

Mitochondrial Genes Reveal *Triatoma jatai* as a Sister Species to *Triatoma costalimai* (Reduviidae: Triatominae)

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Abstract. *Triatoma jatai* was described using a set of morphological structures from specimens collected in Paranã municipality of Tocantins State, Brazil. Under a Bayesian framework and using two mitochondrial genes (*16S* and *COI*), phylogenetic analysis recovered *T. jatai* as a sister species to *Triatoma costalimai* with higher genetic distances than between other well-recognized species. Our results agree with previous suggestions based on morphometric analysis. In the light of the non-monophyly of Matogrossensis subcomplex, the inclusion of *T. jatai* shall be considered for reevaluating this group.

The recently described Chagas disease vector, *Triatoma jatai*, occurs in rocky outcrops and currently presents its distribution restricted to the municipality of Paranã, Tocantins State, north of Brazil.¹ The natural environment where *T. jatai* occurs is a target of continuous environmental degradation, such as deforestation and burning activities.

In *T. jatai* description, the authors observed a close relationship with *Triatoma costalimai*. These two species can be differentiated by the size, general color and shape of wings, connexivum, intersegmental sutures, and genital structures, which made it possible to refine the taxonomic key built in 2012² for the inclusion of *T. jatai*. In addition to these morphological similarities, they observed morphometric closeness, suggesting that it should be included in the Matogrossensis subcomplex of the Infestans complex, which previously included *Triatoma baratai*, *T. costalimai*, *Triatoma deaneorum*, *Triatoma guazu*, *Triatoma jurbergi*, *T. matogrossensis*, *Triatoma vandae*, and *Triatoma williami*.³

In the article that brought up *T. jatai* to science, Gonçalves and others¹ evidenced its occurrence in sympatry with *T. costalimai* in limestone outcrops. A close relationship between these two species was also suggested by wing morphometrics.¹ Thus, in this study, two mitochondrial genes (*16S* and *COI*) were sequenced and analyses were run under a Bayesian framework to evaluate the phylogenetic relationships between *T. jatai* and other South American triatomines.

The wild samples of *T. jatai*, *T. costalimai*, and *Triatoma sordida* used in this study are shown in Table 1 and their GenBank accession numbers in Table 2. Evaluate the phylogenetic position of *T. jatai*, we included species representing the six subcomplexes defined in 2009,³ whenever available in GenBank, we added samples from the same

isolate. In addition, the choice of species downloaded from GenBank for running the analysis (Table 2) was based on the monophyletic clade of South American *Triatoma*.^{4,5} *Panstrongylus megistus* was set as the outgroup.

Total DNA extraction was performed according to the protocol described by Sambrook and Russel.⁶ From the extracted DNA, *16S* and *COI* fragments were amplified as described by Sainz and others⁷ and purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Life Sciences, Buckinghamshire, UK). 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, Warrington, UK). Resulting sequences were edited with BioEdit (version 7.0.5)⁸ and aligned with ClustalW version 44.⁹ Nucleotide data for *COI* were transformed into amino acid sequences to check the alignment for the presence of pseudogenes.¹⁰

Combined phylogenetic analyses using both genes were run under a Bayesian framework in MrBayes (version 3.1.45).¹¹ We conducted two independent runs of 1 million generations using four Markov chains and sampling trees every 100 generations (discarding the first 25%). The best evolutionary models were chosen by the Akaike information criterion implemented in the jModelTest (version 0.1.1)¹² as follows: for *16S* rDNA, HKY+I+G (nst=2, rates=invgamma) was used; for *COI*, GTR+I+G (nst=6, rates=invgamma) was used, yielding a matrix with 22 taxa and 984 nucleotides. Clade support was estimated by Bayesian posterior probabilities (BPP).

