The complete mitogenome of *Achalarus lyciades* (Lepidoptera: Hesperiidae)

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Abstract

We obtained a complete mitochondrial genome of a skipper butterfly *Achalarus lyciades* (Hesperiidae, Eudaminae) from next generation sequencing reads. The 15,612 bp mitogenome covers 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and an A+T rich region. Its gene order is typical for mitogenomes of Lepidoptera. Phylogenetic analysis placed *A. lyciades* as a sister to *Lobocla bifasciatus*, the only other Eudaminae with available mitogenome.

Keywords

The Hoary Edge (*Achalarus lyciades*) is a skipper butterfly (family Hesperiidae, subfamily Eudaminae) widely distributed over the eastern United States. Its English name comes from a large whitish patch beneath the hindwing near the edge. To better understand the phylogeny of Hesperiidae, we sequenced, assembled and annotated the complete mitogenome of *A. lyciades* from the male voucher NVG-3311 collected in USA: Texas, Sabine Co., Sabine National Forest, 1 mi south of Fairmount, near Fox Hunters' Hill, GPS 31.185394, -93.72992 on 12-Apr-2015. The body was stored in *RNAlater* solution and wings preserved to be deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. Methods for genomic DNA extraction, library construction, next-generation sequencing, and computational procedures followed those we reported previously[1–4]. Using mitogenome of *Choaspes benjaminii* as a reference to "bait" sequence reads, about 2.9% of reads (4,732,966 out of 163,971,978) were extracted by MITObim[5] from the 250 bp library, followed by 15 mer JELLYFISH[6] counting and QUAKE[7] error correction to exclude frequency lower than 1000 reads[4]. The corrected reads were assembled denovo with Platanus. The 5' and 3' end of the 15491 bp Platanus assembly was inspected and extended manually to complete the circular structure[4]. The genome

sequence was annotated using the MITOS web server[8], the predictions were manually curated using other published skipper mitogenomes as references. The assembly quality was assessed by reads mapping and sequence multiple alignment of the PCGs, tRNAs and rRNAs genes with published Hesperiidae mitogenomes for consistency checking.

The complete mitogenome of *A. lyciades* is 15613 bp in length (Genbank: KX249739) and is AT rich, with a base composition of 40.5% A, 41.4% T, 7.3% G, and 10.8% C. It retains the typical insect mitogenome gene set, including 13 PCGs (ND1-6, COX1-3, ND4L, ATP8, ATP6, and CYTB), 22 tRNA genes (two for serine and leucine and one for each of the rest amino acids), 2 ribosomal RNAs (rrnL and rrnS), and an A+T rich D-loop control region. In many Lepidoptera mitogenomes, COX1 gene starts from the codon CGA[9], however, in *A. lyciades*, all PCGs including COX1 use the typical start codon ATN. COX1, COX2 and ND4 have an incomplete stop codon T, and a complete TAA codon is likely formed during mRNA maturation[10,11]. The length of tRNAs ranges from 61 to 73 bp. The size of the two rRNAs are 1378 and 778 bp, respectively. A 452 bp A+T rich region connects rrnS and tRNA-Met.

To phylogenetically place *A. lyciades* within Hesperiidae with available mitogenomes[3,4,12–18], we constructed RAxML[19] maximum likelihood tree rooted with *Papilio glaucus* (Papilionidae) mitogenome[3] (Fig 1). Sister relationship of *A. lyciades* and *Lobocla bifasciatus* is strongly supported, in agreement with the placement of *Lobocla* as a sole Old Word genus in the mostly New World subfamily Eudaminae[20–22]. The tree topology is largely consistent with previous phylogenetic studies[20–22]: Coeliadinae are the sister to all other Hesperiidae; among taxa with available mitogenomes, Tagiadini and Celaenorrhini are sisters, Heteropterinae are the sister to Hesperiinae within which Aeromachini is the sister to the rest. However, bootstrap on mitogenomes is insufficient to support monophyly of Pyrginae, and the topology within the crown Hesperiinae group deviates from that reported previously and needs to be investigated further. In conclusion, the complete mitogenome of *A. lyciades* is the first one for a New Word representative of subfamily Eudaminae essential for further studies of Lepidoptera.

