Triatominae in furnariid nests of the Argentine Gran Chaco

G.A. Marti[1,4]✉, M.G. Echeverria[2], E. Waleckx[3], M.L. Susevich[1], A. Balsalobre[1], and D.E. Gorla[4]

[1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE-CCT-La Plata-CONICET – UNLP) 2 # 584, 1900 La Plata, Argentina, gmarti@cepave.edu.ar
[2]Cátedra de Virología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, (CONICET), Argentina
[3]Institut de Recherche pour le Développement (IRD), Montpellier, France
[4]Centro Regional de Investigaciones Científicas y Transferencias Tecnológicas (CRILAR - CONICET), La Rioja, Argentina

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ABSTRACT: Triatomines (Hemiptera, Reduviidae) are bloodsucking insects involved in the transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease, an important public health problem in Latin America. The triatomine species found in sylvatic habitats generally play a limited epidemiological role compared to domestic species, but they may act as a reinfestation source of dwellings after insecticide spraying and have to be carefully considered in control strategies of Chagas disease transmission. The objectives of this work were to carry out a survey of the sylvatic triatomine species colonizing Furnariidae nests in a typical area of the Chaco region of Argentina during the winter and to study the parasites and natural enemies associated with the collected triatomines. Sixty-three triatomine specimens were collected from Furnariidae nests (*Coryphistera alaudina* and *Phacellodomus sibilatrix*) randomly selected within the study area. Fifty-four were identified as *Psammolestes coreodes*, seven as *Triatoma platensis*, and two as *Triatoma infestans*. Specimens of *T. infestans* and *T. platensis* were found in one nest. The first finding of instar nymphs of *T. infestans x T. platensis* in a sylvatic habitat is reported. For the first time, sylvatic collected specimens of *T. platensis* were found infected by *T. cruzi*. Triatoma virus was found in one *Ps. coreodes* specimen. ***Journal of Vector Ecology* 39 (1): 66-71. 2014.**

Keyword Index: Chagas, Furnariidae nests, *Triatoma infestans, Triatoma delpontei, Triatoma platensis, Psammolestes coreodes.*

INTRODUCTION

The members of Triatominae (Hemiptera, Reduviidae) are bloodsucking insects involved in the transmission of *Trypanosoma cruzi*, the causative agent of Chagas disease, an important public health problem in Latin America, where around seven to eight million people are infected. *Triatoma infestans* Klug, 1834 is the main vector in the Southern Cone countries of South America. This species is mainly domestic and consequently, it has been the main target of large-scale campaigns of vector control based on insecticide spraying for more than 20 years. Thanks to these control efforts, the distribution and abundance of *T. infestans* populations have been strongly reduced, leading to the interruption of the vectorial transmission of the parasite in Uruguay, Chile, Brazil, and parts of Argentina, Bolivia, and Paraguay (Schofield et al. 2006). Nevertheless, primarily sylvatic populations of triatomines, which exhibit a tendency to invade the domestic environment, may act as synanthropic vectors of *T. cruzi* when domestic populations of *T. infestans* have been eradicated (Noireau et al. 2000, Moncayo and Silveira 2009).

In the highly endemic Chagas region of the Argentine Chaco, *Triatoma sordida* Stål 1859, *T. guasayana* Wygodzinsky and Abalos 1949, and *T. garciabesi* Carcavallo et al. 1967, are associated with birds and mammals in the sylvatic environment and can be found inside and around the houses, occupying natural or artificial peridomestic structures (Gürtler et al. 1999). Other ornithophilic species, such as *T. platensis* Neiva 1913 and *T. delpontei* Romaña and Abalos 1947, have a similar distribution restricted to the Gran Chaco region and colonize Furnariidae and Psittaciidae nests, respectively (Brewer et al. 1983). *Psammolestes coreodes* Bergroth 1911, has been found in furnariid nests by Damborsky et al. (2001). *T. infestans* and *T. platensis* have been reported in furnariid nests of *Pseudoseisura lophotes* and *Coryphistera alaudina*, respectively (Brewer et al. 1983). Sylvatic *T. infestans* populations have also been recently found inhabiting rock piles in central Chile (Bacigalupo et al. 2010), in hollow trees in the Bolivian Gran Chaco (in hollow trees), (Noireau et al. 1997, Waleckx et al. 2012), in the Paraguayan Chaco under dry branches and in hollow trees (Rolón et al. 2011), and in the Argentine Chaco where they were found associated with *Amazona aestiva* (Psittaciidae) (Ceballos et al. 2009).

