

# Complete mitochondrial genome of *Basilepta melanopus* Lefèvre, 1893 (coleoptera: chrysomelidae: eumolpinae), a tea pest from Southern China

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## ABSTRACT

The tea pest, *Basilepta melanopus* Lefèvre 1893 (Chrysomelidae), belongs to the subfamily Eumolpinae. In this study, the complete mitochondrial genome sequence of *B. melanopus* from southern China was sequenced using the next-generation sequencing technique, assembled, and annotated using bioinformatics tools. The complete mitochondrial genome was 15,905 bp in length. The overall GC content was 22.51%, in which the percentages for the bases A, T, C, and G were 41.23%, 36.26%, 8.92%, and 13.59%, respectively. Thirty-seven genes were predicted, including 13 protein-coding, 22 transfer RNA, and two ribosomal RNA genes. Phylogenetic analysis based on the complete mitochondrial genome sequences of 18 Chrysomelidae taxa revealed that *B. melanopus* was closely related to *Basilepta fulvipes*.

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*Basilepta melanopus*; next generation sequence; mitochondrial genome; tea pest; eumolpinae; phylogeny

## Introduction

Eumolpinae is a large subfamily of leaf beetles with great diversity and wide distribution. The subfamily contains more than 500 genera, which has more than 7000 species, while most of them reside in the tropical and subtropical regions, especially in the south of China, tropical Africa, tropical America, New Guinea, and New Caledonia (Pierre and Krishna 2008). One of the genera of Eumolpinae, *Basilepta* Baly, is recorded with a total of 240 species, of which more than 70 are recorded in China. The tea pest, *Basilepta melanopus* Lefèvre 1893, has seriously affected tea production in southern China (Cao et al. 2006). The mature female of *B. melanopus* is about 3.2~3.8mm in length and 1.5~2.0mm in width, while the male has a smaller body size. The color of its body and wings are brown and yellow. Small and sparse points can be detected on the surface of its head with a pair of oval and black eyes (Figure 1).



There is no effective and economical way to control the population of *B. melanopus* to reduce its impact on the tea yield (Zhou et al. 2019). Although the pest has been a nuisance to the local tea farm for long, genetic studies on this species are still limited. To expand the genetic information of *B. melanopus*, we have sequenced and assembled the mitochondrial genome of this species. The findings of this study would provide insight into the molecular placement of *B. melanopus* in Eumolpinae. At the same time, the data


generated in this work might aid in the future biocontrol strategy for this insect pest.

## Materials and methods

A mature specimen of *B. melanopus* was collected from Luokeng Town of Jiangmen City, Guangdong Province of China (N24°31'11", E113°20'21"). The dried specimen in powder form was deposited in the Herbarium of Guangdong Academy of Agricultural Sciences (Contact person: Jihua Wang; e-mail: [wangjihua@gdaas.cn](mailto:wangjihua@gdaas.cn)) under the collection number GAAS20220819-10.

Total genomic DNA was carried out using the StarPrep Universal DNA Kit (GenStar Biotech, China), and library preparation with an average insert size of 350 bp was constructed using the VATHS Universal DNA Library Prep Kit for Illumina V3 (Vazyme, China). Sequencing was carried out on an Illumina Novaseq 6000 platform with a 150-bp paired-end mode at Guangzhou Jierui Biotech Ltd, China. Approximately 6.0GB of raw data was generated and filtered using fastp v0.19.5 (Chen et al. 2018) based on default settings. The filtered data was fed into the GetOrganelle v1.6.2e (Jin et al. 2020) pipeline for mitochondrial genome assembly. Gene annotation was conducted using the MITOS beta version (Bernt et al. 2013), and the annotated genome was manually examined and corrected by referring to the annotated

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mitochondrial genome sequence of *Basilepta fulvipes* (GenBank accession number MT627597).

