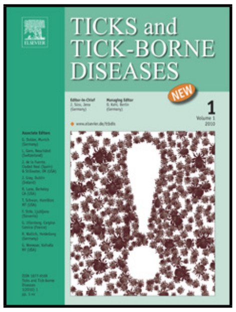

Title: Seasonal dynamics, geographical range size, hosts, genetic diversity and phylogeography of *Amblyomma sculptum* in Argentina

Authors: Evelina L. Tarragona, Patrick S. Sebastian, María N. Saracho Bottero, Emilia I. Martinez, Valeria N. Debárbora, Atilio J. Mangold, Alberto A. Guglielmone, Santiago Nava

PII:	S1877-959X(17)30543-5
DOI:	https://doi.org/10.1016/j.ttbdis.2018.04.009
Reference:	TTBDIS 1023

To appear in:

Received date:	24-11-2017
Revised date:	28-2-2018
Accepted date:	16-4-2018

Please cite this article as: Tarragona, Evelina L., Sebastian, Patrick S., Saracho Bottero, María N., Martinez, Emilia I., Debárbora, Valeria N., Mangold, Atilio J., Guglielmone, Alberto A., Nava, Santiago, Seasonal dynamics, geographical range size, hosts, genetic diversity and phylogeography of Amblyomma sculptum in Argentina.Ticks and Tick-borne Diseases https://doi.org/10.1016/j.ttbdis.2018.04.009

Seasonal dynamics, geographical range size, hosts, genetic diversity and phylogeography of *Amblyomma sculptum* in Argentina.

Evelina L. Tarragona[a*], Patrick S. Sebastian[b], María N. Saracho Bottero[a], Emilia I. Martinez[c], Valeria N. Debárbora[d], Atilio J. Mangold[a], Alberto A. Guglielmone[a], Santiago Nava[a]

[a] Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, and Consejo Nacional de Investigaciones Científicas y Técnicas, CC 22, CP 2300, Rafaela, Santa Fe, Argentina

[b] Parasitology Unit, University of Hohenheim, Emil-Wolff-Straße 34, 70593 Stuttgart, Germany

[c] Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Sargento Cabral 2139, CP3400, Corrientes Capital, Argentina

[d] Laboratorio de Biología de los Parásitos. Facultad de ciencias exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste. Av. Libertad 5470 CP 3400, Corrientes Capital, Argentina

[*]Corresponding author at. Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, CC 22, CP 2300 Rafaela, Santa Fe, Argentina. Tel.: +54 03492440121; Fax: +54 03492440114. E-mail address**:** tarragona.evelina@inta.gob.ar (E.L. Tarragona)

Abstract

The aim of this work was to generate knowledge on ecological aspects of *Amblyomma sculptum* in Argentina, such as seasonal dynamics, geographical range size, hosts, genetic diversity and phylogeography. Adult and immature *A. sculptum* ticks were collected in different localities of Argentina to know the geographical range size and hosts. The genetic diversity of this tick was studied through analyses of 16S rDNA sequences. To describe the seasonal dynamics, free-living ticks were monthly collected from October 2013 to October 2015. *A. sculptum* shows a marked ecological preference for Chaco Húmedo eco-region and “Albardones” forest of the great rivers in the wetlands in the Chaco Biogeographical Province, and for Selvas Pedemontanas and Selva Montana in the Yungas Biogeographical Province. This species has low host specificity, and it has large wild and domestic mammals as principal hosts to both immature and adult stages. *Amblyomma sculptum* is characterized by a one-year life cycle. Larvae peak in early winter, nymphs peaked during mid-spring, and adults during late summer and mid-summer. The genetic divergence was low and the total genetic variability was attributable to differences among populations. This fact could be associated to stochastics process linked to micro-habitat variations that could produce a partial restriction to gene flow among populations. The geographic regions do not contribute much to explain the *A. sculptum* population genetic structure, with an ancestral haplotype present in most populations, which gives rise to the rest of the haplotypes denoting a rapid population expansion.

Keywords: *Amblyomma sculptum*, seasonal dynamics, geographical range size, genetic diversity, Argentina.

1. Introduction

The hard tick *Amblyomma sculptum* Berlese, 1888 belongs to the *Amblyomma cajennense* complex with other five species, namely *Amblyomma tonelliae* Nava, Beati and Labruna, 2014*, Amblyomma cajennense* sensu stricto (Fabricius, 1787), *Amblyomma mixtum* Koch, 1844*, Amblyomma patinoi* Labruna, Nava and Beati, 2014 and *Amblyomma interandinum* Beati, Nava and Cáceres, 2014 (Nava et al., 2014). According to previous information, all stages of *A. sculptum* are catholic feeders with a wide range of mammal hosts, including humans (Nava et al., 2017). This tick has a distribution ranging from the humid areas of northern Argentina (foothill jungle of Yungas and humid areas of Chaco Biogeographic Provinces sensu Morrone (2006)), Bolivia and Paraguay to Brazil in areas belonging to the Cerrado, Pantanal and Brazilian Atlantic Forest Provinces sensu Morrone (2006) (Nava et al., 2014; Martins et al., 2016). In Argentina, *A. sculptum* has a parapatric distribution with other species from the *A. cajennense* species complex, *A. tonelliae* (Estrada-Peña et al., 2014), which is distributed in dry areas of the Chaco Biogeographic Province in Argentina, Bolivia and Paraguay (Nava et al., 2014; Tarragona et al., 2015b). Estrada-Peña et al. (2014) have stated that environmental variables rather than hosts determine the distributional ranges of *A. tonelliae* and *A. sculptum*. These authors showed that the distribution of *A. sculptum* overlaps with that of *A. tonelliae* in northern Argentina; parapatry occurs in regions with lower Land Surface Temperature (LST) and very high Normalized Difference Vegetation Index (NDVI) relative to allopatric regions.

Both *A. sculptum* and *A. tonelliae* are aggressive to humans and domestic mammals and they have been incriminated as potential vectors of pathogenic microorganisms for domestic animals and humans (Guedes et al., 2005; Widmer et al., 2011; Soares et al., 2012; Alves et al., 2014; Labruna et al., 2014; Nunes et al. 2015; Tarragona et al., 2015a; Melo et al., 2016; Tarragona et al. 2016). Fatal cases of spotted fever in humans due to the infection with *Rickettsia rickettsii* were reported in localities of Jujuy and Salta Provinces, Argentina, and ticks of the *A. cajennense* species complex were recognized as the possible vectors (Ripoll et al., 1999; Paddock et al., 2008; Seijo et al., 2016). These cases of spotted fever in humans occurred in the area where *A. sculptum and A. tonelliae* have parapatric distribution (Estrada-Peña et al., 2014). Both species could be the vector incriminated in the transmission of *R. rickettsii,* since experimental studies have proved that

A. tonelliae and *A. sculptum* (named as *A. cajennense*) have vectorial competence to transmit this pathogenic microorganism (Soares et al., 2012; Tarragona et al., 2016). Additionally, specimens of *A. sculptum* were found naturally infected with *Rickettsia amblyommatis* (named as ´*Candidatus* Rickettsia amblyommii´) and *Rickettsia bellii* (Alves et al., 2014; Melo et al., 2016; Sebastian et al., 2017), and *A. tonelliae* with *R. amblyommatis (*named as ´*Candidatus* R. amblyommii´) and *Rickettsia* sp. strain El Tunal (Tarragona et al., 2015a) in Argentina.

Vectorial competence, seasonality, geographic range size, host usage, population genetic and habitat requirement are among the principal intrinsic variables of ticks shaping their role as parasites *per se* and in the cycles of tick-borne pathogens (Sonenshine and Mather, 1994). In this sense, and because the epidemiological relevance of *A. sculptum*, the aim of this work was to study aspects of its ecology as geographical range size and natural hosts, seasonal dynamics, genetic diversity and phylogeography.

2. Materials and methods

2.1. Geographic range size and hosts.

The geographic range size and hosts of *A. sculptum* in Argentina were determined analyzing records of adult and immature ticks collected in 48 localities (Table 1). Data were obtained from Ortiz et al. (2011), Nava et al. (2014) and by examination of the tick collections of INTA Rafaela and Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Santa Fe, Argentina. All records of *A. sculptum* analysed during this work corresponded to localities of the Yungas (YBP) and Chaco (CBP) Biogeographic Provinces as defined by Morrone (2006). In Argentina, the YBP is divided into four vegetation strata which correspond to altitudinal gradient strata: I) Selvas Pedemontanas (from 400 to 700 masl); II) Selva Montana (from 700 to 1500 masl); III) Bosque Montano (from 1500 to 3000 masl); IV) Pastizales de altura (above 2500 masl) (Burkart et al., 1999; Brown et al., 2001). In turn, the area contained within the CBP is divided in two eco-regions, namely Chaco Seco and Chaco Humedo (Burkart et al., 1999).

