

# Complete mitogenome and phylogenetic analysis of *Hyalinocerus flavoscutatus* Cai and Shen 1998 (Hemiptera: Eurymelinae: Idiocerini)

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## ABSTRACT

This study presents the initial sequencing and characterization of the complete mitochondrial genome (mitogenome) of *Hyalinocerus flavoscutatus*, making the first comprehensive exploration of the mitogenome in the *Hyalinocerus*. Utilizing next-generation sequencing techniques, we identified a circular DNA molecule spanning 15,307 bp. The mitogenome comprises 13 protein-coding genes, two ribosomal RNA genes, 22 transfer RNA genes, and a primary non-coding region. Maximum likelihood phylogenetic evaluation, based on 13 protein-coding genes and two ribosomal RNA genes, robustly supports *H. flavoscutatus* as the basal group within Idiocerini. This research unveils valuable insights into the mitogenome of *H. flavoscutatus* and enhances our understanding of phylogenetic placement within the broader context of related tribes.

## ARTICLE HISTORY

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## Introduction

The genus *Hyalinocerus* Zhang and Li (2012) belongs to the tribe Idiocerini of the subfamily Eurymelinae. Until 2020 genus *Hyalinocerus* just contains one species - *Hyalinocerus nigrimaculatus* (Zhang and Li 2012). But along with Xue and Zhang revision of the phylogeny of genus *Amritodus* Anufriev (1970), "*Amritodus flavoscutatus* Cai and Shen (1998)" was transferred from *Amritodus* to *Hyalinocerus* as "*Hyalinocerus flavoscutatus* Cai and Shen (1998)". *H. flavoscutatus* was first described in the Henan Province which located central and eastern of mainland China (Figure 1), often feed on Anacardiaceae (Anufriev 1970). Based on analysis of three genes (*28S rRNA*, *16S rRNA*, *COX1*) and 47 morphological characters, Xue and Zhang reconstructed the phylogeny of *Amritodus* and related idiocerines, include *H. flavoscutatus* and *H. nigrimaculatus*, the results supported that the two were sister groups, which support the transfer of this species to *Hyalinocerus* (Xue and Zhang 2020). However until now the mitogenome data for *H. flavoscutatus* in the NCBI database is absence. To address this discrepancy, the present study undertook the first sequencing, annotation, and characterization of the mitogenome of *H. flavoscutatus*. This effort holds significance as it contributes crucial data for future research.

## Materials and methods



Specimens of adult *H. flavoscutatus* were collected from Baoxing Country, Yaan City, Sichuan Province, China, on


August 28, 2022 (30.3300°N, 102.5400°E), and preserved them in Animal Collection of Chuzhou University (no. GZCI99, Yan Dong, [dongyan\\_bio@126.com](mailto:dongyan_bio@126.com)).

Total genomic DNA was extracted from muscle tissue using the QIAamp DNA Micro Kit (50), and sequencing was conducted on the Illumina Novaseq 2500 platform. Mitogenome assembly utilized Geneious Prime 2023.2.1 (Chan and Lowe 2019). while preliminary annotation employed the MITOS web server (Grant and Stothard 2008) and NCBI BLAST, then checked tRNA locations by tRNAscan-SE (Lowe and Chan 2016). The datasets for the phylogenetic tree included 31 species, and the reconstruction relied on 13 PCGs and two rRNAs. Excluding the two outgroups *Alobaldia tobacae* and *Yanocephalus yanonis*, there are 10 species from Macropsini and 18 from Idiocerini. MAFFT 7 was used for genes aligning (Kato and Standley, 2013) and trimAl 1.4.1 for genes trimming (Capella-Gutiérrez et al., 2009), then concatenate individual genes using MEGA X (Kumar et al., 2018). The maximum likelihood analyze were conducted with IQ-TREE (Nguyen et al. 2015), and ML phylogeny was reconstructed with 1000 ultrafast bootstrap replicates (Hoang et al. 2018).

