Study of the life cycle of *Amblyomma dubitatum* (Acari: Ixodidae) based on field and laboratory data

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Abstract   The life cycle of *Amblyomma dubitatum* was described based on the seasonal distribution of all parasitic stages and the development periods of engorged ticks under different conditions of photoperiod and temperature. All stages were found active along the entire year in the study area. Larvae peaked from May to July, nymphs peaked from July to October, and females peaked from November to March. This pattern represents a life cycle with one generation per year with most of the ticks reaching adulthood during the warmest months. The analysis of the effect of the photoperiod on the development of *A. dubitatum* showed no indication of morphogenetic diapause. Exposure of ticks to field conditions indicates a delay in metamorphosis of immature stages, in the oviposition of females and in the incubation of eggs, which were associated with low winter temperatures. The results indicate that though *A. dubitatum* has a one year life cycle, more than one cohort can co-exist within the same population in a certain interval of time. Finally, the potential role of small rodents as hosts for larvae and nymphs of *A. dubitatum* is confirmed.

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Introduction

Ticks (Acari: Ixodida) are hematophagous ectoparasites of terrestrial tetrapods with capacity to transmit disease agents to domestic and wild animals, and also to humans (Jongejan and Uilenberg 2004; Guglielmone et al. 2014). The knowledge of the life cycle is important not only to understand demographic patterns of ticks, but also to evaluate the potential influence of these parasites on host ecology and as vectors of infectious diseases. In this sense, the spatial and temporal risk of tick-borne disease is associated with the distribution, abundance and seasonal dynamics of vector ticks (Randolph 1999, 2008).

Amblyomma dubitatum is a South American tick with most of the records concentrated in the north-east of Argentina, south-east of Brazil, east of Paraguay and north half of Uruguay (Nava et al. 2010). The capybara *Hydrochoerus hydrochaeris* is the principal host for both immature and adult stages of *A. dubitatum*, but recent findings suggest that other mammals as small rodents (Caviidae and Cricetidae) and marsupials (Didelphidae) can be alternative hosts for the larvae and nymphs of this tick (Dantas-Torres et al. 2010; Nava et al. 2010; Debárbora et al. 2012). Also, adults of *A. dubitatum* were occasionally found in large mammals as cattle, horses, *Tapirus terrestris*, *Mazama gouazoubira*, *Tayassu pecari*, *Axis axis* and *Sus scrofa* (Nava et al. 2010; Debárbora et al. 2012), and there are records of all parasitic stages of *A. dubitatum* biting humans in Argentina, Brazil and Uruguay (Labruna et al. 2007; Nava et al. 2010). Although the role of *A. dubitatum* as vector of human diseases is undetermined, Labruna et al. (2004b) found specimens of this tick (namely as *Amblyomma cooperi*) infected with *Rickettsia parkeri* strain COOPERI in Brazil.

Previous studies have described the developmental rates of *A. dubitatum* ticks feeding on different hosts under laboratory conditions (Almeida et al. 2001; Labruna et al. 2004b), and there are some works where information on seasonality of *A. dubitatum* in Brazil was provided (de Souza et al. 2006; Szabó et al. 2007; Toledo et al. 2008). However, the integrative study of ecological and biological aspects of ticks such as seasonal dynamics, natural hosts and factors affecting the developmental phase of the free-living stages, must be performed in order to better understand the life cycle of these parasites under natural conditions. Therefore, the goal of this work is to describe the life cycle of *A. dubitatum* by integrating field and laboratory data on seasonal distribution and developmental rates of all stages of *A. dubitatum* in Argentina. Because it was hypothesized that small rodents (Caviinae and Sigmodontinae) can be relevant for the life cycle of *A. dubitatum* due to the potential role as alternative hosts for immature stages (Dantas-Torres et al. 2010; Nava et al. 2010; Debárbora et al. 2012), the natural infestation of these mammals with larvae and nymphs of *A. dubitatum* was also evaluated.

