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Original article

Distribution and genetic variation of *Amblyomma triste* (Acari: Ixodidae) in Argentina

Alberto A. Guglielmone[a,*], Santiago Nava[a], Mariano Mastropaolo[b], Atilio J. Mangold[a]

[a] *Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Consejo Nacional de Investigaciones Científicas y Técnicas CC 22, CP 2300 Rafaela, Santa Fe, Argentina*
[b] *Cátedra de Parasitología y Enfermedades Parasitarias, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Kreder 2805, CP 3080 Esperanza, Santa Fe, Argentina*

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ABSTRACT

A study was conducted to investigate the distribution of *Amblyomma triste* in Argentina under the hypothesis that this tick prevails in riparian localities along the Paraná River and adjacent humid environments from 34°30′ S to 25°20′ S, approximately. Ticks were collected from mammals and vegetation in those environments from November 2008 to October 2012. Additionally, genetic variation was tested from Argentinean, Brazilian, Chilean, and Uruguayan populations of *A. triste* by comparing sequences of 16S rDNA mitochondrial gene. The hypothesis was not confirmed because *A. triste* were collected at 36°16′ S, well beyond the southern limit predicted, and the distribution along the banks of the Paraná River was not continuous. The northernmost population of *A. triste* within Argentina was found at 25°42′ S. Still undetermined abiotic factors and plant communities may play a role in modulating the abundance of *A. triste* because host availability does not appear to be a restriction factor. The genetic variation among *A. triste* populations from Argentina, Brazil, and Uruguay indicates that they belong to a unique taxon that is considered bona fide *A. triste* (type locality Montevideo, Uruguay) while it is unclear if the Chilean population of *A. triste* is conspecific with the other populations investigated in this study. It would be of importance to compare those genetically homogeneous populations with other populations of alleged *A. triste*, especially populations established in the Nearctic Zoogeographic Region in Mexico and USA.

Introduction

Amblyomma triste has an ample distribution in the Neotropical Zoogeographic Region where it was found in Argentina, Brazil, Colombia, Ecuador, Paraguay, Peru, Uruguay, and Venezuela (Guglielmone et al., 2003). The range of *A. triste* includes now Nearctic localities in Mexico and the USA (Guzmán Cornejo et al., 2006; Mertins et al., 2010), while its distribution in the neotropics has been expanded into northern Chile (Abarca et al., 2012). Nevertheless, the diagnoses of this tick species is difficult, and it has been historically confused with *Amblyomma maculatum* and *Amblyomma tigrinum* (Estrada-Peña et al., 2005), and the morphological separation between *A. triste* and *A. maculatum* is especially complex even for adult ticks (Mendoza Uribe and Chávez Chorocco, 2004; Estrada-Peña et al., 2005; Mertins et al., 2010). Therefore, several records of *A. triste* should be considered provisional, and a morphological and molecular reevaluation of specimens diagnosed as *A. triste* in different tick collections might be useful to better understand its ecology.

The deer *Blastocerus dichotomous*, with an original distribution in humid habitats from southern Amazonia in Bolivia, Brazil, and Peru to the delta of Paraná River in Argentina (Márquez et al., 2006), is considered to be the ancestral host for adults of *A. triste* (Szabó et al., 2007), and indeed almost all bona fide records of this tick in Argentina, southern and central Brazil, and Uruguay correspond with riparian localities where *B. dichotomus* is extant or extinct. This hypothesis implies that populations of *A. triste* in northern Brazil, Chile, Colombia, Ecuador, Mexico, the USA, and Venezuela are derived from the ancestral population and sustained by hosts different to *B. dichotomus*, stressing the need for a whole study of this taxon throughout its range. In any situation, adults of *A. triste* are found on a variety of medium-sized to large wild and domestic mammals including humans as reviewed by Nava et al. (2011).

