Do not judge a book by its cover: would *Triatoma tibiamaculata* (Pinto, 1926) belong to *Triatoma* Laporte, 1832, or to *Panstrongylus* Berg, 1879, with misleading homoplasies?

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**Abstract**

**Background:** *Triatoma tibiamaculata* is a species distributed in ten Brazilian states which has epidemiological importance as it has already been found infecting household areas. The taxonomy of this triatomine has been quite unstable: it was initially described as *Eutriatoma tibiamaculata*. Later, the species was transferred from the genus *Eutriatoma* to *Triatoma*. Although included in the genus *Triatoma*, the phylogenetic position of *T. tibiamaculata* in relation to other 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* using phylogenetic and chromosomal analysis.

**Methods:** Chromosomal (karyotype) and phylogenetic (with mitochondrial and nuclear markers) analyses were performed to assess the relationship between *T. tibiamaculata* and *Panstrongylus* spp.

**Results:** The chromosomal and phylogenetic relationship of *T. tibiamaculata* and *Panstrongylus* spp. confirms the transfer of the species to *Panstrongylus* with the new combination: *Panstrongylus tibiamaculatus*.

**Conclusions:** Based on chromosomal and phylogenetic characteristics, we state that *P. tibiamaculatus* comb. nov. belongs to the genus *Panstrongylus* and that the morphological features shared with *Triatoma* spp. represent homoplasies.

**Keywords:** Chagas disease vector, Triatomines, Taxonomy, *Panstrongylus tibiamaculatus* comb. nov

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**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 etiological agent of Chagas disease [1]. Chagas disease is a neglected disease that affects about 8 million people and puts another approximately 25 million at risk of infection [1]. The main way to
minimize the incidence of new cases is based on the control of vector populations [1], the studies related to these insects being of extreme importance for public health once they can generate results to help vector control programs in the prophylaxis of Chagas disease.

Systematics has contributed to the correct identification of triatomines and consequently to the surveillance activities of vector control programs [2, 3]. However, in the face of evolutionary events (cryptic speciation and phenotypic plasticity [4]) and associated taxonomic problems, in most cases, with classical taxonomy [5, 6] (based on the morphological characterization of the species [3, 6]), > 190 synonymizations have occurred in the Triatominae subfamily [7]. This highlights the importance of integrative taxonomy for the description of new species [6], as performed by Dorn et al. [8], Lima-Cordón et al. [9] and Alevi et al. [10].

Currently, 157 species are described in the subfamily Triatominae (with 154 extant species and three fossil species), grouped into 18 genera and 5 tribes [6–12]. In Brazil, > 60 species are distributed among the following genera: Alberprosenia Martínez & Carcavallo, 1977, Belminus Stål, 1859, Microtriatoma Prosen & Martínez, 1952, Parabalminus Lent, 1943, Cavernicola Barber, 1937, Psammolestes Berghoef, 1911, Rhodnius Stål, 1859, Eratyrus Stål, 1859, Panstrongylus Berg, 1879, and Triatoma Laporte, 1832 [7]. Rhodnius, Triatoma and Panstrongylus are the most important from an epidemiological point of view [13].

The genera Rhodnius and Triatoma have been considered paraphyletic [13]. Panstrongylus was initially considered monophyletic based on morphological data [2]; however, Marcilla et al. [14], using the internal transcribed spacer 2 (ITS-2) nuclear marker, suggested that Panstrongylus was polyphyletic. Later, several phylogenetic analyses indicated this genus is paraphyletic once species of Panstrongylus are grouped with species of Nesotriatoma Usinger, 1944, and T. tibiamaculata (Pinto, 1926) [13, 15–17].

Triatoma tibiamaculata is distributed in ten Brazilian states [7] and has epidemiological importance as it has already been found infecting household areas [18] and colonizing peridomiciliar environments [19]. The taxonomy of this triatomine was quite unstable because Pinto [20], based only on morphological characteristics, initially described this species in the genus Eutriatoma Pinto, 1926, highlighting that it had intermediate characteristics between Rhodnius and Triatoma. Later, the species was transferred from the genus Eutriatoma to Triatoma [21, 22].

Although grouped in Triatoma, the phylogenetic position of T. tibiamaculata in relation to the other species of this genus has always been uncertain once this triatomine was grouped in all phylogenies with the genus Panstrongylus [13, 15–17], rescuing T. tibiamaculata and P. megistus (Burmeister, 1835) as sister species [13, 16, 17]. Based on this, Gardim et al. [16] suggested a review of the generic status of T. tibiamaculata, highlighting that this species possibly belongs to Panstrongylus.

