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Ecology and genetic variation of *Amblyomma tonelliae* in Argentina

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Abstract. The ecology of *Amblyomma tonelliae* (Ixodida: Ixodidae), including its seasonal distribution and the development periods of each stage, was investigated during a study carried out over two consecutive years in northwestern Argentina. In addition, the genetic variation of this tick was studied through analyses of 16S rDNA sequences. *Amblyomma tonelliae* has a 1-year lifecycle characterized by a long pre-moult period in larvae with no development of morphogenetic diapause. Larvae peak in abundance during late autumn and early winter; nymphs peak in abundance in spring, and adults do so from late spring to early summer. *Amblyomma tonelliae* shows a marked ecological preference for the driest areas of the Chaco ecoregion. In analyses of 16S rDNA sequences in genes from different populations of *A. tonelliae*, values for nucleotide diversity and the average number of nucleotide differences showed genetic diversity within this species to be low. No significant differences were found in comparisons among populations.

Key words. *Amblyomma tonelliae*, biology, lifecycle, population ecology.

Introduction

Ticks are blood-feeding ectoparasites of worldwide distribution with the capacity to transmit more agents of disease to animals and humans than any other arthropod (Sonenshine & Roe, 2014). About 208 species of tick have been described as distributed across the Neotropical biogeographic region, among which the ixodid *Amblyomma* represents the genus with the highest species richness (Barros-Battesti *et al*., 2013; Guglielmone *et al*., 2014). Some of the species of greatest medical and veterinary importance belong to *Amblyomma*, which are common parasites of humans and domestic mammals and act as vectors of pathogenic organisms (Guglielmone *et al*., 2003; Labruna, 2009).

Amblyomma cajennense (Fabricius) was considered for a long time to be a tick species of sanitary importance with a wide distribution from the southern U.S.A. to northern Argentina, but recent studies have demonstrated that this taxon is in fact a complex of six species, namely, *A. cajennense sensu stricto*, *Amblyomma sculptum* Berlese, *Amblyomma mixtum* Koch, *Amblyomma tonelliae* Nava, Beati and Labruna, *Amblyomma patinoi* Labruna, Nava and Beati, and *Amblyomma interandinum* Beati, Nava and Cáceres (Beati *et al*., 2013; Nava *et al*., 2014). This new taxonomic scheme is supported by biological, molecular and morphological evidence (Labruna *et al*., 2011; Mastropaolo *et al*., 2011; Beati *et al*., 2013; Nava *et al*., 2014). Furthermore, the distributions of each of the six species appear to be associated with particular ecological areas (Beati *et al*., 2013; Estrada-Peña *et al*., 2014). In Argentina, immature and adult specimens of the *A. cajennense* complex infest livestock massively (Guglielmone & Nava, 2013) and are second only to *Amblyomma neumanni* Ribaga in their frequency of occurrence on humans (Guglielmone *et al*., 1991). This represents significant information because fatal cases of spotted fever in humans, caused by infection with *Rickettsia rickettsii*, have

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been confirmed in parts of Jujuy Province and ticks of the *A. cajennense* complex are recognized as vectors of this *Rickettsia* (Ripoll *et al*., 1999; Paddock *et al*., 2008).

Amblyomma tonelliae is one of the species of the *A. cajennense* complex and is distributed in dry areas of the Chaco biogeographic province *sensu* Morrone (2006) in Argentina, Bolivia and Paraguay (Nava *et al*., 2014). This tick is a catholic feeder and has been recorded parasitizing humans and wild and domestic mammals, such as cattle, horses, dogs, *Tayassu pecari* (Link), *Tayassu tajacu* (Linnaeus), *Catagonus wagneri* (Rusconi) (all: Artiodactyla: Tayassuidae), *Tamandua tetradactyla* (Linnaeus) (Pilosa: Myrmecophagidae), *Hydrochoerus hydrochaeris* (Linnaeus) (Rodentia: Caviidae), *Puma concolor* (Linnaeus) (Carnivora: Felidae) and *Mazama* sp. (Artiodactyla: Cervidae) (Nava *et al*., 2014). Further, specimens of *A. tonelliae* were recently found to be infected with '*Candidatus* Rickettsia amblyommii' and *Rickettsia* sp. strain El Tunal in northwestern Argentina (Tarragona *et al*., 2015).

