hybrids and each parental species using ANOVA. Data between males and females (without distinction of species/hybrids) were also compared using Student's t-test. The results were considered to be statistically significant when $p \leq 0.05$. Analyzes were conducted in Jasp 0.16.2 [37]. All animal experiments were conducted in accordance with the Guidelines for the Treatment of Experimental Animals according to the ethical issues approved by the Ethics Committee for Animal Use of the FCFAR/UNESP, Brazil (CEUA/FCF/CAr n° 18/2019) and the National Council for Animal Experiment Control of the FCFAR/UNESP, Brazil (CIAEP/CONCEA n° 02.0082.2019).

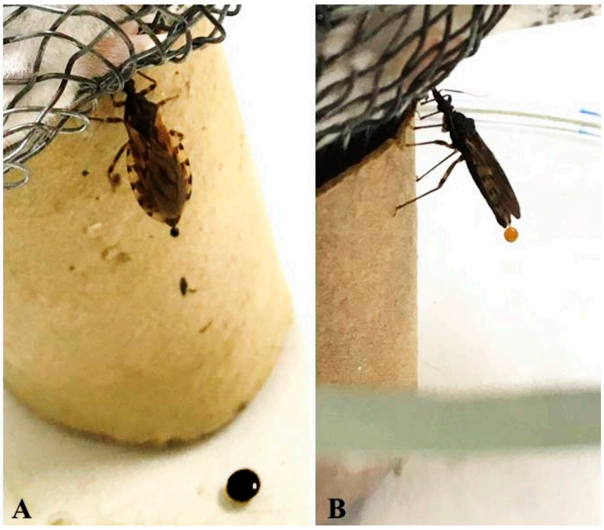

Figure 1. Hybrids defecating (**A**) and urinating (**B**) during blood feeding.

3. Results and Discussion

Morphological [8,38–40], morphometric [38], genetic [39], cytogenetic [8,9,38,41], molecular [39], behavioral [42], and epidemiological [16,20] aspects have already been studied in hybrids of Chagas disease vectors. The morphological characterization of the female genitalia of the hybrids resulting from the cross between *T. rosai* ♀and *T. sordida* ♂showed that in dorsal view (Figure 2A,D,G), *T. rosai* pattern (tenth segment form) and *T. sordida* pattern (ninth segment central form and eighth segment form) was observed; in posterior view (Figure 2B,E,H), *T. sordida* pattern (central portion of the ninth segment) and intermediate pattern (shape and length of the tenth segment) was notified, and in ventral view (Figure 2C,F,I), only the *T. sordida* pattern (line that divides the seventh and eighth gonocoxites segment and gonapophysis and shape of the eighth gonocoxites) was segregated.

