


The complete mitochondrial genome of the pirate butterfly *Catacroptera cloanthe* (Stoll, 1781) (Insecta: Lepidoptera: Nymphalidae: Kallimini)

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ABSTRACT

The pirate butterfly *Catacroptera cloanthe* (Stoll, 1781) (Nymphalidae: Kallimini) is a monotypic genus of butterfly that occupies grassland and savanna habitats in Sub-Saharan Africa, and exhibits seasonal variation. Genome skimming by Illumina sequencing allowed the assembly of a 78.8% AT-rich complete circular mitogenome of 15,204 bp from *C. cloanthe*. The mitogenome has a typical butterfly gene order consisting of 13 protein-coding genes, two rRNAs, 22 tRNAs, and a control region. *Catacroptera cloanthe* *COX1* begins with an atypical CGA start codon, while *COX2*, *ND3*, *ND4*, and *ND5* end with incomplete T or TA stop codons, completed by the addition of the poly-A tail during mRNA processing. Bayesian phylogenetic reconstruction placed *Catacroptera cloanthe* as sister to *Mallika jacksoni* in the monophyletic tribe Kallimini, which was consistent with previous phylogenetic hypotheses.

ARTICLE HISTORY

Received 7 May 2021
Accepted 14 January 2022

KEYWORDS

Illumina sequencing;
mitogenomes; Lepidoptera;
Nymphalidae; Kallimini

The pirate butterfly *Catacroptera cloanthe* (Stoll, 1781) exhibits seasonal variation and occupies grassland and savanna habitats in Sub-Saharan Africa (Henning et al. 2009; Williams 2020; Woodhall 2020). It was originally placed in the genus *Papilio* but later reclassified as the monotypic genus (a genus containing only one species), *Catacroptera* (Karsch 1894). The larval host plants, geographic distribution, and seasonal variation are well documented in this species (Williams 2020; Woodhall 2020). Based on molecular phylogenetic analysis, *C. cloanthe* has been placed as the sister taxon to another monotypic butterfly genus, the Jackson's leaf butterfly, *Mallika jacksoni* (Wahlberg et al. 2005, 2009). Here, I report the complete mitochondrial genome sequence of *C. cloanthe* from specimen Cclo2016.1, collected in Sibwesa, Mpanda, Tanzania (GPS 6.59103S, 30.65727E) in February 2016. It was pinned, spread, and deposited in the Wallis Roughley Museum of Entomology, University of Manitoba (<http://www.wallisroughley.ca/>), Jason Gibbs, Jason.Gibbs@umanitoba.ca) with voucher number WRME0507737.

A DNEasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany) were used to extract DNA from a single specimen leg (McCullagh and Marcus 2015). DNA was sonicated and a fragment library was prepared as described previously (Peters and Marcus 2017) using a NEBNext Ultra II DNA Library Prep Kit. Then Illumina NovaSeq6000 (San Diego, CA) sequencing was performed (Marcus 2018). The mitogenome of *Catacroptera cloanthe* (GenBank accession no. MW722786) was assembled using Geneious version 10.2.6 by mapping the SRA library (15,236,066 paired 150 bp reads; GenBank SRA PRJNA706380) to a *Mallika jacksoni* reference mitogenome (Lepidoptera: Nymphalidae, MT704828) (Alexiuk et al. 2020a),

using the medium sensitivity settings for five iterations. Mitogenome annotation was performed by aligning the *Catacroptera* genome with the previously annotated and published mitogenome from *Mallika jacksoni* (Alexiuk et al. 2020a) in Geneious version 10.2.6, and protein-coding genes were identified based on sequence homology.

The assembled circular mitogenome of *C. cloanthe* was 15,204 bp and composed of 8011 paired reads with the nucleotide composition: 39.3% A, 13.2% C, 7.9% G, and 39.5% T. The gene structure and order in *C. cloanthe* are typical of the arrangement found in most butterfly mitogenomes (Park et al. 2016). The *C. cloanthe* protein-coding gene start codons include: ATG (*ATP6*, *COX2*, *COX3*, *CYTB*, *ND1*, *ND4*, and *ND4L*), ATT (*ND2* and *ND6*), ATC (*ATP8* and *ND3*), CGA, an atypical *COX1* start codon found in many other insects (Liao et al. 2010), and ATA (*ND5*), a start codon infrequently used in insect mitochondria but is frequently used in other animal groups (Okimoto et al. 1990; Han et al. 2016; Alexiuk et al. 2020b). The mitogenome contains three protein-coding genes (*COX2*, *ND3*, and *ND5*) with single-nucleotide (T) stop codons, and one protein-coding gene (*ND4*) with a two-nucleotide (TA) stop codon completed by post-transcriptional addition of 3' A residues. All structures of the tRNAs were verified using ARWEN version 1.2 (Laslett and Canback 2008) and have typical cloverleaf secondary structures with the exception for trnS (AGN) whose dihydrouridine arm is replaced by a loop, whereas the control region and mitochondrial rRNAs are typical for Lepidoptera (McCullagh and Marcus 2015).