The same haplotype was found for all three *T. jatai* specimens for *16S*, on the other hand, none of the three specimens exhibited the same haplotype for *COI*, but with only five segregating sites. It is in agreement with the faster rate of mutation for *COI* gene.¹³ Phylogenetic analysis recovered *T. jatai* as a sister species to *T. costalimai* with high clade support (BPP = 98%; Figure 1). Results from both genes were congruent, revealing that *T. jatai* and *T. costalimai* exhibit higher *p*-distances than between valid species. For *16S*, the *p*-distances between *T. jatai* and *T. costalimai* were all 0.025. A greater differentiation between *T. jatai* and *T. costalimai* was observed for *COI* (*P* = 0.097–0.100). For both gene fragments, the distances between *T. jatai* and *T. costalimai* were greater than the ones within *Rubrovaria* (*Triatoma rubrovaria*,

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TABLE 1
Samples of triatomines used in this work

Subcomplex ³	Species	Origin
Matogrossensis	<i>Triatoma jatai_03</i>	Paraná, TO
	<i>T. jatai_05</i>	Paraná, TO
	<i>T. jatai_16</i>	Paraná – TO
	<i>Triatoma costalimai_06</i>	Aurora do Tocantins, TO
	<i>T. costalimai_09</i>	Aurora do Tocantins, TO
	<i>Triatoma williami_04</i>	Barra do Garças, MT
Sordida	<i>T. williami_05</i>	Barra do Garças, MT
	<i>Triatoma sordida_PAR03</i>	Paranaíba, MS
	<i>T. sordida_ITA24</i>	Itaobim, MG

TO, MT, MS, and MG are Tocantins, Mato Grosso, Mato Grosso do Sul, and Minas Gerais states, respectively.

Triatoma circummaculata, and *Triatoma caracavalloii*) and Infestans subcomplexes (*Triatoma infestans*, *Triatoma platensis*, and *Triatoma delpontei*).

When a taxonomic unit is described, it is important to undertake studies to increase knowledge for the entity in all grounds. Phylogenetic analysis of *T. jatai* based on mitochondrial genes demonstrated the close genetic relationship between *T. jatai* and *T. costalimai*, as it has been initially evidenced by morphological characters and geometric morphometrics of wings.¹ Despite the distinctions in taxa set, the topologic position for the remaining species in our tree was similar to the source from where they were downloaded,^{4,5} as expected.

Evaluating the evolutionary relationships of newly described or sylvatic species is crucial for understanding their vector role in Chagas disease epidemiology.^{4,5,14} In addition, the taxonomic validity of closely related species is sometimes a matter of questioning,^{15,16} and molecular approaches must accomplish a good dataset on distinct grounds. The large number of mutations that *T. jatai* have accumulated in regard to *T. costalimai* suggests a longer term separation than among members of

TABLE 2

Accession codes from GenBank sequences of *Triatoma* and outgroup species used in the phylogenetic analysis

Subcomplex ³	Species	16S	COI	
Brasiliensis	<i>Triatoma brasiliensis</i>	KC248985	KC249318	
	<i>Triatoma sherlocki</i>	KC249068	KC249377	
Infestans	<i>Triatoma infestans</i>	KC249014	KC249348	
	<i>Triatoma platensis</i>	KC249363	KC249047	
	<i>Triatoma delpontei</i>	KC249332	KC249001	
Maculata	<i>Triatoma maculata</i>	AF324524	AF449139	
	<i>Triatoma jatai_03</i>	KT601153	KT601162	
Matogrossensis	<i>T. jatai_05</i>	KT601154	KT601163	
	<i>T. jatai_16</i>	KT601155	KT601164	
	<i>Triatoma costalimai_06</i>	KT601151	KT601160	
	<i>T. costalimai_09</i>	KT601152	KT601161	
	<i>Triatoma williami_04</i>	KT601156	KT601165	
	<i>T. williami_05</i>	KT601157	KT601166	
	<i>T. costalimai</i>	KC571993	KC249327	
	<i>Triatoma matogrossensis</i>	KC249036	KC249359	
	<i>T. matogrossensis</i>	KC249038	KC249361	
	<i>Triatoma guazu</i>	KC571994	KC608984	
	<i>Triatoma vandae</i>	KC571997	KC608989	
	Rubrovaria	<i>Triatoma caracavalloii</i>	KC248990	KC249322
		<i>Triatoma circummaculata</i>	KC248994	KC249323
<i>Triatoma rubrovaria</i>		KC249066	KC249375	
Sordida	<i>Triatoma guasayana</i>	KC249342	KC249010	
	<i>Triatoma sordida</i>	KC249077	KC249386	
	<i>T. sordida PAR03</i>	KT601158	KT601167	
	<i>T. sordida ITA 24</i>	KT601159	KT601168	
Outgroup	<i>Panstrongylus megistus</i>	KC248975	KC249312	

