

## Mitochondrial genome of *Arcynopteryx dichroa* (McLachlan, 1872) (Plecoptera: Perlodidae) and phylogenetic analysis

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### ABSTRACT

To better understand the diversity and phylogeny of Perlodidae, we sequenced and annotated the complete mitochondrial genome (mitogenome) of *Arcynopteryx dichroa*. This mitogenome was 16,215 bp long and encoded 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA unit genes (rRNAs), and a control region like other plecopteran. The nucleotide composition of *A. dichroa* mitochondrial genome obviously biases toward A and T. The A + T content of the whole mitochondrial genome, the PCGs, tRNAs, rRNAs, and the control region were: 69.3%, 67.6%, 69.8%, 71.3%, and 78.7%. Phylogenetic relationship based on the concatenated sequences of 13 PCGs and two ribosomal RNAs showed that the family Perlodidae and Chloroperlidae are sister relationship, family Perlidae being the sister group to the clade (Perlodidae + Chloroperlidae). The monophyly of the family Perlodidae is well supported but the subfamily Perlodinae and Isoperlinae are not monophyletic groups.

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Klapálek (1909) established the subfamily Perlodinae of Plecoptera, the members of Perlodinae usually possess large-sized and conspicuously colored (Li and Murányi 2015). As we know, the Perlodinae has high genetic diversity but low species-level diversification (Li and Murányi 2015; DeWalt and Ower 2019; DeWalt et al. 2021). The Perlodinae is distributed in the Holarctic with a few species in the Oriental Region, this subfamily includes 152 species in 49 genera with more than 20 monotypic genera and only three genera include more than ten species (DeWalt and Ower 2019; Chen 2019a, 2019b, 2020; Huo et al. 2020; Huo and Du 2020; DeWalt et al. 2021). Only two mitochondrial genomes (*Perlodes* sp. and *Pseudomegarcys japonica*) in subfamily Perlodinae have been sequenced and published previously (Chen et al. 2018). In this study, we sequenced the third complete mitochondrial genome of *Arcynopteryx dichroa* (McLachlan, 1872) in Perlodinae with the GenBank accession number MW588036. The male adult specimen of *A. dichroa* was collected from Dillingham City, Alaska, United States (coordinate as follows: N°60.339, W°159.369) on July 13, 2010. A voucher specimen (No. VHL-0256) was deposited in absolute ethanol and kept at  $-20^{\circ}\text{C}$  in the Entomological Museum of the Henan Institute of Science and Technology (contact person: Wang Ying, email: [wangying198586@163.com](mailto:wangying198586@163.com)), Henan Province, China. Specimens were identified by Wei-Hai Li (HIST). Genomic DNA was extracted with the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) from the identified adult's muscle. The

mitogenome of *A. dichroa* was generated by amplification of overlapping PCR fragments (Primer information listed in the [Supplementary Material](#)). BioEdit version 7.0.5.3 (Hall 1999) was used to align all used mitochondrial genomes. The mitochondrial genome was annotated by the MITOS Web Server (Bernt et al. 2013) and the PCGs and rRNAs were identified with other published Plecoptera mitochondrial genomes. MEGA version 7.0 software was used to calculate the nucleotide composition, and the skew values were obtained with formulas:  $\text{AT-skew} = [A - T]/[A + T]$  and  $\text{GC-skew} = [G - C]/[G + C]$ . MAFFT was used to align the 13PCGs and concatenate them excluding the stop codons. Maximum likelihood (ML) analysis was performed by using RAxML version 8 (Stamatakis 2006).

