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## ESTABLISHMENT OF *AMBLYOMMA MACULATUM* (GULF COAST TICK) IN ARKANSAS, U.S.A.

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*Amblyomma maculatum* Koch (Gulf Coast tick) has been reported throughout the southeastern United States, California, and Arizona (Goddard & Norment 1983) and on various animals including cattle, deer, mesocarnivores, and ground dwelling birds (Tugwell & Lancaster 1962). The tick is a pest of economic and pathogenic concern since it readily infests cattle (Semtner & Hair 1973), and is the vector of *Cowdria ruminantium* Cowdry (the causative agent of Heartwater disease) (Uilenburg 1982) and *Rickettsia parkeri* (the causative agent of newly described spotted fever) (Paddock et al. 2004). Previous confirmations of *R. parkeri* in *A. maculatum* included specimens from Georgia, Florida, Kentucky, Mississippi, and South Carolina (Sumner et al. 2007). *Amblyomma maculatum* was initially thought to reside within 160 km of the Atlantic and Gulf Coasts (Bishop & Hixson 1936), but it has been found in 18 eastern counties of Oklahoma (Semtner & Hair 1973) and in Washington County and Ashley County in northwest Arkansas (Lancaster 1973). It may have become redistributed with livestock movement (Goddard & Norment 1983) and cattle egrets migration (USDA APHIS 2008).

As a part of a larger study, investigating ticks on canines and deer (Trout & Steelman *in press*), veterinarians and the Arkansas Game and Fish Commission removed ticks from hosts and stored them in 100% ethanol. Collection date, location, and host were recorded, and collections were submitted to the Veterinary Entomology laboratory at the University of Arkansas where they were identified to species, sex, life stage, and engorgement with keys from Arthur (1961), Lancaster (1973), Goddard & Norment (1985).

To confirm the species identity, 9 identified *A. maculatum* were subjected to phylogenetic analyses based on DNA sequences of a portion of the mitochondrial 16S mt rDNA gene. Extraction and PCR amplification procedures were similar to Trout et al. (2009). Briefly, individual specimens were cut longitudinally with a sterile razor blade and subjected to the Qiagen Dneasy Insect Protocol (Qiagen Inc. Rohm and Haas Company, Valencia, CA). The DNA extractions were assessed by PCR with mitochondrial primers 16S+2 and 16S-1 per the methods previously described (Black & Piesman 1994). Reaction products were analyzed with gel electrophoresis. Positive reaction products were purified and concentrated with minicolumns according to the manufacturer's instructions (Wizard PCR preps, Promega) and sent to the University of Arkansas Medical Sciences

DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. Sequences were aligned with Clustal in Bioedit 5.09 (Hall 1999). Bayesian Evolutionary Analysis Sampling Trees (BEAST) version 1.4.2 software (Drummond & Rambaut 2003) was used to obtain phylogenetic trees with the GTR + G model. To create the consensus tree, 4 Markov chains ran for 10<sup>6</sup> generations with a burn-in of 2 x 10<sup>4</sup>. Phylogenetic relationships were compared to other *Amblyomma* ticks and 1 *Argas* tick obtained from GenBank, as follows: *Amblyomma americanum* Say (L34314), *A. americanum* (L34313), *A. aureolatum* (Pallas) (AF541254), *A. cajennense* Fabricius (L34317), *A. dubitatum* Neumann (DQ858954), *A. glauerti* Keirans, King & Sharrad (U95853), *A. hebraeum* Koch (L34316), *A. maculatum* Koch (AY375442), *A. ovale* Koch (AF541255), *A. parvum* Arag (EU306154), *A. triste* Koch (AY498563), *A. tuberculatum* Marx (U95856), *A. variegatum* Fabricius (L34315), and *Argas brevipes* Banks (U95863). All 9 *A. maculatum* specimens were confirmed with Bayesian analysis and GenBank comparisons. Seven haplotypes of the 16S mt rDNA were identified which were 100% homologous with NCBI GenBank accession numbers DQ076315-DQ76321 collected from Kansas, Texas, and Oklahoma (Ketchum et al. 2009). All of the haplotypes were placed into 1 clade suggesting that the group is monophyletic.

