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

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Triatoma tibiamaculata **(Pinto, 1926) (Hemiptera, Triatominae): um clássico** ***Triatoma*** **Laporte, 1832 ou um** ***Panstrongylus*** **Berg, 1879 com homoplasias?**

Botucatu
2022

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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
Coorientador: Dr. Jader de Oliveira

Botucatu
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B624t

Bittinelli, Isadora de Freitas

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40 f. : tabs., fotos

Dissertação (mestrado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Botucatu

Orientador: Kaio Cesar Chaboli Alevi

Coorientador: Jader de Oliveira

1. Triatomíneos. 2. Taxonomia. 3. Cruzamentos experimentais. 4. Sistemática filogenética. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, Botucatu. Dados fornecidos pelo autor(a).

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Botucatu
25 de fevereiro de 2022

Agradecimentos

Antes de tudo, agradeço aos membros da minha família, por sempre estarem dispostos a procurar o melhor ensino para mim, por acreditarem na minha capacidade e por todo o incentivo que foi necessário para que eu chegasse até aqui.

Do mesmo modo agradeço aos meus cachorros, que também fazem parte da família e são tão importantes quanto. Obrigada por terem complementado minha vida e terem me ajudado a passar por momentos difíceis.

À minha melhor amiga Luiza, que tem tornado essa jornada chamada vida muito mais divertida e leve.

Ao meu orientador, Prof. Dr. Kaio Cesar Chaboli Alevi por ter me acolhido mais uma vez como uma de suas orientandas. E ao meu coorientador Dr. Jader de Oliveira.

Aos integrantes do Insetário de Triatominae por todo o suporte ao longo desses dois anos. À instituição UNESP pela estrutura e por toda a contribuição que nos permite tanto desenvolvimento.

À banca examinadora, Prof. Dr. Kaio Cesar Chaboli Alevi, Profa. Dra. Maria Tercília Vilela de Azeredo Oliveira, e Prof. Dr. Luis Lenin Vicente Pereira, por terem aceitado o convite para avaliar a dissertação.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela concessão da bolsa de Mestrado e pelo apoio à pesquisa.

Agradeço também a todas as pessoas que de alguma forma contribuíram com o meu desenvolvimento pessoal e profissional. Muito obrigada por terem me ajudado nessa trajetória.

RESUMO

O gênero *Panstrongylus* foi inicialmente considerado como monofilético, a partir de dados morfológicos. Dados moleculares com o marcador nuclear ITS-2 sugeriram o polifiletismo de *Panstrongylus* spp. Posteriormente, diversas análises filogenéticas demonstraram que esse gênero é um grupo parafilético, uma vez que *Panstrongylus* spp. foi agrupado com *Nesotriatoma* spp. e *Triatoma tibiamaculata* no grupo *P. megistus*. A taxonomia de *T. tibiamaculata* é bastante instável, pois inicialmente foi descrita no gênero *Eutriatoma*, destacando que ela apresentava características intermediárias entre *Rhodnius* e *Triatoma*. Em 1940, essa espécie foi agrupada no gênero *Triatoma* e no subgênero *Eutriatoma* e, posteriormente, *E. tibiamaculata* e *T. (E.) tibiamaculata* foram sinonimizadas com *T. tibiamaculata*. Embora agrupada no gênero *Triatoma*, a posição filogenética de *T. tibiamaculata* em relação às espécies desse gênero sempre foi incerta, uma vez que esse triatomíneo foi agrupado em todas as filogenias com os gêneros *Panstrongylus* e/ou *Nesotriatoma*, resgatando *T. tibiamaculata* e *P. megistus* como espécies irmãs. Com base no exposto, avaliou-se o *status* genérico de *T. tibiamaculata*, por meio de análises filogenéticas, cromossômica e cruzamentos experimentais. A relação cromossômica e filogenética de *T. tibiamaculata* com *Panstrongylus* spp., bem como a incompatibilidade reprodutiva intragenérica com *Triatoma* spp., confirmam a mudança do *status* genérico da espécie para *Panstrongylus tibiamaculatus* comb. nov. Assim, com base em características genéticas, filológicas e evolutivas, confirmamos que *T. tibiamaculata* é um *Panstrongylus* (*P. tibiamaculatus* comb. nov.**)** e que as características morfológicas compartilhadas com *Triatoma* spp. representam homoplasias decorrentes da convergência evolutiva.

Palavras-chave: triatomíneos; taxonomia; cruzamentos experimentais; sistemática filogenética

