


The complete mitochondrial genome of *Chlorogomphus shanicus* Wilson, 2002 (Anisoptera: Chlorogomphidae), an endemic species in South China

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ABSTRACT

In this study, the complete mitochondrial genome of *Chlorogomphus shanicus* Wilson, 2002 was reported, and the maximum-likelihood (ML) phylogenetic tree was constructed using 13 protein-coding genes (PCGs). The total length of the mitogenome of *C. shanicus* was 15,497 bp. Twelve PCGs started with ATN codons, except *cox1* began with TTG codon. Most transfer RNA genes (tRNAs) were predicted to fold in a typical cloverleaf structure, except the *trnS1* (gct), which lacked a dihydrouridine arm that had been simplified to a loop. The phylogenetic tree showed that Anisoptera was split into two clades, and revealed that *C. shanicus* was closely related to *Cordulegaster boltonii* (Donovan, 1807) which is endemic to Europe.

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Introduction

South China is one of the distribution centers of dragonfly diversity in the world and hosts many endemic species. *Chlorogomphus shanicus* Wilson, 2002 (Wilson 2002) is an endemic species in the region. The species is recorded in Guangdong and Hunan provinces and narrowly distributed around the Nanling Mountains (preferring forests from 500 to 1000 m) (Zhang 2019). *Chlorogomphus* is a large genus of Chlorogomphidae, which was once classified into Cordulegastridae but afterward separated into Chlorogomphidae (Carle 1995). High potential of dispersal and a narrow species range make *C. shanicus* be ideal species for the study of endemism. However, the species is very limitedly studied and no genetic information is available. Here, we reported the complete mitogenome of the *C. shanicus*, and performed an exhaustive phylogenetic analysis to provide further molecular information for future research on Chlorogomphidae.

Materials and methods

The larval specimen (Figure 1) was collected from Chuanlongxia Stream of Nankunshan Provincial Nature Reserve, Guangdong Province, China (23°38'47N, 113°50'36E) on 8 July 2021, and deposited in the Institute of Hydrobiology, Jinan University, Guangzhou, China under the voucher number NKS7 (<https://hydrobio.jnu.edu.cn/1459/list.htm>, Ningning Liu, osss@jnu.edu.cn). Genomic DNA of the specimen was extracted from muscle tissues by a genomic

DNA extraction kit (Sangon, Shanghai, China), and applied to a Next-Generation Sequencing (NGS) library construction for Illumina sequencing. We got 10x genome data, a total of 15 Gb raw data for assembly. MitoZ 2.4 (Meng et al. 2019) was used for mitogenome assembly, and the MITOS web server (Bernt et al. 2013) was used to annotate the mitogenome and predict the secondary structure of transfer RNA (tRNA). Thirty-one Anisoptera species and one outgroup (Zygoptera: Calopterygidae, *Matrona basilaris* Selys, 1853) were downloaded from National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov>). Thirteen protein-coding genes (PCGs) were concentrated to construct the phylogenetic tree. Finally, a maximum-likelihood phylogenetic tree was constructed using IQ-TREE (Nguyen et al. 2015) under an Edge-linked partition model for 100,000 ultrafast (Minh et al.



