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MITOGENOME REPORT

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Complete mitochondrial genome of the *Themus foveicollis* (Coleoptera: Cantharidae)

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ABSTRACT

The genus *Themus* Motschulsky, 1858 is one of the largest genera of beetle family Cantharidae, however, no complete mitochondrial genome is available in the public database so far. Here, we sequenced and analyzed the first complete mitochondrial genome of *Themus*, with *T. foveicollis* as the representative species. The mitochondrial genome was 16,469 bp in size with 37 genes including 13 protein-coding genes (PCGs), 22 transfer *RNA* genes, and two ribosomal *RNA* genes, as well as a non-coding region, which are arranged in the same way as the hypothetical ancestral insect. It exhibited a typical high A + T bias and a higher proportion of bases A and C than T and G (A: 41.1%, T: 37.5%, G: 8.6%, C: 12.9%). The phylogenetic trees of Elateroidea were reconstructed based on 13 PCGs sequences using the Bayesian inference (BI) and maximum-likelihood (ML) analyses to investigate its phylogenetic position. The results showed that *Themus* was consistently grouped with the remaining genera of Cantharidae and placed in a monophyletic clade of Cantharinae in high supporting values, which are congruent with the morphological classification. The newly sequenced mitochondrial genome in this study constitutes important reference data for reconstructing phylogeny of the family Cantharidae or the superfamily Elateroidea.

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1. Introduction

Cantharidae, commonly known as soldier beetles, is occurring on all the world's habitable continents (Delkeskamp 1977, 1978). The cantharid beetles spend the vast majority of their individual life spans as larvae occurring in micro-habitats with relative high humidity, such as beneath leaf litter, under stones, in loose soil, or under loose bark of decaying logs (Ramsdale 2010). The larvae prey on various invertebrates, including earth worms, slugs, and other insects (Balduf 1935; Janssen 1963; Langenstück et al. 1998), so they act on an important role in the ecosystem. *Themus* Motschulsky, 1858 is one of the largest genera of Cantharidae, with over 250 species widely distributed in the Palearctic and Oriental regions (Švihla 2008). To date, research on the genus *Themus* is limited to morphological classification (Yang et al. 2012, 2013). Although it has a high species diversity, no mitochondrial genome of *Themus* is available in the public database now (National Center for Biotechnology Information [NCBI], https://www.ncbi.nih.gov, accessed on 18 February 2023). The lack of genetic information limits our investigation in the phylogenetic relationship of this genus, thereby hindering our understanding of the evolution of Cantharidae or Elateroidea. Fortunately, we successfully sequenced and annotated the complete mitochondrial genome of *Themus foveicollis* (Fairmaire 1900) recently, and it is necessary for us to report it herein.

Themus foveicollis is a member restricted to eastern China (Fujian) (Kazantsev and Brancucci 2007). The adults are frequently met on the flowers or shrubs, sometimes can be attracted by lights in the evening. They are easily recognized

Figure 1. The male habitus of *Themus foveicollis* in dorsal view. Scale bar: 2 mm. The picture was taken by Ya Kang.

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Table 1. Information on the mitogenomes of the species used in this study.

Superfamily	Family	Species	GenBank no.	References
Elateroidea	Cantharidae	*Cantharis plagiata*	MT364421	Xi et al. (2020)
		Chauliognathus opacus	FJ613418	Sheffield et al. (2009)
		Lycocerus asperipennis	MN255352	Wang et al. (2019)
		Rhagonycha fulva	MK692551	Unpublished
		Themus foveicollis	OQ621596	This study
	Cerophytidae	Cerophytidae sp.	KX035161	Unpublished
	Elateridae	*Agriotes hirayamai*	MG728108	Lin et al. (2018)
		Agrypnus binodulus	OP452714	Unpublished
		Limonius californicus	KT852377	Gerritsen et al. (2016)
		Melanotus cribricollis	MK792748	Wang and Liu (2019)
		Sinelater perroti	OM772654	Unpublished
	Eucnemidae	*Melasis buprestoides*	KX087315	Unpublished
		Microrhagus sp.	OK143440	Unpublished
	Lampyridae	*Bicellonychia lividipennis*	KJ922151	Amaral et al. (2016)
		Curtos fulvocapitalis	MW582616	Li et al. (2022)
		Cyphonocerus sanguineus	MW365445	Yuan et al. (2021)
		Diaphanes citrinus	MH651351	Yang and Fu (2019)
	Lycidae	*Dictyoptera aurora*	JX412733	Unpublished
		Lycostomus sp.	MN264644	Liu et al. (2019)
		Platerodrilus sp.	KU878647	Uribe and Gutiérrez-Rodríguez (2016)
	Phengodidae	*Brasilocerus* sp.	KJ938490	Amaral et al. (2016)
		Phrixothrix hirtus	KM923891	Amaral et al. (2016)
	Rhagophthalmidae	*Rhagophthalmus giganteus*	MK292104	Chen et al. (2019)
		Rhagophthalmus ohbai	AB267275	Li et al. (2007)
Dryopoidea	Callirhipidae	*Horatocera niponica*	KX035160	Unpublished
	Dryopidae	*Dryops luridus*	KT876888	Linard et al. (2016)
	Heteroceridae	*Heterocerus parallelus*	KX087297	Unpublished

Note: These species used in this study are the complete mitochondrial genomes.