The integration of data on the biology, population dynamics, geographical distribution and genetic variation of a species is necessary in order to understand vector and vector-borne disease dynamics. Therefore, as *A. tonelliae* has relevance both as a parasite and as a potential vector of pathogenic microorganisms, we present a study on the ecology and genetic variation of this species in Argentina through analyses of both field and laboratory data.

Materials and methods

The field study was performed in El Tunal (25°13′ S, 64°22′ W), Salta Province, Argentina. This area lies within the Chaco ecoregion (Burkart *et al*., 1999) and is characterized by thorny, semi-deciduous forests formed principally of *Schinopsis lorentzii* Engl., *Aspidosperma quebracho-blanco* Schltdl., *Ziziphus mistol* Griseb., *Prosopis nigra* (Griseb.) Hieron and *Prosopis kuntzei* Harms. A variable percentage of the total area of a forest is occupied by shrubs that originated as a consequence of overgrazing by cattle and through the introduction of exotic pastures such as *Panicum maximum* var. *Gatton panic.* The climate is markedly seasonal, with annual rainfall of 600–700 mm concentrated from October to March (spring and summer).

The seasonal distribution of all parasitic stages of *A. tonelliae* was determined by the monthly examination of cattle over 2 years. Twenty-four counts of engorged larvae, engorged nymphs and females were performed from January 2010 to December 2011 on one side of each of five cows. In addition, questing ticks (adults and immature stages) were collected by monthly drag-sampling of the vegetation using a 1.0 × 1.5-m white cloth flag. Dragging was performed for periods of 30 min, during which collected ticks were removed from drags every 10 m. These samplings were made simultaneously with those of ticks on cattle. Spearman's rank correlation was used to evaluate the correlation between ticks collected monthly on cattle and vegetation, respectively. Ticks were identified according to Martins *et al*. (2014) and Nava *et al*. (2014) and were also compared with known laboratory-reared material deposited in the tick collection of the Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina. Additionally, engorged larvae and nymphs collected on cattle were allowed to moult into nymphs and adults, respectively, at 25 °C and 83–86% relative humidity (RH), in order to facilitate the accurate morphological determination of their species.

To study the developmental phases of *A. tonelliae*, the pre-moult periods of larvae and nymphs, pre-oviposition period of females, and minimum incubation period of eggs were recorded under field and laboratory conditions. The ticks used in these experiments originated from the progeny of engorged females collected from cattle in the study area. These females were fed on rabbits. In the field, engorged ticks were enclosed in stainless steel wire mesh envelopes (5 × 5 cm) placed on the ground under grass cover which protected them from direct solar irradiation. Only one female was deposited per envelope, whereas immature stages were kept in groups of 10 specimens. The temporal pattern of the exposures was designed with reference to the seasonal trend of each stage detected in samples of ticks in both parasitic and non-parasitic phases.

A laboratory study was designed to determine the effect of photoperiod on the development of free-living stages of *A. tonelliae*. Engorged ticks of each stage were weighed and kept in the laboratory at 25 °C and 83–86% RH, under three photoperiods (LD 12 : 12 h, LD 14 : 10 h, and LD 10 : 14 h), which represent the natural seasonal variation of the photoperiod in the study area. The trials were designed as follows: (a) on the day of detachment, three groups of engorged females were assigned randomly to the LD 12 : 12 h, LD 14 : 10 h and LD 10 : 14 h photoperiod regimes and the pre-oviposition periods of females and minimum incubation periods of eggs were recorded; (b) after hatching, larvae of each group were kept for 30 days under the original photoperiod regime and fed on rabbits, after which the subsequently engorged larvae originating from each photoperiod regime were divided into three subgroups, of which one was maintained under the original photoperiod regime and the other two were distributed to the other two regimes; (c) nymphs developing from the engorged larvae were subdivided as described in (b) for larvae, and (d) females obtained from the engorged nymphs of each photoperiod regime were kept for 30 days under the original photoperiod and then fed on rabbits. The subsequently engorged females developing from each photoperiod regime were divided into three subgroups, of which one subgroup from each regime was maintained under the conditions of its original photoperiod regime and the other two were distributed among the other regimes. As a result of this design, nine subgroups of larvae, nymphs and adults exposed to different conditions of photoperiod before and after feeding were evaluated. Statistical differences among photoperiod regimes were tested with an analysis of variance (ANOVA) followed by an *a posteriori* Tukey test (Zar, 1999). The *t*-test was used to compare weights and pre-moult periods in nymphs moulting to males and females, respectively.

