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Abstract:	Triatomines are the vectors of Trypanosoma cruzi, the etiologic agent of Chagas disease. Besides Triatoma infestans, the most important T. cruzi vector in the Gran Chaco region, other triatomine species associated with sylvatic birds and mammals are the responsible of the maintenance of the wild cycle of T. cruzi. The present study aimed at evaluating the house invasion by sylvatic triatomine species in rural communities La Rioja (Argentina), and its association with environmental variables. House invasion by flying adult triatomines was recorded by trained community, collected over 377 houses distributed over 73 localities, between October 2014 and February 2015. The result of the study showed that house invasion by adult triatomines if highly frequent, shown as 26.3% of infested houses in 53% of the localities. Seven sylvatic triatomine species were collected, being T. guasayana and T. garciabesi the most abundant ones. House invasion by triatomine species showed no spatial aggregation and was not associated with temperature, precipitation or vegetation cover at the spatial scale considered in the study. House invasion by triatomines highly concerns the rural communities. Besides constituting a latent risk, the presence of these species interferes negatively with the vigilance activities of the provincial program of Chagas disease.

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Invasion of rural houses by wild Triatominae in the arid Chaco

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ABSTRACT: Triatomines are the vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, the main endemic disease affecting 5-7 million people in Latin America. Besides *Triatoma infestans,* the most important *T. cruzi* vector in the Gran Chaco region, other triatomine species associated with sylvatic birds and mammals are responsible for the maintenance of the wild cycle of *T. cruzi*. The present study aimed at evaluating the house invasion by sylvatic triatomine species in rural communities of the Los Llanos region (La Rioja, Argentina), and its association with environmental variables. House invasion by flying adult triatomines was recorded by an appropriately trained community, which collected over 377 houses distributed over 73 localities of a 56600 km^2 study region, between October 2014 and February 2015. The result of the study showed that house invasion by adult triatomines is highly frequent: 26.3% houses were infested in 53% of the localities. Seven sylvatic triatomine species were collected, being *T. guasayana* and *T. garciabesi* the most abundant ones. House invasion by triatomine species showed no spatial aggregation and was not associated with temperature, precipitation or vegetation cover at the spatial scale considered in the present study. House invasion by triatomines highly concerns the rural communities that frequently confound them with the epidemiologically important *T. infestans*. Besides constituting a latent risk (although low), the presence of these species interferes negatively with the vigilance activities of the provincial program of Chagas disease.

Keyword Index: Triatoma guasayana, Triatoma garciabesi, environmental variables.

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INTRODUCTION

Chagas disease is a vector-borne tropical disease that affects the most vulnerable populations across Latin America. At present, an estimated 7-8 million people are infected with *Trypanosoma cruzi* and more than 25 million people are at risk of contracting Chagas disease (WHO 2015). Insects of the Triatominae subfamily (Hemiptera: Reduviidae) are the vectors of *T. cruzi* (Lent and Wygodzinsky 1979). Among Triatominae, only a few species were successful in establishing domestic colonies, thus potentially becoming involved in the transmission of *T. cruzi* to humans (Noireau et al. 2009).

Of the 140 triatomine species, 12 occur in the Gran Chaco of Argentina (Gorla and Noireau 2010). Within this region, no Triatominae species have been recorded establishing colonies within houses except *Triatoma infestans*, the main vector species of *T. cruzi* to humans (WHO 1991). The arid Gran Chaco in central Argentina has been historically hyperendemic for Chagas disease. However, in the southern area, vector control interventions decreased significantly house infestation in the rural communities during '90s (Porcasi et al. 2007). With the reduction of the *T. infestans* intradomestic infestation, rural communities frequently report invasion of houses by flying triatomines and claim the health system for insecticide applications. Sometimes, these flying triatomines are *T. infestans* dispersing from peridomestic ecotopes, but on occasions, these are non- *T. infestans* triatomines (Abrahan et al. 2011). As part of the protocol of provincial health agencies in charge of Chagas disease vector control, non *T. infestans* triatomines are not targets of control interventions using pyrethroid insecticides. Consequently, finding non *T. infestans* adults inside houses does not promote an insecticide application, as it is assumed that these adults do not represent an epidemiological risk for the transmission of *T. cruzi* to humans.