Materials and methods

The study under field conditions was performed in Estancia "Rincón del Socorro" (28°42′S 57°29′W), Corrientes Province, northeastern Argentina. This site is located in a large wetland, Esteros del Iberá, which is characterized by swamps and marshlands that connect an extensive system of shallow lakes (Neiff and Poi de Neiff 2006). Masses of floating vegetation, known locally as "embalsados," are present in the lagoons, where *Schoenoplectus californicus*, *Thypha* spp., *Fuirena robusta* and *Cyperus giganteus* are the dominant plants (Neiff et al. 2011). Terrestrial environment is principally characterized by the presence of shrubland forests and temporarily flooded grasslands. Monthly mean
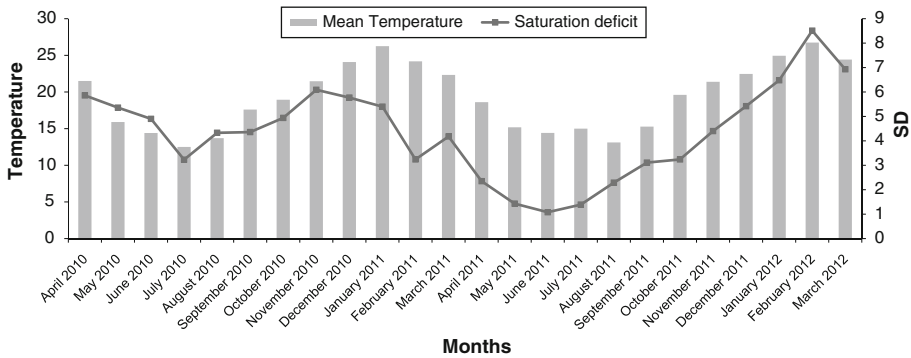
Fig. 1 Monthly mean temperature (°C) and saturation deficit (SD) (mmHg) at the study area from April 2010 to March 2012

temperature (C°) and saturation deficit (mmHg) obtained during the entire study period are presented in Fig. 1. Climate data were recorded using HOBO® data loggers (U23-002 Pro v2).

The seasonal dynamics of all parasitic stages of *A. dubitatum* was determined by monthly examination of *H. hydrochaeris*, which were captured by shot during a program to control overpopulation. Counts of engorged larvae, engorged nymphs and females were made from April 2010 to March 2012. Three specimens of *H. hydrochaeris* were captured per month. The taxonomic determination of larvae, nymphs and adults of *A. dubitatum* collected on *H. hydrochaeris* was made following Joan (1930), Guglielmone and Viñabal (1994) and Martins et al. (2010), and by comparison with known laboratory-reared material deposited in the tick collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina. Prevalence (number of hosts infested/number of hosts examined), mean (with 95 % bootstrap confidence intervals) and median (with first and third quartiles) of the infestation were calculated for each parasitic stage.

The development phases of *A. dubitatum* were studied at both laboratory and field experiments. The ticks used were originated from the progeny of engorged females collected on *H. hydrochaeris* in the study area that were fed on guinea pigs (larvae and nymphs) and rabbits (adults). The laboratory trials were designed to determine the effect of photoperiod on the development of free-living stages of *A. dubitatum*. The engorged ticks were kept in the laboratory at 25° C and 83–86 % relative humidity, at three daily photoperiods (12 h light (L)–12 h dark (D), 14 L–10 D, and 10 L–14 D) that represent the natural seasonal variation of the photoperiod in the study area. The experiments were designed as follow: (1) On the day of detachment, three groups of engorged females were assigned randomly to either 12 L–12 D, 14 L–10 D, and 10 L–14 D photoperiod regimes. Pre-oviposition period of females and minimum incubation period of eggs were recorded. (2) After hatch, larvae of each group were kept by 30 days at the original photoperiod regime and then they were fed on guinea pigs, and the resulting engorged larvae originated from each photoperiod regime were divided into three subgroups. One subgroup from each regime was exposure under the original photoperiod regime, and the other two were distributed among the other regimes. (3) The nymphs obtained from the engorged larvae were subdivided as described in point 2 for larvae. (4) Females obtained from the engorged nymphs of each photoperiod regimes were kept by 30 days at the original photoperiod

regime and then they were fed on rabbits. The resulting engorged females originated from each photoperiod regime were divided into three subgroups, one subgroup from each regime was exposed under the original photoperiod regime, and the other two were distributed among the other regimes. Thus, according to the photoperiodic conditions to which each stage was exposed before and after feeding, nine subgroups of larvae, nymphs and adults of *A. dubitatum* were evaluated (see Tables 2, 3, 4). Analysis of variance (ANOVA) was employed to test differences among photoperiod regimes, followed by an a *posteriori* Tukey test (Zar 1999). Linear correlation coefficient (*r*) was applied to examine relationships between the weight of females and the pre-oviposition period, and between weight and pre-moult period of larvae and nymphs.

Under field condition, the effect of different temperatures and photoperiods on the development phases was determined throughout exposures of engorged ticks (larvae, nymphs and females of *A. dubitatum*) in the study area. The exposures were designed in order to obtain data from each season. The dates of each exposure are as follow: engorged larvae, April, June, August, September, November, February; engorged nymphs, November, December, January, August, September, March; engorged females, January, April and June (see details in Figs. 3 and 4). Pre-moult period of larvae and nymphs, pre-oviposition period of females and minimum incubation period of eggs were the biological parameters recorded.

