

MITOGENOME REPORT



First report of the complete mitochondrial genome of *Glyptotendipes pallens* (Meigen, 1804) (Diptera: Chironomidae) from Hebei Province, China

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ABSTRACT

Glyptotendipes pallens (Meigen, 1804) serves as a significant bioindicator for monitoring freshwater ecosystems. Despite the performance of COI barcode analyses on *G. pallens*, the mitogenome of this taxon remains unassembled and unanalyzed. In this study, we provide one newly sequenced complete mitogenome of *G. pallens* for the first time. The mitogenome is 16,063 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 phylogenetic analysis strongly supports *G. pallens* and *G. tokunagai* as sister species.

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Introduction

Chironomid (Diptera) larvae are widely regarded as an exceptional indicator for monitoring the quality of aquatic environments, owing to their extensive distribution, rich species diversity, abundant population, sensitivity, and remarkable adaptability (Ferrington 2008). Because of the diverse species and varying morphological traits among Chironomidae, traditional morphological identification can be cumbersome. In these cases, molecular data serve as a robust and practical tool for molecular identification and phylogenetic investigations of chironomids (Ekrem et al. 2007; Lin et al. 2015; Ekrem et al. 2018; Lin et al. 2020; Lin, Liu, et al. 2022; Lin, Zhao, et al. 2022). The typical insect mitochondrial genome (mitogenome) is a circular, double-stranded molecule that ranges in size from 14 to 20 kb, encoding a total of 37 genes, including 13 protein-coding genes, two ribosomal RNA genes, and 22 transfer RNA genes, as well as a control region (Wolstenholme 1992; Boore 1999). Owing to its small genome size, maternal inheritance, minimal sequence recombination, and rapid evolutionary rates (Brown et al. 1979; Cuore and Kocher 1999), the mitogenome stands as a potent marker for phylogenetic and evolutionary analyses in insects (Miao et al. 2020; Ge et al. 2022, 2023; Ma et al. 2022; Zhang et al. 2023). Benefiting from the advances of high-throughput sequencing technology, a growing number of complete mitogenomes have been sequenced in Chironomidae, and they are extensively employed for mitochondrial structure comparisons and phylogenetic analyses at various

taxonomic levels of chironomids (Beckenbach 2012; Kong et al. 2021; Zheng et al. 2021; Jiang et al. 2022; Lin, Liu, et al. 2022; Lin, Zhao, et al. 2022; Liu et al. 2022; Zheng et al. 2022; Fang et al. 2023; Qi et al. 2023; Zhang et al. 2023; Li et al. 2024).

Before this study, mitogenomes of *Glyptotendipes* Kieffer (Chironomidae: Chironominae: Chironomini) were scarcely available, thereby restricting our comprehension of their mitochondrial structure and phylogenetic pattern. Herein, we provide one newly sequenced complete mitogenome of *Glyptotendipes pallens* (Meigen, 1804) for the first time.

Materials and methods

Collection, DNA extraction, and sequencing

The larva of *Glyptotendipes pallens* (Figure 1) was collected from the Baiyangdian Lake in Hebei Province, China (38.920140°N, 115.886086°E) on 18 April 2024. During the sampling process, the larva was preserved in 85% ethanol. The voucher specimen was deposited at the College of Fisheries and Life Science, Shanghai Ocean University, China (<https://smxy.shou.edu.cn/>, Xiaolong Lin, lin880224@gmail.com) under the voucher number BYD285. Genomic DNA was extracted from the larva's thorax (without gut) using the Universal Genomic DNA Kit (CWBIO, Taizhou, China). The DNA library, with an insert size of 350 bp, was sequenced using the NovaSeq Xplus-PE150 sequencing strategy at Novogene Co., Ltd. (Beijing, China). Eight Gigabases clean

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data were obtained through the use of Trimmomatic v0.32 (Bolger et al. 2014). The larva was identified through DNA barcoding using the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007).

Assembly, annotation, and structural analysis of the mitochondrial genome

NOVOPlasty v4.3.1 (Dierckxsens et al. 2017) was used to assemble mitogenome. The mitogenome annotation was conducted by MITOS2 v2.1.7 (Bernt et al. 2013), then

mitogenome map (Figure 2) was performed using the CG View server v1.0 (Grant et al. 2023). The annotated mitochondrial genome sequence was imported into Geneious Prime (Biomatters, Auckland, New Zealand), and the annotation table was exported. This table was then uploaded to the CGView Server, which generated the mitochondrial genome map. The read coverage depth map of *G. pallens* was generated using R v4.3.2 (Team, 2014). First, the assembled mitogenome was indexed with minimap2 v2.28 (Li 2018). Sequencing reads were then mapped to the assembled

Table 1. Taxonomic information, GenBank accession numbers, and references of mitochondrial genomes used in the study.

