

# Phylogenetic Relationships of the *Spinolai* Complex and Other Triatomini Based on Mitochondrial DNA Sequences (Hemiptera: Reduviidae)

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## Abstract

In this study, we performed a phylogenetic analysis of 35 species of the tribe Triatomini by means of available 16S ribosomal DNA and cytochrome *b* (*Cyt b*) gene sequence data, adding taxa of the *spinolai* complex, to clarify phylogenetic relationships of this complex and related triatomines. The phylogenetic analysis suggests a monophyletic clustering of the *spinolai* complex related to the South American species of triatomines.

**Key Words:** Triatominae vectors—*Spinolai* complex—Phylogeny—Cytochrome *b* gene.

## Introduction

WITHIN THE SUBFAMILY TRIATOMINAE (Hemiptera: Reduviidae), the genus *Mepraia* is endemic from the semiarid and arid regions of northern and central Chile. It is mainly found amongst rock piles, but occasionally it enters and colonizes domestic and peridomestic habitats (Frías et al. 1998). This genus was first described with the inclusion of *Triatoma spinolai* Porter 1934, as *Mepraia spinolai* by Mazza et al. (1940), synonymized by Lent and Wygodzinsky (1979), but then revalidated by Lent et al. (1994). Lent and Wygodzinsky (1979) described the *spinolai* complex as a taxonomic group composed of *Triatoma eratyrsiformis* Del Ponte 1929, *Triatoma breyeri* Del Ponte 1929, and *Triatoma (Mepraia) spinolai* Porter 1934. *T. eratyrsiformis* and *T. breyeri* occur in Argentina, and are geographically separated from *spinolai* by the Andean Range (Lent and Wygodzinsky 1979). Carcavallo et al. (2000) described the *breyeri* complex formed by *breyeri* and *eratyrsiformis*, as a group related to the *T. (M.) spinolai*, while Hypsa et al. (2002), on the basis of molecular studies using 16S rDNA, postulated that this complex should be assigned to the *Mepraia* genus. Recently, Schofield and Galvão (2009) indicated that is necessary to establish the relationships within the *spinolai* complex to decide the inclusion or exclusion of *Mepraia* from the genus *Triatoma*.

Until 1998, *Mepraia spinolai* was the only species of the genus, distributed in coastal and interior valleys from Chile between parallels 18° and 34° S (Lent and Wygodzinsky

1979). However, on the basis of karyotype, morphological characters, and experimental crosses, coastal desert populations between parallels 18° and 26° S were ranked as a new species named *Mepraia gajardo* (Frías et al. 1998). The remaining populations, from 26° to 34° S, distributed in the interior mountains from the Atacama to the Metropolitan Regions, maintain the name *M. spinolai* (Frías et al. 1998). Recently, genetic studies using nuclear and mitochondrial markers on *Mepraia* sp. populations suggest the possible existence of introgression due to past hybridization events or the retention of ancestral polymorphisms, in the geographical intersection zone between these two taxa (Calleros et al. 2010). The goal of our study is to clarify the systematic uncertainties of *Mepraia* sp. and the Argentinian *T. eratyrsiformis* and *T. breyeri*. To this end, we constructed a phylogeny of Triatominae species belonging to the Triatomini tribe (Lent and Wygodzinsky 1979) by means of currently available mitochondrial gene sequence data of 16S ribosomal DNA and cytochrome *b* (*Cyt b*), adding the missing sequence data of the *spinolai* complex.

## Materials and Methods

### Insect collection

Collection of *M. gajardo* specimens was carried in the locality of Caleta Vitor (Arica and Parinacota Region, 18° 45' 45" S, 70° 20' 34" W), while the *M. spinolai* were collected in the locality of Til Til (Metropolitan Region, 33° 06' 19" S, 70° 55' 53"

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W). In addition, we obtained *Triatoma eratyrisiformis* specimens from Salinas de Bustos (30° 15'50" S, 67° 36'23" W), and *Triatoma breyeri* from Patquia Viejo (30° 2'36" S, 66° 52'46" W). Both locations belong to Department of Independencia, Province of La Rioja, Argentina. Insects were collected manually; then they were dissected in the laboratory and their legs were kept in 70% ethanol at -20°C.

