Infection, Genetics and Evolution 24 (2014) 157–166

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Towards a phylogenetic approach to the composition of species complexes in the North and Central American *Triatoma*, vectors of Chagas disease

Nicholas M. de la Rúa [a], Dulce M. Bustamante [b], Marianela Menes [b], Lori Stevens [a], Carlota Monroy [b], C. William Kilpatrick [a], Donna Rizzo [a], Stephen A. Klotz [c], Justin Schmidt [d], Heather J. Axen [a], Patricia L. Dorn [e,*]

[a] University of Vermont, Burlington, VT, USA
[b] Universidad de San Carlos, Guatemala City, Guatemala
[c] University of Arizona, Tucson, AZ, USA
[d] Southwestern Biological Institute, Tucson, AZ, USA
[e] Loyola University New Orleans, New Orleans, LA, USA

ARTICLE INFO

Article history:
Received 11 December 2013
Received in revised form 16 March 2014
Accepted 19 March 2014
Available online 26 March 2014

Keywords:
Triatominae
Chagas disease
Systematics
ITS-2
Morphometry
Species complexes

ABSTRACT

Phylogenetic relationships of insect vectors of parasitic diseases are important for understanding the evolution of epidemiologically relevant traits, and may be useful in vector control. The sub-family Triatominae (Hemiptera:Reduviidae) includes ~140 extant species arranged in five tribes comprised of 15 genera. The genus *Triatoma* is the most species-rich and contains important vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease. *Triatoma* species were grouped into complexes originally by morphology and more recently with the addition of information from molecular phylogenetics (the four-complex hypothesis); however, without a strict adherence to monophyly. To date, the validity of proposed species complexes has not been tested by statistical tests of topology. The goal of this study was to clarify the systematics of 19 *Triatoma* species from North and Central America. We inferred their evolutionary relatedness using two independent data sets: the complete nuclear internal transcribed spacer-2 ribosomal DNA (ITS-2 rDNA) and head morphometrics. In addition, we used the Shimodaira–Hasegawa statistical test of topology to assess the fit of the data to a set of competing systematic hypotheses (topologies). An unconstrained topology inferred from the ITS-2 data was compared to topologies constrained based on the four-complex hypothesis or one inferred from our morphometry results. The unconstrained topology represents a statistically significant better fit of the molecular data than either the four-complex or the morphometric topology. We propose an update to the composition of species complexes in the North and Central American *Triatoma*, based on a phylogeny inferred from ITS-2 as a first step towards updating the phylogeny of the complexes based on monophyly and statistical tests of topologies.

1. Introduction

1.1. Background

The Triatominae are a sub-family of highly diverse Reduviidae that have evolved hematophagy of vertebrates. They can transmit *Trypanosoma cruzi*, a hemoflagellate protozoan and the causative agent of Chagas disease. Although there are approximately 140 extant species in five tribes within the sub-family, only a couple dozen species within three of the 15 genera (*Triatoma*, *Panstrongylus*, and *Rhodnius*) are associated with the majority of *T. cruzi* transmission to humans (World Health Organization, 1991; Schofield and Galvão, 2009). These three genera are from two tribes: *Triatoma* and *Panstrongylus* from Triatomini and *Rhodnius* from the Rhodniini. The most species-rich and widely distributed is the genus *Triatoma* with ~80 species found across South, Central, and North America including some islands of the Gulf of Mexico and three species found in the Old World.

Triatoma species are divided into three groups (Schofield and Galvão, 2009). The Dispar group includes the high altitude species

* Corresponding author. Tel.: +1 504865 3672; fax: +1 504865 2920.
E-mail address: dorn@loyno.edu (P.L. Dorn).

http://dx.doi.org/10.1016/j.meegid.2014.03.019
1567-1348/

of the Andean region of South America, and the Infestans group includes all other South American species. The Rubrofasicata group includes the North and Central American species along with those from the Old World and islands of the Gulf of Mexico. Recent molecular work has revealed that the Rubrofasicata group form a well-supported clade, separate from the sister South American *Triatoma* (Hypsa et al., 2002).

1.2. The species complexes of the North and Central American *Triatoma*

Within the Rubrofasciata group, there are 29 species of *Triatoma* in North and Central America and the Islands of the Gulf of Mexico arranged in four species complexes that are defined "as species that share extensive morphological similarities" (Carcavallo et al., 1999). The species complex concept has been widely used in the Triatominae and has been a useful way to discuss related species. Molecular phylogenetic analyses support some complex assignments, showing complexes that are recovered as well-supported monophyletic clades, e.g. Brasiliensis, Infestans, Rubida, and the Phyllosoma complex (Hypsa et al., 2002; Monteiro et al., 2004; Pfeiler et al., 2006; Martinez et al., 2006). In addition, molecular work has revealed that some "species" include cryptic species, so are best described as species complexes (Bargues et al., 2008; Dorn et al., 2009).

The currently accepted systematic hypothesis includes four complexes: Phyllosoma, Protracta, Lecticularia, and Flavida (Table 1). Assignment of species to complexes was initially based on morphology with later revisions based on phylogenetic inference. However, not all species assigned to a particular complex appear in monophyletic clades and this four-complex systematic hypothesis has not been tested by statistical tests of topologies (Schofield and Galvão, 2009).