The non *T. infestans* triatomines of the Gran Chaco are associated with sylvatic birds and mammals but in some cases they colonize peridomestic structures. *T. garciabesi*, *T. guasayana* and *T. sordida* are frequently observed in corrals and chicken coops (Canale et al. 2000). Other species have been only recorded in sylvatic ecotopes, associated with birds and mammals (*T. breyeri*, *T. rubrovaria*, *T. eratyrusiformis*, *T. platensis*, *T. delpontei*, *Panstrongylus guentheri, Pa. geniculatus* and *Psammolestes coreodes)* (Bar et al. 1993, Bar et al. 2002, Patterson et al. 2009, Canale et al. 2000, Noireau and Dujardin 2001, Marti et al. 2014*)*. Cactaceous plants, grass and scrub constitute the normal habitat for sylvatic

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triatomine species that occasionally fly into domicile. These triatomine species play a role in the maintenance of the sylvatic cycle of *T. cruzi* transmission (Bar et al. 2002, Ceballos et al. 2006, Cecere et al. 2015), and represent a persistent low risk for the transmission of *T. cruzi* to human beings.

The purpose of this study was to evaluate the occurrence of sylvatic Triatominae invasions by active flight into rural houses of the semiarid Gran Chaco in Argentina and to estimate their relationship with environmental variables. The study was guided by two main questions a) is the distribution of sylvatic triatomines invasions of the arid Gran Chaco associated with environmental variables? and b) are invasions more frequent in houses nearer to vegetated areas that could harbor triatomine hosts than in houses distant from vegetated areas?.

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MATERIALS AND METHODS

Study area

The study was carried out in the southern extreme of Los Llanos, La Rioja Province, Argentina (31°21 S, 66°35 W), over an area of 56585 km^2. Los Llanos is a semi-arid plain region with widespread location of over-dispersed rural communities, where 40% of the houses are located in localities with ≤ 8 houses (Gorla et al. 2009). Most of traditional houses are constructed with adobe walls and roofs are made of a multi-layered structure of mud and local vegetation. In these rural communities, people live under a system of economic subsistence, mainly based on the use of domestic animals (goats, chickens, pigs and cattle in a few places). The main peridomestic structures in the study area are chicken coops and goat corrals. The chicken coops are commonly constructed with wood, sticks, bricks and/or wire around the perimeter. The roofs may have branches, wood, leather or plastic. Chicken coops are 10-20m far from the houses. Goat corrals usually have a more homogeneous construction, with interlaced sticks or tree branches as walls and a roofed area for calves. The distance between the house and goat corrals varies between 30 and 100 m.

In 2008, the provincial Government of La Rioja, Argentina, started a program to improve the living conditions of rural communities by building new houses meant to replace all the

traditional ones. Since 2012, electric lines have been extended into the rural communities, together with the installation of water resources infrastructure in several areas.

A total of 377 rural houses (new and traditional) distributed over 73 localities in the Departments Rosario Vera Peñaloza, Ángel Vicente Peñaloza and San Martin (south of La Rioja province) were included in the sample used to record the house invasion by adult Triatominae between October 2014 and February 2015 (spring and summer seasons). For operational reasons, a locality was defined as a group of houses separated by more than 2 km from another group. All the houses included in the sample are periodically evaluated by the Programa Chagas La Rioja. Those found infested by *T. infestans* receive a standard application of a suspension concentrate formulation of a pyrethroid insecticide, using manual sprayers by technicians of Programa Chagas La Rioja. All rural houses were georeferenced with a hand-held Garmin Legend GPS and identified with an individual code that was marked on a metal plaque attached to an external wall. Entomologic data, evaluation and spraying date, family data, type and number of domestic animals, and construction details of each house were recorded on standard forms and entered into a spatial database that constitutes the geographic information system of the Programa Chagas La Rioja.

