



Check for updates

GENOME NOTE

Identification of the exuviae of larva from *Teinopalpus aureus* Mell, 1923 using the complete mitochondrial genome

[version 1; peer review: 2 approved with reservations]

Lei-Lei Liu¹, Li-Hua Zhang², Guo-Hang Weng¹, Wei-Qin Wang², Xi-Cheng Gong³, Shan-Biao Wang³, Shu-Sheng Zhang^{1,4} 

¹The Management Center of Wuyanling National Natural Reserve in Zhejiang, Taishun, Zhejiang, 325500, China²Taishun County Environmental Protection Bureau, Taishun, Zhejiang, 325500, China³Taishun County Tourism Development Investment Limited Company, Taishun, Zhejiang, 325500, China⁴Key Lab of Wildlife Biotechnology, Conservation and Utilization of Zhejiang Province, Zhejiang Normal University, Jinhua, Zhejiang, 321004, China

V1 First published: 31 Oct 2022, 11:1232
<https://doi.org/10.12688/f1000research.121947.1>

Latest published: 31 Oct 2022, 11:1232
<https://doi.org/10.12688/f1000research.121947.1>

Abstract

Teinopalpus aureus Mell (Lepidoptera: Papilionidae) is distributed throughout China, Vietnam and Laos, and is listed as a Class I species in China. To identify whether the exuviae of larva is belonging to *Teinopalpus aureus* Mell, 1923 or not, and to compare the gene structure and genetic differences among the known populations, ten mitogenomes of *T. aureus* from the exuviae of larva collected in the *Michelia maudiae* were sequenced. This method of sequencing the mitogenomes of exuviae of larva can give us the chance to monitor the conservation of rare butterflies.

Ten mitogenomes of *T. aureus* showed typical gene arrangements and contained 13 protein-coding genes (PCGs), two ribosomal RNA genes (12S rRNA and 16S rRNA), 22 transfer RNA (tRNA) genes, and a non-coding control region (D-loop). The two haplotypes with one base different in *T. aureus* were found. We also conducted phylogenetic analyses including all different populations of *T. aureus* to assess the phylogenetic relationship of *T. aureus*. The lengths of the 12S rRNA and 16S rRNA genes from both haplotypes were 776 base pairs (bp) and 1,334 bp, respectively. The genetic distance of the ten samples was calculated as 0-0.000065 on the basis of the whole mitogenomes. *T. aureus* found in Taishun, Zhejiang province, China had a close phylogenetic relationship with the clade of *T. aureus* found in Pingshan, Jiangxi province, China, which was supported by neighbour-joining analysis.

Keywords

Golden Kaiserihind, Mitogenome, Phylogeny

Open Peer Review

Approval Status  

	1	2
version 1		
31 Oct 2022	view	view

1. **Chang-Bae Kim**, Sangmyung University, Seoul, South Korea

2. **Xue-yan Li**, Chinese Academy of Sciences, Kunming, China

Any reports and responses or comments on the article can be found at the end of the article.



This article is included in the **Genomics and Genetics** gateway.

Corresponding author: Shu-Sheng Zhang (zhangshusheng323@163.com)

Author roles: **Liu LL:** Conceptualization, Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Zhang LH:** Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing; **Weng GH:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing; **Wang WQ:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing; **Gong XC:** Conceptualization, Data Curation, Formal Analysis, Writing – Review & Editing; **Wang SB:** Conceptualization, Data Curation, Methodology, Writing – Original Draft Preparation; **Zhang SS:** Conceptualization, Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Key Lab of Wildlife Biotechnology, Conservation and Utilization of Zhejiang Province, Zhejiang Normal University (2022-013).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Liu LL *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Liu LL, Zhang LH, Weng GH *et al.* **Identification of the exuviae of larva from *Teinopalpus aureus* Mell, 1923 using the complete mitochondrial genome [version 1; peer review: 2 approved with reservations]** F1000Research 2022, 11:1232 <https://doi.org/10.12688/f1000research.121947.1>

First published: 31 Oct 2022, 11:1232 <https://doi.org/10.12688/f1000research.121947.1>

Introduction

Because of its beautiful appearance and relative rarity in China, *Teinopalpus aureus* Mell, 1923 (Lepidoptera: Papilionidae) distributed in China, Vietnam and Laos is listed as a Class I species in China (Morita 1998; Masui 1999; Xing *et al.* 2019; Huang *et al.* 2015), which is one of three highest classifications for protection of endangered insect species in China. If we can identify the larva of *T. aureus* and obtain the mitochondrial genome using the exuviae of larva, we can determine its habitat sites and compare the genetic diversity of *T. aureus* using non-damage sampling to protect it in the larva stage.

