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Phylogeny of *Triatoma sherlocki* (Hemiptera: Reduviidae: Triatominae) Inferred from Two Mitochondrial Genes Suggests Its Location Within the *Triatoma brasiliensis* Complex

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Abstract. The phylogenetic position of *Triatoma sherlocki* within triatomines group was inferred by analyzing mtDNA fragments of *Cyt B* and *16S* ribosomal RNA by using maximum parsimony and Bayesian analysis. Despite being differentiated from members of the *T. brasiliensis* complex on morphologic grounds, molecular phylogenetic analysis suggests *T. sherlocki* is a member of this complex; moreover, it was placed as a sister species of *T. melanica*. These suggestions were supported by robust credibility rates. Hence, we show evidence for the paraphyletic group of the "*Triatoma brasiliensis* complex," which should be composed of *T. brasiliensis brasiliensis*, *T. brasiliensis macromelasoma*, *T. juazeirensis*, *T. melanica*, and *T. sherlocki*.

INTRODUCTION

The great importance of the Triatominae, a subfamily of blood-sucking bugs, in public health terms, derives from its capacity to transmit *Trypanosoma cruzi,* the etiologic agent of Chagas disease.[1] The overall prevalence of human infection is estimated at 15 million cases. Approximately 28 million inhabitants of Latin America are at risk of contracting this infection.[2]

Vector transmission is still considered the main route of human infection; therefore, most of the effort to prevent Chagas disease turns on the interruption of this transmission by controlling domestic vectors with pyrethroid insecticides. The Brazilian National Health Foundation (FUNASA) has used intense insecticide spraying since the 1990s in Brazil. An important achievement of this program has been the interruption of *T. cruzi* transmission by *Triatoma infestans*.[2,3]

The last checklist of triatomines included 137 valid species, distributed in 19 genera and 6 tribes.[4] However, these numbers do not reflect the actual scenario. Continuous changes take place in light of taxonomic reviews, and new species are described. At the time of writing 143 species and 18 genera have been recognized.[5–12]

Triatoma sherlocki Papa and others, 2002 is a highly endemic and wild species, described from specimens collected in the Santo Inácio district, of the town of Gentio do Ouro, Bahia state, Brazil.[13] This species was formerly recorded as a subspecies of *T. brasiliensis* Neiva, 1911 (*T. brasiliensis santinacensis*, Rui Cerqueira; unpublished data).

After the successful vector control program for *T. infestans*, *T. brasiliensis* became the main vectorial threat in semi-arid areas of northeastern Brazil.[2,4,14]

Triatoma brasiliensis shows remarkable chromatic variation across its geographic distribution.[15] This characteristic led this "species" to be subjected to a multi-disciplinary study of its morphology, biology, ecology, isoenzymes, and mitochondrial DNA variation.[14,16–20] It was concluded that *T. brasiliensis* is in fact a complex of species based on three allopatric evolutionary units: *T. brasiliensis* Neiva, 1911, *T. brasiliensis melanica* Neiva and Lent, 1941 and *T. brasiliensis macromelasoma* Galvão, 1956.[15]

Molecular markers based on DNA have led to considerable progress in the understanding of genetic relatedness, phylogeny, and population dynamics in triatomines during the last 10 years.[21–24] However, these studies have not included rare, wild, or newly described species. Because *T. sherlocki* is a newly described species, the aim of this study is to clarify the consistency of the morphologic information used to suggest that *T. sherlocki* is a species closely related to *T. brasiliensis*.

MATERIALS AND METHODS

Insects. *Triatoma sherlocki* (Figure 1A) samples used in this study were taken from a colony maintained at the Triatominae Insectarium of the São Paulo State University (UNESP, Araraquara, Brazil). The colony was generated from 26 specimens collected in a wild and rock upland environment, near the village of Santo Inácio (11°06′55″ S and 42°46′30″ W) in the district of Gentio do Ouro, state of Bahia, Brazil, in 2003 (Figure 2).

Triatoma brasiliensis (Figure 1B) specimens used were from a colony founded from 52 specimens collected in rural houses in São João do Piauí, State of Piauí (8°21′29″ S and 42°14′48″ W) in 1984 and maintained since then at the same Insectarium.

