Complete mitochondrial genomes of *Papilio nephelus chaon* and *Papilio epycides* (Lepidoptera: Papilionidae: Papilioninae) and phylogenetic analysis

Zhentian Yan\(^a\), Mingjuan Lu\(^a\), Site Luo\(^b\), Shulin He\(^a\), Wenbo Fu\(^a\), Xueqian Wang\(^a\), Zhenhua Fan\(^a\), Danlan Hu\(^a\) and Bin Chen\(^a\)

\(^a\)Chongqing Key Laboratory of Vector Insects; Institute of Entomology and Molecular Biology, Chongqing Normal University, Chongqing, China; \(^b\)School of Life Sciences, Xiamen University, Xiamen, China

**ABSTRACT**

The complete mitochondrial genome (mitogenome) sequences of *Papilio nephelus chaon* and *Papilio epycides* were sequenced by Illumina and analyzed in this study. They are 15,287 bp and 15,012 bp in size, respectively, and contain 13 protein-coding genes (PCGs), 22 tRNA genes (tRNAs), 2 rRNA genes (rRNAs), and 1 AT-rich control region (CR). The phylogenetic relationships of 56 species in the Papilionidae were inferred based on concatenated nucleotide sequences by using Maximum Likelihood with the selected best-fit model GTR + F + R6. The phylogenetic analysis showed that *P. nephelus chaon* and *P. epycides* were located in the genus *Papilio*. This study provides a basis for further study on mitogenome and phylogenetics of the Papilionidae.

*Papilio nephelus chaon* can be found from Nepal, Sikkim, Assam to China and from Myanmar, Thailand, Cambodia to Indonesia (Zhou 1998; Wu 2001; Wu and Xu 2017; Jiang et al. 2019). *P. nephelus* wings can be used as templates to fabricate functional composite materials-metal and carbon based metal with 3-D antireflection quasi-periodicity micro structure (Cai et al. 2016). *Papilio epycides* (Hewitson 1864) is distributed in Southwest China, India, Malaysia and Indonesia (Wu 2001; Wu and Xu 2017). The mitogenome data have been used to infer and analyze the phylogenetic relationships of butterflies (Qin 2017) as reliable molecular markers due to the features of maternal inheritance, stable gene composition, relative conserved gene sequence, and low recombination rate (Cameron et al. 2007; Lavrov 2007). But the complete mitogenome sequences of *P. nephelus chaon* and *P. epycides* have not been reported. In this study, we sequenced and annotated the complete mitogenomes of *P. nephelus chaon* (Genbank Number: MZ353681) and *P. epycides* (Genbank Number: MZ501807) and deduced the phylogenetics of both species and other 54 species based on mitogenomes in Papilionidae.

Adult of *P. nephelus chaon* and *P. epycides* were collected by sweep nets in Tiefo Village of Baima Town (107°20′28′′E, 29°13′26′′N), Wulong District, and Nanshan Botanical Park (106°63′77′′E, 29°56′10′′N), Nanan District in Chongqing, China. Voucher specimens were deposited in Chongqing Normal University (CQNU) (accession number 20180701001 and 20200401016, Zhentian Yan, 525201877@qq.com). The genomic DNA was extracted by using TIANamp Genomic DNA Kit (TIANGEN, Beijing, China) and the DNA library was prepared by using the Illumina Truseq™ DNA Sample Preparation Kit (Illumina, San Diego, USA) according to the manufacturer’s recommendations. The prepared libraries were sequenced paired-end 150 bp on an Illumina Novaseq 6000 platform at Novogene Company (Beijing, China). NGS QC toolkit was used for quality control and to filter the low-quality reads (Patel and Jain 2012). The clean data were used to assemble the complete mitogenome by following the GetOrganelle pipeline with the ‘animal_mt’ parameter (Jin et al. 2020). Genome annotation was performed with the Mitoz annotation module (Meng et al. 2019). The annotated genomes were deposited in GenBank under with accession numbers: MZ353681 and MZ501807.

