



## Divergent environmental preferences and areas of sympatry of tick species in the *Amblyomma cajennense* complex (Ixodidae)



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### ABSTRACT

Four species of Neotropical ticks, *Amblyomma mixtum*, *Amblyomma cajennense*, *Amblyomma tonelliae* and *Amblyomma sculptum* (formerly included in the catch-all name *A. cajennense*), have an allopatric distribution in much of their range, with areas of parapatry for at least two of them. We inferred the abiotic niches of these organisms using coefficients of a harmonic regression of the temperature and the Normalized Difference Vegetation Index (NDVI, reflecting plant stress) from remotely sensed data from MODIS satellites with 0.05° spatial resolution. Combinations of coefficients describing the phenology of these two variables pointed to divergent niche preferences, compatible with previous events of vicariance among the species. *Amblyomma cajennense* has been recorded in areas with small variations in temperature and NDVI. The remaining species were recorded in areas with large variations. The maximum environmental niche overlap was ~73.6% between *A. mixtum* and *A. cajennense* and 73.5% between *A. tonelliae* and *A. sculptum*. Projecting these inferences on the geographical space revealed probable areas of sympatry or parapatry between *A. mixtum* and *A. cajennense* or between *A. tonelliae* and *A. sculptum*, the latter of which was confirmed with field collections. The *A. sculptum* distribution overlaps with that of *A. tonelliae* in northern Argentina and Paraguay; parapatry occurs at one extreme of the conditions occupied by both species. Compared with areas of allopatry, sites with both species had consistently lower temperatures, except for 10–12 weeks during the summer, and higher NDVI values throughout the year. We hypothesise that the overlap between *A. tonelliae* and *A. sculptum* resulted from secondary contact between populations, with *A. sculptum* adapting to sites with high water availability to balance high summer temperatures. Additional surveys of the areas of spatial overlap among these species are necessary to elucidate the forces driving their evolution and their adaptation to the environment.

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### 1. Introduction

*Amblyomma cajennense* (Fabricius, 1787) was previously considered to be a tick taxon widely distributed in the Neotropical and Nearctic regions that feeds on a variety of vertebrate hosts, mostly mammals. Its range was recorded from southern Texas (USA) to northern Argentina in environments as diverse as the dry grasslands of the Chaco region of Argentina and Paraguay, the highlands of the Peruvian Andes, and the tropical forest of French Guiana

(Estrada-Peña et al., 2004). Cross-mating experiments with *A. cajennense* ticks from different areas of South America suggested that this taxon likely consists of a complex of different species (Labruna et al., 2011; Mastropaolo et al., 2011).

Molecular analysis of the genetic diversity within *A. cajennense* collected from its entire distribution range revealed strong genetic structure and demonstrated the existence of clades corresponding to different ecological features (Beati et al., 2013). One clade occurred in the Interandean valleys of Peru, the second clade in the dry part of the Chaco region of Argentina and Paraguay, the third clade in the humid forested areas of northern Argentina and coastal southern Brazil, the fourth clade in the Amazonian region, the fifth clade in the mountainous area of the Eastern cordillera of Colombia, and the sixth clade spanned the Pacific coast of

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Ecuador, through central America to Texas (Beati et al., 2013). Evidence of the morphological separation of these clades and their representation of six different species was recently reported (Nava et al., 2014). That investigation re-described three species (*A. cajennense* (Fabricius, 1787), *Amblyomma mixtum* (Koch, 1844), and *Amblyomma sculptum* (Berlese, 1888)), and three additional taxa were described (*Amblyomma tonelliae*, Nava, Beati, and Labruna, 2014; *Amblyomma patinoi*, Labruna, Nava, and Beati, 2014; and *Amblyomma interandinum*, Beati, Nava, and Cáceres, 2014). Nava et al. (2014) also provided an extensive review of the synonymies of these taxa in the scientific literature.

In addition to the morphological and molecular differences among these species of ticks, it is of interest to examine the environmental niches associated with these newly recognised taxa and to determine how the restricted gene flow driving speciation resulted in preferences for “regions” of the abiotic space that are diverse in terms of temperature, humidity and seasonal patterns. Such an analysis would provide a better understanding of the abiotic preferences of the species in the *A. cajennense* complex. A preliminary analysis of these preferences (Estrada-Peña et al., 2014) was based on the coarse description of biomes in the Neotropical-Nearctic regions of the species still included in the catch-all name *A. cajennense*.

It is necessary to define the gradient of variability in the environmental niches in which each species has been recorded, to quantify niche overlaps and to deduce probable areas of allopatry or sympatry/parapatry for species in which gene flow is restricted (Beati et al., 2013). The current investigation summarises the results of such studies of the abiotic variables that define the niches of *A. cajennense* sensu stricto (s.s.), *A. mixtum*, *A. tonelliae* and *A. sculptum*. Our study did not primarily aim to project climate inferences on the geographical range, and therefore we did not extract conclusions about the factors that drive the distribution of this complex of species. We sought to capture information about abiotic niches in terms of our current knowledge of the distribution of these species, with the understanding that future collections and re-examination of existing records will improve such assessment. Further, we sought to characterise the probable parapatric distribution of *A. sculptum* and *A. tonelliae* in parts of their ranges.

## 2. Materials and methods

### 2.1. Compilation of reliable records of the *A. cajennense* group

A total of 29 confirmed records of *A. cajennense* s.s., 140 of *A. mixtum*, 256 of *A. sculptum* and 103 of *A. tonelliae*, were used for this study. Information was obtained from the literature and from the Tick Collection of Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela, Argentina. The recorded distribution of each species appears in Fig. 1. The taxonomic status of these records was re-assessed after the description or re-description of the cluster of species in *A. cajennense* (Nava et al., 2014). More than 300 additional records of *A. cajennense* sensu lato (s.l.) exist in the literature (Guglielmone et al., 2003; Estrada-Peña et al., 2004), but they have not been re-assessed, leading to their exclusion from the current study. The records of *A. patinoi* and *A. interandinum* are also included in Fig. 1. These limited data do not allow further study of the environmental preferences of these species, as analysis of so few records would violate the critical assumption of random collection of specimens throughout the niche (Peterson et al., 2008).