#### Mitochondrial DNA extraction, amplification, and sequence alignment

For DNA extraction, legs from each specimen were cut and macerated in an Eppendorf tube and incubated overnight with protease K, followed by DNA extraction with the EZNA Tissue DNA<sup>®</sup> kit according to manufacturer's instructions. A 663-bp fragment of the mitochondrial *Cyt b* gene was amplified via PCR using the primers and conditions as described (Monteiro et al. 2003). Verification of successful amplification was assessed by agarose gel electrophoresis. The amplified fragment was sequenced using the PCR primers in an internal service information <http://www.macrogem.com/>. Sequences were edited using the programs Bioedit 7.0.8.0 (Hall 1999) and aligned using ClustalW as implemented in Bioedit (Hall 1999). After alignment, sites that showed nucleotide substitutions were re-examined by visual inspection of each individual fluorogram data. We obtained 4 sequences that represent the 4 taxa of the *spinolai* complex, GenBank accession numbers JN102358–JN102361. The accession numbers of available mitochondrial gene sequence are shown in Table 1. All the 16S and *Cyt b* gene sequences obtained were concatenated manually. Unknown sequences in some taxa were filled in the matrix with missing data symbols following (Wiens and Morrill 2011).

#### Phylogenetic analyses

Maximum likelihood (ML) and maximum parsimony (MP) algorithms were used to infer phylogenetic trees. ML analysis was inferred using the online platform PhyML 3.0 (Guindon and Gascuel 2003). The best-fitting model of nucleotide substitution was selected using the Akaike information criterion implemented in the program JmodelTest 0.1.1 (Posada 2008). These results gave a best fit for the GTR+G (G=0.2060, -lnL=8298.59467) nucleotide substitution model. Nodal supports were estimated by the bootstrap method (Felsenstein 1985) with 1000 replicates using PhyML 3.0 (Guindon and Gascuel 2003). MP analyses were performed using PAUP\* 4.0b10 (Swofford 2003) with the heuristic search option, TBR algorithm, and 1000 replicates for the bootstrap method (Felsenstein 1985). Trees were visualized using the FigTree v1.1.2 program, available at <http://tree.bio.ed.ac.uk/software/figtree/>. *Tiarodes venenatus* and *Tapeinus fuscipennis* (Hemiptera; Reduviidae) were used as outgroups, on the basis of their phylogenetic relationships with the Triatomini tribe (de Paula et al. 2005).

#### Results and Discussion

The phylogenetic analysis showed a clustering for the members of the *spinolai* complex, with support of 69% for this node (Fig. 1), which is marginally significant according to Hillis and Bull (1993). Within the complex, *M. gajardoi* and *M. spinolai* show a close relationship with significant nodal

TABLE 1. ACCESSION NUMBERS OF SEQUENCES USED IN THIS ANALYSIS

Taxa	16S rDNA	Cyt b
<b>Outgroup</b>		
Reduviinae		
<i>Tapeinus fuscipennis</i>	AY127044	
<i>Tiarodes venenatus</i>	AY127045	
<b>Ingroup</b>		
Triatomini		
<i>Triatoma dimidiata</i>	AY035448	FN641817
<i>Triatoma pallidipennis</i>	AF045697	DQ198814
<i>Triatoma picturata</i>	AY035447	DQ198817
<i>Triatoma phyllosoma</i>		DQ198818
<i>Triatoma mazzottii</i>	AY035446	DQ198816
<i>Triatoma longipennis</i>		DQ198815
<i>Triatoma recurva</i>	FJ230417	DQ198813
<i>Triatoma sanguisuga</i>	AF045696	HQ141306
<i>Triatoma nitida</i>	AF045702	AF045723
<i>Triatoma rubida</i>	AY185842	DQ198811
<i>Triatoma protracta</i>	AY035444	AF045727
<i>Dipetalogaster maxima</i>	AY035442	AF045728
<i>Triatoma matogrossensis</i>	AY035454	
<i>Triatoma garciabesi</i>	AY035455	
<i>Triatoma sordida</i>	AF021209	AF045730
<i>Triatoma guazu</i>	AY035457	
<i>Triatoma williami</i>	AY035458	
<i>Triatoma pseudomaculata</i>	AY035461	
<i>Triatoma klugi</i>	AY035463	
<i>Triatoma rubrovaria</i>	AF021203	GQ398000
<i>Triatoma guasayana</i>	AF021192	
<i>Triatoma patagonica</i>	AY035464	
<i>Triatoma circummaculata</i>	AF021188	
<i>Triatoma brasiliensis</i>	AF021183	AY494164
<i>Triatoma maculata</i>	AY035465	
<i>Triatoma platensis</i>	AF021201	
<i>Triatoma infestans</i>	AF021198	AY062165
<i>Triatoma delpontei</i>	AF028745	HQ333240
<i>Triatoma tibiamaculata</i>	AY035453	
<i>Panstrongylus megistus</i>	AF021177	AF045722
<i>Panstrongylus herreri</i>	AY035452	
<i>Mepraia spinolai</i>	AY035467	JN102358
<i>Mepraia gajardoi</i>		JN102359
<i>Triatoma eratyrisiformis</i>	AY035466	JN102360
<i>Triatoma breyeri</i>		JN102361