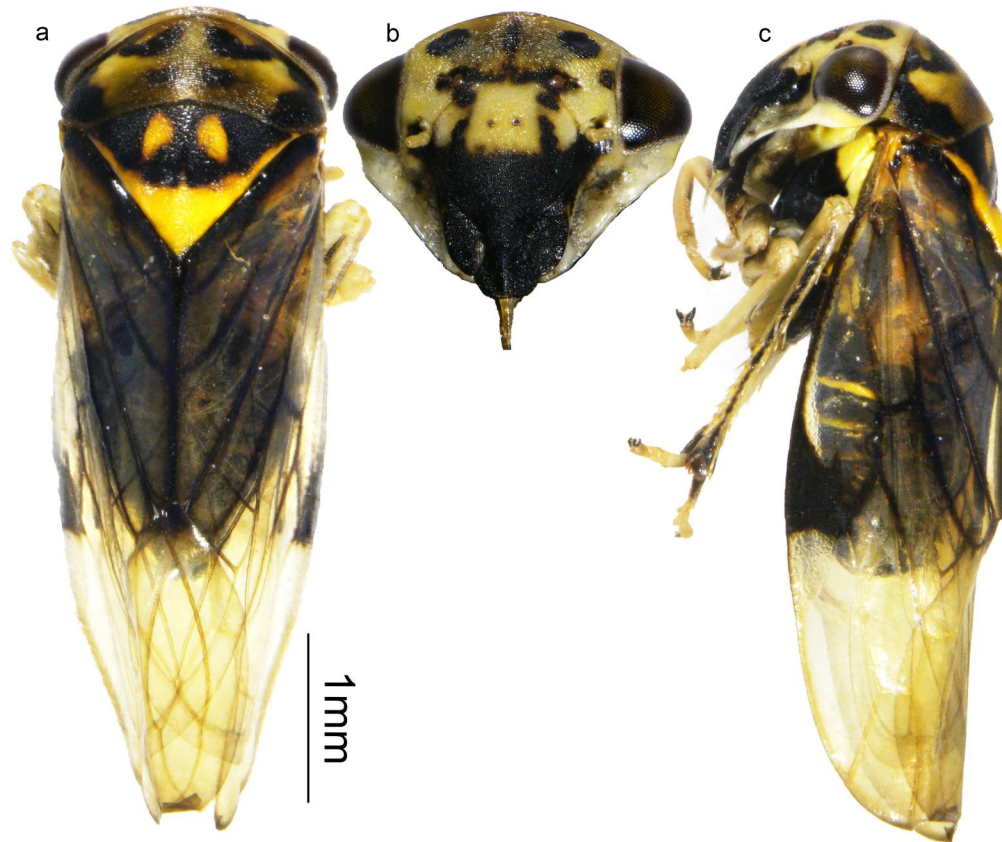
## Results

In this study, 150 Mb clean data was obtained by a single library and produced a final mitogenome for *H. flavoscutatus* with an average sequencing depth of 72× (Figure S1). The mitogenome of *H. flavoscutatus* was assembled into a

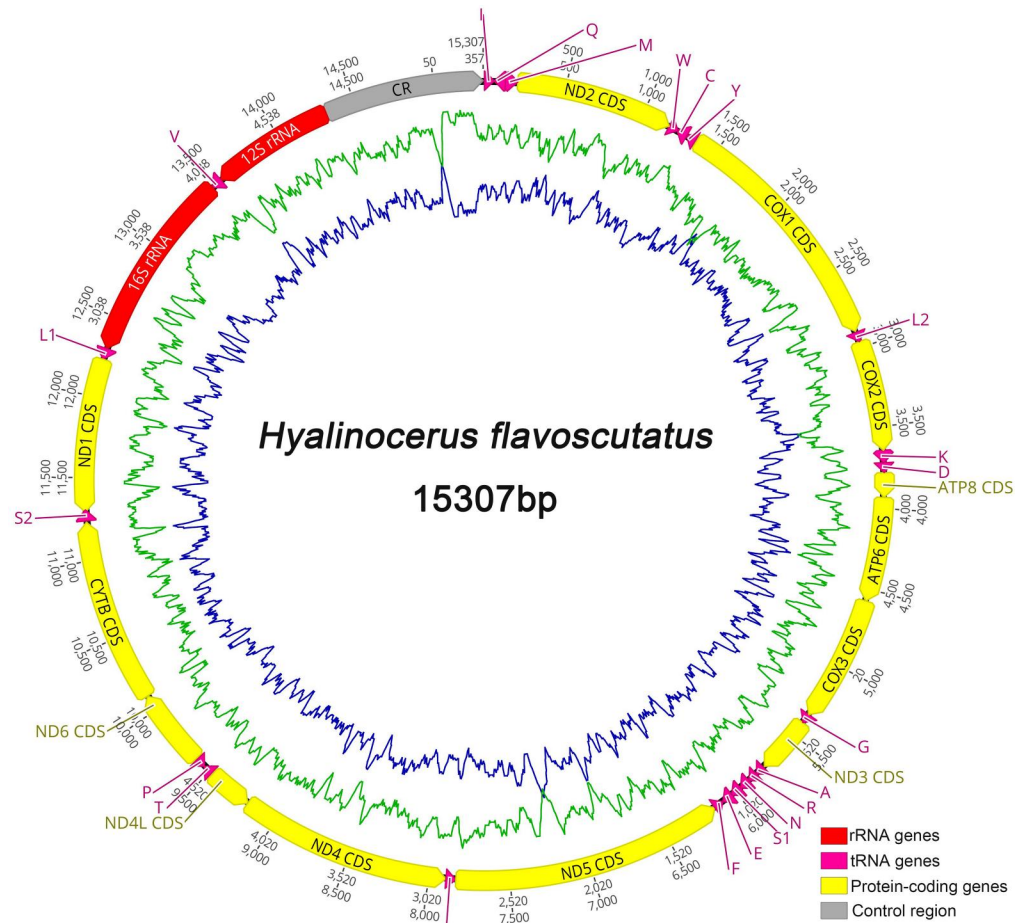
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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2024.2353231>.