### 2.2. Explanatory variables and data preparation

We assumed that the distribution of ticks is shaped by both abiotic (e.g. environmental) and biotic (host) factors; we also assumed

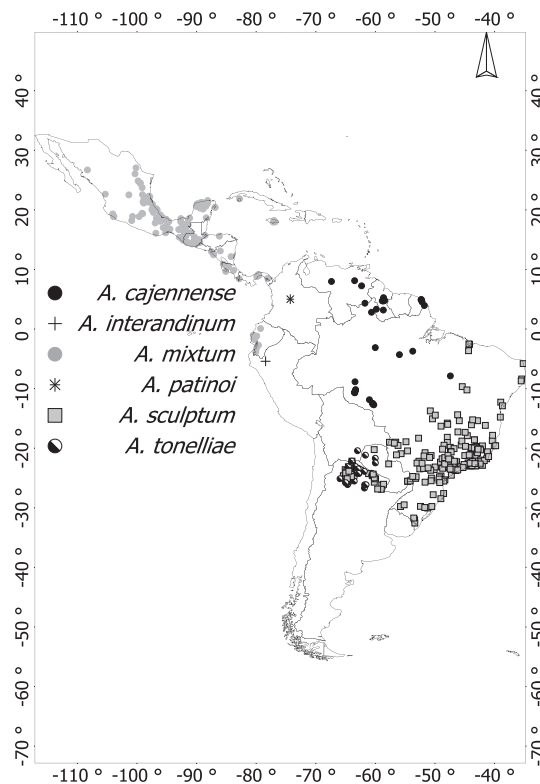


Fig. 1. Geography of the recorded distributions of the species of *Amblyomma cajennense* sensu lato. The area of study includes the coordinates.

that their spread to previously unoccupied areas is driven by host movement and, therefore, the existence of a suitable environment is not causative of the presence of a tick species. Here we sought to evaluate the abiotic component of the niche in order to confirm that the reported morphological and molecular differences between these ticks are reflected in the patterns of niche occupancy. Selection of environmental variables is a critical step in elucidating the niche preferences of an organism (Glass et al., 1995; Guerra et al., 2002). We addressed concerns about the automatic selection of variables by the modelling algorithms, such as the use of collinearly correlated time series of variables and model inflation (Mansfield and Helms, 1982; Legendre, 1993; Segurado and Araujo, 2004; Kuemmerle et al., 2010; Estrada-Peña et al., 2013a), by using the coefficients of the harmonic regression of temporal series of Land Surface Temperature (LST) and Normalized Difference Vegetation Index (NDVI) as explanatory variables. These two datasets were derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) platform (spatial resolution of 0.05°) for the years 2000–2012. It is widely accepted that both temperature and saturation deficits affect the tick life cycle (Estrada-Peña et al., 2013b). In the case of remote sensing, NDVI best captures the reported distribution of some tick species (Estrada-Peña, 1999; Randolph, 2007) because it is a proxy for vegetation stress, therefore reflecting water availability. We used the products MOD11C2 and MOD13C2, which are available at [https://lpdaac.usgs.gov/products/modis\\_products\\_table](https://lpdaac.usgs.gov/products/modis_products_table) (accessed May 2013).

We performed harmonic regression on the MODIS monthly series of variables (Scharlemann et al., 2008; Estrada-Peña et al., 2014) in the R developmental framework (R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL HYPERLINK “<http://www.R-project.org/>” <http://www.R-project.org/>, accessed May 2013) using the packages “raster” (Hijmans and van Etten, 2012. Raster: Geographic data analysis and modelling. R package

version 2.0–41, HYPERLINK <http://CRAN.R-project.org/package=raster>, accessed June 2013) and “TSA” (Kung and Ripley, 2012. TSA: Time Series Analysis. R package version 1.01. 2012, <http://CRAN.R-project.org/package=TSA>, accessed March, 2013). Five coefficients for LST and five for NDVI were extracted from the averaged monthly values of the MODIS-derived images. These coefficients are referred to as “environmental variables” in this investigation because they represent the environmental or abiotic niches that may be occupied by the target species. The first coefficient (LST1 or NDVI1) is a measure of the average values of the variable in a pixel; the second and the third coefficients contain information about the slope and duration, respectively, of the spring rise in environmental values; the fourth and the fifth coefficients describe the negative slope and duration of decrease, respectively, of environmental values in autumn. A complete description of these data layers has been already published (Estrada-Peña et al., 2014). The set of coefficients of the harmonic regressions is publicly available at <http://antigonefp7.eu/our-results/remotely-sensed-variable-data/>.

### 2.3. Abiotic niches and model building

We first determined which environmental variables had enough statistical significance to discriminate the distributions of the four target species. We used a multi-logistic regression of the records against the 10 environmental variables (five for LST and five for NDVI). We used the Akaike information criterion (AIC) to select the best set of environmental variables that, in combination, discriminated among the records of the four species with the minimum misclassification rate.