Triatomines that are usually found in bird nests are not infected with *T. cruzi*, as birds are refractory to the parasite. Consequently, they play only a secondary epidemiological role. However, it is well known that some nests are inhabited by rodents (mice) and/or marsupials (marmosas) during the winter season for protection and feeding (unpublished observations). These opportunistic inhabitants are reservoirs of *T. cruzi*, involved in the sylvatic cycle of the protozoan. Infested bird nests could thus represent potential infestation sources of domestic and peridomestic structures of rural houses of the Gran Chaco (Noireau 2009) and could consequently represent an epidemiological threat.

Very few studies exist on parasites and pathogens of Triatominae in the Chagas endemic region of Argentina. The available information is restricted to *Blastocrithidia triatomae*, *T. cruzi, Beauveria bassiana, Paecilomyces lilacinus,* and Triatoma virus in domestic and peridomestic structures (Marti et al. 2005, 2009). In all these studies, triatomine collections were carried out during the fall, spring, or summer. Consequently, no information is available for the winter, a period during which all bird nests are abandoned (except those of *Myopsitta* spp) and are used by mammals as refuges, thus representing an indirect potential link between birds and the circulation of *T. cruzi* in the sylvatic cycle.

The strong reduction of the domestic infestation by *T. infestans* after successful vector control interventions during the past decades increased the relative importance of the secondary triatomine species, especially those involved in the circulation of *T. cruzi* through the sylvatic cycle. Aiming at improving the knowledge of triatomine species associated with birds and their ecology, the objectives of this work were (i) to carry out a survey of triatomine species colonizing Furnariidae nests in a typical area of the Chaco region of Argentina during winter and (ii) to study the parasites and natural enemies associated with the collected triatomines.

MATERIALS AND METHODS

Study area

The study area is located within the Gran Chaco, between latitudes 17° and 33° S and longitude 65° and 60° W. The Gran Chaco is a vast plain that extends through northern Argentina, southeastern Bolivia, northwestern Paraguay, and a small area of southwestern Brazil. It stretches for about 1,500 km from north to south, and about 700 km from east to west. The climate is subtropical with 450 mm annual rainfall and temperature ranging between 13 and 28° C, with an absolute maximum of 43° C (Cabrera and Willink 1973). The ecoregion is characterized by patches of primary and secondary forest (*Schinopsis balansae*, *Aspidosperma quebracho*, *Prosopis kuntzei*, *Zizyphus mistol*, *Prosopis* sp, *Copernicia australis,* bromeliads, and opuntias), as described by Cabrera (1976), alternating with crop fields and dispersed human dwellings. The region has experienced a long-term ecosystem degradation since the end of the 19th century, leading to high levels of poverty in the rural communities. With typical mud-and-thatch rural houses, it is hyperendemic for Chagas and other neglected diseases. The main challenges for the Chagas control programs of the region are quick reinfestation of domestic and peridomestic structures after residual insecticide spraying and the lack of a sustainable surveillance system for sparse and remote rural populations (Gürtler et al. 2007).

Samples for this study were obtained during July, 2006 (winter), in the localities of Tres Estacas, El Picazo, El Palmar, and Las Leonas (26° 50' S - 61° 40' W and 27° 08' S - 61° 32' W), located in Chacabuco and General Pinedo of the Chaco province (Argentina) (Figure 1).

Triatomine collection

Triatomines were collected in Furnariidae stick nests (diameter of 25-30 cm) located on generally dry trees between two to six m high along domestic animal tracks that started from rural houses. Nests belonged to two species: *Coryphistera alaudina* and *Phacellodomus sibilatrix* . Nests of both species are visually similar and are abandoned during the winter months (July - September). All sampled nests were located more than 300 m away from any domestic or peridomestic structure and the habitats were all considered strictly sylvatic. Triatomine specimens were collected in areas of primary forest, near crop fields, and near the cities of Tres Estacas, El Picazo, and Las Leonas. Sampled nests in El Palmar were found in an area with patches of primary and secondary forest. The average distance between nests was measured using DIVA GIS 7.5.0).