Subfamily	Species	Accession no.	Reference
Chironominae	<i>Chironomus nipponensis</i>	MZ747092	Shen et al. (2022)
Chironominae	<i>Chironomus plumosus</i>	ON838252	Fang et al. (2023)
Chironominae	<i>Dicrotendipes pelochloris</i>	ON838257	Fang et al. (2023)
Chironominae	<i>Einfeldia</i> sp.	ON838255	Fang et al. (2023)
Chironominae	<i>Glyptotendipes tokunagai</i>	MZ747091	Fang et al. (2023)
Chironominae	<i>Polypedilum nubifer</i>	MZ747090	Xiao et al. (2022)
Chironominae	<i>Stictochironomus juncai</i>	OP950226	Zhang et al. (2023)
Chironominae	<i>Stictochironomus rosenschoeldi</i>	OP950224	Zhang et al. (2023)
Chironominae	<i>Synendotendipes impar</i>	OP950223	Zhang et al. (2023)
Chironominae	<i>Stenochironomus baishanzuensis</i>	OL742441	Zheng et al. (2022)
Chironominae	<i>Stenochironomus okialbus</i>	OL753645	Zheng et al. (2022)
Chironominae	<i>Endochironomus albipennis</i>	OP950227	Zhang et al. (2023)
Chironominae	<i>Endochironomus pekanus</i>	OP950219	Zhang et al. (2023)
Chironominae	<i>Phaenopsectra flavipes</i>	OP950216	Zhang et al. (2023)
Chironominae	<i>Polypedilum heberti</i>	OP950225	Zhang et al. (2023)
Chironominae	<i>Sergentia baueri</i>	OP950220	Zhang et al. (2023)
Chironominae	<i>Microchironomus tabarui</i>	MZ261913	Kong et al. (2021)
Chironominae	<i>Glyptotendipes pallens</i>	PQ093791	this study
Orthoclaadiinae	<i>Rheocricotopus villiculus</i>	MW373526	Zheng et al. (2021)
Orthoclaadiinae	<i>Synorthoclaadius bifidus</i>	OP006248	Li et al. (2024)
Orthoclaadiinae	<i>Cricotopus flavozonatus</i>	OP006247	Li et al. (2024)



Figure 1. A photo of the *Glyptotendipes pallens*. Photograph was taken by Xiaolong Lin.