From the records for the ticks, adequate algorithms were used to extract the ranges for each environmental variable to which each species is linked, thus evaluating species occupancy and overlap for each environmental variable. The term “overlapping” is not used here in a spatial sense; it only refers to the common preferences of two or more species for a range of values of the environmental variables. We developed models for each species using the environmental variables that best explained their environmental niche, as determined via training with the available records. The Maximum Entropy Approach using the MaxEnt computer program for modelling the geographic distributions of species (Philips and Dudík, 2008) was employed to generate models for the species studied. This machine-learning modelling method has recently gained attention for its favourable performance in comparison to other modelling methods (Elith and Graham, 2009). We used only linear relationships (Merow et al., 2013) in our models. We explored a range of regularisation parameters, in accordance with published recommendations (Elith and Graham, 2009), to choose a final regularisation parameter of 2. Sampling bias was not addressed due to the inherent difficulties in its calculation from historical records that did not specifically include sampling efforts. Models were trained with 70% of the records and evaluated against the remaining 30%. Model reliability was determined by calculating the area under the curve, a metric for evaluating diagnostic procedures that yields a single measure of model reliability that is independent of any particular choice of threshold value (Swets, 1988). Since its first proposal as an appropriate method to estimate the accuracy of species distribution models, several reports have described its use in this field of research (Pearce and Ferrier, 2000; Manel et al., 2001; McPherson et al., 2004). However, other studies have criticised its indiscriminate application (Lobo et al., 2008; Peterson et al., 2008).

A requirement for the strict evaluation of niche overlap is the comparison of the preferred range of the niche variables against their available ranges for the complete geographical background. This comparison was performed separately for each environmental

variable and species of tick and quantified with the D statistic of the package “phyloclim” (Heibl and Calenge, 2013. phyloclim: Integrating phylogenetics and climatic niche modelling. R package version 0.9–4. <http://CRAN.R-project.org/package=phyloclim>, accessed June 2013).

### 2.4. Collections of specimens in areas of parapatry

Nava et al. (2014) previously showed that at least two species in the complex, *A. sculptum* and *A. tonelliae*, are distributed in parapatry in a rather large area of northern Argentina. We aimed to better capture the environmental features underlying species coexistence. However, such areas of parapatry cannot be resolved at the resolution of the data included in this study; each single pixel of the remotely sensed data presents them as areas of sympatry (i.e. both species coexist in the same pixel even if a frontier in distribution exists). We specifically aimed to demonstrate whether areas of parapatry are associated with environmental conditions intermediate to those for areas of allopatry or whether sites allowing permanent parapatric populations have extreme conditions (i.e. in the upper or lower range of the environmental conditions of the species in allopatry). We thus performed additional collections of the two species in areas of northern Argentina in the Provinces of Chaco, Formosa, Jujuy, Salta and Santiago del Estero. We next compared the abiotic environments (LST and NDVI) of regions in which the species’ ranges overlapped (areas with potential sympatry). This analysis allowed us to evaluate whether the abiotic conditions of allopatric sites are similar, intermediate, or extreme in comparison with parapatric areas. We explicitly rejected the hypothesis of biotic drivers of parapatry, since collections at these sites showed that both species use the same range of hosts (unpublished data).

## 3. Results

### 3.1. Niche occupancy of species in the *A. cajennense* complex

Table 1 contains the details of the statistical significance of the environmental variables that best captured the reported distribution of the four target species. A logistic regression indicated that the four coefficients of the harmonic regression of LST and the first three coefficients of the harmonic regression of NDVI significantly differed over the distribution ranges of the four species. The model

**Table 1**

Results from a logistic regression of the records of the four target species in the complex *Amblyomma cajennense* and the remotely sensed variables used to describe their environmental preferences. Variables are the first five coefficients of a harmonic regression of the Land Surface Temperature (LST1–LST5) or the Normalized Difference Vegetation Index (NDVI1–NDVI5). Significant differences were found for four of the variables describing the phenology of temperature and for three of the variables describing the variability in NDVI. These significant variables were retained to describe the environmental niche of the species. There were 528 observations, with a generalised  $R^2$  of 0.9532 and a misclassification rate of 0.087. The Akaike information criterion was 212.32.

Variable	F	
LST1	14.0024	0.0029 <sup>a</sup>
LST2	105.8661	<0.0001 <sup>a</sup>
LST3	155.7153	<0.0001 <sup>a</sup>
LST4	0.9091	<0.0001 <sup>a</sup>
LST5	4.2310	0.122
NDVI1	16.8101	0.0008 <sup>a</sup>
NDVI2	46.1983	<0.0001 <sup>a</sup>
NDVI3	21.5445	<0.0001 <sup>a</sup>
NDVI4	6.2912	0.098
NDVI5	8.1340	0.231

<sup>a</sup> Statistically significant ( $P < 0.005$ ).

using these seven environmental variables yielded the lowest rate of misclassifications (8.7%). The MaxEnt model built with these environmental variables and trained on 70% of the records for the four species produced areas under the curve of 0.99 for *A. mixtum* and *A. cajennense* and 0.97 for *A. sculptum* and *A. tonelliae*. The spatial projections made by this model on the environmental conditions of the geographical background appear in Fig. 2. This analysis is not an explicit evaluation of the probable range of colonisation of the ticks, but a projection of the areas that are expected to have a climate suitable for their persistence. *Amblyomma mixtum* was associated with areas of dry and moist Meso-America and the Meso-American Seasonal Highlands, with large areas of suitability in the Caribbean. The Madrean semi-desert was not predicted to be suitable for *A. mixtum*. *Amblyomma cajennense* would encounter suitable environmental conditions in large regions of Amazonia, Orinoquia and Guianian, with low but positive suitability in the Caribbean and the Meso-American highlands. It is of interest that both *A. cajennense* and *A. mixtum* are expected to have large areas of environmental similarity in regions of the Caribbean and the Meso-American Biological Corridor, where suitable environments were projected for both species. *Amblyomma sculptum* was predicted to colonise the biomes of the Atlantic forest and the Chaco in areas of Brazil, Paraguay and northern Argentina. *Amblyomma tonelliae* had the smallest spatial niche, which was predicted to extend into Paraguay, Bolivia and northern Argentina in the central dry Chaco. Parts of the range that