A total of 232 *A. maculatum* were collected in Arkansas between 2006 and 2009 (Table 1, Fig. 1). We identified *A. maculatum* collected from 22 canines (*Canis lupus familiaris* L.), 50 white-tailed deer (*Odocoileus virginianus* (Boddaert)), 1 feline (*Felis catus* L.), and 1 cow (*Bos taurus* L.) from Arkansas between I-III-2006 and I-VI-2009. The collection sites were then georeferenced in ArcMap 9.3.1 (ESRI Redlands CA) to map the distribution. Previous reports of *A. maculatum* included a specimen in a 1950s drag sample from Washington County and in a 1960s cattle sample from Ashley County (Lancaster 1973). Eads (2001) found 13 Gulf Coast ticks between May and Jul of 2000 at the Arkansas National Post, located in Arkansas County in the southeast region of the state. We believe this tick's distribution across the state suggests the tick has established itself compared to the few numbers and limited distribution mentioned by Koch (1982). The calculated Moran's I for *A. maculatum* on the canines was 0.01 (0.47 standard deviation) and 0.46 (1.19 standard deviation) on deer, indicating the patterns were neither clustered nor dispersed, but random across

TABLE 1. IDENTIFIED *AMBLIOMMA MACULATUM* TICKS COLLECTED FROM ARKANSAS MAMMALS.

Host	No. Hosts Infested/ No. Hosts Sampled (% infestation)	Life Stage				Total (e)
		Larva (e <sup>1</sup> )	Nymph (e)	Male (e)	Female <sup>2</sup> (e)	
Canine	23/156 (15%)	0	8 (2)	95 (2)	9 (0)	113 (4)
White-tailed deer	50/250 (20%)	0	8 (0)	46 (10)	41 (27)	95 (37)
Cow	1/1 (100%)	0	0	12 (8)	11 (5)	23 (13)
Feline	1/16 (6%)	0	0	0	1 (1)	1 (1)
Total	74/422 (17.5%)	0	16 (2)	153 (20)	62 (33)	232 (55)

<sup>1</sup>Number of engorged specimens.<sup>2</sup>One adult specimen was damaged and could not be properly sexed.

### Distribution of *Amblyomma maculatum*

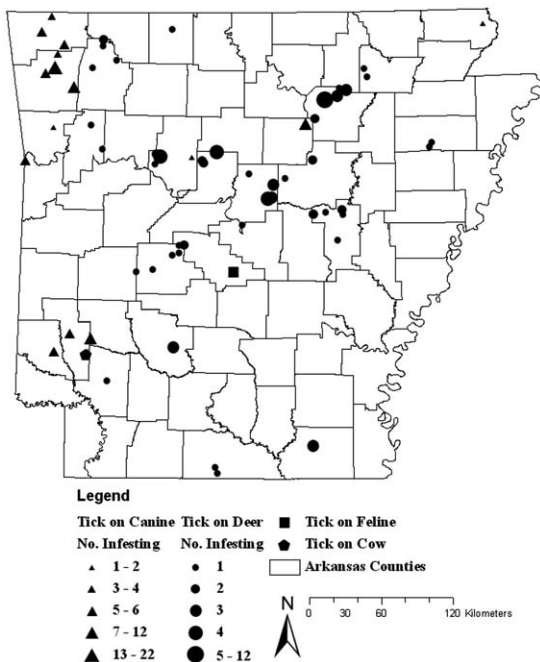


Fig. 1. Geographical distribution of *Amblyomma maculatum* from canines (triangles), white-tailed deer (circles), feline (square), and cow (pentagon) in Arkansas. The larger the symbol the greater the number of ticks found on that animal.

the state. Previous reports considered this tick's distribution as "sporadic" since they are dispersed with livestock shipments (Goddard & Paddock 2005; Goddard 2007).

### SUMMARY

The Gulf Coast tick was found throughout Arkansas on wild and domesticated animals; conse-

quently, this tick has established itself in Arkansas. Bayesian analyses confirmed the presence of this species. Because *Amblyomma maculatum* is the primary vector of *Rickettsia parkeri* and the secondary vector of *Cowdria ruminantium*, additional studies on the distribution of this tick should be investigated as well as its relation to human diseases.

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