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

**Methods**

**Type of material examined**

*Eutriatoma tibiamaculata* Pinto, 1926, syntype. Determined: Pinto, C. 1926, Collected: Travassos, L. 16.XII.1926., Location: Angra dos Reis, Rio de Janeiro, Brazil, deposited in the Entomological Collection of the Instituto Oswaldo Cruz (CEIOC), Rio de Janeiro, Brazil.

**Molecular analysis**

For molecular analysis, the genomic DNA of five specimens of *P. lignarius* (Walker, 1873) (from Porto Velho, Rondônia, Brazil), *P. lutzi* (Neiva & Pinto, 1923) (from Irecê, Bahia, Brazil) and *T. tibiamaculata* (from Mogi Guará, São Paulo, Brazil) 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 cytochrome b (*cytb*) and internal transcribed spacer 1 (ITS-1), as described in the literature [23, 24]. 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 of several molecular markers for 17 taxa available in GenBank (Table 1), which were aligned in the MEGA X program [25] using the Muscle method [26]. 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 had been 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 [16]).
The alignments were concatenated by name using the Seaview4 program [27], resulting in an alignment with 7993 nucleotides, which was converted in Mesquite 3.2 [28]. 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 [29] (Table 2). For the phylogenetic reconstruction by Bayesian inference, the data were submitted to MrBayes 3.2 [30] 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%. Tracer v. 1.7 [31] was used to verify the stabilization (ESS values > 200) of the sampled trees, and the generated phylogenetic tree was visualized and edited in the FigTree v.1.4.4 program [32].

Cytogenetic analysis
Triatoma tibiamaculata (from Mogi Guaçu, São Paulo, Brazil), P. megistus (from Araraquara, São Paulo, Brazil), P. lignarius (from Porto Velho, Rondônia, Brazil) and P. lutzii (from Irecê, Bahia, Brazil) 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. [33]), and cytogenetic analyses were applied to confirm the karyotype of the species using the lacto-acetic orcein technique [33, 34]. The slides were examined using Jenaval light microscopy (Zeiss) coupled to a digital camera and the Axios Vision LE 4.8 image analyzer system, with a 1000-fold increase.

Results
Phylogenetic analysis
Phylogenetic reconstruction with cytb and ITS-1 combined with several mitochondrial and nuclear DNA sequences was deposited in GenBank (16S, 18S, 28S, COI, COII, ITS-2 and 12S) rescued T. tibiamaculata with Panstrongylus spp. (Fig. 1) in a clade distinct from

| Table 1 | GenBank accession number for each marker used in the phylogenetic analysis |
|---------|-----------------------------|
| Species | Molecular markers |
| 16S     | 18S | 28S | cytb | COI | COII | ITS-1 | ITS-2 | 12S |
| Panstrongylus genus | | | | | | |
| P. chinai | JX400960 | | | | | | | |
| P. geniculatus | AF39493 | KX109907 | KX109903 | | | | | |
| P. howardi | JX400969 | | | | | | | |
| P. lignarius | AU185833 | JQ9897584 | KX109906 | | | | | |
| P. lutzii | KC249069 | KC249135 | KC249227 | KC249307 | KC249401 | | | |
| P. megistus | KC249075 | AJ243336 | KC249141 | KC249232 | KC249312 | KC249403 | | |
| P. rufotuberculatus | KY178239 | AJ241955 | | | | | | |
| P. tibiamaculatus comb. nov | KC249089 | KC249127 | KC249214 | KC249296 | KC249389 | KC249485 | ON262109 | |
| P. pygmaeus | KC24978 | KC249142 | KC249234 | | | | | |
| Brasiliensis subcomplex | | | | | | |
| T. brasiliensis | KC249889 | AJ241957 | KC249145 | KC249239 | KC249318 | KC249413 | KJ125138 | |
| T. bahiensis | KT347298 | | | | | | | |
| T. juazeirensis | KC249026 | KC249173 | AY94169 | KF826892 | KJ125150 | |
| T. lenti | K57678 | K576789 | K576791 | |
| T. melanica | KC249041 | KC249183 | AY336527 | KC249041 | KC249461 | KJ125147 | |
| T. petrociotreus | KY5407 | KY54075 | KY54074 | | |
| T. scheschini | EU489057 | KC249205 | EU489058 | KC249087 | KC249478 | KJ125149 | |
| Outgroup | | | | | | |
| Rhodnius prolixus | AJ241962 | AF435860 | AF45718 | AF449138 | | | |

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

Table 3 Cytogenetic characteristics of *P. tibiamaculatus* comb. nov. and *Panstrongylus* spp.

