The complete mitochondrial genome of the African leaf butterfly *Kallimoides rumia* (Insecta: Lepidoptera: Nymphalidae)

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**ABSTRACT**

The African leaf butterfly *Kallimoides rumia* Doubleday, 1849 (Nymphalidae), lives in the understory of Afrotropical primary forests. Genome skimming with Illumina sequencing of *K. rumia* permitted assembly of a complete circular mitogenome of 15,234 bp consisting of 80.9% AT nucleotides, 22 tRNAs, 13 protein-coding genes, 2 rRNAs and a control region. *Kallimoides rumia* COX1 features an atypical start codon (CGA). Genes ATP6, COX1, COX2, ND2, ND4 and ND5 feature incomplete stop codons, completed by adding 3’ A residues to the mRNA. Phylogenetic reconstruction places *K. rumia* as a distinct lineage, not closely related to *Kallima*, consistent with previous phylogenetic hypotheses.

The African leaf butterfly, *Kallimoides rumia*, is native to the understory of Afrotropical primary forests (Nyafwono et al. 2014). Shirózu and Nakaniishi (1984) reclassified *K. rumia* from the leaf-mimicking Asian butterfly genus *Kallima* Doubleday (1849) into the monotypic genus *Kallimoides* based on morphology, but further phylogenetic placement within subfamily Nymphalinae has been uncertain (Larsen 2005). More recent molecular phylogenetic work places *Kallimoides* as sister to the monotypic African genus *Vanessula* (Wahlberg et al. 2009). Here we report the complete mitochondrial genome sequence of *K. rumia* from specimen Krum 2016.1, collected in Abiak Owo, Nigeria (GPS 4.689 N, 8.267E) in June 2016, that was pinned, spread and deposited in the Walls Roughley Museum of Entomology, University of Manitoba (voucher WRME050773).

DNA was prepared from a specimen leg using a DNEasy Blood and Tissue kit (Qiagen, Düsseldorf, Germany) with slight modifications to the standard protocol as described in McCullagh and Marcus (2015). DNA was sheared by sonication and a fragment library was prepared as previously described (Peters and Marcus 2017), before sequencing by Illumina NovaSeq6000 (San Diego, California) (Marcus 2018). Mitogenome assembly of *K. rumia* (Genbank accession MT704827) was performed in Geneious 10.1.2, by mapping the resulting sequence library to a *Junonia stygia* (Lepidoptera: Nymphalidae) reference mitogenome (MN623383) (Living Prairie Mitogenomics Consortium 2020) using 5 iterations of the medium sensitivity settings of Geneious 10.1.2. The mitogenome contains five protein-coding genes (COX1, COX2, ND2, ND4, ND5) with single-nucleotide (T) stop codons, and one protein-coding gene (*ATP6*) with a two-nucleotide (TA) stop codon completed by post-transcriptional addition of 3’ A residues. The locations and structural determinations of tRNAs used ARWEN v.1.2 (Laslett and Canback 2008), tRNAs have typical cloverleaf secondary structures except for *tRNA* (AGN) where a loop replaced the dihydrouridine arm. The mitochondrial rRNAs and control region are typical for Lepidoptera (McCullagh and Marcus 2015).

Phylogenetic reconstruction used complete mitogenomes from *K. rumia*, and 38 additional mitogenomes from tribes Junonini, Kallimini, Nymphalini, and outgroup Melitaeini within subfamily Nymphalinae (Hamilton et al. 2020; Lalonde and Marcus 2019a, 2019b; Lalonde and Marcus 2020; McCullagh and Marcus 2015; Peters and Marcus 2017). Mitogenome sequences were aligned in CLUSTAL Omega (Sievers et al. 2011) using the default settings. The aligned sequences were then analyzed by parsimony and maximum
likelihood searches performed in PAUP* 4.0b8/4.0d78 (Swofford 2002) (Figure 1). For the maximum likelihood analysis, a GTR + G optimal model (G = 0.2250) was identified by jModeltest 2.1.7 software (Darriba et al. 2012), followed by a likelihood ratio test (Huelsenbeck and Rannala 1997).

Phylogenetic analysis places *K. rumia* within its own monotypic genus and supports the hypothesis of Shirōzu and Nakanishi (1984) that separated *Kallimoides* from *Kallima*. Hypotheses grouping *Kallimoides* with *Vanessa* could not be addressed due to lack of mitogenome data from *Vanessa* (Kodandaramaiah and Wahlberg 2007; Wahlberg et al. 2005).

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**Disclosure statement**

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

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**Data availability statement**

The data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov, reference numbers MT704827 and MT704830.

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