The distribution of *A. tonelliae* in Argentina was determined using locality records published in Nava *et al*. (2014) and by examination of the tick collection at INTA Rafaela. All localities were plotted against the ecological regions described by Burkart *et al*. (1999) (Fig. 1). Genetic variation in populations

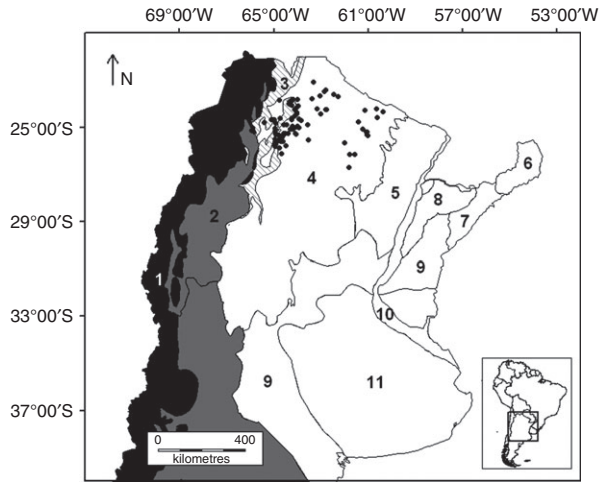

Fig. 1. Argentinean ecoregions according to Burkart *et al*. (1999): 1, Altos Andes; 2, Puna; 3, Selva de las Yungas; 4, Chaco Seco; 5, Chaco Húmedo; 6, Selva Paranaense; 7, Campos y Malezales; 8, Esteros del Iberá; 9, Espinal; 10, Delta e Islas del Paraná; 11, Pampa. Closed circles indicate sites of *Amblyomma tonelliae* collections.

of *A. tonelliae* was assessed using sequences of the mitochondrial 16s rRNA gene. DNA was extracted from adult specimens belonging to populations from nine geographic locations (Table 1), which are representative of the distribution of this tick species in Argentina. Forty-one ticks identified as *A. tonelliae* following Nava *et al*. (2014) were included in the analysis. DNA extraction and polymerase chain reaction (PCR) amplification following the methodology described by Mangold *et al*. (1998a, 1998b) were performed to obtain an approximately 410-bp fragment of the mitochondrial 16S rRNA gene. The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual editing where necessary, and aligned with the program ClustalW (Thompson *et al*., 1994).

Phylogenetics analysis to determine relationships among 16S rDNA gene haplotypes was performed using the maximum likelihood (ML) method in MEGA5 (Tamura *et al*., 2011). The best substitution model for the phylogenetic tree constructed with 16S sequences was the GTR (generalized time-reversible) model obtained using gamma distribution (+G), which was determined with the Akaike information criterion as implemented in MODELTEST Version 3.7 (Posada & Crandall, 1998). Support for the topologies was tested by bootstrapping over 1000 replications. Sequences of *Amblyomma americanum* (Linnaeus) (GenBank accession no. L34314), *A. sculptum* (GenBank accession no. KM519934) and *A. mixtum* (GenBank accession no. KM519935) were chosen as outgroups. Sequences were also used to build a neighbour-joining (NJ) distance-based phylogenetic tree. The total number of segregating sites, nucleotide diversity [defined as the average proportion of nucleotide differences between all possible pairs of sequences in the sample (Hartl & Clark, 1997)], number of haplotypes (h), haplotype diversity [defined as the probability that two random sequences are different (Rozas, 2009)] and average number of nucleotide differences were calculated using DnaSP Version 5.10 (Rozas *et al*., 2005). One haplotype was considered to differ from another haplotype when they differed by one base.

Results

Larvae of *A. tonelliae* were collected on cattle from April to June; collections peaked in number in May and June (late autumn and early winter). Nymphs were collected on cattle from June to December, with the peak infestation occurring from September to November (spring). Females were collected on cattle throughout the year and peaked in abundance from December to February (late spring and early summer) (Fig. 2). Larvae were detected on vegetation from April to July, nymphs from July to December, and females from October to May (Fig. 3). Immature and adult ticks collected in both parasitic and non-parasitic phases showed similar patterns of seasonality (Figs 2 and 3). Spearman's rank correlations between monthly abundances of ticks collected on cattle and vegetation, respectively, were positively significant for all stages (larvae: $r_s = 0.74$ and $P < 0.0001$; nymphs: $r_s = 0.67$ and $P = 0.002$; females: $r_s = 0.65$ and $P < 0.002$). Specimens of *Rhipicephalus* (*Boophilus*) *microplus* Canestrini (Ixodida: Ixodidae) (immatures and adults), *A. neumanni* (immatures and adults) and *Amblyomma parvum* Aragão (adults) were also found on the cattle examined during the study period.