support values, close to *T. breyeri* and *T. eratyrisiformis*. Considering this phylogeny, we suggest that the species of the *spinolai* complex form a monophyletic group and that *Mepraia* corresponds to a clearly established genus and the phylogenetic relationship between *T. eratyrisiformis* and *T. breyeri* with *M. spinolai* and *M. gajardoi* reframes the debate to include these species in a single genus as proposed (Hypsa et al. 2002). According to our phylogeny, it would not be appropriate to synonymize *Mepraia* with *Triatoma* because a clear node supporting the genus *Triatoma* is lacking. In fact, species from the genera *Panstrongylus* and *Dipetalogaster* are also included within the genus *Triatoma*, suggesting a polyphyletic origin. However, it is necessary to include more gene sequence data from other species of the genus *Triatoma* to perform a more complete phylogenetic analysis.

The detected clustering of the *spinolai* complex members agrees with the classical studies proposed by morphological studies (Lent and Wygodzinsky 1979, Moreno et al. 2006).

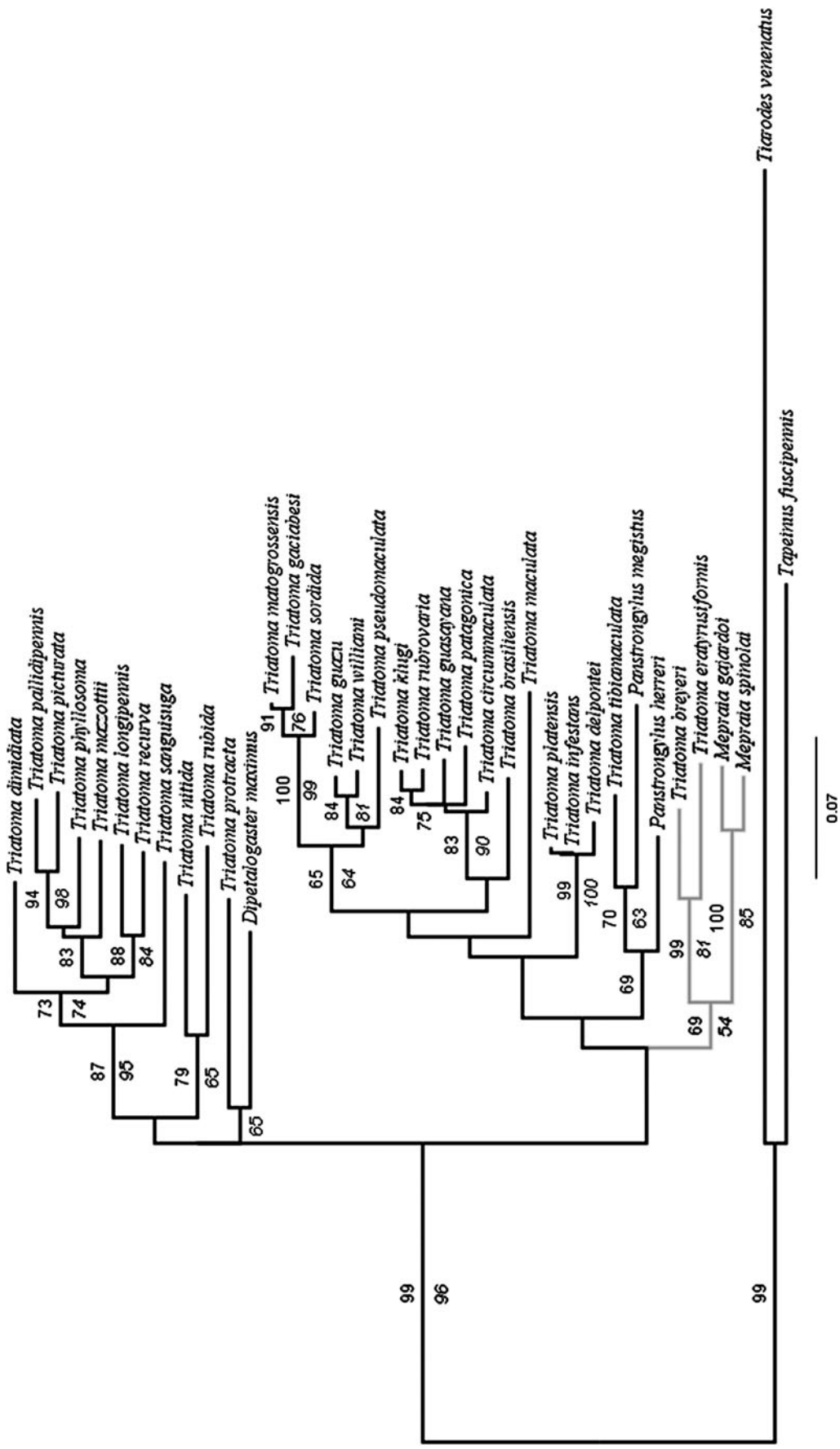


FIG. 1. Maximum likelihood (ML) phylogenetic tree of Triatomini species based on 16S ribosomal DNA and *Cyt b* concatenated gene sequences, inferred with the model GTR + G (G = 0.2060, -lnL = 8298.59467). Numbers above the branch are the ML support values derived from bootstrap resampling with 1000 replicates and numbers under the branches are parsimony bootstrap values. Gray branches indicate the *spinolai* complex members.

Moreno et al. (2006) compared the phenotype of the antennae among the species of the *spinolai* complex and found that *Mepraia* species and *T. eratyrsiformis* shared similarities in bristles and receptors that are not present in *T. breyeri*. In the same vein, it has been reported