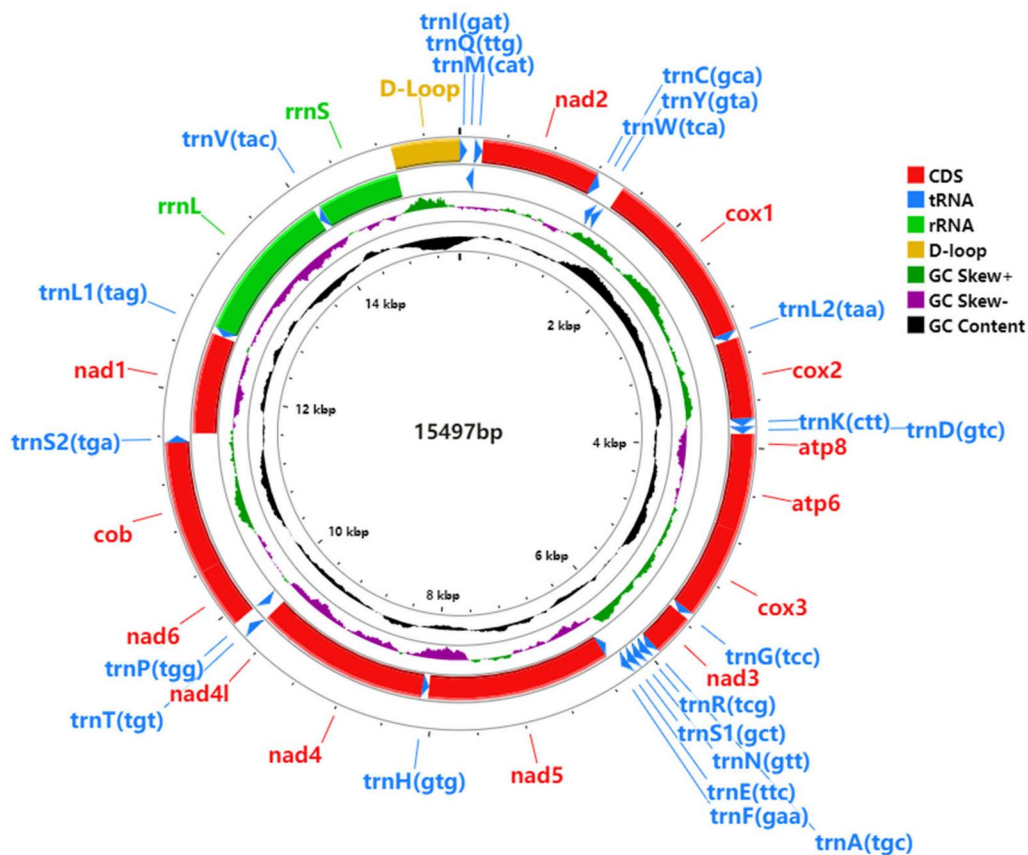
Figure 1. A larval specimen of *C. shanicus* collected at Chuanlongxia Streams of Nankunshan Provincial Nature Reserve, Guangdong Province, China. The photo was taken by Jian Liao using a Canon SLR 500D.

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Table 1. Mitochondrial genomes for *C. shanicus*, 31 Anisoptera species, and one outgroup (*M. basilaris*) presented in NCBI.

Species	Family	Accession number	Reference
<i>Orthetrum chrysis</i> (Selys, 1891)	Libellulidae	NC032048	Yong et al. (2016)
<i>Orthetrum testaceum</i> (Burmeister, 1839)	Libellulidae	NC032050	Yong et al. (2016)
<i>Orthetrum glaucum</i> (Brauer, 1865)	Libellulidae	NC032047	Yong et al. (2016)
<i>Orthetrum sabina</i> (Drury, 1773)	Libellulidae	NC032049	Yong et al. (2016)
<i>Libellula angelina</i> Selys, 1883	Libellulidae	NC050696	Unpublished
<i>Libellula quadrimaculata</i> Linnaeus, 1758	Libellulidae	NC060538	Unpublished
<i>Neurothemis fulvia</i> (Drury, 1773)	Libellulidae	NC053718	Unpublished
<i>Crocothemis</i> sp.	Libellulidae	NC042691	Guan et al. (2019) and Ożana et al. (2022)
<i>Sympetrum</i> sp.	Libellulidae	NC042732	Guan et al. (2019) and Ożana et al. (2022)
<i>Sympetrum striolatum</i> (Charpentier, 1840)	Libellulidae	MT075809	Feng et al. (2020)
<i>Acisoma panorpoides</i> Rambur, 1842	Libellulidae	MN046207	Cao et al. (2019)
<i>Acisoma panorpoides</i> Rambur, 1842	Libellulidae	NC042689	Guan et al. (2019)
<i>Nannophya pygmaea</i> Rambur, 1842	Libellulidae	NC039410	Jeong et al. (2018)
<i>Pantala flavescens</i> (Fabricius, 1798)	Libellulidae	NC056355	Unpublished