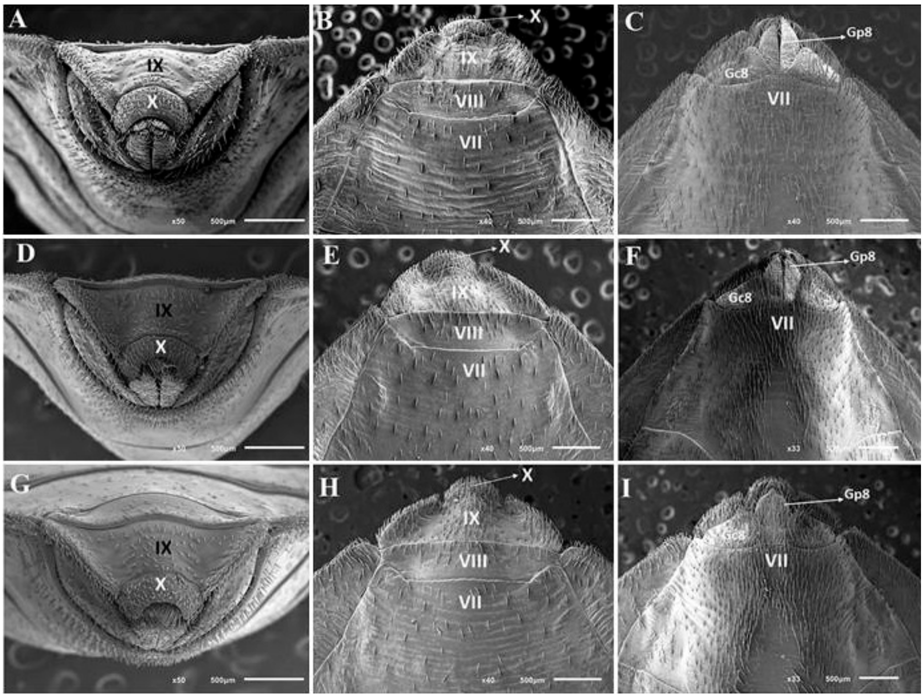

Figure 2. External female genitalia of *T. rosai* (**A**–**C**) from hybrids resulting from the cross between *T. rosai* ♀and *T. sordida* ♂(**D**–**F**) and *T. sordida* (**G**–**I**). Gc8: Gonocoxite VIII; Gp8: gonapophysis VIII; IX, VII and IX: sternites and X: segment.

The morphological characterization of the female genitalia of the hybrids resulting from the cross between *T. sordida* ♀and *T. rosai* ♂showed that in dorsal view (Figure 3A,D,G), *T. sordida* pattern (central form of the ninth segment and form of the tenth segment) and *T. rosai* pattern (form of the eighth segment) were observed; in posterior view (Figure 3B,E,F), only *T. sordida* pattern (central portion of the ninth segment and shape and length of the tenth segment) was notified, and in ventral view (Figure 3C,F,I), only *T. rosai* pattern (line dividing the seventh segment and the eighth gonocoxites and gonapophysis and form of the eighth gonocoxites) was segregated.

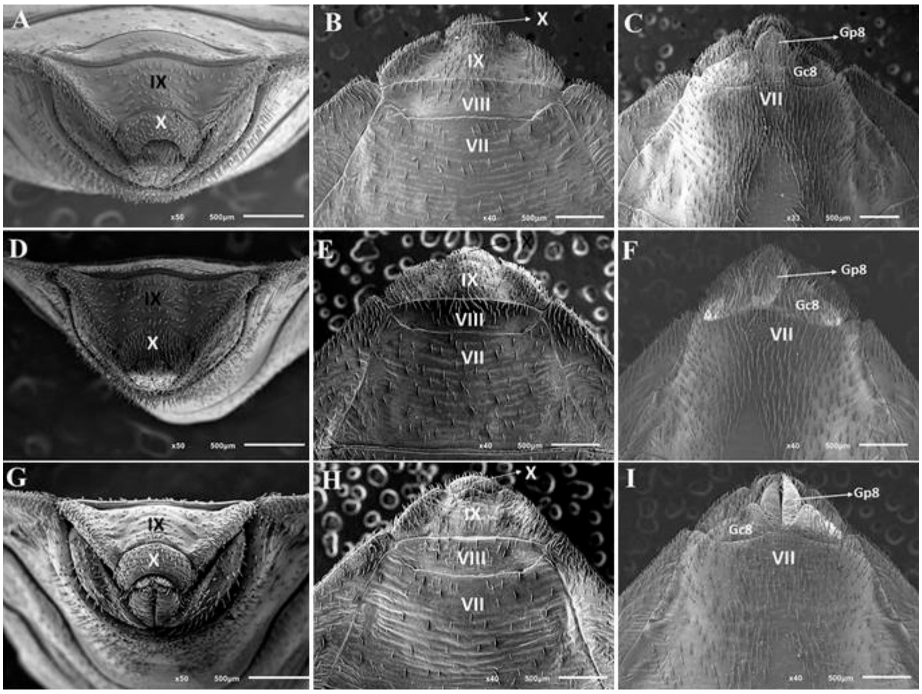

Figure 3. External female genitalia of *T. rosai* (**A**–**C**) from hybrids resulting from the cross between *T. sordida* ♀and *T. rosai* ♂(**D**–**F**) and *T. sordida* (**G**–**I**). Gc8: Gonocoxite VIII; Gp8: gonapophysis VIII; IX, VII and IX: sternites and X: segment.