Bird nests were collected from the trees using an aluminum rod with a hook in one side or by hand using a 6 m ladder. Afterwards, the nests were carefully dissected in a white plastic tray over a white blanket. In order to facilitate insect collection, tetramethrin 0.2% was used occasionally as a dislodging agent, after total disassembly of the nests.

Triatomine identification

Triatomines were collected using metallic forceps and transported individually (to avoid cross contamination of the samples with parasites and pathogens that might be present) to the laboratory in sterile plastic containers with folded pieces of paper inside and capped with a fine screen mesh. The containers were stored inside an expanded polystyrene container to avoid high temperature during the traveling time. The insects were then identified according to Lent and Wygodzinsky (1979) and maintained at a temperature of 28±1° C, 45% ± 5% relative humidity and a photoperiod of 12:12 (light:dark). All nymphs that arrived alive to the laboratory were fed on hens to adulthood in order to confirm the species identity. Some specimens that could not be identified with certainty by external macro-morphological characters were identified by sequencing of the nuclear ribosomal DNA ITS-2 after DNA extraction from legs, according to previous descriptions (Waleckx et al. 2011, Quisberth et al. 2011). The purification and direct sequencing of both strands of the DNA amplicons were performed by MACROGEN in Seoul, South Korea. Sequences were aligned and corrected using BioEdit version 7.0.9. Corrected fragments of 454 or 456 bp were resolved. BLAST (http://blast.ncbi.nlm.nih.gov/Blast) was used to determine the best identity with sequences deposited in GenBank. The sex ratio of *Ps. coreodes* adult specimens was analyzed with a chi-square test.

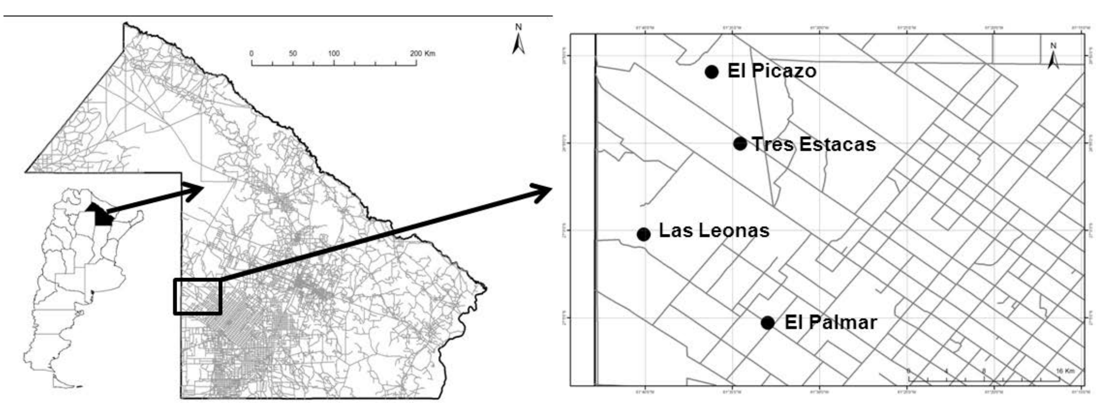

Figure 1. Location of the study sities: 1-Tres Estacas, 2- El Picazo, 3- Las Leonas and 4- El Palmar.

Table I. Number of triatomine specimens collected by age class and species in the four localities sampled. References: M: male, H: female, N3, N4, N5: 3rd, 4th, 5th instar nymphs, respectively.

