Ecological characterization of a tick community across a landscape gradient exhibiting differential anthropogenic disturbance in the Atlantic Forest ecoregion in Argentina

D. LAMATTINA[1], J. M. VENZAL[2], S. A. COSTA[1], J. P. ARRABAL[1], S. FLORES[1,3], P. E. BERROZPE[1,3], D. GONZÁLEZ-ACUÑA[4], A. A. GUGLIELMONE[3,5] and S. NAVA[3,5]

[1]Instituto Nacional de Medicina Tropical (INMeT), Ministerio de Salud de la Nación, Puerto Iguazú, Argentina, [2]Laboratorio de Vectores y Enfermedades Transmitidas, Facultad de Veterinaria, CENUR Litoral Norte-Salto, Universidad de la República, Salto, Uruguay, [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, [4]Departamento de Ciencia Animal, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile and [5]Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Rafaela, Argentina

Abstract. An ecological analysis of a tick (Ixodida: Ixodidae) community across a landscape gradient presenting differential anthropogenic disturbance in the Atlantic Forest ecoregion in Argentina was performed. Ticks were collected from vegetation and hosts between September 2014 and August 2016. A total of 12 697 free-living ticks and 3347 specimens from hosts were collected, including 317 ticks infesting humans. The values obtained show considerable species diversity in the forest environment accompanied by low equitability. The similarity index derived from a comparison of forest and agricultural environments was higher than that calculated by comparing forest and urban environments. The data suggest that although a cycle of one generation per year is apparent in some species, more than one cohort may co-exist within the populations of some of these species. Well-marked patterns of the seasonal distribution of free-living tick species emerged in environments with no anthropic modification. The results indicate that forest environments are more suitable habitats than agricultural and urban environments for many species of native tick, but are unsuitable for exotic species that have successfully established in environments that have been modified by man.

Key words. Ixodidae, diversity, environmental gradient, host preferences, lifecycle, population ecology.

Introduction

Ticks are vectors of a great variety of human and animal pathogens. They can transmit protozoa, viruses, bacteria and fungi, and contribute to the development of toxicosis, myiasis and secondary infections (Jongejan & Uilenberg, 2004). In addition to the availability of suitable hosts, the presence and abundance of ticks are associated with environmental factors that determine moments and ecological niches that are more or less suitable, and are responsible for the seasonality of different stages of ticks (Belozerov, 1982; Estrada-Peña, 2008). Knowledge and understanding of the behaviours of tick populations and their relationships with certain environmental factors are necessary to assess the animal and human risk for contracting tick-borne diseases (Knap *et al.*, 2009).

The province of Misiones in Argentina harbours large fragments of the Paranaense Rainforest, the largest and most biodiverse ecoregion with the greatest endemism of the Atlantic

Correspondence: Daniela Lamattina, Instituto Nacional de Medicina Tropical, Jujuy y Neuquén s/n, Puerto Iguazú, Misiones CP 3370, Argentina. Tel.: + 54 3757 425002; E-mail: daniela.lamattina@gmail.com

Forest ecoregion complex (Burkart *et al.*, 1999; Plací & Di Bitetti, 2005), which is shared with the neighbouring countries of Brazil and Paraguay. This Argentinean region also has a developed tourist industry and receives over one million visitors per year. Several tick species are established in the Paranaense Rainforest in Argentina (Lamattina *et al.*, 2014, 2016; Nava *et al.*, 2017), but their ecology is poorly understood, a fact that may undermine the suitability of strategies for the control of ticks and related diseases.

Understanding tick ecology contributes to the prevention and control of tick-borne pathogens of medical and veterinary relevance. Therefore, the objective of this study was to perform an ecological analysis of the tick community across a landscape gradient presenting differential anthropogenic disturbance in the Atlantic Forest ecoregion in Argentina.

Materials and methods

Study area

Misiones Province contains part of the Paraná forest and *Araucaria angustifolia* (Bertol.) Kuntze forest areas, within the Paraná sub-region in the Neotropical biogeographic region (Morrone, 2006). The area is included within the Atlantic Forest ecoregion. The ecoregion has a subtropical climate, with average annual temperatures of 16–22 °C and rainfall ranging from 1000 mm to 2200 mm per year (Plací & Di Bitetti, 2005). The intensification of deforestation and the expansion of agriculture in the last 50 years have resulted in large variations in the region's environment (Plací & Di Bitetti, 2005). Northern Misiones currently includes many nationally and provincially protected areas of semi-deciduous tropical forest, which are generally bounded by vast areas of agricultural and livestock production. In relation to the latter, there is a more or less pronounced gradient towards urban environments that include settlements of between 225 (Cabure-í) and 41 000 (Puerto Iguazú) inhabitants [Instituto Provincial de Estadística y Censos de Misiones (IPEC), 2010].

The present study was carried out in northern Misiones, where three habitats with different characteristics were identified: (a) natural forest environments; (b) agroecosystems with adjacent forest patches, and (c) green areas in urban environments.

Tick collection

Free-living ticks were collected from vegetation each month from September 2014 to August 2016, in the following three environments.

Natural forest environments. Four sites corresponding to primary forest environments were selected in protected areas of the Department of Iguazú in northeast Misiones. Three sites (Macuco, Ñandú and Apepú) were located in Iguazú National Park (INP) and the fourth was located in Puerto Península Provincial Park (PPPP) (Fig. 1). At each site, three active animal trails were sampled once per month for 1 h by sliding a 1.0 × 1.5-m white cloth over the vegetation in search of ticks in free-living stages. The cloth was checked every 10 m and the attached ticks were collected.

Agroecosystems. Four sites with silvopastoral cattle breeding systems were selected. Three of these (Jacobo, Otto and Werle) were located in the northeast of the province, in the Department of General Belgrano, and the fourth was located in the rural zone of the city of Puerto Iguazú, in northwest Misiones (Fig. 1). At each site, pastures of 20 000 m² were sampled for 30 min once per month using the same method of sliding a cloth over vegetation.