A. triste is a tick of public health relevance in Argentina because Nava et al. (2008) found specimens infected with *Rickettsia parkeri*, while Romer et al. (2011) reported clinical cases of rickettsiosis by *R. parkeri* in humans with a history of tick bites in localities where *A. triste* prevails. Some aspects of the ecology of *A. triste* have been studied in a locality (34°11′ S 58°50′ W) in the southern range of its distribution in Argentina where clinical cases of rickettsiosis by *R. parkeri* occurred (Nava et al., 2011). In brief, adult ticks were found from early winter to mid-summer with a peak in

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[*] Corresponding author. Tel.: +54 3492 44121; fax: +54 3492 440114.
E-mail address: guglielmone.alberto@inta.gob.ar (A.A. Guglielmone).

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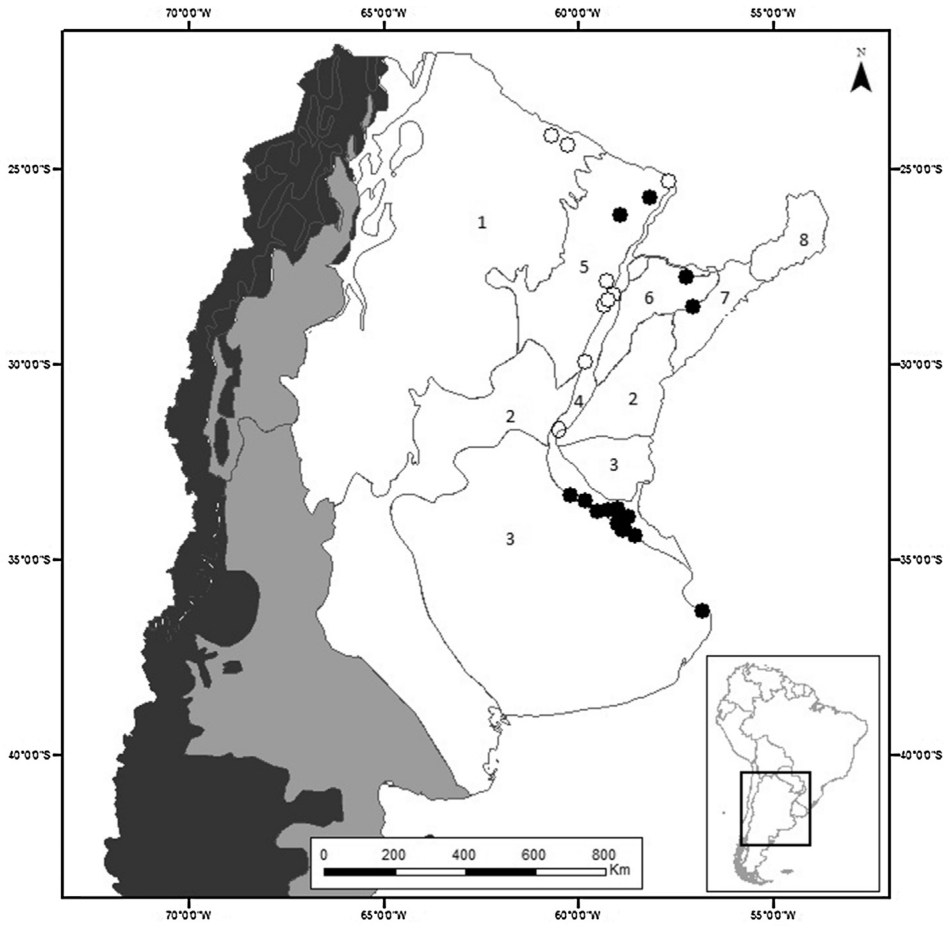

Fig. 1. Argentina ecoregions according to Burkart et al. (1999): (1) Chaco seco, (2) Espinal, (3) Pampa, (4) Delta e Islas del Paraná, (5) Chaco Húmedo, (6) Esteros del Iberá, (7) Campos y Malezales, (8) Paranaense. Open circles represent localities where *Amblyomma triste* has not been detected, close circles indicate localities where *A. triste* has been collected.

late winter to mid-spring, while larva and nymphs were found from December to June with a peak of abundance in summer. Main hosts for larvae and nymphs were rodents of the subfamily Sigmodontinae (*Akodon*, *Oligoryzomys*, *Oxymycterus*, and *Scapteromys*) with a potential role for *Cavia* (Rodentia: Caviidae), while Aves are rarely infested with subadults of *A. triste*. Main hosts for adult ticks were cattle, dog, horse, *B. dichotomus*, and *Hydrochoerus hydrochaeris*. Recently, Debárbora et al. (2012) found wild *Sus scrofa* and *Monodelphis dimidiata* (Didelphimorphia: Didelphidae) as hosts for adults and subadults of *A. triste* in Argentina.