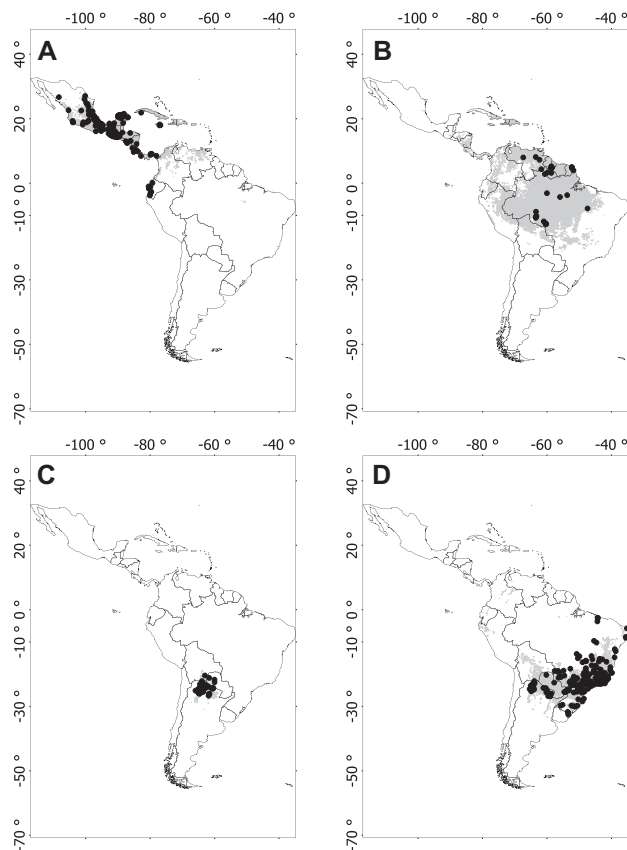
were predicted to be environmentally suitable for *A. tonelliae* fall within the range suitable for *A. sculptum*.

Predicted environmental niche occupancy by the four tick species is displayed in Fig. 3. Niche occupancy is the portion of the available abiotic conditions that is “preferred” by the tick, according to niche reconstruction using field records which report the preferences of each species (range 0–1) toward defined portions of the complete range of values of each environmental variable. It is interesting to note that in this analysis, the four species were restricted to a narrow range of average annual temperature (Fig. 3A); every species most highly preferred a portion of the niche that was less than 5 degrees Kelvin (K). However, the set of preferred environmental niches differed in terms of other environmental variables related to temperature that describe seasonality. The four species also exhibited large differences in niche occupancy according to NDVI, but they largely overlapped in coefficient NDVI4 (Fig. 4). This observation is in agreement with the results of the logistic regression, which failed to detect differences in the ability of this variable to significantly discriminate among the niches of the four species. Niche overlap, or the number of habitat preferences shared by the different species, appears in Fig. 5. As expected from visual inspection of the results in Figs. 3 and 4, maximal niche overlap occurred for LST1 (Fig. 5A) and NDVI4 (Fig. 5H). Maximum niche overlap was detected between *A. mixtum* and *A. cajennense* (average: 73.6%) as well as *A. tonelliae* and *A. sculptum* (average: 73.5%). Other species were associated with a smaller degree of abiotic overlap (65% for *A. cajennense* and *A. sculptum*, 60% for *A. cajennense* and *A. tonelliae*, and 68% for *A. mixtum* and *A. sculptum*).

Fig. 6 shows the seasonal pattern (weekly values) of the environmental variables, as reconstructed from the coefficients of the harmonic regression that defined the abiotic preferences of the four target species. In this analysis, *A. sculptum* was recorded in areas with relatively warm mean temperatures (>300 K for weeks 21–41 (week 1 beginning 01 January)). The lowest temperature range (<295 K) was recorded for weeks 24–36. The seasonal pattern of temperature deduced for *A. cajennense* encompasses a range of higher temperatures (297–305 K) without a well-defined winter period. *Amblyomma tonelliae* was predicted to be present in sites with the highest temperature (~310 K) and with a sustained period of low mean temperature (weeks 11–26). *Amblyomma mixtum* was predicted to colonise areas with the highest seasonal variability in temperature (~292–306 K). *Amblyomma mixtum* preferred sites with sustained high temperatures (~300–305 K, weeks 1–20). Reconstructed seasonality based on NDVI suggested clear differences between species. *Amblyomma sculptum* and *A. tonelliae* were predicted to appear in sites with sharp seasonality in NDVI, with a deep minimum around week 41 (accentuated in *A. tonelliae*). *Amblyomma mixtum* was predicted to be present in areas with an inverted pattern of NDVI phenology because it is prevalent in the northern hemisphere, with NDVI values higher than those recorded for *A. sculptum* and *A. tonelliae*. *Amblyomma cajennense* was predicted to colonise sites with low NDVI variability, with a relative minimum around weeks 9–13, consistent with predictions based on LST.

