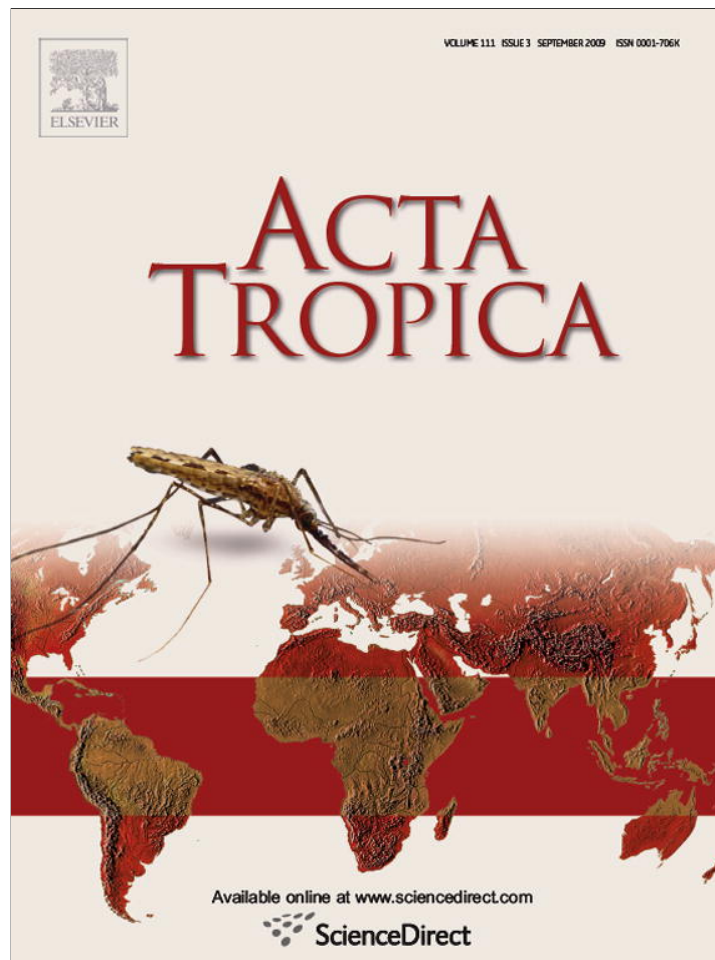


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Ecology of *Amblyomma neumanni* (Acari: Ixodidae)

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ARTICLE INFO

Article history:

Received 23 February 2009

Received in revised form 25 April 2009

Accepted 27 April 2009

Available online 7 May 2009

Keywords:

Amblyomma neumanni

Seasonality

Life cycle

Distribution

Genetic variation

ABSTRACT

The life cycle of *Amblyomma neumanni* was described studying the seasonal distribution of free-living stages and parasitic phases during two consecutive years. Development periods of engorged ticks under different photoperiod conditions were recorded. Larvae of *A. neumanni* have the peak of abundance in autumn. Nymphs reach the peak in winter. Females were collected on cattle from autumn to late spring. The seasonal distribution pattern of females showed a bimodal curve, with a peak in autumn and other during early and middle spring. The engorged females exposed at shortest photoperiod regimen (10 h light–14 h dark) under both laboratory and field conditions undergo morphogenetic diapause, expressed as a delay in the oviposition. It is concluded that females of *A. neumanni* that feed and copulate in autumn undergo morphogenetic diapause, and they will lay eggs in spring, simultaneously with the females that feed and copulate in this season. Climate niche analysis shows that adequate suitability for *A. neumanni* depends mainly from temperature (mean, absolute maximum and minimum, and mean temperature in wettest and driest quarters) as well as from rainfall in warmest and coldest quarters. Sequences of 16S rDNA gene belonging to different populations of *A. neumanni*, showed no intraspecific genetic differentiation.

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1. Introduction

Ticks are parasites with medical and veterinary importance because they have capacity to transmit pathogenic micro-organism and cause paralysis, toxicosis, irritation and allergy to domestic animals and man (Jongejan and Uilenberg, 2004). In the Neotropical zoogeographic region, approximately 50% of the hard ticks species belong to the genus *Amblyomma*, and with few exceptions, they are distributed principally in tropical and sub-tropical areas, where several species of this genus have been found parasitizing humans and domestic mammals (Guglielmone et al., 2003).

One of the Neotropical *Amblyomma* species with sanitary relevance is *Amblyomma neumanni*. Although this tick has been recorded from Colombia, most findings correspond to the north of Argentina, where it is prevalent in the Chaqueño Serrano district of the Chaco Phytogeographic Province (Estrada-Peña et al., 2005; Guglielmone and Nava, 2006). In this country, *A. neumanni* is the species most commonly reported on humans, and the parasitism of this tick in cattle and horses is a common event (Guglielmone et al., 1991, 2006; Guglielmone and Nava, 2006). Furthermore, *Rickettsia bellii* and *R. amblyommii* were detected in *A. neumanni* from Córdoba province, Argentina (Labruna et al., 2007), and Gaido et al.

(1995) established that this tick species is an experimental vector of *Anaplasma marginale* to cattle.

A. neumanni is a catholic feeder. Actually, cattle can support its complete life cycle in nature (Nava et al., 2006a), but both adults and immature stages of *A. neumanni* have also been frequently collected on other large mammals such as *Mazama guazoubira*, peccaries, horses, goats, sheep, domestic pigs, and there are a few records on dogs and on the wild Canidae *Cerdocyon thous* and *Lycalopex gymnocercus* (Guglielmone and Nava, 2006). The diagnoses of *A. neumanni* are wrong in several keys for this genus as already discussed in Guglielmone and Hadani (1985) and Estrada-Peña et al. (2005) who present the relevant features to identify *A. neumanni* correctly. Nevertheless, Voltzit (2007) presents morphological features (and host) of alleged *A. neumanni* that correspond to *A. parvitarsum*.

Concerning the life cycle, Aguirre et al. (1999) carried out a study on the development of all stages of *A. neumanni* under laboratory conditions with immature ticks fed on rabbits and adults on calves. These authors found that the time necessary to complete the life cycle, was almost twice more than that required by other *Amblyomma* species recorded in the same phytogeographic domain and exposed to similar conditions. They concluded that the long pre-oviposition and incubation periods determined the long duration of the life cycle of this tick species. Studying the seasonal distribution of *A. neumanni* in Salta Province, Argentina, Guglielmone et al. (1981) found larvae in autumn and winter, nymphs in winter and spring, and adults in autumn, winter and spring, with the peaks of adults in autumn and winter varying according to ecological region

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and year. Also in Salta, in the work of Guglielmo et al. (1990), larvae were detected from May to October, with the peak of abundance in autumn, the nymphs from April to December, with the maximum abundance in winter, and the adults from April to December, with the peak in late autumn and early winter.