Engorged larvae, nymphs and females of *A. tonelliae* were exposed under field conditions in autumn (May), spring (September) and summer (January), respectively. This pattern of exposures was designed with consideration of the peak

Table 1. Geographic populations of *Amblyomma tonelliae* from Argentina included in the analysis of 16S rDNA sequences.

Province	Locality	Latitude	Longitude	Sequences, *n*	16S rDNA haplotype code
Salta	La Estrella	23°49′S	64°4′W	6	II, III
Salta	Las Tortugas	24°16′S	64°5′W	5	II
Salta	Virgilio Tedin	25°3′S	64°59′W	5	II
Salta	El Tunal	25°13′S	64°22′W	5	II
Jujuy	Saladillo	24°2′S	64°15′W	5	II
Jujuy	Palma Sola	23°58′S	64°18′W	4	II
Santiago del Estero	Parque Nacional Copo	25°55′S	61°43′W	4	I, IV
Formosa	El Simbolar	24°16′S	61°8′W	3	I
Chaco	Castelli	25°56′S	60°37′W	4	I

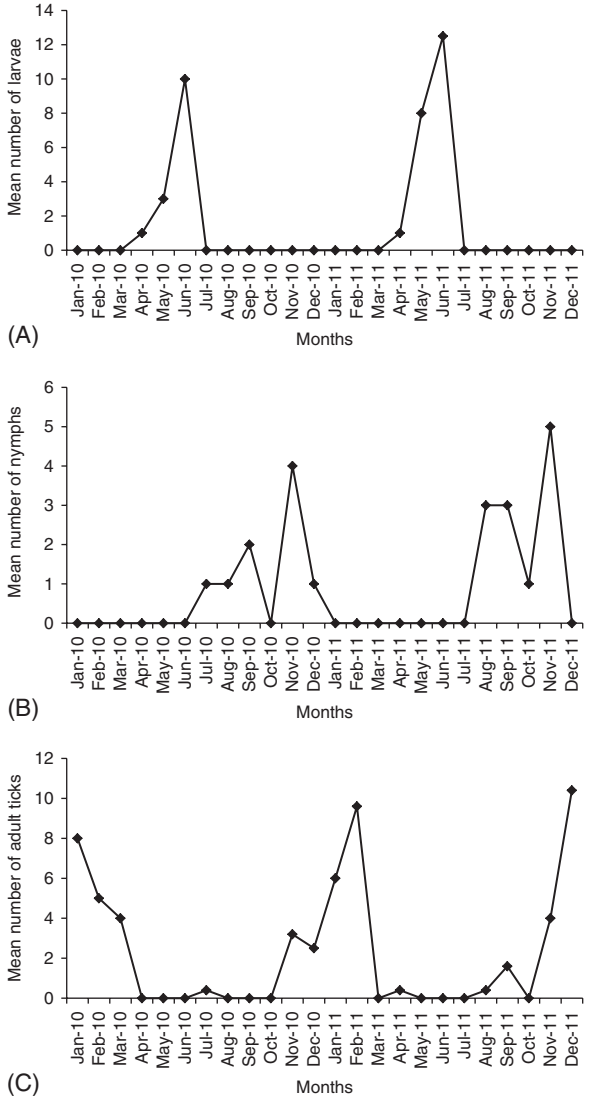

Fig. 2. Seasonal distributions of *Amblyomma tonelliae* ticks collected on cattle from January 2010 to December 2011: (A) larvae; (B) nymphs, and (C) adults.

