Infection, Genetics and Evolution 23 (2014) 74–79

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Short communication

Multiple mitochondrial genes of some sylvatic Brazilian *Triatoma*: Non-monophyly of the *T. brasiliensis* subcomplex and the need for a generic revision in the Triatomini

Sueli Gardim [a], Carlos E. Almeida [a], Daniela M. Takiya [b], Jader Oliveira [a], Renato F. Araújo [c], Regina M.B. Cicarelli [a], João A. da Rosa [a,*]

[a] Department of Biological Sciences, School of Pharmaceutical Sciences, São Paulo State University (UNESP), Araraquara, Sao Paulo, Brazil
[b] Department of Zoology, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[c] Programa de controle da Dengue em Itabuna, Secretaria de Saúde do Estado da Bahia, Brazil

ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 14 January 2014
Accepted 20 January 2014
Available online 4 February 2014

Keywords:
Chagas disease
Phylogeny
Taxonomic revision
Triatominae

ABSTRACT

Multiple fragments of mitochondrial DNA genes (cytochrome *b*, cytochrome oxidase I, and 16S rDNA) were used to evaluate the phylogenetic relationships among *Triatoma melanocephala*, *Triatoma tibiamaculata*, *Triatoma vitticeps*, and other members of *Triatoma brasiliensis* subcomplex under a Bayesian framework and maximum parsimony criterion. With the addition of new sequences of *T. tibiamaculata* and *T. vitticeps*, *Triatoma juazeirensis*, *Triatoma melanica* and the newly sequenced *T. melanocephala,* the three first sylvatic species, *T. melanocephala*, *T. tibiamaculata* and *T. vitticeps*, were strongly recovered into a clade separate from the other with the remaining *Triatoma* species from South America, such as the members of *T. brasiliensis* subcomplex. *Panstrongylus megistus* was recovered as a sister to *T. tibiamaculata*, whereas *T. vitticeps* was a sister to *T. melanocephala.* This study revealed the non-monophyly of the *T. brasiliensis* subcomplex, and the polyphyly of *Triatoma* was reinforced by the placement of these three sylvatic species with *Dipetalogaster*, *Meccus, Mepraia*, and *Panstrongylus*. The results herein shown highlight the need of generic revision in Triatomini.

1. Introduction

Chagas disease affects 7–8 million people worldwide, mostly in Latin America, yet no vaccine has been developed. It is caused by a protozoan parasite, *Trypanosoma cruzi,* transmitted to humans mainly by blood-sucking bugs, the triatomines. Vector transmission is still considered the main mode of infection in Brazil. Therefore, most efforts against Chagas disease focus on the interruption of its natural transmission by controlling domiciliary vector populations with pyrethroid insecticides (Vinhaes and Dias, 2000; WHO, 2013). However, the epidemiological importance of these Chagas disease vectors is continually changing. *Triatoma sherlocki*, for example, was described in 2002 as a sylvatic species, and was recently found invading and colonizing human domiciles in Bahia State (Papa et al., 2002; Almeida et al., 2012); and several other species can be mentioned in this context (e.g., Costa, 1999; Costa and Lorenzo, 2009; Schofield and Galvão, 2009).

These changes in vector importance from an epidemiological standpoint are in part a reflection of an increase of studies on triatomine diversity, especially in Brazil. After the first reported human cases of the disease by Chagas (1909), studies on vector species reservoirs and description of new triatomine species started to increase. Currently, the subfamily Triatominae consists of 147 species and 18 genera, of which sixty-five species occur in Brazil (Galvão et al., 2003; Poinar, 2005; Costa et al., 2006; Galvão and Ângulo, 2006; Bérenger and Blanchet, 2007; Costa and Felix, 2007; Martínez et al., 2007; Sandoval et al., 2007; Jurberg et al., 2009, 2013; Rosa et al., 2012, Gonçalves et al., 2013; Alevi et al., 2013a). More recently, phylogenetic approaches have been used to understand evolutionary relationships among these species (Marcilla et al., 2001, 2002; Bargues et al., 2008, 2010; Costa et al., 2013; Costa and Lorenzo, 2009; Schofield and Galvão, 2009; Gurgel-Gonçalves et al., 2012; Rosa et al., 2012; Gardim et al., 2013), but most of these studies lack from good taxa or character sampling to produce a robust phylogeny. A robust phylogenetic hypothesis can be used as the basis for an informative and

* Corresponding author. Address: Laboratório de Parasitologia, Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas/UNESP, Rod. Araraquara-Jaú, Km 1, Araraquara, SP CEP 14801-902, Brazil. Tel.: +55 16 33016945; fax: +55 16 33016940.
E-mail address: joaoaristeu@gmail.com (J.A. da Rosa).

http://dx.doi.org/10.1016/j.meegid.2014.01.024
1567-1348

predictive classification for the group and highlight sylvatic species with potential to become epidemiologically important.