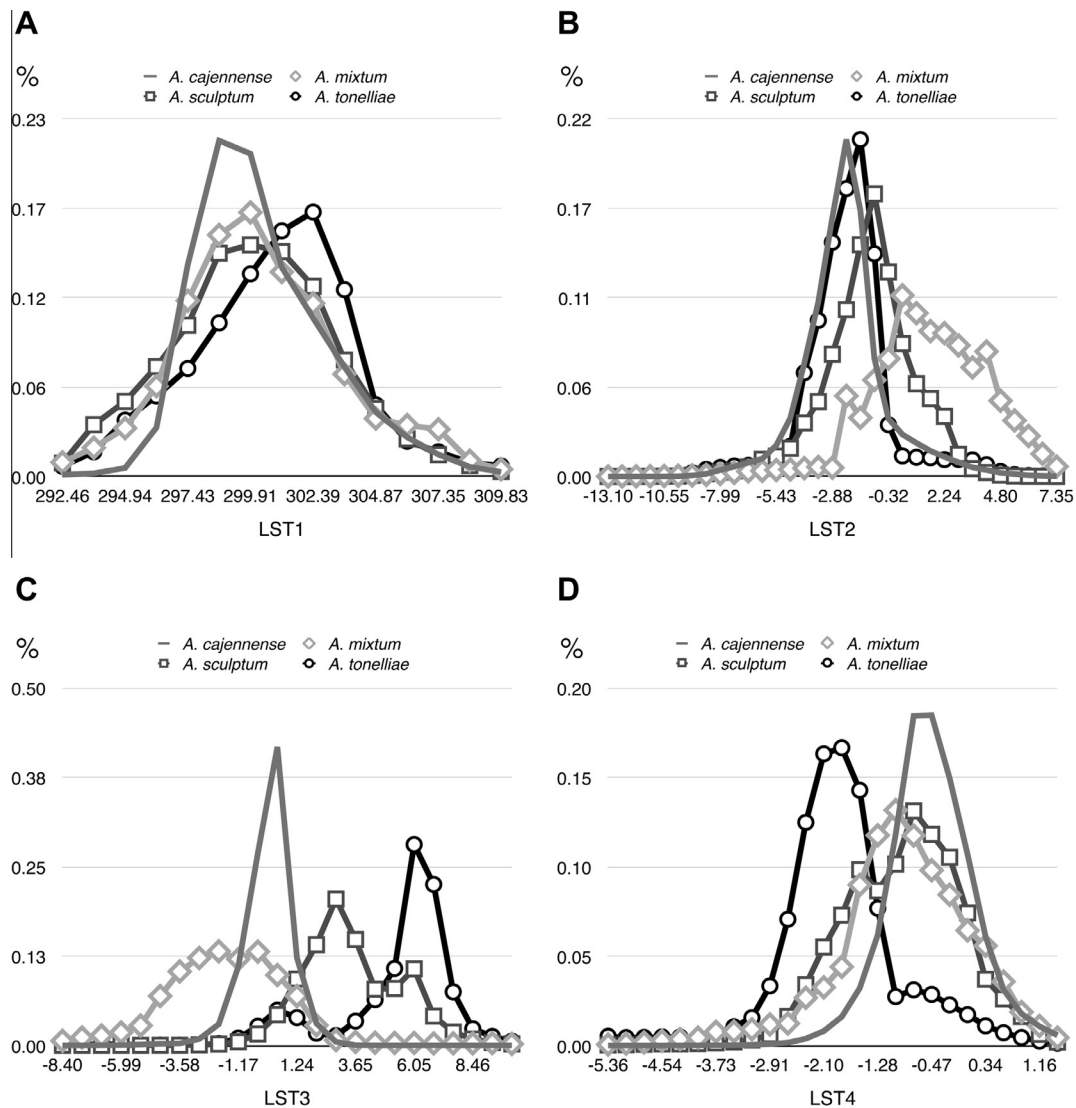
### 3.2. Parapatric distribution of *A. sculptum* and *A. tonelliae*

We addressed the parapatric distribution of *A. sculptum* and *A. tonelliae* in northern Argentina by evaluating environmental seasonality at the resolution of our study (5.8 km) and by comparing sites associated with allopatry or parapatry (Fig. 7). Sites where both species were collected together had a different phenology of LST than sites in which the two species were in allopatry. In sites containing parapatry, LST was lower for most of the year (until approximately week 41), and the average minimum temperatures



**Fig. 2.** Projections of the preferred environmental niches of the four tick species obtained using the best discriminatory variables (the first four coefficients of the harmonic regression of Land Surface Temperature (LST) and Normalized Difference Vegetation Index (NDVI)). (A) *Amblyomma mixtum*; (B) *Amblyomma cajennense*; (C) *Amblyomma tonelliae*; (D) *Amblyomma sculptum*. Light gray areas are the projections of the models onto areas of adequate environmental suitability. Black dots represent the actual records for each species.





**Fig. 3.** Niche occupancy by *Amblyomma cajennense*, *Amblyomma mixtum*, *Amblyomma sculptum* and *Amblyomma tonelliae* based on Land Surface Temperature (LST). The four coefficients of the harmonic regression of LST (LST1–LST4 in A to D, respectively) are plotted as the percentage of occupancy in the ranges of each variable. A is in degrees Kelvin, but the remaining figures are unitless.