Triatoma melanica (Figure 1C) specimens were obtained from a colony maintained at Instituto Oswaldo Cruz (FIOCRUZ). The founders of this last colony were collected in the district of Espinosa (14°55′34″ S and 42°49′09″ W), a town in the state of Minas Gerais, among rock piles in a rocky wildness.

DNA extraction, PCR amplification, cloning, and sequencing. All six legs from one specimen of each species were cut with a sterile needle and ground in a microcentrifuge tube with a clean crusher. The total genomic DNA was extracted from single specimens by the standard phenol/chloroform technique and stored at −4°C until further use.[25]

Two target mitochondrial DNA fragments were amplified by polymerase chain reaction (PCR), resulting in a 414-bp fragment of the *Cyt B* gene and a 373-bp fragment of the *16S* large subunit ribosomal RNA region of the mitochondrial

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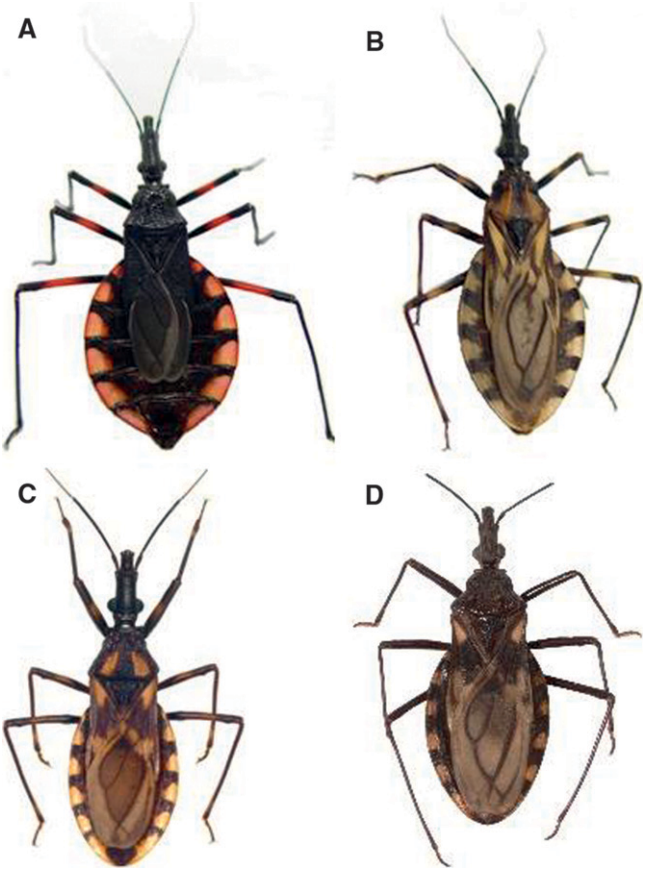

FIGURE 1. **A**, *T. sherlocki*; **B**, *T. brasiliensis*; **C**, *T. melanica*; **D**, *T. juazeirensis*.

gene. The DNA fragments were amplified with the following primer combinations: *Cyt B* (F: 5′-GGA CAA ATA TCA TTT TGA GGA GCA ACA G-3′) and (R: 5′-ATT ACT CCT CCT AGC TTA TTA GGA ATT G-3′) and *16S* mitochondrial gene (F: 5′-CRC CTG TTT AAC AAA AAC AT-3′ and R: 5′-AAA AAA ATT ACG CTG TTA TCC CTA AAG TAA-3′), performed in a PTC–100 thermal cycler (MJ Research).[21] The following reactions conditions were used: denaturation at 94°C for 5 minutes and then 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute, followed by a 72°C extension for 7 minutes and storage at 4°C indefinitely, for the both gene fragments.