Phylogenetic analysis was carried out using complete mitochondrial genome sequences from 19 Chrysomelidae taxa, including *B. melanopus*, with two closely related species

from Cerambycidae as outgroup. Multiple sequence alignment was conducted using MAFFT v7.453 (Katoh et al. 2019). A maximum-likelihood (ML) tree was constructed using RAxML v8.2.12 (Stamatakis 2014), in which the branch nodes were calculated under 1,000 bootstrap replicates. Based on



Figure 1. *Basilepta melanopus*. Photo taken and edited by Jihua Wang.

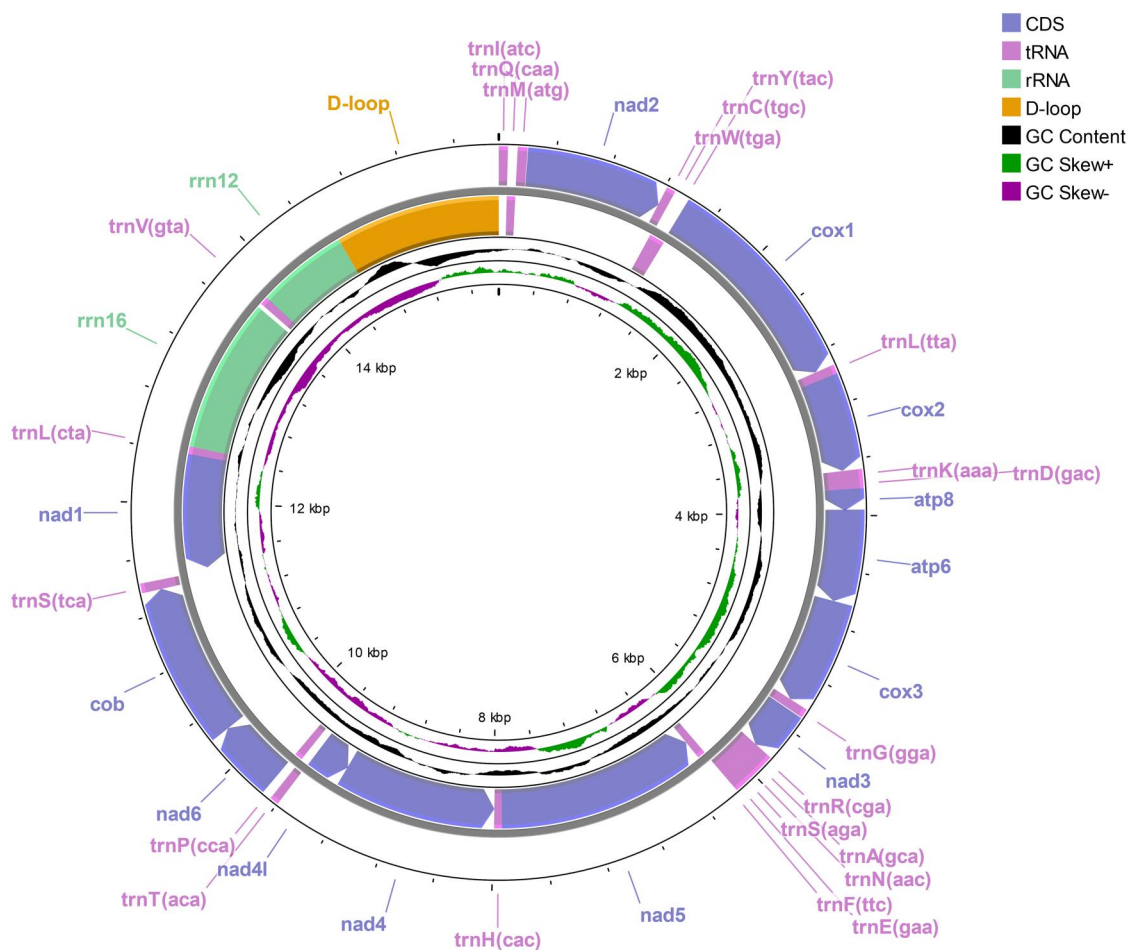
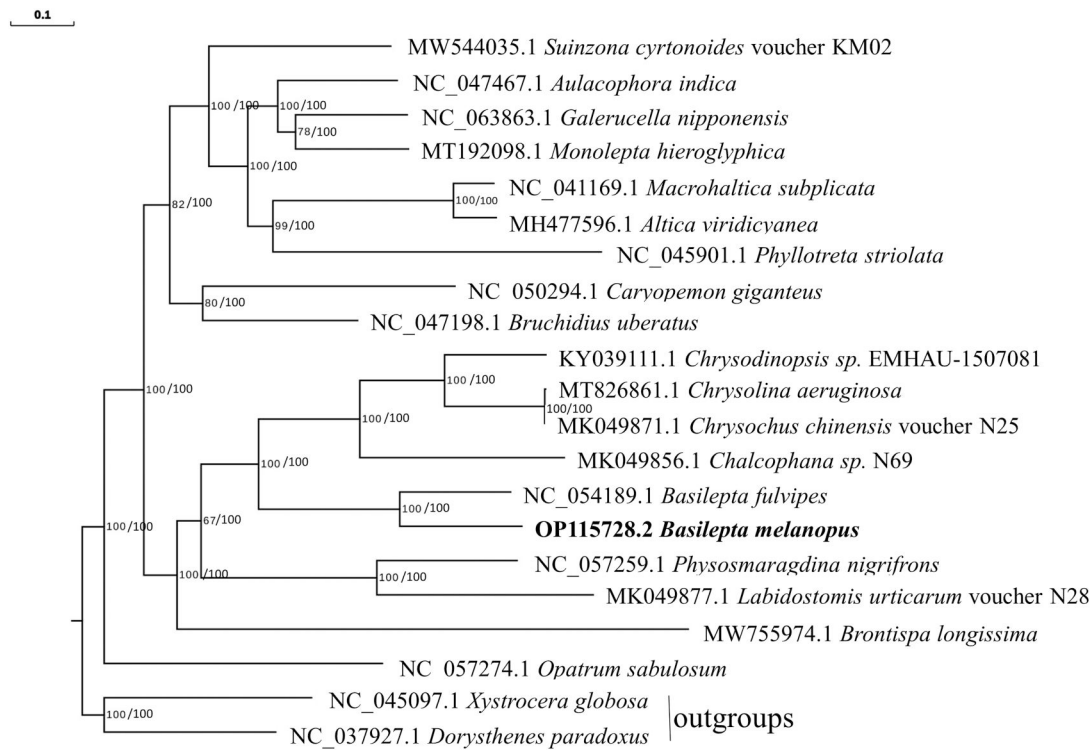