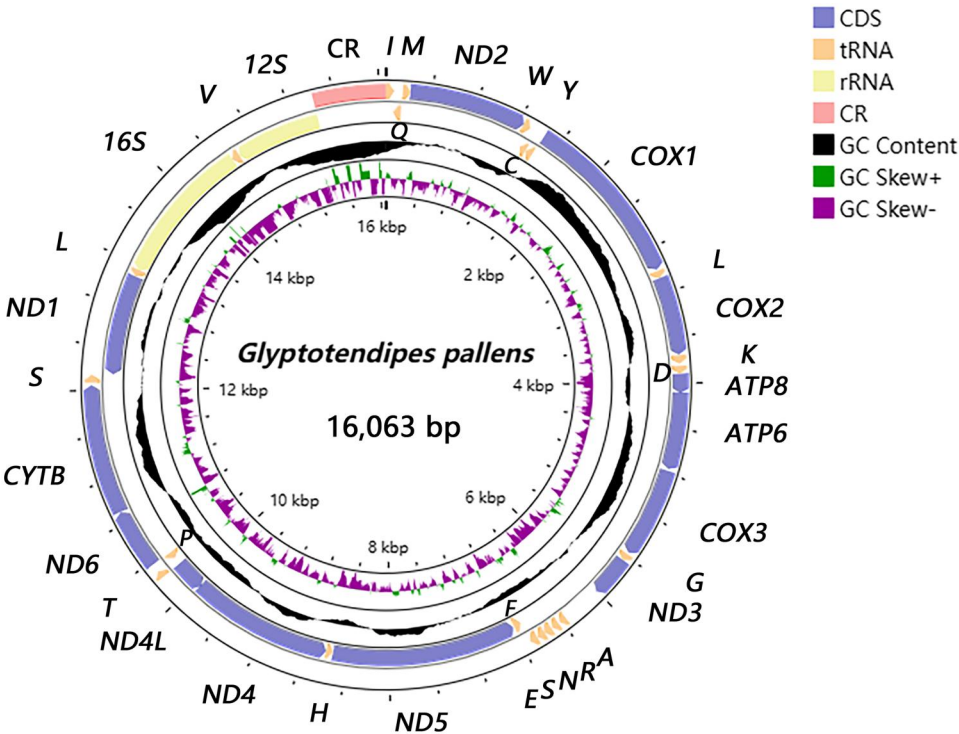


Figure 2. Mitogenome map of *Glyptotendipes pallens*. The names of PCGs and rRNAs are indicated by standard abbreviations, while names of tRNAs are represented by a single letter abbreviation. The first circle shows the gene arrangement, and arrows indicate the orientation of gene transcription. Blue, yellow, orange, and red arrows refer to PCGs, rRNAs, tRNAs, and control region, respectively. The second circle indicates the GC content. The third circle shows the GC-skew. The innermost circle shows the sequence length.

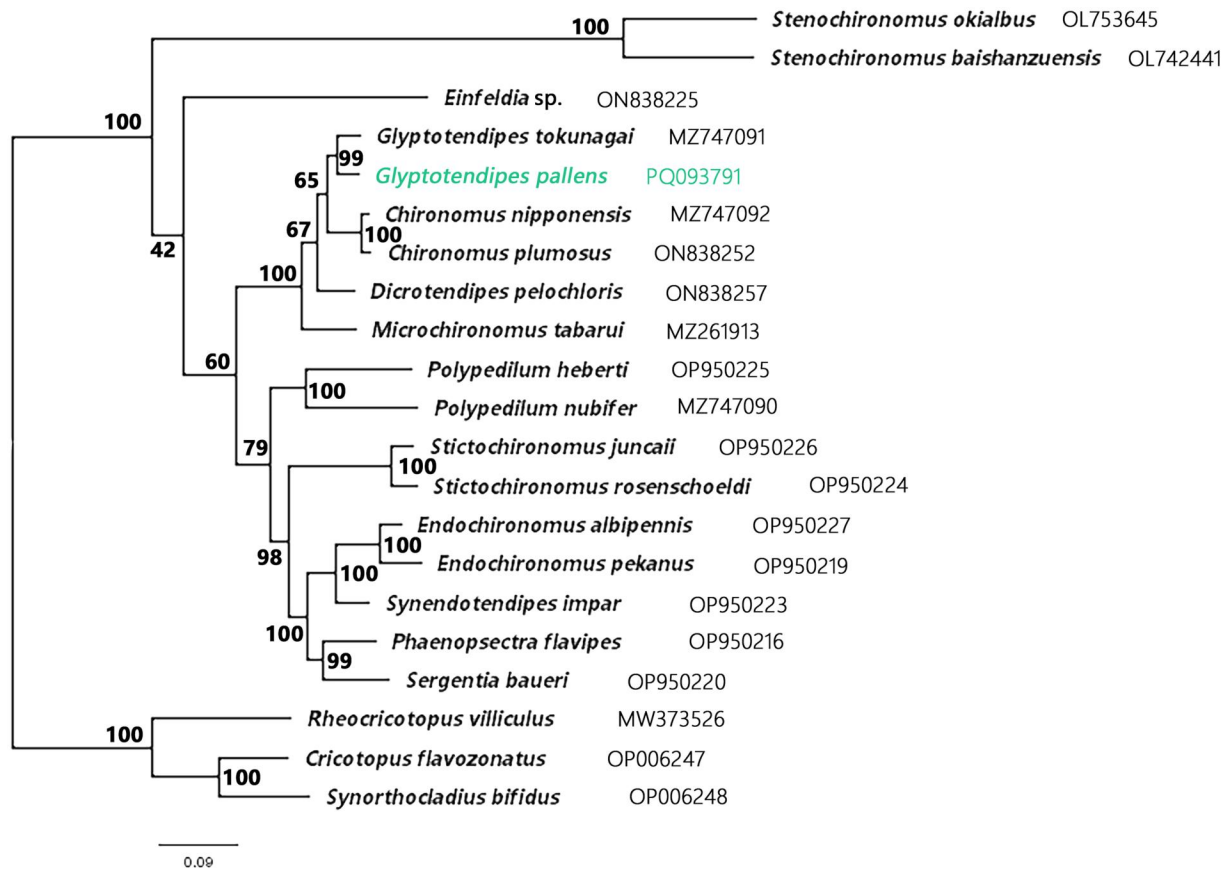


Figure 3. Phylogenetic tree of mitogenomes inferred from amino acids using maximum-likelihood (ML) method. Numbers at the nodes were ML bootstrap values.

mitogenome, and coverage depth was calculated using samtools v1.10 (Li et al. 2009). The depth file 'alin.sort.depth' was finally visualized using ggplot2 v3.5.1 in R. The read coverage depth map of *G. pallens* is shown in [Supplementary Figure S1](#).