No ethical approval was obtained for this study as we used wild exuviate gathered from the Wuyanling National Nature Reserve.

Methods

Ten exuviae of larva of *T. aureus* (No. ZJWYL20200601TP01- ZJWYL20200601TP10) were collected from wild host plants, *Michelia maudiae*, in Zhejiang Wuyanling National Nature Reserve, Taishun, Zhejiang province, China and stored in 100% absolute ethyl alcohol in the Natural Museum of Wuyanling National Nature Reserve, China.

A total of ten genomic DNAs from exuviae of larva (No. ZJWYL20200601TP01- ZJWYL20200601TP10) were extracted from partial exuviae using QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA) according to the manufacturer instructions, and stored at -20 °C refrigerator in Key Lab of Wildlife Biotechnology, Conservation and Utilization of Zhejiang Province.

The mitochondrial genome was obtained by the Sanger method. Ten mt genomes were amplified by polymerase chain reaction (PCR) by using the normal primers (Zhang *et al.* 2018; Guan *et al.* 2021; Xu *et al.* 2021; Yu *et al.* 2021) and six specific primers according to the reported mitochondrial genomes of *T. aureus* (Huang *et al.* 2015). This study used both normal polymerase chain reaction (PCR) and long-and-accurate PCR (LA PCR) methods with Takara Taq or Takara LA Taq DNA polymerase (Takara, Dalian, China). Normal PCR (or Long PCR) was performed in a 50 µL reaction mixture consisting of 32.5 µL (or 26.5 µL) of sterilized distilled water; 5 µL MgCl₂, 25 mM; 5 µL 10 × PCR Buffer (or 5 µL 10 × LA PCR Buffer); 4 µL (or 8 µL) dNTP, 2.5 mM; 1 µL (or 2 µL) of each primer, 5 µmol; 1 µL DNA template; 0.5 µL Takara Taq enzyme (or Takara LA Taq enzyme) DNA polymerase (Takara Biomedical, Japan). Fragments were amplified using Takara Taq enzyme *via* normal PCR or Takara LA Tag enzyme: initial denaturation for 3 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 1 min or 3 min at 46–58 °C, and 50–90 s at 72 °C, and a subsequent 10 min final extension step at 72 °C. All PCR reactions were used in Applied Biosystems Veriti instrument (Singapore). PCR products were purified using the Axygen agarose-out kit (Axygen, Hangzhou, China), and sequenced using ABI 3730 system by primer walking with two-directions.

To further discuss the phylogenetic relationship of *T. aureus* found in Zhejiang Wuyanling National Nature Reserve, a total of 13 mitogenomes were analyzed, including 11 mt genomes of *T. aureus* downloaded from NCBI (Qin *et al.* 2012; Huang *et al.* 2015; Zou *et al.* 2021) and two mt genomes of *T. imperialis* (Huang *et al.* 2016) as outgroups (Figure 1). To align the all complete mt genomes, we used Clustal W in Mega 7.0 (Kumar *et al.* 2016). We constructed a Neighbour-Joining phylogenetic tree with the parameters as below: bootstrap replications (1000), substitution model (Kimura 2-parameter model), Rates among sites (Gamma distributed), other parameters used default parameters.

Results

The two haplotypes with one base different in control region were found. The two obtained whole mt genome haplotypes were deposited in the National Center for Biotechnology Information with accession numbers OL449691 and OL449692.

The two mt genome haplotypes of *T. aureus* in majority-strand was 15,243 base pairs (bp) in length with negative AT-skew and GC-skew, which were -0.0025 and -0.2376, respectively, which showed typical lepidopterous gene structure and contained 13 protein-coding genes (PCGs), two ribosomal RNA genes (12S rRNA and 16S rRNA), 22 transfer RNA (tRNA) genes, and one control region (D-loop). One base T replaced C in control region between two haplotypes was found. The start codons of PCGs were ATG (in COX2, COX3, ATP6, ATP8, ND1, ND4, ND4L, and Cytb), ATT (in ND2, ND3, ND5, and ND6), and CGA (in COX1). The stop codons of PCGs were TAA (in ND2, ATP6, ATP8, COX3, ND1, ND5, ND6, and Cytb), TAG (in ND4L), and the incomplete stop codon T- (in COX1, COX2, ND3, and ND4). The gene arrangement was identical to the reported mt genome of *T. aureus wuyiensis* from Lee (Zou *et al.* 2021). The overall nucleotide composition of A, T, C and G in majority-strand was 39.8%, 40.0%, 12.5%, 7.7%, respectively. The lengths of the 12S rRNA and 16S rRNA genes from two haplotypes were 776 bp and 1,334 bp, respectively. The genetic distance of between two haplotypes and other reported population of *T. aureus* based on Kimura 2-parameter model was calculated as 0.00007-0.01013 on the basis of the whole mitogenomes. According to genetic distance, we can identify that ten exuviae