To assess the role of wild rodents as alternative hosts for *A. dubitatum*, specimens of the subfamilies Caviinae and Sigmodontinae were captured in two-days trapping session using four linear grids each one with 25 Sherman live-trap-type baited with food pellets and seeds, and one trapping linear grids with 50 Tomahawk live-trap-type baited with carrots. The samples were carried out in spring 2010, autumn 2011, winter 2011, spring 2011 and summer 2012. Sigmodontinae rodents were killed in the field, preserved in individual nylon bags with 96 % ethanol, and examined for ticks later in the laboratory. Specimens of Caviinae were checked for ticks, identified in the field and marked by a cutting the hair on their backs and released. Prevalence, mean and median (with first and third quartiles) of the infestation were obtained for each rodent species. To examine the distribution of immature of *A. dubitatum* within each host population, the index of discrepancy (*D*) (Poulin 1993, 2007) and the parameter *k* (Krebs 1999) were estimated by using Quantitative Parasitology 3.0 (Rózsa et al. 2000). In D, 0 constitutes null aggregation (all hosts with equal level of infestation) and 1 complete aggregation (all members of a parasite population on one individual host). The non parametric test of Kruskal–Wallis followed by Dunn's test was used to compare the frequency distributions of number of ticks of each stage among seasons, and prevalence were compared by using Fisher's exact test. Sigmodontines were identified by U.F.J Pardiñas (Centro Nacional Patagónico (CONICET), Puerto Madryn, Argentina), and cavies were determined following Redford and Eisenberg (1992) and Díaz (2000).

Results

A total of 7,695 larvae, 3,206 nymphs and 1,871 females of *A. dubitatum* were recorded on 72 specimens of *H. hydrochaeris*. Prevalence, mean and median of immature stages and females of *A. dubitatum* collected on *H. hydrochaeris* per month are shown in Table 1. In the two years, the seasonal distribution of the three parasitic stages of *A. dubitatum* showed a similar pattern. Larvae were found from January to November with the peak of abundance from May to July (mid-autumn to mid-winter) (Table 1, Fig. 2a). Nymphs and

Table 1 Percentage of prevalence (*P*), mean (with 95 % bootstrap confidence intervals) and median (with first and third quartiles) of all parasitic stages of *Amblyomma dubitatum* monthly collected on *Hydrochoerus hydrochaeris* in Corrientes Province, Argentina