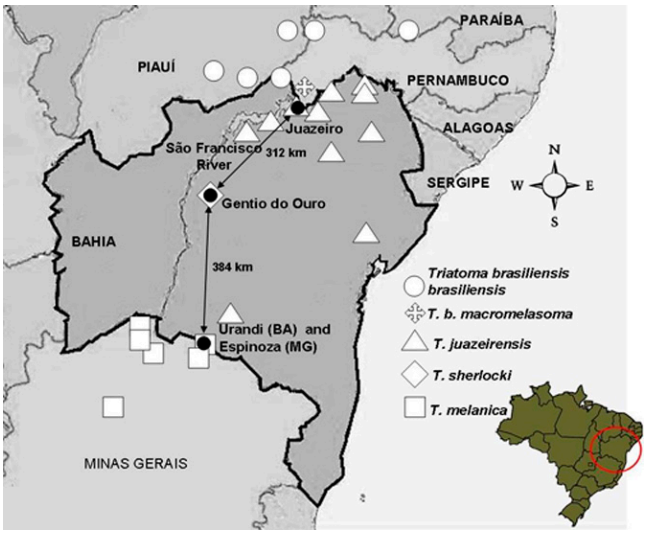

FIGURE 2. Brazil northeastern region with distribution of *T. brasiliensis* complex according to Costa et al. (2002). This figure appears in color at www.ajtmh.org.

The resulting amplicons were separated on a 1.5% agarose gel, and DNA fragments were extracted from the gel with an Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Upsala, Sweden), following the manufacturer's instructions, cloned into the pGEM-T vector (Promega, Madison, WI), and sequenced in an ABI PRISM 377 DNA Sequencer (Perkin Elmer, Wellesley, MA), using the Big Dye Terminator v1.0 Cycle Sequencing kit (Applied Biosystems, Foster City, CA).

Phylogenetic analysis. Sequences of DNA generated in this study, as well as other sequences from the GenBank, were edited with the program BioEdit 7.0.5 and aligned with the program ClustalW, using default parameters.[26,27] Because indels are common for the *16S* fragment, a pilot test was performed including more species in the alignment. However, no region of ambiguity was detected, and the pilot tree based on maximum parsimony (MP) analysis exhibited similar profile already shown by Garcia and Powell[28] and Garcia and others.[22]

The alignments were cropped in accordance with the shortest sequence and analyzed for Kimura 2-parameter distance producing neighbor-joining and phylogenetic trees through the software MEGA 3.0, whereas MP analysis was conducted using PAUP 4.0b10.[29,30] Clade support was estimated by 1,000 replications for both analyses. MP analyses were used with TBR (tree-bisection-reconnection) branch swapping. The TBR is the default search strategy in both PAUP* and MrBayes. It defines the branch lengths, being largely applied and exhibiting a satisfactory degree of reliability of branch lengths.

The choices of taxa to build the trees were made according to availability in the GenBank and to each question. We also used information provided by other authors to choose the most related taxa and the outgroups.[21,22,28]

The phylogeny was also analyzed by Bayesian inference with independent runs of Markov chains. Clade support was estimated using a Markov chain Monte Carlo (MCMC) algorithm performed by the program MrBayes version 3.1.[31] The first analysis was run for 1,000,000 generations, with sampling every 250 generations; it continued running until establishing the average SD of split frequencies. We used estimates of likelihood settings by using MrModeltest and chose the Akaike information criterion (AIC)[32] to obtain the best model for each gene and their posterior probability of credibility. The AIC indicates the best model to be applied to a given gene fragment (*16S* and *Cyt B*, in our case), by measuring of the goodness of fit of all possible statistical models.[33]

Thus, we used different models for the different partitions indicated by AIC. For *Cyt B*, we used the general model of DNA substitution with gamma-distributed rate variation across sites (GTR + G), assuming that all six substitution types have separate rate ratios (nst = 6), and three-codon partition. For the *16S* fragment, we used the GTR model with two substitution types that only distinguishes between transitions and transversions (nst = 2), a proportion of sites being invariant (rates = propinv). The default random tree option was used to begin the analysis. Log-likelihood values from four simultaneous MCMC chains stabilized at 8,000,000 generations, where the average SD of split frequencies was 0.004.

Summarizing the sampled parameter values by using the sump command, we observed that trees sampled during the first 2,000,000 generations of the run did not converge onto a stationary point, showing very different trees. Therefore, those first trees were discarded from the analysis, corresponding to 25% of our samples to avoid having a negative influence in the posterior probability values. *Triatoma infestans* and *T. sordida* were used to root the trees.