ABSTRACT

The genus *Panstrongylus* was initially considered to be monophyletic based on morphological data. Molecular data with nuclear marker ITS-2 suggested the polyphyly of *Panstrongylus* spp. Subsequently, several phylogenetic analyzes demonstrated that this genus is a paraphyletic group, since *Panstrongylus* spp. was grouped with *Nesotriatoma* spp. and *Triatoma tibiamaculata* in the *P. megistus* group. The taxonomy of *T. tibiamaculata* is quite unstable, as it was initially described in the genus *Eutriatoma*, highlighting that it had intermediate characteristics between *Rhodnius* and *Triatoma*. In 1940, this species was grouped into the genus *Triatoma* and the subgenus *Eutriatoma*, and later, *E. tibiamaculata* and *T. (E.) tibiamaculata* were synonymized with *T. tibiamaculata*. Although grouped in the genus *Triatoma*, the phylogenetic position of *T. tibiamaculata* in relation to the species of this genus has always been uncertain, since this triatomine was grouped in all phylogenies with the genera *Panstrongylus* and/or *Nesotriatoma*, rescuing *T. tibiamaculata* and *P. megistus* as sister species. Based on the above, the generic *status* of *T. tibiamaculata* was evaluated through phylogenetic, chromosomal and experimental crosses analysis. Thechromosomal and phylogenetic relationship of *T. tibiamaculata* with *Panstrongylus* spp., as well as the intrageneric reproductive incompatibility with *Triatoma* spp., confirm the change in the generic *status* of the species to *Panstrongylus tibiamaculatus* comb. nov. Thus, based on genetic, philological and evolutionary traits, we confirm that *T. tibiamaculata* is a *Panstrongylus* (*P. tibiamaculatus* comb. nov.) and that the morphological traits shared with *Triatoma* spp. represents homoplasies resulting from evolutionary convergence.

Keywords: triatomines; taxonomy; experimental crosses; phylogenetic systematics

SUMÁRIO

1 INTRODUÇÃO

A doença de Chagas é uma enfermidade negligenciada causada pelo protozoário *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) que atinge cerca de oito milhões de pessoas e coloca em risco de infecção, aproximadamente, outras 25 milhões (WHO, 2020). A principal forma de minimizar a incidência de novos casos se fundamenta no controle das populações de vetores (insetos hematófagos da subfamília Triatominae) (WHO, 2020), sendo os estudos relacionados com esses insetos de extrema importância para a saúde pública, uma vez que podem gerar subsídios para auxiliar os programas de controle de vetores na profilaxia da doença de Chagas.

Atualmente, existem 157 espécies descritas na subfamília Triatominae (sendo 154 espécies vivas e três espécies fósseis), agrupadas em 18 gêneros e cinco tribos (Tabela 1) (ALEVI et al., 2020, 2021; GALVÃO, 2020; DALE et al., 2021; ZHAO et al., 2021). No Brasil, existem mais de 60 espécies agrupadas nos gêneros *Alberprosenia* Martínez & Carcavallo, 1977, *Belminus* Stål, 1859, *Microtriatoma* Prosen & Martínez, 1952, *Parabelminus* Lent, 1943, *Cavernicola* Barber, 1937, *Psammolestes* Bergroth, 1911, *Rhodnius* Stål, 1859, *Eratyrus* Stål, 1859, *Panstrongylus* Berg, 1879 e *Triatoma* Laporte, 1832 (GALVÃO, 2003).

Os gêneros *Triatoma*, *Rhodnius* e *Panstrongylus* são os mais importantes do ponto de vista epidemiológico para a doença de Chagas (JUSTI et al., 2014). O gênero *Panstrongylus* foi inicialmente considerado como monofilético a partir de dados morfológicos (LENT e WYGODZINSKY, 1979). Marcilla et al. (2002), por meio do marcador nuclear ITS-2, sugerem o polifiletismo de *Panstrongylus* spp. Posteriormente, diversas análises filogenéticas apontam o gênero *Panstrongylus* como um grupo parafilético, uma vez que *Panstrongylus* spp. foi agrupado com *Nesotriatoma* spp. e *T. tibiamaculata* (Pinto, 1926) no grupo *P. megistus* (Figura 1) (HYPSA et al., 2002; GARDIM et al., 2014; JUSTI et al., 2014, 2016).

Tabela 1. Tribos, gêneros e espécies agrupados na subfamília Triatominae.

Tribos	**Gêneros**	**Espécies**
Alberproseniini	*Alberprosenia*	2
Bolboderini	*Belminus*	9
	Bolbodera	1
	Microtriatoma	2
	Parabelminus	2
Cavernicolini	*Cavernicola*	2
Rhodniini	*Psammolestes*	3
	Rhodnius	21
Triatomini	*Dipetalogaster*	1
	Eratyrus	2
	Hermanlentia	1
	Linshcosteus	6
	Mepraia	3
	Nesotriatoma	3
	Panstrongylus	15
	Paratriatoma	2
	Triatoma	81
	Paleotriatoma	1
Total		**157**

Fonte: Produzida pela autora.

Figura 1 - Filogenia representando a relação entre *Panstrongylus* spp., *Nesotriatoma* spp. e *T. tibiamaculata*.

Fonte: Adaptado de Justi et al. (2016).