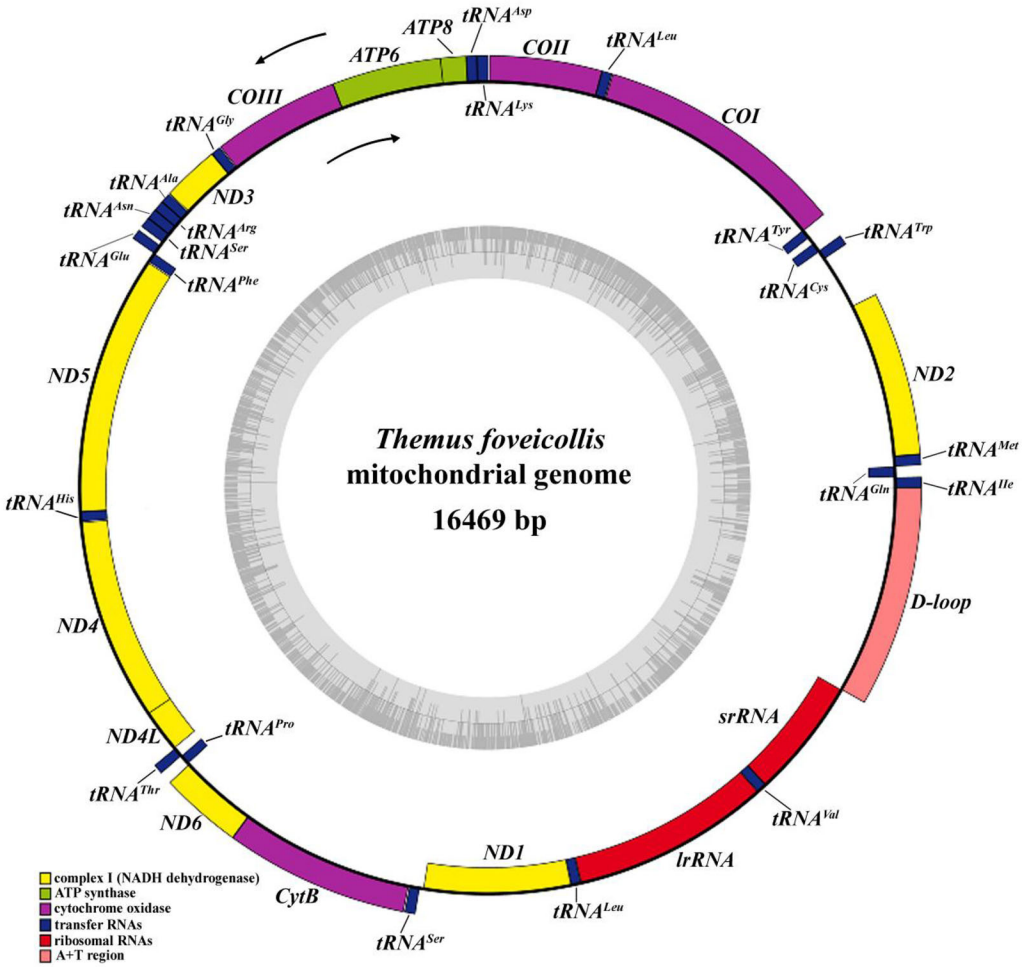

Figure 2. Mitogenome map of *Themus foveicollis* sequenced in this study. Different colors indicate different types of genes and regions. Genes outside the black circle are located on the J-strand in counterclockwise, and those inside the black circle are located at the N-strand in clockwise.