| Months	Larvae			Nymphs			Females		
	P (%)	Mean (CI 95 %)	Median (1Q–3Q)	P (%)	Mean (CI 95 %)	Median (1Q–3Q)	P (%)	Mean (CI 95 %)	Median (1Q–3Q)
January	50	17 (2.6–36)	8 (0–32)	66.7	2.8 (1–5.3)	2.5 (0–4)	100	74.1 (46.3–103.5)	73 (36–108)
February	83.3	58.3 (22.3–123.1)	29 (20–99)	83.3	6.5 (2–18.1)	3 (1–6)	100	53 (35.5–93.6)	45 (33–54)
March	100	29.3 (12.5–64.6)	17.5 (6–35)	100	9.8 (5.3–13.3)	10.5 (7–13)	83.3	47.5 (19.3–84.3)	37 (14–88)
April	100	62.1 (24.5–113)	41 (17–130)	83.3	10.8 (3.5–20.1)	6.5 (3–20)	50	1.6 (0.3–3.1)	1 (0–3)
May	100	540.3 (203–1,308.1)	327 (50–648)	100	49 (8.5–106.6)	14.5 (4–104)	83.3	10.5 (4–17.5)	10 (2–18)
June	100	105.5 (78.3–127.6)	104 (84–131)	100	29.3 (11.8–47.3)	25.5 (5–56)	50	6.8 (1.3–17.1)	2 (0–8)
July	100	353.5 (138–705.8)	292 (30–457)	100	94.1 (62.5–121.3)	94 (76–116)	50	9 (1.6–23.3)	4.5 (0–10)
August	83.3	90.1 (27–160.5)	77 (4–170)	100	148.8 (88.6–276.3)	114 (65–160)	100	4.1 (2.3–6)	4 (2–6)
September	50	22 (0.6–52)	2 (0–56)	83.3	92.6 (24.3–193.8)	61 (2–143)	83.3	9.1 (3.6–18.8)	7.5 (1–12)
October	33.3	4 (0–11.6)	0 (0–2)	66.7	80.8 (17–156.3)	49.5 (0–166)	83.3	6 (1.8–11.5)	3 (1–13)
November	16.7	0.1 (0–0.3)	0 (0–0)	66.7	5.3 (2–8.3)	6 (0–8)	100	52.3 (29.1–77.6)	49.5 (27–74)
December	0	0 (0–0)	0 (0–0)	83.3	4.1 (1.5–6.6)	4 (1–7)	100	37.5 (19.3–51.8)	40.5 (19–58)
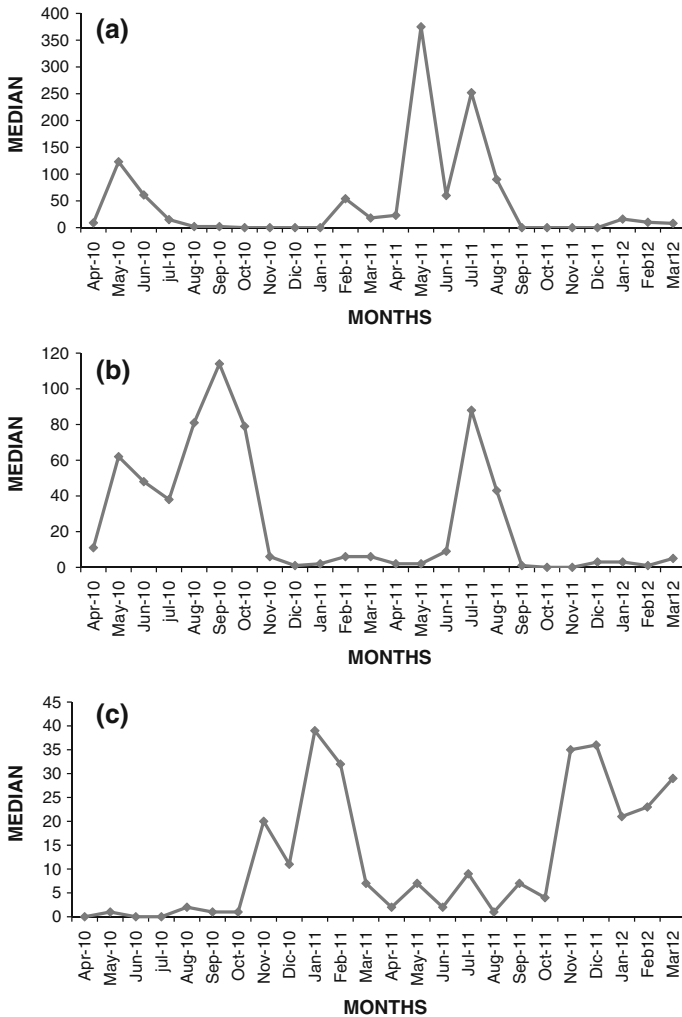
Fig. 2 Seasonal distribution of *Amblyomma dubitatum* ticks collected on *Hydrochoerus hydrochaeris* in Corrientes Province, Argentina. **a** Larvae; **b** nymphs; **c** females

females were collected along the entire year, nymphs peaked from July to October (mid-winter to mid-spring), and females peaked from November to March (late spring to late summer) (Table 1, Figs. 2b, c).

The results of the laboratory analysis on the effect of the photoperiod on the development of each tick stage are showed in Tables 2 (larvae), 3 (nymphs) and 4 (females and eggs). The weight (mg) and pre-moult period (days) of engorged larvae and nymphs of *A. dubitatum* that were fed on rabbits and maintained in the laboratory at the nine combinations of photoperiods regimens are showed in Table 2 for larvae and in Table 3 for nymphs. Although there were statistically significant differences among the pre-moult period of larvae exposed to the different photoperiods (Table 2), the magnitude of the differences does not appear to have biological significance because they ranged between

Table 2 Pre-moult period (days) and weight (mg) of larvae of *Amblyomma dubitatum* maintained in the laboratory at 25 ± 1 °C and 85 ± 5 % relative humidity under different photoperiod regimens