Sequences obtained in this study are given in bold.

Infestans subcomplex. Morphological features evaluated for the sympatric *T. jatai* and *T. costalimai*¹ are supported by our results and confirm the status of *T. jatai*. What is more, by using 143 field samples of *T. jatai*, 113 of *T. costalimai* and other triatomine species, these authors addressed the close relationship between *T. jatai* and *T. costalimai*, in agreement with our results.

Mitochondrial genes are easy to sequence and have historically provided great contribution for phylogenetic reconstruction among triatomines.^{4,10,14} However, Mas-Coma and Bargues¹³ brought up some limitation for inferences on closely related taxa based on mitochondrial genes, particularly when dealing with sympatric or parapatric species without a clear barrier of isolation. These limitations rely mainly on introgression (sometimes followed by mitochondrial selection), complex population structure, and sex-biased gene flow. Taking into account some weakness of mitochondrial genome for inferences, the further use of nuclear markers (e.g., nuclear ribosomal DNA) is important. It is worth mentioning that females of *T. jatai* exhibit shorter wings, and are probably unable to fly, as observed by Almeida and others for *Triatoma sherlocki*,¹⁷ also a brachypterous species. The sessile characteristic for females must be taken into account for further population genetic studies.

Recently, Alevi and others¹⁸ described the Matogrossensis subcomplex cytogenetically and observed that the species of this subcomplex have the same cytogenetic characteristics as the species of the Rubrovaria subcomplex. Gardim and others¹⁰ and Justi and others⁵ have already brought up the non-monophyly of the Matogrossensis subcomplex. Hence, *T. jatai* shall be considered for reevaluating this group. Because some clades did not have high clade support within this subcomplex,¹⁰ we recommend the use of longer genes, a more complete species set, and multiple molecular markers, also containing nuclear genes. We also strongly recommend studies on the biological cycle, reproductive compatibility between *T. jatai* and *T. costalimai* as well as morphological studies of nymphs and antennal phenotype.

Received May 28, 2015. Accepted for publication November 3, 2015.

Published online January 19, 2016.

Note: Supplemental table appears at www.ajtmh.org.

Financial support: The study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Fundação de Amparo à Pesquisa do Estado de São Paulo (process nos. 2010/50.355-1, 2010/17027-0, and 2011/22378-0, FAPESP, Brazil).

Disclosure: Ana Laura Carbajal de La Fuente is a member of the “Consejo Nacional de Ciencia y Técnica de la República Argentina” (CONICET) Researcher’s Career.

