AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

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

Amanda E. Brenner,[a,b] **Rahul Raghavan**[a,b]

[a]Department of Biology, Portland State University, Portland, Oregon, USA
[b]Department 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 Metastriata 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 Metastriata and Australasian Prostriata hard ticks, *A. maculatum*'s mitochondrial genome contains two copies of the

Citation Brenner AE, Raghavan R. 2021. Complete mitochondrial genome sequence of the Gulf Coast tick (*Amblyomma maculatum*). Microbiol Resour Announc 10:e00431-21. https://doi.org/10.1128/MRA.00431-21.

Editor Jason E. Stajich, University of California, Riverside

Address correspondence to Rahul Raghavan, rahul.raghavan@utsa.edu.

Received 8 May 2021
Accepted 25 August 2021
Published 23 September 2021

TABLE 1 *Amblyomma maculatum* mitochondrial genome characteristics

Characteristic	Value
GenBank accession no.	MW719251
AT content (%)	78.75
No. of protein coding genes	13
(+) strand	9
(−) strand	4
Start codon usage	
ATT	6
ATG	6
ATA	1
Stop codon usage	
TAA	10
T--	3
ENc[a]	35.19
No. of tRNAs	22
(+) strand	13
(−) strand	9
Position of putative regulatory elements	
Control region (ancestral)	8796–9100
Control region (derived)	14411–14745
Tick boxes	5827–5844
	6828–6845

[a] ENc, effective number of codons for all protein coding genes.

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.

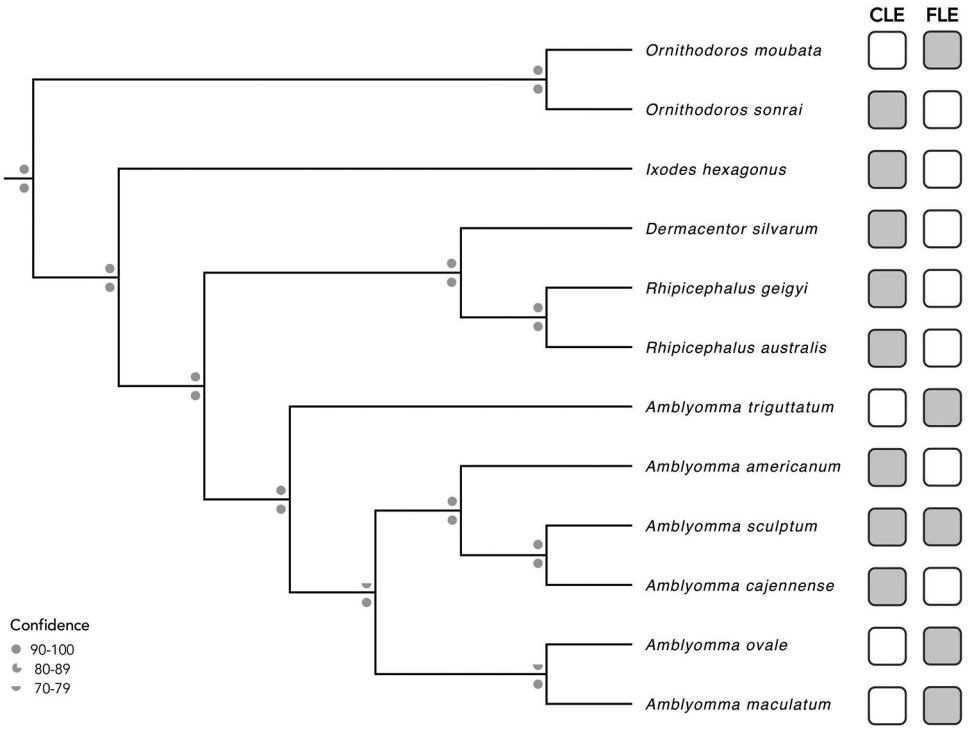

FIG 1 Distribution of primary endosymbionts in ticks. Maximum likelihood and Bayesian trees were generated using 13 mitochondrial protein coding gene sequences from 12 tick species with fully sequenced mitogenomes. The nucleotide sequences of the 13 protein coding genes were aligned individually using global MAFFT v7.475 (15) and then concatenated. GBlocks v0.91b (16) was used to cull ambiguously aligned regions, and jModelTest2 v2.1.10 (17) was used to select the appropriate model (GTR+I+G). The final tree is based on 10,488 nucleotide positions. The maximum likelihood tree was generated using RAxML v8.2.12, and the Bayesian tree was produced using MrBayes v3.2.7 (18, 19). Bootstrap support and posterior probabilities are depicted above and below the branch points, respectively. The gray and white boxes indicate the presence and absence, respectively, of endosymbionts in each tick (4, 5, 20).