Photoperiod (before feeding/ after feeding)	n	Weight (mg) Mean ± SD (range)	Pre-moult period (days) Mean ± SD (range)*
10 L–14 D/10 L–14 D	250	1.1 ± 0.1 (0.8–1.3)	14.2[d] ± 0.1 (12–22)
10 L–14 D/12 L–12 D	180	1.1 ± 0.1 (0.8–1.3)	15.3[c] ± 0.2 (12–23)
10 L–14 D/14 L–10 D	114	1.0 ± 0.1 (0.8–1.3)	14.0[d] ± 0.2 (12–22)
12 L–12 D/10 L–14 D	61	1.0 ± 0.1 (1–1.1)	21.7[ab] ± 0.3 (16–31)
12 L–12 D/12 L–12 D	221	1.1 ± 0.0 (1.0–1.2)	21.2[b] ± 0.2 (16–34)
12 L–12 D/14 L–10 D	252	1.0 ± 0.1 (1.0–1.2)	22.5[a] ± 0.1 (17–35)
14 L–10 D/10 L–14 D	605	1.1 ± 0.0 (0.9–1.2)	12.9[e] ± 0.0 (12–21)
14 L–10 D/12 L–12 D	321	1.2 ± 0.0 (0.9–1.5)	12.5[f] ± 0.0 (12–19)
14 L–10 D/14 L–10 D	311	1.0 ± 0.1 (0.8–1.3)	12.4[f] ± 0.0 (12–19)

* ANOVA with Tukey's test. Numbers not sharing superscripts are significantly different ($P < 0.05$)

n Number of ticks, *L* light, *D* dark

14.0 and 22.5 days. The same occurs in the case of the pre-moult period of the nymphs (Table 3), where the differences were also significant but the values ranged from 20.1 to 22.6 days. No statistically significant differences were found in the pre-oviposition period of females and minimum incubation period of eggs (Table 4). Pre-moult periods of immature stages and pre-oviposition period of females were lesser than one month, and the incubation period of the eggs was between 36.8 and 41.2 days. Correlations between weight and pre-moult period of larvae and nymphs and pre-oviposition period of females were not significant (larvae: r = 0.10, $P = 0.09$; nymphs: r = 0.11, $P = 0.16$; females: r = 0.07, $P = 0.41$).

Engorged larvae, nymphs and females of *A. dubitatum* were exposed in the study area during the four seasons of the year in order to evaluate the effect of temperature and photoperiod on the developmental phases under field conditions. Maximum and minimum values (expressed in days) obtained for each biological parameter are showed in Figs. 3 (pre-moult period of larvae and nymphs) and 4 (pre-oviposition period of females and incubation of eggs). In all cases, the higher values were observed in the exposures of winter. Pre-moult period of larvae were between 23 and 60 days in most of the exposures, with the exception of the exposures of winter, where the values ranged from 80 to 130 days (Fig. 3a). The results for nymphs were similar to those of larvae (Fig. 3b). The maximum values were recorded in the exposure of winter (70–100 days), while in the remaining exposures the values were between 25 and 50. The pre-oviposition period of engorged females was always lesser than one month, with the exception of some females exposed in winter where the oviposition was retarded (Fig. 4a). Incubation period of eggs was superior to 30 days only in some eggs masses exposed in winter (Fig. 4b).

Rodents belonging to three species were captured in the study area: 30 *Akodon azarae*, 6 *Oligoryzomys flavescens* (Cricetidae, Sigmodontinae) and 28 *Cavia aperea* (Caviidae, Caviinae). Quantitative data of the infestation of *A. dubitatum* subadults on rodents are presented in Table 5. Larvae were found on the three rodent species. Non significant differences were found among rodents species when prevalence and tick distributions on hosts were compared (Fisher's exact test, $P = 0.35$, Kruskal–Wallis, $P = 0.17$). *D* values

Table 3 Pre-moult period (days) and weight (mg) of nymphs of *Amblyomma dubitatum* maintained in the laboratory at 25 ± 1 °C and 85 ± 5 % relative humidity under different photoperiod regimens

Photoperiod (before feeding/ after feeding)	n	Weight (mg) Mean ± SD (range)	Pre-moult period (days) Mean ± SD (range)*
10 L–14 D/10 L–14 D	47	18.2 ± 0.6 (9.9–25.8)	22.2^{ab} ± 0.4 (18–28)
10 L–14 D/12 L–12 D	45	18.3 ± 0.6 (11–25.9)	20.1^{abc} ± 0.2 (18–28)
10 L–14 D/14 L–10 D	46	17.8 ± 0.7 (7–28.2)	22.3^{ab} ± 0.3 (18–28)
12 L–12 D/10 L–14 D	42	17.2 ± 0.7 (9.5–25.5)	21.6^{abc} ± 0.2 (20–27)
12 L–12 D/12 L–12 D	46	17.6 ± 0.7 (8.9–26.8)	20.5^{c} ± 0.2 (18–27)
12 L–12 D/14 L–10 D	49	18.2 ± 0.7 (8.4–30.3)	22.6^{a} ± 0.2 (20–31)
14 L–10 D/10 L–14 D	39	18.9 ± 0.8 (9.2–28.5)	22.3^{ab} ± 0.3 (20–26)
14 L–10 D/12 L–12 D	38	19.1 ± 0.7 (10.3–27.4)	20.5^{c} ± 0.2 (17–25)
14 L–10 D/14 L–10 D	37	19.4 ± 0.6 (11.6–25.4)	22.3^{ab} ± 0.2 (21–27)
Field/10 L–14 D	16	24.8 ± 1.3 (17.7–35.8)	21.7^{abc} ± 0.4 (19–25)
Field/12 L–12 D	17	20.6 ± 0.8 (15.9–25.4)	20.6^{bc} ± 0.4 (18–25)
Field/14 L–10 D	15	26.2 ± 0.7 (19.4–30.8)	22.1^{abc} ± 0.6 (19–27)

