

Rickettsia bellii infecting *Amblyomma sabanerae* ticks in El Salvador

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Four *Amblyomma sabanerae* ticks collected from a turtle (*Kinosternon* sp.) in San Miguel, El Salvador, were found by molecular analysis to be infected by *Rickettsia bellii*. We provide the first report of *Rickettsia bellii* in Central America, and the first report of a *Rickettsia* species in El Salvador.

Keywords: *Rickettsia bellii*, *Amblyomma sabanerae*, El Salvador, Central America

A recent review reported 13 *Rickettsia* species occurring in Latin America and Caribbean.¹ Among these species, *Rickettsia bellii* is the one that has been found in the largest number of tick species, being the following ixodid species in Brazil and Argentina: *Amblyomma aureolatum*, *Amblyomma dubitatum*, *Amblyomma humerale*, *Amblyomma incisum*, *Amblyomma neumanni*, *Amblyomma nodosum*, *Amblyomma oblongoguttatum*, *Amblyomma ovale*, *Amblyomma scalpturatum*, *Amblyomma tigrinum*, *Haemaphysalis juxtakochi*, and *Ixodes loricatus*.¹ *R. bellii* is also known to occur in North America, where it was reported infecting either argasid or ixodid ticks.² On the other hand, *R. bellii* has never been reported in Central America.

In July 2010, four tick specimens were collected from a naturally infested turtle (*Kinosternon* sp.) in San Miguel, El Salvador. The ticks were all females, attached to the turtle carapace (Fig. 1). Ticks were removed, placed in a vial containing absolute ethanol, and sent to the laboratory for analyses. All ticks were identified as *Amblyomma sabanerae* following Fairchild *et al.*³ and Jones *et al.*⁴

The four ticks were individually submitted to DNA extraction by the guanidine isothiocyanate-phenol technique, as previously described.⁵ A 'blank' tube containing no tick sample was included in the DNA extraction. Samples were tested individually by PCR using primers CS-78 and CS-323 targeting a 401-bp fragment of the rickettsial gene *gltA*, as previously described.⁶ One negative control tube containing water was included, and also a positive control tube containing DNA of *Rickettsia parkeri* strain NOD, previously isolated in Vero cells from *Amblyomma nodosum* ticks

in our laboratory.⁷ Samples that yielded visible amplicons of the expected size by the *gltA* PCR were further tested by a second PCR assay using primers Rr190.70p and Rr190.602n targeting a 532-bp fragment of the rickettsial gene *ompA*, as previously described.⁸ In addition, to confirm tick taxonomic identification, DNA samples were individually tested by a PCR assay using primers T1B and T2A targeting a 360-bp fragment of the tick 12S rRNA mitochondrial gene, as previously described.⁹ PCR products were submitted to direct DNA sequencing in an automated ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The BLAST program (National Center for Biotechnology Information, Bethesda, MD, USA) was used to compare appropriate similarities of the rickettsial or tick mitochondrial partial sequence generated in the current study.

The four *A. sabanerae* female ticks yielded amplicons of the expected size by the *gltA* PCR. Both the DNA extraction blank tube and the negative control tube yielded no visible amplicon. The four ticks were negative by PCR targeting the *ompA* gene. The products of the *gltA* PCR were DNA sequenced. Through blast analysis, these four *A. sabanerae* ticks were found infected with a *Rickettsia* 100% (320/320) identical to *Rickettsia bellii* strain AT, previously detected in *Amblyomma tigrinum* from Argentina (EU826511), and 99.4% (324/326) similar to at least seven corresponding sequences of *R. bellii* from different tick species in Brazil (EU567181, DQ865204, AY362703, DQ146481), Argentina (DQ517288), and the USA (CP000087, RBU59716). The four ticks were molecularly confirmed to be *A. sabanerae*; their individual 12S rRNA edited sequences (345-bp) were identical to each other and 98.3% (339/345) similar to GenBank accession number AY766150, which according to a recent study, refers to *A. sabanerae*.¹⁰ The DNA sequences of *R. bellii* (*gltA*) and *A. sabanerae* (12S rRNA) from El Salvador generated in the present study