The study of the segregation of phenotypic characteristics in Triatominae has been carried out for over 50 years [43]. Both segregation patterns similar to those observed for hybrids of *T. sordida* and *T. rosai*, as well as divergent patterns were characterized in the genus *Triatoma* Laporte, 1832: hybrids resulting from the crosses between *T. b. brasiliensis* Neiva, 1911 ♀ x *T. lenti* Sherlock & Serafim, 1967 ♂, *T. juazeirensis* Costa & Felix (2007) ♀ x *T. lenti* ♂, and *T. melanica* Neiva & Lent, 1941 ♀ x *T. lenti* ♂showed segregation of characteristics of both parental species [40], hybrids resulting from the cross between *T. lenti* x *T. sherlocki* Papa et al. (2002) and between *T. juazeirensis* x *T. sherlocki* showed intermediate characteristics [38,42], hybrids resulting from the crosses between *T. lenti* ♀ x *T. juazeirensis* ♂, *T. b. macromelasoma* Galvão, 1956 ♀ x *T. lenti* ♂, *T. lenti* ♀ x *T. melanica* ♂, and *T. infestans* and *T. rubrovaria* (Blanchard, 1843) showed a specific pattern of *T. lenti*, *T. lenti*, *T. melanica*, and *T. rubrovaria*, respectively [40,43].

Morphological studies on hybrids have taxonomic, evolutionary and epidemiological importance [7–10,38–45]. Recently, Pinotti et al. [40] analyzed the phenotypic segregation in hybrids of *T. brasiliensis* subcomplex and, based on the observation of different patterns (intermediate, of both parents or just one parent), they highlighted the importance of integrative taxonomy for the correct identification of Chagas disease vectors grouped in the subcomplex if natural hybridization events occur. In addition, in the studies presented by Almeida et al. [42] who crossed the brachypterous *T. sherlocki* with the macropterous *T. juazeirensis*, the hybrids presented intermediate patterns, which provided greater fitness than the parents in the home invasion process (since they can do this either walking or flying).

Cytogenetic analyzes of *T. sordida* and *T. rosai* hybrids (both gender combinations) showed regular metaphases, with 100% pairing between the homologous chromosomes (Figure 4A,B). In general, phylogenetically related species show a higher degree of homeology between chromosomes in metaphase I [46]. This can be observed, for example, for the hybrids of the species of the monophyletic *T. brasilieinsis* subcomplex [41]. Although the post-zygotic barrier characterized for the cross between *T. sordida* and *T. rosai* is the infeasibility of the hybrid [7], the reproductive barrier characterized among the species of the *T. brasiliensis* subcomplex is the hybrid collapse [38]. This event was characterized by chromosome pairing errors observed in second-generation hybrids (F2), which resulted in the formation of nonviable gametes [8–13].

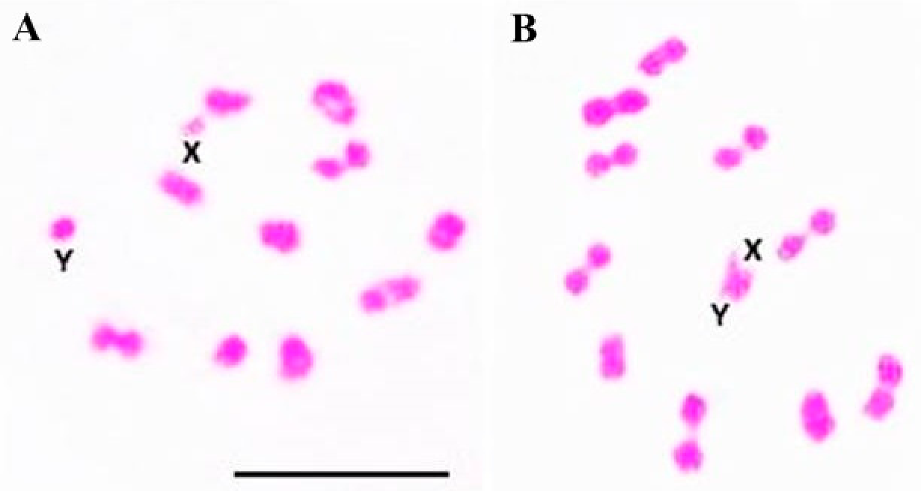

Figure 4. Metaphases I of hybrids resulting from crosses between *T. sordida* ♀and *T. rosai* ♂(**A**) and *T. sordida* ♂and *T. rosai* ♀(**B**). Note 100% pairing between homeologous chromosomes. X: X sex chromosome, Y: Y sex chromosome. Bar: 10 μm.