* ANOVA with Tukey's test. Numbers not sharing superscripts are significantly different ($P < 0.05$)

n Number of ticks, *L* light, *D* dark

Table 4 Pre-oviposition period (days) and weight (mg) of females of *Amblyomma dubitatum* maintained in the laboratory at 25 ± 1 °C and 85 ± 5 % relative humidity under different photoperiod regimens

Photoperiod (before feeding/after feeding	n	Weight (mg) Mean ± SD (range)	Pre-oviposition period Mean ± SD (range)*	Egg incubation period Mean ± SD (range)*
10 L–14 D/10 L–14 D	18	443.7 ± 36.9 (181.9–817.1)	9.0^{a} ± 0.6 (4–13)	38.8^{a} ± 1.2 (27–46)
10 L–14 D/12 L–12 D	16	476.5 ± 39.1 (254.7–686.4)	8.8^{a} ± 0.5 (5–12)	40.9^{a} ± 0.9 (35–46)
10 L–14 D/14 L–10 D	15	500.6 ± 43.3 (280.8–906.9)	11.0^{a} ± 1.4 (5–26)	38.9^{a} ± 1.7 (28–54)
12 L–12 D/10 L–14 D	11	573.5 ± 78.8 (128.6–933.9)	9.6^{a} ± 0.9 (5–15)	39.8^{a} ± 1.9 (31–51)
12 L–12 D/12 L–12 D	10	508.5 ± 69.3 (227.2–796.9)	9.7^{a} ± 0.8 (6–14)	39.1^{a} ± 1.3 (34–48)
12 L–12 D/14 L–10 D	9	443.4 ± 55.9 (142.7–669.8)	10.2^{a} ± 1.1 (7–17)	40.0^{a} ± 2.7 (31–53)
14 L–10 D/10 L–14 D	16	563.1 ± 43.1 (372.3–901.8)	7.8^{a} ± 0.5 (5–12)	38.2^{a} ± 1.3 (31–50)
14 L–10 D/12 L–12 D	10	390.7 ± 56.1 (179.9–743.2)	8.6^{a} ± 0.7 (4–13)	38.5^{a} ± 1.7 (32–49)
14 L–10 D/14 L–10 D	13	515.2 ± 68.1 (139.9–80.6)	10.2^{a} ± 0.9 (6–17)	41.0^{a} ± 1.7 (29–51)

Egg incubation period under the same conditions is also showed

n Number of ticks, *L* light, *D* dark

* ANOVA with Tukey's test. Numbers not sharing superscripts are significantly different ($P < 0.05$)

close to 1 and *k* less than 0.5[1] indicate a high level of aggregation. Nymphs were collected on *A. azarae* and *C. aperea*. The level of infestation in *C. aperea* was higher than in *A. azarae*. Differences in prevalence and tick distributions between *C. aperea* and *A. azarae*

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[1] Because all measures of aggregation are very sensitive to the size of the sample (Poulin 2007), the results on *O. flavescens* should be considered carefully due to the low number of specimens analyzed in comparison with *C. aperea* and *A. azarae*.
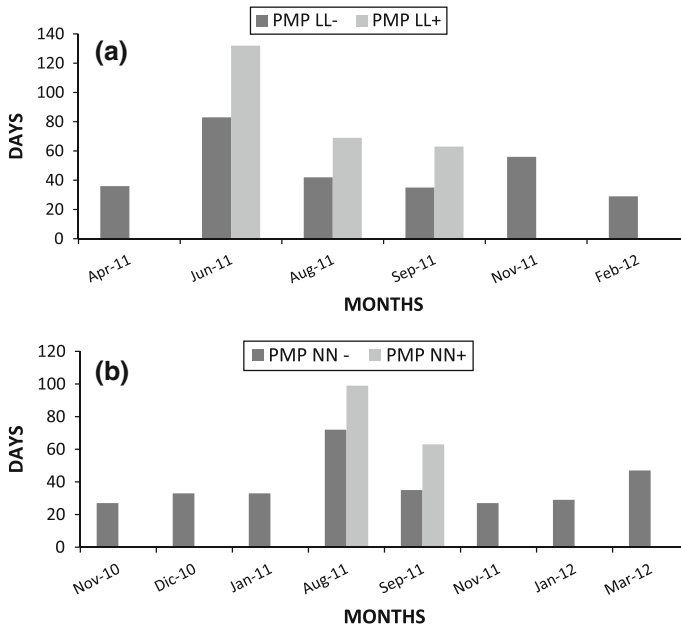
Fig. 3 Pre-moult period (minimum: PMP−, maximum: PMP+) of *Amblyomma dubitatum* larvae (**a**) and nymphs (**b**) under field conditions in Corrientes Province, Argentina

