

The complete mitochondrial genome of *Pachylophus rufescens* (de Meijere, 1904) (Diptera:Chloropidae)

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ABSTRACT

Pachylophus belongs to the subfamily Chloropinae, the second most diverse subfamily of Chloropidae. However, there have been few complete mitochondrial genomes of Chloropinae reported in the public database. Consequently, we sequenced and annotated the complete mitochondrial genome of *Pachylophus rufescens* (de Meijere, 1904). The whole mitochondrial genome is 17,926 bp in length, consisting of 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs). The mitochondrial genome exhibits high A + T bias, accounting for 79.7% of its entirety. All PCGs start with ATN codon and end with TAN or incomplete stop codon TA or single T. The Maximum likelihood phylogenetic tree revealed a close relationship between *Pachylophus* and *Cetema*. This study contributes to the expansion of the mitochondrial genome library of Chloropinae, providing a valuable resource for gaining insights into the evolutionary history of Chloropidae.

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Chloropinae is the second most speciose subfamily within the family Chloropidae. It encompasses 75 genera and over 1000 species worldwide, and several taxa have been reported as feeding on monocots (Cherian 2015; Deeming 2018). Species diagnosis and phylogenetic analysis within Chloropinae have consistently depended on morphological approaches (Riccardi and Amorim 2020). With the development of molecular systematics, molecular data has become an effective means for species delimitation and phylogenetic analysis (Cameron 2014; Luo et al. 2018). However, due to the deficiency of mitochondrial genome data, the comprehensive analysis for Chloropinae has not been conducted. To date, only five Chloropinae mitochondrial genomes have been documented on NCBI (Wang et al. 2021; Song et al. 2022; Liu et al. 2024).


The genus *Pachylophus* Loew belongs to the tribe Meromyzini of Chloropinae. It comprises 35 known species, of which 26 species are distributed in the Afrotropical Realm and only 10 species in the Oriental, Australian, and Palearctic Realms (Nartshuk 2012; Deeming 2018). They are ovoviviparous with larvae found in the stems of grasses and cereals (Deeming 2018). *Pachylophus rufescens* (de Meijere, 1904) is found widely in Asia and Australia (Kanmiya 1983; Spencer 1986; Nartshuk 1993). In this study, we elucidate the complete mitochondrial genome of *P. rufescens* and conduct a phylogenetic analysis of Chloropidae.

Materials and methods

The specimens of *Pachylophus rufescens* used for this study were collected from Yiliang (104.27E, 27.78 N), Yunnan, China by Sihan Li on 15 July 2023 and diagnosed by Jiuzhou Liu (Figure 1). Specimens are deposited in Entomological Museum of China Agricultural University (CAU) (<https://www.cau.edu.cn/>, contact person: Ding Yang; e-mail: dyangcau@126.com, Accession number: CAU0010025). *P. rufescens* is an invertebrate species not classified as endangered or protected in China or any other country. As a result, ethical approval or other relevant permissions were not necessary for this study.

The genomic DNA was extracted from the thoracic muscle tissues using the DNeasy DNA Extraction kit (QIAGEN). Illumina sequencing libraries were constructed using VAHTS Universal Plus DNA Library Prep Kit for Illumina V2 with a 400 bp insert size and then sequenced on the illumina X plus platform using 150 bp paired-end (PE150) sequencing. Raw read data were filtered and trimmed using Fastp (Chen et al. 2018) with default parameters. The outcomes obtained from high-quality reads (~6 GB) were employed for assembly and annotation using MitoFinder (Allio et al. 2020), followed by manual verification through Geneious. The mitochondrial genome map was visualized using Proksee (<https://proksee.ca>). The sequencing depth and coverage map for the

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mitochondrial genome of *P. rufescens* was generated following the protocol by Ni et al. (2023) and presented in Figure S1.

To discuss the phylogenetic status of *P. rufescens*, all available Chloropid mitochondrial genomes were download from NCBI, including five species of Chloropinae, five species of Oscinellinae, five species of Rhodesiellinae and two species of

Siphonellopsinae. In addition, we also selected two Milichiidae species as outgroup. PhyloSuite v1.2.3 was used for protein coding genes extractions from mitochondrial genomes and matrix preparation (Zhang et al. 2020). Each PCG was aligned using the MAFFT module under the “-auto” strategy (Kato and Standley 2013). All ambiguously aligned sites were removed using Gblock (Castresana 2000). Alignments of individual genes were concatenated to build datasets. Based on 13 PCGs matrix (partitioned by gene), we conducted a maximum likelihood (ML) phylogenetic analysis using IQ-TREE (Nguyen et al. 2015). The phylogenetic trees generated in this study were visualized using tvBOT (Xie et al. 2023).