Urban environments. Four sites were selected in green areas of the city of Puerto Iguazú. Three of these contained small patches of native forest with specimens of *Cedrela fissilis* Vellozo, *Balfourodendron riedelianum* (Engler) Engler and *Patagonula americana* Linnaeus, among others, and the fourth consisted of a pasture with specimens of *Solanum granulosum leprosum* Dunal, *Baccharis* sp. Linnaeus, and others (Fig. 1). In each site, areas of 5000 m² were sampled once per month for 30 min by sliding a cloth over the vegetation.

The differences between sites in sampling times reflected differences in terrain and the areas available for collecting samples in the different environments. The distances travelled in all three environments during the sampling periods were estimated to be similar. The difference between the sizes of the urban and rural environments sampled reflected the existence of animal trails in rural environments that facilitated travel over greater distances within 30 min, whereas in the urban environments sampling cloths were slid over practically the entire area for 30 min because no animal trails were visible.

Six wild animal capture campaigns were carried out in, respectively, February, June, August and December 2015, and February and June 2016. In each trapping session in PPPP, 150 Sherman traps (24 × 9 × 8 cm) were arranged in three transects of 50 traps with distances of at least 200 m between transects and 2 m between traps. In INP, 50 cage traps (40 × 15 × 15 cm) were distributed in five transects of 10 traps with distances of at least 50 m between transects and 2 m between traps. In INP, cage traps were supplemented by 12 mist nets and 14 Tomahawk traps (60 × 30 × 30 cm) separated from one another by at least 10 m in a total area of 30 000 m². Mist nets were activated from dawn to sunset and the birds caught were identified according to Narosky & Yzurieta (2003) and Clements *et al.* (2016); the traps were activated for three consecutive days and nights. Small rodents and opossums were identified by Dr Pablo Teta (Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina). Other mammals were determined according to Canevari & Vaccaro (2007).

In addition, once per month from September 2014 to August 2016, five canines and 10 bovines in agroecosystems were randomly selected and carefully examined for attached ticks. Using the same schedule, five canines were randomly selected in the urban areas and meticulously examined. Occasionally ticks
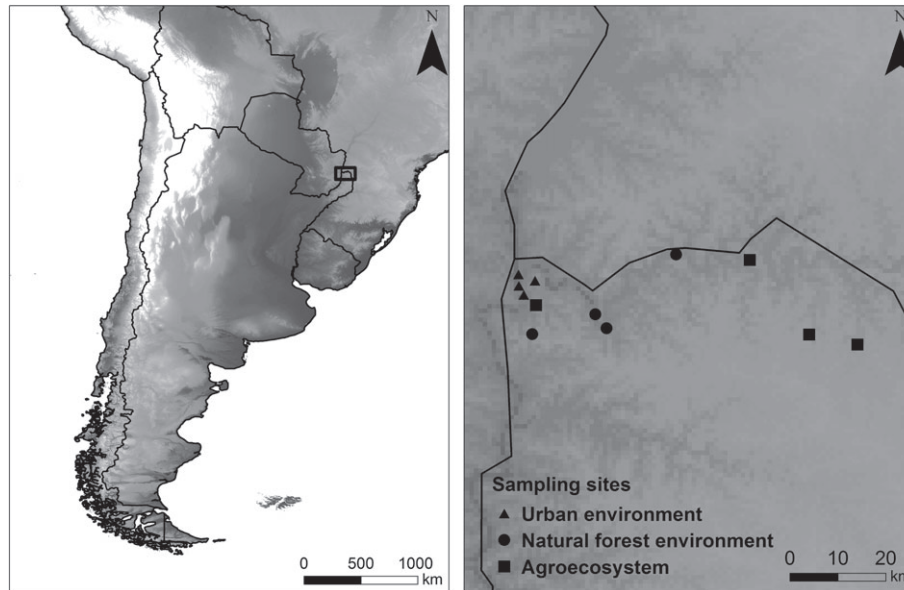
Fig. 1. Study area and sampling sites in northern Misiones Province, Argentina.

were collected from animals killed by traffic on routes that cross protected areas and from researchers who donated ticks that had attached to their bodies.

The sampling sessions scheduled for September 2015 and March and July 2016 were not carried out as a result of severe weather and damage to the transport intended for this purpose.

The collected ticks were placed in tubes containing 96% ethanol and deposited in the National Institute of Tropical Medicine in Puerto Iguazú. The specimens were identified according to their morphological characters in line with Barros-Battesti *et al.* (2006), Martins *et al.* (2014) and Nava *et al.* (2017), and, in the case of larvae, by comparisons with known laboratory-reared material deposited in the tick collection of the Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina.

Morphological identifications of larvae of *Amblyomma brasiliense* Aragão, *Amblyomma coelebs* Neumann and *Amblyomma incisum* Neumann (Ixodida: Ixodidae) were confirmed by analyses of sequences of a 410-bp fragment of the mitochondrial 16S rRNA gene, which were obtained from representative specimens using a polymerase chain reaction protocol described by Mangold *et al.* (1998a, 1998b).

Statistical analysis

Using the online iNEXT software (Chao *et al.*, 2016), based on the rarefaction and extrapolation curves with Hill numbers for q = 0 (for species richness) (Chao *et al.*, 2014), coverage estimates were obtained for each environment dataset in order to assess for the completeness of samples and then to compare the diversity of samples that were equally complete despite differences in sample sizes.

SpadeR software (Chao *et al.*, 2015) was used to obtain the Chao1 species richness estimator (Chao, 1984; Chao & Chiu, 2016) for the tick assemblies found in each environment using the formula:

$$Chao1 = S + \left(\frac{n-1}{n} \right) \left(\frac{f1 \, (f_1 - 1)}{2 \, (f_2 + 1)} \right)$$

where, for each tick assembly, S is the total number of species observed, n is the total number of tick specimens collected, f_1 is the number of singletons (species of which only one specimen was collected) and f_2 is the number of doubletons (species of which only two specimens were collected). In this way, the number of species expected with more exhaustive sampling was robustly calculated, taking into account rare species to estimate the number of undetected species.

To evaluate the level of uncertainty of predicting the species to which an individual chosen randomly from one of the assemblies would belong, the Shannon–Wiener index, which takes into account the relative abundances of all species found in each environment, was calculated:

$$H'_e = - \sum p_{ie} * \ln p_{ie}$$

where H_e' is the Shannon entropy index of each tick assembly e and p_{ie} is the relative abundance of the species i in the assembly e.