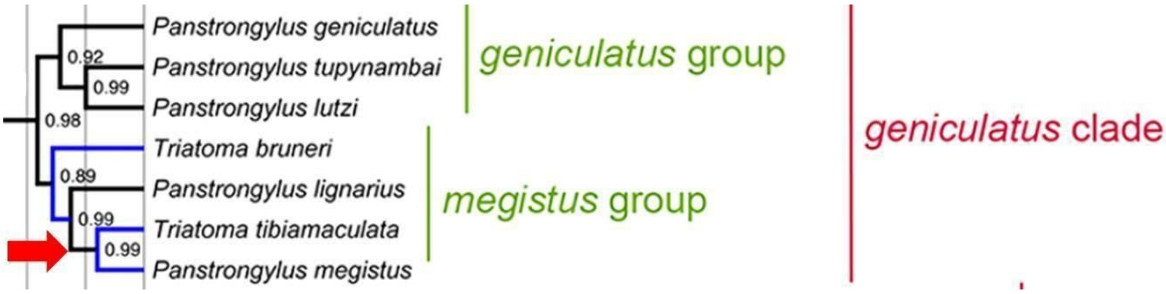
Triatoma tibiamaculata (Figura 2) é uma espécie distribuída em 10 estados brasileiros e apresenta importância epidemiológica, pois já foi encontrada infectada em regiões domiciliares (COURA, 1966). A taxonomia desse triatomíneo é bastante instável. Pinto (1926), com base em características morfológicas, descreveu inicialmente essa espécie no gênero *Eutriatoma*, destacando que ela apresentava características intermediárias entre *Rhodnius* e *Triatoma*. Costa-Lima (1940) agrupa essa espécie no gênero *Triatoma* e no subgênero *Eutriatoma* Pinto, 1926. Já Del Ponte (1930) sinonomiza *E. tibiamaculata* e *T. (E.) tibiamaculata* com *T. tibiamaculata*.

Figura 2 - Fêmea de *T. tibiamaculata*. Barra: 5 mm.

Fonte: Fornecida pelo Dr. Cleber Galvão.

Embora agrupada no gênero *Triatoma*, a posição filogenética de *T. tibiamaculata* em relação às espécies desse gênero sempre foi incerta, uma vez que esse triatomíneo foi agrupado em todas as filogenias com os gêneros *Panstrongylus* e/ou *Nesotriatoma* (HYPSA et al., 2002; GARDIM et al., 2014; JUSTI et al., 2014, 2016), resgatando *T. tibiamaculata* e *P. megistus* como espécies irmãs (Figura 2) (GARDIM et al., 2014; JUSTI et al., 2014, 2016). Além disso, Gardim et al. (2014) sugerem uma revisão do *status* genérico de *T. tibiamaculata* destacando que possivelmente essaespécie seja um *Panstrongylus*.

Figura 3 - Filogenia demonstrando que *T. tibiamaculata* e *P. megistus* são espécies irmãs (seta).



Fonte: Adaptado de Justi et al. (2016).

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o *status* genérico de *T. tibiamaculata*, por meio de análises filogenéticas, cromossômicas e cruzamentos experimentais.

2.2 Objetivos específicos

a) Analisar a relação evolutiva entre *T. tibiamaculata* com *Triatoma* spp. e *Panstrongylus* spp., por meio de análise filogenética com marcadores mitocondriais e nucleares;

b) Realizar análises cariotípicas em *T. tibiamaculata* e *Panstrongylus* spp.;

c) Realizar cruzamentos experimentais entre *T. tibiamaculata* e *Triatoma* spp., com o intuito de 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.

3 RESULTADOS

Artigo científico aceito para publicação na revista internacional *Parasites & Vectors*.

Don't judge a book by its cover: would *Triatoma tibiamaculata* (Pinto, 1926) be aclassic *Triatoma* Laporte, 1832 or a *Panstrongylus* Berg, 1879 with homoplasies?

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ABSTRACT

Background. *Triatoma tibiamaculata* is a species distributed in 10 Brazilian states which has epidemiological importance, as it has already been found infected in household areas. The taxonomy of this triatomine is quite unstable: based on morphological characteristics, was initially described as *Eutriatoma tibiamaculata*. Posteriorly, was changed to the genus *Triatoma* including the subgenus *Eutriatoma* and, finally, synonymized with *T. tibiamaculata*. Although grouped in the genus *Triatoma*, the phylogenetic position of *T. tibiamaculata* in relation to species of this genus has always been uncertain, once this triatomine was grouped in all phylogenies with the genus *Panstrongylus*, rescuing *T. tibiamaculata* and *P. megistus* as sister species. Thus, we evaluated the generic status of *T. tibiamaculata*, through phylogenetic and chromosomal analysis and experimental crosses. **Methods.** Chromosomal (karyotype) and phylogenetic (with mitochondrial and nuclear genes) analyzes were performed to assess the relationship between *T. tibiamaculata* and *Panstrongylus* spp. In addition, experimental crosses were performed between *T. tibiamaculata* and *Triatoma* spp. to assess whether there is reproductive compatibility**. Results.** The chromosomal and phylogenetic relationship of *T. tibiamaculata* with *Panstrongylus* spp., as well as intrageneric reproductive incompatibility with *Triatoma* confirm the change of generic status to *Panstrongylus tibiamaculatus* comb. nov. **Conclusions.** Thus, based on genetic, phylogenetic and evolutionary characteristics, we confirm that *T. tibiamaculata* should be changed to *Panstrongylus* genus as *P. tibiamaculatus* comb. nov. and that the morphological features shared with *Triatoma* represent homoplasies arising from evolutionary convergence.

Keywords: Chagas disease vector, triatomines, taxonomy, evolutionary convergence

Background

The members of the subfamily Triatominae (Hemiptera, Reduviidae) are hematophagous insects of great epidemiological importance, as they act as vectors of the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), the etiologic agent of Chagas disease (CD) [1]. CD is a neglected disease that affects about eight million people and puts them at risk of infection, approximately, another 25 million [1]. The main way to minimize the incidence of new cases is based on the control of vector populations [1], being the studies related to these insects of extreme importance for public health, once can generate subsidies to help vector control programs in the prophylaxis of CD.

