

Complete mitochondrial genome of *Papilio elwesi* and its phylogenetic analyses with other swallowtail butterflies (Lepidoptera, Papilionidae)

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ABSTRACT

The complete mitochondrial genome of *Papilio elwesi* was sequenced and annotated using high-throughput sequencing in the present study. The mitochondrial genome (mitogenome) is a circular molecule of 15,082 bp in length, containing 37 genes (13 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes) and a putative control region. We performed mitogenomic phylogenetic analyses of 34 swallowtail species employing methods of Bayesian inference and maximum-likelihood. Our results further confirm that *P. elwesi* is a member of the subgenus *Pterourus* in *Papilio sensu lato*.

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Papilio elwesi; high-throughput sequencing; mitochondrial genome; systematic position

Papilio elwesi Leech (2009) is a large-size, valuable and rare species of swallowtail butterflies in the family Papilionidae of Lepidoptera. The species was first described by Leech (2009) and occurs only from Southwest to Southeast mainland China and Vietnam (Lien 2014; Wu et al. 2015). Adults of this species are regarded as mimicking *Atrophaneura* butterflies in wing patterns (Tsukada and Nishiyama 1982), their early-instar larvae mimic bird droppings, late-instar larvae may imitate snakes with remarkable eyespots, and pupae look like dry sticks in appearance (Figure 1). Although much attention has been drawn to the taxonomy, lifecycle, and conservation biology of *P. elwesi* (Huang et al. 2009; Zhou et al. 2011), the systematic position of this species is still debatable. Morphologically, *P. elwesi* and its closest related relative *P. maraho* have always been assigned to *Agehana* Matsuyama (1936) as an independent Asian genus because of the distinct adult features with two veins present in the broad boot-like tail of hindwing (Matsuyama 1936), but these two species have been grouped into American subgenus *Pterourus* in the large genus *Papilio sensu lato*, supported by molecular data of several DNA loci (Wu et al. 2015). In our study, the complete mitogenome of *P. elwesi* was identified, which would be useful for further phylogenetic relationships, genetic and conservation studies of this species.

An adult individual of *P. elwesi* was collected from Fengyangshan National Nature Reserve, Zhejiang Province, China in July 2019 (119°16'11"E, 27°87'79"N). The specimen with a voucher number KW0001 was deposited in the Insect Collection of Department of Entomology, South China Agricultural University, Guangzhou, China (Min Wang, minwang@scau.edu.cn). The total genomic DNA of *P. elwesi* was

extracted from the legs using a TIANamp Genomic DNA Kit (Tiangen, Beijing, China) following the manufacturer's protocol. DNA library was prepared using a NEBNext ultra DNA Library Prep Kit (New England Biolabs, Ipswich, MA). The whole mitogenome of *P. elwesi* was sequenced on the Illumina HiSeqX platform. This species does not require ethical approval in China.

The complete mitogenome of *P. elwesi* is a closed, circular DNA molecule 15,082 bp in length, with 37 coding genes (two rRNA genes, 22 tRNA genes, and 13 protein-coding genes) and a non-coding A+T rich region, and the gene arrangement and orientation are similar to those of the typical mitogenomes of Lepidoptera (Wang et al. 2015; Chen et al. 2020). Among the mitogenome, 23 genes and A+T rich regions are encoded on the major strand (J-strand), and the other 14 genes are encoded on the minor strand (N-strand). The PCGs of mitogenome encode a total of 3725 amino acids, which account for 78.10% of *P. elwesi*. All PCGs are initiated by typical ATN codons (atp8, nd2, and nd5 with ATT; atp6, cox2, cox3, nd1, nd4, and nd4l with ATG; nd6 with ATC, nd3, and cytb with ATA), except cox1 gene which utilizes a start codon by CGA, such encoded mode is common in Lepidoptera (Peng et al. 2017; Sun et al. 2020). Ten of PCGs contain the complete termination codons, either TAA (nd2, atp8, atp6, cox3, nd3, nd5, nd4l, nd6, and cytb) or TAG (nd1), whereas 'T' is an incomplete termination codon for the remaining genes. The 22 tRNAs range from 60 bp (tRNA^{Ser}(AGA)) to 71 bp (tRNA^{Lys}) in length, and appears highly A+T biased. All tRNAs have the typical cloverleaf secondary structures, except for tRNA^{Ser}(AGA), which lacks a