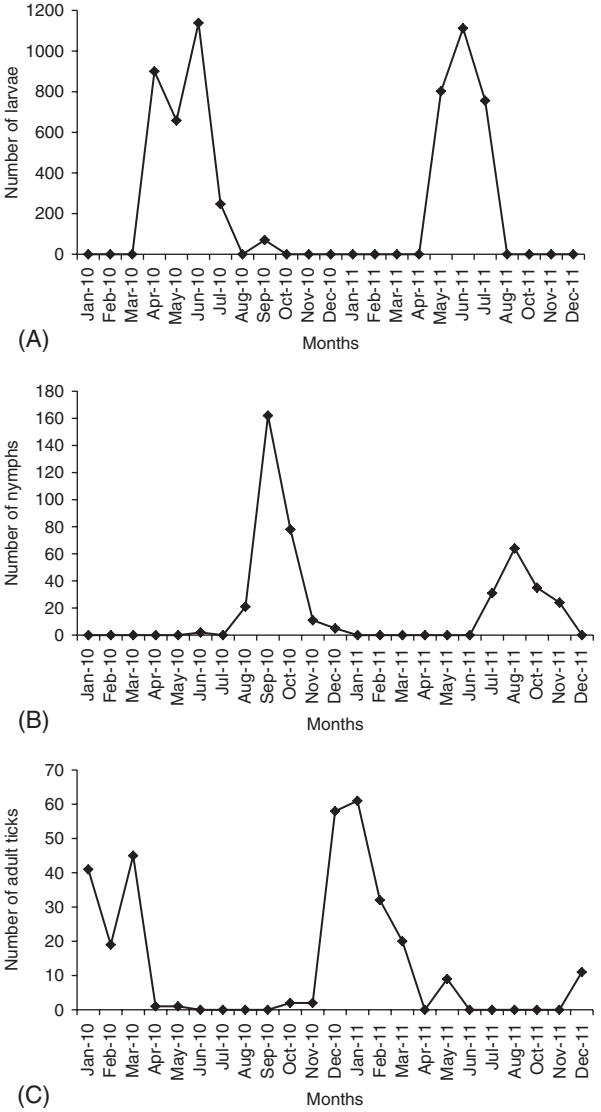

Fig. 3. Seasonal distributions of *Amblyomma tonelliae* ticks collected from vegetation from January 2010 to December 2011: (A) larvae; (B) nymphs, and (C) adults.

in abundance detected for each stage in both parasitic and non-parasitic phases (Figs 2 and 3). The minimum pre-moult periods of larvae ($n = 170$) and nymphs ($n = 230$) under field conditions were 61 days and 40 days, respectively. The minimum pre-oviposition period of females ($n = 136$) exposed in January was 12 days and the minimum incubation period of eggs was 47 days.

The biological parameters of *A. tonelliae* ticks maintained in the laboratory under different photoperiods are shown in Table 2 (pre-moult period of engorged larvae), Table 3 (pre-moult period of engorged nymphs) and Table 4 (pre-oviposition period of females). In some cases statistically significant differences in the pre-moult periods of larvae and nymphs exposed to different photoperiods were detected (Tables 2 and 3). However, the magnitude of these differences does not appear to have biological significance because they ranged from only 14.1 to 16.3 days in larvae, and 19.3 to 23.5 days in nymphs. The same occurs in the pre-oviposition period of females and minimum incubation period of eggs (Table 4). The pre-oviposition period of females ranged from 8.9 to 13.5 days, and the incubation period of eggs ranged from 38.3 to 43.9 days. The difference in the mean ± standard deviation (SD) engorgement weight of nymphs moulting to males (13.3 ± 2.0 mg, $n = 161$) and females (18.1 ± 1.6 mg, $n = 174$), respectively, was statistically significant ($P < 0.0001$), but the difference between the mean ± SD pre-moult periods of nymphs moulting to males (21.3 ± 1.9 days) and females (20.9 ± 1.5 days), respectively, was not significant ($P = 0.13$).

Records of *A. tonelliae* in Argentina were restricted to the ecoregions of Chaco Seco and Selva de las Yungas (Fig. 1), but most of the findings reported applied to the Chaco Seco

Table 2. Pre-moult period and weight of larvae of *Amblyomma tonelliae* maintained in the laboratory at 25 ± 1 °C and 85 ± 5% relative humidity under different photoperiods.

Light period (before/after feeding)	Larvae, *n*	Weight, mg, mean ± SD (range)	Pre-moult period, days, mean ± SD (range)∗
10 h/10 h	150	0.98 ± 0.09 (0.8–1.1)	14.2^{a} ± 0.7 (14–16)
10 h/12 h	150	0.92 ± 0.07 (0.7–1.1)	14.9^{b} ± 0.9 (14–17)
10 h/14 h	150	0.94 ± 0.10 (0.7–1.1)	15.5^{bc} ± 0.6 (14–16)
12 h/10 h	150	0.93 ± 0.09 (0.8–1.0)	16.3^{c} ± 0.9 (14–18)
12 h/12 h	117	0.94 ± 0.09 (0.8–1.1)	14.5^{ab} ± 0.9 (14–16)
12 h/14 h	150	0.87 ± 0.07 (0.8–1.0)	14.2^{a} ± 0.7 (13–15)
14 h/10 h	128	0.94 ± 0.09 (0.8–1.1)	14.1^{a} ± 0.6 (14–15)
14 h/12 h	150	0.90 ± 0.09 (0.6–1.0)	14.2^{a} ± 0.8 (14–16)
14 h/14 h	113	0.90 ± 0.07 (0.8–1.1)	14.5^{ab} ± 0.9 (14–16)

∗ANOVA with Turkey's test.
Different superscript letters indicates statistically significant differences.
SD, standard deviation.

