

Spotted Fever Group Rickettsiae in Immature *Amblyomma maculatum* (Acari: Ixodidae) from Mississippi¹

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J. Entomol. Sci. 51(4): 329–331 (October 2016)

Key Words *Amblyomma maculatum* immatures, spotted fever group rickettsiae, Mississippi

Very little is known about the vector potential posed by larval and nymphal *Amblyomma maculatum* Koch (Gulf Coast ticks) to humans or other vertebrate species. *Rickettsia parkeri* Lackman, the causative agent of American boutonneuse fever in humans, which is transmitted by the Gulf Coast tick (Parker et al. 1939, Public Health Rep. 54: 1482–1484; Goddard 2004, Infect. Med. 21: 207–210; Paddock et al. 2004, Clin. Infect. Dis. 38: 805–811) has been detected in Mississippi on several occasions (Paddock et al. 2008, Clin. Infect. Dis. 47: 1188–1196; Ferrari et al. 2012, Emerg. Infect. Dis. 18: 1705–1707; Ekenna et al. 2014, J. Miss. State Med. Assoc. 55: 216–219), as well as other states in the United States and several countries in South America. The role played by immatures in the ecology of *R. parkeri* has only recently been considered. Another spotted fever group rickettsia (SFGR), “*Candidatus Rickettsia andeanae*,” occurs in Gulf Coast ticks from North and South America (Blair et al. 2004, J. Clin. Microbiol. 42: 4961–4967; Paddock et al. 2010, Appl. Environ. Microbiol. 76: 2689–2696; Ferrari et al. 2012). It is unknown if “*Ca. R. andeanae*” is a pathogen of humans; however, it is possible that this SFGR may play a role in the natural history of *R. parkeri* by rickettsial interference (Paddock et al. 2015, Ticks Tick Borne Dis. 6: 297–302).

Rickettsia parkeri is transmitted transovarially under laboratory conditions (Wright et al. 2015, Ticks Tick Borne Dis. 6: 568–573), and immature stages of *A. maculatum* have been shown to attach to humans (Goddard 2002, J. Agromed. 8: 25–32; Portugal and Goddard 2015, J. Med. Entomol. doi: 10.1093/jme/tjv185). To our knowledge, neither *R. parkeri* nor “*Ca. R. andeanae*” have ever been detected in questing immature specimens of *A. maculatum* (Fornadel et al. 2011, Vector Borne Zoonotic Dis. 12: 1535–1538; Florin et al. 2013, Syst. Appl. Acarol.

¹Received 08 April 2016; accepted for publication 06 June 2016.

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18: 27–29; Nadolny et al. 2014, Ticks Tick Borne Dis. 5: 53–57). This study was initiated to document rickettsial infection in questing (unfed) immature Gulf Coast ticks to better assess the relative risk posed by these stages in the transmission of *R. parkeri* to humans.

Immature *A. maculatum* ticks were collected at Grand Bay National Wildlife Refuge in Jackson Co., MS, a site known for abundant adult stages of this species. Collections were made by drag cloth once per month from October to May for 2 yr (2012–2014). In addition to the drag cloth, a swab specifically designed to collect immature and low-questing ticks was used to sample underbrush and rodent trails (Portugal and Goddard 2015, Syst. Appl. Acarol. 20: 20–24). All specimens collected were immediately placed in 95% ethanol and transported to Mississippi State University for identification using standard keys (Keirans and Litwak 1989, J. Med. Entomol. 26: 435–448; Keirans and Durden 1998, J. Med. Entomol. 35: 489–495). Identifications were confirmed by Dr. Richard G. Robbins formerly at the Armed Forces Pest Management Board in Washington, DC.

Field-collected specimens were removed from ethanol, allowed to air-dry, and transferred to individual 1.5-ml microcentrifuge tubes. Genomic DNA was extracted from triturated ticks using a DNA Minikit (Qiagen, Valencia, CA) and eluted in a final volume of 50 μ L. Each extract was tested using a conventional polymerase chain reaction (PCR) assay targeting a 336-bp portion of the tick mitochondrial 12S ribosomal DNA gene with primers T1B/T2A (Beati and Keirans 2001, J. Parasitol. 87: 32–48) and screened for presence of *Rickettsia* spp. DNA using a real-time TaqMan assay targeting a 74-bp segment of the rickettsial *gltA* gene (Stenos et al. 2005, Am. J. Trop. Med. Hyg. 73: 1083–1085). Samples that tested positive by the *gltA* PCR assay were subsequently tested using a hemi-nested PCR assay that generates a 470-bp amplicon of the rickettsial outer membrane protein *ompA* (Regenry et al. 1991, J. Bacteriol. 173: 1576–1589; Roux et al. 1996, J. Clin. Microbiol. 34: 2058–2065). All amplicons were gel-purified and sequenced in both directions. Nucleotide sequences were aligned using Sequencher 5.1 (Gene Codes Corporation, Ann Arbor, MI) and compared to GenBank data using Basic Local Alignment Search Tool (BLAST) analysis.

A total of 13 questing immature *A. maculatum* ticks comprising 12 nymphs and 1 larva were collected and evaluated. Molecular analysis of the 12S ribosomal RNA gene corroborated the taxonomic identification of *A. maculatum* for all samples. Three (25.0%) of 12 *A. maculatum* nymphs yielded amplicons with the *gltA* and *ompA* PCR assays. BLAST analysis revealed 100% identity of two of them (16.6%) with the corresponding sequence of *R. parkeri* strain Portsmouth (CP003341). The third nymphal tick contained DNA with 100% identity with the corresponding sequence of “*Ca. Rickettsia andeanae*” (KF179352). The single larval specimen was negative for infection with SFGR.

To our knowledge, this is the first confirmation of SFGR infection in field-caught, unfed immature *A. maculatum*. This study is limited by small sample size; however, immature *A. maculatum* are only rarely collected as questing specimens (Goddard 2007, J. Vector Ecol. 32: 157–158; Portugal and Goddard 2015; Portugal and Goddard 2016, J. Entomol. Sci. 51: 1–8) and are almost always obtained from vertebrate hosts. The infection rate of nymphs with *R. parkeri* reported here (16.6%) is similar to that reported in adult *A. maculatum* collected in the same area (15.2%) (Ferrari et al. 2012). There are some clinical data that indicate that immature *A.*

maculatum may be involved in transmission of *R. parkeri* rickettsiosis (Paddock et al. 2008). Further investigation is warranted to elucidate the role(s) of both larval and nymphal *A. maculatum* in transmission of this agent to humans and other vertebrates.