| Panstrongylus spp. | Karyotype | Autosomal number | Sex determination system | FISH (45S rDNA) |
|--------------------|-----------|-----------------|--------------------------|----------------|
| *P. chinai*        | 2n = 23\(^a\) | 20\(^a\) | X1X2Ya                     | The largest autosomal par\(^9\) |
| *P. geniculatus*   | 2n = 23\(^a\) | 20\(^b\) | X1X2Ya                     | The largest autosomal par\(^9\) |
| *P. howardi*       | 2n = 23\(^b\) | 20\(^b\) | X1X2Ya                     | The largest autosomal par\(^1\) |
| *P. lignarius*     | 2n = 23\(^b\) | 20\(^b\) | X1X2Ya                     | The largest autosomal par\(^9\) |
| *P. lutzi*         | 2n = 24\(^c\,d\) | 20\(^c\,d\) | X1X2X3Ya\(^c\,d\) | The largest autosomal par\(^1\) |
| *P. megistus*      | 2n = 21\(^e\) | 18\(^e\) | X1X2Y\(^e\)                 | The largest autosomal par\(^9\) |
| *P. rufotuberculatus* | 2n = 23\(^a\) | 20\(^f\) | X1X2Y\(^a\)                 | The largest autosomal par\(^h\) |
| *P. tibiamaculatus* comb. nov. | 2n = 23\(^f\) | 20\(^f\) | X1X2Y\(^f\)                 | The largest autosomal par\(^9\) |
| *P. tupynambai*    | 2n = 23\(^f\) | 20\(^f\) | X1X2Y\(^f\)                 | – |

\(^a\) Crossa et al. [35]
\(^b\) Panzera et al. [36]
\(^c\) Santos et al. [37]
\(^d\) Alevi et al. [38]
\(^e\) Schreiber and Pellegrino [39]
\(^f\) Panzera et al. [40]
\(^g\) Panzera et al. [41]
\(^h\) Pita et al. [42]
\(^i\) Panzera et al. [43]
Triatoma spp., demonstrating that *T. tibiamaculata* is a species of *Panstrongylus*.

**Chromosomal analysis**

The confirmation of the karyotype of the species *T. tibiamaculata*, *P. megistus*, *P. lignarius* and *P. lutzi*, when combined with literature data [35–40], 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, *T. tibiamaculata* and all species of *Panstrongylus* present markings in a pair of autosomes [41–43] (Table 3), confirming that *T. tibiamaculata* is a species of *Panstrongylus*.

**Generic transfer**

Kingdom Animalia Linnaeus, 1758, Phylum Arthropoda von Siebold, 1848, Class Insecta Linnaeus, 1758, Order Hemiptera Linnaeus, 1758, Suborder Heteroptera Latreille, 1810, Family Reduviidae Latreille, 1807, Subfamily Triatominae Jeannel, 1919, Tribe Triatomini Jeannel, 1919, Genus *Panstrongylus* Berg, 1879, Species *Panstrongylus tibiamaculatus* (Pinto, 1926) comb. nov. (Fig. 2).

- *Eutriatoma tibiamaculata* Pinto, 1926 (p. 134, Figs. C–E [20]).
- *Triatoma (Eutriatoma) tibia-maculata* (Lima, 1940) (p. 199, Fig. 383 [22]).
- *Triatoma tibiamaculata* (Pinto, 1926) (p. 902, Fig. 2 [21]).

*Panstrongylus*: the genus name comes from the Greek "pan" means whole, and "strongylus" means round, plump, burly, a reference to the insect’s robust, rounded body [44].

*tibiamaculatus*: the specific epithet comes from the Latin "tibia" and "maculatus," and the combination means stained tibias, a reference to the insect’s tibiae being totally "stained" in orange [44].

The change of the specific epithet "*tibiamaculata*" to "*tibiamaculatus*" was carried out based on Art. 31.2 of the International Code of Zoological Nomenclature (ICZN) [45] since "Panstrongylus" is masculine—because (i) the ending ‘-us’ usually indicates masculine words; (ii) the ICZN requires that the specific epithet be of the same grammatical gender as the generic epithet, for example, the species of the genus *Panstrongylus* are all male, as *P. geniculatus* (Latreille, 1811), *P. lignarius* and *P. rufotuberculatus* (Champion, 1899), and so is the genus; (iii) the Portuguese versions of Latin words retain the grammatical gender: if the term "strongyl" is masculine, so is *Panstrongylus* [46]—and "*tibiamaculatus*" is a latinized adjective.