Knowledge on ecology of ticks of medical and veterinary importance is essential to design control strategies and to make epidemiological inferences about tick-borne diseases. Besides, the misidentification of medically important species in cryptic complexes can have serious negative consequences, and the analysis of DNA sequences is a widely used approach to detect the presence of cryptic taxa (Bickford et al., 2007). Because mitochondrial DNA sequences (mtDNA) evolve rapidly and show non-recombinational inheritance, they can be used to construct an intraspecific phylogeny of haplotypes (gene genealogy), allowing in that way the identification of cryptic species complexes and the inference of a population structure along a certain distribution (Avise, 2004). In this sense, sequences of mtDNA have been widely used in studies on intraspecific genetic variation of tick species belonging to different genera (Nava et al., 2009).

The purposes of this work were to study the life cycle in nature of *A. neumanni* of both parasitic and non-parasitic stages, and to carry out an analysis about the potential area of suitable habitat of this tick in Argentina, through a study of the climate niche. In addition, a preliminary analysis of the intraspecific genetic variation of *A. neumanni* using 16 rDNA sequences was carried out to detect the possibility of the occurrence of cryptic species complexes along its distribution in Argentina.

2. Materials and methods

2.1. Life cycle

The seasonal distribution of all parasitic stages of *A. neumanni* was determined by monthly examination of cattle. The counts of engorged larvae, engorged nymphs and females were performed from January 2005 to December 2006 on one side of 5 cows in Dean Funes (30°22'S 64°22'W), northwest of Córdoba Province, Argentina. The study area is located in the Chaqueño Serrano district of the Chaco Phytogeographic Province (Cabrera, 1994), its altitude is 500–600 m above sea level and the vegetation is characterized by forests of *Lithraea ternifolia*, *Schinopsis haenkeana*, *Condalia montana*, *Aspidosperma quebracho blanco*, *Prosopis nigra* and *Cercidium australe*, by the bushes *Flourensia campestris*, *Acacia caven*, *Baccharis flabellata*, *Croton sarcopetalus*, *Buddleja cordobensis*, *Larrea divaricata*, *Mimozyanthus carinatus*, *Celtis pallida* and *Geoffroea decorticans*, and a herbaceous stratum of perennial grasses. Fig. 1 shows the total monthly rain and mean monthly temperature during the sampling period, and Fig. 2 includes the monthly photoperiod by the use of the light intensity records of the 15th day of each month. For the statistical analysis the number of collected ticks on cows was duplicated, and the prevalence (P), median (M), and first and third quartiles (1Q–3Q) were obtained for each tick stage. Also in Dean Funes, the seasonal activity of host-finding ticks was studied by collecting both immature and adults of *A. neumanni* from vegetation, following the methodology described by Schulze et al. (1997), where two transects of 500 m were walked every month by an operator bearing a white overall (this technique was selected because the type of vegetation in the sampling sites precluded the use of dragging or flagging techniques). These samplings were made simultaneously with those of ticks on cattle, and Spearman's rank correlation was used to evaluate the correlation between the ticks collected monthly on cattle and from vegetation.

To assess aspects of the life cycle related to developmental phases in the environment, the engorged ticks monthly collected on cattle were weighed and kept in the laboratory at 25 °C and 83–86%

relative humidity, at three daily photoperiods, namely, 12 h light (L)–12 h dark (D), 14 h L–10 h D, and 10 h L–14 h D. These regimes were selected to be representative of the seasonal variations in the photoperiod that occur naturally along the year in the study area (see Fig. 2). Moulting success and pre-moult period of larvae and nymphs, pre-oviposition period and number of eggs laid by females, and minimum incubation period were recorded. Additionally, engorged ticks were exposed to field conditions in Dean Funes inside cylindrical tubes made of stainless-steel as described by Sutherst et al. (1978). Only one female was used per single tube while immatures were kept in groups of 5 specimens. The tubes were placed under the grass, protected from sun. Analysis of variance (ANOVA) was employed to test differences among photoperiod regimes, followed by an *a posteriori* Tukey's test (Zar, 1999). Pearson's correlation indices (r) were used to detect relationships between the weight of females and the number of eggs laid, between the weight of females and the pre-oviposition period, and between weight and pre-moult period of larvae and nymphs. The deterministic coefficient (r^2) for the significant ($P < 0.01$) correlations was indicated.

2.2. Climate niche model

A climate niche model for *A. neumanni* was created using MaxEnt version 3.0 (Phillips et al., 2004). MaxEnt creates species distributional models by combining presence of only data with layers of explanatory variables using a statistical approach known as maximum entropy. It finds a unique probability distribution that assumes the least biased distribution encoding the given information in the records. MaxEnt was running using point locality information from specimens collected in north-western Argentina. We used the WorldClim bioclimatic variables, which are biologically relevant temperature and precipitation layers (Hijmans et al., 2005) created by interpolating observed climate stations around the world, using a thin-plate smoothing spline set at a resolution of approximately 1 km, over the 30-year period from 1960 to 1990 (Hijmans et al., 2005). The climate variables included in such a data set are consecutively numbered and referred as follows: 1, annual mean temperature; 2, mean diurnal range; 3, isothermality; 4, temperature seasonality; 5, max temperature of warmest month; 6, min temperature of coldest month; 7, temperature annual range; 8, mean temperature of wettest quarter; 9, mean temperature of driest quarter; 10, mean temperature of warmest quarter; 11, mean temperature of coldest quarter; 12, annual precipitation; 13, precipitation of wettest month; 14, precipitation of driest month; 15, precipitation seasonality; 16, precipitation of wettest quarter; 17, precipitation of driest quarter; 18, precipitation of warmest quarter; 19, precipitation of coldest quarter. The modelling algorithm also provided with information about the most significant variables involved in the delineation of the climate envelope of the tick. An additional ANOVA was carried out over these significant variables, looking for statistically significant differences between sites where the tick is present and those where the tick is absent. The absence points included locations where the tick has been sampled but not recorded, and provides a comparable set of absence records, with the same collecting pressure, to ensure comparability. To avoid problems derived from points of absence near to sites of presence, only absence points located at a minimum of 10 km of the presence points were included. This analysis was based on a set of records compiled from the literature and from the unpublished collections made by the authors.