Acknowledgments

References

1. Cong Q, et al. Complete genomes of Hairstreak butterflies, their speciation, and nucleo-mitochondrial incongruence. Sci Rep. 2016; 6:24863. [PubMed: 27120974]

2. Cong Q, et al. Speciation in Cloudless Sulphurs Gleaned from Complete Genomes. Genome Biol Evol. 2016; 8:915–931. [PubMed: 26951782]

3. Shen J, Cong Q, Grishin NV. The complete mitochondrial genome of Papilio glaucus and its phylogenetic implications. Meta Gene. 2015; 5:68–83. [PubMed: 26106582]

4. Cong Q, Grishin NV. The complete mitochondrial genome of Lerema accius and its phylogenetic implications. PeerJ. 2016; 4:e1546. [PubMed: 26788426]

5. Hahn C, Bachmann L, Chevreux B. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads--a baiting and iterative mapping approach. Nucleic acids research. 2013; 41:e129. [PubMed: 23661685]

6. Marcais G, Kingsford C. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics. 2011; 27:764–770. [PubMed: 21217122]

7. Kelley DR, Schatz MC, Salzberg SL. Quake: quality-aware detection and correction of sequencing errors. Genome biology. 2010; 11:R116. [PubMed: 21114842]

8. Bernt M, et al. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 2013; 69:313–319. [PubMed: 22982435]

9. Kim MI, et al. Complete nucleotide sequence and organization of the mitogenome of the red-spotted apollo butterfly, Parnassius bremeri (Lepidoptera: Papilionidae) and comparison with other lepidopteran insects. Molecules and cells. 2009; 28:347–363. [PubMed: 19823774]

10. Ojala D, Montoya J, Attardi G. tRNA punctuation model of RNA processing in human mitochondria. Nature. 1981; 290:470–474. [PubMed: 7219536]

11. Boore JL. Animal mitochondrial genomes. Nucleic acids research. 1999; 27:1767–1780. [PubMed: 10101183]

12. Wang K, Hao J, Zhao H. Characterization of complete mitochondrial genome of the skipper butterfly, Celaenorrhinus maculosus (Lepidoptera: Hesperiidae). Mitochondrial DNA. 2013

13. Kim MJ, Wang AR, Park JS, Kim I. Complete mitochondrial genomes of five skippers (Lepidoptera: Hesperiidae) and phylogenetic reconstruction of Lepidoptera. Gene. 2014; 549:97–112. [PubMed: 25058696]

14. Wang AR, Jeong HC, Han YS, Kim I. The complete mitochondrial genome of the mountainous duskywing, Erynnis montanus (Lepidoptera: Hesperiidae): a new gene arrangement in Lepidoptera. Mitochondrial DNA. 2014; 25:93–94. [PubMed: 23586336]

15. Wang J, James John Y, Xuan S, Cao T, Yuan X. The complete mitochondrial genome of the butterfly Hasora anura (Lepidoptera: Hesperiidae). Mitochondrial DNA. 2015:1–2.

16. Jiang W, et al. Complete mitochondrial DNA genome of Polytremis nascens (Lepidoptera: Hesperiidae). Mitochondrial DNA A DNA MappSeq Anal. 2016; 27:3131–3132. [PubMed: 25690054]

17. Shao L, Sun Q, Hao J. The complete mitochondrial genome of Parara guttata (Lepidoptera: Hesperiidae). Mitochondrial DNA. 2015; 26:724–725. [PubMed: 24460163]

18. Hao J, et al. The Complete Mitochondrial Genome of Ctenoptilum vasava (Lepidoptera: Hesperiidae: Pyrginae) and Its Phylogenetic Implication. Comparative and functional genomics. 2012; 2012:328049. [PubMed: 22577351]

19. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006; 22:2688–2690. [PubMed: 16928733]

20. Warren AD, Ogawa JR, Brower AVZ. Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperiidae (Lepidoptera: Hesperioidea). Cladistics. 2008; 24:642–676.

21. Warren AD, Ogawa JR, Brower AVZ. Revised classification of the family Hesperiidae (Lepidoptera: Hesperioidea) based on combined molecular and morphological data. Systematic Entomology. 2009; 34:467–523.