Systematics has contributed to the correct identification of triatomines and, consequently, to the entomoepidemiological activities of vector control programs [2,3]. However, in the face of evolutionary events (cryptic speciation and phenotypic plasticity [4]) and taxonomic problems associated, in most cases, with classical taxonomy [5] (based on the morphological characterization of the species [3]), more than 190 synonymizations occurred in the Triatominae subfamily [6], highlighting the importance of integrative taxonomy for the description of new species (as performed by Dorn et al. [7], Lima-Cordón et al. [8] and Alevi et al. [9]).

Currently, there are 157 species described in the subfamily Triatominae (with 154 living species and three fossil species), grouped into 18 genera and five tribes [7- 11]. In Brazil, there are more than 60 species grouped in the *Alberprosenia* Martínez & Carcavallo, 1977, *Belminus* Stål, 1859, *Microtriatoma* Prosen & Martínez, 1952, *Parabelminus* Lent, 1943, *Cavernicola* Barber, 1937, *Psammolestes* Bergroth, 1911, *Rhodnius* Stål, 1859, *Eratyrus* Stål, 1859, *Panstrongylus* Berg, 1879 and *Triatoma* Laporte, 1832 [6], being *Rhodnius*, *Triatoma* and *Panstrongylus* genera the most important from an epidemiological point of view [12].

The genera *Rhodnius* and *Triatoma* are considered paraphyletic [12]. *Panstrongylus* was initially considered monophyletic based on morphological data [2], however, Marcilla et al. [13], using the Internal Transcribed Spacer 2 (ITS-2) nuclear marker, suggested that *Panstrongylus* spp. was polyphyletic. Posteriorly, several phylogenetic analyzes pointed this genus as paraphyletic, once *Panstrongylus* spp. was grouped with *Nesotriatoma* spp. and *T. tibiamaculata* (Pinto, 1926) [12, 14-16].

Triatoma tibiamaculata is a species distributed in 10 Brazilian states [6] which has epidemiological importance, as it has already been found infected in household areas [17] and colonizing peridomiciliar environments [18]. The taxonomy of this triatomine was quite unstable, because Pinto [19], based on morphological characteristics, initially described this species in the genus *Eutriatoma* Pinto, 1926, highlighting that it had intermediate characteristics between *Rhodnius* and *Triatoma*. Posteriorly, Del Ponte [20] synonymizes *E. tibiamaculata* and *T. (E.) tibiamaculata* with *T. tibiamaculata*, however, Costa-Lima [21] neglected this change, citing again the species into the *Eutriatoma* subgenus.

Although grouped in the genus *Triatoma*, the phylogenetic position of *T. tibiamaculata* in relation to species of this genus has always been uncertain, once this triatomine was grouped in all phylogenies with the genus *Panstrongylus* [12, 14-16], rescuing *T. tibiamaculata* and *P. megistus* as sister species [12,15,16]. Based on this, Gardim et al. [15] suggested a review of the generic status of *T. tibiamaculata*, highlighting that this species is possibly a *Panstrongylus*.

Thus, we evaluated the generic status of *T. tibiamaculata*, through phylogenetic and chromosomal analysis and experimental crosses.

Methods

Molecular analysis

For molecular analysis, the genomic DNA of five *P. lignarius*, *P. lutzi* and *T. tibiamaculata* was extracted from gonads using the DNeasy Blood and Tissue kit (QIAGEN®). Amplification of the fragments was performed by polymerase chain reaction (PCR), using primers targeting the Cytochrome B (Cyt B) [22], and Internal Transcribed Spacer 1 (ITS-1) genes [23] described in the literature. The amplified PCR products were visualized by electrophoresis in 1% agarose gel and later purified using the GFX PCR DNA & Gel Band Kit (GE Healthcare and Life Technology®) according to the manufacturer's instructions. Subsequently, this material was submitted for direct sequencing on an ABI 3730 DNA Analyzer (Life Technologies) sequencer, from the Research Center on the Human Genome and Stem Cells, University of São Paulo (USP), Brazil.

The gene sequences obtained were grouped with sequences available in GenBank of several molecular markers for 17 taxa (Table 1), which were aligned in the MEGA X program [24] using the Muscle method [25]. For the alignment of ITS-1 and ITS-2, the sequences of the *brasiliensis* subcomplex species are only available concatenated (Table 1), thus, the sequences for the other species were previously concatenated and then aligned with species of the *brasiliensis* subcomplex (representatives of the *Triatoma* genus of the *brasiliensis* subcomplex were used in the phylogeny because *T. tibiamaculata* was initially considered in this subcomplex based on morphological data and geographic distribution [15]).

The alignments were concatenated by name using the Seaview4 program [26], resulting in an alignment with 7993 nucleotides, which was converted in Mesquite 3.2 [27]. Data were partitioned for each molecular marker and the best model for each one (lowest Akaike Information Criterion value) was determined in the jModeltest 2 program [28] (Table 2).

For the phylogenetic reconstruction by Bayesian inference, the data were submitted to MrBayes 3.2 [29] in an analysis with 100 million generations. Trees were sampled every 1000 generations, in two independent runs (each with four Markov chains) and burn-in adjusted to 25%. The Tracer v. 1.7 [30] was used to verify the stabilization (ESS values above 200) of the sampled trees and the generated phylogenetic tree was visualized and edited in the FigTree v.1.4.4 program [31].

