The validity of the subspecies, *Teinopalpus aureus wuyiensis* Lee, from complete mitochondrial genome

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**ABSTRACT**

The complete mitochondrial genome (mtgenome) was determined from the emerged-pupa stage (Noninvasive sampling) of *T. aureus wuyiensis* Lee. It was 15,234 base pairs in length and contained 13 protein-coding genes (PCGs), 2 RNA genes, 22 tRNA genes, and a control region. By taking *Meandrusa sciron* and *Teinopalpus imperialis* as outgroups, a maximum-likelihood phylogenetic tree was constructed among five geographical populations of *Teinopalpus aureus* based on 13 PCGs and two RNA genes. Our results showed that the WYS, MHS and PS populations, locating at or closing to Wuwishan Mountain-range, were in one cluster; while the JLS (locating at Nanling Mountain-range) and DYS (locating at Dayaoshan Mountain close to Wuyishan Mountain-range) populations belonged to another cluster. It supported well the subspecies of *T. aureus wuyiensis*, and suggested that the genetic relationship between *T. a. guangxiensis* and the nominal subspecies of *T. a. aureus* were closer enough to combine into one subspecies.

The butterfly of golden kaiserihind, *Teinopalpus aureus*, is endemic to tropical and subtropical regions of Asia (Zhou 1994; Igarashi 2001; Wu and Xu 2017), it has been listed as Red Species of IUCN since 1985 (Gimenez Dixon 1996), and also being as one of the First-class of National Key Protected Animals in China since 1989. *Teinopalpus aureus* is one of flagship species to insect-diversity conservation in East Asia (Wang et al. 2020), so it has attracted lots of attention worldwide, from entomologists (e.g., Igarashi 2001; Collard 2007; Zeng et al. 2008) as well as conservation biologists (Wang et al. 2018; Xing et al. 2019), environmental protectors (Li et al. 2013) and (local to national) governments.

*Teinopalpus aureus* was first recorded on a mountain near Lianping (see Figure S1) in northern Guangdong Province, southern China, and it was treated as a subspecies of *T. imperialis*, but soon adjusted as a new species (Mell 1923). Actually, *T. aureus* is a distinct species based on lots of evidences from morphological characteristics (Zhou 1994; Wu and Xu 2017), molecular sequences (Qin et al. 2012; Huang et al. 2015; Huang et al. 2016) and ecological traits (Igarashi 1987; 2001; Zeng et al. 2008, 2012, 2014; Lin et al. 2017). The second finding of *T. aureus* is occurred in Wuwishan of Fujian (another mountain in southern China, WYS) in 1980s (Zhou 1994), more than 60 years later from the first record. But since then, similar findings are occurred subsequently in Wuwishan of Hainan island (the third mountain in 1992, WZS), Dayaoshan of Guangxi (the forth mountain in 1994, DYS), Jiulianshan of Jiangxi (a mountain 15 km to Lianping in the north in 1996, JLS, see Figure S1) (Lin et al. 2017), and so on, which thus promote the intraspecific taxonomy of *T. aureus* (Zhou 1994). In view of above findings from different isolated mountains or regions, the subspecies is then identified and named correspondingly, like the nominal subspecies of *T. a. aureus* Mell mainly from Nanling Mountain-range (e.g. JLS population, see Figure S1) (Zhou 1994), *T. a. wuyiensis*, Lee mainly from Wuwishan Mountain-range (e.g. WYS population, see Figure S1) (Li and Zhu 1992; Zhou 1994), *T. a. guangxiensis* Chou et Zhou mainly from Guangxi (e.g. DYS population, see Figure S1) (Zhou 1994), and *T. a. hainani* Lee mainly from Wuwishan Mountain-range (e.g. WZS population), etc. (Li and Zhu 1992; Zhou 1994; Zhong et al. 2000). However, due to (1) the number of specimens used, such as with only female or male specimens, is totally limited in subspecies taxonomy, and then leading to incomplete description in morphology; (2) in particular, there is a lack of other supporting information like genetic, ecological divergence, the intraspecific taxonomy of *T. aureus* is still controversial.