Members of *Triatoma* are grouped into complexes and subcomplexes based on morphological similarities, geographic distribution, epidemiological importance, phylogenetic relationships, and other features. At the moment, there is no consensus about the features that define complexes (Usinger et al., 1966; Lent and Wygodzinsky, 1979; Costa and Lorenzo, 2009; Rosa et al., 2012). In an attempt to consider most classifications proposed based on different rationales, Schofield and Galvão (2009) grouped together *Triatoma melanocephala*, with *Triatoma petrochiae*, *Triatoma lenti*, *Triatoma brasiliensis*, *Triatoma juazeirensis*, *Triatoma melanica*, and *T. sherlocki* in the *T. brasiliensis* subcomplex of the *Triatoma infestans* complex. They also tentatively placed *Triatoma tibiamaculata* and *Triatoma vitticeps* in this group based on morphological similarities, but point out that based on 16S rDNA sequences and cytogenetics they were not found to be closely related to members of the *T. brasiliensis* subcomplex. To date, only the last four (*T. brasiliensis*, *T. juazeirensis*, *T. melanica*, and *T. sherlocki*) were recovered as a monophyletic group based on the analysis of the mitochondrial genes cytochrome *b* (Cytb) and 16S rDNA (16S) (Monteiro et al., 2004; Mendonça et al., 2009). Given that DNA samples for sylvatic species are difficult to be obtained, species complexes and subcomplexes definition has been chiefly based on morphological and ecological features; and for this reason, Cytb, cytochrome oxidase I (COI), and 16S sequences of mitochondrial DNA were obtained and used to evaluate the phylogenetic relationships among *T. melanocephala*, *T. tibiamaculata*, *T. vitticeps*, and other triatomines, with focus on members of *T. brasiliensis* subcomplex.

2. Material and methods

Insects for DNA extraction were randomly obtained from colonies (CTA) maintained at the Triatominae Insectarium of the Department of Biological Sciences, School of Pharmaceutical Sciences, São Paulo State University (Araraquara, Brazil). Details about the origin of insects can be found in Table 1. Multiple specimens were used for gene sequencing of *T. tibiamaculata* ($N = 3$), *T. vitticeps* ($N = 3$) and specimens of three geographic occurrence points of *T. melanocephala* distribution ($N = 3$ for each), due to the reasonable geographic distance among sites (22–80 km), herein called 1, 2, and 3. In addition, we deposited new sequences for *T. juazeirensis* and *T. melanica* (COI and 16S) extracted from a single specimen for each species (see Table 2).

The species set choice for the analysis was based on (i) a broad taxon sampling representing species of different genera of Triatomini, previously recovered as related to the species of interest (members of *T. brasiliensis* subcomplex) and (ii) availability of sequences for the genes studied for these species in GenBank. Therefore, after an explorative search, not all species chosen had all three markers available for the analysis.

Total DNA extraction was performed according to the protocol described by Sambrook and Russell (Gaunt and Miles, 2002). From the extracted DNA, 16S and COI fragments were amplified as described by Mello (1982), and for Cytb as by Hypsa and collaborators (2002), and purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Life Sciences). Purified products were subjected to a sequencing reaction using BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and were analyzed in the ABI PRISM® 377 DNA Sequencer (Applied Biosystems). Additional sequences of Cytb and 16S and COI deposited at GenBank were added to the analysis, including several representative species of South and North American species complexes and subcomplexes. *Rhodnius prolixus* were used to root the resulting phylogenies (Table 2).

Sequences were edited with BioEdit 7.0.5 and aligned with ClustalW (Larkin et al., 2007). Nucleotide data for Cytb and COI were transformed into aminoacid sequences to check the alignment. Phylogenetic analyses of concatenate 16S, Cytb, and COI sequences were run under a Bayesian framework in MrBayes 3.1 (Huelsenbeck and Ronquist, 2001; 2 independent runs, 4 chains, and 1 M gens) and under a maximum parsimony criterion in PAUP* 4.0b10 (Swofford, 1998; *hsearch*, 1000 random addition replicates with TBR branch swap, gaps treated as "?"). The following evolutionary models were chosen for the three partitions using the Akaike Information Criterion in MrModeltest (Nylander, 2004): HKY + G for 16S rDNA; GTR + I + G for Cytb; and for COI was used HKY + I + G. Clade support was estimated by Bayesian posterior probabilities (BPP) and 1,000 parsimony bootstrap pseudoreplicates (BP).

3. Results

Sixteen new sequences were obtained (five for 16S, five for Cytb, and six for COI) and aligned with others available at GenBank (Table 2) and cropped according to the shorter sequences. The alignment constructed for the phylogenetic analysis included 256 bp of 16S (95 variable sites, including 79 parsimony informative), 313 bp of Cytb (141 variable sites, including 128 parsimony informative), and 202 bp of COI (87 variable sites, including 76 parsimony informative), totalizing 771 bp evaluated. However, sequences deposited in the GenBank were much longer, with variable length.

A single haplotype for each gene was found for *T. tibiamaculata* and *T. vitticeps*. Each population of *T. melanocephala* also exhibited a single haplotype for each gene. Interspecific pairwise divergences (uncorrected p-distances) between *T. melanocephala* specimens varied in 16S sequences from 0.8% (between 1 vs. 3) to 1.2% (2 vs. 3 and 1), in Cytb sequences from 0.3% (between 1 vs. 3) to 4.1% (2 vs. 1), and in COI sequences from 4.8% (between 1 vs 2) to 10.8% (3 vs. 2). Given that 1 and 2, 2 and 3, and 1 and 3 are localities distant by 73.9 km, 80.4 km, and 22.0 km, the variation found

Table 1
Number and data of colonies studied.