2.3. Genetic variation

To infer, in a preliminary way, the intraspecific variation of *A. neumanni*, DNA was extracted from specimens belonging to six

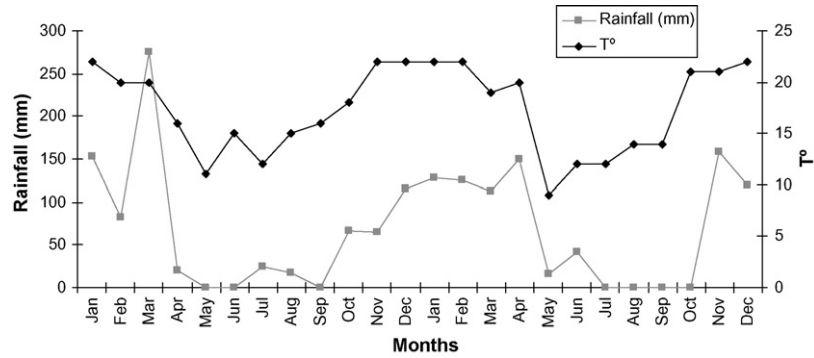


Fig. 1. Monthly rainfall and mean temperature (T°C) at the study area from January 2005 to December 2006.

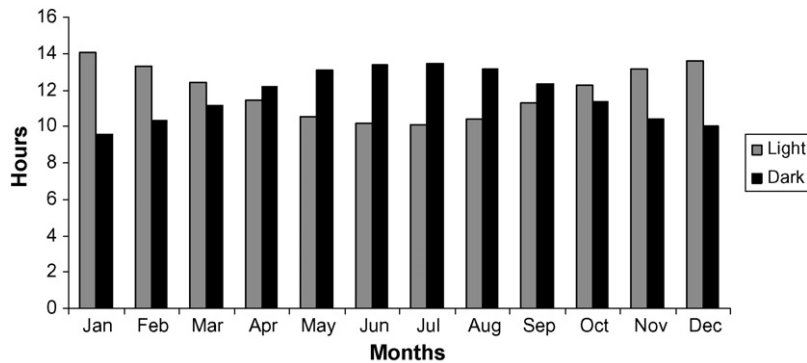


Fig. 2. Monthly photoperiod at the study area.

geographic populations, which are representative of the distribution of this tick species in Argentina. The sampled populations correspond to the localities of Dean Funes (30°22'S 64°22'W; Córdoba Province), Santa Bárbara (24°15'S 64°24'W; Jujuy Province), Cruz Quemada (25°02'S 64°58'W; Salta Province), Siete de Abril (26°16'S 64°29'W; Tucumán Province), Las Tipas (27°22'S 65°43'W; Tucumán Province) and Villa Ojo de Agua (29°29'S 63°41'W; Santiago del Estero Province) (Fig. 3). Five ticks per locality identified as *A. neumanni* by using the taxonomic keys of Guglielmono and Viñabal (1994) and Estrada-Peña et al. (2005) were included in the analysis. DNA extraction and polymerase chain reaction (PCR)

amplification followed the methodology described by Mangold et al. (1998), and the 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, Argentina) to obtain a ca. of 400-bp fragment of the mitochondrial 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. Phylogenetics analyses to determine the relationships among 16S rDNA gene haplotypes were performed with the maximum parsimony (MP) and neighbour-joining distance (NJ) criterions. The MP analysis was made using the heuristic search procedure, with close neighbour-interchange (CNI) at search level 3 in which the initial trees were obtained with the random addition of taxa (10 replicates), gaps were excluded and branch supports were assessed using bootstrap analysis with 1000 replications. A NJ tree was generated from the Tamura–Nei model, gaps were excluded in the pairwise comparison, and support for the NJ topology was tested by bootstrapping over 1000 replications. Pairwise comparison of sequences and the phylogenetics analyses were executed with the program Mega 4.0 (Tamura et al., 2007), and a sequence of *Ixodes loricatus* (IL) was used as outgroup.



Fig. 3. Administrative divisions of Argentina, showing the sample localities of the *Amblyomma neumanni* ticks used for the study of genetic variation.

3. Results

3.1. Life cycle

Prevalence and $M \pm 1Q-3Q$ of immature stages and females of *A. neumanni* collected on cattle per month are shown in Table 1. Larvae of *A. neumanni* were detected on cattle from middle and late

Table 1

Percentage of prevalence (P) median (M), first and third quartiles (1Q–3Q) of all parasitic stages of *Amblyomma neumanni* monthly collected on cattle between January 2005 and December 2006 in Dean Funes, Córdoba Province, Argentina.

Month	Larvae		Nymphs		Females	
	P	M (1Q–3Q)	P	M (1Q–3Q)	P	M (1Q–3Q)
January 2005	0	0	0	0	0	0
February 2005	40	0 (0–2)	0	0	0	0
March 2005	60	2 (0–2)	0	0	0	0
April 2005	100	178 (100–232)	0	0	20	0 (0–1)
May 2005	100	90 (82–93)	100	20 (15–27)	100	92 (66–100)
June 2005	100	22 (5–32)	100	76 (63–144)	100	14 (11–20)
July 2005	60	2 (0–2)	100	10 (9–41)	100	6 (6–12)
August 2005	0	0	100	14 (11–18)	80	8 (3–13)
September 2005	0	0	100	50 (36–157)	100	80 (43–175)
October 2005	0	0	80	20 (10–32)	100	462 (390–1382)
November 2005	0	0	40	0 (0–2)	100	238 (168–364)
December 2005	0	0	0	0	100	10 (4–20)
January 2006	0	0	0	0	0	0
February 2006	40	0 (0–2)	0	0	0	0
March 2006	40	0 (0–2)	0	0	20	0 (0–1)
April 2006	100	56 (32–94)	40	0 (0–2)	100	2 (0–7)
May 2006	100	102 (49–247)	80	10 (2–35)	100	144 (106–243)
June 2006	100	6 (4–55)	100	26 (18–77)	100	26 (15–38)
July 2006	40	0 (0–2)	100	60 (37–99)	100	10 (5–30)
August 2006	0	0	80	2 (2–6)	100	64 (48–102)
September 2006	0	0	40	0 (0–5)	100	364 (234–410)
October 2006	0	0	40	0 (0–2)	100	90 (41–144)
November 2006	0	0	0	0	100	4 (4–6)
December 2006	0	0	0	0	100	1 (0–1)