<i>Trithemis aurora</i> (Burmeister, 1839)	Libellulidae	MW789008	Unpublished
<i>Hydrobasileus croceus</i> (Brauer, 1867)	Libellulidae	NC025758	Tang et al. (2014)
<i>Brachythemis contaminata</i> (Fabricius, 1793)	Libellulidae	NC026305	Yu et al. (2014)
<i>Pseudothemis zonata</i> (Burmeister, 1839)	Libellulidae	NC053716	Unpublished
<i>Cordulia aenea</i> (Linnaeus, 1758)	Corduliidae	JX963627	Unpublished
<i>Somatochlora hineana</i> Williamson, 1931	Corduliidae	MG594801	Unpublished
<i>Macromia amphigena</i> Selys, 1871	Macromillidae	MZ504971	Unpublished
<i>Macromia manchurica</i> Asahina, 1964	Macromillidae	MZ504972	Unpublished
<i>Macromia daimoji</i> Okumura, 1949	Macromillidae	NC041425	Kim et al. (2018)
<i>Epophthalmia elegans</i> (Brauer, 1865)	Macromillidae	NC046756	Wang et al. (2019)
<i>Asiagomphus coreanus</i> (Doi & Okumura, 1937)	Gomphidae	NC061968	Park et al. (2022)
<i>Gomphus vulgatissimus</i> (Linnaeus, 1758)	Gomphidae	NC060537	Unpublished
<i>Davidius lunatus</i> (Bartenef, 1914)	Gomphidae	NC012644	Unpublished
<i>Ophiogomphus cecilia</i> (Fourcroy, 1785)	Gomphidae	MT410835	Unpublished
<i>Ictinogomphus</i> sp MT 2014	Gomphidae	KM244673	Tang et al. (2014)
<i>Anax imperator</i> Leach, 1815	Aeshnidae	NC031821	Feindt et al. (2016)
<i>Anax parthenope</i> (Selys, 1839)	Aeshnidae	NC050976	Ma et al. (2020)
<i>Cordulegaster boltonii</i> (Donovan, 1807)	Cordulegastriidae	NC063611	Unpublished
<i>Matrona basilaris</i> Selys, 1853	Calopterygidae	MK722304	Lan et al. (2019)
<i>Chlorogomphus shanicus</i> Wilson, 2002	Chlorogomphidae	OP572413	This study