Figure 2. Mitochondrial genome map of *B. melanopus*.



**Figure 3.** Phylogenetic analysis based on the complete mitochondrial genome sequence of 19 chrysomelidae taxa (MW544035.1: Cho and Kim 2021; NC\_047467.1: Wang et al. 2020; NC\_063863.1: unpublished; MT192098.1: Li et al. 2020; NC\_041169.1: unpublished; MH477596.1: unpublished; NC\_045901.1: Zu and Yan 2019; NC\_050294.1: Wu et al. 2020; NC\_047198.1: Zhang et al. 2020; KY039111.1: Song et al. 2018; MT826861.1: unpublished; MK049871.1: Nie et al. 2021; MK049856.1: unpublished; NC\_054189.1: Liu et al. 2020; NC\_057259.1: Yang et al. 2019; MK049877.1: Nie et al. 2021; MW755974.1: unpublished; NC\_057274.1: Feng et al. 2020), with *xystrocera globosa* (NC\_045097.1: Wang et al. 2019) and *dorysthenes paradoxus* (NC\_037927.1: Liu et al. 2018) of cerambycidae as outgroup. The values for the bootstrap support and posterior probability for each branch node are as indicated.

ModelFinder in IQ-TREE v2.0.3 (Minh et al. 2020), the optimum nucleotide substitution model that is most suitable for the dataset was the general time reversible model (GTR) with invariable site (+I) plus discrete Gamma model (+G) (=GTR + G + I). For the Bayesian inference (BI) tree, the optimum nucleotide substitution model, which was determined by ModelFinder, was the GTR with empirical AA frequencies (+F) +G+I (=GTR + F + G + I). The BI tree was constructed using MrBayes v3.2 (Ronquist et al. 2012), in which the Markov chain Monte Carlo was conducted for 1,000,000 generations with reading sampled every 100 cycles.

## Results

The average coverage depth of the mitochondrial genome assembly was 1921 $\times$ , ranging from 458 to 7,999 $\times$  (Figure S1). The complete circularized mitochondrial genome was 15,905 bp in length and was predicted with 37 genes, including 13 protein-coding (PCGs), 22 transfer RNA, and two ribosomal RNA genes (Figure 2). Among these genes, four PCGs (i.e. *cox1*, *cox2*, *cox3*, and *nad4*) were detected with gene editing events. There were four start codons in the mitochondrial genome of *B. melanopus*, including ATA (detected in *nad2*, *nad3*, *nad4*, *nad4l*, and *nad6*), ATT (detected in *atp8*, *cob*, *cox1*, *nad1*, and *nad5*), ATC (detected in *cox2*), and ATG (detected in *atp6*, and *cox3*). The D-loop region ranged from 14,561 to 15,905 sites. The overall GC content was 22.51%, in which the percentages for the bases A, T, C, and G were 41.23%, 36.26%, 8.92%, and 13.59%, respectively.

Both the maximum likelihood (ML) and Bayesian inference (BI) analyses produced the same tree topology. However, only the ML tree was included in Figure 3, which displayed the bootstrap support value and posterior probability for each branch node. The complete mitochondrial genome sequence analysis of the 19 selected Chrysomelidae taxa led to a fully resolved phylogenetic relationship (bootstrap support  $\geq 75\%$ , posterior probability  $\geq 0.95$ ). The findings indicate that *B. melanopus* is closely related to *B. fulvipes*.

## Discussion

It is the first study conducted on *B. melanopus*. After comparing the mitochondrial genome sequence of *B. melanopus* to the published sequence of *B. fulvipes* (Liu et al. 2020), the two species appear to have similar genome size, gene order, and gene content. It is worth noting that gene editing events were detected in the mitochondrial genome of *B. melanopus*. Although there are no phylogenetic analyses that include *B. melanopus*, a previous study on *Basilepta* by Gómez-Zurita et al. (2005) used three ribosomal genes (mtDNA 16S as well as nrDNA 18S and 28S) to examine the phylogenetic relationship among samples. However, the study included only three *Basilepta* species, namely *B. multicostata*, *B. nitida*, and *B. wallacei*, and did not resolve the phylogenetic relationship among all species examined. Despite the limited sample size used in this study, the complete mitochondrial genome sequence was used to examine the phylogenetic relationship among members of Chrysomelidae. This genetic data might offer valuable

insights into the systematic evolution of this understudied genus and species. They could serve as a valuable resource for future studies, including their potential contribution toward the biocontrol management of this insect pest.

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## Ethics statement

This study was permitted and ethically approved by the Guangdong Academy of Agricultural Sciences. The study was supported and assisted by the Tianyang Plant Protection Station.

## Authors' contributions

Experiment design and data acquisition: YL, YF, WC & JW. Data analysis and submission: YL & JW. Manuscript - drafting: YL, YF & WC. Manuscript- revision: YL & JW. Photo-taking: JW. All authors approved the final version of the manuscript and agreed to be released for all aspects of this work.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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## Data availability statement

The mitogenome sequence data in this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/nucleotide/OP115728.2/>. Raw sequencing reads used here have been deposited in the SRA database of NCBI under accession number SRR20980349. The associated 'BioProject' and 'Bio-Sample' numbers are PRJNA867501 and SAMN30202747, respectively.

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