To assess for the beta diversity or dissimilarity between environments, SpadeR was employed to calculate multiple community similarity measures with the Horn index. Comparisons between data for each assembly were made by selecting q = 1 to incorporate species abundances in the analysis, without focusing on dominant species, with a bootstrap of 1000 replications.

The abundances of each tick species and stage found on vegetation were compared in each type of environment, between

the two sampling years, between seasons and between months. The non-parametric Mann–Whitney–Wilcoxon test was used in comparisons between the first and second sampling years and the Kruskal–Wallis test was used in comparisons between seasons and months. The same tests were used to compare tick abundances in parasitic life stages (including on human hosts); in this instance, the mean abundances of the most abundant ticks found on the host species with the highest number of captured individuals were also calculated.

Results

Spatial analysis

A total of 12 697 free-living ticks were collected from vegetation, of which 11 990 were found in forest environments and 707 in agroecosystems. Of ticks collected from hosts, 1953 specimens were collected in forest environments, 970 in agroecosystems and 107 in urban environments; a total of 317 ticks were collected from humans across the three environments. In forest environments, 415 wild animals were examined. In agricultural environments, 105 canines and 210 bovines were examined. In urban sites, 105 canines were examined.

In forest environments, 10 tick species were present on 30 host species. These included *A. brasiliense*, *A. coelebs*, *A. incisum*, *Amblyomma longirostre* (Koch), *Amblyomma ovale* Koch, *Amblyomma dubitatum* Neumann, *Amblyomma calcaratum* Neumann, *Haemaphysalis juxtakochi* Cooley and *Haemaphysalis leporispalustris* Packard (Ixodida: Ixodidae), and *Ixodes schulzei* Aragão and Fonseca (Ixodida: Ixodidae). In the free-living phase, only six of these species were found, including *A. brasiliense*, *A. coelebs*, *A. incisum*, *A. longirostre*, *A. ovale* and *H. juxtakochi*. In agroecosystems three species were found on three host species, including *A. ovale*, *Rhipicephalus sanguineus* sensu lato and *Rhipicephalus microplus* Canestrini (Ixodida: Ixodidae). In this environment the species found in free-living stages were *A. brasiliense*, *A. ovale* and *R. microplus*. In urban environments only *R. sanguineus* s.l. ticks were found on canines and humans.

Morphological determinations of larvae of *A. incisum*, *A. brasiliense* and *A. coelebs* were confirmed by the analysis of sequences of the 16S rRNA gene. Comparisons of the sequences obtained from larvae of *A. incisum* (GenBank accession no. MG791917), *A. brasiliense* (GenBank accession no. MG791916) and *A. coelebs* (GenBank accession no. MG791918) with those of these three species available in GenBank showed higher similarities than 98%, confirming the identifications based on morphological characters. The abundances of each tick species in free-living and parasitic phases, in addition to the prevalence of each captured host species, are presented in Tables 1 and 2. The most abundant species in forest environments on both vegetation and hosts was *A. coelebs*, which accounted for 82.9% of all specimens collected from vegetation and 83.2% of those collected from hosts. In agroecosystems, *R. microplus* was the most abundant species, which represented 99.3% (larvae) of all ticks collected from vegetation and 95.1% (all parasitic stages) of those collected from hosts. In urban environments, no specimens were

found on vegetation and *R. sanguineus* s.l. was the only tick species collected from hosts.

The extrapolation curves showed that increasing the number of individuals in the samples would not increase the number of species or sample completeness. The sample coverage estimates of the reference samples of each set were found to be in the range of 0.9986–1. The species richness of the tick assembly from forest environments, estimated with Chao1, was 10.33, and was higher than those found in the agricultural and urban environments, which were 3.00 and 1.00, respectively.

Shannon–Wiener indices were obtained for free-living tick assemblies for each environment. The mean ± standard deviation (SD) index was 0.647 ± 0.00004 for the forest environment, 0.046 ± 0.0003 for the agroecosystem environment and 0 for the urban environment. For the parasitic tick assemblies, the mean ± SD indices obtained were 0.72 ± 0.0002 for the forest environment, 0.222 ± 0.0004 for the agroecosystems, and 0 for the urban environment.

Comparisons of the relative abundances (Horn) of species between the three environments, with bootstraps of 1000 replications, for parasitic tick assemblies, provided a mean ± standard error (SE) similarity estimation of 0.0355 ± 0.0049 [95% confidence interval (CI) 0.0259–0.045] and an average pairwise similarity of 0.028. The mean ± SE similarity index between the forest and agricultural environments was 0.043 ± 0.004 (95% CI 0.034–0.051), and that between the agricultural and urban environments was 0.041 ± 0.011 (95% CI 0.020–0.063). The Horn index for the forest and urban environments was 0. For ticks in free-living stages, a mean ± SE similarity index of 0.0338 ± 0.0147 (95% CI 0.005–0.0627) was obtained between the forest and agroecosystem assemblies.

Temporal dynamics

The comparisons between years using the Mann–Whitney non-parametric test for all tick species collected from vegetation and from hosts revealed no significant differences between the first and second years of sampling ($P > 0.05$). The results of comparing tick abundances between seasons (summer vs. autumn vs. winter vs. spring) using the Kruskal–Wallis test were not significant for almost all tick species collected from hosts, with the exception of *A. incisum* larvae (differences between spring and summer abundances vs. autumn abundances, $P = 0.02$, presenting a peak in autumn) and *R. sanguineus* nymphs and adults ($P = 0.02$ and $P = 0.005$, respectively). The latter species was collected only from hosts and showed significant differences in the abundances of nymphs in autumn and winter vs. that in summer, and a very marked difference in adult abundances in autumn and winter vs. spring and summer. By contrast, comparisons of abundances by season showed significant differences for all species collected from vegetation in the forest environment ($P < 0.05$), except *A. longirostre*, of which only a single individual was collected (Table 3). Although *A. brasiliense* was present during the entire year, peaks in its abundance were observed in larvae in summer and autumn, in nymphs in winter and in adults in spring. *Amblyomma coelebs* larvae and nymphs were present during all seasons, although

Table 1. Abundances of tick species in parasitic and free-living stages collected in northern Misiones Province between September 2014 and August 2016. Numbers of infested individuals of each host species are shown in parentheses.