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Insect collection

Owners of each house selected for this study received a briefing about the objective of the study, were invited to participate and were shown the type of triatomine species that would eventually invade the house. Each owner received a labeled self-sealing plastic bag with a clear explanation of the procedure for collecting and keeping the insects in the plastic bags. This insect collection method was used and identified as one of the most sensitive sampling technique for domestic triatomines, especially for low density populations (Cecere et al. 2004, Abad-Franch et al. 2011). Two weeks after the plastic bags distribution, the research team returned to each house to collect the bags. This procedure was carried out in October 2014 (late spring) and February 2015 (late summer). Triatomines collected in the bags were classified and data recorded into the database including species (Lent and Wygodzinsky, 1979) age class, sex, number of insects, house number and collection site (intradomestic or peridomestic).

Data analysis

A house was recorded as infested when the plastic bag contained one or more triatomine specimens. House infestation for each species was calculated taking into consideration the percentage of houses infested by a particular species within a locality or groups of localities. Spatial aggregation of house infestation by species was estimated using the satscan statistics in order to identify eventual clusters of high house infestation, compared with the regional average. The unit of analysis was the locality, using a circular shape and a Poisson model for cluster detection (Kulldorf 1997).

To study the relationship between the occurrence of house invasion and environmental variables, the ecological niche modeling approach was used. Response variables were abundance and occurrence of each triatomine species. Predictor variables included the 20 temperature- and precipitation-derived variables and elevation from the *Worldclim* database (www.worldclim.org), and vegetation cover (see below). *Worldclim* variables and elevation were formatted as raster files with a pixel size of 1 km and were used to fit generalized linear models to estimate triatomine abundance (count data) and occurrence (binary variable). The former was modeled using a Poisson distribution and the latter, with a binomial distribution. Variable colinearity was estimated using the *vif* function of the *car* package of R (https://cran.r-project.org/web/packages/car/car.pdf); variables with vif>10 were discarded from the models. Goodness of fit was calculated using the *pROC* package.

In order to verify the hypothesis that houses nearer vegetated areas would report higher frequency of triatomine invasions, vegetation cover classified as shrub or forest surrounding studied houses was estimated in concentric circles of 100, 500 and 1000 mts. Vegetation cover was estimated from the normalized difference vegetation index (NDVI) calculated using Landsat 8 imagery, at a 30x30 meters nominal spatial resolution. Landsat 8 scenes covering the study area (path and row 230-082, 231-081 and 231-082) of September and October 2014 were downloaded from www.earthexplorer.usgs.gov. The atmospheric Dark Object Subtraction method (DOS1 correction, (Chavez Jr, 1988) was applied to the raw bands using the semi-automated classification (SCP) plugin of QGIS (qgis desktop 2.4). NDVI was calculated using the standard equation (B5-B4)/(B5+B4), where B5 and B4 are Landsat 8 bands 5 (infrared) and 4 (red) respectively.

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RESULTS

A total of 8 Triatominae species were collected by house owners in the study area. *T. infestans* was the most abundantly collected, and results on this species will be reported elsewhere. From now on, reference to collected triatomines will include all triatomine species collected during the study except *T. infestans*. The other 7 species, peridomestic and/or sylvatic triatomines are listed in Table 1. Of the 377 houses included in the study, 26.3% were infested and were distributed in 39 localities. All collected specimens were adults that flew into the houses from either the sylvatic environment and/or peridomestic structures. The most abundant species were *T. guasayana* (Figure 1) and *T. garciabesi* (Figure 2), of which 211 and 139 adult specimens were collected, respectively. *T. guasayana* females invaded houses more frequently than males (sex ratio F:M> 1, P< 0.05), whereas a higher number (marginally significant) of *P. guentheri* males invaded houses (Table 1). House invasion by active flight occurred mainly during late spring, as 90.8% of the specimens were collected in November/December.

The most frequent species invading houses was *T. guasayana*, found in 19.4% of the studied houses and 46.6% of the studied localities. The second most frequent was *T. garciabesi*, found in 12.2% of the houses and 35.6% of the localities (Table 1). The occurrence of house invasion showed no aggregation at the level of localities (measured by the SatScan statistic) neither for *T. guasayana* nor *T. garciabesi*. Table 2 shows the very low fit of the derived models. Table 3 (supplementary data) shows average and standard deviation values of the variables included in the modeling estimation for *T. guasayana* and *T. garciabesi*. Minor differences between environmental values in presence and absence locations, within and among species, which in case might explain the lack of significance of the tested models, were found.