All locality records were plotted against two biogeographic schemes: The first one, designed in this study, corresponds to a combination of the eco-regions of Argentina presented by Burkart et al. (1999) and the Biogeographic Provinces delimited by Morrone (2006). This procedure resulted in the following scheme: 1, Altos Andes; 2, Puna; 3, Selva de las Yungas; 4, Chaco Seco; 5, Chaco Húmedo; 6, Selva Paranaense; 7, Campos y Malezales; 8, Espinal; 9, Delta e Islas del Paraná; 10, Pampa (see Figure 1). The second one corresponds to the scheme of the wetlands of the Chaco Seco eco-region presented by Ginzburg et al. (2005) (see Figure 2). This scheme was incorporated in the analysis because allows a more accurate description of the ecological preferences of *A. sculptum* within the Chaco Seco eco-region. The Q-GIS 2.18.5 program (Geographic Information System for Data Analysis Species Distribution, http://www.qgis.org/es/site/) was used in both cases. The QGIS is a cross-platform free and open-source desktop geographic information system (GIS) was queried for georeferenced data (i.e. those with latitude/longitude coordinates) and allows to created vectors layer that in our case would be biogeographic regions of Argentina (biogeographic schemes) and then were plotted georeferenced occurrent data in those.

2.2. Seasonal dynamics.

The field study to determine the seasonality of *A. sculptum* was carried out in the private reserve “Eco- Portal de Piedra”, located in Villamonte (24°05'39.6"S 64°23'54.0"W), Jujuy Province, Argentina (REPP). This site belongs to the YBP, which is characterized by a subtropical climate with annual rainfalls concentrated from November to March (late spring to late summer).

Questing ticks (adults and immature stages) were monthly collected from October 2013 to October 2015 by using a 1x1.50 m white cloth flag. Dragging was performed for a period of 30 minutes by two operators, checking and removing ticks from drags every 10 minutes. Ticks were determined following Martins et al. (2014), Nava et al. (2014) and by comparison with known laboratory reared material deposited in the tick collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina. Temperature (°C) and relative humidity were measured during the entire study period by using HOBO data loggers (U23-002 Pro v2), and they were used to calculate saturation deficit according to the formula presented by Randolph and Storey (1999).

2.3 Genetic diversity and phylogeography.

Genetic variation of *A. sculptum* was assessed using sequences of the mitochondrial 16s rRNA gene. DNA was extracted from adult specimens belonging to populations from 14 geographic locations (Table 2), which are representative of the distribution of this tick species in Argentina. DNA extraction and polymerase chain reaction (PCR) were made following the methodology described by Mangold et al. (1998a, b) in order to obtain a ca. 410-bp fragment of the mitochondrial 16S rRNA gene. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual editing whenever necessary, and aligned with the program ClustalW (Thompson et al., 1994). The group of sequences obtained from ticks collected in the same geographic location was considered as belonging to the same population. A haplotype network was constructed with the program Network 5.0 (Bandelt et al., 1999) to obtain a graphical representation of intraspecific genealogical relationships. Molecular genetic variability explained by different hierarchical levels of organization (within populations and between populations) was estimated by the analysis of molecular variance (AMOVA) based on 1000 permutations and the fixation rate (Fst). This rate determines the genetic differentiations between pairs of populations, whose values significantly different from 0 indicate low gene flow or interruption of the same as it approaches to 1. These procedures were executed with the program Arlequin 3.11 (Excoffier et al., 2005), and the statistical significance of the indices was determined by the non-parametric methods described by Excoffier et al. (1992).

Phylogenetic analyses to determine the relationships among haplotypes of the 16S rDNA gene were performed using the maximum likelihood (ML) method with the program Mega 5 (Tamura et al., 2011). To construct the ML tree, the best fitting substitution model was determined with the Akaike Information Criterion using the ML model test implemented in MEGA 5 (Tamura et al. 2011). Support for the topologies was tested by bootstrapping over 1000 replications. Sequences of *Amblyomma americanum* (Linnaeus, 1758), *A. tonelliae* and *A. mixtum* (GenBank accession nos. L34314, KF179349, KM519935) were chosen as outgroups. The nucleotide diversity (π) [defined as the average proportion of nucleotide differences between all possible pairs of sequences in the sample (Hartl and Clark, 1997)], number of haplotypes, haplotype diversity (h) [defined as the probability that two random sequences are different (Rozas, 2009)] and average number of nucleotide differences were calculated using DnaSP Version 5.10 (Librado and Rozas, 2009). Haplotypes were considered distinct when differing by at least one base pair. The most frequent and most connected haplotype was regarded as being ancestral following the criteria explained by Posada and Crandall (2001).

3. Results

3.1. Geographic range size and host.

Specimens of *A. sculptum* were found in the provinces of Chaco (8 localities), Corrientes (1 locality), Formosa (14 localities), Jujuy (7 localities) and Salta (18 localities). The geographic range size of *A. sculptum* in Argentina is framed in three different areas (Figures 1 and 2): 1) the western distribution, which includes the Selvas Pedemontanas and Selva Montana of the YBP and the transition between the YBP and the Chaco Seco eco-region (see Figure 1) ; 2) the distribution in the “Albardones” forest (riparian forest) along the Bermejo, Teuco and Pilcomayo rivers in the middle north of Chaco Seco eco-region (see Figures 1 and 2); 3) the east distribution, in the Chaco Húmedo eco-region (see Figure 1).

The natural wild hosts for adults and immature stages of *A. sculptum* recorded for Argentina in this work are mammals belonging to the families Cervidae, Myrmecophagidae, Tayassuidae, Canidae, Caviidae, Tapiridae and Chinchillidae (Table 1). In addition, adults and immature of *A. sculptum* were recorded parasitizing domestic mammals as cattle, horses, dogs, domestic pigs and mules, and also humans (Table 1).

3.2. Seasonal dynamics.

Questing larvae of *A. sculptum* were detected on vegetation from March (late summer) to September (late winter) during the first year and from April (mid-autumn) to August (mid-winter) in the second year (Figure 3A). The peak of larval abundance was observed at July (early winter) in both years. Questing nymphs were detected from June to January, with the peak of abundance at October (mid spring) in both years (Figure 3B). Finally, questing adults were detected on vegetation from August to April during the two-year sampling period, with the peaks of abundance in December (early summer) and February (mid-summer) in the first and second year of sampling, respectively (Figure 3C).

The records of weekly mean temperature and saturation deficit during the study period are showed in Figure 3 in relation to the seasonal variation in the number of *A. sculptum* specimen of each parasitic stage.

3.3. Genetic diversity and phylogeography.

A total of 41 sequences of the mitochondrial 16S rRNA gene of *A. sculptum* ticks collected in 14 localities of Argentina and four sequences (obtained from GenBank) of *A. sculptum* from Brazil (Chapada dos Guimarães, Pirizal and Poconè state Mato Grosso (GenBank accession nos. KT722808, KT238826 and KJ557134) and Perdizes, Minas Gerais state (GenBank accession no. KP686061) were analysed (Table 2). Five haplotypes were found: haplotype I, Haplotype II, Haplotype III, Haplotype IV, Haplotype V (GenBank accession nos. MG460313, MG460314, MG460315, MG460316 and KT722808, respectively) (Figure 4). As expected, phylogenetically they are grouped in the same monophyletic group (Figure 5). The topology of the haplotype network (Figure 4) and its relationship with the geographic distribution of the populations shown the following pattern: 1) an ancestral haplotype (haplotype I) with high frequency (present in eleven populations from Argentina and in two from Brazil) that gives rise to haplotypes II, III and IV; 2) haplotype II is present in three populations from Argentina; 3) haplotype III is present in two populations from one population of Argentina and one from Brazil; 4) haplotype IV was obtained from a single individual from Argentina; 5) haplotype V belonging to individuals from one populations of Brazil.

The genetic divergence among the 16S sequences of *A. sculptum* from Argentina was low, because it never exceeded 0.5%. The values of h and π were 0.386 and 0.00117, respectively. The mean frequency of each nucleotide base was 2.44% for cytosine, 0.61% for thymine, 49.39% for adenine and 47.56% for guanine. The mean number of nucleotide differences for all sequences was 0.45253. The 65.21% of the total genetic variability was attributable to differences among populations and the 34.79% of the total variability corresponded to differences within populations. The Fst value was 0.65.

4. Discussion

The results of this study showed that *A. sculptum* has a life cycle with one generation per year. Larvae were active from late summer to late winter with the peak of abundance in early winter, and nymphs were found from early winter to early summer with the peak of abundance in spring. Adults were found on vegetation throughout all year, but the peak of abundance occurred in early and mid-summer. Adult ticks predominate during the rainy season and immature stages do so in the dry season. With this life cycle structure, the females oviposition occurs during rainy seasons, allowing eggs during incubation periods to not be exposed for desiccation stress. Eggs are particularly sensible to high levels of saturation deficit, which has harmful effects during the incubation period of hard tick's eggs (Randolph, 1999; Sutherst and Bourne, 2006). Therefore, this behaviour of *A. sculptum* may prevent oviposition under unfavourable environmental conditions for the development of eggs and survival of larvae.

With some minor variations, the pattern of seasonal dynamics of *A. sculptum* in northern Argentina is similar to those previously described for other species of the *A. cajennense* complex in Argentina (*A. tonelliae*), Brazil (*A. sculptum*) and United States (*A. mixtum*) (Oliveira et al., 2000; Labruna et al., 2002; Oliveira et al., 2003; Beck et al., 2011; Tarragona et al., 2015a), and it is in line with previous studies in Brazil (Aragão 1936) and Argentina (Guglielmone et al., 1981, 1990) dealing with *A. cajennense* s. l. The more remarkable difference is observed regarding *A. mixtum*, which has a prolonged activity of larvae and nymphs throughout the year (Beck et al., 2011). Another noteworthy issue that should be taken into account is the modulation of the life cycle by the development of diapause. Labruna et al. (2003) and Cabrera and Labruna (2009) described a behavioral diapause in the unfed larva of *A. cajennense* s.l. (probably *A. sculptum*) in Brazil during spring and summer, which leads them to become active simultaneously during autumn. Also, Tarragona et al. (2015a) have stated that the life cycle of *A. tonelliae* in northern Argentina is characterized by a long pre-moult period of larvae in autumn under field conditions. Therefore, it is probable that these biological processes that act in the development of the larvae also have relevance to regulate the biological cycle of *A. sculptum* in the north of Argentina.