Cytogenetics analysis

Triatoma tibiamaculata, *P. megistus*, *P. lignarius* and *P. lutzi* males were dissected, the testes were removed and stored in methanol: acetic acid solution (3: 1). Slides were prepared by the cell crushing technique (as described by Alevi et al. [32]) and the cytogenetic analyzes were applied with the aim of confirm the karyotype of the species, using the Lacto-Acetic Orcein technique [32,33]. The slides were examined in light microscopy Jenaval (Zeiss), coupled to the digital camera and the Axio Vision LE 4.8 image analyzer system, with a 1000-fold increase.

Experimental crosses

Experimental reciprocal crosses were conducted between *T. tibiamaculata* and other triatomines from South America of the genus *Triatoma*: *T. brasiliensis* Neiva, 1911 of the *brasiliensis* subcomplex, *T. infestans* (Klug, 1834) of the *infestans* subcomplex, *T. pseudomaculata* Corrêa & Espínola, 1964 of the *pseudomaculata* subcomplex, *T. rubrovaria* (Blanchard, 1843) of the *rubrovaria* subcomplex and *T. melanocephala* Neiva & Pinto, 1923 of the *vitticeps* subcomplex. The insects used in the experiments 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 Correia et al. [34] and Mendonça et al. [35]: the insects were sexed as 5th instar nymphs [36], 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) and kept at room temperature (average of 24°C [37]) and relative humidity average of 63% [37]). Weekly, the eggs were collected, counted, and separated in new jars to assess the hatch rate.

Results

Phylogenetic analysis

Phylogenetic reconstruction with Ctyb and ITS-1 genes combined with several mitochondrial and nuclear genes deposited in the GenBank (16S, 18S, 28S, COI, COII, ITS-2 and 12S) rescued *T. tibiamaculata* with *Panstrongylus* spp. (Figure 1) in a clade distinct from *Triatoma* spp.

Chromosomal Analysis

The confirmation of the karyotype of the species *T. tibiamaculata*, *P. megistus*, *P. lignarius* and *P. lutzi*, when combined with literature data [37-42], demonstrates that, except for *P. megistus* and *P. lutzi*, *T. tibiamaculata* and all other species of *Panstrongylus* have the same diploid chromosome set (2n = 23 chromosomes) (Table 3). In addition, based on FISH data in the literature, it was possible to observe that *T. tibiamaculata* and all species of *Panstrongylus* present markings in a pair of autosomes (Table 3).

Reproductive Compatibility Analysis

Experimental crosses showed that there are pre-zygotic barriers that prevented the formation of hybrids between all experimental crosses (Table 4). Although it is not possible to define which barrier is present, interspecific copulas were observed (Figure 2), which rules out the prezygotic barrier of mechanical isolation.

Generic reclassification

Kingdom Animalia Linnaeus, 1758, Phylum Arthropoda von Siebold, 1848, Class Insecta Linnaeus, 1758, Order Hemiptera Linnaeus, 1758, Family Reduviidae Latreille, 1807, Subfamily Triatominae Jeannel, 1919, Tribe Triatomini Jeannel, 1919, Genus *Panstrongylus* Berg, 1879, Species *Panstrongylus tibiamaculatus* comb. nov. (Pinto, 1926) (Figure 3).

Synonyms

Eutriatoma tibiamaculata Pinto, 1926, *Triatoma (Eutriatoma) tibia-maculata* (Lima, 1940) and *Triatoma tibiamaculata* (Pinto, 1926).

Discussion

The chromosomal and phylogenetic relationship of *T. tibiamaculata* with *Panstrongylus* spp., as well as intrageneric reproductive incompatibility confirms the change of generic status to *Panstrongylus tibiamaculatus* comb. nov. Thus, the genus *Panstrongylus* becomes composed of 16 species, namely, *P. chinai* (Del Ponte, 1929), *P. diasi* Pinto & Lent, 1946, *P. geniculatus* (Latreille, 1811), *P. guentheri* Berg, 1879, *P. hispaniolae* Poinar, 2013, *P. howardi* (Neiva, 1911), *P. humeralis* (Usinger, 1939), *P. lenti* Galvão & Palma, 1968, *P. lignarius* (Walker, 1873), *P. lutzi* (Neiva & Pinto, 1923), *P. martinezorum* Ayala**,** 2009, *P. megistus* (Burmeister, 1835), *P. mitarakaensis* Bérenger & Blanchet, 2007, *P. rufotuberculatus* (Champion, 1899), *P. tupynambai* Lent, 1942 and *P. tibiamaculatus* comb. nov. [3].

As already mentioned, phylogenetic studies have shown, since 2002, the relationship between *P. tibiamaculatus* comb. nov. and *Panstrongylus* spp. (more specifically, *P. megistus*) [12,14-16] demonstrating that these taxa share common ancestry. Justi et al. [16], based on phylogenetic reconstruction associated with geological events suggest that the ancestral population that gave rise to *P. tibiamaculatus* comb. nov. and *P. megistus* was distributed along the former connection between the Amazon Forest and the Atlantic Forest and, later, with the climate changes caused by the Andean uplift that resulted in the disappearance of this connection, a vicariance event occurred that resulted in the speciation of *P. tibiamaculatus* comb. nov.and *P. megistus*.