RESULTS

***Cytochrome b* (*Cyt B*).** The amplified fragment of the *Cyt B* region produced a sequence with 414 bp for both *T. sherlocki* and *T. brasiliensis*. The alignment was performed with the following species: *T. sherlocki*, *T. brasiliensis*, *T. brasiliensis brasiliensis*, *T. brasiliensis macromelasoma*, *T. juazeirensis*, *T. melanica* H, *T. infestans,* and *T. sordida.* The resulting consensus sequence had 399 bp, being shortened to the length of the *T. sordida* sequence. This alignment exhibited 120 (30%) polymorphic sites and 132 substitutions (30 transversions and 102 transitions). GenBank accession numbers and references with details about the species are shown in Table 1. Third-base modifications corresponded to 77% of the 120 polymorphic sites, resulting in 12 non-synonymous substitutions (10%) among the set of species chosen (Table 2).

The neighbor-joining tree built with the set of taxa cited above, based on the Kimura 2-parameter distance model, showed that *T. sherlocki* was placed as the sister species of the newly described *T. melanica* (Figure 3A). The robustness of credibility rates was increased when *T. sordida* was taken out of the analysis (all > 73), permitting analysis of a fragment of 414 bp. The uncorrected p-distances between *T. sherlocki* and the two haplotypes of *T. melanica* were 0.085 and 0.094. These distances were not appreciably lower than those between *T. sherlocki* and *T. juazeirensis* (0.094–0.114).

The phylogenetic closeness between *T. sherlocki* and *T. melanica* was also observed from the genetic translation of the sequences of the species involved. The sequence of 133 amino acids derived for the *Cyt B* DNA sequence exhibited a polymorphism in only one amino acid between these two species.

The *Cyt B* haplotype that we obtained here for *T. brasiliensis* from Ceará was the same one designated by Monteiro and others[20] as haplotype "v," agreeing with the subspecific taxonomic characteristics of *T. brasiliensis brasiliensis* and with the geographic distribution of this taxon.

Bayesian analysis with this gene showed strong support for the allocation of *T. sherlocki* inside the *T. basiliensis* complex (posterior probability [pp] = 99) and for its placement as a sister species to *T. melanica* (pp = 97%).

TABLE 1
Accession numbers and references with details about the species available at GenBank

Species	Accession number	
	Cytochrome b	16S
T. sherlocki	EU489058	EU489057
T. brasiliensis CTA 076	unpublished	Unpublished
T. brasiliensis brasiliensis a	AY336524	—
T. brasiliensis macromelasoma w	AY336525	—
T. juazeirensis E	AY494167	—
T. juazeirensis F	AY494144.1	—
T. melanica H	AY336527	Unpublished
T. melanica I	AY494170.1	—
T. infestans	AF045721	AF021198
T. soridda	AF045730	AF021209

Large subunit ribosomal RNA (*16S*). The amplification of the mtDNA for 16S rRNA generated a fragment with 373 bp for *T. sherlocki* and *T. melanica*, and 374 bp for *T. brasiliensis*. After aligning our sequences with the ones from *T. infestans* and *T. sordida,* we had a consensus sequence of 316 bp. This alignment exhibited 45 substitutions, of which 28 were transitions, 17 were transversions, and 41 were polymorphic sites (13%; Table 3). Accession numbers codes and references with details about the species are shown in Table 1.

A p-distance tree was built using the Kimura 2-parameter model, where *T. sherlocki* and *T. melanica* were again placed as sister species, with a low bootstrap value (71). However, for this gene, *T. sherlocki* was also relatively well supported (bootstrap = 81) as a member of the *T. brasiliensis* complex (Figure 3B). These fragment provided few informative sites, and further analysis (MP and Bayesian) did not increase the robustness of credibility for this gene alone. As observed for *Cyt B*, *T. sordida* and *T. infestans* were the outer species, *T. infestans* being a little closer (p-distances) to the *T. brasiliensis* complex (Figure 3B).

Bayesian analysis with *Cyt B* and concatenated data. Only *Cyt B* was considerably informative considering our question, and the Bayesian tree built this genes (Cyt b and 16S) and the representative members of *T. brasiliensis* complex placed *T. sherlocki* as a sister to *T. melanica* (Figure 4) with 97% of posterior probability.