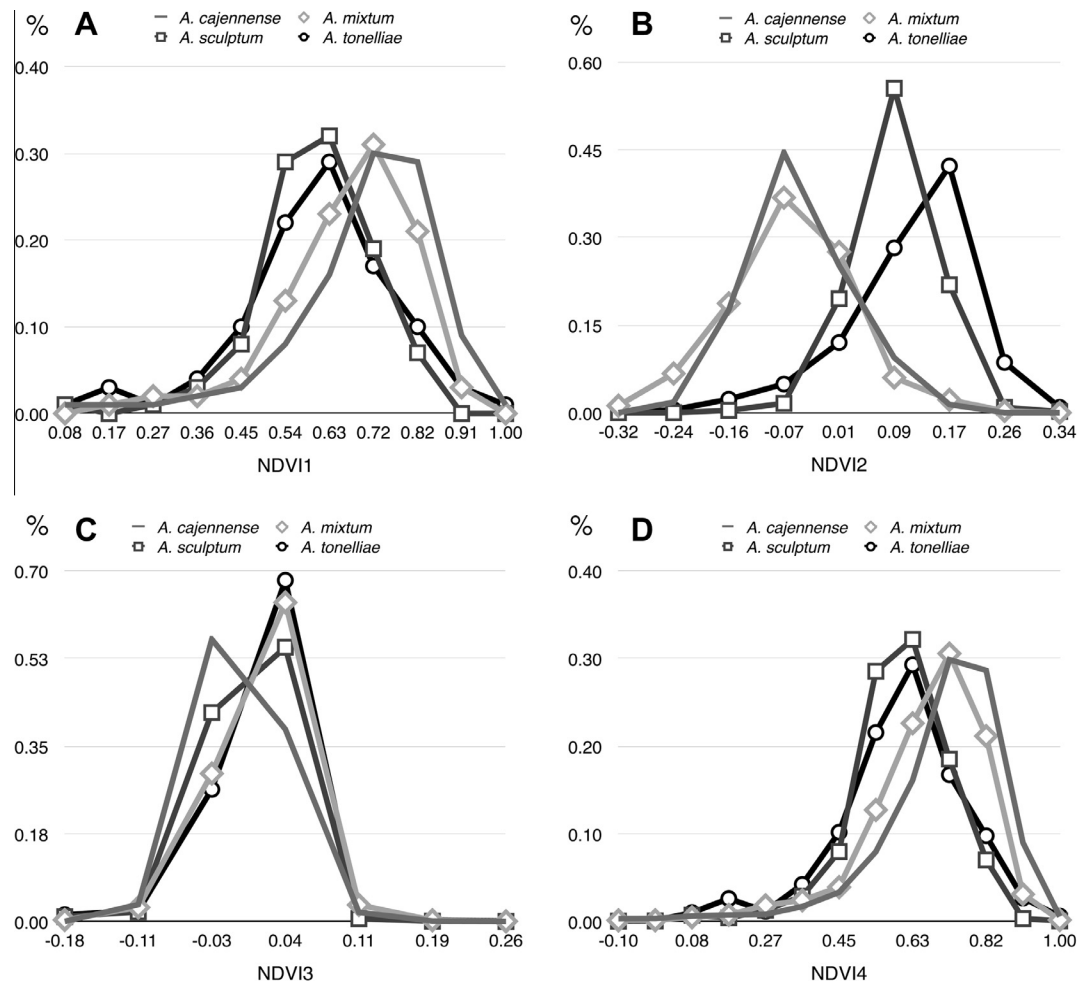
were below the minimum LST recorded for sites with allopatry. From these collections and the explanatory variables in the working resolution, both species shared the habitat only where NDVI values were well above the values recorded for sites of allopatry. Therefore, the parapatric habitat is not intermediate to the conditions preferred by both species in allopatry, but rather exists at the extreme of these conditions. NDVI values in areas of parapatry were 15–20% higher than the average NDVI values in allopatry. In summary, parapatry occurs in regions with lower LST and very high NDVI relative to allopatric regions.

#### 4. Discussion

A group of ticks in the Nearctic and Neotropic, formerly included under the catch-all name of *A. cajennense*, is now recognised as a cluster of species with morphological, reproductive and molecular differences (Labruna et al., 2011; Mastropaolo et al., 2011; Beati et al., 2013; Nava et al., 2014). These differences underlie the recognition of six taxa that have been recently described or re-described (Nava et al., 2014). Here we demonstrated that four

species of ticks in this complex have well-differentiated environmental (abiotic) niches, in agreement with previous findings on the molecular and morphological differences among these species. Two species described by Nava and co-workers (2014), *A. interandinum* and *A. patinoi*, could not be included in this study due to the lack of an adequate number of records; their inclusion would have violated assumptions about the randomness of collections (Peterson et al., 2008).

Our analyses of niche occupancy and niche overlap show that these species are predicted to have divergent patterns of environmental preferences, which can be detected via a set of environmental variables based on remotely sensed features of temperature and vegetation. These environmental patterns thus describe abiotic conditions that are critical during certain periods of the tick life cycle for adequate population turnover. The use of coefficients of harmonic regression instead of raw environmental variables has been demonstrated to greatly improve the development of correlative models because (i) they represent the complete phenology of the abiotic niche without introducing too many variables that inflate the model, (ii) they lack internal issues of collinearity, and (iii) they represent variables with ecological meaning