Species	Number (CTA)[a]	Origin	Initiated	Coordinates
T. juazeirensis	207	Juazeiro - BA	01/12/2007	-9 42′ 63.43637″, -40 50′ 31.55027″
T. melanica	206	Urandi - BA	29/07/2008	-14 76′ 40.25299″, -42 64′ 99.30749″
T. melanocephala	221 (1)[b]	Bom Jesus da Serra – BA	26/04/2010	-14 22′ 04.46160″, -40 30′ 52.55281″
	219 (2)[b]	Jequié – BA	08/10/2009	-13 51′ 03.75834″, -40 04′ 52.22281″
	220 (3)[b]	Poções – BA	17/11/2009	-14 31′ 01.94880″, -40 22′ 43.37040″
T. tibiamaculata	195	Mogi-Guaçu – SP	17/06/1986	-22 22′ 14.51637″, -46 56′ 16.73298″
T. vitticeps	198	Guarapari - ES	01/11/1979	-20 39′ 01.41478″, -40 30′ 25.29000″

[a] CTA - Colony number registration.
[b] 1, 2 and 3 - different collection spots of *T. melanocephala*.

Table 2
Accession GenBank codes of mitochondrial genes (16S, Cytb and COI fragments) of triatominaes used in this study. Sequences obtained in this study are in bold. Species placements under groups recovered in the phylogenetic analysis (Fig. 1) are also given. Species marked with asterisks did not have the 3 genes deposited in the GenBank. Sequences obtained in this study are in bold.

(Sub)complex[a]	Species	Groups	16S	Cytb	COI
T. brasiliensis	*T. brasiliensis*	A	EU827222	FJ623064	AF021186
	T. juazeirensis	A	**KF769453**	AY494169	**AF826892**
	T. melanica	A	**KF769454**	AY336527	**AF826891**
	T. melanocephala 1	C	**KF769452**	**KF826898**	**AF826893**
	T. melanocephala 2	C	**KF769450**	**KF826899**	**AF826894**
	T. melanocephala 3	C	**KF769451**	**KF826900**	**AF826895**
	T. sherlocki	A	EU489057	EU489058	KC608987
	T. tibiamaculata	C	AY185843	**KF826897**	**AF826890**
	T. vitticeps	C	EU827202	**KF826896**	AF021219
T. dimidiata	*T. dimidiata*	C	AY062138	JN585910	AF301594
T. infestans	*T. delpontei*	D	AF324520	HQ333241	FJ439768
	T. infestans	D	EU143699	AY702023	AF021199
	T. platensis∗	D	AF028744	–	AF021202
T. lecticularia	*T. rubida*	C	AY185842	DQ198810	DQ198800
T. maculata	*T. maculata*	B	EU827231	KC608977	AF449139
	T. pseudomaculata	B	EU827225	KC608979	KC608986
T. matogrossensis	*T. guazu*	B	KC571994	KC608976	KC608984
	T. matogrossensis	B	KC571995	KC608978	KC608985
	T. vandae∗	B	KC571997	–	KC608989
	T. williami	B	KC571998	KC608981	KC608990
T. phyllosoma (=*Meccus*)	*Mec. longipennis*∗	C	–	DQ198815	DQ198804
	Mec. mazzotti	C	AY035446	AY859421	DQ198805
T. rubrovaria	*T. circummaculata*∗	B	AF021188	–	AF021191
	T. rubrovaria	B	AF021203	GQ398003	AF021204
T. sordida	*T. garciabesi*∗	B	AY185835	–	EF451041
	T. sordida	B	KC571996	KC608980	KC608988
M. spinolai (=*Mepraia*)	*Mep. gajardoi*∗	C	–	JN102359	–
	Mep. spinolai∗	C	AY035467	JN102358	–
	T. eratyrusiformis∗	C	AY035466	JN102360	–
Outgroups	*Rhodnius neglectus*	–	AF045704	AF045716	EU444068
	Rhodnius prolixus	–	AF045707	EF011726	AF449138
	Dipetalogaster maximus∗	C	–	AF045728	–
	Panstrongylus herreri (=*P. lignarius*)∗	C	AY185833	–	AF449141
	Panstrongylus megistus	C	AF045701	AF045722	AF021179

(-) Not available.
[a] Schofield and Galvão, 2009.

in these intraspecific pairwise divergences are in accordance with geographic linear distances.

The resulting Bayesian consensus (burnin = 10%, mean of lnL of postburnin trees = −6581.46 [ESS = 1891.43]) is shown in Fig. 1, and clades also present in the strict consensus of the three most parsimonious trees (length = 1350, CI = 0.37, RI = 0.57, RC = 0.21) are shown in the same figure. Phylogenetic analyses conducted suggest the division of Triatominae in four clades, named A, B, C, and D with high support, but the present dataset does not give strong evidence concerning the relationships amongst these clades (Fig. 1). Most South American *Triatoma* were recovered in clades D (*T. infestans* subcomplex), A (*T. brasiliensis* subcomplex), and B (*T. maculata, T. matogrossensis, T. rubrovaria*, and *T. sordida* subcomplexes). However, the newly sequenced species, *T. melanocephala, T. tibiamaculata*, and *T. vitticeps*, were recovered with strong support within clade C (BPP = 99%), which also includes different triatomine genera (*Dipetalogaster* and *Panstrongylus*), species groupings of *Triatoma* from North America, the *T. phyllosoma* subcomplex and *T. lecticularia* complex (the former also sometimes treated as *Meccus*), and the Chilean *T. spinolai* complex (also sometimes treated as *Mepraia*). Concerning the species of interest within clade C, *T. vitticeps* was recovered as sister to populations of *T. melanocephala* with high support (BPP = 100%, BP = 98). Furthermore, Bayesian inference recovered *T. tibiamaculata* as sister to *P. megistus* with strong support (BPP = 99%). Terminals of clade C definitely show higher pairwise divergences than the ones between terminals of species complexes in other clades.

