Phylogenetic analysis of the complete mitochondrial genome of the Blomfild’s Beauty butterfly Smyrna blomfildia (Fabricius 1781) (Insecta: Lepidoptera: Nymphalidae: Nymphalini)

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ABSTRACT
The Blomfild’s Beauty butterfly Smyrna blomfildia (Fabricius 1781) (Lepidoptera: Nymphalidae: Nymphalini) is a sexually dimorphic species found in Mexico, Central, and South America. Males are territorial and are more vibrantly colored than females. Genome skimming by Illumina sequencing allowed the assembly of a complete circular mitochondrial genome (mitogenome) of 15,149 bp from S. blomfildia consisting of 83.9% AT nucleotides, 13 protein-coding genes, 22 tRNAs, two rRNAs, and a control region in the typical butterfly gene order. The S. blomfildia COX1 gene features an atypical start codon (CGA) while ATP6, COX1, COX2, CYTB, ND1, ND3, ND4, and ND5 display partial stop codons completed by the addition of 3′ A residues to the mRNA. Bayesian phylogenetic reconstruction places Smyrna as a member of the tribe Nymphalini and sister to a clade containing genera Araschnia, Vanessa, Polygonia, and Aglais, which differs from its classic taxonomic placement in tribe Coeini.

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 using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, Massachusetts) as previously described (Peters and Marcus 2017), before sequencing by Illumina NovaSeq6000 (San Diego, California) (Marcus 2018). The mitogenome of S. blomfildia (Genbank MZ151338) was assembled and annotated using Geneious Prime 2021.1.1 from an SRA library of 18,400,288 paired 150 bp reads (Genbank SRA PRJNA729786) using a Baeotus beotus reference mitogenome (Lepidoptera: Nymphalidae, MW566598) (Lalonde 2021). The S. blomfildia nuclear rRNA repeat (Genbank MZ198233) was also assembled and annotated using a B. beotus (MW571038) reference sequence. The rRNA repeat sequence is increasingly recognized as being very useful for phylogenetic comparisons based on nuclear markers (Dodsworth 2015; Coissac...
et al. 2016; Marcus 2018; Krehenwinkel et al. 2019), so we have chosen to release it here.

The *S. blomfildia* circular 15,149 bp mitogenome assembly was composed of 24,120 paired reads with nucleotide composition: 34.5% A, 10.6% C, 5.5% G, and 49.4% T. The gene composition and order in *S. blomfildia* is typical of the arrangement found in most butterfly mitogenomes (Park et al. 2016). The *S. blomfildia* protein coding gene start codons include: ATG (*ATP6*, *COX2*, *CYTB*, *ND1*, *ND4*, *ND4L*), ATT (*ATP8*, *ND2*, *ND5*, *ND6*), ATC (*ND3*), and CGA, an atypical *COX1* start codon that is also found in the *COX1* gene of many other insects (Liao et al. 2010). The mitogenome contains four protein-coding genes (*COX1*, *COX2*, *ND4*, *ND5*) with single-nucleotide (T) stop codons, and four protein-coding gene (*ATP6*, *CYTB*, *ND1* *ND3*) with two-nucleotide (TA) stop codons completed by post-transcriptional addition of 3′ A residues. All structures of the tRNAs were verified using ARWEN v.1.2 (Laslett and Canback 2008) and have typical cloverleaf secondary structures with the exception for trnS (AGN) where the dihydrouridine arm is replaced by a loop, whereas the control region and mitochondrial rRNAs are typical for Lepidoptera (McCullagh and Marcus 2015).

Phylogenetic reconstruction (Figure 1) was completed using the complete mitogenome of *S. blomfildia* and 37 other mitogenomes from the family Nymphalidae. Sequences were aligned in CLUSTALX 2.1 (Thompson et al. 1997; Larkin et al. 2007) and analyzed using Bayesian Inference with the GTR + I + G model (model selected using jModeltest 2.1.1 (Darriba et al. 2012)) in Mr. Bayes version 3.2.7 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Phylogenetic analysis places *Smyrna* as a member of the tribe Nymphalini and sister to a clade containing genera *Araschnia*, *Vanessa*, *Polygonia*, and *Aglais*, confirming the findings of some previous molecular phylogenetic analyses (Wahlberg et al. 2005; Wahlberg and Wheat 2008). Placing *Smyrna* in tribe Nymphalini is also supported by larval morphological characters (Muyschodt and Muyschodt 1978). This differs from the classical taxonomic placement of *Smyrna* with *Baeotus beotus* in the tribe Coeini based on adult morphology (Muyschodt and Muyschodt 1978) and supported by a different molecular phylogenetic analysis (Wahlberg et al. 2009). Based on our results, we agree with prior researchers who have reclassified *Smyrna* in tribe Nymphalini.

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Disclosure statement

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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] under the accession nos. MZ151338 and MZ198233. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA729786, SRX10874928, and SAMN19163223 respectively.

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