The incongruence length difference (ILD) test allowed the two mtDNA fragments studied here to be combined.[34] The close relationship between *T. sherlocki* and the members of *T. brasiliensis* complex observed in previous results was confirmed with a very slight robustness for MP analysis. However, for Bayesian analysis, we observed 100% of credibility to support this branch (Figure 4).

DISCUSSION

Triatoma sherlocki (Figure 1A) is a sylvatic species collected and studied for the first time by Cerqueira (1982) (unpublished data), being originally considered a subspecies of *T. brasiliensis*: *T. brasiliensis santinacensis*. The specimens used for its description were collected ~250 m from a small town (Gentio do Ouro), district of Santo Inácio, in the Chapada Diamantina range, an extended plateau rich in rock formations that rises from 400 to 2,000 m above sea level in the central region of Bahia state. Since its description, it has not been found in any other part of northeastern Brazil (Figure 2). According to Cerqueira (1982) (unpublished data), the habitats of *T. sherlocki* are rocky ecotypes without vegetation, around an elevation of 470 m above sea level, where sylvatic lizards and rodents (*Trichomys cunicularius, Kerodon rupestris*) are common. This species has peculiar characteristics, such as shorter fore and hind wings (brachypterous) and red-orange spots or stripes in the connexivum and legs, respectively (Figure 1A).[13]

Triatoma brasiliensis (Figure 1B) was described from specimens collected in Caicó, Rio Grande do Norte state, in northeastern Brazil.[35] Lent and Wygodzinsky[15] considered *T. brasiliensis melanica* Neiva and Lent, 1941 and *T. brasiliensis macromelasoma* Galvão, 1956, as belonging to the same species as *T. brasiliensis*. Costa[35] reported that *T. brasiliensis* was in fact composed of four distinct populations: *brasiliensis* (Caicó,

TABLE 2

Variable sites in 399 bp of the mitochondrial Cyt B gene for eight species of bugs

	3	6	9	12	13	18	21	24	27	30	31	33	36	39	42	48	54	55	60	63	69	72	75	78	81	84	87	88	99	101	102	103	105	108	111	117	120	123	129	132	135	136	138	139	141	144	148	150	153	154	156	157	159	161	162	163	168	177	180
T. sherlocki	T	T	C	T	C	T	C	C	T	T	C	A	T	T	A	A	A	C	A	A	C	C	T	T	T	T	C	C	C	T	T	G	T	C	C	T	A	C	C	T	C	G	C	C	C	G	A	C	C	C	C	C	T	T	C	T	T	A	C
T. brasiliensis CTA 076	.	.	.	.	T	.	.	.	C	C	.	.	.	C	G	.	.	.	.	.	.	.	.	C	.	.	T	T	.	.	.	.	C	T	.	C	.	A	T	C	T	.	.	.	.	A	G	.	T	.	A	.	A	.	.	C	C	.	T
T. bras. brasiliensis a	.	.	.	.	T	.	.	.	C	C	.	.	.	C	G	.	.	.	.	.	.	.	.	C	.	.	T	T	.	.	.	.	C	T	.	C	.	A	T	C	T	.	.	.	.	A	.	.	T	.	A	.	A	.	.	C	C	.	T
T. bras. macromelasoma w	.	.	.	.	T	.	.	.	C	C	.	.	.	C	G	.	G	.	.	.	.	.	.	C	.	.	T	T	.	.	.	.	C	.	.	C	.	A	T	C	T	.	.	.	.	A	.	.	.	.	T	.	A	.	.	C	C	.	T
T. juazeirensis	.	.	.	.	.	.	.	T	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	T	T	.	.	.	A	C	.	.	.	.	A	.	C	T	.	T	.	.	A	.	T	T	.	A	.	.	C	.	C	C	.	.
T. melanica	C	.	T	.	.	.	.	.	A	C	T	.	C	.	.	G	.	.	.	.	.	T	.	.	C	C	T	.	T	.	.	.	C	.	T	C	.	A	T	.	.	.	.	.	.	A	.	.	T	.	T	.	A	.	.	C	C	.	T
T. infestans	.	.	.	C	.	.	T	T	A	C	T	.	A	C	.	.	.	T	G	.	.	T	A	.	C	C	A	T	.	.	.	.	C	.	.	C	C	A	T	.	.	.	A	T	A	A	.	T	T	.	A	T	A	.	T	.	.	.	A
T. sordida	.	C	.	C	.	C	.	.	A	C	.	G	C	.	G	.	.	T	.	G	T	.	A	C	C	C	.	T	T	A	C	.	C	.	.	C	C	.	.	C	T	A	.	.	.	A	.	.	.	T	A	.	A	.	.	C	C	C	A