Geographic range size of *A. sculptum* in Argentina is restricted to the Chaco Húmedo eco-region in the CBP, Selvas Pedemontanas and Selva Montana of YBP and "Albardones" forest of the great rivers in the wetlands of CBP (Figures 1 and 2). The finding of *A. sculptum* in Corrientes province corresponds to the most southern distribution record for this species. This new record was done in patch of forest characteristics of the Chaco Húmedo eco-region, surrounded by marshlands that contain water most of the time (Carnevali, 1994). Two hypotheses could be explaining these results. The first is associated to propagation of ticks in a new territory through movement of domestic animals. At the site where *A. sculptum* was collected, water buffalos were introduced more than ten years ago from the Formosa province, where *A. sculptum* is established (Ivancovich and Luciani, 1992; Sebastian et al., 2017; this work). It is possible that water buffalos translocated from Formosa have carried ticks to Corrientes Province, and *A. sculptum* has found a new system that is ecologically suitable for its development. This situation is similar to that of the cattle tick *Rhipicephalus microplus* in West Africa, where it was recently introduced to a previously tick-free area through movement of cattle from South Africa and Brazil to West Africa (Madder et al., 2011). The second hypothesis is related to an error by omission due to the relatively smaller sampling effort carried out in the suitable ecological site for *A. sculptum* in Corrientes province compared with the rest of the Argentinean regions where this tick is also present. Ivancovich and Luciani (1992) have recorded *A. cajennense* (probably *A. sculptum*) nymphs on the bird *Rhynchotus rufescens* in Corrientes Province (El Sombrero, 27°42'12.9"S 58°46'11.5"W), but one of us (SN) has examined this sample (sample number: 364, data: 1/9/1972) and the nymphs determined as *A. cajennense* by Ivancovich and Luciani (1992) correspond in fact to *Amblyomma tigrinum*.

The *A. sculptum* populations detected in the middle north of Chaco Seco eco-region were found in forests adjacent to large watercourses, known as “Albardones” forests. The “Albardones” forest bordering rivers such as Teuco and Bermejo has peculiar vegetation differing from the typical vegetation of the Chaco Seco eco-region. According to Biani et al. (2006), the Bermejo river would be the main recruitment route for the jungle species, since this river transports seeds, fruits and young plants from the Humid subtropical montane of the YBP, located in the upper part of the Bermejo Basin. Thus, the vegetation of the “Albardones” is more similarity with the vegetation of the Selvas Pedemontanas and Selva Montana than with the rest of the Chaco Seco forest. The Selvas Pedemontanas, Selva Montana, “Albardones” forests and Chaco Húmedo eco-region share microclimatic characteristics that allow the ingression of *A. sculptum* populations within areas of the Chaco Seco eco-region, which observed at macro-scale, appear to be unsuitable for this tick species. This study has not been considered the deforestation process of Selvas Pedemontanas and Chaco forest. This phenomenon, principally associated to agricultural expansion, is a significant process of environmental modification in the north of Argentina (Brown et al., 2009; Gasparri and Grau, 2009). Therefore, retrospective longitudinal studies comparing the occurrence of ticks and annual satellite images of past decades are necessary to elucidate whether the anthropic modification in some biogeographical areas could be responsible for the modifications of geographic distribution range of *A. sculptum* and others species from the *A. cajennense* complex as *A. tonelliae*.

The host records reported in this study and in previous works (Nava et al., 2014; Martins et al., 2016) reinforce the conclusion of Nava and Guglielmone (2013) that *A. sculptum* (named as *A. cajennense*), is a generalist tick with low host specificity. In addition, *A. sculptum* has demonstrated ability to adapt to recently introduced host species such as cattle and horses, which were introduced in the Americas not before 400 years ago. This statement is strengthened by the fact that the species of the *A. cajennense* complex were already differentiated in South America about 5 million years ago (Beati et al., 2013), when most of the current wild and domestic hosts of *A. sculptum* were not present in South America.

The analysis of the 16S sequences have shown that the genetic diversity of this species is low compared to the intra-specific genetic diversity of 16S sequences from other *Amblyomma* species such as *A. americanum* from the United States (Mixson et al., 2006; Trout et al., 2010) and *A. parvum* from Argentina and Brazil (Nava et al., 2008) but similar to the genetic diversity of *A. tonelliae* from Argentina (Tarragona et al., 2015b). The intra-specific differences in the 16S sequences were lesser than 0.5%, and low nucleotide diversity and relatively high haplotype diversity were found. Two hypotheses could explain these results. The first is based on the possibility of an association with a rapid population expansion after a bottleneck. The expansion of a population increases the retention of new mutations (Watterson, 1984) and is characterized by a common haplotype to most individuals that coexists with other very low frequency haplotypes derived from it and which differ only in one or a few mutations (Slatkin and Hudson, 1991, Rogers and Harpending, 1992). In the particular case of the South American populations of the *A. cajennense* species complex, this expansion may have occurred between late Miocene to late Pliocene (11-3 million years) (Beati et al. 2013). This rapid population expansion from the refuge areas may also have resulted in secondary contact between *A. sculptum* and its related species (*A. tonelliae)* in areas ecologically suitable for both species (Beati et al., 2013). The second hypothesis refers to the fact that low diversity could be associated with the sampling size using in this study. Sjogren and Wyoni (1994) suggest that a limited sample size may generate the risk of finding "monomorphisms" in true polymorphic populations, omitting the real genetic diversity of the species.

The AMOVA analysis and Fst values indicate that *A. sculptum* has a population genetic structure where the 65.21% of the total genetic variability is related to differences among populations. In Argentina, all parasitic stages of *A. sculptum* are mostly associated to large wild and domestic mammals with a high vagility in an area without natural geographical barriers. Some authors have suggested that cattle parasites are generally characterized by low genetic differentiation between populations, because host movement allows a high gene flow (Hilburn and Sattler, 1986a, Blouin et al., 1995, Rosenthal, 2008). This situation has been described for other *Amblyomma* species that use large mammals as hosts for all parasitic stages such as *A. americanum* (Hilburn and Sattler, 1986b), *Amblyomma neumanni* Ribaga, 1902 (Nava et al., 2009) and *Amblyomma variegatum* (Beati et al., 2012). Thus, the degree of genetic structure detected in this work for *A. sculptum* is not associated to low host vagility. Similar genetic variability was reported by Bitencourth et al. (2017) in *A. sculptum* populations of the Cerrado biogeographic provinces sensu Morrone (2006) of Brazil. Beati et al. (2013) suggest that host availability is not a limiting factor for the dispersion of the species belonging to the *A. cajennense* complex. Thus, the population genetic structure could be associated to a stochastics process linked to micro-habitat variations that could produce a partial restriction to gene flow, but further studies using molecular markers with higher mutation rates, as for example microsatellites, are necessary to confirm this subject. The hypothesis that the genetic structure of *A. sculptum* in Argentina is a result of the habitat fragmentation due to anthropogenic factors such as changes in land use in the area of distribution of this species in northern Argentina (Gasparri and Grau, 2009; Malizia et al., 2012) was discarded because it is not sufficient on the time scale to produce gene flow disruption taking into account the estimation of the 16S mitochondrial gene mutation rate for *Amblyomma* ticks (Mixon et al., 2006).

Amblyomma sculptum is a usual parasite of domestic mammals as cattle and horses, and it is the principal vector of *R. rickettsii* in South America (Labruna et al., 2014). Cases of human spotted fever by *R. rickettsii* in Argentina (Ripoll et al., 1999; Paddock et al., 2008; Seijo et al., 2016) have been reported in areas within the distributional range of *A. sculptum*. It is expectable that the issues described in this work could be useful to understand epidemiological aspects related to the deleterious effects of *A. sculptum* on the hosts and about the dynamics of the tick-borne pathogens transmitted by this tick.

Acknowledgements

We acknowledge the financial support of INTA-EEA Rafaela, Asociación Cooperadora INTA Rafaela, Agencia Nacional de Promoción Científica y Tecnológica (PICT 2011-1298 and PICT 2013-526). We thank Oscar Warnke, Mario Wuattier, José M. Venzal and Carlos Cuñado Strelkov for help during field work. Ticks collected from Parque Nacional Chaco, Parque Nacional El Impenetrable, Parque Nacional Pilcomayo and Parque Nacional El Rey were performed in accordance to permission given by Administración de Parques Nacionales. We thank the logistic support of Silvia Strelkov and Carlos Cuñado from Eco-Portal de Piedra Reserva Natural Privada, Joaquin Tillous from Estancia El Bagual and Alejandro DiGiacomo from Reserva Natural Privada El Bagual. Ticks collected from Parque Provincial Pampa del Indio was performed in accordance to permission given by Chaco Province.

