



## First complete mitogenome of *Axarus fungorum* (Albu, 1980) from Guizhou Province, China (Diptera, Chironomidae)

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### ABSTRACT

*Axarus fungorum* (Albu, 1980) exhibits certain adaptations to different aquatic environments, appearing as an important evaluation element for freshwater quality monitoring. In this study, complete mitogenome of *A. fungorum* was provided for the first time to define the systematic and phylogenetic history of this taxon. The whole mitogenome is 15,696 bp long with high A + T content that consists of 13 protein-coding genes, 22 tRNA genes, two rRNA genes, and a noncoding control region. ML analysis showed support for monophyly of Chironominae and close relationship between *A. fungorum* and *Chironomus* generic genera.

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Chironomids; mitochondrial genome; phylogeny





*Axarus fungorum* (Albu, 1980) belongs to Chironominae, a subfamily under the Chironomidae, one of the most abundant invertebrate taxa in freshwater ecosystems with more than 6300 valid species. Chironomid larvae are considered as an excellent indicator for monitoring aquatic environment quality due to their wide distribution, high species diversity, large population, sensitivity, and adaptability (Ferrington 2008). Due to species diversity and variable morphological features within Chironomidae, the traditional morphological identification is inconvenient. In such instances, mitogenomic data can be considered as powerful and convenient material for molecular identification and phylogenetic studies for Diptera (e.g. Yan et al. 2019; Li et al. 2020; Zhang et al. 2022). However, complete mitogenomes are still scarce for Chironomidae (Beckenbach 2012; Kim et al. 2016; Deviatiiarov et al. 2017; Kong et al. 2021; Lei et al. 2021; Zheng et al. 2021, 2022; Fang et al. 2022). In the present study, we have provided complete mitochondrial genome of *A. fungorum* for the first time.

Fresh and adult male individuals of *A. fungorum* were collected from Meitan, Guizhou, China (27.828857°N, 107.5955098°E) on 8 June 2020. The DNeasy Blood and Tissue kit (QIAGEN Sciences, Valencia, CA) was used to isolate total genomic DNA from the muscle tissues of head and thorax. The DNA and voucher specimen of *A. fungorum* has been deposited in the College of Fisheries and Life Science, Shanghai Ocean University, Shanghai, China (<https://www.shou.edu.cn>, Xiao-Long Lin, [lin880224@gmail.com](mailto:lin880224@gmail.com)) under the voucher number DLC28. COI of *A. fungorum* (GenBank

accession: MN521232) was used as bait to iterate and assemble the mitogenome of *A. fungorum*. DNA fragments with 350 bp insert size were sequenced by Illumina Nova6000 (PE150, Illumina, San Diego, CA) platform using pair-end strategy at Novogene Co., Ltd. (Cambridge, UK). Four Gb clean data were obtained from the library by trimming using Trimmomatic (Bolger et al. 2014). IDBA-1.1.1 (Peng et al. 2012) software package was employed to assemble the data. The bait sequence of COI (Crampton-Platt et al. 2015) was used in the BLAST program (Altschul et al. 1990) to compare with the mitogenome of *A. fungorum*. The percentage of match rate was found as 100% from the blast result. The mitogenome annotation was conducted as previously described by Zheng et al. (2020).