Locality	Species	No. of triatomines	No. of nests with triatomines / total No. of nests (%)
El Palmar	*Ps. coreodes*	29 (7M,9H,8N5,4N4,1N3)	8/19 (42.1%)
	T. platensis	7 (1M,2H, 4N5)	4/19 (21%)
	T. infestans	2 (2N5)	1/19 (5.3%)
3 Estacas	*Ps. coreodes*	6 (4M,2H)	2/7 (28.6%)
El Picazo	*Ps. coreodes*	8 (3M,4H,1N4)	3/8 (37.5%)
Las Leonas	*Ps. coreodes*	11(3M,4H,2N5, 2N4)	2/11 (18.2%)
TOTALS		63 (17M,17H,11N5,11N4,1N3)	19/45 (42.2%)

Detection of parasites and natural enemies

Feces from the triatomine specimens were obtained by applying pressure to the abdominal region of each insect. A drop of the material was collected and examined under an optical microscope at 400X to identify eventual trypomastigotes and epimastigotes forms of *T. cruzi.*

The detection of TrV infection in fecal samples was performed using a reverse transcription-polymerase chain reaction (RT-PCR) and electron microscopic analysis as described by Marti et al. (2008).

To detect entomopathogenic fungal infection, the field-collected triatomines that died in the laboratory within 20 days post-collection were held in a moist chamber consisting of a sterile Petri dish with a disc of filter paper moistened with sterile distilled water and incubated at 22 °C according to Marti et al. (2005). Identification and isolation of fungal species was according to Lecuona (1996).

RESULTS

Triatomine collection and identification

A total of 45 bird nests was analyzed, among which 19 were colonized by at least one triatomine specimen (42.2%). Twelve out of the positive 19 nests were collected in El Palmar, three in El Picazo, two in Tres Estacas, and two in Las Leonas (Table 1). The average distance between nests in the same locality was 1.8 km ±0.21 SE.

A total of 63 triatomines was collected, including 39 adults and 24 nymphs. Sixty-one of them were identified using standard taxonomical keys based on macro-morphological characters. A total of 54 specimens was identified as *Ps. coreodes,* seven as *T. platensis,* and two as *T. infestans* (Table 1). *Ps. coreodes w*as found in all four study sites, in 15 out of the 19 positive nests, at an average density of 2.7-5.5 insects per nest (nymphs and adults). It was the predominant species throughout the study. The age distribution included 36 adults, ten 5th instar nymphs, seven 4th instar nymphs, and one 3rd instar nymph. The sex ratio of *Ps. coreodes a*dults showed no significant difference from 1:1 (χ^2= 0,81 d.f =2, p>0,05).

Of four 5th instar nymphs found in one particular nest in El Palmar, two were morphologically identified as *T. platensi*s, and two were impossible to classify using the morphological taxonomic keys because they had mixed characters between *T. platensi*s and *T. infestans*. The specimens morphologically identified as *T. platensis* and the unclassified specimens were further processed to be identified by molecular techniques. After ITS-2 sequencing and use of BLAST, one of the two *T. platensis* specimens was confirmed as *T. platensis*; indeed, the resolved sequence was identical to the one found in two different ITS1-5.8S-ITS2 composite haplotypes previously reported for *T. platensis* from Uruguay and Argentina (accession n° AJ576061 and AJ576062) (Bargues et al. 2006). The ITS-2 sequence of the other specimen morphologically identified as *T. platensis* could not be obtained. The other two specimens were identified as *T. infestans*: one presented an ITS-2 sequence identical to accession no. AJ575052 and AY860387, previously reported for *T. infestans* collected in Argentina and Brazil, respectively (Bargues et al. 2006, Martinez et al. 2006); the ITS-2 sequence of the other specimen presented a maximum identity (99%,corresponding to one mutation and one indel) with sequences previously reported for *T. infestans* from the Bolivian Chaco (accession n° HQ333214) (Quisberth et al. 2011) and from Argentina, Brazil, Chile, Paraguay, and Uruguay (accession n°AJ576054) (Bargues et al. 2006).

Screening of parasites and natural enemies

T. cruzi was found in two 5th instar nymphs of *T. platensis* obtained from a *C. alaudina* nest in El Palmar. Triatoma virus was found in one *Ps. coreodes* from El Palmar. No entomopathogenic fungi were found in the triatominae bugs.