ACKNOWLEDGMENT

This project was supported in part by the NIH grant AI126385 to R.R.

REFERENCES

1. Smith TA, Driscoll T, Gillespie JJ, Raghavan R. 2015. A *Coxiella*-like endosymbiont is a potential vitamin source for the Lone Star tick. Genome Biol Evol 7:831–838. https://doi.org/10.1093/gbe/evv016.
2. Gerhart JG, Moses AS, Raghavan R. 2016. A *Francisella*-like endosymbiont in the Gulf Coast tick evolved from a mammalian pathogen. Sci Rep 6: 33670. https://doi.org/10.1038/srep33670.
3. Gerhart JG, Dutcher HA, Brenner AE, Moses AS, Grubhoffer L, Raghavan R. 2018. Multiple acquisitions of pathogen-derived *Francisella* endosymbionts in soft ticks. Genome Biol Evol 10:607–615. https://doi.org/10.1093/gbe/evy021.
4. Binetruy F, Buysse M, Lejarre Q, Barosi R, Villa M, Rahola N, Paupy C, Ayala D, Duron O. 2020. Microbial community structure reveals instability of nutritional symbiosis during the evolutionary radiation of *Amblyomma* ticks. Mol Ecol 29:1016–1029. https://doi.org/10.1111/mec.15373.
5. Duron O, Binetruy F, Noël V, Cremaschi J, McCoy KD, Arnathau C, Plantard O, Goolsby J, Pérez de León AA, Heylen DJA, Van Oosten AR, Gottlieb Y, Baneth G, Guglielmone AA, Estrada-Peña A, Opara MN, Zenner L, Vavre F, Chevillon C. 2017. Evolutionary changes in symbiont community structure in ticks. Mol Ecol 26:2905–2921. https://doi.org/10.1111/mec.14094.
6. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
7. Peng Y, Leung HCM, Yiu SM, Chin FYL. 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28:1420–1428. https://doi.org/10.1093/bioinformatics/bts174.
8. Boratyn GM, Camacho C, Cooper PS, Coulouris G, Fong A, Ma N, Madden TL, Matten WT, McGinnis SD, Merezhuk Y, Raytselis Y, Sayers EW, Tao T, Ye J, Zaretskaya I. 2013. BLAST: a more efficient report with usability improvements. Nucleic Acids Res 41:W29–W33. https://doi.org/10.1093/nar/gkt282.
9. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol 69:313–319. https://doi.org/10.1016/j.ympev.2012.08.023.
10. Chen Z, Xuan Y, Liang G, Yang X, Yu Z, Barker SC, Kelava S, Bu W, Liu J, Gao S. 2020. Precise annotation of tick mitochondrial genomes reveals multiple copy number variation of short tandem repeats and one transposon-like element. BMC Genomics 21:488. https://doi.org/10.1186/s12864-020-06906-2.
11. Burger TD, Shao R, Beati L, Miller H, Barker SC. 2012. Phylogenetic analysis of ticks (Acari: Ixodida) using mitochondrial genomes and nuclear rRNA genes indicates that the genus *Amblyomma* is polyphyletic. Mol Phylogenet Evol 64:45–55. https://doi.org/10.1016/j.ympev.2012.03.004.
12. Shao R, Barker SC, Mitani H, Aoki Y, Fukunaga M. 2005. Evolution of duplicate control regions in the mitochondrial genomes of metazoa: a case study with Australasian *Ixodes* ticks. Mol Biol Evol 22:620–629. https://doi.org/10.1093/molbev/msi047.
13. Montagna M, Sassera D, Griggio F, Epis S, Bandi C, Gissi C. 2012. Tick-box for 3′-end formation of mitochondrial transcripts in Ixodida, basal chelicerates and *Drosophila*. PLoS One 7:e47538. https://doi.org/10.1371/journal.pone.0047538.

14. Brenner AE, Muñoz-Leal S, Sachan M, Labruna MB, Raghavan R. 2021. *Coxiella burnetii* and related tick endosymbionts evolved from pathogenic ancestors. Genome Biol Evol 13:evab108. https://doi.org/10.1093/gbe/evab108.
15. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010.
16. Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 56:564–577. https://doi.org/10.1080/10635150701472164.
17. Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772. https://doi.org/10.1038/nmeth.2109.
18. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
19. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029.
20. Gofton AW, Doggett S, Ratchford A, Oskam CL, Paparini A, Ryan U, Irwin P. 2015. Bacterial profiling reveals novel "*Ca. Neoehrlichia*," *Ehrlichia*, and *Anaplasma* species in Australian human-biting ticks. PLoS One 10: e0145449. https://doi.org/10.1371/journal.pone.0145449.