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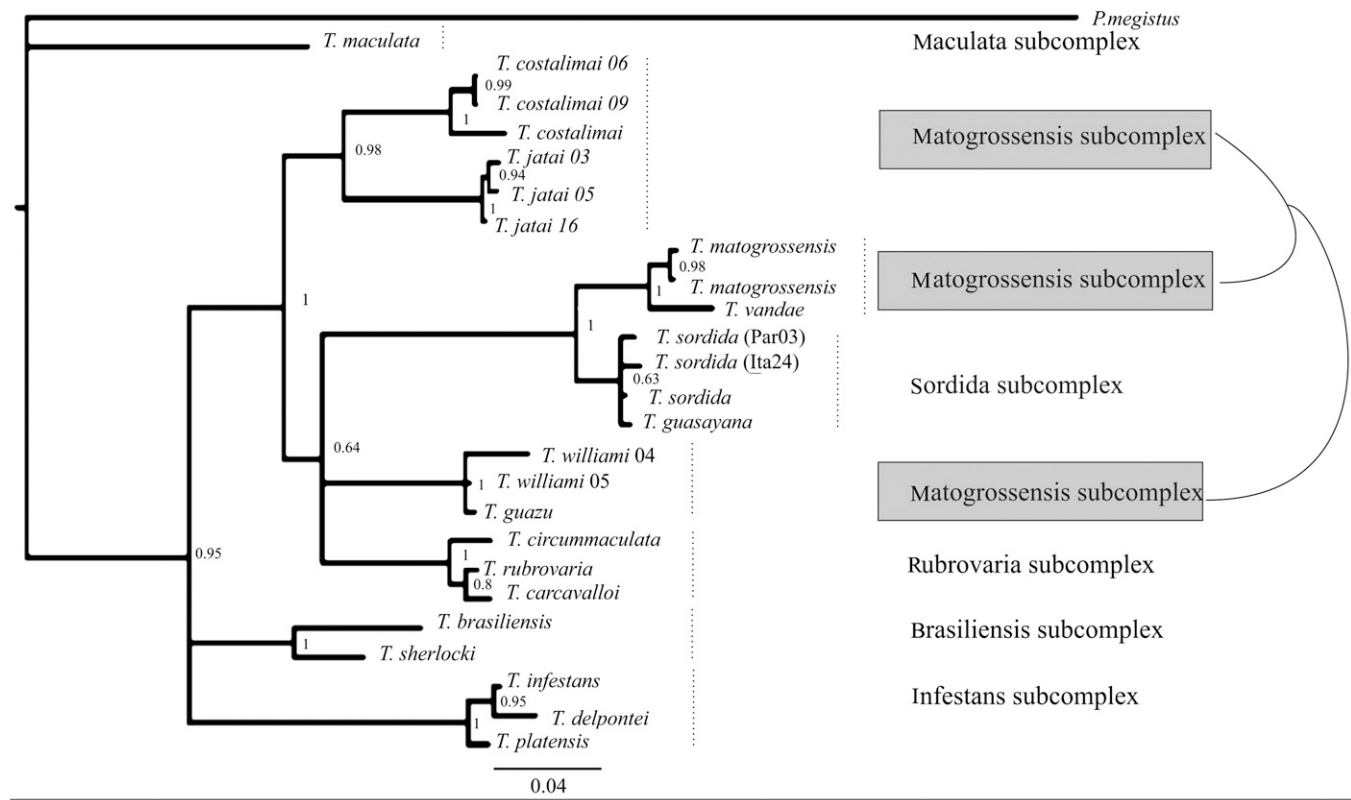


FIGURE 1. Bayesian inference consensus of the combined analysis of sequences of *Triatoma* species focused on *Triatoma jatai* based on *16S* and *COI* genes. Molecular evolution models for each partition were HKY+I+G for *16S* (444 bp) and GTR+I+G for *COI* (492 bp). Numbers above the nodes indicate Bayesian posterior probabilities. *Panstrongylus megistus* was selected as out-group.