DISCUSSION

The results obtained in this study show a high prevalence of *Ps. coreodes* in furnariid nests of the Argentine Chaco. This species is not epidemiologically relevant because it is present only in bird nests, presumably does not participate in the sylvatic transmission cycle of *T. cruzi*, and does not colonize domestic and peridomestic structures (Damborsky et al. 2001). The high prevalence of *Ps. coreodes* in El Palmar is similar to the results obtained by Damborsky et al. (2001), who found 39.5% of this species in the province of Corrientes, northeast Argentina, in furnariid nests. The average population density of the species is low, as observed for other sylvatic triatominae species of the Gran Chaco, contrasting with the frequently high average density reported for *Rhodnius* spp in palm trees (Braga Stehling Dias et al. 2008). During a year-long study in the same province of Corrientes, Bar et al. (1999) found 53.8% of the furnariid nests were colonized by *Ps. coreodes*. In our study, the age structure of *Ps. coreodes* population specimens showed 66% adult. According to Bar et al. (1999), *Ps. coreodes* populations found in bird nests in the province of Corrientes are composed mainly of adult specimens (63%) and according to Gurgel-Gonçalves and Cuba (2011), in *Ps. tertius* populations found in bird nests in the Caatinga ecoregion of Brazil, adult specimens represented 48% of the collections. The age distribution of *Ps. tertius* and *Ps. coreodes,* therefore, contrasts with other triatomine bug populations in which immature stages are predominant and adults generally account for less than 5% under either experimental or natural conditions (Gurgel-Gonçalves and Cuba 2011). Nevertheless, the high frequency of adults and 4th and 5th instar nymphs found in this study (Table 1) might be associated with the strong seasonal reproduction cycle that is interrupted in April (autumn in South America). Indeed, most of the young instar nymphs die during the winter months, while specimens of the 4th and 5th instar nymphs and adults are more resistant to cold weather and fasting. As observed by Gurgel-Gonçalves and Cuba (2011), a high proportion of adults in *Ps. tertius* populations from *Phacellodomus* nests could be caused by a combination of high longevity and low dispersal of adult bugs from the nests, in combination with marked seasonal reproduction.

Gorla and Schofield (1989) reported that during the colder months (May to September) in the southern Gran Chaco, *T. infestans* nymphs do not molt because of the low temperatures. Under field conditions throughout the year, the population structure presents a relevant peak of emergence of adults during the summer months (December-January), followed by a minor peak during winter months (June-July), which are correlated with the development time (six months) from egg to adult (Schofield 1980). The same authors also observed that the variation of the population size is strongly related with seasonality, as cold months temperature are usually below the 16° C threshold that interrupts the *T. infestans* development cycle (Gorla 1992).

To date, field studies on *Psammolestes* species during winter are scarce (Bar et al. 1999, Gurgel-Gonçalves and Cuba 2011), although it is possible that their populations behave similarly to the *T. infestans* populations under field conditions, receiving similar influences of temperature seasonality and the existence of a threshold temperature of zero development.

The second most prevalent species in this study was *T. platensis* (21.0% in El Palmar), similar to the results obtained by Damborsky et al. (2001) in Corrientes Province (Argentina) (13.2%) in bird nests.

The third species collected in this study was *T. infestans,* already found in bird nests during the 1930s and in Furnariidae nests during the 1980s (Turienzo and Di Iorio 2007) in Argentina. Brewer et al. (1983) and Ronderos et al. (1980) reported *T. infestans* finding in two *Myopsitta monachus* nests (approximately 250 and 350 m from a rural house, respectively) and in two *C. alaudina* nests (approximately 50 and 1,100 m from rural houses, respectively). *T. infestans* was also reported in *Amazona aestiva* nests (Marti[5], Ceballos et al. 2009). The present study is the most recent report of *T. infestans* presence in Furnariidae nests in Argentina at a distance of 1,250 m from the nearest house and 900 m from a goat corral. It contrasts with the absence of reports of sylvatic *T. infestans* findings in bird nests in the Bolivian Chaco, despite the prolonged collection effort of Noireau and colleagues (Waleckx et al. 2012). Moreover, the presence of a *T. infestans* 5th instar nymph, together with a *T. platensis* 5th instar nymph is relevant because it indicates that sylvatic nests can be effectively colonized by *T. infestans*.