summer to early winter, with the peak of abundance in April and May, nymphs were collected from late autumn to early spring, with the peak of infestation in winter, and females were collected on cattle from autumn (April–May) to late spring (November–December) (Fig. 4). In the two years, the seasonal distribution pattern of females showed a bimodal curve, with a peak in May and other during early and middle spring. Larvae were detected on vegetation from April to July, nymphs from May to November with the peak of abundance in June and July, and females from May to November with the peak of abundance in October in the two years (Fig. 5). Spearman's rank correlation between monthly abundance of each stage on cattle and vegetation was positively significant (larvae: $r_s = 0.79$ and $P < 0.0001$; nymphs: $r_s = 0.89$ and $P < 0.0001$; females: $r_s = 0.83$ and $P < 0.0001$).

The weight (mg) and pre-moult period (days) of engorged larvae and nymphs of *A. neumanni* collected on cattle and maintained in the laboratory at three different photoperiods are shown in Table 2. The pre-moult period of larvae exposed under field conditions in May ranged between 22 and 27 days, and that of the nymphs exposed under field conditions in June and July between 29 and 38 days. Non-significant correlations were found between the weight

of immature stages and pre-moult period, under every condition of photoperiod and considering the total sample (Table 3).

The engorgement weight, pre-oviposition period and number of eggs laid by the females of *A. neumanni*, together with the minimum incubation period are presented in Table 4. The engorged females collected on cattle in the field and exposed at shortest photoperiod regimen (10 h light–14 h dark) under laboratory conditions, presented a statistically significant delay in the oviposition with respect to the other groups of females (ANOVA, $P < 0.01$). The information obtained from engorged females exposed under field conditions indicated that those collected on cattle in May oviposited in September (25%)–October (75%), while females collected in June and July oviposited in October (100%). All the females collected in September oviposited in November (40%)–December (60%), and those females collected in December oviposited in January (100%). The minimum incubation period under field conditions was 73.8 ± 6.1 days. There was a significant positive correlation between weight of females and number of eggs laid but not between the weight of females and the pre-oviposition period (Table 3).

3.2. Climate niche analysis

The climate niche model revealed a potential distribution as included in Fig. 6 in which both the actual records of the ticks and the contour of potential distribution are outlined over a background of the altitude. Analysis of climate niche of *A. neumanni* shows a strong dependence of a given set of variables, derived from temperature (mean, absolute maximum and minimum, and mean temperature in wettest and driest quarters) as well as from rainfall in warmest and coldest quarters. The presence of the tick is not related to the total yearly rainfall. A comparison between positive and negative sites for the presence of *A. neumanni* reveals that the species prefers colder sites ($P = 0.005$). Absolute maximum temperature experienced by populations of *A. neumanni* is around 30°C , while it is present at sites with only 4°C of minimum temperatures (Table 5). Concerning rainfall, it is interesting to notice that the tick needs a high amount of rain both in the warmer month and quarter, while it tolerates lower ranges of rain at both coldest and driest

Table 2

Weights and pre-moult periods of engorged larvae and nymphs of *Amblyomma neumanni* collected in the field on cattle. Ticks were maintained at 25°C and 83–86% relative humidity, at three daily photoperiods regimens: 12 h light (L)–12 h dark (D), 14 h L–10 h D, and 10 h L–14 h D.

Photoperiod	n	Weight (mg) Mean \pm SD (range)	Pre-moult period (days) Mean \pm SD (range)
Larvae			
12 L–12 D	24	0.96 \pm 0.22 (0.7–1.4)	12.54 ^b \pm 1.93 (9–15)
14 L–10 D	24	1.05 \pm 0.22 (0.7–1.4)	14.54 ^{ab} \pm 0.95 (13–16)
10 L–14 D	24	1.00 \pm 0.13 (0.8–1.3)	16.54 ^a \pm 1.58 (14–20)
Nymphs			
12 L–12 D	25	10.28 \pm 1.47 (8.9–12.7)	18.86 ^a \pm 0.94 (17–21)
14 L–10 D	25	11.03 \pm 2.31 (6.1–14.9)	19.07 ^a \pm 1.21 (17–21)
10 L–14 D	25	10.67 \pm 1.34 (9.3–12.9)	20.70 ^a \pm 1.33 (17–25)

SD: standard deviation.

^a ANOVA. Numbers not sharing superscripts are significantly different ($P < 0.01$).

