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The complete mitochondrial genome of *Choroterpides apiculata* (Ephemeroptera: Leptophlebiidae) and its phylogenetic relationships

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**ABSTRACT**

The complete mitochondrial genome of *Choroterpides apiculata* (Ephemeroptera: Leptophlebiidae) is typically a circular molecule of 15,199 bp in length, containing 37 genes (13 protein-coding genes, 22 tRNAs, and two rRNAs) and one control region. The overall A + T content of the whole genome is 74% and the A + T content of the control region is 79.7%. In Bayesian inference and maximum-likelihood phylogenetic trees using 24 species from 13 families of Ephemeroptera, the monophyly of the families Isonychiidae, Heptageniidae, Vietnamellidae, Leptophlebiidae, Caenidae, and Baetidae were highly supported and *C. apiculata* was a sister group to *Habrophlebiodes zijinensis*.

Species of the Leptophlebiidae family (Insecta: Ephemeroptera) are distributed all over the world, including in China. The phylogenetic relationships of Ephemeroptera are still disputed based on morphological and/or molecular studies (Hebert et al. 2003; Ogden and Whiting 2005; Zhang et al. 2008; Ogden et al. 2009; Simon and Hadrys 2013; Li et al. 2014; Misof et al. 2014; Cai et al. 2018). There was only one mitogenome of species (*Habrophlebiodes zijinensis*) in Leptophlebiidae and so its monophyly could not be tested. Thus, it is necessary to sequence more mitogenomes of Leptophlebiidae species to discuss its phylogenetic relationship within Ephemeroptera.

Samples of *Choroterpides apiculata* were collected from Jinggu (23°57′38″ N, 100°51′47″ E), Yunnan province, China on 28 August 2018 and identified by Dr. JY Zhang. The sample (YN20180828) was identified and stored at a –40°C freezer in the Animal Specimen Museum, College of Life Sciences and Chemistry, Zhejiang Normal University, China. Total genomic DNA was extracted from leg tissue of individual samples using Ezup Column Animal Genomic DNA Purification Kit (Sangon Biotech Company, Shanghai, China) and stored in the Zhang’s laboratory. Universal primers (Wang et al., 2019; Zhang, Cai, et al. 2018; Zhang, Yu, et al. 2018) were used to amplify some partial fragments. The mitogenome was deposited in GenBank with the accession number MN807287.

The complete mitogenome of *C. apiculata* was a circular molecule of 15,199 bp in length, including 13 protein-coding genes (PCGs), 22 tRNAs, two rRNAs, and one control region. The gene arrangement and orientation coincided with those of typical mayfly species. The overall base composition of the major strand was as follows: A (34.5%), T (39.5%), G (10.1%), and C (15.9%). All 13 PCGs used the standard mitochondrial start codon ATN. Eleven PCGs terminated with a complete stop codon (TAA), whereas COX2 and ND4 showed an incomplete terminal T—.

Phylogenetic trees were constructed from the 13 PCGs using two methods: Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) and maximum-likelihood (ML) using RAxML 8.2.0 (Stamatakis 2014). *Siphlonurus chinensis* was used as the outgroup according to the method of Li et al. (2014) and Xu et al. (2020). The mitogenomes of 23 Ephemeroptera species were downloaded from GenBank (Zhang et al. 2008; Li et al. 2014; Tang et al. 2014; Zhou et al. 2016; Cai et al. 2018; Gao et al. 2018; Ye et al. 2018; Xu et al. 2020) and used to investigate the phylogenetic relationships. In order to select the conserved regions of nucleotides, each alignment was performed by Gblock 0.91 b (Castresana 2000) using the default settings. In BI and ML phylogenetic trees, the monophyly of the families Isonychiidae, Heptageniidae, Vietnamellidae, Leptophlebiidae, Caenidae, and Baetidae were supported, whereas the monophyly of Siphlonuroidea failed (Figure 1). Leptophlebiidae was supported as a sister group to the clade Caenidae + (Teloganodidae + Baetidae), which also agreed with the results of Cai et al. (2018), Gao et al. (2018), Ye et al. (2018), and Xu et al. (2020). *Choroterpides apiculata* was supported as the sister group to *H. zijinensis*. 

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Figure 1. Phylogenetic tree of the relationships among 24 species of Ephemeroptera, including Charoterpides apiculata (MN807287) based on the nucleotide dataset of the 13 mitochondrial protein-coding genes (PCGs). Siphluriscus chinensis was used as the outgroup. The numbers above branches specify posterior probabilities as determined from BI (left) and bootstrap percentages from ML (right). The GenBank numbers of all species are shown in the figure.

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