There is only one molecular study on triatomine hybrids, in which the authors analyzed the relationship between *T. longipennis* Usinger 1939, *T. pallidipennis* Stal, 1872, *T. picturata* Usinger 1939 and their experimental hybrids through the Cytochrome C Oxidase Subunit I (*COI*) gene [39]. We performed molecular studies with the *CytB* (Figure 5) and ITS-1 (Figure 6) molecular markers in *T. sordida*, *T. rosai* and in the experimental hybrids: the mitochondrial marker related the hybrids with *T. sordida* (Figure 5) and the nuclear marker related the hybrids with *T. rosai* (Figure 6).

Mitochondrial genes are maternally inherited [47], so it was expected that in the resulting phylogeny of *CytB* the hybrids would group together with the respective female species used in the cross (Figure 5). However, as mentioned above, both hybrids clustered with *T. sordida*. The knowledge of gene segregation in triatomine hybrids is still uncertain, as Davila-Barboza et al. [39], when analyzing hybrids resulting from the cross between *T. picturata* ♀and *T. pallidipennis* ♂for the *COI* gene, observed that these organisms were not directly related to the parental species, but with *T. longipennis* and with hybrids resulting from the cross between *T. longipennis* ♀and *T. pallidipennis* ♂and between *T. longipennis* ♀and *T. picturata* ♂. On the other hand, nuclear genes show genetic recombination [48,49], which justifies the randomness of the hybrids in the phylogeny. However, with the analysis of ITS-1, both hybrids were closer to *T. rosai* (Figure 6), demonstrating that there was probably a dominance of segregation of the genotypic characteristics of this parental species in the hybrids.

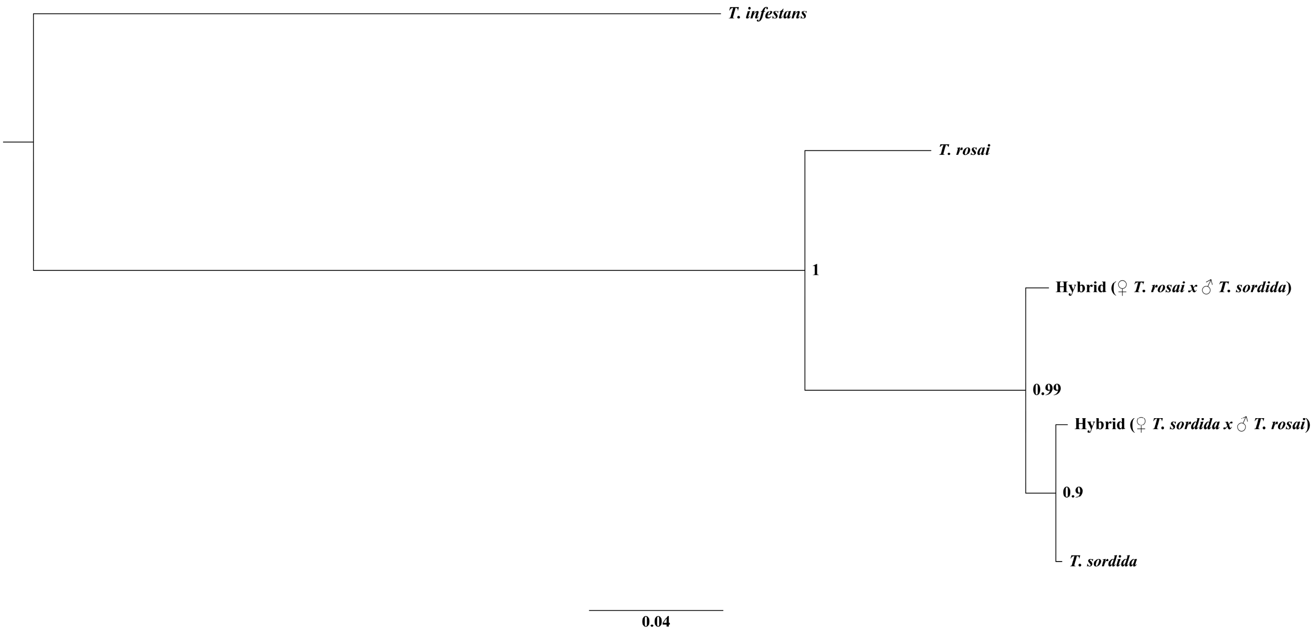

Figure 5. Phylogenetic relationship between *T. rosai*, *T. sordida*, and experimental hybrids with the *CytB* gene. The numbers in the nodes indicates the posterior probability.