Table 3. Pre-moult period and weight of nymphs of *Amblyomma tonelliae* maintained in the laboratory at 25 ± 1 °C and 85 ± 5% relative humidity under different photoperiods.

Light period (before/after feeding)	Nymphs, *n*	Weight, mg, mean ± SD (range)	Pre-moult period, days, mean ± SD (range)∗
10 h/10 h	50	17.5 ± 0.7 (9.7–24.4)	20.2^{de} ± 0.1 (19–22)
10 h/12 h	49	17.1 ± 0.9 (5.9–23.9)	20.3^{cd} ± 0.1 (19–22)
10 h/14 h	50	16.7 ± 1.1 (9.3–24.0)	22.9^{ab} ± 0.1 (21–27)
12 h/10 h	40	17.6 ± 1.0 (11.1–25.7)	21.0^{c} ± 0.1 (19–24)
12 h/12 h	41	17.8 ± 1.0 (10.9–25.4)	20.5^{cd} ± 0.1 (19–24)
12 h/14 h	41	15.9 ± 1.5 (9.8–24.3)	23.5^{a} ± 0.2 (21–29)
14 h/10 h	22	14.1 ± 0.9 (9.1–22.2)	19.3^{e} ± 0.1 (18–21)
14 h/12 h	22	15.2 ± 1.3 (9.3–21.8)	20.0^{de} ± 0.3 (18–26)
14 h/14 h	22	16.2 ± 2.1 (6.0–20.5)	22.5^{b} ± 0.3 (20–25)

∗ANOVA with Turkey's test.
Different superscript letters indicates statistically significant differences.
SD, standard deviation.

ecoregion and its areas of transition with the Selva de las Yungas ecoregion. Forty-one 16S rDNA sequences of *A. tonelliae* ticks collected from different populations in Argentina were analysed. The sequences consisted of only four unique haplotypes (GenBank accession nos: haplotype I, KM507359; haplotype II, KM507360; haplotype III, KM507361; haplotype IV, KM507362), which were clustered in a monophyletic clade (Table 1, Fig. 4). Neighbour-joining reconstructions showed similar topologies and are not shown. The total number of segregating sites and values for haplotype diversity, nucleotide diversity and the average number of nucleotide differences for all sequences were three, 0.45, 0.001 and 0.53, respectively.

Discussion

The results of this study show that *A. tonelliae* has a lifecycle of one generation per year. Larvae are active from early autumn to mid-winter and peak in abundance in late autumn. Nymphs are found from early winter to spring and peak in early spring, and females are found during spring and summer and peak in early and mid-summer. Adult ticks predominate during the rainy season and immature stages do so in the dry season. Consequently, the oviposition of females occurs in the rainy season. This finding has biological significance because low RH has been shown to be harmful to eggs of ticks (Randolph, 1999; Sutherst & Bourne, 2006). Thus, this behaviour in *A. tonelliae* may preclude oviposition under environmental conditions that are unfavourable for egg development. Field studies in Argentina and southern Brazil have shown species of the *A. cajennense* complex also to have a lifecycle of one generation per year and a similar seasonal pattern. In northwestern Argentina, Guglielmone *et al*. (1990) found larvae to be concentrated between June and August, nymphs to predominate from August to November, and adults to peak in abundance between November and January. With a few variations, results on the seasonality of *A. cajennense* sensu lato (probably *A. sculptum*) obtained in southern Brazil were similar to those found in Argentina. Labruna *et al*. (2002) found larvae concentrated between April and June, nymphs between June and October, and adults between October and March. Oliveira *et al*. (2000, 2003) found high abundances of larvae between April and July, nymphs to be concentrated from June to October, and adults to prevail between September and March. The same pattern of seasonal distribution was described by Szabó *et al*. (2007). All of these studies show that species of the *A. cajennense* complex have similar natural lifecycles in the southern ranges of their distributions. In the Nearctic region (Texas, U.S.A.), the overall pattern of seasonality recorded for another species of the *A. cajennense* complex, *A. mixtum* (named as *A. cajennense*) (Beck *et al*., 2011), was similar to those patterns described in Argentina and Brazil. The principal difference refers to a more extended period of activity in larvae and nymphs throughout the year in the case of *A. mixtum* (Beck *et al*., 2011).