Vegetation cover (as measured by the Landsat 8 NDVI) around houses invaded by triatomines was not different from vegetation cover around houses not invaded by triatomines. However, *T. garciabesi* was more frequent where there is an intermediate vegetation cover (NDVI values between 0.25 and 0.35) near the house (<= 100 mts) and there is intermediate vegetation cover (NDVI between 0.3 and 0.4) within a 1000 mts circle

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around the house. *T. guasayana* is more frequent where there is low vegetation cover (NDVI < 0.25) near the house (<= 100 mts) and there is intermediate vegetation cover (NDVI between 0.25 and 0.35) within a circle of 1000 mts around the house. No species were collected in houses where vegetation cover is high (NDVI> 0.4) (Table 4).

DISCUSSION

Studies on sylvatic triatomines of the Gran Chaco were usually based on active search captures of sylvatic and/or peridomestic ecotopes and/or the use of light traps (Abrahan et al 2011; Vazquez-Prokopec et al. 2004). This study reports for the first time the invasion of domestic premises by sylvatic triatomines, arriving at rural houses during the active dispersal process, using a triatomine collection technique based on the community participation. Our results show that the number of adult triatomines reaching rural houses in Los Llanos Riojanos is high, compared to the number of individuals captured in Santiago del Estero using light traps (Vazquez-Prokopec et al. 2004; Abrahan et al. 2011). Seven species (other than *T. infestans*) arrive frequently at rural houses, during the first part of the warm season.

T. guasayana and *T. garciabesi* were the most recurrent species approaching the houses, with higher abundance of the former, unlike the findings of Canale et al. 2000 that collected almost 5 times more *T. garciabesi* than *T. guasayana*. *T. guasayana* has been more frequently found associated with peridomestic structures of rural houses, such as goat corrals, piled materials or orchard fences (Canale et al. 2000) and adults were collected near light sources (Wisnivesky-Colli et al. 1993). This study shows that *T. guasayana* females were more abundant early than later in the season. However, other studies show dominance of different sexes at the beginning or at the end of the warm season. *T. garciabesi* was most frequently found below the bark of *Prosopis* spp trees, where chickens usually spend the night (Canale et al. 2000).

No evidence of spatial aggregation of house invasion by either *T. guasayana* or *T. garciabesi* was found in this study. It is remarkable that none of the environmental variables were able to describe the distribution and/or the abundance of the most frequently collected species (*T. guasayana* and *T. garciabesi*). Many other studies showed the contrary, though, being temperature- and rainfall- related variables mainly frequently found associated with

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the distribution of Triatominae species. The absence of relationship in this case suggests that either none of the variables is affecting the species distribution (unlikely) or that the study area is environmentally very homogeneous for the variables included in the modeling estimation, as it is shown by the very similar values of environmental variables in sites with and without the occurrence of the most frequent species. Although models for the presence and/or abundance of *T. infestans* show strong association with peridomestic features (animals, corrals, deposits etc.), this is not expected in this case, as wild triatomines rarely colonize the peridomestic area and find refuge and blood source in the wild environments.

We found no evidence of increasing frequency of house invasion in areas with high vegetation cover. However, a higher number of *T. guasayana* was found in houses with nearby and distant (<1000 m) intermediate vegetation cover. *T. garciabesi* was found in houses with nearby low and distant (<1000 m) intermediate vegetation cover.

Triatoma infestans is still the most important vector of *Trypanosoma cruzi* in the Gran Chaco region. Once responsible for 80% of the new *T. cruzi* infections in the Southern Cone countries of South America (because of the very abundant populations that existed within the rural houses), it now dropped over 90% of its original distribution (Schofield et al. 2006). The occupation of *T. infestans* niche, once controlled, does not occur since wild triatomine species of the Gran Chaco rarely colonize rooms of rural houses and they have much lower vectorial capacity than *T. infestans* on the transmission of *T. cruzi*.