The Phyllosoma complex, of greatest epidemiological importance in North and Central America, is comprised mostly of species endemic to Mexico. These taxa have posed many difficult systematic problems. Originally, species of the Phyllosoma complex were recognized as sub-species of *Triatoma phyllosoma* (Usinger, 1944) based on laboratory crosses in which fertile hybrids were reared (Mazzotti and Osorio, 1942). However, Lent and Wygodzinsky (1979) raised the sub-species to specific status rejecting the experimental design, scope, and interpretations of the crosses. ITS-2 (nuclear rDNA) and *cytochrome* b (mtDNA) sequence data have been used to clarify the relationships among the Phyllosoma (Martinez et al., 2006). Recent molecular and morphometric analyses, combined with data from experimental laboratory crosses have uncovered systematic intricacies of the Phyllosoma suggesting that many of the groups are morphotypes with chromatic and genetic variants, and might be more appropriately designated as sub-species (Martinez-Hernandez et al., 2010). In addition, placement of one species, *Triatoma ryckmani*, within the Phyllosoma is tentative as it is a poorly studied sylvatic species of Central America (Carcavallo et al., 2000; Dujardin et al., 2000).

Triatoma dimidiata, a highly polymorphic species of the Phyllosoma complex, is the most epidemiologically important species in Central America (reviewed in Dorn et al. (2007)). Multiple metrics have been used to assess variation across its geographic range; this taxon appears to be polytypic with an as yet unresolved number of cryptic species and/or subspecies (Bargues et al., 2008; Dorn et al., 2009, 2007; Bustamante et al., 2004; Monteiro et al., 2013). *T. dimidiata* form a well-differentiated and supported clade within the Phyllosoma, which may include *Triatoma hegneri*, an island variant (Martinez et al., 2006; Bargues et al., 2008; Marcilla et al., 2001). This has led some to propose a Dimidiata sub-complex (Schofield and Galvão, 2009), although the definition of a sub-complex is unclear.

The Protracta complex, as described in the four-complex hypothesis, is comprised of species ranging from North to Central America including a few species of low to moderate epidemiological importance. The complex also includes some rare and consequently less studied sylvatic species such as *Triatoma incrassata* and *Triatoma neotomae*. *Triatoma protracta* is a polymorphic species that is usually found in close association with wood rat (*Neotoma*) nests across the southwestern United States; due to habitat encroachment, human encounters are becoming more frequent (Klotz et al., 2010). Although only 23 cases of autochthonous transmission of *T. cruzi* have been reported in the United States (Cantey et al., 2012), many more cases of allergic reactions including anaphylaxis from *T. protracta* salivary proteins have been reported in the southwestern United States (Klotz et al., 2010). While classically described as sylvatic species, human blood meals in *T. protracta* from California have been reported (Stevens et al., 2012).

The Lecticularia complex includes species found in the southern United States and northern Mexico. With regards to epidemiological importance, *Triatoma gerstaeckeri*, *Triatoma sanguisuga*, and *Triatoma lecticularia* have been implicated as the vectors of autochthonous transmission in the United States (Woody and Woody, 1955; Schiffler et al., 1984; Ochs et al., 1996; Herwaldt et al., 2000; Dorn et al., 2007); and all three species are found in close association with homes in rural areas of Texas and Louisiana (Beard et al., 2003; Kjos et al., 2009; Cesa et al., 2011; de la Rua et al., 2011). Human blood meals are common in *Triatoma rubida* from Arizona and California (Stevens et al., 2012; Klotz et al., 2014). Many species within the Lecticularia complex show morphological variation across their broad geographic range including *T. rubida* and *T. sanguisuga*. Studies of species within the Lecticularia complex are few, but phylogenetic inference using *cyt* b supported sub-specific status for populations of *T. rubida* (Pfeiler et al., 2006); and the population of *T. sanguisuga* responsible for the sixth case of autochthonous transmission in the United States was found to be hyper-variable by *cyt* b and 16S (de la Rua et al., 2011). *Dipetalogaster maximus*, a species of the Baja peninsula, is the only species of its genus, separated due to extreme size as well as additional apomorphic characters (Lent and Wygodzinsky, 1979). *D. maximus* was sister to *T. lecticularia* by a fragment of the mitochondrial *cyt* b gene, but not by a phylogeny inferred by concatenated fragments of mitochondrial *cyt* b and nuclear ITS-2; the latter placed *D. maximus* in close affiliation with *Triatoma barberi* and *T. protracta* (Espinoza et al., 2013).

The Flavida complex is comprised of species found only in Cuba and Jamaica. *Triatoma flavida* and *Triatoma bruneri*, both endemic to Cuba, were synonymized, but recent molecular data supports the elevation of each to specific status (Fraga et al., 2011). Included in this complex is *Triatoma obscura*, a poorly studied species of Jamaica.

Table 1
Currently accepted "four-complex hypothesis" of species complexes of North and Central American *Triatoma*.

Complex	Species
Phyllosoma	*T. dimidiata*, *T. hegneri*, *T. brailoviskyi*, *T. gomeznunezi* *T. bassolsae*, *T. bolivari*, *T. longipennis*, *T. mazzottii*, *T. mexicana*, *T. pallidipennis*, *T. phyllosoma*, *T. picturata*, *T. ryckmani*
Flavida	*T. flavida*, *T. bruneri*, *T. obscura*
Protracta	*T. barberi*, *T. incrassata*, *T. neotomae*, *T. nitida*, *T. peninsularis*, *T. protracta*, *T. sinaloensis*
Lecticularia	*T. gerstaeckeri*, *T.indictiva*, *T. lecticularia*, *T. recurva*, *T. rubida*, *T. sanguisuga*

Adapated from Schofield and Galvão (2009).