Host species	Hosts, n Examined	Infested	*Amblyomma brasiliense* Larvae	Nymphs	Adults	*Amblyomma calcaratum* Adults	*Amblyomma coelebs* Larvae	Nymphs	Adults	*Amblyomma incisum* Larvae	Nymphs	Adults	*Amblyomma dubitatum* Nymphs	*Amblyomma longirostre* Larvae	Nymphs	Adults
Didelphimorphia																
Didelphis aurita	36	34	1 (1)	2 (2)			221 (20)	519 (34)		1 (1)						
Didelphis albiventris	1	1					17 (1)	35 (1)								
Monodelphis americana	1	0														
Philander frenatus	3	0														
Cryptonanus chacoensis	1	0														
Carnivora																
Nasua nasua	80	74	1 (1)	15 (13)	1 (1)		183 (23)	310 (65)								
Cerdocyon thous	2	2						3 (1)								
Leopardus pardalis	2	2					4 (2)	2 (1)								
Eira barbara	2	2					36 (1)	59 (2)								
Puma concolor	1	1														
Puma yagouaroundi	1	1														
Dogs	210	74														
Pilosa																
Tamandua tetradactyla	2	2		4 (1)		1 (1)		6 (1)								
Rodentia																
Dasyprocta azarae	9	9	10 (3)	3 (3)			89 (7)	67 (9)								
Akodon cf. *A. montensis*	19	3														
Oligoryzomys cf. *O. nigripes*	5	1														
Sooretamys angouya	1	0														
Nectomys squamipes	4	1														
Oxymycterus cf. *O. misionalis*	1	0														
Lagomorpha																
Sylvilagus brasiliensis	3	3						3 (2)								
Artiodactyla																
Mazama americana	2	2	8 (1)	3 (2)			2 (1)									
Cattle	210	198														
Perissodactyla																
Tapirus terrestris	2	2		1 (1)	74 (1)		4 (1)	52 (1)			14 (1)					
Birds	237	20						6 (5)						12 (8)	7 (6)	
Human		73	25 (8)	6 (6)			150 (30)	100 (54)	3 (3)	13 (3)	14 (12)		1 (1)			
Vegetation			275	598	298		9145	640	26	341	148	37				1

Host species	Hosts, n Examined	Infested	*Amblyomma ovale* Larvae	Nymphs	Adults	*Haemaphysalis juxtakochi* Larvae	Nymphs	Adults	*Haemaphysalis leporispalustris* Adults	*Ixodes schulzei* Adults	*Rhipicephalus microplus* Larvae	Nymphs	Adults	*Rhipicephalus sanguineus* s.l. Nymphs	Adults
Didelphimorphia															
Didelphis aurita	36	34	21 (2)	1 (1)		1 (1)	2 (1)								
Didelphis albiventris	1	1													
Monodelphis americana	1	0													
Philander frenatus	3	0													
Cryptonanus chacoensis	1	0													
Carnivora															
Nasua nasua	80	74	7 (5)	1 (1)	52 (21)	27 (9)	15 (12)								
Cerdocyon thous	2	2			3 (1)										
Leopardus pardalis	2	2													
Eira barbara	2	2													
Puma concolor	1	1			4 (1)										
Puma yagouaroundi	1	1			1 (1)										
Dogs	210	74			38 (17)								4 (3)	43 (23)	74 (37)
Pilosa															
Tamandua tetradactyla	2	2													
Rodentia															
Dasyprocta azarae	9	9	2 (1)			2 (2)									
Akodon cf. *A. montensis*	19	3	5 (2)	2 (1)											
Oligoryzomys cf. *O. nigripes*	5	1		1 (1)											
Sooretamys angouya	1	0													
Nectomys squamipes	4	1								2 (1)					
Oxymycterus cf. *O. misionalis*	1	0													
Lagomorpha															
Sylvilagus brasiliensis	3	3							2 (1)						
Artiodactyla															
Mazama americana	2	2				9 (2)	1 (1)								
Cattle	210	198									66 (24)	182 (114)	670 (198)		
Perissodactyla															
Tapirus terrestris	2	2			3 (2)			1 (1)							
Birds	237	20	1 (1)			5 (2)									
Human		73			2 (2)	1 (1)								1 (1)	1 (1)
Vegetation			2	5	24	78	63	20				702			

Table 2. Ticks collected on birds in Iguazú National Park between September 2014 and August 2016.