**Figure 2.** Representation of *C. shanicus* mitogenome: the total length was 15,497 bp, which was divided into 37 genes, including 13 PCGs, 22 tRNAs, two rRNA, and D-loop.

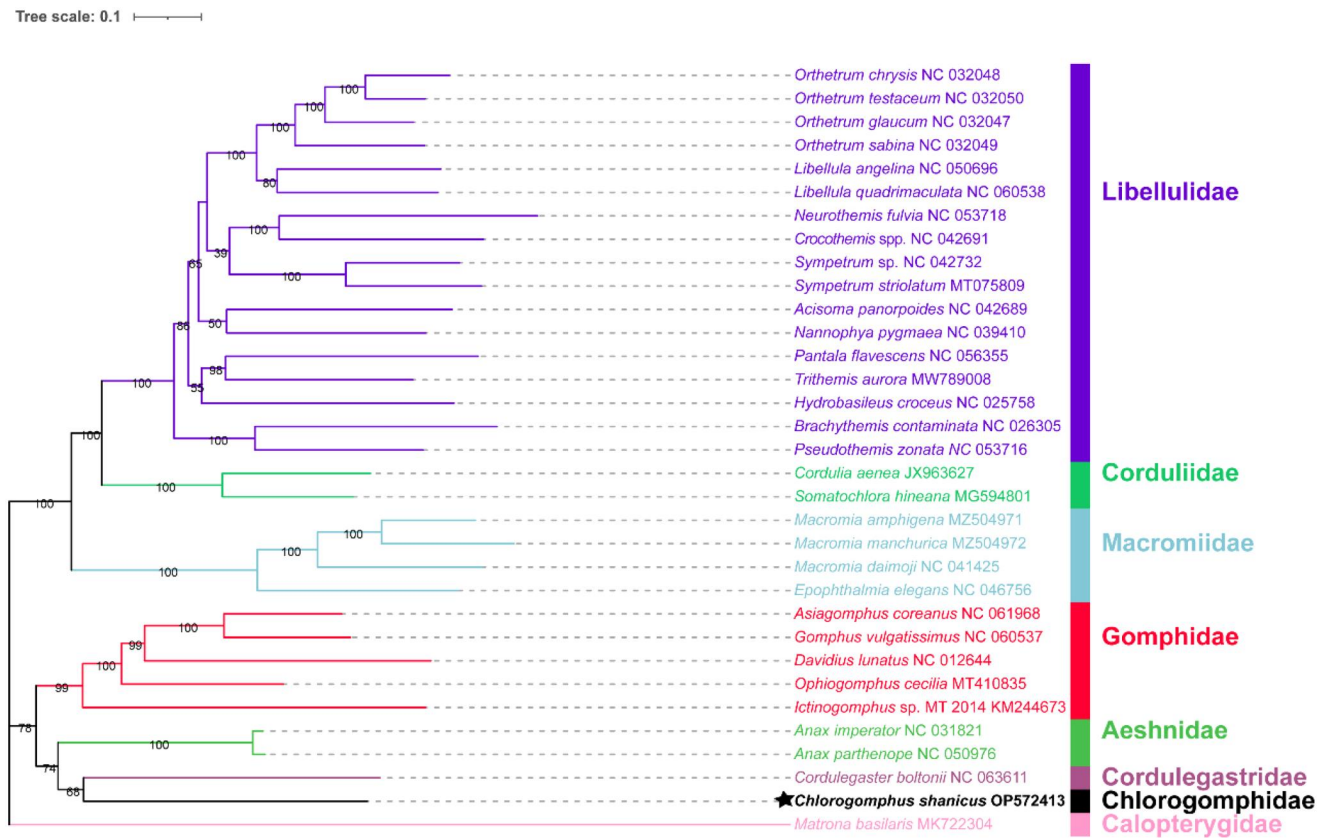


Figure 3. The ML phylogenetic tree based on 13 PCGs of *C. shanicus*, other 31 Anisoptera species, and one outgroup (*M. basilaris*). The numbers on the nodes are bootstrap values from 100,000 replicates. The species under study is bolded and highlighted by star.

2013) bootstraps, as well as the Shimodaira–Hasegawa-like approximate likelihood-ratio test (Guindon et al. 2010) by PhyloSuite v1.2.2 (Zhang et al. 2020) (Table 1).

Results and discussion

The complete mitogenome of *C. shanicus* (GenBank accession number: OP572413) contains 13 PCGs, 22 tRNA genes, two ribosomal RNA genes (rRNAs), and a control region (D-loop), with a total length of 15,497 bp (Figure 2). Except *cox1* beginning with TTG codon, 12 PCGs started with ATN codons (*nad2*, *nad3*, and *nad5* with ATT, *cox2*, *atp6*, *cox3*, *nad4L*, and *nad1* with ATG, *atp8*, and *nad6* with ATC), nine PCGs terminated with a complete codon (*atp8*, *atp6*, *nad4L*, *nad1*, *nad2*, *nad6*, and *cob* with TAA, *nad3*, and *nad4* with TAG); however, *cox1*, *cox3*, *nad5*, and *nad6* ended with an incomplete codon (*cox1*, *cox2*, and *nad5* with T, *cox3* with TA). tRNAs had lengths from 65 bp to 72 bp, and could be folded in a typical cloverleaf structure, except the *trnS1* (*gct*), which lacked a dihydrouridine arm that had been simplified to a loop. The 12S rRNA (*rrnS*) was 776 bp in length, and 16S rRNA was 1421 bp.

The phylogenetic tree (Figure 3) was consistent with morphological classification. It showed that Anisoptera was split into two clades, and *C. shanicus* was shown as a sister taxon to *Cordulegaster boltonii*, suggesting a close phylogenetic relatedness between Cordulegastridae and Chlorogomphidae. *C. boltonii* is endemic to Europe, with the only populations outside in the north of Morocco and Algeria. The two species

are endemic to two different continents but close in phylogeny, suggesting that further investigation is necessary. Phylogenetic trees revealed that all families are monophyletic groups.

Conclusions

In this study, we reported the complete mitogenome of *C. shanicus*, which was 15,497 bp in length, containing 13 PCGs, 22 tRNA, two rRNA, and one D-loop region as a typical mitogenome. The phylogenetic tree we constructed showed Anisoptera was split into two clades, and *C. shanicus* was a sister taxon to *C. boltonii*, indicating that Chlorogomphidae was more closely related to Cordulegastridae, which was consistent with morphological classification. The phylogenetic tree revealed that all families are monophyletic groups. Our works provided the phylogenetic information of Chlorogomphidae at the mitochondrial genome level for inferring the phylogenetic relationship of Anisoptera species.

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Author contributions

Haojie Wang: conceptualization, fieldwork, analysis, and writing-original draft. Lu Wang: methodology, analysis, and writing-review. Jian Liao and

Bo-Ping Han: conceptualization, supervision, and writing-review and editing. All authors approve the published version of the manuscript, and agree to be accountable for all aspects of the work.

Ethical approval

This study includes no endangered animals and does not require ethical approval to collect samples.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that supported the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/> under the accession no. OP572413. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA886846, SRR21799098, and SAMN31143302, respectively.

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