Species found in lower frequency in this study, including *T. delpontei* and *T. platensis*, are commonly associated with psittacid (the former) and furnariid (the latter) nests (Brewer et al. 1978, Marti et al. 2014). These species have very low epidemiological importance, as they are associated with birds (refractory to *T. cruzi* infection), therefore they are very rarely found infected. However, *T. platensis* can be found colonizing peridomestic structures of rural houses and hybrids with *T. infestans* have been found (Marti et al. 2014). *P. guentheri* has been found in peridomestic structures associated with rodents and didelphids (Lent and Wygodzinsky 1979), whereas *T. eratyrusiformis* is an aggressive species that inhabits mountainous areas, rocky environments and stone fences in peridomestic areas where it is associated with edentates and rodents. These mammals can show very high infection prevalence of *T. cruzi*, as in the case of *Microcavia australis* (Cecere et al. 2015).

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Main limitations of this study relate with the extension of the insect collection period, of only one year and the heterogeneity in the insect collection ability that could vary among house owners. This study is in progress and insect collection within the study area is planned for a second warm season.

Although the detected triatomine species do not pose a high epidemiological risk, they represent a source of concern for the rural communities. People frequently request the intervention of the provincial vector control program. Since resources available for the vector control agency are frequently scarce, it rarely responds to the request of rural communities after house invasion by adult triatomines. Moreover, on many occasions these house invasions are associated with sylvatic triatomine species with low epidemiological importance, and therefore spraying is not done. This lack of response to the community request discourages systematic reports and reduces the chances of detecting house invasion by *T. infestans*, the species with the highest epidemiological importance for the transmission of *T. cruzi* in the area.

The invasion of rural houses by wild triatomines, with lower epidemiological importance than *T. infestans*, is a frequent event during the beginning of the warm season. As they are easily confused with *T. infestans*, teaching the rural communities to differentiate the main triatomine species present in the area might improve the vigilance system of Chagas disease.

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Table 1. Number of males and females invading houses by species. Sex ratio informs the significance of a 1:1 ratio null hypothesis, NS: not significant, between brackets the confidence interval 95% of male percentage.

species	males	females	Sex ratio 1:1	N° localities	N° Houses
Triatoma garciabesi	81	58	NS	26	46
Triatoma guasayana	80	131	P = 0.0004 (31.3-44.8)	34	73
Triatoma eratyrusiformis	12	16	NS	5	12
Panstrongylus guentheri	11	3	P = 0.032 (49.2 – 95.4)	7	9
Triatoma platensis	4	4	NS	3	4
Triatoma breyeri	0	1	-	1	1
Triatoma delpontei	0	1	-	1	1

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Table 2. Goodness global linear models of fit of presence (binomial link) or abundance (Poisson link) models for *T. garciabesi* and *T. guasayana.* Env. Vars: Significant environmental variables remaining in the model after removing those with vif>1, Approx R sq: Approximate R squared, d.f degrees of freedom, bio: annual mean temperature, bio3:isothermality, bio15: precipitation seasonality.

	Null deviance (d.f.)	**Residual deviance (d.f.)**	**Approx. R sq**	**Env. Vars**
Garciabesi presence	259.3 (376)	244.45 (361)	0.057	none
Garciabesi abundance	608.9 (376)	545.2 (373)	0.103	bio1, bio3, bio15
Guasayana presence	358.9 (376)	335.1 (361)	0.06	none
Guasayana abundance	865.3 (376)	837.5 (372)	0.032	bio15

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Table 4. Number of *Triatoma guasayana* and *Triatoma garciabesi* collected in houses, surrounded by varying values of vegetation cover (as measured by NDVI) around the house in concentric circles of 100, 500 and 1000 meters. NDVI: Normalised Difference Vegetation Index calculated from Landsat 8 imagery.