The current study was conducted to better understand the distribution of *A. triste* in Argentina under the hypothesis that this tick prevails in riparian localities along the river Paraná and adjacent humid environments from 34°30′ S to 25°20′ S, approximately.

Materials and methods

Ticks were randomly collected in Argentinean localities by parasitologists of the Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria (INTA), and complemented with tick samples sent by collaborators or from veterinarians and physicians when there were suspicious of tick-borne diseases of animals or humans. This routine work was accompanied with collections of ticks along the Paraná River and adjacent riparians environments from November 2008 to October 2012 with emphasis on the ecoregion Delta e islas of Paraná River as presented in Burkart et al. (1999), but also in a few localities in the ecoregions of Chaco Seco, Chaco Húmedo, Esteros del Iberá, and a locality in the Pampa ecoregion (Fig. 1). All localities were marshlands with a known history to sustain populations of *B. dichotomus* currently or in the past, with the exception of the locality in the Pampa ecoregion (a marshland where *B. dichotomous* was never established). Ticks were collected from domestic and wild mammals, but flagging and dragging techniques were also applied to obtain *A. triste* from vegetation at each locality visited. Collected ticks were deposited in the INTA Tick Collection with the exception of specimens collected from *Akodon azarae* and *Necromys temchuki* in Reserva El Bagual (see Table 1) that have been deposited in the collection maintained in Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina under accession numbers FO00006, FO0007, FO00009, and FO00010. The specimens of *A. triste* collected in this study plus those specimens collected in the works of Nava et al. (2005, 2011) and Debárbora et al. (2012) were used to compile localities and hosts for this tick in Argentina.

DNA was extracted from specimens belonging to 7 geographic populations representative of *A. triste* in Argentina to infer the intraspecific variation on a preliminary basis. The sampled populations were from the localities of Dársena Paraná Guazú (33°54′ S, 58°54′ W; Buenos Aires Province), INTA Delta (34°11′ S, 58°50′ W; Buenos Aires Province), Reserva Natural Otamendi (34°15′ S, 58°52′ W; Buenos Aires Province), Reserva Natural Campos del Tuyú (34°15′ S, 58°52′ W; Buenos Aires Province), Esteros del Iberá (29°00′ S, 57°30′ W; Corrientes Province), and Reserva El Bagual (26°10′ S, 58°56′ W, Formosa Province). Ticks were determined according to Estrada-Peña et al. (2005), and DNA extraction and polymerase chain reaction (PCR) amplification were carried out

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Table 1
Argentinean localities where *Amblyomma triste* were collected from mammals and vegetation (coordinates, INTA Tick Collection accession numbers, hosts, and tick stages found). M, male; F, female; N, nymph; L, larva.