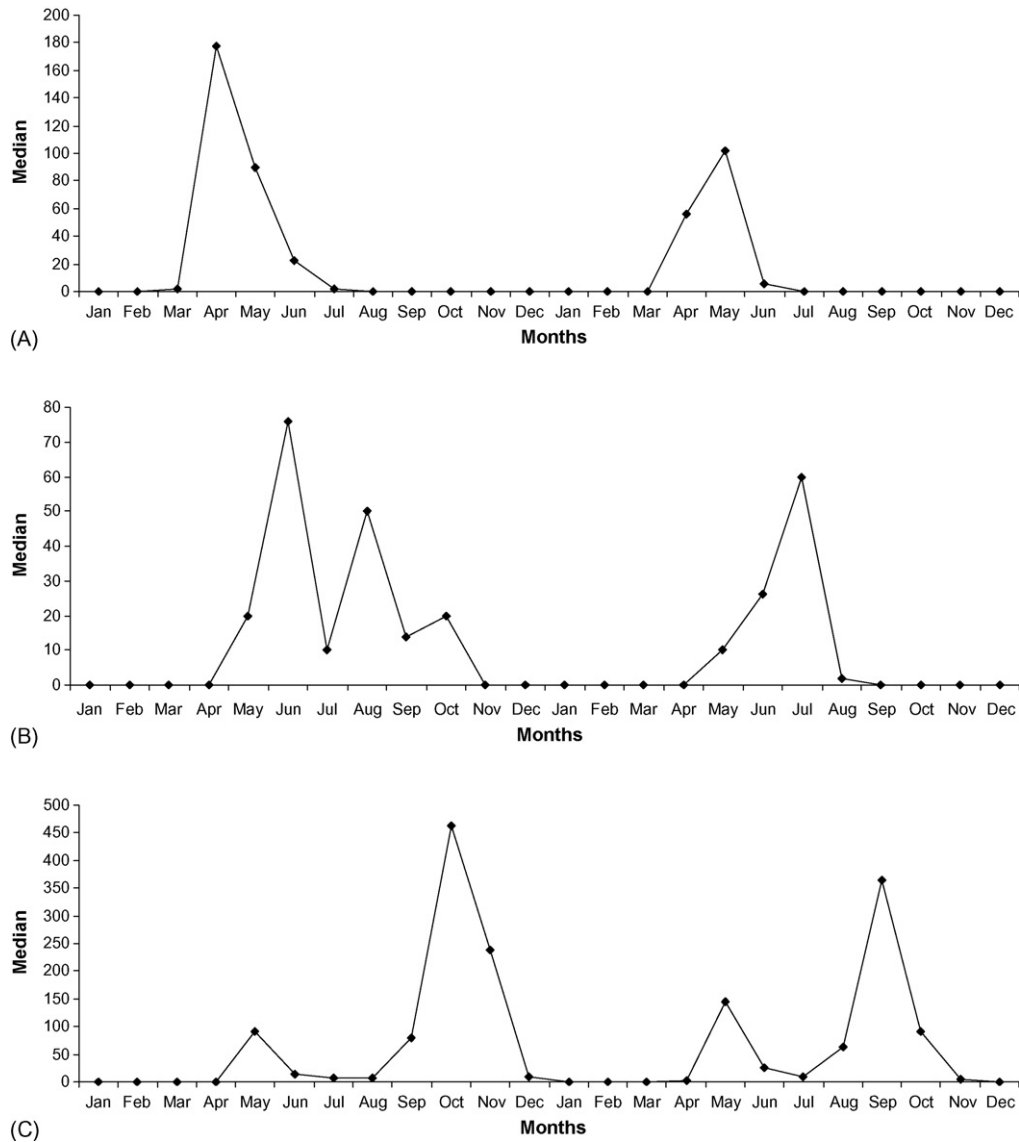


Fig. 4. Seasonal distribution of *Amblyomma neumanni* ticks collected on cattle in Dean Funes, Córdoba Province, Argentina, from January 2005 to December 2006. (A) Larvae; (B) nymphs; (C) adults.

periods. In other words, the tick seems to be sensible to evaporation, since it prefers high rain values at the season of high temperatures.

3.3. Genetic variation

Comparative analysis of the mitochondrial 16S rDNA sequences of *A. neumanni* ticks belonging to different Argentinean populations, showed that the intraspecific divergences not exceed 0.5% (0–0.5%) (Table 6). In the phylogenetic analysis, the trees obtained with MP and NJ (Fig. 7) showed similar topologies, without clades correlated with the geographic origin of the populations analyzed.

4. Discussion

In Dean Funes, the life cycle of *A. neumanni* in nature is characterized by the presence of larvae from late summer to early winter, with the peak of abundance in autumn, and by nymphs present in winter and early spring, with the peak in winter. These nymphs develop into the adult ticks that are in the environment and parasitizing their hosts during winter and spring. With the exception of

very few larvae collected on cattle in late summer, no *A. neumanni* ticks were detected in this season.

There were no significant differences in the pre-moult period among the groups of nymphs exposed at different photoperiods. Although the differences for this biological parameter were significant among the groups of larvae, it is unlikely that these differences have biological significance, because the extremes of range differed by 4.0 days. This result plus the data obtained with the ticks exposed under field conditions showed that the development of immature stages of *A. neumanni* probably is not regulated via diapause.

On the other hand, engorged females were strongly affected by the photoperiod regimens. Statistically significant differences were found in pre-oviposition period between engorged females exposed at different photoperiods in the laboratory. The engorged females exposed at shortest photoperiod regimen (10 h light–14 h dark) undergo morphogenetic diapause, expressed as a delay in the oviposition. Day length at temperate latitudes predicts future seasons more reliably than any other environment cue (Bradshaw and Holzapfel, 2007). Morphogenetic diapause is strongly connected with photoperiod and is defined as a delay of some steps in development of ticks, which makes it possible for the appearance of