	T. guasayana			*T. garciabesi*		
NDVI	**100**	**500**	**1000**	**100**	**500**	**1000**
<0.25	101	13	16	31	3	3
0.25-0.30	46	76	39	30	21	6
0.30-0.35	34	64	94	35	34	47
0.35-0.40	17	42	43	13	51	42
>0.40	6	9	12	4	4	15

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FIGURE LEGENDS

Figure 1: Distribution of house infestation by *Triatoma guasayana* in the study area. 1: Ángel Vicente Peñaloza Department, 2: Rosario Vera Peñaloza Department, 3: San Martin Department. Circles indicate location of studied rural houses. White circles: un-infested house; black circles infested house with number of *T. guasayana* proportional to circle diameter.

Figure 2: Distribution of house infestation by *Triatoma garciabesi* in the study area. 1: Ángel Vicente Peñaloza Department, 2: Rosario Vera Peñaloza Department, 3: San Martin Department. Circles indicate location of studied rural houses. White circles: un-infested house; black circles infested house with number of *T. garciabesi* proportional to circle diameter.

For Review Only

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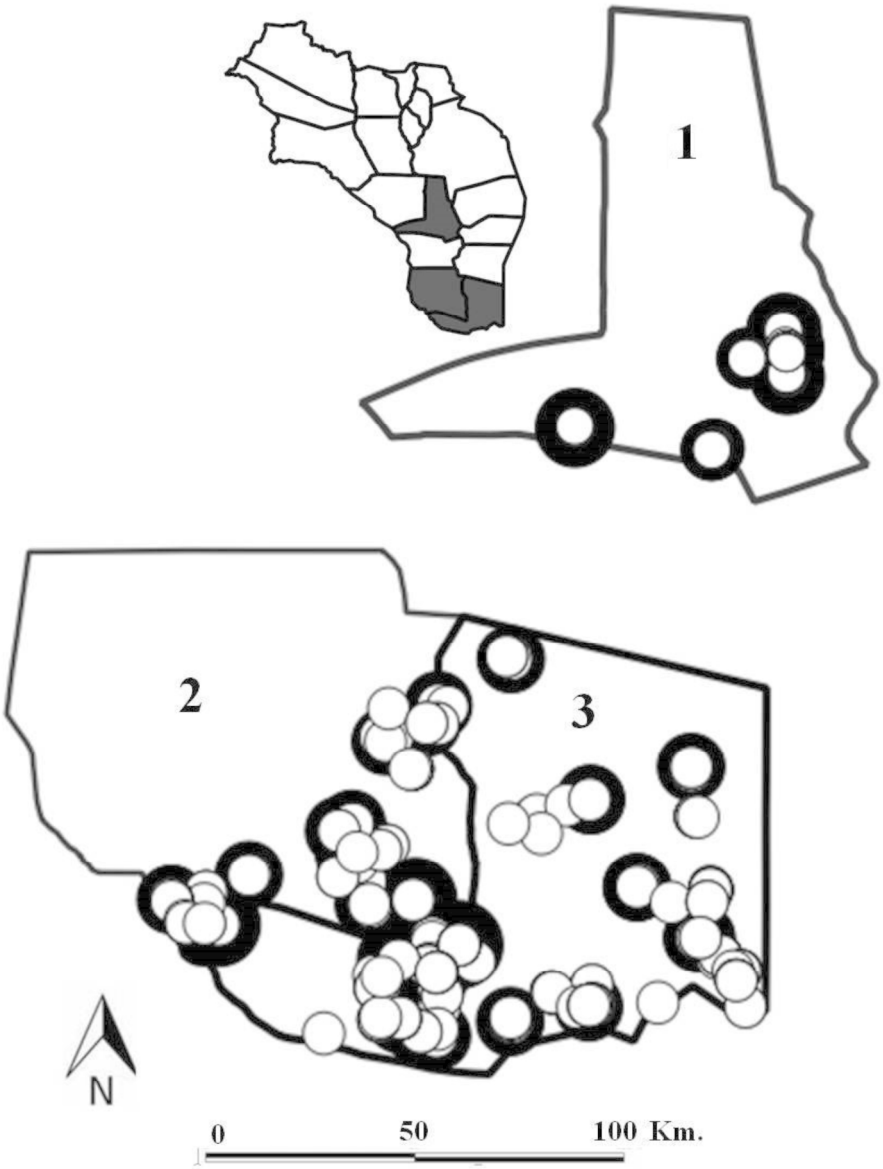