were significant (Fisher's exact test, $P < 0.01$; Kruskal–Wallis, $P < 0.01$). In the case of nymphs, the level of aggregation in *C. aperea* was lower than in *A. azarae*. The three rodent species were also found parasitized by larvae and nymphs of *Amblyomma triste*.

Discussion

The seasonal distribution of *A. dubitatum* is characterized by distinct sequential peaks of activity for each parasitic stage. Larvae reach the peak of abundance in autumn and early winter, nymphs in winter and early spring, and females peaked from mid-spring to late summer. This pattern represents a life cycle with one generation per year with most of the ticks reaching adulthood during the warmest months, which is similar to that reported for other Neotropical species of *Amblyomma* such as *A. cajennnense* and *A. parvum* (Guglielmone et al. 1990; Labruna et al. 2002; Oliveira et al. 2003; Nava et al. 2008). However, both immature and adults stages of *A. dubitatum* were found parasitizing *H. hydrochaeris* along the whole year (the only exception is the absence of larvae in December), and exposures in the field of immature and adult ticks (Figs. 3 and 4) showed that moulting, oviposition and hatching of eggs occur throughout the year, independently of the season. These results indicate that though *A. dubitatum* has a one year life cycle, more than one cohort can co-exist within the same population in a certain interval of time. The presence of different cohorts in the same year has already been suggested for other hard tick species, as for example *Ixodes ricinus* (Walker 2001), *Dermacentor variabilis* (Burg 2001), *Amblyomma neumanni* (Nava et al. 2009a) and *Amblyomma tigrinum* (Nava et al. 2009b).

The seasonal distribution of *A. dubitatum* observed in northeastern Argentina is in part similar to the pattern described by de Souza et al. (2006) in southeastern Brazil, since these
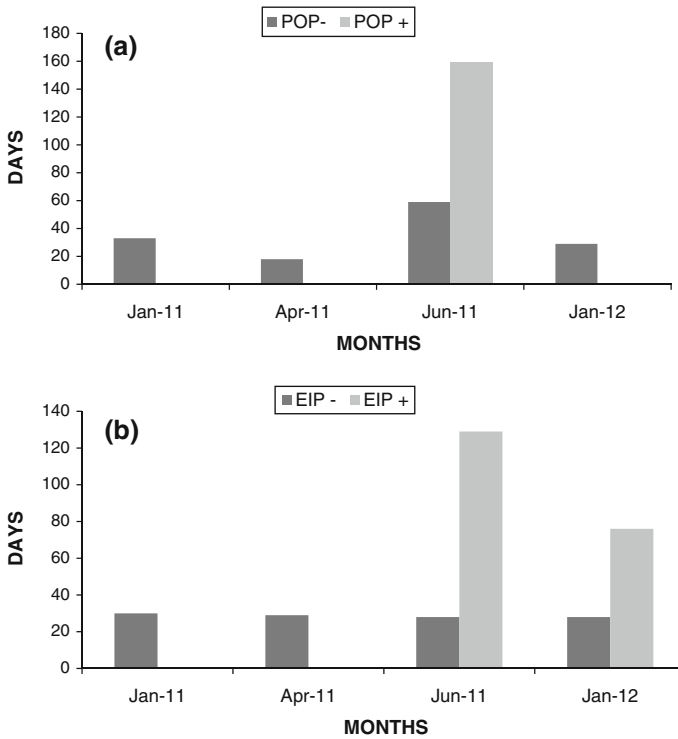
Fig. 4 Pre-oviposition period of *Amblyomma dubitatum* females (**a**) (minimum: POP−, maximum: POP+) and egg incubation period (**b**) (minimum: EIP−; maximum EIP+) under field conditions in Corrientes Province, Argentina