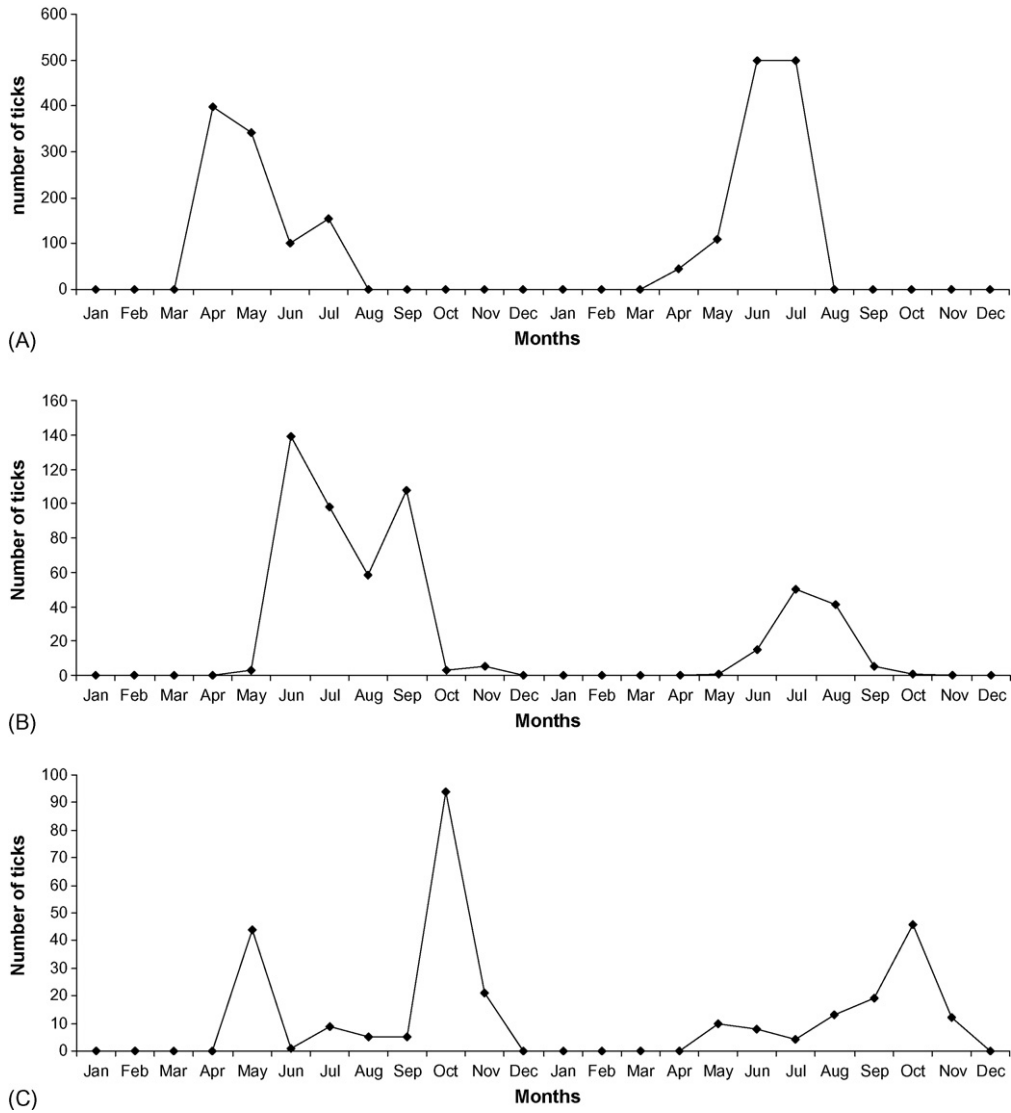


Fig. 5. Seasonal distribution of free-living *Amblyomma neumanni* ticks collected from vegetation in Dean Funes, Córdoba P rovince, Argentina, from January 2005 to December 2006. (A) Larvae; (B) nymphs; (C) adults.

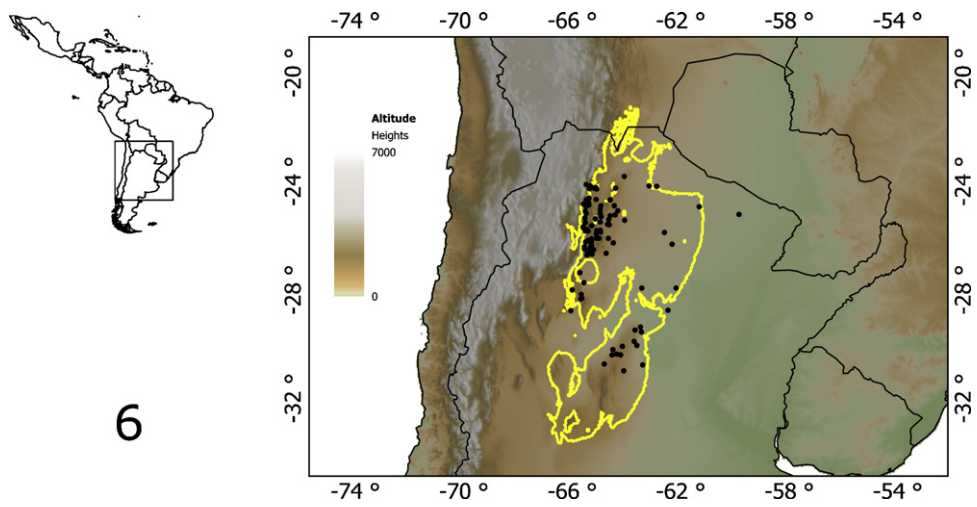


Fig. 6. Expected distribution of suitable climate for permanent populations of *A. neumanni*, covering a wide region (see inset at top, left) of pre-Andean Argentina, as well as some areas further at south-east. Points represent the actual records of the tick and the colored contour its potential distribution.

Table 3
Correlation (*r*) of biological parameters of immature and females of *Amblyomma neumanni* collected on cattle and maintained in the laboratory at 25 °C and 83–86% relative humidity, at three daily photoperiods: 12 h light (L)–12 h dark (D), 14 h L–10 h D, and 10 h L–14 h D.

<i>n</i>	Photoperiod	<i>x</i>	<i>y</i>	<i>r</i>	<i>r</i> ^{2a}
Larvae		Weight (mg)	Pre-moult period (days)		
24	12 h (L)–12 h (D)			0.26	NA
24	14 h (L)–10 h(D)			0.54	NA
24	10 h (L)–14 h(D)			0.14	NA
72	Total sample			0.23	NA
Nymphs		Weight (mg)	Pre-moult period (days)		
25	12 h (L)–12 h (D)			0.05	NA
25	14 h (L)–10 h(D)			0.05	NA
25	10 h (L)–14 h (D)			0.05	NA
75	Total sample			0.07	NA
Females		Weight (mg)	Pre-oviposition period		
36	12 h (L)–12 h (D)			0.20	NA
44	14 h (L)–10 h (D)			0.18	NA
46	10 h (L)–14 h (D)			0.35	NA
126	Total sample			0.12	NA
Females		Weight (mg)	No. of eggs laid		
36	12 h (L)–12 h (D)			0.83	0.70
44	14 h (L)–10 h (D)			0.79	0.54
46	10 h (L)–14 h (D)			0.85	0.91
126	Total sample			0.86	0.75

The deterministic coefficient (*r*²) for the significant correlations (*P* < 0.01) is indicated.

^a NA: not applicable.

Table 4
Mean, standard deviation (SD) and range of the engorgement weight, pre-oviposition period and number of eggs laid of the engorged *Amblyomma neumanni* females collected on cattle and maintained in the laboratory at 25 °C and 83–86% relative humidity, at three daily photoperiods: 12 h light (L)–12 h dark (D), 14 h L–10 h D, and 10 h L–14 h D. Minimum egg incubation period is also indicated.