4. Discussion

4.1. The status of the T. brasiliensis subcomplex

Among the triatomines, *Triatoma* includes the highest number of described species and these are subdivided into complex and subcomplexes. The definition of the *T. brasiliensis* subcomplex by Schofield and Galvão (2009) was more recently questioned by cytogenetic studies in Alevi et al. (2012a). In this study, authors proposed that *T. melanocephala* should be excluded from this subcomplex because their karyotype structure matches with species of *Panstrongylus, Mepraia, Meccus*, and other *Triatoma* species from North America. In addition, the inclusion *T. tibiamaculata* and *T. vitticeps* in the *T. brasiliensis* subcomplex by Schofield and Galvão (2009) was already uncertain based on previous cytogenetic information (Panzera et al., 1998).

Based only on distance analyses of 16S sequences (parsimony analysis did not recover it), Hypsa et al. (2002) had previously found the close relationship of *T. tibiamaculata* to *P. megistus* and not to *T. brasiliensis*. More recently, Mendonça et al. (2009) sequenced fragments of Cytb and 16S to study the placement of

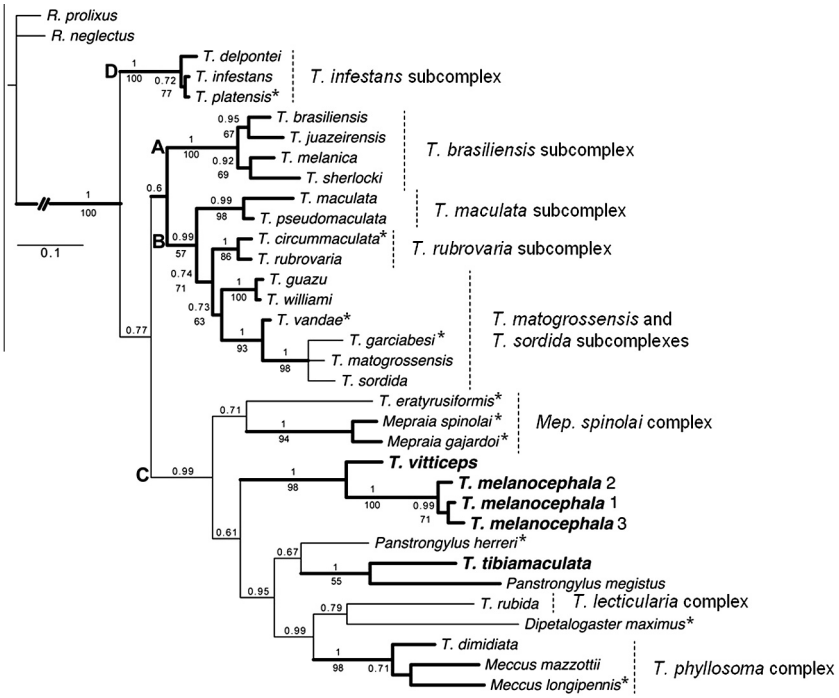

Fig. 1. Bayesian inference consensus of the combined analysis of sequences of triatomines used for the phylogenetic reconstruction. Molecular evolution models for each partition were HKY + G for 16S rDNA; GTR + I + G for Cytb; and for COI was used HKY + I + G. Thick clades represent those also recovered by parsimony analysis. Values above the clades indicate Bayesian posterior probabilities (BPP) whereas those below indicate parsimony bootstrap (PB). Species marked with asterisks did not have the three gene fragments for the analysis.

T. sherlocki in the *T. brasiliensis* subcomplex, but although the species was recovered with high clade support as related to *T. brasiliensis*, *T. juazeirensis* (without 16S data), and *T. melanica*, the study of only four species included in the subcomplex and usage of only a couple *Triatoma* species (*T. sordida* and *T. infestans*) as outgroups, did preclude this dataset to be good test of monophyly of this group. With the present dataset, we herein confirmed their previous suggestion.

We feel that with the inclusion of the new sequences generated herein and the addition of several species belonging to different *Triatoma* groupings, we have a more robust dataset to test this hypothesis. Our results do agree with authors above-cited since *T. tibiamaculata*, and the newly sequenced *T. melanocephala* and *T. vitticeps* were strongly recovered in a clade separate from the other clades with the remaining species of *Triatoma* from South America. Therefore the *T. brasiliensis* subcomplex, as currently defined by Schofield and Galvão (2009) was not recovered as a monophyletic unit, and should probably be redefined to include only *T. brasiliensis, T. juazeirensis*, *T. melanica, T. sherlocki, T. petrochiae,* and *T. lenti*, although the position of last two species was never evaluated with evidence other than morphology (Schofield and Galvão, 2009; Lent and Wygodzinsky, 1979) and cytogenetic information (Panzera et al., 2000; Alevi et al., 2012b; Alevi et al., 2013b). Relationships among members of *T. brasiliensis* subcomplex have been mainly tested based on biologic features. As such, cross-mating experiments have detected the reproductive compatibility among *T. brasiliensis*, *T. juazeirensis*, *T. melanica*, and *T. sherlocki* (Costa et al., 2003; Correia et al., 2013). On the other hand, *T. petrochiae*, and *T. lenti* failed to produce hybrids with *T. brasiliensis* (Espínola, 1971; Heitzmann-Fontenelle, 1984).