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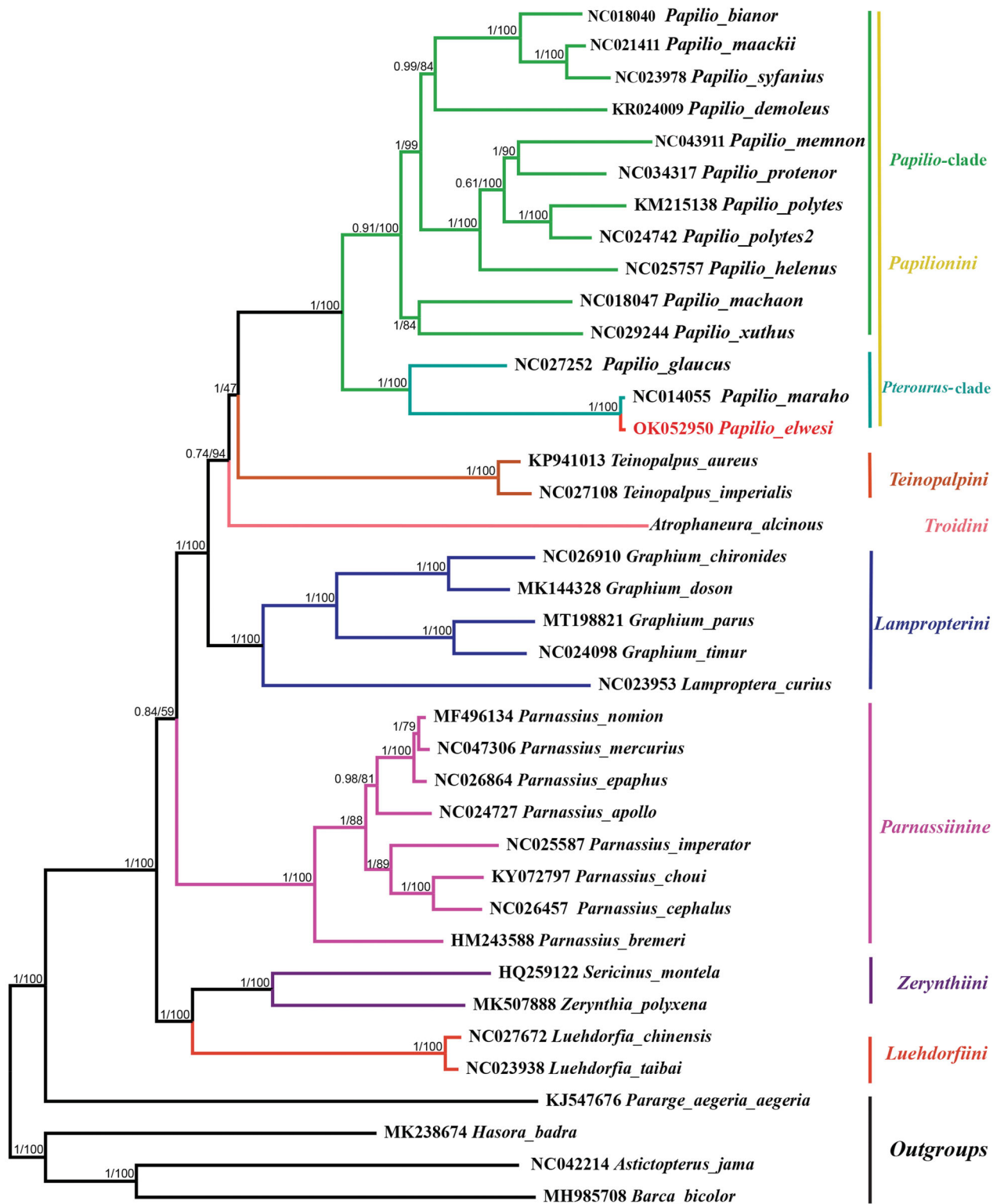


Figure 1. Phylogenetic tree using Bayesian inference (BI) and maximum-likelihood (ML) analysis. Number in each branch indicate Bayesian's posterior probabilities (BPP) and bootstrap value (BS) based on ML analyses. Dot on nodes means this branch: PP/BS = 1/100.

dihydrouridine (DHU) arm. The 12S rRNA(780 bp) and 16S rRNA (1373 bp) were separated by tRNA^{Val}.

Thirty-three related Papilionid species and four outgroups were used to reconstruct phylogenetic tree based on 13 PCG sequences of 38 specimens through Bayesian inference (BI)

and maximum-likelihood (ML) methods. The topologies reconstructed by both ML and BI methods are identical, with high support values. In the phylogenetic trees, the family Papilionidae is comprised of 34 species, representing two subfamilies (*Papilioninae* + *Coliadinae*). The subfamily

Papilioninae includes four clades (Papilionini + Teinopalpini + Troidini + Lampropterini), and the subfamily Coliadinae consists of three clades (Parnassiini + Zerynthiini + Luehdorfiini). The phylogenetic position of Parnassiini + Zerynthiini is controversial. In this study, Parnassiini + Zerynthiini is not a monophyletic group, which is consistent with some previous studies (Caterino and Sperling 1999; Omoto et al. 2004).

In our analyses, *P. elwesi* and *P. maraho* form a clade that is sister to an American species *Papilio glaucus* in the *Pterourus*-clade of *Papilio sensu lato*. This result further supports the opinion of Liu et al. (2017). This work provides essential DNA molecular data for further phylogenetic and evolutionary analyses of swallowtail butterflies. Further studies, with more comprehensive sampling, are needed to clarify their taxonomic status.

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Authors contributions

Conception and design: Houshuai Wang, Fangzhou Ma. Materials collection: Min Wang, Houshuai Wang. Analysis and interpretation of the data: Jiamin Liang. The drafting of the paper: Jiamin Liang. Revising it critically for intellectual content: Min Wang, Houshuai Wang. The final approval of the version to be published: Houshuai Wang, Jiamin Liang. All authors agree to be accountable for all aspects of the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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 no. OK052950. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA780793, SRR16954894, and SAMN23176799, respectively.

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