Order	Family	Host species	I/E, *n*	Amblyomma coelebs Nymphs	Amblyomma longirostre Larvae	Amblyomma longirostre Nymphs	Amblyomma ovale Larvae	Haemaphysalis juxtakochi Larvae
Accipitriformes	Accipitridae	*Rupornis magnirostris*	0/2					
Columbiformes	Columbidae	*Leptotila verreauxi*	0/9					
Strigiformes	Strigidae	*Megascops choliba*	0/1					
Caprimulgiformes	Caprimulgidae	*Nyctidromus albicollis*	0/1					
	Trochilidae	*Thalurania furcata*	0/1					
		Thalurania glaucopis	0/9					
		Amazilia versicolor	0/9					
		Phaethornis eurynome	0/2					
Trogoniformes	Trogonidae	*Trogon rufus*	0/2					
Coraciiformes	Alcedinidae	*Chloroceryle americana*	0/1					
	Momotidae	*Baryphthengus ruficapillus*	1/2	1		1		
Piciformes	Picidae	*Celeus flavescens*	0/1					
		Picumnus temminckii	0/2					
	Ramphastidae	*Pteroglossus castanotis*	0/1					
Psittaciformes	Psittacidae	*Pionus maximiliani*	0/1					
Passeriformes	Thamnophilidae	*Dysithamnus mentalis*	0/3					
	Furnariidae	*Automolus leucophthalmus*	1/3		2			
		Dendrocolaptes platyrostris	0/1					
		Dendrocincla fuliginosa	1/8		1			
		Philydor atricapillus	1/1			1		
		Philydor rufum	0/1					
		Xenops minutus	0/3					
		Sittasomus griseicapillus	0/8					
	Tyrannidae	*Platyrinchus mystaceus*	1/4		1			
		Myiornis auricularis	0/1					
		Mionectes rufiventris	0/3					
		Cnemotriccus fuscatus	0/10					
		Poecilotriccus plumbeiceps	0/1					
		Pitangus sulphuratus	0/4					
		Corythopis delalandi	0/5					
		Leptopogon amaurocephalus	1/10		1			
	Pipridae	*Chiroxiphia caudata*	1/5			1		
		Pipra fasciicauda	1/23		3			
	Tityridae	*Schiffornis virescens*	0/1					
	Vireonidae	*Vireo olivaceus*	0/1					
	Corvidae	*Cyanocorax chrysops*	0/11					
	Turdidae	*Turdus albicollis*	3/8		4			
		Turdus rufiventris	3/12	4				
		Turdus amaurochalinus	0/4					
		Turdus leucomelas	4/22	1		3		5
	Parulidae	*Basileuterus culicivorus*	0/6					
		Myiothlypis leucoblephara	0/1					
	Thraupidae	*Trichothraupis melanops*	1/12			1		
		Dacnis cayana	0/3					
		Tersina viridis	0/3					
		Tachyphonus coronatus	1/2				1	
	Emberizidae	*Arremon flavirostris*	0/1					
	Cardinalidae	*Habia rubica*	0/2					
	Icteridae	*Cacicus haemorrhous*	0/2					
	Fringillidae	*Chlorophonia cyanea*	0/2					
		Euphonia violácea	0/4					
		Euphonia pectoralis	0/1					
		Euphonia chlorotica	0/1					

I/E, infested/examined.

Table 3. Comparison[*] of abundances of free-living ticks by season in the forest environment, northern Misiones Province, 2014–2016.

Species	Stage	Summer	Autumn	Winter	Spring
Amblyomma brasiliense	Larvae	A	A–B	B	B
	Nymphs	A	A–B	B–C	C
	Adults	A	A–B	B–C	C
Amblyomma coelebs	Nymphs	A–B	B–C	C	A
Amblyomma incisum	Larvae	A	B–C	A–B	A
Amblyomma ovale	Adults	A–B	A	A	B
Haemaphysalis juxtakochi	Larvae	A	A–B	A–B	B
	Nymphs	A–B	B–C	C	A
	Adults	A	A	B	A–B

[*]Means with a common letter present no significant differences ($P > 0.05$).

the larvae showed higher abundances in spring and summer, and nymphs were more abundant during winter. Larvae of *A. incisum* collected from vegetation showed peaks in autumn and winter, whereas nymphs were more abundant only in winter. *Amblyomma ovale* larvae were found on vegetation in summer and autumn, nymphs in autumn and winter, and adults mostly in spring. Larvae of *H. juxtakochi* were more abundant in autumn and summer, nymphs in winter and adults in spring. The patterns of seasonality in these and other tick species are shown in Fig. 2.

Adults of *A. brasiliense* and *A. coelebs* were the only stages to show significant differences between months ($P = 0.049$ and $P = 0.047$, respectively). Figure 2 shows monthly variations in abundances of the tick species found most frequently on vegetation and comparisons with variations in the mean abundances of the same species found on their main hosts. As *Nasua nasua* (Linnaeus), *Didelphis aurita* (Wied-Neuwied) and cattle were the most commonly examined hosts, and both the prevalence and mean abundance of several tick species were highest in these host species, these were taken into account to compare seasonal peaks in tick abundances on vegetation and on hosts.

Discussion

In the present study, a total of 16 044 ticks belonging to 12 species were collected from vegetation and hosts in three distinct environments in northern Argentina. The presence of all tick species found in this study had been previously reported in this area (Boero & Delpietro, 1971; Ivancovich & Luciani, 1992; Nava *et al.*, 2012; Lamattina *et al.*, 2014, 2016). The immature stages of most species presented lower levels of host specificity than adult stages, in agreement with Nava & Guglielmone (2013) and Esser *et al.* (2016). For example, adults of *A. ovale* were found almost exclusively on carnivores, whereas immature forms were found on didelphid marsupials, rodents, carnivores and passerine birds. In the same way, adults of *A. coelebs* and *A. brasiliense* were found almost exclusively on *Tapirus terrestris* (Linnaeus), whereas immature stages were found to be host generalist. With respect to birds, the most commonly captured birds were passerines, within which the genus *Turdus* presented the highest prevalence of tick infestation (21.7%). The human seems to be a host that occasionally harbours different

ixodid species, as observed in this study, in agreement with Guglielmone *et al.* (2006).

The sample coverage estimators of each sampling set showed that about 100% of the entire population of individuals in each tick assembly belonged to the species represented in the samples. The Chao1 species richness estimator of the tick assembly from the forest environment was the only estimator to show a small difference with the species richness found in this study, which reflected high numbers of singletons and doubletons. The estimates obtained for the agroecosystem and urban environments did not differ from the species richness found in this study.

The differences among the forest, agricultural and urban environments can be explained by means of species richness data and the proportional abundances of each species. The data showed considerable species diversity in the forest environment accompanied by low equitability because collections in this environment included a high proportion of rare species represented by low numbers of individuals.

The Horn biodiversity similarity indices among the three free-living tick assemblies and between the two parasitic tick assemblies were low. The forest and agricultural environments, and the agricultural and urban environments, respectively, as well as being contiguous (they contain patches of vegetation of similar structures), were found to share some of the reported hosts of ixodids, such as *D. aurita* in the first case and dogs in the second. Therefore, it is understandable that their similarity indices were higher than that calculated for the forest and urban environments. Nevertheless, the wide differences observed between environments indicate that the establishment of some species of tick in a given environment depends not only on the presence of hosts, but on the suitability of the habitat in terms of other environmental factors such as microclimatic conditions and vegetation composition. In any case, the findings of this study indicate that forest environments are more suitable habitats than agricultural and urban environments for many native species of tick, but are unsuitable for *R. sanguineus* and *R. microplus*, which are exotic species that have established successfully in environments that have been modified by human activities and in which their principal hosts, dogs and cattle, respectively, prevail.