Based on our evidence, we herein propose to exclude *T. tibiamaculata*, *T. melanocephala*, and *T. vitticeps* from the *T. brasiliensis* subcomplex, and that the inclusion of *T. petrochiae* and *T. lenti* should be waiting phylogenetic studies including these sylvatic species, also difficult to be collected. However, we shall not propose a placement of these species in the triatomine classification at this moment, because we feel that to accomplish this, a future analysis should include a good sampling of non-South American *Triatoma* groupings and other triatomine genera.

4.2. Reinforcing the need for a generic revision of the Triatomini

Hypsa et al., (2002) based on the analysis of 16S sequences have previously recovered the polyphyly of *Triatoma* with respect to other triatomine genera, such as *Linshcosteus*, *Dipetalogaster*, *Eratyrus*, and *Panstrongylus* species. The polyphyly was mostly caused by the position of members of North American *Triatoma* groupings, such as the *T. phyllosoma* and *T. protracta* subcomplexes and *Meccus*, the Antillean *T. flavida* complex (sometimes treated as *Nesotriatoma*), the Chilean *Mepraia*, and the mostly Oriental *T. rubrofasciata* complex. Furthermore, as corroborated in this work with the addition of Cytb and COI sequences, the South American *T. tibiamaculata* also does contribute to this polyphyly, having been recovered as related to *Panstrongylus*. In the analyses of Hypsa and collaborators (2002), however, 16S rDNA alone was not useful in positioning *T. vitticeps*, but the parsimony analysis of combined 16S, 12S rDNA, and COI placed this species as related to the South American clade. In the present study, the inclusion of Cytb, combined with 16S and COI, groups with strong support this species to the newly sequenced *T. melanocephala*, and contrastingly places this clade together with the North America/Oriental clade. The growing accumulation of sequence data, generating more robust phylogenetic hypotheses, increasingly reinforces the need for a generic revision for the tribe. This revision should address not only the definition of species complexes and subcomplexes, but also of which species are included in other triatomine genera. In particular to this study, *Panstrongylus* was recovered as paraphyletic in relation to *T. tibiamaculata*, and this finding should be given more attention, as morphological studies should underline a possible future transfer of this species to *Panstrongylus.* Out of the 32

species included in this studied, only ten did not have sequences for all three markers (16S, Cytb, and COI). We understand that this may result in a phylogenetic signal loss, which might have been responsible in part for the low support in the placement of these species, thus these results must be interpreted with caution. Nevertheless, a separate analysis without these ten species, recovers an identical topology for the species of interest (members of the *T. brasiliensis* subcomplex) and similar associated clade support.

4.3. Eco-epidemiological implications

Triatoma vitticeps is nowadays recognized one of the potential Chagas disease vectors and, recently human cases of Chagas disease transmission has been attributed to this vector in Rio de Janeiro State throughout domiciliary colonization (Gonçalves et al., 1988; Gonçalves et al., 2000; dos Santos et al., 2005; Lorosa et al., 2008). On the other hand, *T. melanocephala* seems to remain exclusively sylvatic (Galvão et al., 2003; Gurgel-Gonçalves et al., 2012) in the Northeastern Brazil semi-arid. Nevertheless, given the close relationship found herein between these two species, we suggest continuously monitoring *T. melanocephala*.

Panstrongylus megistus has been incriminated as one of the most important Chagas disease vector in semi-arid zones of Brazil in the past (Dias et al., 2000), being eliminated from this non-native area due to a huge and costly effort of interventions of Brazilian Public Health Authorities. In fact, Dias and collaborators (2000) stated that *P. megistus* was passively carried out by humans, and that its native range was within the Atlantic Tropical Rainforest (Forattini, 1980). The close relationship between *P. megistus* and *T. tibiamaculata*, 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 (Santana et al., 2011). The capacity of *P. megistus* to transmit Chagas disease and to be spread out by humans, also calls for intensifying the monitoring of *T. tibiamaculata* domiciliary invasions.

5. Conclusions

We propose the exclusion of *T. melanocephala*, *T. tibiamaculata*, and *T. vitticeps* from the *T. brasiliensis* subcomplex. The three sylvatic Brazilian triatomines herein focused were recovered with strong support based on three mitochondrial markers (Cytb, COI, and 16S) and two different methods of tree reconstruction as closely related to other triatomine genera and *Triatoma* species with North American distribution. This study corroborates previous suggestions of polyphyly for *Triatoma* and stresses the need of a revision of the *Triatoma* and related genera taking into account phylogenetic results. Because of the epidemiological scenario of triatomine domiciliation is dynamic, we suggest attention to *T. melanocephala*, which was recovered as sister species to *T. vitticeps*, a known vector of Chagas disease.