Photoperiod	<i>n</i>	Engorgement weight Mean ± SD (range)	Pre-oviposition period Mean ^a ± SD (range)	Egg incubation period Mean ^a ± SD (range)	Number of eggs laid Mean ^a ± SD (range)
12 h (L)–12 h (D)	36	402.60 ± 75.57 (245–704.45)	15.72 ^b ± 3.90 (12–38)	47.75 ^b ± 1.46 (45–50)	1397.9 ^a ± 194.17 (601–2050)
14 h (L)–10 h (D)	44	352.81 ± 88.72 (151–523.50)	21.74 ^b ± 7.23 (13–35)	51.57 ^b ± 4.71 (43–59)	1204.2 ^a ± 312.67 (474–2165)
10 h (L)–14 h (D)	46	367.65 ± 89.03 (105–626)	78.00 ^a ± 12.72 (52–97)	61.13 ^a ± 10.67 (37–85)	1199.1 ^a ± 143.84 (489–1915)

^a ANOVA. Numbers not sharing superscripts are significantly different (*P* < 0.01).

later stages to be synchronized with appropriate season (Belozero, 1982). Therefore, the conclusion is that females of *A. neumanni* that feed and mate in autumn and early winter undergo morphogenetic diapause initiated by shortening days; they will lay eggs in spring, simultaneously with the females that feed and mate in this season. As a result, oviposition of *A. neumanni* is synchronized to coincide with the onset of rainy season in spring and early summer that characterizes the north of the Córdoba province (Fig. 1). This fact may have a biological significance, since it has been proven that both low temperatures and relative humidity are harmful to eggs and larvae of several tick species (Knülle and Rudolph, 1982; Randolph, 1999; Randolph and Storey, 1999; Sutherst and Bourne, 2006; Yoder et al., 2008). The pre-oviposition period observed in the ticks under field conditions support this conclusion, because all engorged females exposed in autumn and winter laid eggs in spring or early summer. This structure of the life cycle of *A. neumanni* agrees with the results achieved with the climate niche analysis, because it shows that the tick prefers high rain values at the season of high temperatures.

The incubation periods of *A. neumanni* eggs were rather long for an ixodid tick, independently of the photoperiod regimen. As expected for ticks, there is a positive correlation between weight of engorged females and number of eggs laid. Long pre-oviposition and egg incubation periods reported in this study with female ticks feeding under natural conditions are in agreement with the results found by Aguirre et al. (1999) for *A. neumanni* feeding in the laboratory. Accordingly, there is strong evidence to conclude that the life cycle of *A. neumanni* is regulated via morphogenetic diapause of engorged females and by a long embryogenesis, with the long

period of incubation explaining the interval between the peak of females and the peak of larvae.

An important matter to resolve is the origin of the females that constitute the peak of May. It is speculated that the females of *A. neumanni* that conform the peak during autumn are formed by specimens unable to found a host in the previous spring and undergo behavioural diapause in summer, or by adults ticks that entered in behavioural diapause after ecdysis in spring. The development of summer diapause in *A. neumanni* was already suggested by Guglielmo and Hadani (1982) and Guglielmo et al. (1990) being based on the absence of active ticks in this season, and the

Table 5
Values of significant reported as significantly variables between sites where *Amblyomma neumanni* is present and sites where the tick has been sampled but not found.

Variable	Absent	Present
Annual mean temperature	21.4 (2.3)	18.3 (2.1)
Max temperature warmest month	33.9 (2.0)	30.2 (2.7)
Min temperature coldest month	7.4 (3.0)	4.2 (2.4)
Mean temperature wettest quarter	26.2 (2.1)	22.9 (2.3)
Mean temperature driest quarter	15.8 (3.0)	13.0 (2.1)
Mean temperature warmest quarter	26.6 (2.7)	23.2 (2.5)
Mean temperature coldest quarter	15.6 (2.7)	12.6 (2.0)
Precipitation of wettest quarter	317.8 (57.8)	350.6 (81.5)
Precipitation of driest quarter	26.5 (18.7)	18.0 (14.0)
Precipitation of warmest quarter	312.9 (56)	342.0 (77.1)
Precipitation of coldest quarter	33.8 (26.4)	19.5 (16.7)

Table shows mean values and standard deviation between parentheses. Values for temperature derived variables are in degree centigrade; values for rainfall related variables are millimeters of water.

Table 6
Pairwise nucleotide divergence (%) among 16S rDNA sequences of *Amblyomma neumanni* ticks collected in different localities of Argentina.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
1	-																														
2	0.3	-																													
3	0.3	0	-																												
4	0.3	0	0	-																											
5	0.5	0.3	0.3	0.3	-																										
6	0.3	0	0	0	0.3	-																									
7	0.3	0	0	0	0.3	0	-																								
8	0.3	0	0	0	0.3	0	0	-																							
9	0.3	0	0	0	0.3	0	0	0	-																						
10	0.3	0	0	0	0.3	0	0	0	0	-																					
11	0.3	0	0	0	0.3	0	0	0	0	0	-																				
12	0.3	0	0	0	0.3	0	0	0	0	0	0	-																			
13	0.3	0	0	0	0.3	0	0	0	0	0	0	0	-																		
14	0.5	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	-																	
15	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	-																
16	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	-															
17	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	-														
18	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	-													
19	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	-												
20	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	-											
21	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	-										
22	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	-									
23	0.5	0.3	0.3	0.3	0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	-								
24	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	-							
25	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	-						
26	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	-					
27	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0	-				
28	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	-			
29	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-		
30	0.3	0	0	0	0.3	0	0	0	0	0	0	0	0	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	

1-5 (GenBank: FJ965564-FJ965568): Dean Funes, Córdoba Province; **6-10** (GenBank: FJ965589-FJ965593): Santa Bárbara, Jujuy Province; **11-15** (GenBank: FJ965579-FJ965583): Cruz Quemada, Salta; **16-20** (GenBank: FJ965574-FJ965578): Siete de Abril, Tucumán Province; **21-25** (GenBank: FJ965584-FJ965588): Las Tipas, Tucumán Province; **26-30** (GenBank: FJ965569-FJ965573): Villa Ojo de Agua, Santiago del Estero Province.