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SUPPLEMENTAL TABLE 1

Uncorrected pairwise divergences between taxa

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Uncorrected <i>p</i> -distances for the <i>16S</i> mitochondrial rDNA																									
1	<i>Triatoma costalimai_06</i>																								
2	<i>T. costalimai_09</i>	0.000																							
3	<i>Triatoma jatai_03</i>	0.025	0.025																						
4	<i>T. jatai_05</i>	0.025	0.025	0.000																					
5	<i>T. jatai_16</i>	0.025	0.025	0.000	0.000																				
6	<i>Triatoma matogrossensis</i>	0.046	0.046	0.053	0.053	0.053																			
7	<i>Triatoma williamsi_04</i>	0.032	0.032	0.048	0.048	0.048	0.050																		
8	<i>T. williamsi_05</i>	0.032	0.032	0.048	0.048	0.048	0.050	0.000																	
9	<i>Triatoma sordida_PAR03</i>	0.039	0.039	0.046	0.046	0.046	0.021	0.043	0.043																
10	<i>T. sordida_ITA24</i>	0.039	0.039	0.046	0.046	0.046	0.021	0.043	0.043	0.000															
11	<i>Triatoma brasiliensis</i>	0.041	0.041	0.050	0.050	0.050	0.062	0.053	0.055	0.055															
	KC248985_KC249318																								
12	<i>Triatoma sherlocki</i>	0.037	0.037	0.039	0.039	0.039	0.059	0.048	0.048	0.048	0.021														
	KC249068.1_KC249377																								
13	<i>Triatoma maculata</i>	0.037	0.037	0.046	0.046	0.046	0.060	0.039	0.039	0.053	0.053	0.044													
	AF324524.1_AF449139.1																								
14	<i>Triatoma circummaculata</i>	0.041	0.041	0.055	0.055	0.055	0.053	0.048	0.048	0.046	0.055	0.046	0.060												
	KC248994.1_KC249323																								
15	<i>Triatoma rubrovaria</i>	0.037	0.037	0.046	0.046	0.046	0.053	0.048	0.048	0.046	0.041	0.055	0.009												
	KC249066.1_KC249375																								
16	<i>Triatoma carvalhoi</i>	0.041	0.041	0.050	0.050	0.050	0.057	0.048	0.048	0.055	0.050	0.046	0.060	0.014	0.005										
	KC248990.1_KC249322																								
17	<i>Triatoma infestans</i>	0.041	0.041	0.048	0.048	0.048	0.066	0.066	0.066	0.064	0.055	0.057	0.053	0.048	0.053										
	KC249014_KC249348																								
18	<i>Triatoma platensis</i>	0.053	0.053	0.055	0.055	0.055	0.062	0.064	0.064	0.059	0.055	0.050	0.055	0.055	0.050	0.055									
	KC249363_KC249047																								
19	<i>Triatoma delponiei</i>	0.041	0.041	0.048	0.048	0.048	0.066	0.066	0.066	0.064	0.055	0.057	0.053	0.048	0.053	0.030									
	KC249332_KC249001.1																								
20	<i>T. sordida</i>	0.041	0.041	0.048	0.048	0.048	0.018	0.041	0.041	0.002	0.057	0.050	0.046	0.062	0.066	0.066									
	KC249077.1_KC249386																								
21	<i>Triatoma guasayana</i>	0.046	0.046	0.053	0.053	0.053	0.014	0.046	0.046	0.007	0.062	0.055	0.060	0.053	0.057	0.062	0.071	0.066	0.071	0.005					
	KC249342_KC249010																								
22	<i>T. matogrossensis</i>	0.046	0.046	0.053	0.053	0.053	0.007	0.050	0.050	0.016	0.062	0.060	0.060	0.053	0.053	0.057	0.064	0.062	0.064	0.014	0.011				
	KC249036.1_KC249361																								
23	<i>T. costalimai</i>	0.020	0.020	0.032	0.032	0.032	0.067	0.044	0.044	0.063	0.063	0.056	0.048	0.052	0.056	0.044	0.044	0.060	0.067	0.060	0.063	0.071	0.068		
	KC571993_KC249327																								
24	<i>Panstrongylus megistus</i>	0.094	0.094	0.087	0.087	0.087	0.117	0.108	0.108	0.115	0.092	0.089	0.094	0.108	0.099	0.103	0.094	0.094	0.094	0.117	0.122	0.113	0.124		
	KC248975_KC249312																								