The observations made under laboratory conditions on the developmental rate of immature stages (pre-moult period) and females (pre-oviposition period and minimum incubation period of eggs) of *A. tonelliae* were similar to those presented by Guglielmone *et al*. (1992), who conducted a laboratory study with a tick colony of *A. cajennense s.l.* originating from ticks collected at a site in Argentina (Cruz Quemada, Salta Province) near El Tunal and located in the same phytogeographic province. The biggest differences do not exceed values (expressed as means) of 3.3, 3.1, 6.4 and 5.8 days for the pre-moult periods of larvae and nymphs, pre-oviposition period of females and minimum incubation period of eggs, respectively. As expected for ticks, in both studies nymphs moulting to females were heavier than those moulting to males. Data on the developmental phases of *A. tonelliae* obtained with the field exposures of engorged ticks are adjusted appropriately to data for the seasonal distribution of each stage because they explain the temporal interval among the peaks of each stage. Minimum values for the pre-oviposition period of females (12 days) and minimum incubation period of eggs (47 days) under field conditions were similar to those found under laboratory conditions, independent of photoperiod regimen (Table 4). Conversely, minimum pre-moult periods of larvae (61 days) and nymphs (40 days) under field conditions were longer than the pre-moult periods of larvae and

Table 4. Pre-oviposition period and weight of females of *Amblyomma tonelliae*, and incubation periods of eggs maintained in the laboratory at 25 ± 1 °C and 85 ± 5% relative humidity under different photoperiods.

Light period (before/after feeding)	Females, *n*	Pre-oviposition period, days, mean ± SD (range)∗	Weight, mg, mean ± SD (range)	Egg incubation period, days, mean ± SD (range)∗
10 h/10 h	10	11.5^{ab} ± 2.2 (9–15)	506.2 ± 43.1 (336–791)	42.1^{ab} ± 1.3 (40–45)
10 h/12 h	11	13.0^{a} ± 2.2 (9–17)	524.4 ± 41.9 (377–685)	39.0^{cd} ± 0.5 (38–40)
10 h/14 h	10	13.5^{a} ± 2.2 (9–17)	451.9 ± 35.6 (288–632)	42.3^{ab} ± 0.6 (41–43)
12 h/10 h	9	12.7^{a} ± 2.3 (7–17)	447.8 ± 35.8 (291–600)	42.0^{ab} ± 0.7 (41–44)
12 h/12 h	15	10.7^{ab} ± 2.0 (7–16)	539.9 ± 24.7 (398–705)	40.2^{bcd} ± 1.8 (37–44)
12 h/14 h	8	11.5^{ab} ± 2.0 (7–14)	525.1 ± 52.2 (302–665)	41.2^{bc} ± 1.4 (38–44)
14 h/10 h	10	8.9^{b} ± 1.5 (7–15)	554.2 ± 24.9 (455–712)	43.9^{a} ± 1.0 (42–45)
14 h/12 h	10	9.4^{b} ± 2.1 (7–15)	514.7 ± 31.5 (375–658)	38.3^{d} ± 1.2 (36–41)
14 h/14 h	17	9.5^{b} ± 1.3 (7–14)	526.3 ± 21.7 (400–727)	41.5^{b} ± 1.4 (38–45)

∗ANOVA with Turkey's test.
Different superscript letters indicates statistically significant differences.
SD, standard deviation.

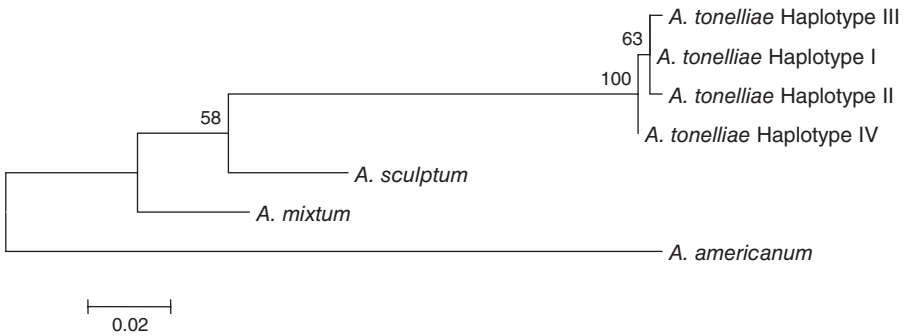

Fig. 4. Maximum-likelihood tree constructed from 16S rDNA sequences of *Amblyomma tonelliae*. Numbers represent bootstrap support generated from 1000 replications. Details of each sequence are presented in Table 1.