With respect to the temporal distribution of ixodid tick abundances, there were no significant differences between findings
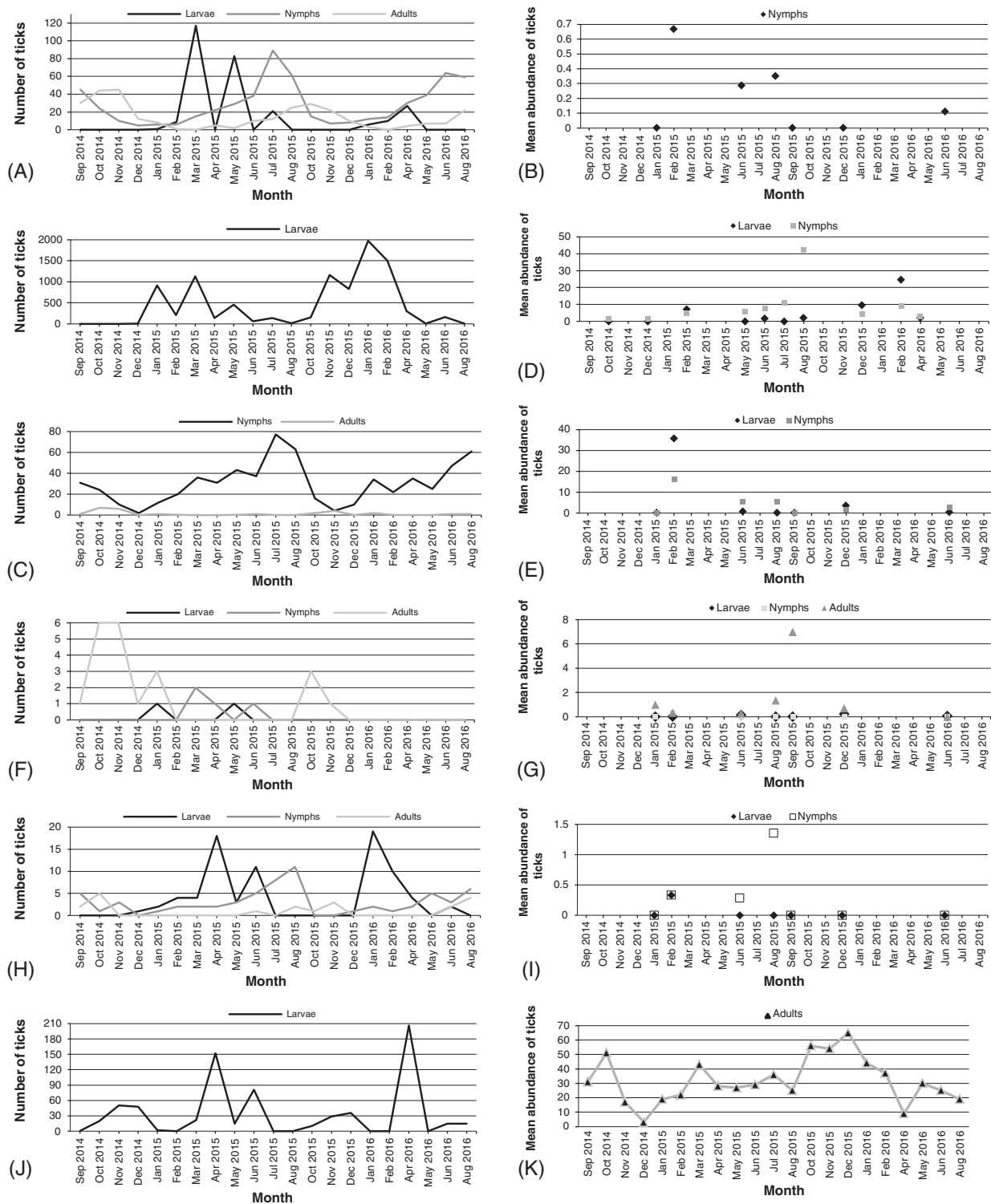
Fig. 2. Monthly distributions of the different stages of tick species found on vegetation and on hosts in northern Misiones Province, Argentina, 2014–2016. (A) *Amblyomma brasiliense* abundance on vegetation. (B) *A. brasiliense* mean abundance on *Nasua nasua*. (C) *Amblyomma coelebs* abundance on vegetation. (D) *A. coelebs* mean abundance on *Didelphis aurita*. (E) *A. coelebs* mean abundance on *N. nasua*. (F) *Amblyomma ovale* abundance on vegetation. (G) *A. ovale* mean abundance on *N. nasua*. (H) *Haemaphysalis juxtakochi* abundance on vegetation. (I) *H. juxtakochi* mean abundance on *N. nasua*. (J) *Rhipicephalus microplus* (larvae) abundance on vegetation. (K) *R. microplus* (adults) mean abundance on cattle.

for the first and second years of sampling. The findings suggest that some species, such as *A. coelebs*, *A. brasiliense* and *A. ovale*, may show a cycle of one generation per year but that more than one cohort may co-exist within their populations because specimens of different stages were found throughout the year on both vegetation and hosts. These results agree with findings in other species such as *Ixodes ricinus* (Linnaeus) in southwest Scotland (Walker, 2001), *Amblyomma neumanni* Ribaga in the northwest of the province of Córdoba, Argentina (Nava *et al.*, 2009), and *A. dubitatum* in the province of Corrientes, Argentina (Debárbora *et al.*, 2014). Other species, such as *H. juxtakochi*, present cycles of a single generation per year.

In the environments with no anthropic modification, well-marked patterns of seasonal distribution in free-living tick species were seen, with significant differences between seasons in the abundances of all species found. *Amblyomma brasiliense* presented a characteristic pattern in all stages, with significant differences between summer and winter, as was also observed in nymphs of *A. coelebs* and nymphs and adults of *H. juxtakochi*. In *A. incisum* larvae and *A. ovale* adults, significant differences were observed mainly between autumn and spring, whereas *H. juxtakochi* larvae presented significant differences only between summer and spring. Seasonality and other parameters were evaluated individually by tick stage in order to avoid biases based on the amount of larvae collected in clusters. However, the number of larvae found in a cluster differed by species (e.g. larvae in clusters of *A. coelebs* were more numerous than larvae in clusters of *A. brasiliense*, which, in turn, were more numerous than larvae in clusters of *A. incisum*) and, given that this factor may be significant in the ecology of a species, it was considered important to ensure this information was not lost by considering clusters of larvae as units in summaries of abundances.