Results

The mitochondrial genome of *P. rufescens* is typically double-stranded circular molecules with 17,926 bp in length, containing 37 genes (13 protein-coding genes, two rRNA genes and 22 tRNA genes) and a control region (CR) (Figure 2). The mitochondrial genome nucleotide composition of *P. rufescens* was 41.8% of A, 12.4% of C, 7.8% of G and 37.9% of T, and with an A + T bias of 79.7%. All PCGs start with ATN codon



Figure 1. Reference image of *Pachylophus rufescens* (de Meijere, 1904). This image was photographed by Jiuzhou Liu.

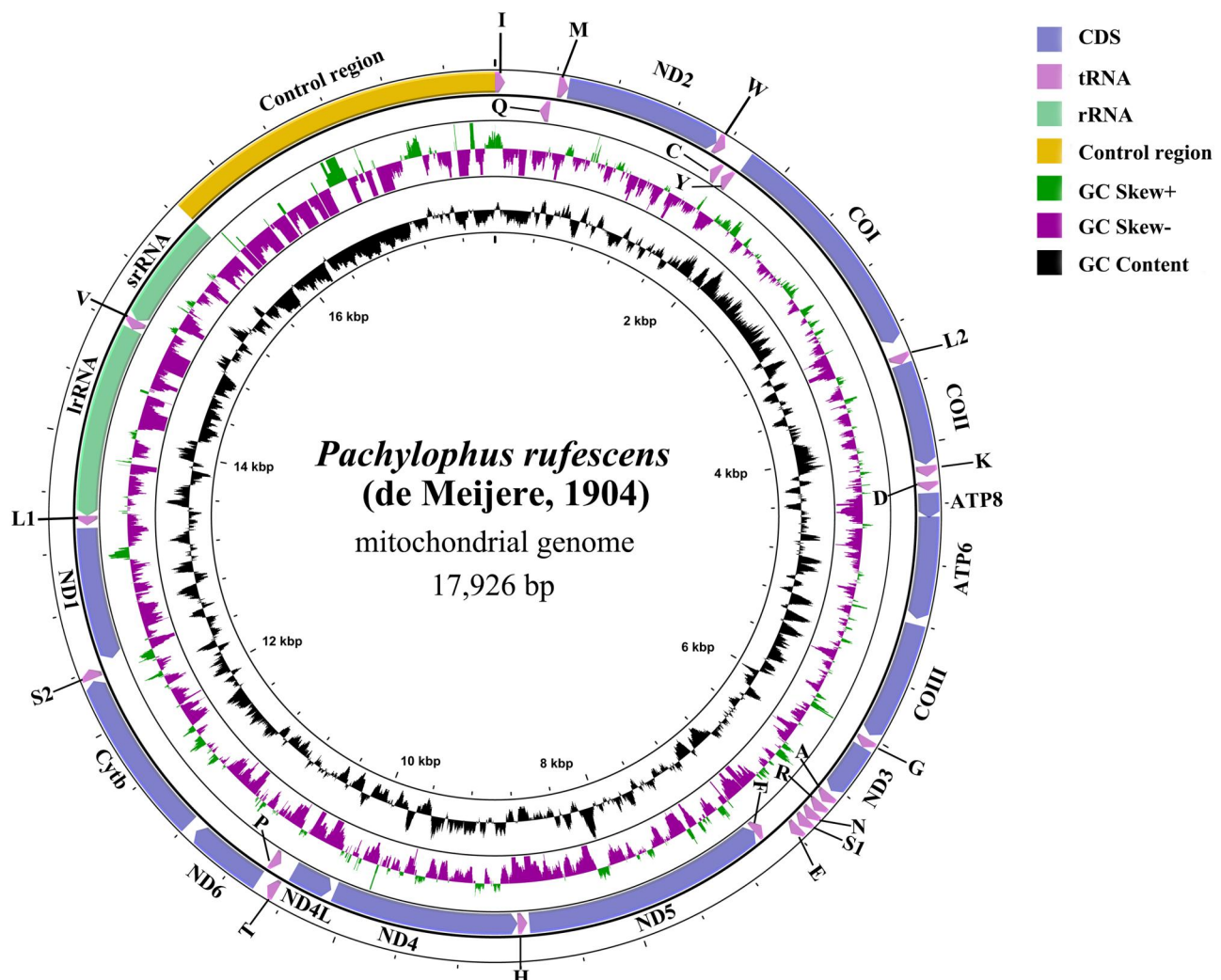


Figure 2. Graphical map of the complete mitochondrial genome of *Pachylophus rufescens* (de Meijere, 1904).