The complete mitogenomes of *P. nephelus chaon* and *P. epycides* are 15,287 bp and 15,012 in length, containing 37 typical mitogenome genes (13 protein-coding genes, 22 transfer RNAs, and 2 ribosomal RNAs genes). Overall AT content values for the mitogenomes are 80.47% and 80.07%. Both mitogenomes were assembled with high coverage (800 folded). We constructed the phylogenetics of the Papilionidae with the mitogenomes of *P. nephelus chaon* and *P. epycides* and 54 other species in Papilionidae by using maximum likelihood (ML) method implemented in IQ-TREE v2.1.2 and with the *Calingga davidis* (NC_015480) as out-group. Each mitochondrial gene was aligned separately by the MAFFT v7.388 with default settings (Katoh and Standley 2012).
2013). The nucleotide sequences of 13 PCGs were used, and the best model GTR + F + R6 was selected by using ModelFinder (Kalyaanamoorthy et al. 2017; Minh et al. 2020).

The support values of the internal nodes in the phylogeny were inferred with 1000 bootstrapping replicates. The result shows that *P. nephelus chaon* and *P. epycides* were located in a clade including other Papilioninae species (Figure 1). This study provides important information for species identification and phylogenetic position of *Papilio* in the Papilioninae.

**Author contributions**

YZT and CB conceived this study. LMJ, FWB, WXQ, HSL, FZH, and HDL conducted the experiments. YZT, HSL, LST (Site Luo), and CB analyzed the data. YZT and LST (Site Luo) wrote the manuscript. All authors contributed to the writing of the manuscript.

**Ethics statement**

The samples of this study are insects that are not included in the list of protected animals, and we confirm that all the research meets the ethical guidelines, including adherence to the legal requirements of the study country.

**References**

Cai NJ, Zhang W, Tian JL, Zhang D. 2016. The absorption of functional composite material synthesized by chemical synthesis method in the by Hemicia synthesis method in the template of butterfly-wings. Mater China. 35(9):651–657.

**Data availability statement**

The genome sequence data that support the findings of this study are publicly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) under the accession numbers MZ353681 and MZ501807. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA783429, SAMN2329721, SRR17035426 for *P. nephelus chaon* and PRJNA783428, SAMN2329720, SRR17035425 for *P. epycides*.

**Funding**

This work was supported by the Science and Technology Research Program of Chongqing Education Commission of China (No. KJQN201900532).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).
Cameron SL, Lambkin CL, Barker SC, Whiting MF. 2007. A mitochondrial genome phylogeny of Diptera: whole genome sequence data accurately resolve relationships over broad timescales with high precision. Syst Entomol. 32(1):40–59.

Hewitson WC. 1864. Illustrations of new species of exotic butterflies, selected chiefly from the Collections of W. Wilson Saunders and William C. Hewitson. Vol. II. London: Van Voorst.

Jiang YJ, Chen B, Yan ZT. 2019. Butterfly species investigation and floristic analysis in Chengkou County, Chongqing. J Chongqing Normal Univ. 36(6):47–52.

Jin JJ, Yu WB, Yang JB, Song Y, Depamphilis CW, Yi TS, Li DZ. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21(1):1–31.

Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14(6):587–589.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780.

Lavrov DV. 2007. Key transitions in animal evolution: a mitochondrial DNA perspective. Integr Comp Biol. 47(5):734–743.

Meng GL, Li YY, Yang CT, Liu SL. 2019. MitoZ: a toolkit for animal mitochondrial genome assembly, annotation and visualization. Nucleic Acids Res. 47(11):63.

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 37(5):1530–1534.

Patel RK, Jain M. 2012. NGS QC toolkit: a toolkit for quality control of next generation sequencing data. PLoS One. 7(2):e30619.

Qin JM. 2017. The study on mitochondrial genomes and molecular phylogenetics of butterflies. Beijing: Science Press.

Wu CS. 2001. Fauna Scinica, Insecta Vol. 25 (Lepidoptera: Papilionidae: Papilioninae). Beijing: Science Press.

Wu CS, Xu YF. 2017. Butterflies of China. Fuzhou: The Straits Publishing & Distributing Group.

Zhou Y. 1998. Classification and identification of Chinese butterflies. Zhengzhou: Science and Technology of Henan Press.