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235x293mm (300 x 300 DPI)

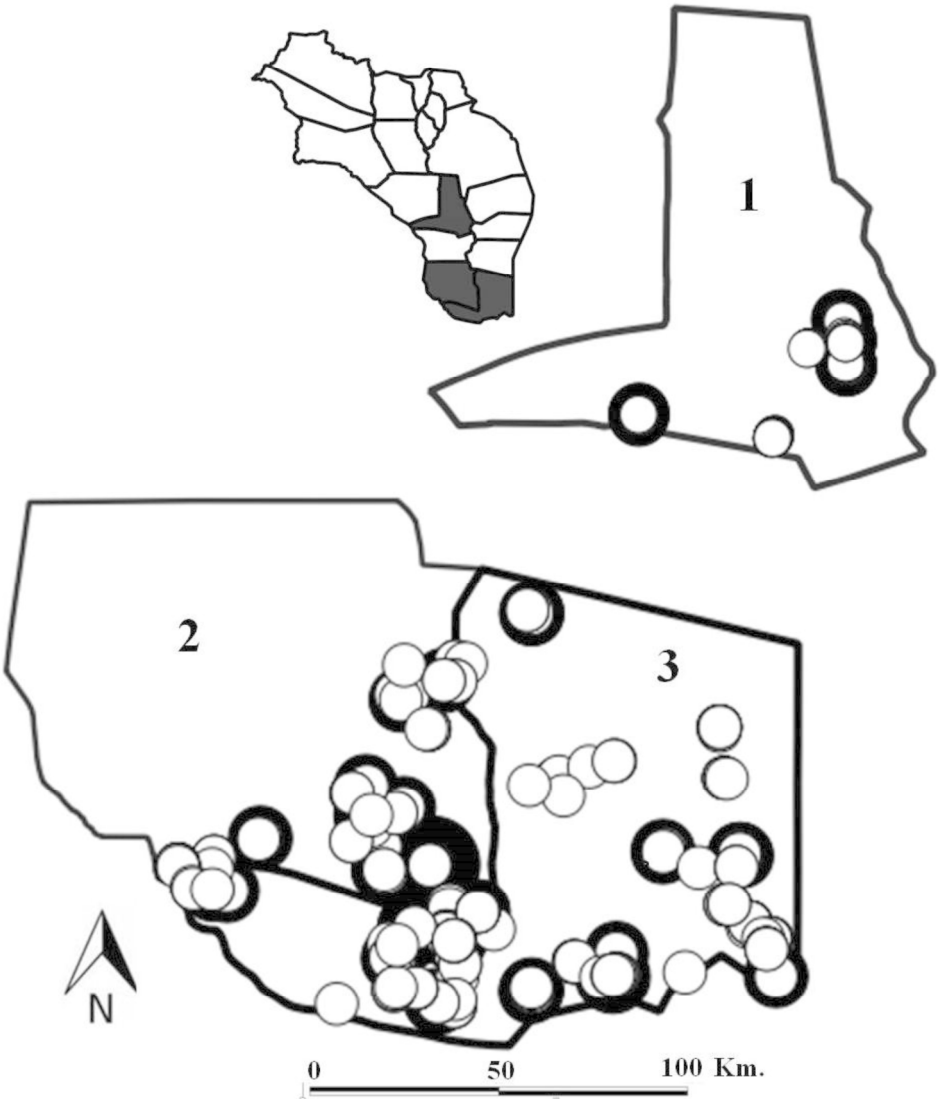

Figure 2: Distribution of house infestation by Triatoma garciabesi in the study area. 1: Ángel Vicente Peñaloza Department, 2: Rosario Vera Peñaloza Department, 3: San Martin Department. Circles indicate location of studied rural houses. White circles: un-infested house; black circles infested house with number of T. garcibesi proportional to circle diameter.
261x295mm (300 x 300 DPI)