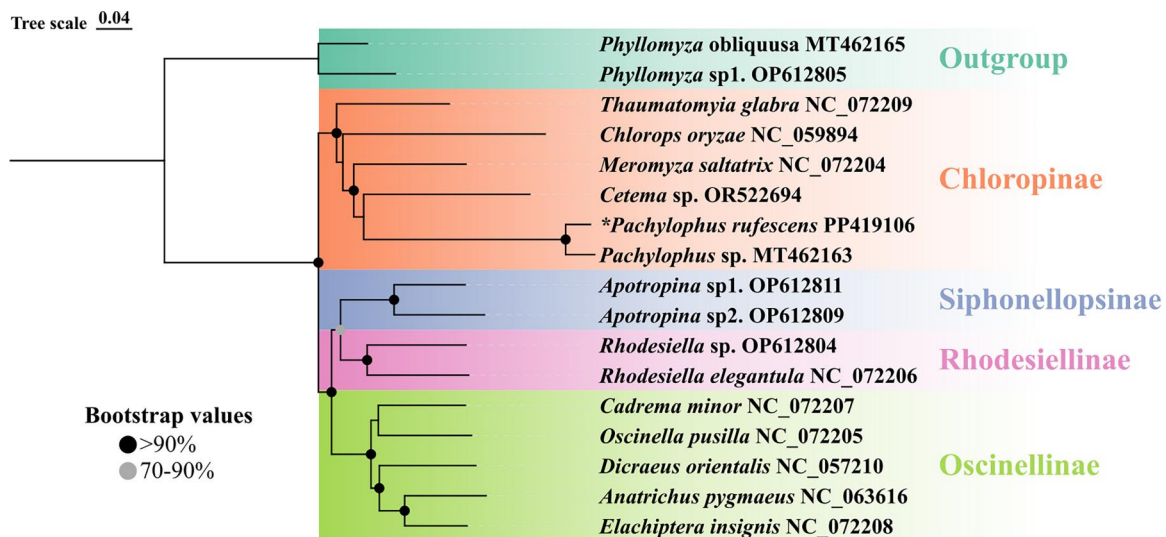


Figure 3. Maximum likelihood phylogenetic tree of Chloropidae species based on 13 protein coding genes. The circle at each node indicates the ML bootstrap values and asterisk represent the new sequenced species.

and end with TAN or incomplete stop codon TA or single T. Maximum likelihood (ML) phylogenetic tree indicated that *P. rufescens* belongs to the subfamily Chloropinae and the *Pachylophus* is closely related to the *Cetema* (Figure 3).

Discussion and conclusions

Mitochondrial genomes have gained widespread use in phylogenetic inference across various dipteran taxonomic lineages due to their uniparental inheritance, lack of introns, high copy numbers, relatively simple structure, conserved gene composition, and rapid evolutionary rate (Ramakodi et al. 2015; Zhang et al. 2019). We have successfully sequenced and annotated the entire mitochondrial genome of *P. rufescens*. The genome content and arrangement of *P. rufescens* align with those of the Chloropidae mitogenomes reported by Liu et al. (2024), including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs). The Maximum Likelihood (ML) results were consistent with those of morphological taxonomy, and all Chloropidae species cluster into one clade and form a sister group with Milichiidae, which is consistent with previous research (Liu et al. 2024). This highlights the promising application of mitochondrial genome data in species taxonomy and phylogeny of Chloropinae. However, the scarcity of high-quality mitochondrial genome data has limited the current research on the phylogeny of the subfamily Chloropinae to a purely morphological level. This study presents a meticulously annotated mitochondrial genome of *P. rufescens*, establishing a solid foundation for future research on the phylogeny of *Pachylophus* and Chloropinae.

Ethical approval

No specific permits were required for the insect specimens collected for this study. The field studies did not involve endangered or protected species. The insect species sequenced is a common Chloropidae species in China and is not included in the 'List of Protected Animals in China'.

Authors' contributions

Conceptualization: Jiuzhou Liu, Xiaodong Cai, Xiaoyan Liu and Ding Yang; methodology: Jiuzhou Liu; formal analysis: Jiuzhou Liu, Xiaodong Cai; investigation: Xiaoyan Liu, Ding Yang; writing original draft preparation: Jiuzhou Liu, Xiaodong Cai; writing review and editing: Xiaoyan Liu, Ding Yang; All authors have read and agreed to the published version of the manuscript.

Disclosure statement

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

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Data availability statement

The genome sequence data that support the finding of this study is openly available in NCBI (<https://www.ncbi.nih.gov>), the accession number is PP419106. The associated BioProject, BioSample and SRA numbers are PRJNA1082890, SAMN40220929, and SRR28201466 respectively.

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