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Vector potential and population dynamics for *Amblyomma inornatum*

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ABSTRACT

We studied the natural life cycle of *Amblyomma inornatum* and its vector potential in South Texas. This tick is distributed throughout South Texas and most of Central America. *A. inornatum* represented 1.91% of the ticks collected by carbon dioxide traps during a study of free-living ticks in the Tamaulipan Biotic Province in South Texas. The life cycle of *A. inornatum* in South Texas showed a clear seasonal pattern consistent with one generation per year. Nymphs emerged in the spring with a peak in February through May. Adults emerged in the summer with a peak in July through September. Detection of *A. inornatum* larvae was negatively correlated with saturation deficit and positively correlated with rain in the previous few months. Adult activity was positively correlated with temperature and rain in the previous five weeks. Using PCR we detected the presence of species related to *Candidatus* Borrelia lonestari, *Borrelia burgdorferi*, *Rickettsia* species (*Candidatus* Rickettsia amblyommii), *Ehrlichia chaffeensis*, and another *Ehrlichia* related to *Ehrlichia ewingii*. Finally we sequenced the mitochondrial 16S rRNA genes and found that *A. inornatum* is most closely related to *Amblyomma parvum*. This is the first report of the life cycle, vector potential and phylogeny of *A. inornatum*.

Introduction

Amblyomma inornatum (Banks, 1909) is found throughout South Texas (Eads and Borom, 1975; Gladney et al., 1977), Mexico (Guzmán-Cornejo et al., 2011) and Central America. The species has been reported as far north as 30° N (Presidio and Bexar County, TX) (Brennan, 1945; Eads, 1951) and as far south as Costa Rica (Alvarez et al., 2000). *A. inornatum* has been found on a wide range of hosts: occasionally on large mammals (Cooley and Kohls, 1944; Guzmán-Cornejo et al., 2011) including *Homo sapiens* Linnaeus, 1758, *Bos taurus* Linnaeus, 1758, *Odocoileus virginianus* Zimmerman, 1780; but has been most commonly reported from a large variety of small mammals and birds (Eads, 1951; Eads and Borom, 1975; Gladney et al., 1977; Guzmán-Cornejo et al., 2011; Samuel and Trainer, 1970). Because *A. inornatum* feeds on humans (the most recent report from a traveler to the United States returning to Ontario, Canada – Nelder et al., 2014), a wide range of mammals, ground birds, and migratory birds (it has been collected far from its natural range off of migratory birds as far north as eastern Canada as reported in Ogden et al., 2008) it is important to understand its vector potential. It should be noted that Texas is a major flyway for migratory birds that could acquire tickborne diseases and/or infect ticks with tickborne diseases from other areas in the Americas. Other than occasional reports of the isolation of *A. inornatum* over the last century there is only a single report on its cytogenetics (Oliver and Osburn, 1985) and a single report on its laboratory life cycle (Gladney et al., 1977). *Amblyomma auricularium* (Conil, 1878), *Amblyomma pseudoparvum* Guglielmone, Mangold and Keirans, 1990, *Amblyomma pseudoconcolor* Aragão, 1908, *Amblyomma parvum* Aragão, 1908 and *A. inornatum* are similar in their morphological characteristics (Cooley and Kohls, 1939; Nava et al., 2008). Prior genetic analysis of this group has not included *A. inornatum*. In this study the population dynamics and phylogenetics of *A. inornatum* are reported for the first time. In addition, *A. inornatum* was tested for *Ehrlichia*, *Rickettsia* and *Borrelia* species which are known to be present in *Amblyomma americanum* (Linnaeus, 1758) and *Amblyomma mixtum* Koch, 1844. These ticks are also present in South Texas where *A. inornatum* is found (Billings et al., 1998; Estrada-Peña et al., 2004; Williamson et al., 2010). It should be noted that *A. mixtum* is the reinstated name of one of the "*Amblyomma cajennense* (Fabricius, 1787) sensu lato" strains from South Texas following observation of developmental features (Guglielmone et al., 1992), crossbreeding incompatibility (Labruna et al., 2011; Mastropaolo et al., 2011),

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and genetic analysis (Beati et al., 2013). These studies resulted in "*A. cajennense* sensu lato" being divided into six different species by Nava et al. (2014). Understanding tickborne disease in South Texas is critical as hundreds of thousands of people entering the United States illegally each year cross through this region on foot. Because of the circumstances of their migration they tend to avoid roads and pass through trails, made by both man and animal, that are potentially targeted by questing *A. inornatum* and *A. mixtum* ticks. As both of these species feed on humans, these individuals are extensively exposed to ticks and tickborne diseases during this time.