Acknowledgements

We thank to Eliane Góes Nascimento from *Secretaria de Saúde do Estado da Bahia* for capturing the *T. melanocephala* specimens used. Financial support: Fapesp - process numbers 2010/50355-1, 2010/17027-0, and 2011/22378 and CAPES - process number 23038.005285/2011-12.

References

Alevi, K.C.C., Mendonça, P.P., Pereira, N.P., Rosa, J.A., Oliveira, M.T.V.A., 2012a. Karyotype of *Triatoma melanocephala* Neiva and Pinto (1923). Does this species fit in the Brasiliensis subcomplex? Infect. Genet. Evol. 12, 1652–1653.

Alevi, K.C.C., Mendonça, P.P., Succi, M., Pereira, N.P., Rosa, J.A., Azeredo-Oliveira, M.T.V., 2012b. Karyotype of *Triatoma lenti* (Hemiptera: Triatominae), a potential Chagas vector. Genet. Mol. Res. 11, 4278–4284.

Alevi, K.C.C., Rosa, J.A., Azeredo Oliveira, M.T.V., 2013a. Mini review: karyotypic survey in Triatominae subfamily (Hemiptera, Heteroptera). Entomol. Ornithol. Herpetol. 2, 106. http://dx.doi.org/10.4172/2161-0983.1000106.

Alevi, K.C.C., Mendonça, P.P., Pereira, N.P., Guerra, A.L., Facina, C.H., Rosa, J.A., Azeredo-Oliveira, M.T.V., 2013b. Distribution of constitutive heterochromatin in two species of triatomines: *Triatoma lenti* Sherlock and Serafim (1967) and *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira & Barata (2002). Infect. Genet. Evol. 13, 301–303.

Almeida, C.E., Oliveira, H.L., Correia, N., Dornak, L.L., Gumiel, M., Neiva, V.L., Harry, M., Mendonça, V.J., Costa, J., Galvão, C., 2012. Dispersion capacity of *Triatoma sherlocki*, *Triatoma juazeirensis* and laboratory-bred hybrids. Acta Trop. 122, 71–79.

Bargues, M.D., Klisiowicz, D.R., Gonzalez-Candelas, F., Ramsey, J.M., Monroy, C., Ponce, C., Salazar-Schettino, P.M., Panzera, F., Abad-Franch, F., Sousa, O.E., Schofield, C.J., Dujardin, J.P., Guhl, F., Mas-Coma, S., 2008. Phylogeography and genetic variation of *Triatoma dimidiata*, the main Chagas disease vector in Central America, and its position within the genus *Triatoma*. PLoS Neglected Trop. Dis. 7, e233.

Bargues, M.D., Schofield, C.J., Dujardin, J.P., 2010. Classification and phylogeny of the Triatominae. In: Telleria, J., Tibayrenc, M. (Eds.), American Trypanosomiasis: Chagas Disease One Hundred Years of Research. Elsevier, London, pp. 117–148.

Bérenger, J.M., Blanchet, D., 2007. A new species of the genus *Panstrongylus* from French Guiana (Heteroptera; Reduviidae; Triatominae). Mem. Inst. Oswaldo Cruz 102, 733–736.

Chagas, C.R.J., 1909. Nova tripanozomiaze humana. Estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi*, n. gen. n. esp, agente etiológico de nova entidade mórbida do homem. Mem. Inst. Oswaldo Cruz 1, 159–218.

Correia, N., Almeida, C.E., Lima-Neiva, V., Gumiel, M., Dornak, L.L., Lima, M.M., Medeiros, L.M., Mendonça, V.J., Rosa, J.A., Costa, J., 2013. Cross-mating experiments detect reproductive compatibility between *Triatoma sherlocki* and other members of the Triatoma brasiliensis species complex. Acta Trop. 128, 162–167.

Costa, J., 1999. The synanthropic process of Chagas disease vectors in Brazil, with special attention to *Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae, Triatominae) population, genetical, ecological, and epidemiological aspects. Mem. Inst. Oswaldo Cruz 94, 239–241.

Costa, J., Felix, M., 2007. *Triatoma juazeirensis* sp. nov. from the state of Bahia, Northeastern Brazil (Hemiptera: Reduviidae: Triatominae). Mem. Inst. Oswaldo Cruz 102, 87–90.

Costa, J., Lorenzo, M., 2009. Biology, diversity and strategies for the monitoring and control of triatomines - Chagas disease vectors. Mem. Inst. Oswaldo Cruz 104, 46–51.

Costa, J., Almeida, C.E., Dujardin, J.P., Beard, C.B., 2003. Crossing experiments detect genetic incompatibility among populations of *Triatoma brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae, Triatominae). Mem. Inst. Oswaldo Cruz 98, 637–639.

Costa, J., Argolo, A., Felix, M., 2006. Redescription of *Triatoma melanica* Neiva & Lent, 1941, new status (Hemiptera: Reduviidae: Triatominae). Zootaxa 1385, 47–58.

Costa, J., Correia, N.C., Lima-Neiva, V., Gonçalves, T.C.M., Felix, M., 2013. Revalidation and redescription of *Triatoma brasiliensis macromelasoma* Galvão, 1956 and an identification key for the *Triatoma brasiliensis* complex (Hemiptera: Reduviidae: Triatominae). Mem. Inst. Oswaldo Cruz 108, 1–5.