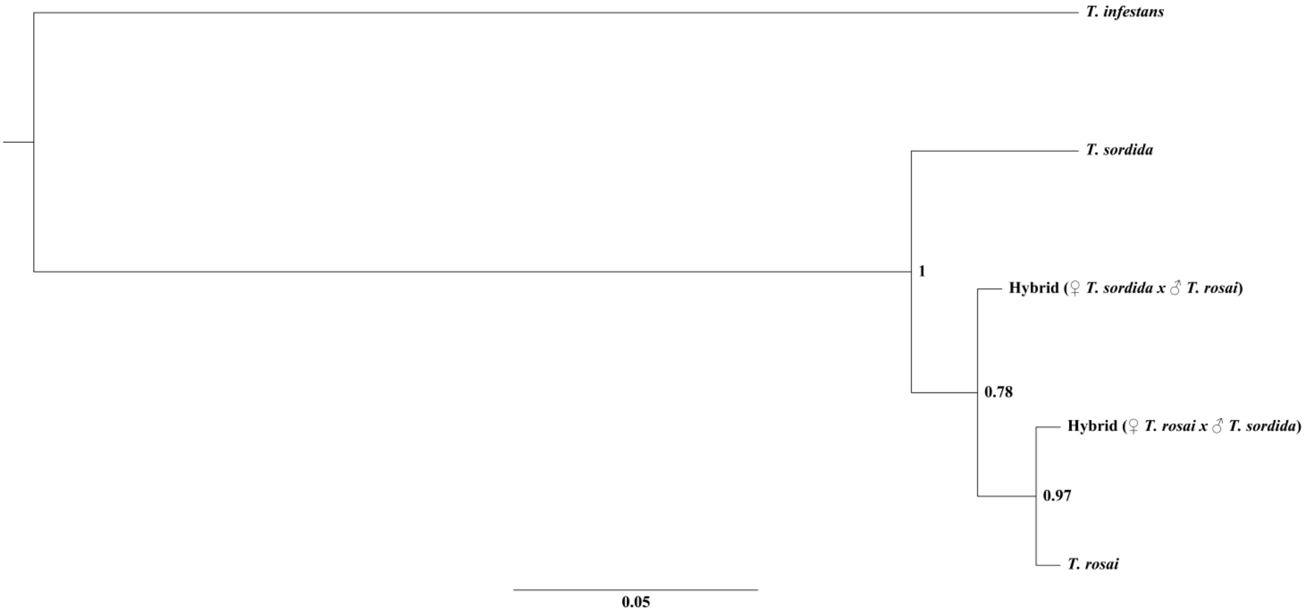

Figure 6. Phylogenetic relationship between *T. rosai*, *T. sordida*, and experimental hybrids with the ITS-1 molecular marker. The numbers in the nodes indicates the posterior probability.

The feeding and defecation pattern of *T. rosai*, *T. sordida* and the experimental hybrids was evaluated (Table 2). Both parents and hybrids defecated during the blood meal (Table 2), however, there was no significant difference between the times of feeding and defecation of the hybrids in relation to the parents (p = 0.595 and p = 0.544, respectively). Despite this, we could observe a significant difference in feeding (p = 0.005) and defecation (0.009) times between males and females (grouping data for each species and hybrids), the shortest times being observed for females. These results are important from an epidemiological point of view, as a good vector of Chagas disease, in general, has a shorter period of time between the beginning of blood ingestion and first defecation, depositing *T. cruzi* while still feeding [50].

Table 2. Mean period of time for feeding and mean period of time after beginning of feeding until defecation (n = 20 in each group).