Reference

Alves, A.S., Melo, A.L.T., Amorin, M.V., Borges, A.M.C.M., Silva, L.G.E., Martins, T.F., Labruna, M.B., Aguiar, D.M., Pacheco, R.M., 2014. Seroprevalence of *Rickettsia* spp. in equids and molecular detection of '*Candidatus* Rickettsia amblyommii' in *Amblyomma cajennense* sensu lato ticks from the Pantanal Region of Mato Grosso, Brazil. J. Med. Entomol. 41, 1252-1257.

Aragão, H.B., 1936. Ixodidas brasileiros e de algunos paízes limitrophes. Mem. Inst. Oswaldo Cruz. 31, 759-843.

Bandelt, H. J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37-48.

Beati, L., Patel, J., Lucas-Williams, H., Adakal, H., Kanduma, E.G., Tembo-Mwase, E., Krecek, R., Mertins, J.W., Alfred, J.T., Kelly, S., Kelly, P., 2012. Phylogeographyand demographic history of *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae), the tropical bont tick. Vector Borne Zoonotic Dis. 12, 514–525.

Beati, L., Nava, S., Burkman, E.J., Barros-Battesti, D., Labruna, M.B., Guglielmone, A.A., Cáceres, A.G., Guzman Cornejo, C., Léon, R., Durden, L.A., Faccini, J.L.H., 2013. *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation. BMC Evol. Biol. 13: 267.

Beck, D.L., Zavala, J., Montalvo, E.O., Quintana, F.G., 2011. Metereological indicators for *Amblyomma cajennense* and populations dynamics in the Tamaulipan biotic province in Texas. J. Vector Ecol. 36, 135–146.

Biani, N.B., Vesprini, J.L., Prado, D.E., 2006. Conocimiento sobre el gran Chaco Argentino en el siglo XX. Ecología y Manejo de los bosques de Argentina, pp. 19.

Bitencourth, K., Amorim, M., De Oliveira, S.V., Caetano, R.L., Voloch, C.M., Gazêta, G.S., 2017. *Amblyomma sculptum*: genetic diversity and rickettsias in the Brazilian Cerrado biome. Med. Vet. Entomol. doi: 10.1111/mve.12249

Blouin, M.S., Yowell, C.A., Courtney, C.H., Dame, J.B., 1995. Host movement and the genetic structure of populations of parasitic nematodes. Genetics. 141, 1007–1014.

Brown, A.D., Grau, H.R., Malizia, L.R., Grau, A., 2001. Argentina. In: Kappelle, M., Brown, A.D. (Eds.), Bosques nublados del Neotrópico. INBIO, Santo Domingo de Heredia, pp 623-658.

Brown, A.D., Blendinger, P.G., Lomáscolo, T., García Bes, P., 2009. Selva Pedemontana de Las Yungas, Historia natural, ecología y manejo de un ecosistema en peligro. Ediciones del Subtrópico. Tucuman, pp. 487.

Burkart, R., Bárbaro, O., Sánchez, R.O., Gómez, D.A., 1999. Eco-Regiones de la Argentina. Administración de Parques Nacionales, Secretaría de Recursos Naturales y Desarrollo Sustentables, Buenos Aires, pp. 43.

Cabrera, R.R., Labruna, M.B., 2009. Influence of photoperiod and temperature on the larval behavioral diapause of *Amblyomma cajennense* (Acari: Ixodidae). J. Med. Entomol. 46, 1303-1309.

Carnevali, R., 1994. Fitogeografía de la provincia de Corrientes: cartas, escalas 1: 500.000 y 1: 1.000. 000. Instituto Nacional de Tecnología Agropecuaria, Corrientes, pp. 324.

Estrada Peña, A., Tarragona, E.L, Vesco, U., De Meneghi, D., Mastropaolo, M., Mangold, A.J., Guglielmone, A.A., Nava, S., 2014. Divergent environmental preferences and areas of sympatry of tick species in the *Amblyomma cajennense* complex (Ixodidae). Int. J. Parasitol. 44, 1081-1089.

Excoffier, L., Smouse, P., Quattro, J., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics. 131, 479-491.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online. 1, 47-50.

Gasparri, N.I., Grau, H.R., 2009. Deforestation and fragmentation of Chaco dry forest in NW Argentina (1972–2007). Forest Ecol. Manag. 258, 913–921.

Ginzburg, R., Adámoli, J., Herrera, P., Torrella, S., 2005. Los Humedales del Chaco: clasificación, inventario y mapeo a escala regional. Insugeo, miscelánea, San Miguel de Tucumán, pp 138.

Guedes, E., Leite, R.C., Prata, M.C.A., Pacheco, R.C., Walker, D.H., Labruna, M.B., 2005. Detection of *Rickettsia rickettsii* in the tick *Amblyomma cajennense* in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem. Inst. Oswaldo Cruz. 100, 841–845.

Guglielmone, A.A., Hadani, A., Mangold, A.J., De Haan, L., Bermudez, A., 1981. Garrapatas (Ixodoidea-Ixodidae) del Ganado bovino en la provincia de Salta: especies y carga en 5 zonas ecológicas. Rev. Med. Vet. 62, 194-205.

Guglielmone, A.A., Mangold, A.J., Aguirre, D.H., Gaido, A.B., 1990. Ecological aspects of four species of ticks found on cattle, in Salta, Northwest Argentina. Vet. Parasitol. 35, 93-101.

Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.

Hartl, D.L., Clark, A.G., 1997. Principles of population genetics. Sinauer Associates, Sunderland, pp. 542.

Hilburn, L.R., Sattler, P.W., 1986a. Are tick populations really less variable and should they be? Heredity. 57, 113–117.

Hilburn, L.R., Sattler, P.W., 1986b. Electrophoretically detectable protein variation in natural populations of the lone star tick, *Amblyomma americanum* (Acari: Ixodidae). Heredity. 56, 67–74.

Ivancovich, J.C., Luciani, C.A., 1992. Las garrapatas de Argentina. Monografía. Mimeo., 95 pp.

Labruna, M.B., Kasai, N., Ferreira, F., Faccini, J.L., Gennari, S.M., 2002. Seasonal dynamics of ticks (Acari: Ixodidae) on horses in the state of São Paulo, Brazil. Vet. Parasitol. 105, 65-77.

Labruna, M.B., Amaku, M., Metzner, J.A., Pinter, A., Ferreira, F., 2003. Larval behavioral diapause regulates life cycle of *Amblyomma cajennense* (Acari: Ixodidae) in southeast Brazil. J. Med. Entomol. 40, 170–178.

Labruna, M.B., Santos, F.C.P., Ogrezewalska, M., Nascimento, E.M.M., Colombo, S., Marcili, A., Angerami, R.N., 2014. Genetic identification of rickettsial isoaltes from fatal cases of Brazilian spotted fever and comparison with *Rickettsia ricktettsii* isolates from the American continents. J. Clin. Microbiol. 52, 3788-3791.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25,1451–1452.

Madder, M., Thys, E., Achi, L., Touré, A., De Deken, R., 2011. *Rhipicephalus* (*Boophilus*) microplus: a most successful invasive tick species in West-Africa. Exp. Appl. Acarol. 53, 139-145.

Malizia, L., Pacheco, S., Blundo, C., Brown, A.D., 2012. Caracterización altitudinal, uso y conservación de las Yungas Subtropicales de Argentina. Ecosistemas 21, 53-73.

Mangold, A.J., Bargués, M.D. & Mas-Coma, S., 1998a. 18S rRNA gene sequence and phylogenetic relationships of European hard-tick species (Acari: Ixodidae). Parasitol. Res. 84, 31–37.

Mangold, A.J., Bargués, M.D., Mas-Coma, S., 1998b. Mitochondrial 16S rRNAsequences and phylogenetic relationships of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). Parasitol. Res. 84, 478–484.

Martins, T.F., Labruna, M.B., Mangold, A.J., Cafrune, M. M, Guglielmone, A.A., Nava, S., 2014. Taxonomic key to nymphs of the genus *Amblyomma* (Acari: Ixodidae) in Argentina, with description and redescription of the nymphal stage of four *Amblyomma* species. Ticks Tick-borne Dis. 5, 753-770.

Martins, T.F., Barbieri, A.R., Costa, F.B., Terassini, F.A., Camargo, L.M., Peterka, C.R., Scofield, A., Artur, K. C., Horta, M.C., Guilloux, A.G.A., Benatti, H.R., Ramírez, D.G., Barros-Battesti, D.M., Labruna, M.B., 2016. Geographical distribution of *Amblyomma cajennense* (sensu lato) ticks (Parasitiformes: Ixodidae) in Brazil, with description of the nymph of *A. cajennense* (sensu stricto). Parasit. Vectors. 9, 186.

Melo, A. L. T., Witter, R., Martins, T. F., Pacheco, T. A., Alves, A. S., Chitarra, C. S., V. Dutra, S.V., Nakazato, L., Pacheco, R.C., Labruna, M.B., Aguiar, D. M., 2016. A survey of tick-borne pathogens in dogs and their ticks in the Pantanal biome, Brazil. Med. Vet. Entomol. 30, 112-116.

Mixson, T.R., Lydy, S.L., Dasch, G.A., Real, L.A., 2006. Inferring the population structure and demographic history of the tick, *Amblyomma americanum* Linnaeus. J. Vector Ecol. 31, 181–192.