Dias, J.C.P., Machado, E.M.M., Fernandes, A.L., Vinhaes, M.C., 2000. Esboço geral e perspectivas da doença de Chagas no Nordeste do Brasil. Cad. Saude Publica 16, 13–34.

Espínola, H.N., 1971. Reproductive isolation between *Triatoma brasiliensis* Neiva, 1911 and *Triatoma petrochii* Pinto & Barreto, 1925 (Hemiptera Reduvvidae). Rev. Brasileira Biologia 31, 278–281.

Forattini, O.P., 1980. Biogeography, origin, and distribution of triatominae domiciliarity in Brazil. Rev. Saúde Pública 14, 265–299.

Galvão, C., Ângulo, V.M., 2006. *Belminus corredori*, a new specie of Bolboderini (Hemiptera: Reduviidae: Triatominae) form Santander, Colômbia. Zootaxa 1241, 61–68.

Galvão, C., Carcavallo, R., Rocha, D.S., Jurberg, J., 2003. Checklist of the current valid species of the subfamily Triatominae Jeannet, 1919 (Hemíptera, Reduviidae) and their geographical distribuition, with nomenclatural and taxonomic notes. Zootaxa 202, 1–36.

Gardim, S., Rocha, C.S., Almeida, C.E., Takiya, D.M., Silva, M.T.A., Ambrósio, D.L., Cicarelli, R.M.B., Rosa, J.A., 2013. Evolutionary relationships of the *Triatoma matogrossensis* subcomplex, the endemic *Triatoma* in Central-Western Brazil, based on mitochondrial DNA sequences. Am. J. Trop. Med. Hyg. 89, 766–774.

Gaunt, M.W., Miles, M.A., 2002. An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Mol. Biol. Evol. 19, 748–761.

Gonçalves, T.C.M., Victorio, V.M.N., Jurberg, J., Cunha, V., 1988. Biologia do *Triatoma vitticeps* (Stal, 1859) em condições de laboratório (Hemiptera: Reduviidae: Triatominae). I. Ciclo evolutivo. Mem. Inst. Oswaldo Cruz 83, 519–523.

Gonçalves, T.C.M., Rocha, D.S., Cunha, R.A., 2000. Feeding patterns of Triatoma vitticeps in the State of Rio de Janeiro, Brazil. Rev. Saúde Pública 34, 348–352.

Gonçalves, T.C.M., Teves-Neves, S.C., dos Santos-Mallet, J.R., Carbajal-de-la-Fuente, A.L., Lopes, C.M., 2013. *Triatoma jatai* sp. nov. in the state of Tocantins, Brazil (Hemiptera: Reduviidae: Triatominae). Memórias do Instituto Oswaldo Cruz 108, 429–437.

Gurgel-Gonçalves, R., Galvão, C., Costa, J., Peterson, A.T., 2012. Geographic distribution of Chagas disease vectors in Brazil based on ecological niche modeling. J. Trop. Med. 2012, 1–15.

Heitzmann-Fontenelle, T.J., 1984. Bionomia comparativa de triatomíneos. VII – *Rhodnius neglectus* Lent, 1954 (Hemiptera, Reduviidae). Mem. Inst. Butantan 48, 183–188.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hypsa, V., Tietz, D., Zrzavy, J., Rego, R.O., Galvão, C., Jurberg, J., 2002. Phylogeny and biogeography of Triatominae (Hemiptera: Reduviidae): molecular evidence of a New World origin of the Asiatic clade. Mol. Phylogenet. Evol. 23, 447–457.

Jurberg, J., Rocha, D.S., Galvão, C., 2009. *Rhodnius zeledoni* sp. nov. afim de Rhodnius paraensis Sherlock, Guitton and Miles, 1977 (Hemiptera, Reduviidae, Triatominae). Biota Neotropica 9, 123–128.

Jurberg, J., Cunha, V., Cailleaux, S., Raigorodschi, R., Lima, M.S., Rocha, D.S., Moreira, F.F.F., 2013. *Triatoma pintodiasi* sp. nov. of the *T. rubrovaria* subcomplex (Hemiptera, Reduviidae, Triatominae). Revista Pan-Amazônica de Saúde 4, 43–56.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 1, 2947–2948.

Lent, H., Wygodzinsky, P., 1979. Revision of the triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas's disease. Bull. Am. Museum Nat. Hist. 163, 123–520.

Lorosa, E.S., Santos, C.M., Jurberg, J., 2008. Foco de doença de Chagas em São Fidélis, no estado do Rio de Janeiro. Rev. Sociedade Brasileira Med. Trop. 41, 419–420.

Marcilla, A., Bargues, M.D., Ramsey, J.M., Gastelum, E.M., Schettino, P.M.S., Abad-Franch, F., Dujardin, J.P., Schofield, C.J., Mas-Coma, S., 2001. The ITS-2 of the nuclear rDNA as a molecular marker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease. Mol. Phylogenet. Evol. 18, 136–142.

Marcilla, A., Bargues, M.D., Abad-Franch, F., Panzera, F., Carcavallo, R.U., Noireau, F., Galvão, C., Jurberg, J., Miles, M.A., Dujardin, J.P., Mas-Coma, S., 2002. Nuclear rDNA ITS-2 sequences reveal polyphyly of *Panstrongylus* species (Hemiptera: Reduviidae: Triatominae), vectors of *Trypanosoma cruzi*. Infect. Genet. Evol. 1, 225–235.