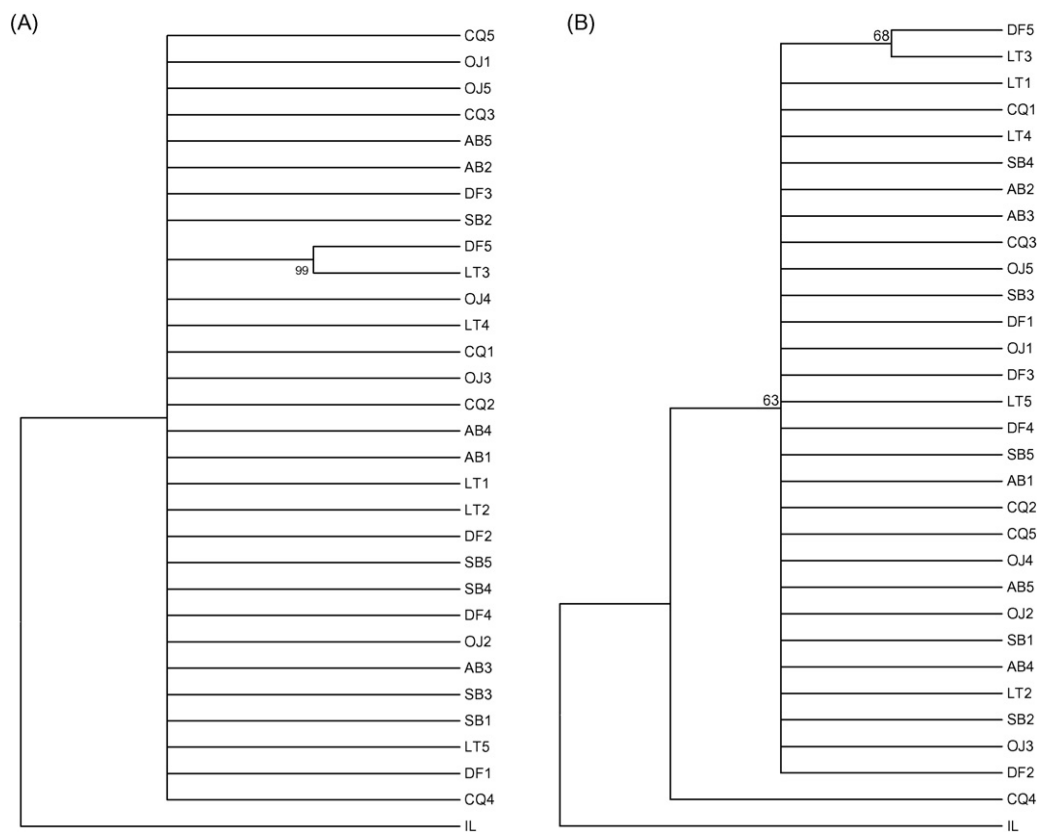


Fig. 7. Maximum parsimony (A) and neighbour-joining (B) trees based on 16S rDNA sequences of *Amblyomma neumanni* ticks collected in different localities of Argentina. Only bootstraps >50% are present. DF: Dean Funes, Córdoba Province; SB: Santa Bárbara, Jujuy Province; CQ: Cruz Quemada, Salta Province; AB: Siete de Abril, Tucumán Province; LT: Las Tipas, Tucumán Province; OJ: Villa Ojo de Agua, Santiago del Estero Province. *Ixodes loricatus* (IL) was used as outgroup (GenBank accession number AY510268).

presence of different ticks cohorts in the same year it has been established for other species such as *I. ricinus* (Walker, 2001) and *Dermacentor variabilis* (Burg, 2001). Future studies focused on the development of behavioural diapause of *A. neumanni* adults during summer and about the possible presence of two cohorts of females in the same year should be carried out.

Previous research on the ecology of *A. neumanni* in Argentina was carried out by Guglielmo et al. (1981, 1990) in Salta province, which is located approximately 650 km to the north of Dean Funes. The principal difference with the results of this work is that the peak of adults in spring was not detected in Salta. Intraspecific variation in the tick seasonality pattern from one area to another, or from year to year in the same locality, is a frequent event which is influenced by external factors that determine the population dynamics of these ectoparasites, as was demonstrated, for example, for *A. americanum* (Kollars et al., 2000a), *A. maculatum* (Teel et al., 1998; Barker et al., 2004), *Rhipicephalus appendiculatus* (Randolph, 1999), *D. variabilis* (Kollars et al., 2000b), *I. kingi* (Salkeld et al., 2006), *I. rubicundus* (Fourie et al., 1996), *I. scapularis* (Ogden et al., 2005), *I. sculptus* (Salkeld et al., 2006) and *I. ricinus* (Talleklint and Jaenson, 1997; Randolph et al., 2002). Moreover, because development and intensity of the diapause are latitudinal-dependent, intraspecific geographic variation in photoperiod responses can occur within same species (Madder et al., 2002; Bradshaw and Holzapfel, 2007). Therefore, extrapolation of the results recorded for a particular area to other geographic points should be made carefully. According to climate niche studies, adequate suitability for *A. neumanni* exists in a rather large area in western Argentina and small portions of southern Bolivia, mainly close to the pre-Andes area, and in sites located south-eastern to this main distribution area.

Considering that variation in time and space of the age structure of a vector (relative proportion of each age class in a population) is a factor that causes fluctuations in its vectorial capacity (Black IV and Moore, 2005), and that the first line of defense against tick bites is to avoid high-risk habitats during peak tick activity periods (Piesman and Esisen, 2008), there is no doubt that the ecological information regarding to seasonal distribution of each tick stage is essential to prevent or to control the tick parasitism. In the case of *A. neumanni*, control may be directed against the larvae. Treatment of cattle with acaricides in autumn would reduce the number of larvae, with the consequent decrease in the number of nymphs and adults originating from those larvae. Furthermore, the application of acaricides in autumn would have effect on the females that reach its peak in May. From a medical point of view, the risk of human infestation with *A. neumanni* ranges between March and December, given that both immature and adults stages are prone to bite humans (Guglielmo et al., 1991; Nava et al., 2006b).

The low degree of genetic divergence observed suggests that there are no elements to suppose that the specimens morphologically identified as *A. neumanni* belong to more than one species. Both MP and NJ trees were similar with a polytomic pattern, and they did not show population genetic subdivision in a regional scale. In *A. neumanni*, the absence of inter-population genetic differentiation probably is due to the inexistence of natural geographic barriers that isolate the populations, and its low host-specificity, and to the high vagility of their principal hosts (wild and domestic ungulates). One expectation is that parasites with broad host range are more likely to experience low genetic differentiation among populations than those with narrow host range (Barrett et al., 2008). In this sense, it is recognized the importance of the migratory potential of the hosts in the modulation of tick population genetics (Mccoy

et al., 2003). *A. neumanni* is a tick species commonly associated to cattle, and it has been demonstrated that broadly distributed livestock parasites usually are characterized by lack of genetic population structure (Blouin et al., 1995; Rosenthal, 2008), because the host movement allows a high gene flow. However, this assumption on the intraspecific variation of *A. neumanni* should be considered as preliminary evidence. Studies with larger sample sizes and the use of other molecular markers are needed to confirm the results obtained in this study.

Acknowledgements

We acknowledge to O. Warnke, J. Martinez and F. Seguro for their support in the field work, and INTA, Asociación Cooperadora INTA Rafaela, Fundación ArgenINTA, and CONICET for their financial assistance to SN, AJM and AAG.

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