Morrone, J.J., 2006. Biogeographic areas and transition zones of Latin American and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. Ann. Rev. Entomol. 51, 467–494.

Nava, S., Guglielmone, A.A., 2013. A meta-analysis of host specificity in Neotropical hard ticks (Acari: Ixodidae). Bull. Entomol. Res. 103, 216-224.

Nava, S., Szabó, M.P.J, Mangold, A.J, Guglielmone, A.A., 2008. Distribution, hosts, 16S rDNA sequences and phylogenetic position of the Neotropical tick *Amblyomma parvum* (Acari: Ixodidae). Ann. Trop. Med. Parasitol. 102, 409-425.

Nava, S., Estrada-Peña, A., Mangold, A.J., Guglielmone, A.A., 2009. Ecology of *Amblyomma neumanni* (Acari: Ixodidae). Act. Trop. 111, 226-236

Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch, 1844 and *Amblyomma sculptum* Berlese, 1888 (Ixodida: Ixodidae). Ticks Tick-borne Dis. 5, 252–276.

Nava, S., Venzal, J.M., González-Acuña, D., Martins, T.F., Guglielmone, A.A., 2017. Ticks of the Southern Cone of America: Diagnosis, Distribution and Hosts with Taxonomy, Ecology and Sanitary Importance. Elsevier Academic Press, London, 352 pp.

Nunes, E.D.C., Vizzoni, V.F., Navarro, D.L., Iani, F.C.D.M., Durães, L.S., Daemon, E., Gazeta, G.S., 2015. *Rickettsia amblyommii* infecting *Amblyomma sculptum* in endemic spotted fever area from southeastern Brazil. Mem. Inst. Oswaldo Cruz. 110, 1058-1061.

Oliveira, P.R., Borges, L.M.F., Lopes, C.M.L., Leite, R.C., 2000. Population dynamics of the free-living stages of *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae) on pastures of Pedro Leopoldo, Minas Gerais State, Brazil. Vet. Parasitol. 92, 295–301.

Oliveira, P.R., Borges, L.M.F., Leite, R.C., Freitas, C.M.V., 2003. Seasonal dynamics of the Cayenne tick, *Amblyomma cajennense* on horses in Brazil. Med. Vet. Entomol. 17, 412–416.

Ortiz, F., Nava, S., Guglielmone, A.A. 2011., Análisis de una colección de garrapatas (Acari: Argasidae, Ixodidae) del norte Argentino. Rev. FAVE. 10, 49-55.

Paddock, C.D., Fernández, S., Echenique, G.A., Summer, J.W., Reeves, W.K., Zaki, S.R., Remondegui, C.E., 2008. Rocky Mountain spotted fever in Argentina. Am. J. Trop. Med. Hyg. 78, 687–692.

Posada, D., Crandall, K.A., 2001. Intraspecific gene genealogies: trees grafting into networks. Trend. Ecol. Evol. 16, 37-45.

Randolph, S.E., Storey, K., 1999. Impact of microclimate on immature tick rodent host interactions (Acari: Ixodidae): implications for parasite transmission. J. Med. Entomol. 36, 741–748.

Ripoll CM, Remondegui CE, Ordoñez G, Arazamendi R, Fusaro H, Hyman MJ, Paddock, C.D., Zaki, S.R., Olson, J.G., Santos-Buch, C.A., 1999. Evidence of Rickettsial spotted fever and ehrlichial infections in a subtropical territory of Jujuy, Argentina. Am. J. Trop. Med. Hyg. 61, 350–354.

Rogers, A.R., Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. J. Mol. Evol. 9, 552-569.

Rosenthal, B.M., 2008. How has agriculture influenced the geography and genetics of animal parasites? Trend. Parasitol. 25, 67–70.

Rozas, J., 2009. DNA sequences polymorphism analysis using DnaSP. In: Posada D. (Ed.), Bioinformatics for DNA Sequence Analysis, Humana Press Springer, New York, pp. 337–350.

Sebastian, P.S., Tarragona, E.L., Saracho Bottero, M.N., Mangold, A.J., Mackenstedt, U., Nava, S., 2017. Bacteria of the genera *Ehrlichia* and *Rickettsia* in ticks of the family Ixodidae with medical importance in Argentina. Exp. Appl. Acarol. 71, 87-96.

Seijo, A., Giamperetti, S., Ortiz-Mayor, S.M., González, M.B., Ortega, E.S., González, R.C., 2016. Fiebre manchada grave por *Rickettsia rickettsii* en turista en el noroeste argentino. Medicina (Buenos Aires). 76, 317-320.

Sjogren, P., Wyoni, P.I., 1994. Conservation genetics and detection of rare alleles in finite populations. Conserv. Biol. 8, 267–270.

Slatkin, M., Hudson, R.R., 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics. 123, 603-613.

Soares, J.F., Soares, H.S., Barbieri, A.M., Cabruna, M.B., 2012. Experimental infection of the tick *Amblyomma cajennense*, Cayenne tick, with *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever. Med. Vet. Entomol. 26, 139-151.

Sonenshine, D.E., Mather, T.N., 1994. Ecological dynamics of tick-borne zoonoses. Oxford University Press. Demand, pp. 441.

Sutherst, R.W., Bourne, A.S., 2006. The effect of desiccation and low temperature on the viability of eggs and emerging larvae of the tick, *Rhipicephalus* (Boophilus) *microplus* (Canestrini) (Ixodidae). Int. J. Parasitol. 36, 193-200.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. Mega5: molecular evolutionary genetics analysis using máximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Tarragona, E.L., Cicuttin, G.L., Mangold, A.J., Mastropaolo, M., De Salvo, N., Nava, S., 2015a. *Rickettsia* infection in *Amblyomma tonelliae*, a tick species from the *Amblyomma cajennense* complex. Ticks Tick-Borne Dis. 6, 173–177.

Tarragona, E.L., Mangold, A.J., Mastropaolo, M., Guglielmone, A.A., Nava, S. 2015b., Ecology and genetic variation of *Amblyomma tonelliae* in Argentina. Med. Vet. Entomol. 29, 297-304.

Tarragona, E.L., Soares, J.F., Costa, F.B., Labruna, M.B., Nava, S., 2016. Vectorial competence of *Amblyomma tonelliae* to transmit *Rickettsia rickettsii.* Med. Vet. Entomol. 30, 410–415.

Thompson, J.D., Higgins, D., Gibson, T.J., 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequenceweighting, position-specific gap penalties andweight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Trout, R.T., Steelman, C.D., Szalanski, A.L., 2010. Population genetics of *Amblyomma americanum* (Acari: Ixodidae) collected from Arkansas. J. Med. Entomol. 47, 152–161.

Watterson, G.A., 1984. Allele frequencies after a bottleneck. Theo. Popul. Biol. 26, 387-407.

Widmer, C.E., Azevedo, F.C.C., Almeida, A.P., Ferreira, F., Labruna, M.B., 2011. Tick-borne bacteria in free-living jaguars (*Panthera onca*) in Pantanal, Brazil. Vector borne Zoonotic Dis. 11, 1001-1005.