In a study performed in São Paulo, Brazil (Szabó *et al.*, 2009), *A. incisum* showed peaks in abundance in nymphs in spring, and in adults in winter and summer, whereas larvae were present only in autumn and winter. Larvae of *A. brasiliense* were more abundant during autumn, and nymphs and adults were less abundant in autumn and summer, respectively. *Amblyomma ovale* adults were more abundant in spring and summer (Szabó *et al.*, 2009). In the present study, *A. incisum* nymphs were more abundant during winter, whereas adults were present throughout the year and showed no significant differences between seasons. Findings in all stages of *A. brasiliense* were similar to those described by Szabó *et al.* (2009), as were those in *A. ovale* adults. This suggests that, in similar environments (in this case, in two Atlantic Rainforest studies), the temporal and spatial dynamics of the different tick species can be expected to be similar, albeit with divergences caused by associations with external factors such as temperature and saturation deficit, as has been reported in, for example, *Amblyomma maculatum* Koch (Barker *et al.*, 2004) and *Ixodes scapularis* Say (Ogden *et al.*, 2005) in the U.S.A.

The absence of significant differences in the temporal distributions of parasitic life stages may reflect the heterogeneity of the wild hosts examined because the current records are derived from catches and from animals killed by vehicle traffic, on which abundances may be underestimated. By contrast, the seasonal distribution of *R. sanguineus s.l.* showed significant differences

in abundance between seasons and a marked increase in abundance in summer. This is in agreement with a great number of works that indicate that successful oviposition, egg hatching and larval and nymphal moulting are unlikely at low temperature conditions (Dantas-Torres, 2010).

In Fig. 2, which shows levels of similarity in abundances of ticks found on vegetation and hosts, there are apparent peaks of greater abundance that correspond between collections of ticks from vegetation and from their main hosts, respectively. Nymphs of *A. brasiliense* showed greater abundance on vegetation and greater mean abundance on *N. nasua* in summer, as did *A. coelebs* larvae, of which mean abundance on *D. aurita* was also greater in summer. By contrast, *A. coelebs* nymphs showed higher abundances on vegetation and a greater mean abundance on *D. aurita* during winter. Adults of *A. ovale*, however, presented peaks in abundance on vegetation and in mean abundance on *N. nasua* in spring. In *H. juxtakochi*, larvae peaked in abundance on vegetation and on *N. nasua* in summer, whereas nymphs did so in winter. Both immature and adult stages of *R. microplus* were detected on hosts but only adults were considered for the analysis of monthly mean abundance. Adults of *R. microplus* were present throughout the year on *Bos taurus* and did not fluctuate much in abundance between seasons. The pattern of seasonality observed for *R. microplus* suggests that this species produces at least four generations per year in the study area.

In conclusion, this study showed that some species are present only in some seasons of the year (*A. ovale*, *H. juxtakochi*) and others are present throughout the year and show seasonal peaks in abundance (*A. brasiliense*, *A. coelebs*, *R. microplus*). In the same way, some species are present in a variety of environments but at greater abundance in some than in others. This information may be useful in the development of strategies for the surveillance and prevention of tick-borne diseases because it provides the possibility of identifying zones and times at which the risk for tick bite is greater.

Knowledge on host usage and the temporal and spatial dynamics of tick abundances is necessary to further understanding of the risk for transmission of tick-borne pathogens. Indeed, tick control strategies should be based on existing information on the spatial and temporal distributions and the climatic and host preferences of ticks. The present work provides information on all of these factors for ticks present in environments of different levels of anthropogenic disturbance in the Atlantic Forest ecoregion of Argentina.

Acknowledgements

References

Barker, R.W., Kocan, A., Ewing, S.A., Wettemann, R.P. & Payton, M.E. (2004) Occurrence of the Gulf Coast Tick (Acari: Ixodidae) on wild and domestic mammals in north-central Oklahoma. *Journal of Medical Entomology*, **41**, 170–178.

Barros-Battesti, D.M., Arzua, M. & Bechara, G.H. (2006) *Carrapatos de Importância Médico-Veterinária da Região Neotropical: um Guia Ilustrado para Identificação de Espécies*. Integrated Consortium on Ticks and Tick-borne Diseases-ICTTD/Butantan, São Paulo, SP.

Belozerov, V.N. (1982) Diapause and biological rhythms in ticks *Physiology of Ticks* (ed. by F.D. Obenchain & R. Galun), pp. 469–500. Pergamon Press, Oxford.

Boero, J.J. & Delpietro, H. (1971) *Amblyomma calcaratum* Neumann, 1899 (Acarina: Ixodidae). Nueva especie para la fauna Argentina. *Revista de Medicina Veterinaria*, **52**, 339–341.

Burkart, R., Bárbaro, N.O., Sánchez, R.O. & Gómez, D.A. (1999) *Eco-regiones de la Argentina. Programa Desarrollo Institucional Ambiental*. Administración de Parques Nacionales, Secretaría de Recursos Naturales y Desarrollo Sustentable, Buenos Aires.

Canevari, M. & Vaccaro, O. (2007) *Guía de Mamíferos del Sur de América del Sur*. LOLA, Buenos Aires.

Chao, A. (1984) Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, **11**, 265–270.

Chao, A. & Chiu, C.H. (2016) Species richness: estimation and comparison. Wiley StatsRef: Statistics Reference Online. https://doi.org/10.1002/9781118445112.stat03432.pub2.

Chao, A., Gotelli, N.J., Hsieh, T.C. *et al.* (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs*, **84**, 45–67.

Chao, A., Ma, K.H., Hsieh, T.C. & Chiu, C.H. (2015) Online Program SpadeR (Species-richness Prediction and Diversity Estimation in R). Program and User's Guide. http://chao.stat.nthu.edu.tw/wordpress/software_download/softwarespader_online/ [accessed on 15 February 2017].