Table 5 Percentage of prevalence (*P*), mean, median (with first and third quartiles), *K* parameter and index of discrepancy (*D*) of the infestation with immature stages of *Amblyomma dubitatum* on rodents in Corrientes Province, Argentina

Hosts	n	*P* (%)	Mean*	Median (1Q–3Q)	*K*	*D*
Larvae						
Akodon azarae	30	56.7	6.7[a]	1 (0–6)	0.23	0.78
Cavia aperea	28	39.3	5.2[a]	0 (0–2)	0.13	0.81
Oligoryzomys flavescens	6	66.7	5[a]	2 (0–7)	0.44	0.56
Nymphs						
Akodon azarae	30	6.7	0.1[a]	0 (0–0)	Not apply	0.91
Cavia aperea	28	64.3	3.6[b]	1 (0–6.5)	0.48	0.64

* Kruskal-Wallis followed by Dunn's test. Numbers not sharing superscripts are significantly different

authors found the peak of abundance of adults (there is not information on immature stages of *A. dubitatum* in the work of de Souza et al. (2006)) in spring and early summer. However, the results of Argentina and those of de Souza et al. (2006) are discordant with

respect to other works also performed in southeastern Brazil. Szabó et al. (2007) also found the peak of nymphs in winter and spring, but the peak of adults described by these authors was in autumn, and Toledo et al. (2008) found the peak of abundance of adults in spring. These dissimilarities could be related to variations in local conditions (host density, microclimatic conditions, vegetation, e.g.) or associated to methodological differences. For example, the analysis of Szabó et al. (2007) was based on four annual samples of free-living ticks (one per season) by using CO_2 traps, de Souza et al. (2006) also used CO_2 traps but they carried out monthly samples of free-living ticks during a period of two years, and the information in northeastern Argentina was obtained from two year monthly samples of all tick stages in the parasitic phase. Further studies will be useful to confirm whether this geographical variation in the seasonality of *A. dubitatum* is related to methodological or ecological issues.

Tick dormancy by diapause is a pre-adaptive behaviour which precedes the onset of unfavourable environmental conditions, and it is principally regulated by photoperiod (Belozerov 1982). There are two types of diapause, behavioural (suppression of host-seeking activity in unfed ticks) and morphogenetic (delay of embryogenesis, in the metamorphosis of larvae and nymphs, and in the oogenesis of engorged females) (Belozerov 1982). In South America, behavioural diapause was described for larvae of *A. cajennense* in Brazil (Cabrera and Labruna 2009) and morphogenetic diapause was recorded for females of *A. neumanni* in Argentina (Nava et al. 2009a). The analysis of the effect of the photoperiod on the development of *A. dubitatum* showed no indication of morphogenetic diapause in the life cycle of this tick. However, the exposures of engorged ticks under field conditions indicate a delay in metamorphosis of larvae and nymphs, in the oviposition of females and in the incubation of eggs, probably associated to the low temperatures of the winter (Figs. 3 and 4), because humidity is not a limiting factor in the study area. It is recognized that low winter temperatures in temperate regions inhibit tick activity (Randolph 2008). This phenomenon is connected with quiescence, other form of dormancy defined as an arrest of development in response to direct action of external limiting factors (Belozerov 2009). Therefore, the delay in the development of *A. dubitatum* in winter can be attributed to the occurrence of quiescence, which enables the adaptation of this tick to the climate seasonality of the northeastern Argentina. Additional field and laboratory experiments are needed to test this hypothesis.

One of the most important components of the life cycle of ticks is the host usage. Usually it was accepted that the immature stages of *A. dubitatum* feed on the same host (*H. hydrochaeris*) that the adults stages. The results of this work support previous hypothesis (Dantas-Torres et al. 2010; Nava et al. 2010; Debárbora et al. 2012) on the potential role of small rodents as hosts for larvae and nymphs of *A. dubitatum*. Sigmodontines and cavies appear to be important hosts for larvae, while cavies are relevant for nymphs. This result is not unexpected. Guglielmone and Nava (2010, 2011) have already stressed the relevance of the rodents of the subfamilies Caviinae and Sigmodontinae in supporting the immature stages of several hard tick species in South America. But besides the ecological meaning of this host-parasite assemblage, the participation of small rodents in the life cycle of *A. dubitatum* could have epidemiological implications. The importance of small rodents as reservoir or amplifier hosts of tick-borne pathogens is documented (Randolph et al. 1999; Szabó et al. 2013). Thus, future studies on the potential capacity of *A. dubitatum* as vector of pathogenic microorganisms need to focus on a system involving *A. dubitatum* ticks, *H. hydrocaheris* and small rodents.

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