Legends to Figures

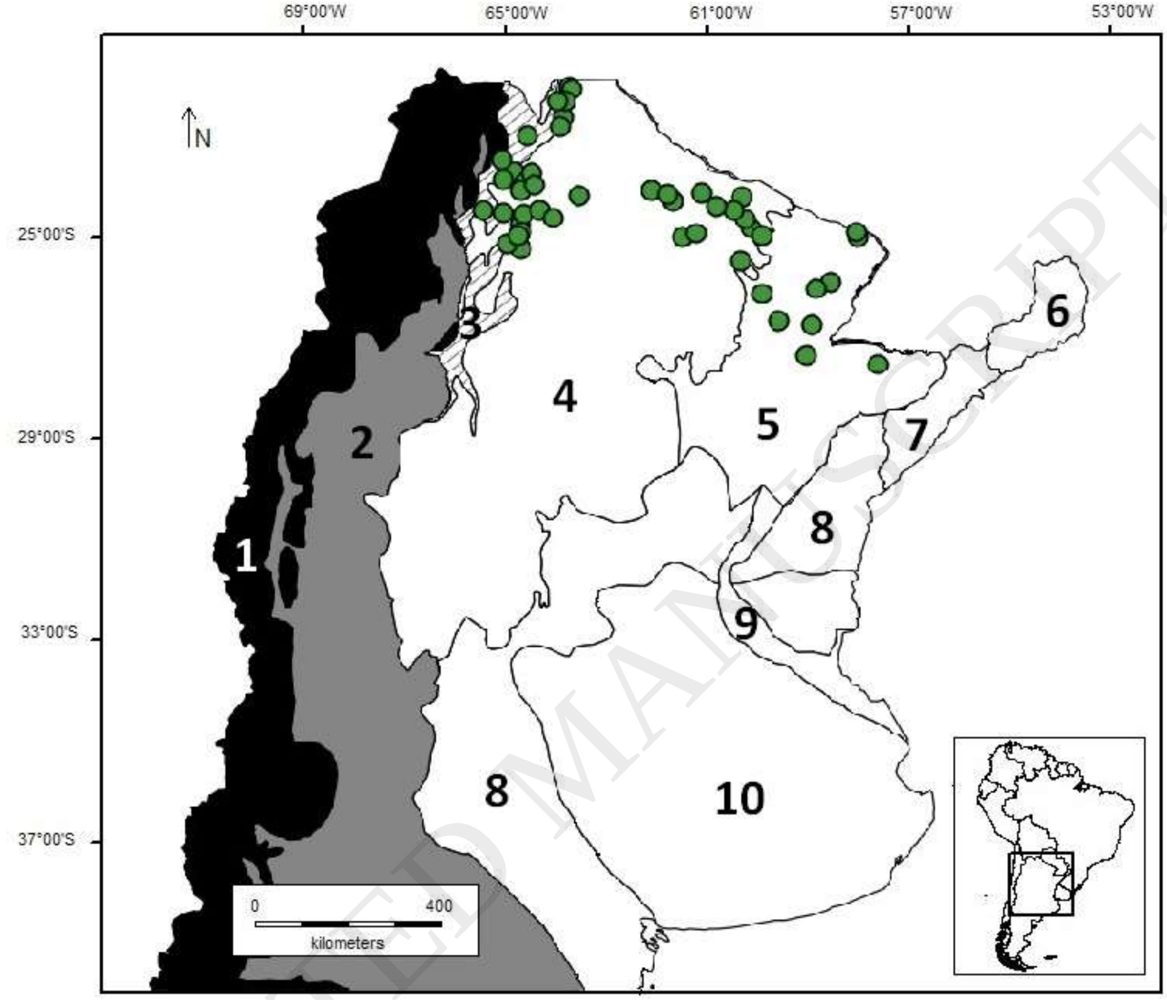

Figure 1. Geographical range of *Amblyomma sculptum* in relation to the combination of the Eco-regions of Argentina by Burkart et al. (1999) and Biogeographic Provinces as defined by Morrone (2006). 1, Altos Andes; 2, Puna; 3, Selva de las Yungas; 4, Chaco Seco; 5, Chaco Húmedo; 6, Selva Paranaense; 7, Campos y Malezales; 8, Espinal; 9, Delta e Islas del Paraná; 10, Pampa. Green points represent locality records of *A. sculptum* in Argentina.

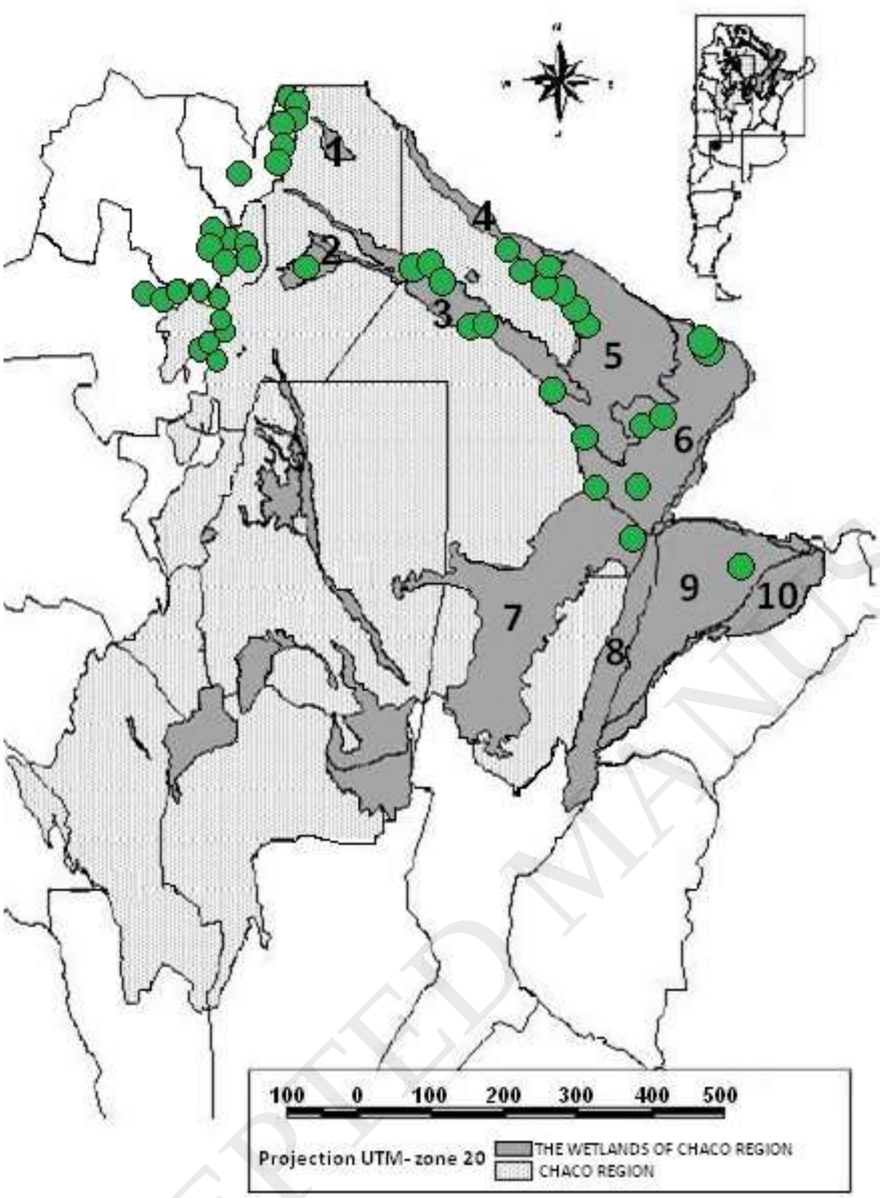

Figure 2. Geographical range of *Amblyomma sculptum* in relation to the Chaco wetlands scheme described by Ginzburg et al. (2005). 1, Derrames del río Itiyuro; 2, Bañados del quirqincho; 3, interfluvio río Teuco-Bermejo; 4, Bañados La Estrella; 5, Bosques cañadas y lagunas; 6, Esteros, cañadas y bosques de albardones; 7, Bajos submeridionales; 8, Planicie de inundación del río Paraná; 9, Bañados y esteros del Iberá; 10, Grandes lagunas del sector oriental del Iberá. Green points represent locality records of *A. sculptum* in Argentina.

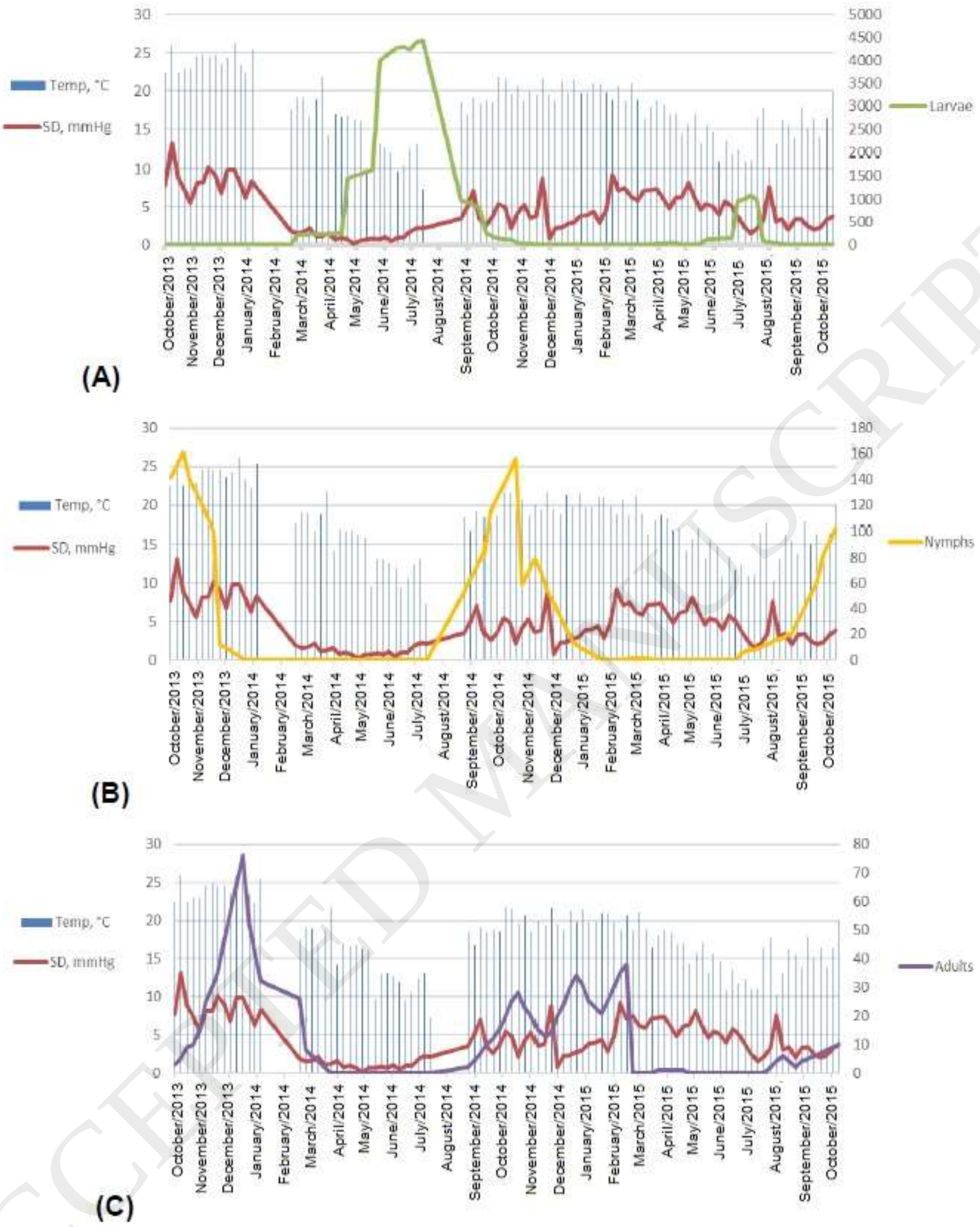

Figure 3. Seasonal distribution of *Amblyomma sculptum* larvae (A), nymphs (B) and adults (C) collected from the vegetation in the Reserva Eco-Portal de Piedra, Jujuy Province, from October 2013 to October 2015. Microclimatic data are also presented (Temp: weekly mean of temperature; SD: weekly mean of saturation deficit).