Uncorrected *p*-distances for *COI* gene of mitochondrial DNA

1	<i>T. costalimai_06</i>																								
2	<i>T. costalimai_09</i>	0.000																							
3	<i>T. jatai_03</i>	0.100	0.100																						
4	<i>T. jatai_05</i>	0.097	0.097	0.009																					
5	<i>T. jatai_16</i>	0.097	0.097	0.006	0.004																				
6	<i>T. matogrossensis</i>	0.129	0.129	0.129	0.123	0.127																			
7	<i>T. williamsi_04</i>	0.139	0.139	0.124	0.120	0.118	0.150																		
8	<i>T. williamsi_05</i>	0.119	0.119	0.108	0.108	0.106	0.129	0.040																	

(continued)

SUPPLEMENTAL TABLE 1
Continued

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
9 <i>T. sordida</i> _PAR03	0.134	0.134	0.121	0.112	0.116	0.061	0.143	0.131																
10 <i>T. sordida</i> _ITA24	0.134	0.134	0.123	0.114	0.117	0.057	0.146	0.138	0.019															
11 <i>T. brasiliensis</i> KC248985_KC249318	0.161	0.161	0.146	0.144	0.148	0.157	0.152	0.125	0.148	0.142														
12 <i>T. sherlocki</i> KC249068.1_KC249377	0.148	0.148	0.139	0.139	0.136	0.166	0.161	0.133	0.163	0.086														
13 <i>T. maculata</i> AF324524.1_AAF49139.1	0.171	0.171	0.171	0.163	0.165	0.171	0.185	0.171	0.165	0.162	0.162	0.164												
14 <i>T. circummaculata</i> KC248994.1_KC249323	0.110	0.110	0.117	0.119	0.116	0.125	0.105	0.091	0.125	0.129	0.134	0.145	0.171											
15 <i>T. rubrovaria</i> KC249066.1_KC249375	0.110	0.110	0.114	0.108	0.112	0.121	0.110	0.093	0.117	0.117	0.123	0.133	0.163	0.027										
16 <i>T. carcavalloi</i> KC248990.1_KC249322	0.108	0.108	0.116	0.114	0.114	0.131	0.116	0.095	0.127	0.127	0.131	0.136	0.171	0.028	0.017									
17 <i>T. infestans</i> KC249014_KC249348	0.153	0.153	0.144	0.148	0.146	0.189	0.165	0.146	0.186	0.182	0.150	0.145	0.186	0.140	0.142	0.144								
18 <i>T. platensis</i> KC249363_KC249047	0.157	0.157	0.140	0.144	0.142	0.186	0.162	0.142	0.182	0.178	0.146	0.139	0.186	0.140	0.142	0.144	0.004							
19 <i>T. delponteii</i> KC249332_KC249001.1	0.152	0.152	0.140	0.144	0.142	0.180	0.152	0.140	0.184	0.176	0.148	0.139	0.179	0.142	0.144	0.146	0.028	0.025						
20 <i>T. sordida</i> KC249077.1_KC249386	0.136	0.136	0.121	0.112	0.116	0.059	0.144	0.136	0.006	0.017	0.148	0.166	0.167	0.123	0.119	0.129	0.184	0.180	0.182					
21 <i>T. guasayana</i> KC249342_KC249010	0.138	0.138	0.127	0.117	0.121	0.059	0.146	0.134	0.009	0.017	0.146	0.169	0.165	0.125	0.121	0.131	0.188	0.184	0.186	0.008				
22 <i>T. matogrossensis</i> KC249036.1_KC249361	0.129	0.129	0.129	0.123	0.127	0.000	0.150	0.129	0.061	0.057	0.157	0.166	0.171	0.125	0.121	0.131	0.189	0.186	0.180	0.059	0.059			
23 <i>T. costalimai</i> KC571993_KC249327	0.032	0.032	0.110	0.102	0.106	0.129	0.148	0.121	0.140	0.142	0.157	0.151	0.171	0.129	0.121	0.119	0.155	0.159	0.157	0.144	0.146	0.129		
24 <i>P. megistis</i> KC248975_KC249312	0.174	0.174	0.169	0.167	0.167	0.189	0.186	0.176	0.188	0.189	0.167	0.186	0.133	0.180	0.182	0.189	0.174	0.176	0.172	0.191	0.189	0.176		