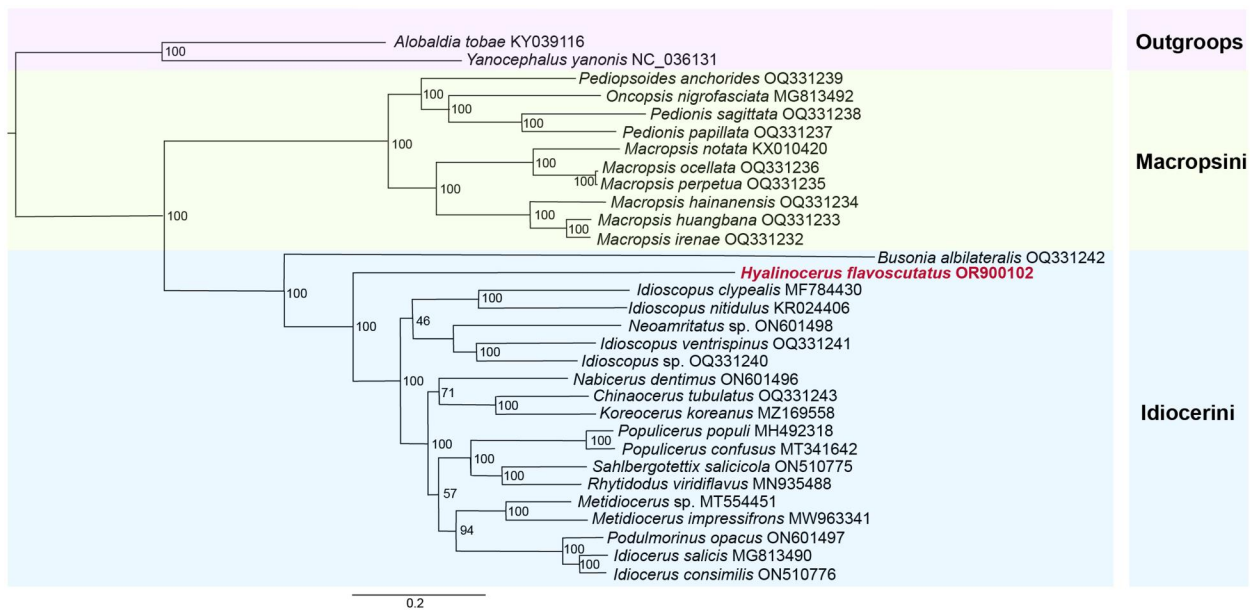
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**Figure 1.** Species reference image of *Hyalinocerus flavoscutatus*; (a) the dorsal view of *Hyalinocerus flavoscutatus*; (b) the ventral view of *Hyalinocerus flavoscutatus*; (c) the lateral view of *Hyalinocerus flavoscutatus* (photo taken by Shijia Zhu).



**Figure 2.** Circular map of the *Hyalinocerus flavoscutatus* mitochondrial genome and graphic representation of AT (green) and GC (blue) content and their changes throughout the genome.