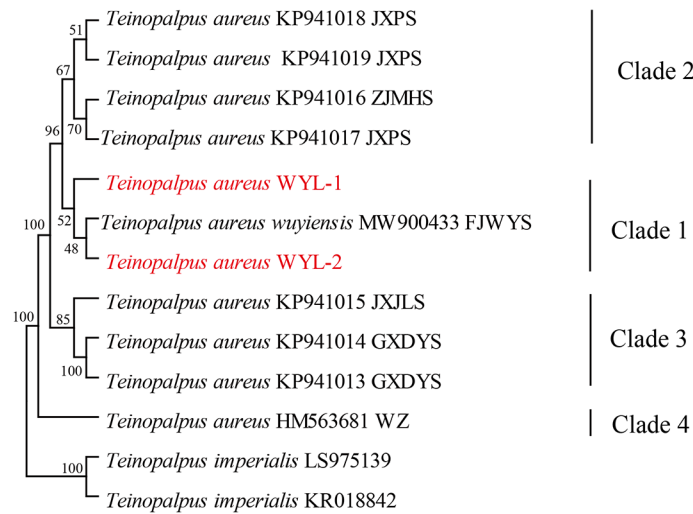


Figure 1. Phylogenetic tree of the relationships among 11 mt genomes of *Teinopalpus aureus* and two mt genomes of *Teinopalpus imperialis* based on the whole complete mitochondrial genomes. Numbers around the nodes are the bootstrap values of NJ. The GenBank numbers and the abbreviations of location information of all species are shown in the figure. JXPS: Pingshan, Jiangxi province; ZJMHS: Meihuashan, Zhejiang province; WYL: Wuyanling, Zhejiang province; JXJLS: Jiulianshan, Jiangxi province; GXDYS: Dayaoshan, Guangxi Zhuang Autonomous Region; WZ: Zhuang Autonomous Region.

of larva is belonging to the exuviae from *T. aureus*. This method using the exuviae to identify species can help us to monitor and protect *T. aureus*.

In NJ tree, two haplotypes from Taishun, Zhejiang province and the sample from Wuyishan, Fujian province were formed one clade 1. Then this clade is closed to the clade 2 from Pingshan and Meihuashan. Clade 1 and clade 2 are sister clade to the clade 3 from Jiulianshan and Dayaoshan. The clade 4 is sister clade to other clade and formed the phylogenetic relationship of (Clade 4 + (Clade 3 + (Clade 1 + Clade 2)).

In this study, the monophyly of *T. aureus* was well supported. The samples collected in Taishun, Zhejiang province can be identified as *T. aureus wuyiensis* because the genetic distance is below 0.00013 and the phylogenetic relationship of the samples collected in Taishun are clustered together with *T. aureus wuyiensis*. The new mitochondrial genomes obtained from the exuviae of larva of *T. aureus* can give us a further understanding of phylogenetic relationships of *T. aureus* to protect it.

Author contributions

All authors were involved in the conception and design, or analysis and interpretation of the data; LL Liu, LH Zhang, GH Weng, WQ Wang, and SS Zhang were involved in the drafting of the paper; all authors were involved in revising it critically for intellectual content; and all authors were involved in the final approval of the version to be published; and that all authors agree to be accountable for all aspects of the work.

Data availability

Source data

The mitochondrial genome data that support the findings of this study are openly available in GenBank of NCBI at [<https://www.ncbi.nlm.nih.gov/nuccore/OL449691> and <https://www.ncbi.nlm.nih.gov/nuccore/OL449692>] under the accession no. OL449691 and OL449692. The mt genome was obtained by the Sanger method, so no associated “BioProject”, “SRA” and “Bio-Sample” numbers should be shown.