Province	Locality	Hosts
Buenos Aires	Baradero (33°45′ S, 59°31′ W) INTA2108	Vegetation F
Buenos Aires	Campana (34°12′ S, 58°54′ W) INTA2041	*Blastocerus dichotomus* M
Buenos Aires	Campo Goyena (33°43′ S, 59°15′ W) INTA2107	Vegetation M
Buenos Aires	Dársena Paraná Guazú (33°54′ S, 58°54′ W) INTA2113	*Hydrochoerus hydrochaeris* MF
Buenos Aires	Close to Dársena Paraná Guazú (33°55′ S, 58°53′ W) INTA2193	Cattle F
Buenos Aires	INTA Delta (34°11′ S, 58°50′ W) INTA2075), INTA2080, 2087, 2082, 2081, 2079, 2104, 2115, 2009, 2065, 1979, 2033, 2198, 2199, 2200, 2201, 2202, 2203	*Oligoryzomys nigripes* L., *Akodon azarae*, *Cavia aperea*, *Oligoryzomys flavescens*, *Oxymycterus rufus*, *Scapteromys aquaticus* LN, cattle, dog, human, vegetation MF
Buenos Aires	Reserva Ecológica Buenos Aires city (34°36′ W, 58°21′ W) INTA2196	Dog F
Buenos Aires	Reserva Natural Otamendi (34°15′ S, 58°52′ W) INTA1978	Vegetation MF
Buenos Aires	Reserva Natural Campos del Tuyú (36°19′ S, 56°50′ W) INTA2183	Vegetation MF
Buenos Aires	San Nicolás (33°20′ S, 60°14′ W) INTA2184	Vegetation F
Buenos Aires	Tigre (34°24′ S, 58°33′ W) INTA2061	Human F
Buenos Aires	Zárate (34°05′ S, 59°00′ W) INTA2093	Vegetation MF
Corrientes	Estancia Rincón del Socorro (28°42′ S, 57°27′ W) INTA accession numbers in process	*C. aperea*, *S. aquaticus* N, *A. azarae*, *Monodelphis dimidiata*, *O. flavescens* NL, wild boar MF
Corrientes	Esteros del Iberá (29°00′ S, 57°30′ W) INTA1914, 2099, 2100, 2128	*B. dichotomus*, vegetation MF, *H. hydrochaeris* MN
Corrientes	Loreto (27°46′ S, 57°16′ W) INTA1901, 1902, 1903, 1904	*O. nigripes* N, *A. azarae*, *Calomys callosus*, *O. rufus* LN
Entre Rios	Islas Lechiguanas (33°29′ S, 59°50′ W) INTA2038, 2045, 2092	Cattle, *H. hydrochaeris* MF
Entre Rios	Isla La Paloma (33°55′ S, 58°44′ W) INTA2114	*H. hydrochaeris* MF
Entre Rios	Paranacito (33°41′ S 59°00′ W) INTA2194	Horse F
Entre Rios	Río Paraná Guazú East bank (33°53′ S, 58°59 ′W) INTA2095	Vegetation MF
Formosa	Colonia Pastoril (25°42′ S, 58°12′ W) INTA2151	*B. dichotomus* F
Formosa	Reserva El Bagual (26°10′ S, 58°56′ W) INTA2096	*A. azarae*, *Necromys temchuki* LN, Vegetation MF

following the methodology described by Mangold et al. (1998). Amplified PCR products were purified using Wizard PCR Preps DNA Purification System (Promega, Madison, WI). The DNA was sequenced at the ImyZA (Instituto de Microbiología y Zoología Agrícola, INTA, Castelar, Buenos Aires) to obtain a ~400-bp fragment of the 16S rDNA gene. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual edition whenever it was necessary, and aligned with the program Clustal W (Thompson et al., 1994). The number of variable nucleotide positions between haplotypes (individuals) was used to calculate pairwise estimates of percent sequence divergence by using the program Mega 4.0 (Tamura et al., 2007) with the Tamura-Nei model. Additionally, 16S rDNA sequences from *A. triste* specimens from Brazil (near Miranda, 20°13′ S, 56°25′ W, Mato Grosso do Sul) and Uruguay (Barra del Arroyo Maldonado, 34°54′ S, 54°52° W, Maldonado) were obtained, and 16S rDNA sequences available in GenBank of *A. triste* from Chile (JN180848) and Brazil (AY498563) and of *A. triginum* (DQ342290) and *A. maculatum* (AY498559) were also included in the analysis.

Results

The localities, hosts, tick stages found and INTA Tick Collection accession numbers for *A. triste* are shown in Table 1. The positive and provisionally negative localities for *A. triste* along the Paraná River, adjacent riparian environments, and marshland in the ecoregions defined by Burkart et al. (1999) are depicted in Fig. 1.