Martínez, E., Chávez, T., Sossa, D., Aranda, R., Benigno, V., Vidaurre, P., 2007. *Triatoma boliviana* sp. n. de los valles subandinos de La Paz, Bolívia (Hemiptera: Reduviidae: Triatominae), similar a *Triatoma nigromaculata* Stål, 1859. Bol. Inst. Invest. Salud y Desarrollo 3, 1–10.

Mello, D.A., 1982. Roedores, marsupiais e triatomíneos silvestres capturados no município de Mambaí-Goiás. Infecção natural pelo *Trypanosoma cruzi*. Rev. Saúde Pública 16, 282–291.

Mendonça, V.J., Silva, M.T.A., Araujo, R.F., Junior, J.M., Junior, M.B., Almeida, C.E., Costa, J., Graminha, M.A.S., Cicarelli, R.M.B., Rosa, J.A., 2009. Phylogeny of *Triatoma sherlocki* (Hemiptera: Reduviidae: Triatominae) inferred from two mitochondrial genes suggests its location within the *Triatoma brasiliensis* complex. Am. J. Trop. Med. Hyg. 81, 858–864.

Monteiro, F.A., Donnelly, M.J., Beard, C.B., Costa, J., 2004. Nested clade and phylogeographic analyses of the Chagas disease vector *Triatoma brasiliensis* in Northeast Brazil. Mol. Phylogenet. Evol. 32, 46–56.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Panzera, F., Scvortzoff, E., Perez, R., Panzera, Y., Hornos, S., Cestau, R., Nicolini, P., Delgado, V., Alvarez, F., Mazzella, M.C., Cossio, G., Martinez, M., Salvatella, R., 1998. Cytogenetics of Triatomines. In: Carcavallo, R.U. (Ed.), Atlas of Chagas Disease Vectors in the Americas. Fiocruz, Rio de Janeiro, pp. 621–664.

Panzera, F., Pérez, R., Nicolini, P., Hornos, P., Costa, J., Borges, E., Diotaiuti, L., Scholfield, J., 2000. Chromosome homogeneity in populations of *Triatoma brasiliensis* Neiva 1911 (Hemiptera–Reduviidae–Triatominae). Cad. Saúde Pública 16, 83–88.

Papa, A.R., Jurberg, J., Carcavallo, R.U., Cerqueira, R.L., Barata, J.M.S., 2002. *Triatoma sherlocki* sp. n. coletada na Bahia, Brasil (Hemiptera, Reduviidae, Triatominae). Entomología y Vectores 9, 133–146.

Poinar, J.R.G., 2005. *Triatoma dominicana* sp. n. (Hemiptera: Reduviidae: Triatominae) and *Trypanosoma antiquus* sp. n. (Stercoraria: Trypanosomatidae), the first fossil evidence of a Triatomine-Trypanosomatid vector association. Vector Borne Zoonotic Dis. 5, 72–81.

Rosa, J.A., Rocha, C.S., Gardim, S., Pinto, M.C., Mendonça, V.J., Ferreira Filho, J.C.R., Carvalho, E.O.C., Camargo, L.M.A., Oliveira, J., Nascimento, J.D., Cilense, M., Almeida, C.E., 2012. Description of *Rhodnius montenegrensis* sp.n. (Hemiptera: Reduviidae: Triatominae) from the state of Rondônia Brazil. Zootaxa 3478, 62–76.

Sandoval, C.M., Pabon, E., Jurberg, J., Galvão, C., 2007. *Belminus ferroae* n. sp. from the Colombian north-east, with a key to the species of the genus (Hemiptera: Reduviidae: Triatominae). Zootaxa 1443, 55–64.

Santana, K.S., Bavia, M.E., Lima, A.D., Guimarães, I.C., Soares, E.S., Silva, M.M., Mendonça, J., Martin, M.S., 2011. Spatial distribution of triatomines (Reduviidae: Triatominae) in urban areas of the city of Salvador, Bahia, Brazil. Geospatial Health 5, 199–203.

Santos, C.B., Ferreira, A.L., Leite, G.R., Ferreira, G.E.M., Rodrigues, A.A.F., Falqueto, A., 2005. Peridomiciliary colonies of *Triatoma vitticeps* (Stal, 1859) (Hemiptera, Reduviidae, Triatominae) infected with *Trypanosoma cruzi* in rural areas of the state of Espírito Santo, Brazil. Mem. Inst. Oswaldo Cruz 100, 471–473.

Schofield, C.J., Galvão, C., 2009. Classification, evolution, and species groups within the Triatominae. Acta Trop. 110, 88–100.

Usinger, R.L., Wygodzinsky, P., Ryckman, R.E., 1966. The biosystematics of Triatominae. Annu. Rev. Entomol. 11, 309–330.

Vinhaes, M.C., Dias, J.C.P., 2000. Doença de Chagas no Brasil. Cad. Saúde Pública 16, 7–12.

WHO, 2013. Fact sheet – Chagas disease (American trypanosomiasis). <http://www.who.int/mediacentre/factsheets/fs340/en/.n.340> (accessed 03.05.13).