The coexistence of different species of Triatominae within the same ecotope, such as *T. sordida* with *Ps. coreodes* and *T. platensis*, is well known (Damborsky et al. 2001). In this work, *T. platensis* and *T. infestans* were found together in the same bird nest in El Palmar, where they probably interbreed. This fact may explain the difficulty of morphological identification for two of the four bugs found in this nest, neither totally "*infestans*", nor "*platensis*". As mentioned by Bargues et al. (2010), the two morphospecies *T. platensis* and *T. infestans* are the same biological entities: they are interfertile, in the laboratory as well as in nature, although their interfertility has not been frequently reported because of their ecological separation. In fact, the ecological specialization provides the strongest argument to distinguish the species (Dujardin et al. 1999, Bargues et al 2010). The current study suggests that the occupation of the same sylvatic ecotope by the two species is perhaps more common than previously thought. Moreover, even with molecular tools, it is also very difficult to distinguish the two species, which are genetically similar (Bargues et al. 2006). In the current study, a fragment of 388 bp of the mtCytB of the four specimens found in the nest was also sequenced, and four different haplotypes were found (data not shown). Surprisingly, the number of mutations between the haplotypes of the two *T. platensis* specimens (five mutations) was greater than between *T. platensis* and *T. infestans* haplotypes (between two and four mutations). Moreover, it has been previously reported that 18 mutations can be found between mtCytB haplotypes of *T. infestans* (Waleckx et al. 2011). These observations show that *T. infestans* and *T. platensis* are not clearly genetically differenciated. Carcavallo et al. (1998) reported around 30 experimentally hybrids. Found naturally in Argentina, was the occurrence of *T. infestans x T. platensis* (Abalos 1948, Ronderos et al. 1980) and *T. infestans x T rubrovaria* hybrids (Carcavallo et al. 1998). The infection of *T. platensis* by *T. cruzi* was reported by Giraldez et al. (1997) and Lent and Wygodzinsky (1979) from domestic habitat collections.

The only flagellates infecting triatomines in the area were either *T. cruzi* or *Blastocrithidia* sp. Although a PCR technique for the identification of *T. cruzi* would have been more appropriate, all recent field studies carried out around the region reported the presence of *T. cruzi* I using PCR techniques (Tomasini et al. 2011), and *Blastocrithidia* was clearly discarded because trypomastigotes were found (absent in *Blastocrithidia*). This led us to conclude that this is the first time that *T. cruzi* was found in a *T. platensis* nymph inside a bird nest.

Diosque et al. (2004) reported *T. cruzi* infection in *Didelphis albiventris* from collections in the same area as the current study and hypothesized that *T. guasayana* could be the main vector of the *T. cruzi* sylvatic cycle. This study shows that *T. infestans, T. platensis,* and their hybrids colonizing bird nests should also be considered as potential vectors of the sylvatic cycle of *T. cruzi*. As

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shown by Schweigmann et al. (1995), *D. albiventris* is an important host of *T. cruzi,* and Damborsky et al. (2001) found *D. albiventris* infected with *T. cruzi* in nests with *T. platensis.*

As shown by Salvatella (1987), *T. platensis* occurs in bird nests and is less important than *T. infestans* for the vectorial transmission of *T. cruzi* to humans, although they were found infected with *T. cruzi* under natural conditions (Giraldez et al. 1997, Lent and Wygodzinsky 1979). Although triatomine species associated with bird nests might be less important than species associated with mammals, because birds are refractory to *T. cruzi* infection, the presence of mammals within the bird nests (using them as winter refuges) calls attention to these triatomine species. Nests dissected in this study were occupied by at least *Graomys griseoflavus chacoensis* (Rodentia) and *Thylamys pusillus* (Marsupialia), both found infected by *T. cruzi* in Paraguay (Yeo et al. 2005) and in Santiago del Estero (Argentina) by Wisnivesky-Colli et al. (1992).

In this study, TrV is the only pathogen virus for Triatomines found in South America to date, and it has been found only in *T. infestans* and *T. sordida* from domiciles and peridomiciles in Argentina (Marti et al. 2009), and in sylvatic *T. infestans, T. delpontei,* and *Ps. coreodes* (Susevich et al. 2012). In this study the natural infection of *Ps. coreodes* is reported. The partial sequence of the virus was compared with other TrV isolates (Susevich et al. 2012).

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