The hypothesis that the southern limit of *A. triste* is about 34°30′ S was erroneous. About 100 adults of *A. triste* were collected from vegetation at Reserva Natural Campos del Tuyú (36°16′ S), a locality visited because a clinical case of suspected rickettsiosis by *R. parkeri* has been diagnosed in a woman after suffering tick bites there. This natural reserve has the particularity to sustain a natural population of the deer *Ozotoceros bezoarticus*, and the ticks were collected from the vegetation along a deer-path in a marsh environment. On the other hand, the northern limit was determined at Colonia Pastoril (25°42′ S, 58°12′ W) where a collaborator collected an *A. triste* female from a road-death *B. dichotomus*, a locality relatively close to the hypothetical northern limit (25°20′ S) of *A. triste* within Argentina. However, no *A. triste* was collected on a visit to a site 30 km north of Colonia Pastoril.

The distribution of *A. triste* along the Paraná River and adjacent riparian localities was not uniform as predicted. All localities visited along the Paraná River in the Province of Buenos Aires were positive for *A. triste*, but the opposite was true for the Provinces of Chaco and Santa Fe. A special effort was made to collect *A. triste* in the vicinities of Romang (29°30′ S, 59°45′ W) because the ecological conditions appeared to be favourable to sustain *A. triste*, including a large population of *H. hydrochaeris* and a few *B. dichotomus* apart from a large cattle population. About 500 ticks were collected by the authors and collaborators, most of them from dogs and *H. hydrochaeris*, but no *A. triste* was found. Dogs were infested with *Rhipicephalus sanguineus* sensu lato, and *H. hydrochaeris* carried *Amblyomma dubitatum*. Flagging and dragging in several points around Romang failed to obtain any tick.

Comparative analysis of the mitochondrial 16S rDNA sequences of *A. triste* ticks belonging to different Argentinean populations plus Brazilian and Uruguayan populations showed that the intraspecific divergences do not exceed 0.7% (0–0.7%) (Table 2). The differences increased to 0.5–1.2% when comparing sequences from Argentina, Brazil, and Uruguay with sequences of *A. triste* from Chile.

Discussion

The predicted distribution of *A. triste* was not confirmed in this study because the southern occurrence at Reserva Natural

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Table 2
Pairwise nucleotide divergence (%) among 16S rDNA sequences from *Amblyomma triste* ticks collected in different localities of Argentina. Sequences of specimens of *A. triste* from Brazil, Chile, and Uruguay were also included in the analysis. EI, Esteros del Ibera; DPG, Dársena Paraná Guazú; REB, Reserva El Bagual; RNO, Reserva Natural Otamendi; RNCT, Reserva Natural Campos del Tuyú; MGS, Matto Grosso do Sul; GBI, GenBank AY498563; MA, Maldonado.

	A	B	C	D	E	F	G	H	I	J	K	L	LL	M	N	O	P	Q	R	S	T
A. triste Brazil GBI (A)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste Uruguay MA (B)	0.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste EI I (C)	0.2	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste DPG (D)	0.2	0.2	0.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste Brazil MGS (E)	0.2	0.2	0.0	0.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste INTA Delta I (F)	0.5	0.5	0.2	0.2	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste REB I (G)	0.5	0.5	0.2	0.2	0.2	0.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste RNO I (H)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste RNO II (I)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0	–	–	–	–	–	–	–	–	–	–	–	–	
A. triste RNCT I (J)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0	0.0	–	–	–	–	–	–	–	–	–	–	–	
A. triste RNCT II (K)	0.5	0.5	0.2	0.2	0.2	0.5	0.5	0.2	0.2	0.2	–	–	–	–	–	–	–	–	–	–	
A. triste REB II (L)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.2	–	–	–	–	–	–	–	–	–	
A. triste REB III (LL)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.2	0.0	–	–	–	–	–	–	–	–	
A. triste INTA Delta II (M)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.2	0.0	0.0	–	–	–	–	–	–	–	
A. triste INTA Delta III (N)	0.5	0.5	0.2	0.2	0.2	0.5	0.5	0.2	0.2	0.2	0.5	0.2	0.2	0.2	–	–	–	–	–	–	
A. triste EI II (O)	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	–	–	–	–	–	
A. triste EI III (P)	0.7	0.7	0.5	0.5	0.5	0.7	0.7	0.5	0.5	0.5	0.7	0.5	0.5	0.5	0.7	0.5	–	–	–	–	
A. triste EI IV (Q)	0.5	0.5	0.2	0.2	0.2	0.5	0.5	0.2	0.2	0.2	0.0	0.2	0.2	0.2	0.5	0.2	0.7	–	–	–	
A. triste Chile (R)	0.5	0.5	0.7	0.7	0.7	1.0	1.0	0.7	0.7	0.7	1.0	0.7	0.7	0.7	1.0	0.7	1.2	1.0	–	–	
A. maculatum (S)	3.1	3.1	2.9	2.9	2.9	3.1	3.1	2.9	2.9	2.9	3.1	2.9	2.9	2.9	3.1	2.9	2.9	3.1	2.6	–	
A. tigrinum (T)	4.1	4.1	3.9	3.9	3.9	3.6	3.6	3.9	3.9	3.9	4.1	3.9	3.9	3.9	3.6	3.9	3.9	4.1	3.6	4.1	–