References

- Guan JY, Zhang ZY, Cao YR, *et al.*: **The complete mitochondrial genome of *Choroterpes (Euthralus) yixingensis* (Ephemeroptera: Leptophlebiidae) and its mitochondrial protein-coding gene expression under imidacloprid stress.** *Gene*. 2021; **800**: 145833–145844.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Huang CB, Zeng JP, Zhou SY: **Complete mitochondrial genomes of *Teinopalpus imperialis* (Lepidoptera: Papilionidae) and phylogenetic relationships analyses.** *Mitochondrial DNA A*. 2016; **27**(4): 2903–2904.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Huang Y, Zhou S, Huang C, *et al.*: **Geophylogenetic analysis of *Teinopalpus aureus* Mell based on re-sequencing of the whole mitochondrial genome.** *Issues Biol. Sci. Pharm. Res.* 2015; **3**(5): 47–56.
- Kumar S, Stecher G, Tamura K: **Mega 7: molecular evolutionary genetics analysis version 7.0 for bigger datasets.** *Mol. Biol. Evol.* 2016; **33**(7): 1870–1874.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Masui A: **Butterflies recently collected from Laos PDR.** *Gekkan-Mushi*. 1999; **338**: 18–23.
- Morita S: **A new subspecies of *Teinopalpus aureus* Mell, 1923 from Vietnam (Lepidoptera: Papilionidae).** *Wallace*. 1998; **4**(2): 13–15.
- Qin F, Jiang G, Zhou S: **Complete mitochondrial genome of the *Teinopalpus aureus guangxiensis* (Lepidoptera: Papilionidae) and related phylogenetic analyses.** *Mitochondrial DNA*. 2012; **23**(2): 123–125.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Xing S, Au TF, Dufour PC, *et al.*: **Conservation of data deficient species under multiple threats: Lessons from an iconic tropical butterfly (*Teinopalpus aureus*).** *Biol. Conserv.* 2019; **234**: 154–164.
[Publisher Full Text](#)
- Xu XD, Guan JY, Zhang ZY, *et al.*: **Insight into the phylogenetic relationships among three subfamilies within Heptageniidae (Insecta: Ephemeroptera) along with low-temperature selection pressure analyses using mitogenomes.** *Insects*. 2021; **12**: 656–674.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Yu DN, Yu PP, Zhang LP, *et al.*: **Increasing 28 mitogenomes of Ephemeroptera, Odonata and Plecoptera support the Chiasmomyaria hypothesis with three different outgroup combinations.** *PeerJ*. 2021; **9**(1): e11402.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zhang LP, Yu DN, Storey KB, *et al.*: **Higher tRNA gene duplication in mitogenomes of praying mantises (Dictyoptera, Mantodea) and the phylogeny within Mantodea.** *Int. J. Biol. Macromol.* 2018; **111**: 787–795.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Zou W, Huang CB, Wang L, *et al.*: **The validity of the subspecies, *Teinopalpus aureus wuyiensis* Lee, from complete mitochondrial genome.** *Mitochondrial DNA B*. 2021; **6**(9): 2589–2591.
[Publisher Full Text](#)

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 27 March 2023

<https://doi.org/10.5256/f1000research.133868.r163936>

© 2023 Li X. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Xue-yan Li

State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China

This manuscript investigated a method to use exuviae of butterfly larva for species identification, which is useful especially for protected species. Nevertheless, the authors should describe more details of methods and explain some following points.

1. What does 'Class I species in China' mean?
2. The authors got ten mt genomes of *T. aureus* by the Sanger methods. Are all these sequences submitted to a public database? GenBank or other database?
3. Figure 1 include 11 mt genomes of *Teinopalpus aureus* and two mt genomes of *Teinopalpus imperialis*. For 11 mt genomes of *Teinopalpus aureus*, which one(s) are from this study? Which ones from published database? The authors said "including 11 mt genomes of *T. aureus* downloaded from NCBI", please make the figure legend to be readable by itself.

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Insect genome

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 09 December 2022

<https://doi.org/10.5256/f1000research.133868.r156619>

© 2022 Kim C. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Chang-Bae Kim

Department of Biotechnology, Sangmyung University, Seoul, South Korea

The manuscript sequenced and analyzed the complete mitogenomes for identification of the exuviae of larva from *Teinopalpus aureus*. However, the authors did not explain why mitogenomes are necessary for this aim.

In the manuscript, the authors focus on describing mitogenome structure but this work was done 10 years ago by Qin *et al.* (2012): "Complete mitochondrial genome of the *Teinopalpus aureus* guangxiensis (Lepidoptera: Papilionidae) and related phylogenetic analyses"¹ and some following studies, compare your data to previous studies.

Moreover, all 10 samples for analyses in this study were collected in one region so there was almost no variation among mitogenome sequences. The authors describe these limitations and further studies.

References

1. Qin F, Jiang GF, Zhou SY: Complete mitochondrial genome of the *Teinopalpus aureus* guangxiensis (Lepidoptera: Papilionidae) and related phylogenetic analyses. *Mitochondrial DNA*. 2012; **23** (2): 123-5 [PubMed Abstract](#) | [Publisher Full Text](#)

Are the rationale for sequencing the genome and the species significance clearly described?

No

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics and bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research