	182	183	186	189	190	196	198	201	209	213	219	228	229	231	234	236	237	242	246	249	250	258	261	267	271	273	276	277	279	282	285	286	291	294	295	297	300	306	309	312	313	315	321	330	333	336	339	345	348	349	351	354	357	363	366	369	372	384	388	390	393
	A	C	T	A	T	T	A	A	C	C	C	C	C	T	T	T	C	T	A	C	T	A	A	T	T	A	A	T	A	C	C	A	T	T	T	A	A	A	A	T	C	A	C	C	C	T	C	C	A	T	A	A	C	C	T	C	A	C	C	A	T
	.	T	.	.	.	.	.	.	T	.	.	T	.	C	.	.	.	C	.	T	C	.	.	.	.	.	.	C	.	T	T	.	.	C	.	.	G	.	.	.	T	.	T	T	T	C	A	.	.	.	.	.	T	T	C	T	.	.	T	.	C
	.	T	.	.	.	.	.	.	T	.	.	T	.	C	.	.	.	C	G	T	C	.	.	.	.	.	.	C	.	T	T	.	.	C	.	.	G	.	.	.	T	.	T	T	T	C	A	.	.	.	.	.	T	T	C	T	.	.	T	.	C
	.	T	.	.	.	.	.	.	T	.	.	T	.	C	.	.	.	C	G	T	C	.	.	.	.	.	G	.	.	T	T	.	.	C	.	.	G	.	.	.	.	.	T	T	T	C	A	T	.	.	.	.	T	T	C	T	.	.	T	.	C
	.	T	.	.	C	.	G	T	T	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	C	C	G	.	.	.	C	.	.	.	.	T	C	A	T	C	C	.	.	.	T	C	T	.	.	.	.	.
	.	T	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	G	.	.	.	.	A	T	.	.	.	.	T	.	C	T	.	.	.	.	.
	G	T	.	C	.	.	.	.	T	.	T	.	.	C	.	A	T	.	.	.	.	.	.	.	.	.	.	.	T	T	.	C	A	C	.	.	.	.	T	.	.	.	T	.	T	C	A	T	.	C	.	T	.	T	C	T	.	.	.	.	C
	G	A	C	G	C	C	.	.	T	T	.	.	T	C	C	.	.	.	G	.	.	G	T	C	C	T	.	C	.	.	.	C	A	C	C	.	.	C	C	C	.	C	T	.	.	C	A	.	.	C	G	C	.	A	C	T	G	T	.	T	C

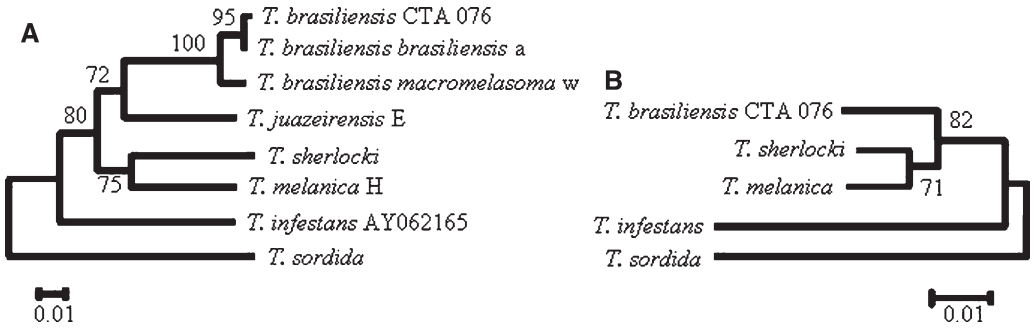

FIGURE 3. Neighbor-joining tree based on Kimura 2-parameter distances among members of *T. brasiliensis* complex, with mtCytB and 16S. (**A**) phylogram resulting from 399 bp sequence alignment of mtCytB; (**B**) phylogram resulting from 316 bp sequence alignment of 16S mitochondrial ribosomal RNA. The values of the nodes indicate the percentage of the posterior probability. *T. sordida* and *T. infestans* were used as outgroups.

