

## Insights into a hotspot in the Brasiliensis subcomplex (Hemiptera, Triatominae) by analysis of D2 domain of the nuclear gene 28S

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**ABSTRACT.** The Brasiliensis subcomplex is a monophyletic group formed by the species *Triatoma brasiliensis brasiliensis*, *T. b. macromelasoma*, *T. juazeirensis*, *T. melanica*, and *T. sherlocki*. However, using cytogenetic data and experimental hybrid crosses, *T. lenti* and *T. petrochiae* were also grouped into this subcomplex. This study aims to analyze the properties of hotspot in the D2 domain of the nuclear gene 28S in all species of the Brasiliensis subcomplex as well as *T. lenti* and *T. petrochiae*. These species show two transversions at position 385 (G↔C and T↔G). We suggest that this mutation in haplotype 4 may be an initial molecular tool that supports the relationship

of these species with the subcomplex. In addition to the transversion at haplotype 4, these species, aside from *T. melanica*, also possess a transversion at position 385 (G↔T) in haplotype 1. Thus, we describe the hotspot mutations of the D2 domain of the nuclear gene 28S for species in Brasiliensis subcomplex as follows: three transversions are present at position 385 of haplotypes 1 and 4, which are shared by members of the subcomplex as well as *T. lenti* and *T. petrochiae*. These transversions may be considered a synapomorphy between these species. However, we emphasize that new phylogenetic studies should be conducted to evaluate whether *T. lenti* and *T. petrochiae* are truly members of the Brasiliensis subcomplex.

**Key words:** *Triatoma lenti*; *Triatoma petrochiae*; Hotspot

## INTRODUCTION

The Triatominae subfamily consists of 150 species that are divided into 18 genera (Alevi et al., 2015). These species can also be grouped into complexes and specific subcomplexes (Lent and Wygodzinsky, 1979; Carcavallo et al., 2000; Schofield and Galvão, 2009). Although complexes and subcomplexes are not valid according to the International Code of Zoological Nomenclature (Carcavallo et al., 2000) they should be considered monophyletic groups (Justi et al., 2014).

Schofield and Galvão (2009) mainly used morphological characteristics and geographical distribution to group nine species in the Brasiliensis subcomplex: *Triatoma brasiliensis* Neiva, 1911; *T. juazeirensis* Costa & Felix, 2007; *T. melanica* Costa, Argolo & Felix, 2006; *T. melanocephala* Neiva & Pinto, 1923; *T. petrochiae* Pinto & Barreto, 1925; *T. lenti* Sherlock & Serafim, 1967; *T. sherlocki* Papa, Jurberg, Carcavallo, Cerqueira & Barata, 2002; *T. tibiamaculata* Pinto, 1926; and *T. vitticeps* Stal, 1859. However, *T. melanocephala*, *T. tibiamaculata* and *T. vitticeps* were excluded from this subcomplex based on cytogenetic (Alevi et al., 2012a, 2013a, 2014a,b) and molecular analyses (Gardim et al., 2014).

All phylogenetic reconstructions of the Brasiliensis subcomplex were conducted with mitochondrial (Cyt b, 16S, COI and COII) and nuclear (18S and 28S) genes. Brasiliensis subcomplex is a monophyletic group formed by the species *T. b. brasiliensis*, *T. b. macromelasoma*, *T. juazeirensis*, *T. melanica*, and *T. sherlocki* (Monteiro et al., 2004; Mendonça et al., 2009; Gardim et al., 2014; Justi et al., 2014). However, we emphasize that *T. lenti* and *T. petrochiae* were not used in any of the phylogenetic analyses described above (perhaps due to difficulties in sample collection). Cytogenetic data (Alevi et al., 2012b, 2013b, 2014a) and experimental hybrid crosses (Mendonça et al., 2014) suggest that *T. lenti* can be considered as a member of this subcomplex.

Monteiro et al. (2004) found independent mutations on the same sites of the Cyt b gene during phylogenetic reconstruction. These sites are high frequency homoplasious sites known as hotspots. Mutation hotspots often reflect a specific mechanism of generating mutations at a particular site and/or unusual properties of a phenotypic selection (Rogozin and Pavlov, 2003).

Thus, this study aims to analyze hotspots in the D2 domain of the nuclear gene 28S in all species of the Brasiliensis subcomplex (*T. b. brasiliensis*, *T. b. macromelasoma*, *T. juazeirensis*, *T. melanica*, and *T. sherlocki*), as well as *T. lenti* and *T. petrochiae*.

## MATERIAL AND METHODS

Seven adult specimens of each species belonging to the Brasiliensis subcomplex (except

for *T. melanica*, where only two specimens could be collected due to difficulties with sample collection) and *T. infestans* (as an outgroup), were obtained from the “Insectary of Triatominae”, Faculdade de Ciências Farmacêuticas (FCFAR/UNESP), Campus of Araraquara, São Paulo, Brazil.