According to the field methods of Zeng (2005), the puparium (emerged-pupa shell) of *T. aureus* were collected from WYS in Huangken of Jianyang city (27.57029N,117.652410E, ...
The complete mitochondrial genome of *Teinopalpus aureus wuyiensis* Lee was 15 234 bp (GenBank accession number MW900433) in length, and contained 13 protein-coding genes (PCGs; ATP6, ATP8, COI-III, ND1-6, ND4L, Cytb), two ribosomal RNAs (12S rRNA and 16SrRNA), 22 transfer RNAs, and a putative control region (D-loop). The sequence of the genome and the direction of the protein-coding genes were similar to most reported mitochondrial genes in lepidopteron insects. In the nucleotide composition, it showed high bias toward A and T, with 79.81% in A and 56.07% in T nucleotides. The ATG start codon was used for most of the PCGs except for three genes; ND1, ND3 and ND4L were terminated with stop codon TAG. The conservative stop codon TAA was shared with most of the PCGs except for three genes; ND1, ND3 and ND4L were terminated with stop codon TAG.

To illustrate the phylogenetic relationships of the complete mt genomes from (1) WYS population (MW900433, from the subspecies of *T. a. wuyiensis* Lee) in this study and from other locations (see Figure S1) in GenBank, including (2) JLS population (KP941015, from the subspecies of *T. a. aureus* Mell) (Huang et al. 2015), (3) DYS population (KP941013; from the subspecies of *T. a. guangxiensis* Chou et Zhou) (Huang et al. 2015), (4) PS population (KP941017.1, from the subspecies of *T. a. wuyiensis* Lee) (Huang et al. 2015, Huang 2016), (5) MHS population (KP941016, from the subspecies of *T. a. aureus* wuyiensis Lee) (Huang et al. 2015), the maximum-likelihood (ML) methods with 1000 bootstrap simulations using MEGA software version 7 (Kumar et al. 2016) were performed to construct the phylogenetic tree basing on the tandem nucleotide sequences of 13 PCGs and two ribosomal RNAs (Sun et al. 2020; Wen et al. 2020; Kim et al. 2021), with *Teinopalpus imperialis* Hope (KR018842) as well as *Meandrusa sciron* Leech (LS975123.1) as the outgroups. The ML tree (Figure 1) showed *T. aureus* and the outgroup of *T. imperialis* were independent of each other, and the intraspecific genetic distance (0.0011 ± 0.00067) of *T. aureus* was also significantly shorter (t = 54.589, df = 13, p < 0.001) than that between (0.0212 ± 0.00067) *T. aureus* and *T. imperialis* in t-test. In the ML tree, there were two clusters among the five geographical populations of *T. aureus*, the three WYS, MHS and PS populations were in one cluster, while another two populations, JLS and DYS, were belonged to another cluster (Figure 1). In effect, the ML tree was consistent well with the mountain-range distribution of the five locations geographically, like the WYS, MHS and PS, all locating at or closing to Wuyishan Mountain-range, while the JLS and DYS, both locating at or closing to Nanling Mountain-range (Figure S1).

Similarly, the genetic distance within (0.0002 ± 0.00012) the subspecies of *T. a. wuyiensis* (including WYS, MHS, and PS populations) was found significantly shorter (t = 6.188, df = 7, p < 0.001) than that between (0.0015 ± 0.00033) *T. a. wuyiensis* and *T. a. aureus* (including JLS and DYS populations). Our results supported well the subspecies of *T. a. wuyiensis*, and suggested that the genetic relationship between *T. a. guangxiensis* and the nominal subspecies of *T. a. aureus* were closer enough to combine into one subspecies (Figure 1). To be noted, continuation of genomic studies is still necessary for clarifying taxonomy of the remaining subspecies.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
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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. MW900433.

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MITOCHONDRIAL DNA PART B