nymphs exposed to the nine different photoperiods under laboratory conditions (Tables 2 and 3). This disparity among the developmental periods of engorged ticks exposed under field and laboratory conditions has been recorded previously in other tick species (Yu *et al*., 2010; Nava *et al*., 2011; Meng *et al*., 2014) and emphasizes that any analysis of laboratory data to infer the duration of lifecycle in ticks in nature should be made with caution. Thus, daily temperature fluctuations (e.g. lower temperatures at night) may hypothetically explain the longer pre-moult periods of larvae and nymphs in the field compared with those maintained under laboratory conditions because the laboratory temperature remained constant during the entire experiment.

Morphogenetic diapause is a pre-adaptive behaviour expressed by a delay in embryogenesis, in the metamorphosis of larvae and nymphs, or in the oogenesis of engorged females, and is principally regulated by photoperiod (Belozerov, 1982). Particularly in the southern cone of South America, the lifecycle of *A. neumanni*, a tick species sympatric with *A. tonelliae* in areas of northern Argentina, has been shown to be regulated by the morphogenetic diapause of engorged females (Nava *et al*., 2009). The presence of morphogenetic diapause in the lifecycle of *A. tonelliae* was investigated in this work by means of laboratory trials but, at least under the conditions in which the experiments were performed, the results showed no indication of morphogenetic diapause at any stage. The integrated analysis of field and laboratory data indicates that *A. tonelliae* has a 1-year lifecycle characterized by a long pre-moult period in larvae with no development of morphogenetic diapause. However, studies carried out in Brazil (Labruna *et al*., 2003; Cabrera & Labruna, 2009) found the lifecycle of *A. cajennense s.l.* (probably *A. sculptum*) to be modulated by the behavioural diapause of larvae born during the spring and summer that initiate simultaneous feeding activity during the autumn. Although the results obtained for *A. tonelliae* under laboratory conditions suggest the absence of morphogenetic diapause, the regulation of the lifecycle of this tick by the development of behavioural diapause in larvae should be tested in further studies.

Amblyomma tonelliae displays a marked ecological preference for the driest areas of the Chaco ecoregion, as previously suggested by Beati *et al*. (2013) and Estrada-Peña *et al*. (2014). Given that *A. tonelliae* is a catholic feeder with a wide host range, it can be concluded that environmental variables rather than hosts determine the distributional ranges of this tick species. Values of nucleotide diversity and the average number of nucleotide differences showed that genetic diversity within *A. tonelliae* is low in comparison with the intraspecific genetic divergence of 16S sequences of other *Amblyomma* species such as *A. americanum* (Linnaeus) (Mixson *et al*., 2006;

Trout *et al*., 2010) and *A. parvum* (Nava *et al*., 2008). All parasitic stages of *A. tonelliae* are commonly associated with cattle in an area without natural geographical barriers and it is recognized that broadly distributed livestock parasites are usually characterized by a low genetic interpopulation differentiation because host movement allows for high gene flow (Hilburn & Sattler, 1986a; Blouin *et al*., 1995; Rosenthal, 2008). A lack of genetic differentiation similar to that among populations of the cattle-associated tick *A. tonelliae* has also been reported for other species of *Amblyomma* that are common parasites of cattle, such as *A. americanum* (Hilburn & Sattler, 1986b) *A. neumanni* (Nava *et al*., 2009) and *Amblyomma variegatum* (Fabricius) (Beati *et al*., 2012).

Considering larvae, nymphs and adults in combination, the results of this work show that *A. tonelliae* ticks actively seek hosts in all seasons (Figs 2 and 3). Because both immature and adult stages of *A. tonelliae* are attracted to humans, cattle and horses, the risk for infestation with *A. tonelliae* in these hosts extends throughout the year. This temporal ubiquity and the aggressive behaviour of all parasitic stages highlight the medical and veterinary importance of *A. tonelliae* as a parasite *per se* and as a potential vector of infectious agents along its geographical distribution in the southern cone of South America.

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