Considering the phylogenetic relationship between *P. tibiamaculatus* comb. nov. and *Panstrongylus* spp. (more specifically, *P. megistus*) [12,14-16], Monteiro et al. [5] highlight that these species probably descend from a common ancestor that colonized the moist Atlantic forests of eastern Brazil south of parallel 7S. The authors signal that *P. megistus* is widespread across the Atlantic forests but also occurs in gallery forests throughout the drier Cerrado and stretches into the semiarid Caatinga, the Chaco, and parts of the Pantanal and Uruguayan savannahs. On the other hand, the authors point out that *P. tibiamaculatus* comb. nov. is associated with palms and bromeliads along a narrow strip of coastal Brazil including the Pernambuco, Bahia, and Serra do Mar coastal moist forests.

Gardim et al. [15] when related phylogenetically *P. tibiamaculatus* comb. nov. and *P. megistus,* evaluated ecoepidemiological issues of these species. The authors pointed out that *P. megistus* has been incriminated as one of the most important CD vectors in semi-arid zones of Brazil in the past, being eliminated from this non-native area due to a huge and costly effort of interventions of Brazilian Public Health Authorities. The authors also emphasize that the close relationship between *P. megistus* and *P. tibiamaculatus* comb. nov. (another Atlantic Forest species) may help to explain the recent finding of the latter species invading human domiciles in the downtown in Salvador, Bahia State, Brazil. In addition, the authors argue that the capacity of *P. megistus* to transmit CD (including in cases of oral contamination that even result in death [47]) and to be spread out by humans, also calls for intensifying the monitoring of *P. tibiamaculatus* comb. nov. domiciliary invasions.

Justi et al. [16] grouped the species of the genus *Panstrongylus* into two groups: *geniculatus* and *megistus*. However, more recent Monteiro et al. [5] considered four groups: *P. rufotuberculatus*, *P. lignarius*, *P. geniculatus* and *P. megistus*. Our results also retrieved four groups, namely, *P. rufotuberculatus* (composed by the species *P. chinai*, *P. rufotuberculatus* and *P. howardi*), *P. lignarius* (composed by *P. lignarius*), *P. geniculatus* (composed by the species *P. geniculatus*, *P. lutzi* and *P. tupynambai*) and *P. megistus* (composed of the species *P. megistus* and *P. tibiamaculatus* comb. nov.).

Although *P. tibiamaculatus* comb. nov. has morphological characteristics that approximate it to *Triatoma* spp. (which led to the misclassification of the species in this genus), the most prominent morphological feature that distinguishes the genus *Panstrongylus* from other triatomines is the short head, with antennae close to the eyes [3]. Justi et al. [12] highlight that the morphological divergences observed between *P. tibiamaculatus* comb. nov. and the other *Panstrongylus* may be due to morphological convergence, because variations in the size of the eyes of *Panstrongylus* spp. have already been reported in the literature [48] and these variations influence the distances between the antennas and the eyes. In addition, the authors point out that during the development of *Panstrongylus* species, nymphs tend to have head shapes similar to *Triatoma* [48], as well as highlighting that North American

Triatoma may exhibit smaller heads and antennas closer to the eyes [49], which makes it difficult to use this parameter as a diagnosis to group the species in the genus *Panstrongylus.*

Some morphological similarities between *P. tibiamaculatus* comb. nov. and the species in the *T. brasiliensis* complex led Schofield and Galvão [49] to group these species in this complex. However, based on chromosomal divergences Alevi et al. [32] proposed the exclusion of the species from this complex. From a karyosystematic point of view, while *P. tibiamaculatus* comb. nov. has 2n = 23 chromosomes (which approximates it to most species of *Panstrongylus*), all South American *Triatoma* species have 2n = 22 (present in the species of the *brasiliensis, infestans, maculata, pseudomaculata, rubrovaria* and *sordida* subomplexes) or 24 chromosomes (present in the *vitticeps* subcomplex species) [50]. Based on the ancestral karyotype of Triatominae (2n = 22) [51], Alevi et al. [52] suggested that during the divergence of the common ancestor of *Panstrongylus* there was a fission in the sex chromosome X, which resulted in the karyotype 2n = 23 (karyotype shared by *P. chinai, P. geniculatus, P. howardi, P. lignarius, P. rufotuberculatus, P. tupynambai* and *P. tibiamaculatus* comb. nov.). However, the authors suggest that during the karyotypic evolution of *Panstrongylus*, two possible punctual events occurred: fusion in a pair of autosomes in *P. megistus* and fission in the sex chromosome X in *P. lutzi*. The karyotypes of *P. megistus* and *P. lutzi* (2n = 21 and 2n = 24, respectively) were observed only in five species of *Triatoma* (*T. nitida* Usinger, 1939, *T. eratyrusiformis* Del Ponte, 1929, *T. melanocephala* Neiva & Pinto, 1923, *T. vitticeps* (Stål, 1859) and *T. breyeri* Del Ponte, 1929 [50]), demonstrating that these evolutionary events occurred independently during the chromosomal evolution of triatomines.

In addition, *P. tibiamaculatus* comb. nov. and all other *Panstrongylus* species (regardless of the number of chromosomes) have 45S rDNA probes restricted to a pair of autosomes [44-46].