**Figure 3.** Maximum-likelihood phylogeny between *Hyalinocerus flavoscutatus* and its close relatives based on the 13 mitochondrial protein-coding and two rRNAs genes. Including *Alobaldia tobae*, *Yanocephalus yanonis* (Song et al. 2017), *Pediopsoides anchoroides*, *Pedionis sagittata*, *Pedionis papillata*, *Macropsis ocellata*, *Macropsis perpetua*, *Macropsis hainanensis*, *Macropsis huangbana*, *Macropsis irenae*, *Idioscopus ventrispinus*, *Busonia albilateralis*, *Idioscopus* sp., *Chinaocerus tubulatus*, *Oncopsis nigrofasciata*, *Macropsis notata* (Wang et al. 2020), *Idioscopus clypealis* (Dai et al. 2017), *Idioscopus nitidulus* (Choudhary et al. 2018), *Nabicerus dentimus*, *Sahlbergottetix salicicola*, *Podulmorinus opacus*, *Idiocerus consimilis* (Tian et al. 2022), *Koreocerus koreanus* (Yu and Li 2021), *Populicerus populi* (Wang et al. 2018), *Populicerus confusus* (Shan et al. 2020), *Rhytidodus viridiflavus* (Xiao et al. 2020), *Metidiocerus* sp. (Di et al. 2020), *Metidiocerus impressifrons* (Ma et al. 2022), *Idiocerus salicis* (Wang et al. 2018). The GenBank accession numbers for the sequences are indicated next to the species names. Numbers near nodes indicate maximum-likelihood bootstrap percentages.

typical circular molecule which was 15,307 bp in length, with a nucleotide composition of 39.4% A, 36.3% T, 13.1% C, and 11.2% G. The mitogenome comprises 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and one control region. In addition, the control region is located between 12S rRNA and *trnI* with a length of 987 bp (Figure 2). Among the 13 PCGs, with a total length of 10,941 bp, constituting 71.5% of the mitogenome, complete stop codons (TAA, TAG) are observed in 10 PCGs (*ATP8*, *ATP6*, *ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, *ND6*, *CYTB* and *COX1*), the remaining three (*COX2*, *COX3* and *ND5*) conclude with an incomplete stop codon (T–). The tRNA genes, totaling 595 bp, exhibit lengths ranging from 61 bp (*trnG*) to 72 bp (*trnK*) and adopt a typical cloverleaf secondary structure, except for one gene (*trnS1*) lacking a dihydrouracil arm (DHU arm) and loop. Both rRNA genes are positioned after *trnV*, with 16S rRNA measuring 1,191 bp and 12S rRNA measuring 762 bp, their A+T contents are 80.9% and 77.3%, respectively.

In the Maximum Likelihood (ML) analysis (Figure 3), strong support indicates that *H. flavoscutatus* belongs to Idiocerinae and does not form sister relationships with any other species, instead nesting within the tribe Idiocerini with high support values.

## Discussion and conclusion

In summary, we present the mitogenome of *H. flavoscutatus* first time, which is the first mitogenome of *Hyalinocerus*. The gene order and composition align with those of other Idiocerini species (Wang et al. 2018; Shan et al. 2020; Ma

et al. 2022). Due to the molecular data for the *H. nigrimaculatus* is lacking, in this study we unable to reappear Xue and Zhang's conclusion. Nevertheless, our findings significantly contribute to understanding the genetic diversity and evolution of Idiocerini, offering valuable insights for future studies in this field.

## Authors' contributions

Shijia Zhu: Conceptualization, analysis and interpretation of the data, writing original draft; Mengrong Guo and Hui Liu: DNA extraction and sequence assembly, annotation and analysis; Jiajia Wang: Funding acquisition, revised the manuscript critically for intellectual content, phylogenetic tree constructing and analysis. All authors agree to be accountable for all aspects of the work.

## Ethical approval

Experiments were performed in accordance with the recommendations of the Ethics Committee of College of Biology and Food Engineering, Chuzhou University. These policies were enacted according to the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

## Disclosure statement

The authors have no conflicts of interest to report. The authors alone are responsible for the content and writing of the paper.

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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. OR900102. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1050025, SRR27143082, and SAMN38724732, respectively.