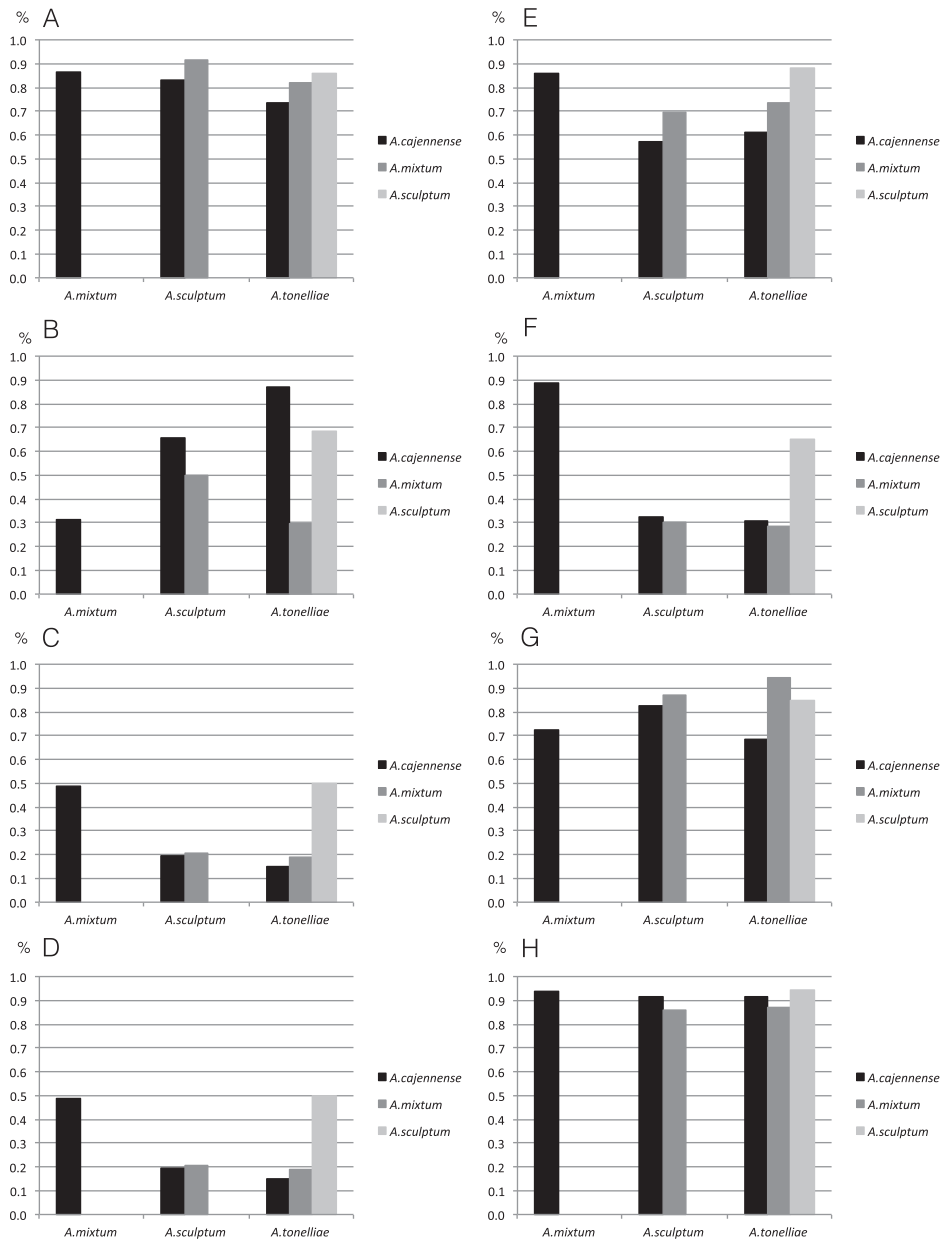
**Fig. 4.** Niche occupancy by *Amblyomma cajennense*, *Amblyomma mixtum*, *Amblyomma sculptum* and *Amblyomma tonelliae* based on NDVI. The four coefficients of the harmonic regression of Normalized Difference Vegetation Index (NDVI1–NDVI4 in A to D, respectively) are plotted as the percentage of occupancy in the ranges of the variable. Values are unitless.

(Estrada-Peña et al., 2013a). We further demonstrated that this methodology captures small but critical details of the environmental niche that could be disregarded if long-term values of a variable (such as average values or annual variability) are the only source of information. Such an approach based on remote sensing clearly outperforms modelling based on interpolated climate (Estrada-Peña et al., 2013a). The precise phenology of the environmental variables that shape the distribution of these organisms can only be captured by transforming raw climate variables while retaining their ecological meaning (Randolph, 2007). A potential limitation of our study is our use of a relatively short series of remotely sensed data (years 2000–2012). Such a short series, which included the only available MODIS data, certainly captures the niches present during that time-frame. We acknowledge that a longer series would better define the abiotic niches of these species but basic questions relating to the coherence among morphological, molecular and environmental correlates, can be drawn from this preliminary study. For example, comparison of the environmental niches showed that overlap may exist in some portions of the distribution ranges of *A. mixtum* and *A. cajennense*, a hypothesis that was not empirically addressed in this study via additional surveys. However, all ticks examined by Nava et al. (2014) from central America and the Caribbean were identified as *A. mixtum*. Such re-examination supports the existence of only *A. mixtum* in the Caribbean but there are literally hundreds of

records of *A. cajennense* s.l. that have not been yet re-examined and confirmed. Therefore, environmental inferences for *A. cajennense* s.s. should be confirmed through further analysis of previous records or deliberate collections.

It is far from clear whether the geographical context of speciation can be neatly subdivided into the three traditional categories of allopatry, sympatry and parapatry (Butlin et al., 2008). Different types of interactions between species and their environment may drive the co-occurrence of related taxa. If abiotic factors are the primary drivers of species ranges, then species should coexist wherever conditions fall within the fundamental niche of both species. For example, if one species requires colder temperatures and the other requires warmer temperatures, then coexistence would occur at intermediate temperatures, and only one species would occur at more extreme temperatures (Chunco et al., 2012). The competitive exclusion principle, a cornerstone of ecology, establishes that species can stably coexist only if they differ in their resource use (Hardin, 1960; Arthur, 1982; Pianka, 2000). Therefore, interacting species experience strong selection to evolve differences in resource use (Schluter, 1994; Pfennig and Pfennig, 2005).

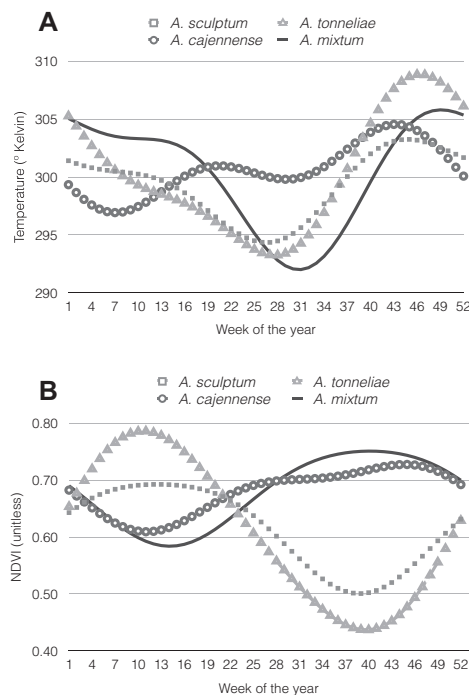
The competitive exclusion principle cannot be applied to the areas of sympatry between *A. sculptum* and *A. tonelliae* because it refers to closely related species evolving in sympatry that experience selective pressure toward different portions of the environmental niche. Molecular evidence indicates that the speciation of