Pita et al. [53] suggest that the chromosomal position of 45S rDNA is variable in Triatominae, although it is conserved among closely related species (such as *P. tibiamaculatus* comb. nov. and *Panstrongylus* spp.). In addition to thegenetic relationships observed between *P. tibiamaculatus* comb. nov. and *Panstrongylus* spp., morphological similarities between fifth-instar female nymphs of *P. megistus* and *P. tibiamaculatus* comb. nov. (more specifically in the structures of the eighth ventral segment, as well as between sensillas) were observed [54]. Furthermore, Nascimento et al. [55] also observed similarities between spermathecae morphology from *P. lignarius, P. megistus* and *P. tibiamaculatus* comb. nov. and Mello et al. [56] observed a relationship between exocorial cells in eggs of *P. tibiamaculatus* comb. nov. with *Panstrongylus*.

The absence of hybrids between *P. tibiamaculatus* comb. nov. and *Triatoma* spp. it resulted from a prezygotic reproductive barrier. Neves et al. [57] highlight that from a systematic point of view, the degree of relatedness between species can be evaluated through the characterization of prezygotic or postzygotic reproductive barriers, i.e., evolutionarily more distant species have prezygotic barriers that prevent the formation of hybrids, while evolutionarily closer species can produce hybrids that will be later declined (hybrid breakdown) by postzygotic barriers. Although the production of intrageneric hybrids between different *Triatoma* species [58] and between different *Panstrongylus* species [59] are relatively common (as long as they have the same number of chromosomes), triatomine species of different genera do not produce hybrids and, if this occurs, questions are raised about the validity of the generic status (just as happened recently for *Meccus* who was synonymized with *Triatoma* [60]).

Conclusion

Thus, based on genetic, phylogetic and evolutionary characteristics, we confirm that *P. tibiamaculatus* comb. nov. is a *Panstrongylus* and that the morphological features shared with *Triatoma* represent homoplasies arising from evolutionary convergence.

Acknowledgments

We appreciate the 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) for financial support.

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Table 1. Genbank accession number for each marker used in the phylogenetics analysis. *gene sequenced for the first time

Species	Molecular markers								
	16S	**18S**	**28S**	**Cytb**	**COI**	**COII**	**ITS-1**	**ITS-2**	**12S**
***Panstrongylus* genus**									
P. chinai				JX400960				AJ306547	
P. geniculatus	AF394593		KX109907	KX109903			AM949585	AJ306543	
P. howardi				JX400969				JX400871	
P. lignarius	AY185833	JQ897584	KX109906	*	AF449141			AJ306549	AY185818
P. lutzi	KC248969		KC249135	KC249227	KC249307	KC249401	*		
P. megistus	KC248975	AJ243336	KC249141	KC249232	KC249312	KC249403	AM949580	AJ306542	AF021178
P. rufotuberculatus	KY748239	AJ421955		JX400989				AJ306546	
P. tibiamaculatus comb. nov.	KC249080	KC249127	KC249214	KC249296	KC249389	KC249485	*		AY185829
P. tupynambai	KC248978		KC249142	KC249234		KC249404			
***T. brasiliensis* Subcomplex**									
T. brasiliensis	KC248985	AJ421957	KC249145	KC249239	KC249318	KC249413	KJ125138		AF021187
T. bahiensis				KT347298					
T. juazeirensis	KC249026		KC249173	AY494169	KF826892		KJ125150		
T. lenti	KY576788			KY576789	KY576791				
T. melanica	KC249041		KC249183	AY336527	KC249041	KC249461	KJ125147		
T. petrocchiae	KY654073			KY654075	KY654074				KY654072
T. sherlocki	EU489057		KC249205	EU489058	KC608987	KC249478	KJ125149		
Outgroup									
Rhodnius prolixus		AJ421962	AF435860	AF045718	AF449138			AJ286888	AF394519

Table 2. Substitution models for each marker.

Molecular markers	Substitution models
16S, CytB	GTR +I +G
12S, 28S, COI, COII	GTR +G
18S	HKY +I
ITS-1 + ITS-2	HKY +G

Table 3. Cytogenetic characteristics of *T. tibiamaculata* and *Panstrongylus* spp.

***Panstrongylys* genus**	**Karyotype**	**Autosomal number**	**Sex determination system**	**FISH (45S rDNA)**
P. chinai	2n = 23[1]	20[1]	X_1X_2Y[1]	The largest autosomal par[7]
P. geniculatus	2n = 23[1]	20[1]	X_1X_2Y[1]	The largest autosomal par[8]
P. howardi	2n = 23[2]	20[2]	X_1X_2Y[2]	The largest autosomal par[9]
P. lignarius	2n = 23[1]	20[1]	X_1X_2Y[1]	The largest autosomal par[7]
P. lutzi	2n = 24[3,4]	20[3,4]	$X1X_2X_3Y$[3,4]	The largest autosomal par[9]
P. megistus	2n = 21[5]	18[5]	X_1X_2Y[5]	The largest autosomal par[7]
P. rufotuberculatus	2n = 23[1]	20[1]	X_1X_2Y[1]	The largest autosomal par[8]
P. tibiamaculatus	2n = 23[6]	20[6]	X_1X_2Y[6]	The largest autosomal par[7]
P. tupynambai	2n = 23[6]	20[6]	X_1X_2Y[6]	-

[1]Crossa et al. [37],[2]Panzera et al. [38], [3]Santos et al. [39], [4]Alevi et al. [40], [5]Schreiber and Pellegrino [41], [6]Panzera et al. [42], [7]Panzera et al. [43], [8]Pita et al. [44], [9]Panzera et al. [45], X: X Sex Chromosome, Y: Y Sex Chromosome.