22. Yuan X, Gao K, Yuan F, Wang P, Zhang Y. Phylogenetic relationships of subfamilies in the family Hesperiidae (Lepidoptera: Hesperioidea) from China. Scientific Reports. 2015; 5:11140. [PubMed: 26059470]
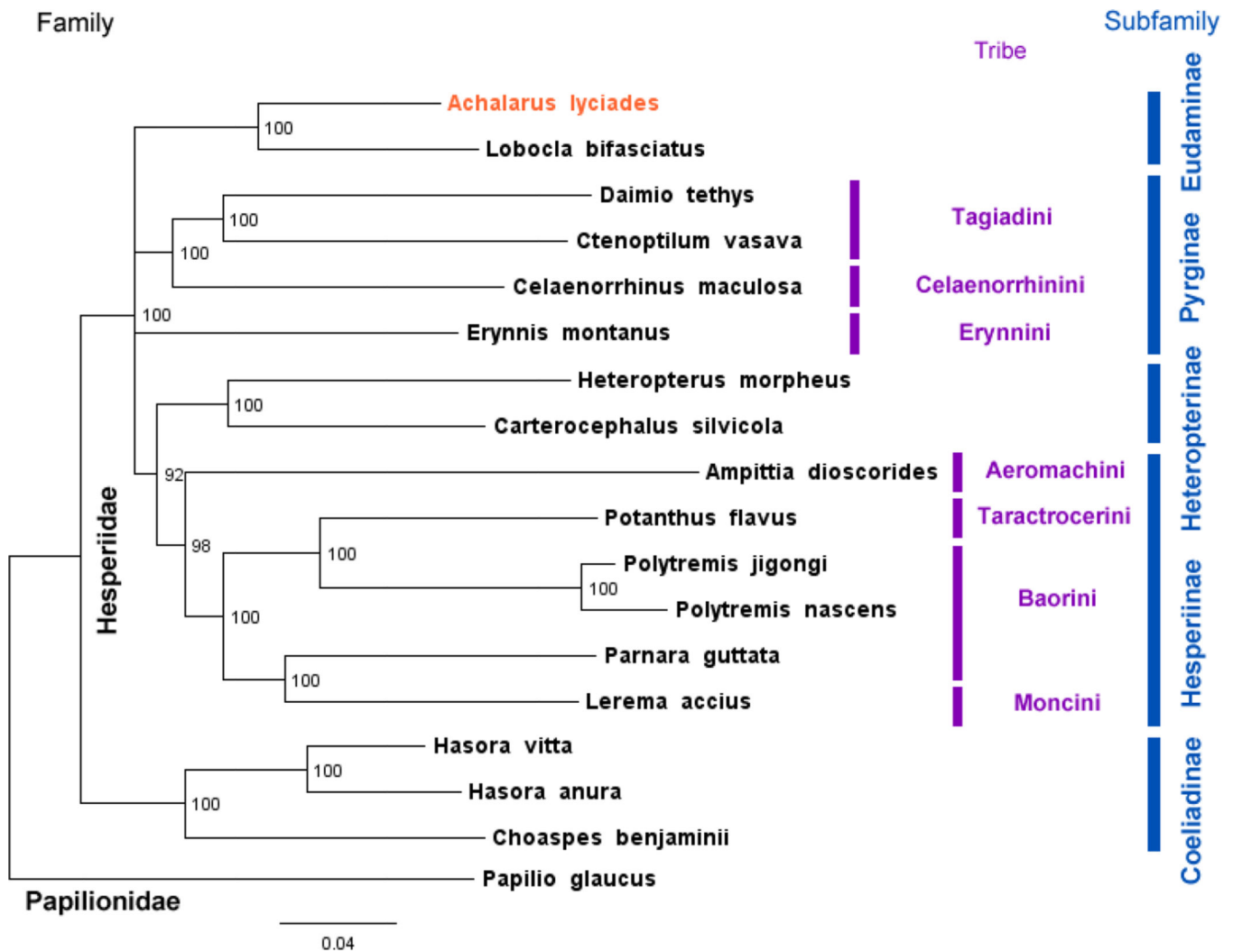
Figure 1.
Maximum likelihood tree of complete mitogenomes of 18 Hesperiidae species rooted with *Papilio glaucus* (Papilionidae). *Achalarus lyciades* with mitogenome reported here is shown first. Numbers by the nodes show bootstrap support values and branches with bootstrap less than 50% are collapsed. GenBank accessions for sequences are: *Ampittia dioscorides* KM102732.1; *Celaenorrhinus maculosa* NC_022853.1; *Daimio Tethys* NC_024648.1; *Erynnis montanus* NC_021427.1; *Hasora anura* NC_027263.1; *Hasora vitta* NC_027170.1; *Heteropterus morpheus* NC_028506.1 *Choaspes benjaminii* NC_024647.1; *Lerema accius* NC_029826.1; *Lobocla bifasciatus* NC_024649.1; *Carterocephalus silvicola* NC_024646.1; *Polytremis jigongi* NC_026990.1; *Polytremis nascens* NC_026228.1; *Potanthus flavus* NC_024650.1; *Parnara guttata* NC_029136.1; *Ctenoptilum vasava* NC_016704.1; *Papilio glaucus* NC_027252.