RN), *melanica* (Espinosa, MG), *macromelasoma* (Petrolina, PE), and *juazeirensis* (Juazeiro, BA). Costa and others,[7] through morphologic and enzymatic studies, concluded that *T. melanica* (Figure 1C) was a new species. Based on the same approaches, 1 year later, Costa and Felix[10] described another species, *T. juazeirensis* (Figure 1D), which had been previously considered only a population of *T. brasiliensis* complex.

This study presents data suggesting that *T. sherlocki* is a member of the *T. brasiliensis* complex by sequencing two distinct mitochondrial genes (Figure 4). In addition, the placement of *T. sherlocki* within the *T. brasiliensis* complex suggests that it is a sister species of *T. melanica*, with strong clade support (98% credibility; Figure 4).

A profile of rates of polymorphic sites observed for other taxa for the ribosomal *16S* and *Cyt B* mtDNA fragments was found here (Tables 2 and 3). Although the *16S* sequence showed polymorphic sites in only 13% (41/316; Table 2) of aligned base pairs, the *Cyt B* gene showed 30% (120/399; Table 1) of polymorphic sites among the following species set: *T. sherlocki*, *T. brasiliensis brasiliensis*, *T. brasiliensis macromelasoma*, *T. melanica*, *T. juazeirensis*, T. *infestans*, and *T. sordida* (Table 3).

Despite being originally considered a subspecies of *T. brasiliensis* based on laboratory crossing experiments (Cerqueira 1982, unpublished data). Posteriorly Papa and others[13] described *T. sherlocki* that presented morphologic distinguishable features of *T. lenti*, suggesting that *T. sherlocki* could be a closely related species. Therefore, considering the unusual morphologic features of *T. sherlocki,* it was not expected to be placed within the *T. brasiliensis* complex, where the members differ among them mainly by a few details in the chromatic pattern (Figure 1). For that reason, *T. melanica* was classified as new taxa considering a peculiar characteristic in male genital: a spongy fossula on the fore tibia.[7] Despite the marked variation in male genital structures among the members of the *T. brasiliensis* complex, this feature by itself was not useful to distinguish these species.[7] On the other hand, *T. sherlocki* can easily be differentiated from all members of the *T. brasiliensis* complex by genital structures, the particular red-orange details, and very small wings (Figure 1A).

Disagreements between morphologic and genetic approaches have also been recorded for other taxa among the triatomines, such as the paraphyletic group of *Triatoma* genera that should include some species of *Panstrongylus* genera and the discussion about *Psammolestes* genera as a closely related genera to *Rhodnius*, despite being very different morphologically (both originally evidenced by Hypsa and others).[36]

All members of the *T. brasiliensis* complex are found in rock piles within the biogeographic zone known as the Caatinga, a mosaic of xerophytic, deciduous, semi-arid thorn scrubs and forest.[37] However, an ecologic niche model for the *T. brasiliensis* complex showed that each species of this complex occupies an environment with particular features, which might have driven the specialization and, therefore, the genetic drift.[38] Despite apparently being physically closer to the geographic area occupied by *T. juazeirensis* (Figure 1D), *T. sherlocki* is genetically closer to *T. melanica*. However, it is necessary point out that the real geographic distribution of *T. sherlocki* was poorly studied, because it was described in a remote region in the center northwestern part of the state of Bahia, Brazil, and its presence was restricted to a very small area. Along this region, there is a gap between the known geographic distributions of the two species of the *T. brasiliensis* complex that occur in this state: *T. melanica* and *T. juazeirensis*. The former is restricted to the southern part of the Brazilian semi-arid area, whereas the latter occupies the middle part, south of São Francisco River, and *T. brasiliensis* is found in the region to the north of São Francisco River.