Table 4. Experimental cross between *T. tibiamaculata* and *Triatoma* spp.

Crossing experiments	Number of eggs	Egg Fertility
***brasiliensis* subcomplex**		
P. tibiamaculatus ♀ x *T. brasiliensis* ♂	60	0%
T. brasiliensis ♀ x *P. tibiamaculatus* ♂	193	0%
***infestans* subcomplex**		
P. tibiamaculatus ♀ x *T. infestans* ♂	93	0%
T. infestans ♀ x *P. tibiamaculatus* ♂	174	0%
***pseudomaculata* subcomplex**		
P. tibiamaculatus ♀ x *T. pseudomaculata* ♂	102	0%
T. pseudomaculata ♀ x *P. tibiamaculatus* ♂	150	0%
***rubrovaria* subcomplex**		
P. tibiamaculatus ♀ x *T. rubrovaria* ♂	159	0%
T. rubrovaria ♀ x *P. tibiamaculatus* ♂	63	0%
***vitticeps* subcomplex**		
P. tibiamaculatus ♀ x *T. melanocephala* ♂	237	0%
T. melanocephala ♀ x *P. tibiamaculatus* ♂	102	0%

Figure 1. Phylogeny obtained by Bayesian approach. *Rhodnius prolixus* was placed as outgroup. The number in the nodes indicates the posterior probability (> 0.5).

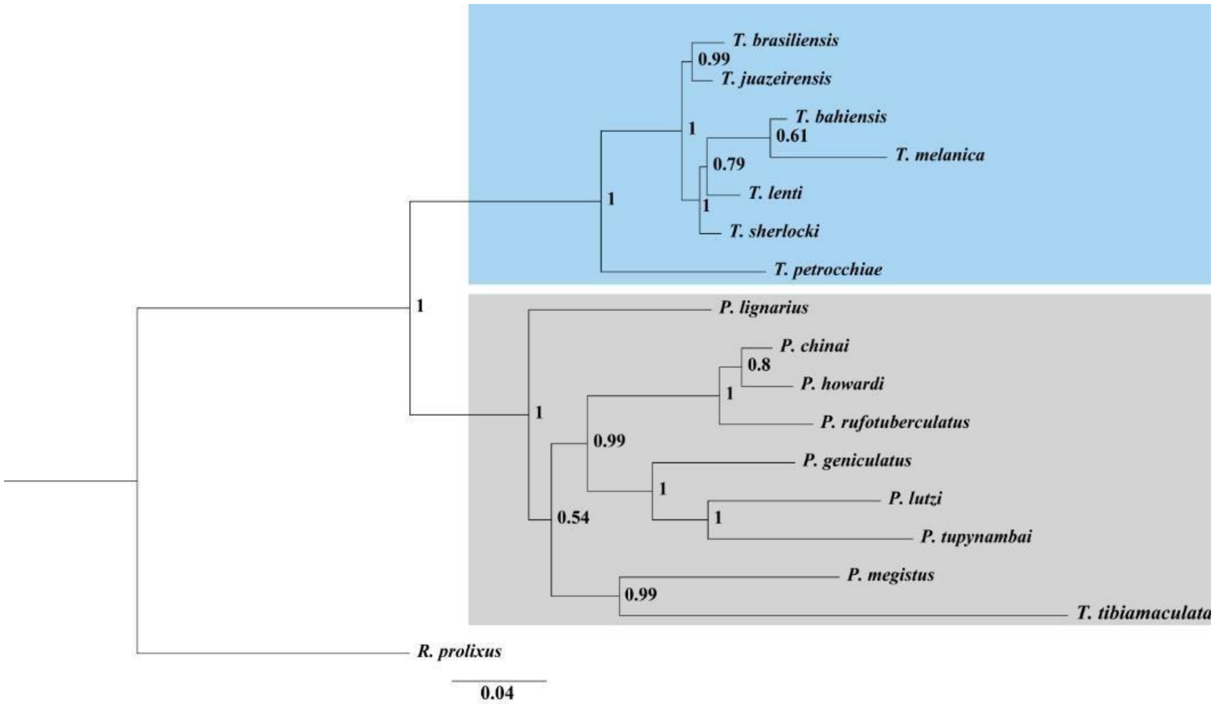

Figure 2. Copulation between male *T. rubrovaria* and female *P. tibiamaculatus* comb. nov.

Figure 3. *Eutriatoma tibiamaculata* holotype deposited in the Herman Lent Collection of the OswaldoCruz Institute, Rio de Janeiro, Brazil. Bar: 6 mm.

4. CONCLUSÃO

Assim, com base em características genéticas, filológicas e evolutivas, confirmamos que *T. tibiamaculata* é uma espécie do gênero *Panstrongylus* (*P. tibiamaculatus* comb. nov.**)** e que as características morfológicas compartilhadas entre *P. tibiamaculatus* comb. nov. e *Triatoma* spp. representam homoplasias decorrentes da convergência evolutiva.

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