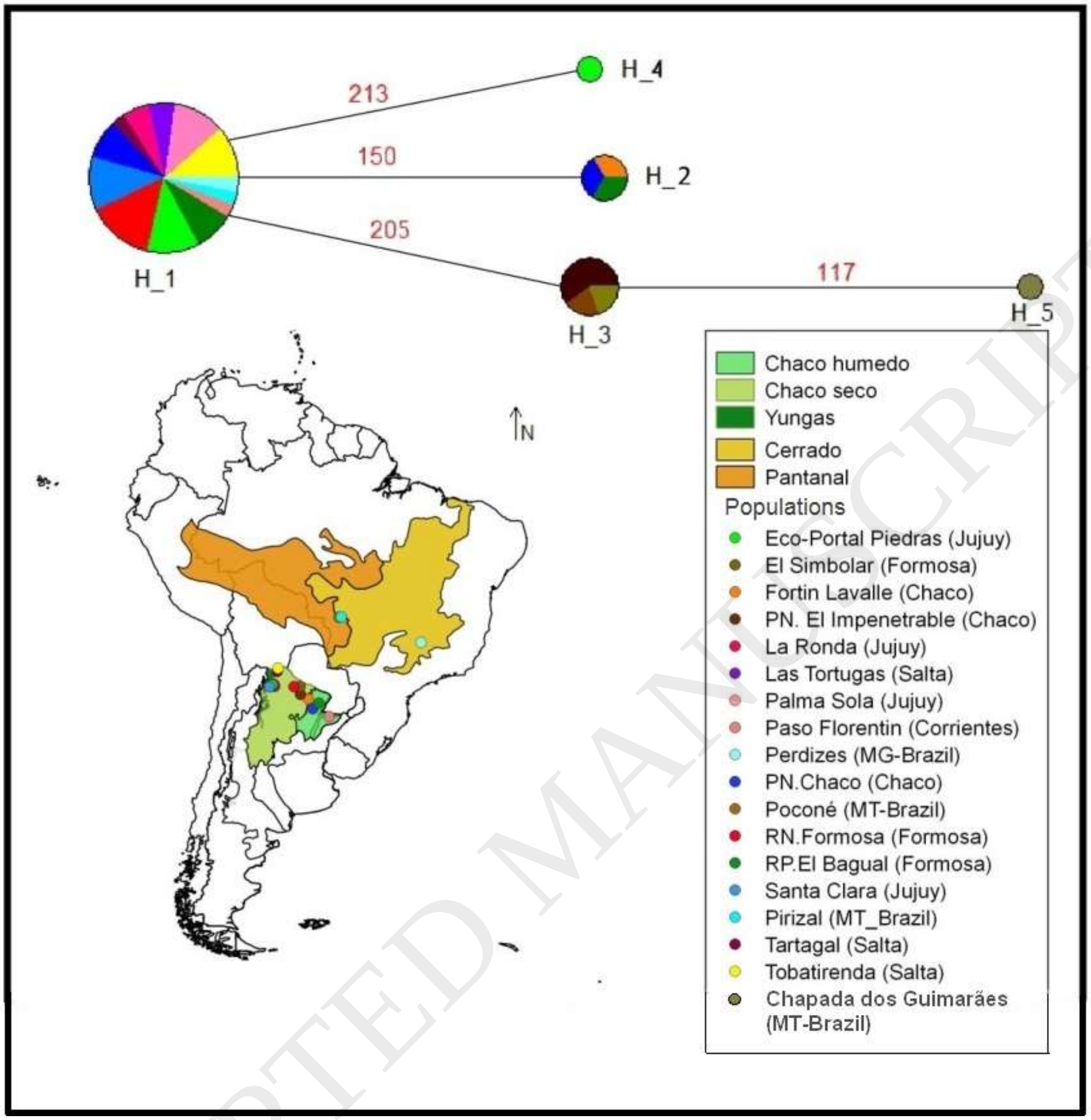

Figure 4. Relationship between the geographical distribution and the genealogy of the five haplotypes of the mitochondrial sequences of the 16S rRNA gene of *Amblyomma sculptum*. The haplotype network reflects the frequency of each haplotype (circle size) and its population composition (each population is represented by a colour). Values in red represent the nucleotide base number where the mutational step occurs. Map is adapted from Eco-regions of Argentina by Burkart et al. (1999) and the Biogeographic Provinces of Latin America and the Caribbean of Morrone (2006).

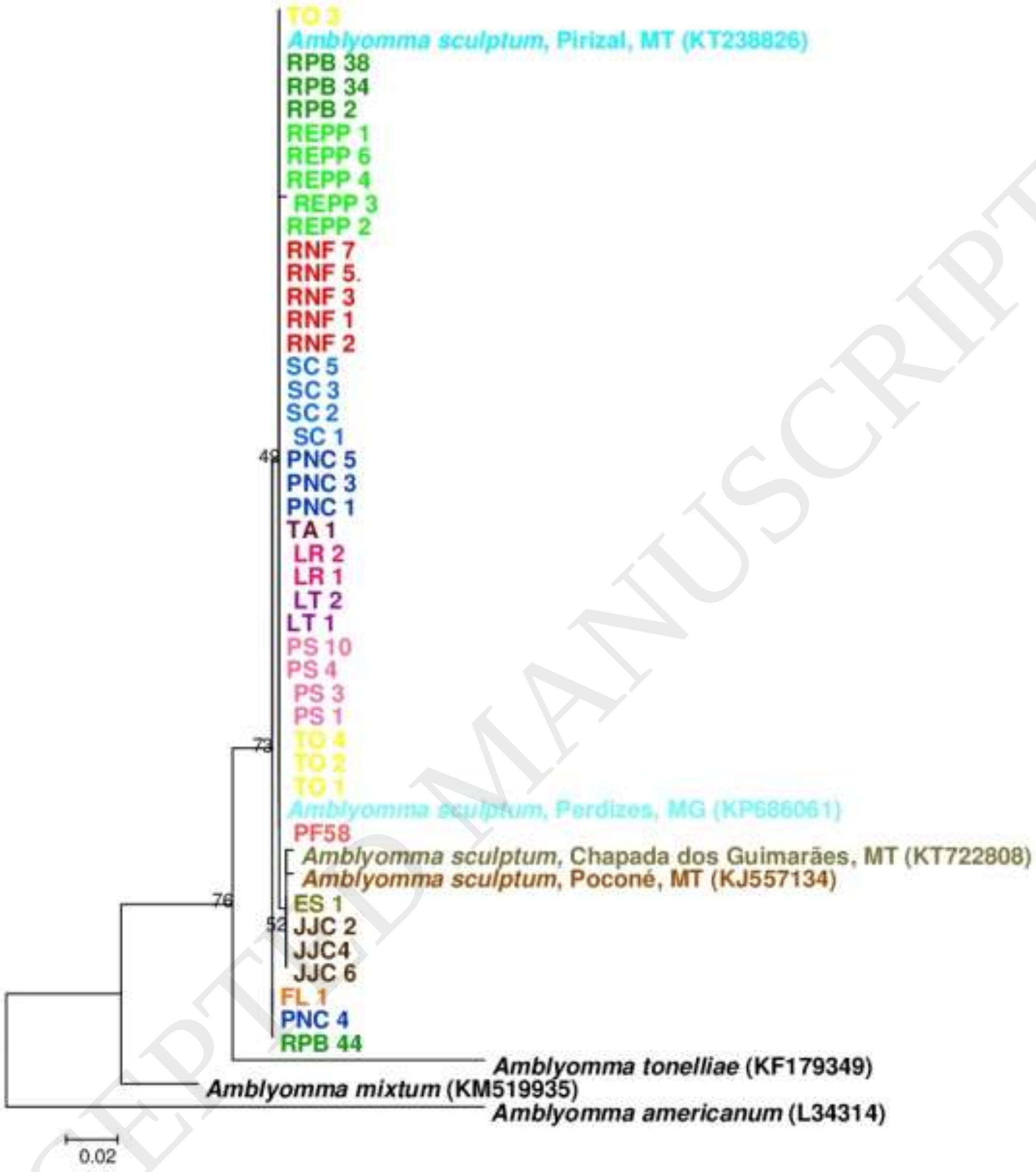

Figure 5: Maximum Likelihood (ML) tree constructed with sequences of the mitochondrial sequences of the 16S rRNA gene of *Amblyomma sculptum*. The numbers represent the bootstrap support generated from 1000 repetitions. The data corresponding to each sequence are presented in Table 2.