Phylogenetic reconstruction was completed using the complete mitogenome of *C. cloanthe*, 29 mitogenomes from

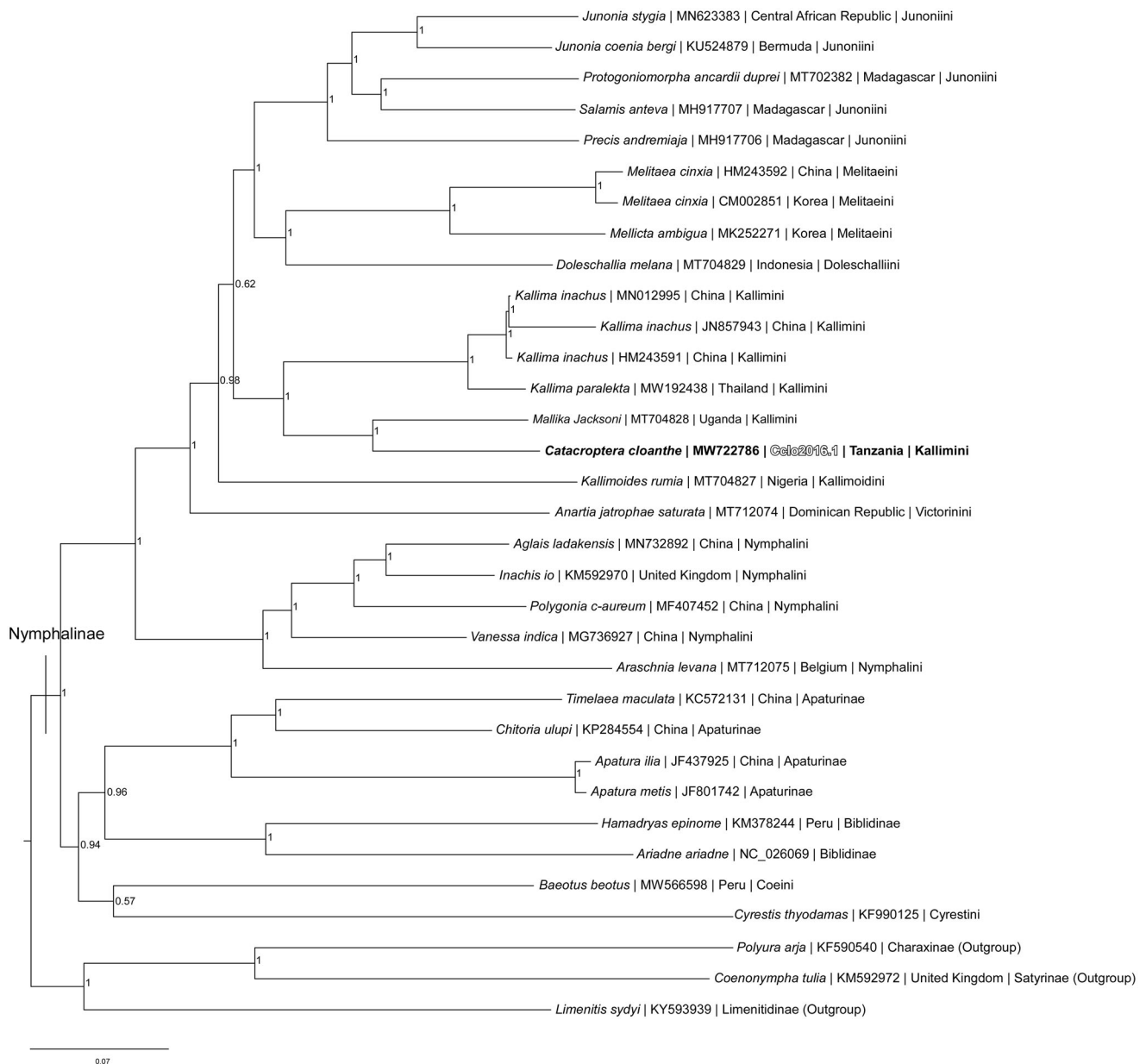


Figure 1. Bayesian Inference phylogeny (GTR + I + G model, average deviation of split frequencies = 0.000639) of the *Catoxoptera cloanthe* mitogenome, 29 additional mitogenomes from 11 tribes within subfamily Nymphalinae, and three outgroup species from other subfamilies within family Nymphalidae (Charaxinae (*Polyura arja*), Satyrinae (*Coenonympha tulia*), and Limenitidinae (*Limenitis sydyi*)) produced by 10 million MCMC iterations in MrBayes with sampling every 1000 generations. The Bayesian posterior probability values determined by MrBayes are given at each node.

the subfamily Nymphalinae, and three outgroup species from other subfamilies (Charaxinae, Limenitidinae, and Satyrinae) within the family Nymphalidae (Wahlberg et al. 2005, 2009; Hamilton et al. 2020; Lalonde and Marcus 2020; Payment et al. 2020; Alexiuk et al. 2020a; Lalonde 2021). Mitogenome sequences were aligned in CLUSTALX version 2.1 (Thompson et al. 1997; Larkin et al. 2007) and analyzed using Bayesian Inference with the GTR + I + G model selected using jModeltest version 2.1.1 (Darriba et al. 2012) in MrBayes version 3.2.7 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012) (Figure 1). Phylogenetic analysis places *C. cloanthe* as the sister taxon to *Mallika jacksoni* within the monophyletic tribe Kallimini, which is consistent with recent phylogenetic analyses (Wahlberg et al. 2005, 2009).

Ethics statement

Ethics approval was not required for this study.

Author Contributions

M.L. contributed to the conception and design of research, the analysis of the and interpretation of the data, drafting, and revising of the manuscript.

Acknowledgments

I would like to thank Jeffrey Marcus for his constructive criticism of this manuscript and Genome Quebec for assistance with library preparation and sequencing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [<https://www.ncbi.nlm.nih.gov>] (<https://www.ncbi.nlm.nih.gov/>) under the accession nos. MW722786 and MW722785. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA706380, SRX10228918, and SAMN18128423, respectively.

Funding

This work received support from the Natural Sciences and Engineering Research Council of Canada (NSERC) under [Grant RGPIN-2016-06012] and the University of Manitoba under the University Research Grants Program.

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