TABLE 3
Variable sites in 316 bp of the mitochondrial *16S* gene for five bugs

	5	6	2	7	7	8	9	9	9	9	9	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3
			5	8	9	8	2	4	6	7	8	9	0	3	7	7	7	8	8	9	9	0	0	0	0	0	1	1	1	3	4	4	6	6	6	7	8	9	0
													0	8	2	5	8	2	3	0	2	0	2	3	6	7	8	2	3	5	9	6	8	0	2	4	5	8	6	6	7
T. sherlocki	T	C	A	G	A	A	T	A	T	T	G	A	G	A	T	–	T	G	G	A	G	A	A	C	T	A	A	G	A	T	A	A	A	T	T	A	T	G	A	G	G
T. melanica	.	.	.	.	.	.	.	.	.	.	.	G	T	.	.	–	C	.	.	.	.	T	.	T	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
T. brasiliensis CTA 076	.	.	.	A	.	.	.	.	.	.	T	.	A	.	.	–	.	.	.	.	.	T	.	.	.	.	.	A	G	.	.	G	.	.	.	.	.	T	.	T
T. infestans	.	T	.	A	.	G	.	.	G	G	.	G	A	G	.	–	.	A	A	G	T	T	G	T	.	.	.	.	.	A	.	.	.	A	.	T	A	A	G	T	T
T. sordida	A	T	G	.	G	T	C	C	.	.	.	.	A	.	C	A	.	A	A	.	.	.	.	T	G	G	G	G	A	.	G	G	G	G	A	G	.	.	.	T	A	.

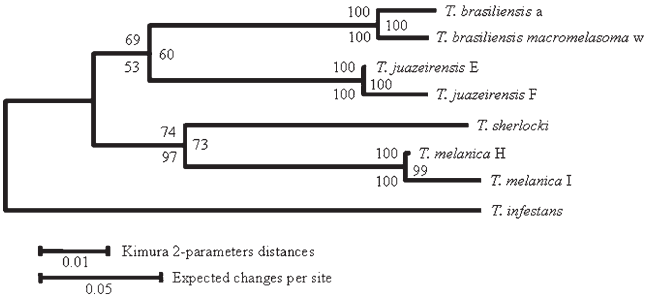

FIGURE 4. Tree derived from maximum likelihood (ML) estimation by Bayesian inference applied to fragments from CytB and 16S. The values over the nodes refer to Kimura 2-parameter distances. Those below the nodes indicate the percentage of clade credibility by Bayesian analysis, while those beside the nodes are the bootstrap value by maximum parsimony.

Among the four members of the *T. brasiliensis* complex, the subspecies *T. brasiliensis brasiliensis* has been considered the main concern in terms of Chagas disease transmission, because this subspecies is the most widespread and shows the highest rates of domestic capture and of natural infection by *T. cruzi.* On the other hand, *T. melanica* has been found exclusively in wild habitats thus far, whereas *T. brasiliensis brasiliensis*, *T. brasiliensis macromelasoma,* and *T. juazeirensis* can be found in both wild and domestic environments.[14] *T. sherlocki* seems to be a sylvatic species, but it eventually invades homes in Santo Inácio (FUNASA, personal communication).

However, the epidemiologic profile of Chagas disease vectors is not static. Some wild vectors are progressively coming closer to human habitation, in the process of domiciliation. For example, since the control of *T. infestans* in the south of Brazil, data from the Chagas Disease Control Program have indicated an increase in the invasion of housing and nearby areas by *T. rubrovaria*, which is becoming the most frequently captured triatomine species in that region.[39–41] A similar pattern has been observed for *R. nasutus* in the state of Ceará, where *T. brasiliensis*, *R. nasutus,* and *T. pseudomaculata* have been frequently captured inside houses.[42,43] Additional studies are needed on *T. sherlocki,* by analyzing other genes, to confirm its phylogenetic position. In addition, it is suggested that the knowledge of this species must be strengthened through morphologic, biologic, ecologic, and biogeographic studies, which could provide valuable information on its taxonomic position and vector capacity.

From our results, the close phylogenetic relationship between *T. sherlocki* and the species of the *T. brasiliensis* complex, especially with the *T. melanica*, inferred from Bayesian trees (Figures 3 and 4) support the hypothesis that *T. sherlocki* can be classified as one specie of the *T. brasiliensis* complex and might indicate possible philogeography from a common ancestry.

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