First report of the complete mitogenome of *Tanypus punctipennis* Meigen, 1818 (Diptera, Chironomidae) from Hebei Province, China

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**ABSTRACT**

*Tanypus punctipennis* Meigen, 1818 is an important bioindicator for freshwater ecosystems monitoring. Although COI barcode analyses have been performed on *T. punctipennis*, the mitogenome of this taxon has not been assembled and analyzed. Here, the complete mitogenome of *T. punctipennis* was sequenced and analyzed to confirm the systematic and phylogenetic history of this species. The mitogenome is 16,215 bp long with high A + T content, and consists of 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a noncoding control region. The phylogenomic analysis supports monophyletic Tanypodinae and close relationship between *T. punctipennis* and *Clinotanypus*. Our results indicate that mitogenomes showed strong signals in phylogenetic reconstructions at the genus level of Tanypodinae.

**KEYWORDS**

Chironomids; bioindicator; mitochondrial genome; phylogeny

**ARTICLE HISTORY**

Received 5 September 2021
Accepted 16 December 2021

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*Tanypus punctipennis* Meigen, 1818 is classified in the subfamily Tanypodinae, Chironomidae, one of the most diverse group of aquatic Diptera with around 6300 described species (P. Ashe, personal communication). Due to their high species diversity and ability to inhabit different types of water bodies, chironomid larvae are important bioindicators for freshwater ecosystem monitoring (Ferrington 2008). Due to variable morphological features, entomologists face great challenges in identifying chironomids. Mitogenomic data have been broadly used in molecular identification and phylogenetic studies of Diptera (e.g. Yan et al. 2019; Li et al. 2020; Miao et al. 2020). However, only a few Chironomidae mitogenomes have been deciphered (Beckenbach 2012; Kim et al. 2016; Deviatiiarov et al. 2017; Kong et al. 2021; Lei et al. 2021; Zheng et al. 2021). In this study, we provide the first complete mitochondrial genome of *T. punctipennis*.

The larva of *T. punctipennis* was collected from Baoding, China (38.320556°N, 115.375000°E) on 9 May 2018. Total genomic DNA was extracted from the muscle tissues of the head and thorax of an adult using the DNeasy Blood and Tissue kit (QIAGEN Sciences, Valencia, CA). The DNA and voucher specimen of *T. punctipennis* is deposited in the College of Life Sciences, Nankai University, Tianjin, China (https://sky.nankai.edu.cn, Xiao-Long Lin, lin880224@gmail.com) under the voucher number XL2604. COI of *T. punctipennis* (GenBank accession: JN887098) was used as bait to iterate and assemble the mitogenome of *T. punctipennis*. The genomic DNA was subsequently pooled with other insect species and sequenced using the Illumina Nova6000 (PE150, Illumina, San Diego, CA) platform with an insert size of 350 bp and a paired-end 150 bp sequencing strategy at Novogene Co., Ltd. (Beijing, China). Four Gb clean data were obtained from the library after trimming using Trimmomatic (Bolger et al. 2014). The software IDBA-1.1.1 (Peng et al. 2012) was employed to assemble the data with similarity set to be 0.98. The mitogenome of *T. punctipennis* was then identified using a Blast search (Altschul et al. 1990) with COI as the bait sequence (Crampton-Platt et al. 2015), and the percentage of identical matches was 100%. The mitogenome annotation was conducted following Zheng et al. (2020).

The mitogenome of *T. punctipennis* is 16,215 bp in length (GenBank accession no. MZ475054), containing 13 protein-coding genes (PCGs), two ribosomal RNA genes, 22 transfer RNA genes, and one noncoding control region. The overall nucleotide composition was 39.5% of A, 36.2% of T, 14.3% of C, 10.1% of G, and 75.7% of A + T content. Most of the 13 PCGs used ATN as the start codon (ATG for ATP6, COII, COIII, CytB, ND4, and ND4L; ATT for ATP8, ND2, ND3, and ND6; GTG for ND1 and NDS), while ACG for COI. The stop codon TAA is assigned to the most PCGs (except TAG for ND4 and a single T for COI). Gene arrangement of the 13 PCGs is identical to that of other known Chironomidae mitogenomes. Nucleotide composition of *T. punctipennis* is similar with other known Chironomidae mitogenomes, with a high A + T bias.

Eleven mitochondrial genomes of Chironomidae and two of Ceratopogonidae available from GenBank were mined for the phylogenetic analysis. The sequences were concatenated with alignments of 13 PCGs using the default settings in MAFFT (Katoh and Standley 2013). The maximum-likelihood
(ML) reconstruction was performed using IQ-TREE (Nguyen et al. 2015) with 1000 bootstraps replicates and the PMSF acid substitution model. In addition, Culicoides arakawae and Forcipomyia makanensis were designated as the outgroups. The result (Figure 1) clearly shows that Chironominae formed a monophyletic group. T. punctipennis is sister to the genus Clinotanypus based on mitogenomics, which is concordant with morphology. This work provides molecular characterizations of T. punctipennis and contributes to the phylogenetic analysis of Chironomidae.

Disclosure statement
No potential competing interest was reported by the authors.

Funding
This study was supported by the National Natural Science Foundation of China [31900344] and the China Postdoctoral Science Foundation [2018M640227].

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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/ under the accession no. MZ475054. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA759705, SRR15694340, SAMN21192202, respectively.

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