	Feeding	**Defecation**
T. sordida ♀	30:29	18:47
T. sordida ♂	32:56	23:09
T. rosai ♀	31:49	22:02
T. rosai ♂	34:27	25:11
Hybrid [1] ♀	32:00	19:14
Hybrid [1] ♂	35:46	24:18
Hybrid [2] ♀	31:17	21:01
Hybrid [2] ♂	36:12	23:15

[1] Resulting from the cross between *T. sordida* ♂and *T. rosai* ♀; [2] resulting from the cross between *T. sordida* ♀and *T. rosai* ♂.

The time interval before beginning of feeding, for feeding, and until defecation for *T. mazzottii* Usinger, 1941, *T. pallidipennis*, and *T. phyllosomus* Burmeister, 1835 and their laboratory hybrids, as well as *T. pallidipennis*, *T. longipennis*, *T. picturata*, and their laboratory hybrids were evaluated [16–20]. According to these data, the hybrid cohorts were more effective vectors of *T. cruzi* than their parental species. In the same way, López et al. [51] analyzed the vector competence of hybrids resulting from the cross between *T. infestans* and *T. platensis* Neiva, 1913 and, based on the blood ingestion velocity, the amount of blood ingested, and the short time required for the production of the first defecation, the hybrid can be considered as a competent *T. cruzi* vector.

4. Conclusions

Based on the above, the hybrids resulting from the cross between *T. sordida* and *T. rosai* presented segregation of phenotypic characters of both parental species, 100% homeology between metaphase chromosomes, phylogenetic relationship with *T sordida* (with the *CytB* gene) and with *T. rosai* (with the ITS-1 molecular marker) and, finally, feeding and defecation patterns similar to the parents, highlighting the possible vector competence of these insects for Chagas disease (because they defecate during a blood meal).

Author Contributions: Conceptualization, R.D.V., F.F.M., M.T.V.D.A.-O., J.D.O., C.G., J.A.D.R. and K.C.C.A.; methodology, R.D.V., F.F.M., K.C.B., A.C.C.G., D.C.C., D.V.D.M., L.M.G.D., I.d.F.B., Y.V.D.R., A.R., J.D.O. and K.C.C.A.; formal analysis, R.D.V., F.F.M., K.C.B., A.C.C.G., D.C.C., L.M.G.D., I.d.F.B., Y.V.D.R., A.R., J.D.O. and K.C.C.A.; investigation, R.D.V., F.F.M., K.C.B., A.C.C.G., D.C.C., D.V.D.M., L.M.G.D., I.d.F.B., Y.V.D.R., A.R., C.G., M.T.V.D.A.-O., J.A.D.R., J.D.O. and K.C.C.A.; resources, K.C.C.A., J.D.O., C.G., J.A.D.R. and M.T.V.D.A.-O.; writing—original draft preparation, R.D.V. and K.C.C.A.; writing—review and editing, R.D.V., F.F.M., K.C.B., A.C.C.G., D.C.C., D.V.D.M., L.M.G.D., I.d.F.B., Y.V.D.R., A.R., C.G., M.T.V.D.A.-O., J.A.D.R., J.D.O. and K.C.C.A.; supervision, K.C.C.A., J.A.D.R. and M.T.V.D.A.-O.; project administration, K.C.C.A., J.A.D.R. and M.T.V.D.A.-O.; funding acquisition, K.C.C.A., C.G., J.A.D.R. and M.T.V.D.A.-O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by São Paulo Research Foundation, Brazil (FAPESP) (Process number 2017/05015-7), Coordination for the Improvement of Higher Education Personnel, Brazil (CAPES)—Finance Code 001, National Council for Scientific and Technological Development, Brazil (CNPq) and Carlos Chagas Filho Research Foundation of the State of Rio de Janeiro (FAPERJ).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

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

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

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4. CONCLUSÕES GERAIS

A análise morfológica da genitália feminina mostrou que as características fenotípicas de *T. sordida* e *T. rosai* segregaram na prole híbrida. As análises citogenéticas mostraram metáfases regulares (sem erros de pareamento entre os cromossomos homeólogos). Do ponto de vista filogenético, o marcador mitocondrial *CitB* relacionou os híbridos com *T. sordida* e o nuclear ITS-1 relacionou os híbridos com *T. rosai*. Por fim, os híbridos apresentaram padrões de alimentação (tempo de hematofagia variando entre 30 minutos e 36 minutos, aproximadamente) e defecação (evacuação realizada durante o repasto sanguíneo) semelhantes aos parentais.

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