The complete mitochondrial genome of *Epeorus herklotsi* (Ephemeroptera: Heptageniidae) from Longquan, Zhejiang, China and its phylogeny

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**ABSTRACT**

The mitochondrial genome of *Epeorus herklotsi* (Ephemeroptera: Heptageniidae) from Longquan, Zhejiang province, China was a circular molecule of 15,499bp in length, containing 13 protein-coding genes, two ribosomal RNAs, 23 transfer RNAs, and a control region. The A+T content of the overall base composition of H-strand is 65.7%. All of the 13 protein-coding genes were begun with ATN as start codon except ND5 using GTG. ATP6, ATP8, COI, COIII, ND1, ND2, ND4L, and ND6 genes were terminated with TAA as stop codon, Cyt b ended with TAG and the other three protein-coding genes ended with an incomplete stop codon (TA or T). In BI phylogenetic trees, the monophyly of the families Caenidae, Heptageniidae, Isonychiidae and Viemamellidae, and the genus *Epeorus* were strongly supported. *Epeorus* is a sister clade to *Parafronurus*, then cluster with *Paegniodes*.

The heptageniid mayflies *Epeorus herklotsi* is widely distributed throughout the rivers of China. For the convenience of identifying this species, we sequenced the mitochondrial genome of *E. herklotsi*. In Ephemeroptera, gene rearrangements were found in six sequences of twenty-three known mayfly mitochondrial genomes with IMQM tRNA cluster found in Heptageniidae (Zhang et al. 2008; Li et al. 2014; Tang et al. 2014; Gao et al. 2018). The phylogenetic relationships of Ephemeroptera still exist disputes in morphological and molecular methods (Kristensen 1981; Ogden and Whiting 2003; Zhang et al. 2008; Simon and Hadrys 2013; Li et al. 2014; Misof et al. 2014; Cai et al. 2018; Ye et al. 2018). Hence, we used the mitochondrial genome of *E. herklotsi* to analyse the characteristics of mitochondrial gene arrangement and to discuss the phylogenetic relationships within Ephemeroptera.

The samples of *E. herklotsi* from Longquan city, Zhejiang province, China were stored at −70°C in our laboratory at Shaoxing People’s hospital. Some DNA fragments were amplified using highly conserved primers (Zhang et al. 2008). After obtaining most part of the mtgenome, we designed species-specific primers for the remaining part with reference to previously determined sequences. All polymerase chain reactions (PCRs) were carried out using a MyCycler Thermal Cycler (Bio-Rad, Hercules, CA). Takara Ex-Taq and LA-Taq Kits (Takara Biomedical, Dalian, China) were used for normal and long PCRs.

The complete mtDNA is 15,499bp in length and contains 13 protein-coding genes, two ribosomal RNAs, 23 transfer RNAs genes, and noncoding regions. The overall base composition of H-strand is as follows: T (33%), C (21.3%), A (32.7%), G (13%), and the A+T content (65.7%). All of the 13 protein-coding genes were begun with ATN as start codon except ND5 using GTG. ATP6, ATP8, COI, COIII, ND1, ND2, ND4L, ND6 genes were terminated with TAA as stop codon, Cyt b ended with TAG and the other protein-coding genes ended with an incomplete stop codon (TA or T). An IMQM tRNA cluster at the upstream of ND2 gene was found, which was consistent with *P. youi* (Zhang et al. 2008) and three species of *Epeorus* (Tang et al. 2014; Gao et al. 2018), while it was different to *Paegniodes cupulatus* with the typical IQM tRNA cluster (Zhou et al. 2016).

Bayesian inference (BI) tree was constructed using the 13 PCGs from 21 species using *S. chinensis* (Li et al. 2014) as outgroup (Figure 1). BI analysis was performed by MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). In the results, the monophyly of the families Caenidae, Heptageniidae, Isonychiidae, Viemamellidae, and the genus *Epeorus* was strongly supported. Isonychiidae was the basal clade to Ephemeroptera excluding Siphlonuridae. The monophyly of the family Siphlonuridae was not supported in our results, because *Siphlonurus* sp. (KM244684) and *S. immanis* (FJ606783) were cluster with *Ameletus* sp. 1 (KM244682) and *Ephemera orientalis*, respectively. Within the Heptageniidae clade, *Epeorus* was a sister clade to *Parafronurus*, then (*Epeorus + Parafronurus*) cluster with *Paegniodes*. Two species of *Epeorus herklotsi* were cluster together.
The complete mitochondrial genome of *E. herklotsi* has been assigned deposited to GenBank with the following accession number MH752075.

Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Nucleotide sequence accession number**

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