Phylogenetic analysis

In total, we chose 18 Chironominae species as ingroup, and three Orthoclaadiinae species as outgroup (Table 1). According to previous studies of chironomid mitogenomes (Jiang et al. 2022; Lin, Zhao, et al. 2022; Li et al. 2024), we used the amino acid sequence translated from the 13 PCGs for phylogenetic analysis. MAFFT v7.526 (Kato and Standley 2013) was used to process the alignment of multiple sequences, and then the sequences were trimmed using trimAl v1.4 (Capella-Gutiérrez et al. 2009). The substitution model was selected by ModelFinder (Kalyaanamoorthy et al. 2017) in IQ-TREE2 v2.3.6 (Minh et al. 2020). The model mtZOA + F + R4 was identified as the best-fit substitution model with 1000 bootstrap replicates. The phylogenetic tree was constructed based on the amino acids of 13 PCGs by maximum-likelihood (ML) analysis using IQ-TREE2.

Results

The double-strand circular mitogenome of *Glyptotendipes pallens* spans a length of 16,063 base pairs, encompassing 13 PCGs, 22 tRNA genes, one 16S rRNA gene, one 12S rRNA

gene, and a noncoding control region. The overall base composition consists of 39.6% adenine (A), 36.9% thymine (T), 14.7% cytosine (C), and 8.8% guanine (G), indicating a high A + T content of 76.5%. The lengths of the 16S and 12S rRNA genes are 1375 bp and 813 bp, respectively. The sizes of the 22 tRNA genes range from 65 to 72 bp. The control region has a length of 641 bp. The most common start codon of the 13 PCGs is ATG in *COX2*, *COX3*, *ATP6*, *ND4*, *CYTb*, and *ND4L*, followed by ATT in *ATP8*, *ND2*, *ND3*, and *ND6*. The start codon of *COX1* and *ND1* is TTG. The start codon of *ND5* is GTG. TAA is used as termination codon of all PCGs.

The phylogenetic tree (Figure 3) clearly shows that *G. pallens* is sister to *G. tokunagai*. The genus *Glyptotendipes* is sister to *Chironomus*.

Discussion and conclusions

In this study, we successfully sequenced the complete mitogenome of *G. pallens* for the first time. Our findings indicate that the nucleotide composition and gene order of *G. pallens* mitogenome are relatively conserved. The phylogenetic analysis strongly supports *G. pallens* and *G. tokunagai* as sister species. Although the sequencing depth map (Figure S1) shows minor variations, such as a dip around 16 kb and lower depth at the start of the genome, these variations do not compromise the overall quality of the assembly. The observed dip in sequencing depth around 16 kb and the relatively lower depth at the start are likely due to technical artifacts or regions with challenging sequencing characteristics,

such as secondary structures or repetitive elements. Despite these variations, the high and consistent depth across the majority of the genome provides strong evidence for the correctness and reliability of the mitogenome assembly (Bernt et al. 2013). Overall, our study significantly expands the library of chironomid mitogenomes.

Acknowledgments

BS, ML, and TW conceived and designed the study. BS, SL, ML, and TW conducted experiment. SL, XL, and TW analyzed data. ML and TW collected sample. BS, ML, SL, TW, and XL wrote the manuscript. All the authors have approved to the published version of the manuscript.

Author contributions

CRediT: **Bingjiao Sun**: Conceptualization, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing; **Mengyu Lv**: Conceptualization, Data curation, Formal analysis, Project administration, Resources; **Tong Wu**: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing; **Shuyi Li**: Formal analysis, Writing – original draft; **Xiaolong Lin**: Formal analysis, Writing – original draft.

Ethical approval

The study did not involve humans or protect animals. In this study, samples of *Glyptotendipes pallens* can be collected without ethical approval or permission.

Disclosure statement

There are no relevant financial or non-financial competing interests to report. The consistent and high sequencing depth across the mitochondrial genome, as shown in Figure S1, confirms the accuracy and completeness of the assembly, which is reported as the complete mitogenome in the Results section.

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Data availability statement

The genome sequence data that support the findings of this study are available in GenBank at <https://www.ncbi.nlm.nih.gov/> under accession no. PQ093791. The associated BioProject, SRA, and Bio-Sample numbers were PRJNA1140420, SRR30003023, and SAMN42831410, respectively.

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