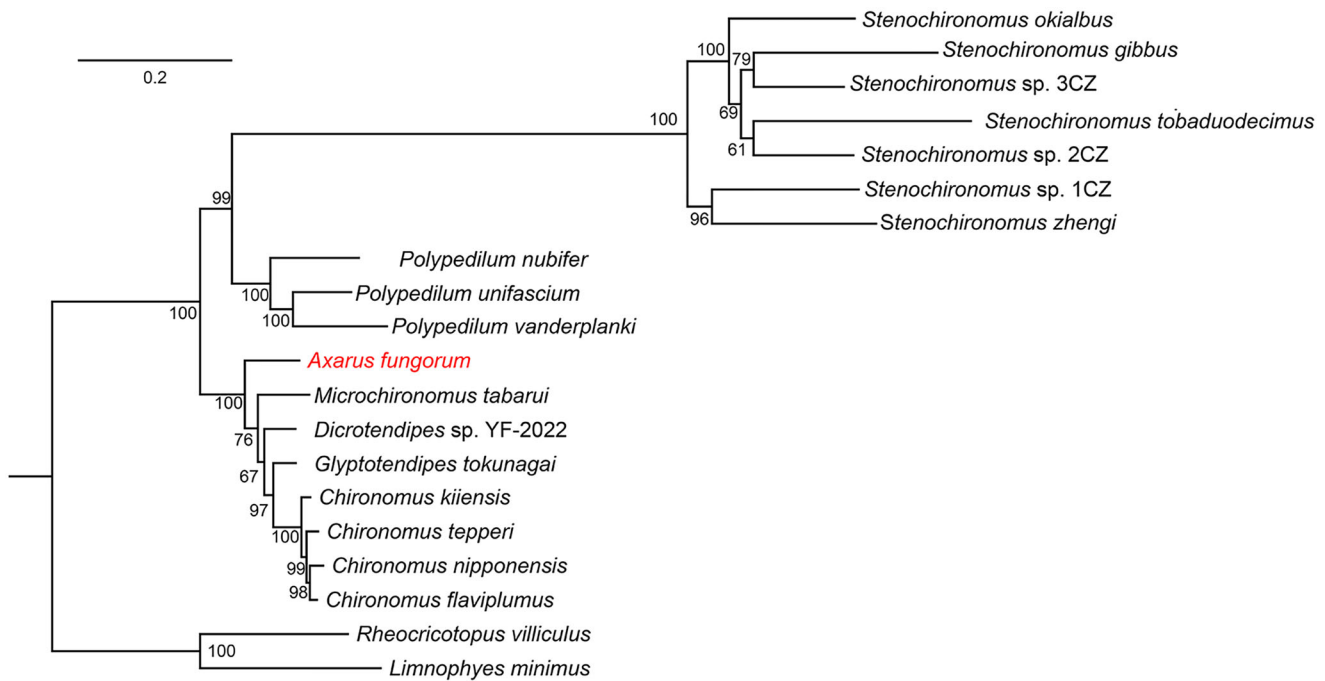
The double-strand circular mitogenome of *A. fungorum* is 15,696 bp in length (GenBank accession no. ON099430) which encodes for 37 genes (13 protein-coding genes, two rRNA genes, and 22 tRNA genes) and a control region. Nucleotides within the mitogenome were distributed as follows: 41.2% A, 38.3% T, 12.2% C, and 8.3% G. The most frequently observed start codons were ATG for ATP6, COII, COIII, CytB, ND4, ND4L and ATT for ATP8, ND2, ND3, ND6, respectively, while GTG for ND5; TTG for COI and ND1. All of the 13 PCGs were terminated with TAA stop codon. Mitogenome organization, nucleotide composition and codon usage were similar to the previously sequenced Chironomidae mitogenomes with a high AT bias (79.5%).

Eighteen mitogenomes of Chironominae and two of Orthocladiinae were mined from GenBank for the

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**Figure 1.** Phylogenetic tree of 20 Chironomidae species based on the concatenated dataset of 13 PCGs using the maximum-likelihood (ML) method. The following sequences were used: *Axarus fungorum* ON099430 (present study), *Chironomus flaviplumus* MW770891 (Park et al. 2021), *Chironomus kiiensis* MZ150770 (Liu et al. 2022), *Chironomus nipponensis* MZ747092 (Shen et al. 2022), *Chironomus tepperi* JN861749 (Beckenbach 2012), *Dicrotendipes* sp. YF-2022 MZ747093 (direct submission), *Glyptotendipes tokunagai* MZ747091 (direct submission), *Limnophyes minimus* MZ041033 (Fang et al. 2022), *Microchironomus tabarui* MZ261913 (Kong et al. 2021), *Rheocricotopus villiculus* MW373526 (Zheng et al. 2021), *Stenochironomus gibbus* OL742440 (Zheng et al. 2022), *Stenochironomus okialbus* OL753645 (Zheng et al. 2022), *Stenochironomus* sp. 1CZ OL753646 (Zheng et al. 2022), *Stenochironomus* sp. 2CZ OL742441 (Zheng et al. 2022), *Stenochironomus* sp. 3CZ OL753647 (Zheng et al. 2022), *Stenochironomus tobaduodecimicus* OL753648 (Zheng et al. 2022), *Stenochironomus zhengi* OL753649 (Zheng et al. 2022), *Polypedilum nubifer* MZ747090 (direct submission), *Polypedilum unifascium* MW677959 (Lei et al. 2021), and *Polypedilum vanderplanki* KT251040 (Deviatiarov et al. 2017).

phylogenetic analysis. Initially, sequences of 13 PCGs were concatenated and then aligned with MAFFT (Kato and Standley 2013) keeping all the settings in default (Kato and Standley 2013). Using 1000 bootstraps and PMSF acid substitution model, we conducted phylogenetic analysis by maximum-likelihood (ML) method with IQ-TREE (Nguyen et al. 2015) considering *Limnophyes minimus* and *Rheocricotopus villiculus* as outgroups. Topologies from the reconstructed tree strongly supported the monophyly of Chironominae, and the sister relationship between *A. fungorum* and the *Chironomus* generic genera (Figure 1).

### Ethics statement

The collection of specimen conformed to the requirement of International ethics, which did not cause damage to the local environment. The process and purpose of this experimental research were in line with the rules and regulations of our institute. There are no ethical issues and other conflicts of interest in this study.

### Author contributions

Yan Qi and Xin Duan were involved in the conception and design, analysis, and interpretation of the data; Ke-Long Jiao and Xiao-Long Lin were involved in the drafting of the paper, revising it critically for intellectual content and the final approval of the version to be published; and the authors agreed to be accountable for all aspects of the work. No potential conflict of interest was reported by the authors.

### Disclosure statement

No potential competing 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/> under the accession no. ON099430. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA820975, PRJNA820975, and SAMN27029903, respectively.

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