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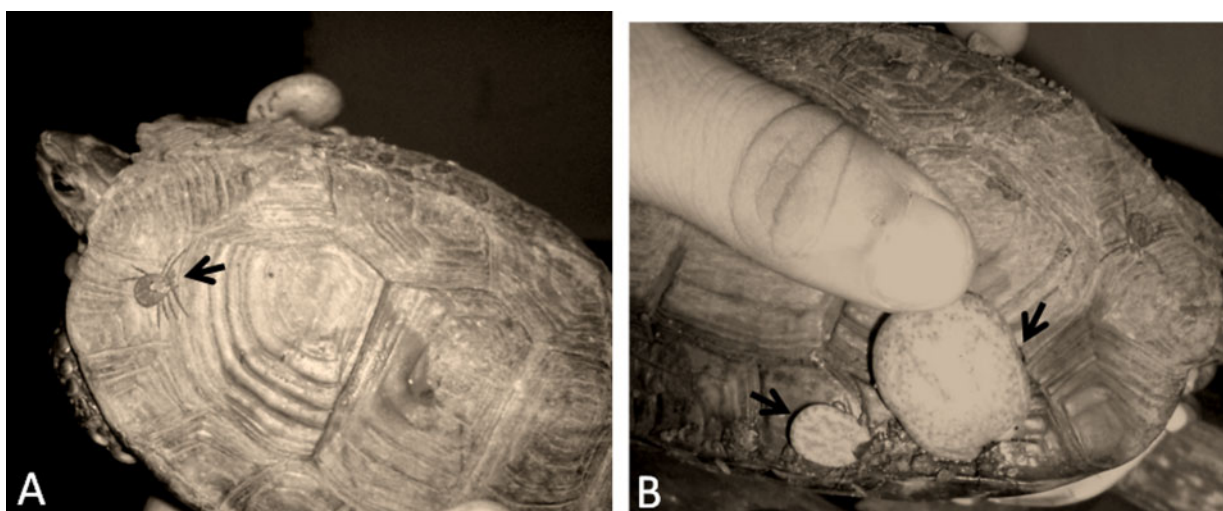


Figure 1 *Amblyomma sabanerae* female ticks attached to the carapace of a turtle *Kinosternon* sp. in El Salvador: (A) one non-engorged female indicated by an arrow; (B) two partially engorged females indicated by arrows.

have been deposited in GenBank under the accession numbers JQ664297 and JQ928695, respectively.

The present report adds the tick species *A. sabanerae* to the growing list of American ticks that have been found infected by *R. bellii*, which now includes 13 *Amblyomma* species in the Neotropical region. In addition, our findings represent the first report of *R. bellii* in Central America, and the first report of a *Rickettsia* species in El Salvador. Previous reports related to *Rickettsia* in El Salvador has been restricted to seroepidemiological studies, indicating that humans in this country were exposed to either a spotted fever group (SFG) or a typhus group (TG) agent.^{11,12} Recent genetic studies have indicated that *R. bellii* is not either a SFG or a TG agent; it has been classified in a basal group together with other genotypes associated mostly with insects.¹³ Therefore, our negative results of the *ompA* PCR are expected, since this PCR protocol has been shown to work only for some members of the SFG rickettsiae.⁸

Vertical transmission of *R. bellii* in ticks is also known to occur.¹⁴ Although we tested a small sample size, all four ticks were shown to be infected. Previously, a 100% infection rate by *R. bellii* was reported in *Amblyomma rotundatum* ticks in Brazil.⁶ Interestingly, both *A. sabanerae* and *A. rotundatum* are commonly found feeding on reptiles.^{3,4,15} Despite a wide distribution of *R. bellii* in the New World,^{1,2} the role of this organism as human or animal pathogen remains unknown.

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