**Fig. 5.** Percentage of niche overlap in each of the four variables for Land Surface Temperature (LST1–LST4, A to D) and the four variables for Normalized Difference Vegetation Index NDVI (NDVI1–NDVI4, E to H) for *Amblyomma cajennense*, *Amblyomma mixtum*, *Amblyomma sculptum* and *Amblyomma tonelliae*. Each bar displays the percentage of one species' niche (defined by the indicated variable) that overlaps with the niches (defined by the indicated variable) of the other species.

the ancestor of *A. tonelliae* and *A. sculptum* was one of the oldest vicariance events for this cluster of species (Beati et al., 2013). Analyses of nuclear and mitochondrial genes provide corroborating evidence that the species in this complex did not experience any sudden demographic event, and that they have been evolving under stable conditions for a long period. Therefore, the simplest explanation is a secondary contact between the two species, which is supported by molecular data. Critically, these comparisons demonstrate the need for integrated studies, since environmental “distances” among the ticks have not been linked with molecular findings. Niche inferences for these two species indicated that temperature is not statistically significant for sites in which *A. tonelliae* and *A. sculptum* are in parapatry, with the exception of a period of approximately 12 weeks in spring and summer, when temperature was intermediate in parapatry in relation to allopatry. This

observation suggests that these 12 weeks are critical for the life cycles of *A. sculptum* and *A. tonelliae*, which will therefore only be present in sites with the appropriate conditions. However, in terms of NDVI, sites in which *A. sculptum* and *A. tonelliae* were predicted to co-exist were not intermediate in relation to sites in which the species were predicted to be in allopatry. NDVI values for areas of parapatry were 15–20% higher than for areas of allopatry throughout the year. We hypothesise that complex (not linear) relationships among abiotic variables may be the primary factors governing co-occurrence between the two species, since collections demonstrated a total coincidence of hosts. We should not disregard the resolution of our analysis (~5.8 km), which is sufficient to reveal general patterns in areas of allopatry and parapatry but too coarse to rigorously define the spatial distributions of these two species. Additional studies would clarify this issue by focusing

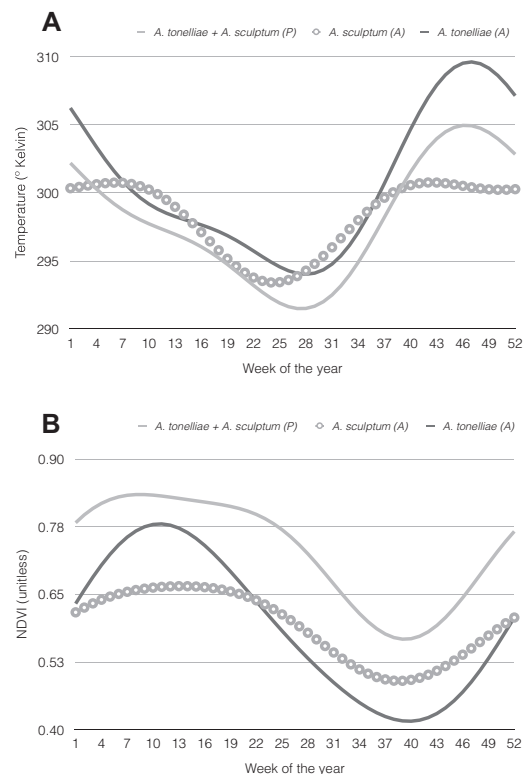


**Fig. 6.** Comparative reconstruction of phenology of the environmental variables for the four species in the *Amblyomma cajennense* group. It is based on (A) Land Surface Temperature (LST) and (B) Normalized Difference Vegetation Index (NDVI). The coefficients of the harmonic regression of the environmental variables were used to reconstruct the averaged series of environmental values in which the four target species, *Amblyomma cajennense*, *Amblyomma mixtum*, *Amblyomma sculptum* and *Amblyomma tonneliae*, have been collected. Week 1 begins on 01 January.

on high-resolution satellite imagery and data from probes recording crucial parameters for tick survival and reproduction, such as temperature and humidity.

A point not explicitly addressed in this study is the probable sympatry between *A. cajennense* s.s. and *A. sculptum*. Environmental modelling suggests that these species may overlap in parts of their range due to the similarities in abiotic variables. This possibility could only be investigated via re-examination of previous collections and new collections at critical areas of overlap; the expected range of *A. sculptum* might be larger than that already known, with areas in Brazil that remain unexplored. Analysis of these records would yield interesting information about the evolution of this complex of species, which was probably driven by abiotic factors, and would enable the association of these findings with the molecular structures of these populations.

This study provided ecological evidence of widely divergent niches in the current ranges of four species in the *A. cajennense* complex. These large differences are compatible with the hypothesised high ecological plasticity of the primitive genetic pool of specimens, which separated due to geological changes in South America that began ~9 million years ago. This study further demonstrated the existence of parapatry of at least two species, which exploited widely different niches compared with niches associated with allopatry. We also proposed hypotheses to explain the unexpected differences in the abiotic niche in areas of allopatry or sympatry. This investigation yielded further support for a methodology that employed harmonic decomposition of a time series of remotely sensed abiotic variables, a more robust approach to explicitly describing the environmental factors driving the distribution of the target species. Available collections of *A. cajennense* s.l. must be re-evaluated to establish the actual range of the species and to uncover further environmental patterns that underlie the distribution of the species in this complex.



**Fig. 7.** Comparative reconstruction of phenology of the environmental variables in areas of allopatry or parapatry of *Amblyomma tonneliae* and *Amblyomma sculptum* in terms of (A) Land Surface Temperature (LST) and (B) Normalized Difference Vegetation Index (NDVI). The coefficients of the harmonic regression of the environmental variables were used to reconstruct the averaged series of environmental values in which *A. tonneliae* and *A. sculptum* are in allopatry (A) or parapatry (P). Week 1 begins on 01 January.

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