Complete Mitochondrial Genome Sequence of the Gulf Coast Tick (Amblyomma maculatum)

Amanda E. Brenner,a,b Rahul Raghavana,b

aDepartment of Biology, Portland State University, Portland, Oregon, USA
bDepartment of Biology, The University of Texas at San Antonio, San Antonio, Texas, USA

ABSTRACT The complete circularized mitochondrial genome sequence of Amblyomma maculatum is 14,803 bp long. It encodes 13 protein coding genes, 2 rRNA genes, 22 tRNA genes, 2 tick box motifs, and 2 control regions. The gene arrangement and content are consistent with those of previously reported Metastrata tick mitochondrial genomes.

While many Amblyomma species have established long-term relationships with Coxiella-like endosymbionts (CLEs), Amblyomma maculatum contains a Francisella-like endosymbiont (FLE) that likely supplements its obligate hematophagous diet with essential vitamins (1–5). In order to understand the dynamics of endosymbiont-tick coevolution, it is necessary to establish the relationships among ticks. In contribution to this effort, in this genome announcement, we describe the complete mitochondrial genome sequence of the Gulf Coast tick (Amblyomma maculatum).

To sequence its mitochondrial genome, a female A. maculatum was procured from the Oklahoma State University Tick Rearing Facility. DNA was extracted from it using a DNeasy blood and tissue kit (Qiagen) and submitted to the Oregon Health & Science University’s Massively Parallel Sequencing Shared Resource. Sequencing libraries were prepared using the TruSeq DNA library kit (Illumina) and sequenced using a paired-end protocol on a HiSeq 2500 instrument (Illumina). FASTQ files were assembled from the base-called files using bcl2fastq v2.20 software (Illumina). This process yielded ~180 million 100-bp read pairs. The reads were trimmed using Trimmomatic v0.39 (leading and trailing q-scores, ≥20; 5-bp sliding window q-scores, ≥25; length, ≥50 bp) and assembled into several thousand contigs using IDBA-UD v1.1.3 (6, 7). A single tick mitochondrial contig was identified among them using BLASTn v.2.6.0 (E value, ≤10−15) (8) and a database of all complete tick mitochondrial genome sequences publicly available in NCBI as of May 2017 (n = 47). All trimmed reads were mapped back to the mitochondrial contig as well as directly to the library of mitochondrial genome sequences, and the mapped reads were pooled and deduplicated, resulting in approximately 64,000 read pairs. These reads were assembled using IDBA-UD v1.1.3, yielding a linear 14,803-bp mitochondrial genome sequence (Table 1). PCR was used to close the genome and to validate the control region sequences; the PCR primer sequences are available on NCBI along with the genome sequence (GenBank accession number MW719251). The final assembly had an average sequencing coverage of 43×.

The A. maculatum mitochondrial genome sequence was annotated using MITOS rev.6b33f95 (9), and in order to perform a manual comparison of the mitochondrial gene arrangement, we reannotated the other 47 mitochondrial genome sequences using the same program. Similar to other Metastrata and Australasian Prostriata hard ticks, A. maculatum’s mitochondrial genome contains two copies of the

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control region, the putative regulatory region for mitochondrial replication and transcription (10) (Table 1). Previous reports indicate that the two regions evolved in synchrony, although the derived sequence is more variable in length (11, 12). In line with these findings, in *A. maculatum* the two control regions share an identical 275-bp segment, which accounts for most of the ancestral control region’s length, while the derived control region spans an additional 30 bp (Table 1). A putative posttranscriptional regulatory element known as the “tick box” is thought to be responsible for the extant Metastriata gene arrangement (10, 13). In *A. maculatum*, two tick box motifs occur as direct tandem repeats flanking a past relocation event (Table 1), whereas some other Metastriata species contain a third inverted tick box sequence (10, 13). It is unclear how prevalent this inverted repeat is among *Amblyomma* species; for instance, *Amblyomma hebraeum* contains the inverted repeat, but *Amblyomma americanum* does not (13).

To elucidate the evolutionary relationship of *A. maculatum* to other ticks, phylogenetic trees were generated using 10,488 bp of unambiguously aligned nucleotides. As illustrated in Fig. 1, the primary endosymbiont present in a tick is not determined solely by the tick phylogeny. CLEs are likely the ancestral tick endosymbiont, but newer CLEs and FLEs with better metabolic capabilities for supplying their hosts with nutrients have likely replaced the original CLEs (2, 14). In sum, the *A. maculatum* mitochondrial genome sequence presented here is expected to improve our understanding of mitochondrial noncoding regions and the evolutionary relationships between ticks and the endosymbionts they carry.

Data availability. The mitochondrial genome sequence for *Amblyomma maculatum* has been deposited in DDBJ/ENA/GenBank under the accession number MW719251. The genome assembly described in this paper is the first version, MW719251.1. The SRA records are available under accession number SRS8901492. The BioSample and BioProject accession numbers are SAMN19069232 and PRJNA728115, respectively.
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