Campos del Tuyú (36°15′ S, 56°50′ W) is well beyond the hypothetical boundary at 34°30′ S, and probably tick populations are established south to this known southern limit. This finding is outside the area originally colonized by *B. dichotomus* according to Márquez et al. (2006), but historically home of another endangered deer, *O. bezoarticus*. Probably, adults of *A. triste* are opportunistic feeders of medium-sized and large mammals prone to enter riparian tick habitats, and *B. dichotomus* was a main, but not exclusive host for *A. triste* in the past.

The continuous distribution along the banks of the Paraná River was not confirmed either. Probably there are continuous populations of *A. triste* in the region sampled, but our techniques were not adequate to demonstrate it. However, it is obvious that tick abundance differs widely among areas infested with *A. triste*. Tick collections have been carried out regularly at INTA Delta (34°11′ S, 58°50′ W), Reserva Natural Otamendi (36°19′ S, 58°52′ W), and Esteros del Iberá (29°00′ S, 57°30′ W). Populations of *A. triste* are obviously more abundant at INTA Delta than in the other 2 localities (Nava, S., unpublished), but factors that govern the differences are unknown.

The ecology of *A. triste* is more complex than expected by the authors. Hosts for subadult and adult tick stages are common throughout the study area, but the tick was not found in several localities where its presence was expected, and it was detected in unexpected localities. If hosts are not a major constraint for tick abundance under the given conditions, abiotic factors might be of importance to determine presence, absence, and abundance of *A. triste* in a particular area, but also plant communities may play a role to favour the establishment of *A. triste* populations because they influence abiotic factors (microclimate) as shown in the extreme examples in Civitello et al. (2008) and Racelis et al. (2012) dealing with invasive plants species in tick habitats. Therefore, studies focused on those variables among localities where *A. triste* is present or absent will have to be carried out to understand its ecology and its involvement as a vector of tick-borne rickettsiosis to humans.

The low degree of genetic divergences observed in the sequences of 16S rDNA gene from ticks of Argentinean, Brazilian, and Uruguayan origin suggests that there are no elements to suppose that the specimens morphologically identified as *A. triste* belong to more than one species. This is probably a consequence of the lack of major geographic barriers and its low host-specificity that allows a high gene flow level among *A. triste* populations. These populations are considered to be bona fide *A. triste* because its type locality is Montevideo, Uruguay (Koch, 1844), where this species is still established (Venzal et al., 2008). The genetic divergence increased when those populations of *A. triste* were compared with a sequence of 16S rDNA gene from Chile, but additional studies are needed to determine if the Chilean tick population is conspecific with Argentinean, Brazilian, and Uruguayan populations. It would be of interest to compare the genetic variation of *A. triste* from Argentina, Brazil, Chile, and Uruguay with populations established north of the Amazonia in the Neotropical Zoogeographic Region, but also with those populations of *A. triste* established in the Nearctic Zoogeographic Region in Mexico and the USA.

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