Materials and methods

Study area, tick collection and meteorological analysis

The primary field site for this study was on the Texas A&M International University Campus in Webb County, Texas, USA (27°57′N 99°44′W) with an elevation of 155 m. The study site has been previously described (Beck et al., 2011). The study area is part of the South Texas Plains vegetation region (Gould, 1975) and Tamaulipan Biotic Province (Dice, 1943). The region is relatively dry (52 cm of precipitation per year), with mild winters and hot summers (mean temperature from 12 °C in the winter to 31 °C in the summer) and slight peaks of rainfall in the spring and fall. Vegetation consisted mostly of thorny woodland and shrub land. Tick surveys were undertaken monthly from March 2005 to November 2008 as previously described (Beck et al., 2011). Ticks were surveyed using carbon dioxide traps. Actively questing and responsive questing ticks were collected using this method. As described in Beck et al. (2011) ticks were identified to species using published keys (Brinton et al., 1965; Cooley, 1938, 1946; Cooley and Kohls, 1944; Jones et al., 1972; Keirans and Durden, 1998; Kohls, 1958; Yunker et al., 1986). It should be noted that only five species of *Amblyomma* are present in South Texas: *A. americanum*, *Amblyomma imitator* Kohls, 1958, *A. inornatum*, *Amblyomma maculatum* Koch, 1844, and *A. mixtum*. *A. inornatum* larvae are easily distinguished from other *Amblyomma* larvae in this region by their short palpi, but must be carefully distinguished from some *Dermacentor* larvae which also have short palpi. Of the *Amblyomma* larvae, only *A. inornatum* larvae were identified to the species level. All larvae were identified by two different researchers. Meteorological data (daily averages and daily total rainfall) was from Laredo International Airport which is less than 2 km from the study site. The saturation deficit was calculated using this equation: $SD = (1 - RH/100)\ 4.9463e^{0.0161T}$, where SD represents the saturation deficit in millimeters of mercury, RH is the daily average relative humidity in percent, and T is the daily average temperature in degrees Celsius (Randolph and Storey, 1999). Statistical analysis was as previously described (Beck et al., 2011) using IBM SPSS for Windows, Version 22 (13 Aug, 2013, IBM, Chicago, IL). Three week means were determined by averaging the daily average temperature, humidity saturation deficit or wind speed for 21 days prior to the trap day. Total rainfall was determined by totaling the daily total rainfall for 21 days prior to the trap day, unless otherwise indicated. Spearman's coefficient of rank correlation was used to study the relationship between tick trap data and meteorological data. For nymphs and adults the density (number of ticks) per trap was determined. For larvae the density per trap is highly variable due to oviposition sites having large numbers of larvae, and other nearby sites having very few larvae. For larvae the percent of traps per trap day that had the presence of larvae on traps was used for statistical analysis. Multiple regression analysis was done with tick density of nymphs or adult per trap/percent of traps per trap day with larvae as the dependent variable and meteorological indicators as the independent variables. Pearson's rho was calculated to determine if the independent variables were correlated with each other.

Detection of Borrelia, Ehrlichia *and* Rickettsia *in* A. inornatum

Ticks were frozen within 1–2 h of collection at −20 °C. Except for the purpose of identification of ticks to species, were kept frozen until they were used for DNA purification. A total of seventy male and female adult *A. inornatum* ticks were used for PCR analysis. The entire tick was utilized for DNA extraction using E.Z.N.A. Mollusc DNA Isolation Kits (Omega Bio-Tek, Inc., Norcross, GA, USA). The protocol of Williamson et al. (2010) was modified as described. Each tick was washed in bleach five times followed by washing five times in deionized water. The tick was then chopped using a sterile razor blade and homogenized in 350 μL MLI buffer, using a microtube pestle. Each tick was ground for 5 min. After adding proteinase K, the samples were incubated at 60 °C for 4 h. Subsequent sample purification was performed according to the manufacturer's protocol.

The samples were then subjected to PCR for amplification of tick 16S rDNA, as well as *Borrelia* spp., *Ehrlichia* spp., and *Rickettsia* spp. genes as described by Williamson et al. (2010). To minimize risk of contamination, barrier tips were used. DNA isolation and PCR were conducted in different locations and at different times. Positive controls were added using separate hoods and pipettors from templates. The primers used in the present study are listed in Table 1. The PCR mixture was a 25 μL reaction volume containing 1.25 units of GoTaq polymerase (Promega, Madison, WI), 1× GoTaq Buffer, 160 ng/μL bovine serum albumin, 1.0 mM $MgCl_2$ 200 μM of each dNTP, 2 pmol primers, and 5 μL of template (1 μL for nested reactions). Amplifications were performed on a Bio-Rad MyCycler thermal cycler (Bio-Rad, Carlsbad, CA) with an initial 5 min denaturation at 95 °C, followed by 46 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A final extension step was performed at 72 °C for 7 min. For the detection of *Borrelia* spp. DNAs, nested amplifications were performed with 1 μl from the initial reaction as a template with an initial 5 min denaturation at 95 °C, followed by 36 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A final extension step was performed at 72 °C for 7 min. For PCR of mitochondrial 16S rDNA, an initial 2 min denaturation at 94 °C was followed by seven cycles with an increased annealing temperature of 0.3 °C per cycle, denaturation 94 °C for 30 s, annealing 47–48.8 °C, extension at 72 °C, followed by 28 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s. A final extension step was performed at 72 °C for 7 min.

PCR products were electrophoresed on 1.6% agarose gels stained with ethidium bromide (EtBr) in a 1× TAE buffer with EtBr. Gels were examined under UV light and results were compared to a known positive control. PCR samples were purified using Spin-Prep PCR Clean-UP Kits (Novagen, La Jolla, CA, USA) according to the manufacturer's protocols. DNA sequencing was performed at Cornell University Core Laboratories Center, at Texas A&M International University and MCLab (San Francisco, CA). The sequences were compared to sequences in the NCBI GenBank using BLAST. Sequences greater than 200 nucleotides long were submitted to GenBank (KM458241–KM458271).

Phylogenetic analysis

For phylogenetic analysis, initial alignments for genes from *Amblyomma*, *Borrelia*, *Ehrlichia*, and *Rickettisia* were each performed with MUSCLE (Edgar, 2004) as implemented by the European Bioinformatics Institute's MUSCLE server (http://www.ebi.ac.uk/Tools/muscle/) using the default settings, with subsequent manual adjustments if needed. Bayesian inference phylogenetic analyses were conducted using MrBayes