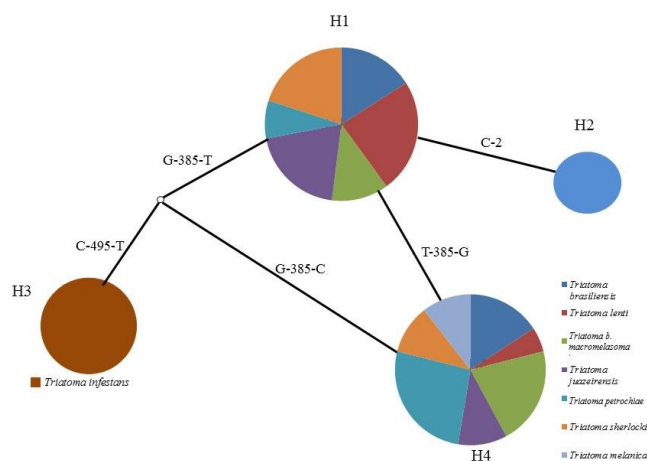
These triatomines were dissected, and the legs were used for extraction of genetic material using the DNeasy Blood and Tissue Kit (QIAGEN) according to the manufacturer instructions. Amplification of variable region D2 in the 28S gene (rDNA) was performed via PCR as previously described by Porter and Collins (1996). The primers used were as follows: forward sequence: 5'-GCGAGTCGTGTTGCTTGATAGTGACG-3' and reverse sequence: 5'-TTGGTCCGTGTTTCAAGACGGG-3'. The cycling parameters are as follows: an initial cycle at 95°C for 2 min; 40 cycles of denaturation (95°C, 30 s), annealing (68°C, 30 s), and extension (72°C, 1 min); and one cycle at 72°C for 5 min. Following electrophoresis, the amplified fragments were purified using the GFX PCR DNA & Gel Band kit (GE Healthcare and Life Technology) according to the manufacturer instructions.

Purified PCR products were subjected to direct sequencing. Samples were sent to the Research Center on the Human Genome and Stem Cells, USP/São Paulo, Brazil. Sequences of all individuals were analyzed by the BioEdit software 7.0.5, and a consensus sequence was obtained for each DNA segment. The sequences were aligned using ClustalW editor.

We constructed a haplotype network using the TCS 1.21 software (Clement et al., 2000) based on the statistical parsimony method (Templeton et al., 1992). Reticulations were resolved according to common theoretical predictions about network structures (Crandall and Templeton, 1993; Posada and Crandall, 2001).

## RESULTS

All species analyzed, except for *T. infestans*, presented hotspot mutations in haplotype 4, which were characterized as two transversions at position 385 (G↔C and T↔G) (Figure 1). With the exception of *T. melanica*, all species showed transversion at position 385 (G↔T) of haplotype 1. In addition to the mutations described above, *T. brasiliensis* showed a transition in haplotype 2, and a transition at position 495 (C↔T) was found in the *T. infestans* outgroup (Figure 1).



**Figure 1.** Parsimony haplotype network showing hotspot mutations in species of the subcomplex Brasiliensis, with *Triatoma infestans* as the outgroup.

## DISCUSSION

The D2 domain of the nuclear gene 28S is frequently used in phylogenetic studies in the Rhodniini tribe (Monteiro et al., 2000, 2003; Díaz et al., 2014). In the Rhodniini tribe, this gene is more conserved as compared with other genes, containing only 9% variable sites (Monteiro et al., 2000). Díaz et al. (2014) observed that the D2 domain of the nuclear gene 28S contained only 12 variable sites in the *pallenscens* group. Monteiro et al. (2003) by means of D2 domain of the nuclear gene 28S corroborates the paraphilia of *R. robustus* because *R. prolixus* and *R. robustus* from the Orinoco region share a derived C in position 360.

For a long time, the species of the Brasiliensis subcomplex were considered to be chromatic variants of *T. brasiliensis* (Costa et al., 1998). Costa et al. (2006) elevated the specific status of *T. b. melanica* to *T. melanica*. Costa and Felix (2007) described *T. juazeirensis* based on the *T. brasiliensis* population in Juazeiro/Bahia. Mendonça et al. (2009) grouped *T. sherlocki* into the Brasiliensis subcomplex based on phylogenetic analyses with mitochondrial genes (16S and Cyt b). Costa et al. (2013) revalidated and re-described the subspecies *T. b. macromelanosoma* based on melanin forms of *T. brasiliensis* encountered at housing (Galvão, 1955).

All species of the Brasiliensis subcomplex have cytogenetic synapomorphies: 20 autosomes plus two sex chromosomes (XY in males and XX in females), C-blocks in one or both chromosomal ends in all autosomal pairs, a large chromocenter made up of both sex chromosomes plus two autosomal pairs, and multiple C-dots spread in the nucleus during early meiotic prophase (Panzera et al., 2000; Alevi et al., 2013a, 2014a). *T. vitticeps*, *T. melanocephala*, and *T. tibiamaculata*, which were excluded from this subcomplex, have been found to have multiple sex systems and different C-heterochromatin patterns (Panzera et al., 2010; Alevi et al., 2012a, 2013a, 2014b).

Hotspot analyses showed that all species belonging to the Brasiliensis subcomplex as well as *T. lenti* and *T. petrochiae* have two transversions at position 385 (G↔C and T↔G). Considering that *T. lenti* and *T. petrochiae* have the same cytogenetic characteristics described for the Brasiliensis subcomplex (Panzera et al., 2000; Alevi et al., 2012b, 2013b, 2014a), it is possible that this mutation in haplotype 4 may be an initial molecular tool that supports inclusion of these species within the subcomplex.

In addition to transversion in haplotype 4, all species except for *T. melanica* also demonstrated transversion at position 385 (G↔T) in haplotype 1. Since *T. melanica* is the most evolutionarily differentiated species within the subcomplex, it may have genetic incompatibility and provide unviable hybrids with other members of the Brasiliensis subcomplex (Costa et al., 2006). It is possible that this hotspot can also be a defining feature of the species within this subcomplex.

In this study, we have illustrated hotspot mutations in the D2 domain of the nuclear gene 28S for species in the Brasiliensis subcomplex, and suggest that three transversions at position 385 of haplotypes 1 and 4 are shared by members of the subcomplex as well as *T. lenti* and *T. petrochiae*. However, we emphasize that new phylogenetic studies should be conducted to evaluate whether *T. lenti* and *T. petrochiae* truly are members of the Brasiliensis subcomplex.

## Conflicts of interest

The authors declare no conflict of interest.

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