that *T. eratyrsiformis* shows multiple sex chromosomes like *spinolai* and *gajardoi*, but unlike most other South American Triatoma species (Panzeria et al. 1998). It is necessary to complete the information provided by cytogenetic studies to have the whole scenario of the relationships of *spinolai* complex.

The monophyletic relationships between the taxa of the *spinolai* complex suggest that the Argentinian *T. breyeri* and *T. eratyrsiformis* could have had a common ancestor with the *Mepraia* genus. It has been proposed that the species of *spinolai* complex were separated with the uplifting of the Andes mountains during the Miocene in the Tertiary geological era about 20 million years ago (Moreno et al. 2006). It would be interesting to perform studies of the most recent common ancestor to estimate dates of divergence among the taxa to be compared with geological events. The *spinolai* complex appears related to South American species of triatomines. The relationship between *M. spinolai* and *T. eratyrsiformis* with South American species of the *infestans* complex were previously reported (Hypsa et al. 2002, de Paula et al. 2005). Interestingly, North American species seems to form a distinctive group from South American species, even though not supported by high bootstrap values, which agrees with de Paula et al. (2005). This topology could be explained by the polyphyly of the Triatomini and the radiation pattern of these species (Schofield and Galvão 2009).

In conclusion, our phylogenetic evidence suggests that all the members of the *spinolai* complex constitute a monophyletic group corresponding to an established genus.

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### Author Disclosure Statement

No conflicts of interest are declared.

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