Table 1. Localities and hosts of *Amblyomma sculptum* in Argentina

Province	Locality	Coordinates	Altitude (meter)	Host	Stage	References
Chaco	El Sauzalito	24°25'S 61°41'W	170	Horse	M	INTA 2308
	La Eduvigis	26°54'S 59°00'W	73	Human	M	FAVE/CH 00001
	Parque Nacional Chaco	26°49'S 59°39'W	80	Environment	MFN	INTA 2369
	Parque Nacional El Impenetrable	25°10'S 61°03'W	138	Horse	MF	INTA 2307
	Parque Provincial Pampa del Indio	26°16'S 59°58'W	100	Environment	MF	INTA 2362
	Resistencia 10 km S	27°31'S 59°07'W	52	*Myrmecophaga tridactyla*	MF	INTA 2366
	Toba Muerto	25°04'S 61°15'W	143	Horse	M	INTA 2309
	Villa Río Bermejito	25°37'S 60°23'W	116	Horse	MF	INTA 2310
Corrientes	Paso Florentín	27°41'S 57°43'W	70	Environment	F	INTA 2282
	Paso Florentín	27°41'S 57°43'W	70	Human	F	INTA 2356
Formosa	Apeadero Bruchard	25°08'S 59°58'W	117	Horse	M	INTA 2313
	Campo La Elisa	25º10'S 58º06'W	76	*Mazama gouazoubira*	F	INTA 2155
	Campo La Elisa	25º10'S 58º06'W	76	*Tapirus terrestris*	MF	INTA 2158
	El Simbolar	24º16'S 61º08'W	156	Environment	M	This study
	Ingeniero Juárez	24°12'S 62°07'W	186	Domestic Pig	MF	Ortiz et al. 2011
	Las Lomitas, 10 km N	24°37'S 60°31'W	136	Environment	N	This study
	Las Lomitas, 30 km NW	24°31'S 60°52'W	145	Horse	MF	INTA 2314
	Pampa de los Hoyales	24°08'S 60°26'W	144	*Myrmecophaga tridactyla*	M	INTA 2370
	Parque Nacional Río Pilcomayo	25º03'S 58º08'W	77	Environment	MFN	INTA 2359
	Pozo del Tigre	24°58'S 60°10'W	120	Horse	MF	INTA 2311
	Pozo del Tigre, road 95 km 1267	24°47'S 60°17'W	125	Horse	M	This study
	Puesto Campo del Cielo	24°20'S 60°22'W	134	Horse	M	INTA 2312
	Reserva Nacional Formosa	24°17'S 61°48'W	178	Horse	MF	INTA 2265

Province	Locality	Coordinates	Altitude (meter)	Host	Stage	References
	Reserva Nacional Formosa	24°17'S 61°48'W	178	Environment	MF	This study
	Reserva Privada El Bagual	26°10'S 58°56'W	75	Environment	M	This study
	Reserva Privada El Bagual	26°10'S 58°56'W	75	Human	M	INTA 2148
	San Hilario	26º03'S 58º38'W	75	*Hydrochoerus hydrochaeris*	MF	Nava et al. 2014
Salta	Apolinario Saravia, 49 km NE	24°19'S 63°31'W	251	Environment	MF	This study
	Campo Durán	22°12'S 63°39'W	488	Horse	MF	INTA 2317
	Coronel Cornejo	22º45'S 63º51'W	443	Cattle	MF	Nava et al. 2014
	El Galpón	25º24'S 64º39'W	525	Domestic Pig	M	INTA 985
	El Oculto	23º06'S 64º32'W	606	Dog	F	Nava et al. 2014
	Estancia González	24º54'S 64º39'W	1270	Cattle	MF	Nava et al. 2014
	General Ballivián	22°56'S 63°53'W	385	Environment	MF	This study
	La Caldera	24º37'S 65º23'W	1356	Human	MF	Nava et al. 2014
	Laguna del Cielo	22°26'S 63°57'W	657	Environment	M	INTA 1330
	Las Lajitas, 40 km W	25º03'S 64º39'W	779	Horse	MFN	Nava et al. 2014
	Lumbreras, 30 km E	25º07'S 64º43'W	703	*Lagostomus maximus*	N	INTA 1368
	Parque Nacional El Rey	24º41'S 64º36'W	1030	*Tapirus terrestris*	MF	Nava et al. 2014
	Parque Nacional El Rey	24º41'S 64º36'W	1030	Domestic Pig	MF	Nava et al. 2014
	Parque Nacional El Rey	24º41'S 64º36'W	1030	Cattle	MF	Nava et al. 2014
	Parque Nacional El Rey	24º41'S 64º36'W	1030	Human	MFN	Nava et al. 2014
	Parque Nacional El Rey	24º41'S 64º36'W	1030	Horse	MF	Nava et al. 2014
	Parque Nacional El Rey	24º41'S 64º36'W	1030	Mule	MF	Nava et al. 2014
	Parque Nacional El Rey	24º41'S 64º36'W	1030	Environment	MF	INTA 2057
	Parque Nacional El Rey	24º41'S 64º36'W	1030	*Mazama gouazoubira*	N	INTA 2358

Table 1. Continued

Province	Locality	Coordinates	Altitude (meter)	Host	Stage	References
	Pocoy	24º45'S 64º01'W	394	Human	N	INTA 1015
	Río Piedras	25º17'S 64º55'W	699	Cattle	F	INTA 1039
	Santa Rita	24º40'S 64º59'W	667	Horse	MF	Nava et al. 2014
	Santa Rita	24º40'S 64º59'W	667	Mule	MF	INTA 297
	Tartagal, 10 km N	22°26'S 63°48'W	563	Horse	MF	INTA 2316
	Tobantirenda	22°08'S 63°42'W	513	Horse	MF	INTA 2315
	Villa Aurelia	24º36'S 64º17'W	584	Cattle	F	Nava et al. 2014
Jujuy	El Piquete	24°13'S 64°39'W	491	*Cerdocyon thous*	L*	INTA 2258
	Ingenio Ledesma	23º50´S 64º47'W	444	Horse	MF	Nava et al. 2014
	Palma Sola, 26 km W	24°03'S 64°34'W	543	Horse	MF	INTA 2318
	Puesto de La Quinta	23°52'S 64°27'W	589	Environment	N	INTA 1988
	Reserva Privada Eco-Portal de Piedra	24°07'S 64°24'W	1130	Cattle	MFN	INTA 2298
	San Pedro, 60 km NW	24º00'S 64º59'W	807	Human	N	INTA 1361
	Santa Bárbara	23°36'S 65°01'W	1289	*Tapirus terrestris*	MF	Nava et al. 2014
	Santa Bárbara	23°36'S 64°44'W	1289	*Tayassu pecari*	M	INTA 1440
	Santa Bárbara	23°36'S 65°04'W	1289	Human	M	INTA 1441

Table 1. Continued

M=male;F=female;N=nymph;L=larval
INTA=Tick collection of the Instituto Nacional de Tecnología Agropecuaria (Rafaela, Santa Fe, Argentina); FCV-UNL=Ixodidos collection of Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral (Esperanza, Santa Fe, Argentina)
*Collected as engorged larvae that moulted to nymphs in the laboratory.

Table 2. Geographic populations of *Amblyomma sculptum* from Argentina included in the analysis of 16S rDNA sequences in this study and four populations of *A. sculptum* from Brazil. The Brazil sequences were taken from Genbank.

Country/Province	**Locality**	**Latitude**	**Longitude**	**Sequences, *n***	**16S rDNA haplotype code**
Argentina					
Chaco	Fortín Lavalle (FL)	25° 41'S	60° 11'W	1	II
Chaco	Parque Nacional Chaco (PNC)	26° 49'S	59° 39'W	4	I, II
Chaco	Parque Nacional El Impenetrable (JJC)	25° 10'S	61° 03'W	3	III
Corrientes	Paso Florentín (PF)	27° 41'S	57°43'W	1	I
Formosa	El Simbolar (ES)	24° 16'S	61° 08'W	1	III
Formosa	Reserva Nacional Formosa (RNF)	24° 17'S	61° 48'W	5	I
Formosa	Reserva Privada El Bagual (RPB)	26° 10'S	58° 56'W	4	I, II
Jujuy	Palma Sola (PS)	24° 01'S	64° 19'W	4	I
Jujuy	La Ronda (LR)	24° 02'S	64° 08'W	2	I
Jujuy	Reserva Privada Eco-Portal de Piedra (REPP)	24° 07'S	64° 24'W	5	I, IV
Jujuy	Santa Clara (SC)	24° 18'S	64° 39'W	4	I
Salta	Las Tortugas (LT)	24° 14'S	64° 07'W	2	I
Salta	Tartagal (TA)	22° 26'S	63° 48'W	1	I
Salta	Tobantirenda (TO)	22° 08'S	63° 42'W	4	I
Brazil					
Mato Groso	Chapada dos Guimarães (KT722808)	15° 27'S	55° 44'W	1	V
Mato Groso	Pirizal (KT238826)	16° 12'S	56° 12'W	1	I
Mato Groso	Poconè (KJ557134)	16° 15'S	56° 37'W	1	III
Minas Gerais	Perdizes (KP686061)	19° 10'S	47° 06'W	1	I