## References

- Anufriev GA. 1970. Description of a new genus *Amritodus* for *Idiocerus atkinsoni* Leth. From India (Hemiptera: cicadellidae). *J Nat Hist.* 4(3): 375–376. doi:10.1080/00222937000770351.
- Cai P, Shen XC. 1998. Eight new species of Idiocerinae from Mt. Funiu in Henan (Homoptera: cicadellidae). *Fauna Taxon Insects Henan.* 2:27–36.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics.* 25(15):1972–1973. doi:10.1093/bioinformatics/btp348. 19505945
- Chan PP, Lowe TM. 2019. tRNAscan-SE: searching for tRNA genes in genomic sequences. *Methods Mol Biol.* 1962:1–14. doi:10.1007/978-1-4939-9173-0\_1.
- Dai RH, Wang JJ, Yang MF. 2017. The complete mitochondrial genome of the leafhopper *Idioscopus clypealis* (Hemiptera: cicadellidae: idiocerinae). *Mitochondrial DNA B Resour.* 3(1):32–33.
- Di XC, Dong HR, Shan LC, Tian LL, Zhang B. 2020. The complete mitochondrial genome of *Metidiocerus* sp. (Hemiptera: cicadellidae: idiocerinae). *Mitochondrial DNA B Resour. Jul.* 11(3):2752–2753.
- Grant JR, Stothard P. 2008. The CGView Server: a comparative genomics tool for circular genomes. *Nucleic Acids Res.* 36(Web Server issue): W181–W184. doi:10.1093/nar/gkn179.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol.* 35(2):518–522. doi:10.1093/molbev/msx281.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 30(4):772–780. doi:10.1093/molbev/mst010.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol.* 35(6):1547–1549. doi:10.1093/molbev/msy096.
- Lowe TM, Chan PP. 2016. tRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Res.* 44(W1): W54–W57. doi:10.1093/nar/gkw413.
- Ma M, Li Z, Guo X, Zhang B. 2022. The complete mitochondrial genome of *Metidiocerus impressifrons* (Kirschbaum, 1868) (Hemiptera: cicadellidae: idiocerinae). *Mitochondrial DNA B Resour.* 7(2):372–373. doi:10.1080/23802359.2021.2008835.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 32(1):268–274. doi:10.1093/molbev/msu300.
- Shan LC, Dong HR, Di XC, Tian LL, Zhang B. 2020. Complete mitochondrial genome of *Populicerus confusus* (Hemiptera: cicadellidae: idiocerinae). *Mitochondrial DNA B Resour.* 5(3):2940–2941. doi:10.1080/23802359.2020.1788440.
- Song N, Cai W, Li H. 2017. Deep-level phylogeny of Cicadomorpha inferred from mitochondrial genomes sequenced by NGS. *Sci Rep.* 7(1):10429. doi:10.1038/s41598-017-11132-0.
- Tian L, Yang W, Si C, Guo X, Zhang B. 2022. Complete Mitogenome Analysis of Five Leafhopper Species of Idiocerini (Hemiptera: cicadellidae). *Genes (Basel).* 13(11):2000. doi:10.3390/genes13112000.
- Wang JJ, Wu YF, Yang MF, Dai RH. 2020. The Phylogenetic Implications of the Mitochondrial Genomes of *Macropsis notata* and *Oncopsis nigrofasciata*. *Front Genet.* 11:443. doi:10.3389/fgene.2020.00443.
- Wang JJ, Yang MF, Dai RH, Li H, Wang XY. 2018. Characterization and phylogenetic implications of the complete mitochondrial genome of Idiocerinae (Hemiptera: cicadellidae). *Biomac.* 08:191.
- Xue QQ, Zhang YL. 2020. Phylogeny and revision of the Oriental leafhopper genus *Amritodus* (Hemiptera: cicadellidae: idiocerini). *Zool J Lin Soc.* 189(4):1438–1463. doi:10.1093/zoolinnean/zlz129.
- Yu K, Li H. 2021. The complete mitochondrial genome of leafhopper *Koreocerus koreanus* (Matsumura, 1915) (Hemiptera: cicadellidae). *Mitochondrial DNA B Resour.* Aug. 9(9):2624–2625.
- Zhang B, Li ZZ. 2012. Hemiptera: cicadellidae: idiocerinae. In: Dai RH, Li ZZ, Jin DC, editors. *Insect fauna from National Natural Reserve of Guizhou Province, China. VII. Insects from Kuankuoshui landscape.* Guiyang: Guizhou Science and Technology Publishing House; p. 201–209.