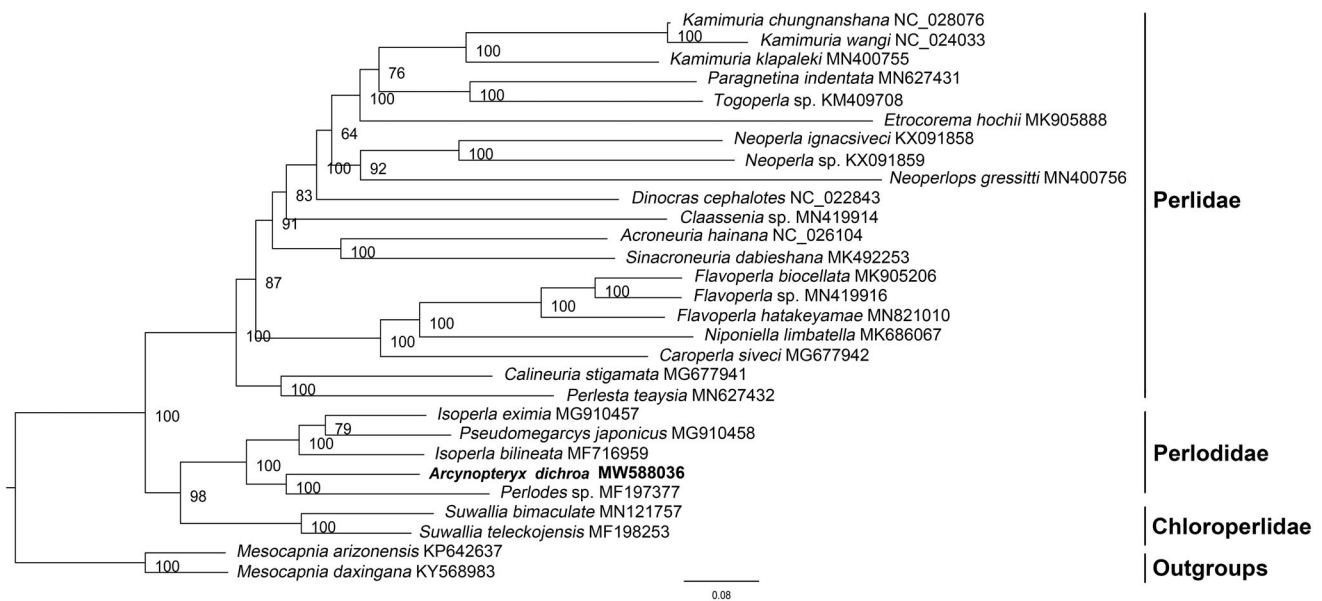
The entire mitochondrial genome of *A. dichroa* was a double strand circular 16,215 bp molecule, which encoded 37 typical genes (22 transfer RNA genes, 13 protein-coding genes, two ribosomal RNA genes) and a 1205 bp-long control region. In this genome, 23 genes were found on the J-strand (majority strand) and 14 genes were on the N-strand (minority strand). The mitochondrial arrangement was identical to that of other published stoneflies as well as the inferred ancestor of insects. There were altogether 48 bp overlapping nucleotides were found in 13 gene pairs, and their length ranged from 1 to 8 bp. There were 48 intergenic nucleotides dispersed in 10 gene gaps, ranging from 1 to 21 bp.

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 Supplemental data for this article is available online at [here](#).

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**Figure 1.** Phylogenetic tree based on the concatenated nucleotide sequences of 13 PCGs and two ribosomal RNAs by maximum-likelihood (ML) methods from the complete mitochondrial genomes of *Arcynopteryx dichroa* and 28 other stoneflies. Scientific name is followed by the accession number for each species.

The A + T content of the whole mitochondrial genomes, PCGs, tRNAs, rRNAs and the control region were all obviously biased toward A and T: 69.3%, 67.6%, 69.8%, 71.3%, and 78.7%, respectively. Eleven PCGs began with the normal start codon ATN, however, *ND1* and *ND5* used TTG and GTG. All PCGs of the mitochondrial genome used complete termination codons (TAA or TAG), but *COI*, *COII*, and *ND5* terminated with an incomplete termination signal T. Each tRNA gene ranged from 65 to 71 bp, all tRNAs could be folded into standard cloverleaf secondary structures, however, the dihydrouridine (DHU) arm of *trnS1* (AGN) was reduced to a small loop like other species in Plecoptera. Large ribosomal RNA and the small ribosomal RNA were 1330 bp and 844 bp long, with A + T content of 72.9% and 68.6%, respectively. The control region followed closely behind the small ribosomal RNA, which was 1205 bp long with the highest A + T content in the mitochondrial genome of *A. dichroa*.

We reconstructed the phylogenetic relationship of Perlodidae based on the concatenated sequences of 13 PCGs and two ribosomal RNAs from 28 published stoneflies and *A. dichroa* mitochondrial genomes (Figure 1), *Mesocapnia arizonensis* and *Mesocapnia daxingana* were served as the outgroup. The maximum-likelihood (ML) method was used to generate the topological tree. In the analyses, the family Perlodidae and Chloroperlidae are sister relationships, family Perlidae being the sister group to the clade (Perlodidae + Chloroperlidae). This result is consistent with the previous phylogenetic relationship (Chen et al. 2018; Wang et al. 2019; Zhao et al. 2020) and the traditional morphological result of Illies (1965). In the present mitochondrial study, the monophyly of the family Perlodidae is well supported but the subfamily Perlodinae and Isoperlinae are not monophyletic groups with the *Pseudomegarcys japonica*, *A. dichroa*, and *Perlodes* sp. not clustered in one clade. In summary, more mitochondrial genomes in future studies are needed to reconstruct a more robust phylogenetic relationship of the Perlodidae.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, reference number MW588036.

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