**Discussion**

The chromosomal and phylogenetic relationship of *Panstrongylus tibiamaculatus* comb. nov. and *Panstrongylus* spp. confirms the change of generic status to this species. Thus, the genus *Panstrongylus* includes 16 species now, namely, *P. chinai* (Del Ponte, 1929), *P. diasi* Pinto & Lent, 1946, *P. geniculatus*, *P. guentheri* Berg, 1879, *P. hispaniolae* Poinar, 2013 (fossil species), *P. howardi* (Neiva, 1911), *P. humeralis* (Usinger, 1939), *P. lenti* Galvão & Palma, 1968, *P. lignarius*, *P. lutzi*, *P. martinezorum* Ayala, 2009, *P. megistus*, *P. mitarakaeensis* Bérenger & Blanchet, 2007, *P. rufotuberculatus*, *P. tibiamaculatus* comb. nov. and *P. tupynambai* Lent, 1942 [3].

As already mentioned, since 2002, phylogenetic studies have shown the relationship between *P. tibiamaculatus* and *P. humeralis* as very close...
P. chinai, P. rufotuberculatus, and P. megistus. Our results also retrieved four groups, namely, Panstrongylus example, is a tool that discriminated and triatomines is the short head, with antennae close to Panstrongylus that distinguishes the genus this genus), the most prominent morphological feature spp. (which led to the misclassification of the species in logical characteristics that approximate it to P. rufotuberculatus, more recently Monteiro et al. [5] considered four groups: P. megistus into two groups: However, related to 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, Monteiro et al. [5] pointed 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. [16] evaluated ecoepidemiological issues related to P. tibiamaculatus comb. nov. and P. megistus. The authors also emphasized that the close relationship between P. megistus and P. tibiamaculatus comb. nov. may help to explain the recent finding of the latter species invading human domiciles in downtown Salvador, Bahia State, Brazil.

Justi et al. [17] grouped the species of Panstrongylus into two groups: geniculatus and megistus. However, more recently 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 of P. chinai, P. rufotuberculatus and P. howardi), P. lignarius (composed of P. lignarius), P. geniculatus (composed of P. geniculatus, P. lutzi and P. tupynambai) and P. megistus (composed of 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]. The geometric morphometric of head, for example, is a tool that discriminated Panstrongylus and Triatoma based on the position of the antennal insertion relative to the eyes [47]. Justi et al. [12] highlighted that the morphological divergences observed between P. tibiamaculatus comb. nov. and the other Panstrongylus may be due to morphological convergence with Triatoma spp., 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 antennae and the eyes.

Some morphological similarities between P. tibiamaculatus comb. nov. and the species in the brasiliensis subcomplex led Schofield and Galvão [49] to group these species in this complex. However, based on chromosomal divergences, Alevi et al. [33] 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 (species of the Brasiliensis, Infestans, Maculata, Pseudomaculata, Rubrovaria and Sordida subcomplexes) or 24 chromosomes (Viticeps 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 sex chromosome X, which resulted in the karyotype 2n = 23 (karyotype shared by P. chinai, P. geniculatus, P. howardi, P. lignarius, P. rufotuberculatus, P. tibiamaculatus comb. nov. and P. tupynambai). However, the authors suggested 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. viticeps (Stål, 1859) and T. breyeri Del Ponte, 1929 [52]), suggesting 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 [41–43]. 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 the genetic 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 setae) 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. [36] recorded a relationship between exocorial cells in eggs of *P. tibiamaculatus* comb. nov. with *Panstrongylus*.

**Conclusion**

Thus, based on chromosomal and phylogenetic characteristics, we state that *P. tibiamaculatus* comb. nov. belongs to the genus *Panstrongylus* and that the morphological features shared with *Triatoma* spp. represent homoplasies.

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**Author contributions**

ISB: Conceptualization, Methodology, Investigation, Writing—Original Draft Preparation and Writing—Review & Editing, IO. Conceptualization, Methodology, Investigation, Data Curation and Writing—Review & Editing, AR: Methodology, Investigation and Data Curation, FFM: Methodology, Investigation and Data Curation, YVR: Methodology, Investigation and Data Curation, AJCG: Methodology, Investigation and Data Curation, GM: Methodology, Investigation and Data Curation, YVR: Methodology, Investigation and Data Curation, ABBO: Methodology, Investigation and Data Curation, FMM: Methodology, Investigation and Data Curation, AR: Methodology, Investigation and Data Curation, ISB: Conceptualization, Methodology, Investigation, Writing—Original Draft Preparation and Writing—Review & Editing, KCCA: Conceptualization, Methodology, Investigation, Data Curation, CG: Conceptualization, Writing—Review & Editing, and Funding acquisition. All authors read and approved the final manuscript.

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**Availability of data and materials**

GenBank accession numbers of sequences generated in this study: *P. tibiamaculatus* ITS-1 (ON262109), *P. lutzi* ITS-1 (ON262110) and *P. lignarius* cytb (ON262111).

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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