Chao, A., Ma, K.H. & Hsieh, T.C. (2016) iNEXT (iNterpolation and EXTrapolation) Online: Software for Interpolation and Extrapolation of Species Diversity. Program and User's Guide. http://chao.stat.nthu.edu.tw/wordpress/software_download/inext-online/ [accessed on 15 February 2017].

Clements, J.F., Schulenberg, T.S., Iliff, M.J., Roberson, D., Fredericks, T.A., Sullivan, B.L. & Wood, C.L. (2016) The eBird/Clements checklist of birds of the world: v2016. http://www.birds.cornell.edu/clementschecklist/download/ [accessed on 15 March 2017].

Dantas-Torres, F. (2010) Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Parasites & Vectors*, **3**, 26–36.

Debárbora, V., Mangold, A.J., Oscherov, E.B., Guglielmone, A.A. & Nava, S. (2014) Study of the life cycle of *Amblyomma dubitatum* (Acari: Ixodidae) based on field and laboratory data. *Experimental and Applied Acarology*, **63**, 93–105.

Esser, H.J., Herre, E.A., Blüthgen, N., Loaiza, J.R., Bermúdez, S.E. & Jansen, P.A. (2016) Host specificity in a diverse Neotropical tick community: an assessment using quantitative network analysis and host phylogeny. *Parasites & Vectors*, **9**, 372–385.

Estrada-Peña, A. (2008) Climate, niche, ticks, and models: what they are and how we should interpret them. *Parasitology Research*, **103**, 87–95.

Guglielmone, A.A., Beati, L., Barros-Battesti, D.M. *et al.* (2006) Ticks (Ixodidae) on humans in South America. *Experimental and Applied Acarology*, **40**, 83–100.

Instituto Provincial de Estadística y Censos de Misiones (IPEC) (2010) Censo Nacional de Población, Hogares y Viviendas, Argentina. http://www.ipecmisiones.org/censo-2010 [accessed on 15 March 2017].

Ivancovich, J.C. & Luciani, C.A. (1992) Las Garrapatas de Argentina. Monografías de la Asociación Argentina de Parasitología Veterinaria, Buenos Aires.

Jongejan, F. & Uilenberg, G. (2004) The global importance of ticks. *Parasitology*, **129**, 3–14.

Knap, N., Durmiši, E., Saksida, A., Korva, M., Petrovec, M. & Avšič-Županc, T. (2009) Influence of climatic factors on dynamics of questing *Ixodes ricinus* ticks in Slovenia. *Veterinary Parasitology*, **164**, 275–281.

Lamattina, D., Tarragona, E.L., Costa, S.A., Guglielmone, A.A. & Nava, S. (2014) Ticks (Acari: Ixodidae) of northern Misiones Province, Argentina. *Systematic and Applied Acarology*, **19**, 393–398.

Lamattina, D., Venzal, J.M., Guglielmone, A.A. & Nava, S. (2016) *Ixodes schulzei* Aragão & Fonseca, 1951 in Argentina. *Systematic and Applied Acarology*, **21**, 865–867.

Mangold, A.J., Bargues, M.D. & Mas-Coma, S. (1998a) 18S rRNA gene sequences and phylogenetic relationships of European hard-tick species (Acari: Ixodidae). *Parasitology Research*, **84**, 31–37.

Mangold, A.J., Bargues, M.D. & Mas-Coma, S. (1998b) Mitochondrial 16S rRNA sequences and phylogenetic relationships of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). *Parasitology Research*, **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 and Tick-borne Diseases*, **5**, 753–770.

Morrone, J.J. (2006) Biogeographic areas and transition zones of Latin America and the Caribbean Islands based on panbiogeographic and cladistic analyses of the entomofauna. *Annual Review of Entomology*, **51**, 467–494.

Narosky, T. & Yzurieta, D. (2003) Aves de Argentina y Uruguay: Guía para la Identificación. Vazquez Mazzini Editores, Buenos Aires.

Nava, S. & Guglielmone, A.A. (2013) A meta-analysis of host specificity in Neotropical hard ticks (Acari: Ixodidae). *Bulletin of Entomological Research*, **103**, 216–224.

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

Nava, S., Mastropaolo, M., Venzal, J.M., Mangold, A.J. & Guglielmone, A.A. (2012) Mitochondrial DNA analysis of *Rhipicephalus sanguineus sensu lato* (Acari: Ixodidae) in the Southern Cone of South America. *Veterinary Parasitology*, **190**, 547–555.

Nava, S., Venzal, J.M., González-Acuña, D., Martins, T.F. & Gugliel-
mone, A.A. (2017) *Ticks of the Southern Cone of America. Diagnosis,
Distribution, and Hosts with Taxonomy, Ecology and Sanitary Impor-
tance*. Academic Press, London.

Ogden, N.H., Bigras-Poulina, M., O'Callaghan, C.J. *et al.* (2005) A
dynamic population model to investigate effects of climate on geo-
graphic range and seasonality of the tick *Ixodes scapularis*. *Interna-
tional Journal for Parasitology*, **35**, 375–389.

Plací, G. & Di Bitetti, M. (2005) Situación ambiental en la Ecor-
región del Bosque Atlántico del Alto Paraná (Selva Paranaense) *Una
visión de biodiversidad para la Ecorregión del Bosque Atlántico
del Alto Paraná: Diseño de un Paisaje para la Conservación de la
Biodiversidad y Prioridades para las Acciones de Conservación*, pp.
197–225. World Wildlife Fund, Washington, DC.

Szabó, M.P.J., Labruna, M.B., Garcia, M.V. *et al.* (2009) Ecological
aspects of the free-living ticks (Acari: Ixodidae) on animal trails
within Atlantic rainforest in south–eastern Brazil. *Annals of Tropical
Medicine and Parasitology*, **103**, 57–72.

Walker, A.R. (2001) Age structure of a population of *Ixodes ricinus*
(Acari: Ixodidae) in relation to its seasonal questing. *Bulletin of
Entomological Research*, **91**, 69–78.