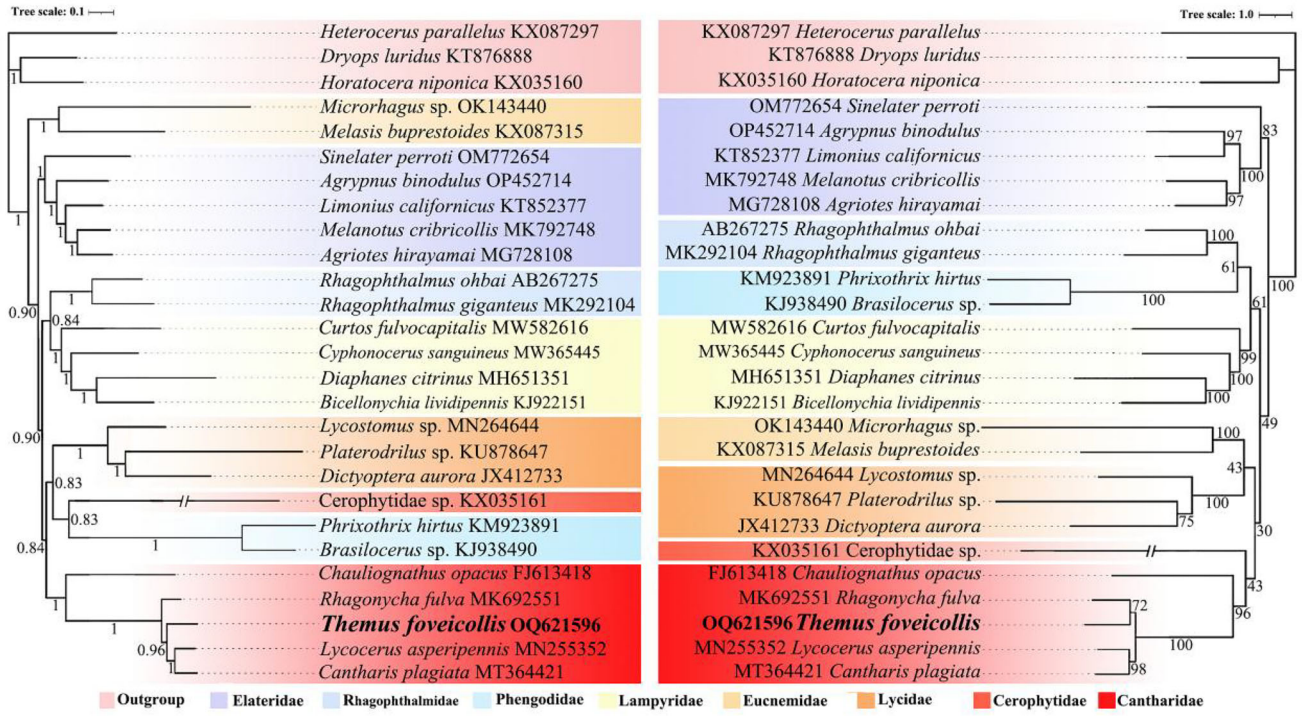

Figure 3. Phylogenetic trees of Elateroidea produced from the ML (right) and BI (left) analyses based on PCGs dataset. The numbers on the branches represent bootstrap (BS) (right) and posterior probability (PP) (left), respectively.

by the large-sized body (ca. 13 mm in length), black antennae but yellow at basal antennomeres, green and metallic shining elytra, subquadrate, and yellow pronotum, as well as yellow legs except for black tarsi (Figure 1).

In this study, the characteristics of the mitogenome of *T. foveicollis* were analyzed, and the phylogenetic trees were produced to investigate its phylogenetic position within Elateroidea. The mitogenome produced here will thus be an important reference data for reconstructing phylogeny of the family Cantharidae or the superfamily Elateroidea.

2. Materials

The material was collected in Fengyang Mountain (N:27°56′21″, E:119°12′58″; 2 May 2008) from Zhejiang Province. The sampling material was preserved in 100% ethanol at −20 °C. The specimen was deposited at the Museum of Hebei University, Baoding, China (MHBU) (http://museum.hbu.cn/, Yuxia Yang, yxyang@hbu.edu.cn) under the voucher number 2-CA138.

3. Methods

3.1. Mitochondrial genome assembly and annotation

Total DNA was extracted from the thoracic muscle using a DNeasy Blood & Tissue kit. The mitogenome was sequenced using an Illumina Novaseq 6000 platform. High-quality reads were assembled using an iterative De Bruijn graph *de novo* assembler, the IDBA-UD toolkit (Peng et al. 2012). The gene *COI* was amplified through polymerase chain reaction using universal primers (Table S1) as 'reference sequence' to target mitochondrial scaffold. The assembled mitogenome sequence was annotated using Geneious version 2019.2 (Kearse et al. 2012) with the sequence of *Lycocerus asperipennis* (Wang et al. 2019) as a reference. Protein-coding genes (PCGs) were determined by identifying open reading frames, and the secondary structure and positions of 22 tRNAs were predicted using MITOS WebServer (http://mito.bioinf.uni-leipzig.de/index.py) (Bernt et al. 2013). Genomic sequence data was registered in the NCBI database under accession no. OQ621596. Mitogenomic circular map was drawn using the Draw Organelle Genome Maps (OGDRAW) (https://chlorobox.mpimp-golm.mpg.de/ OGDraw.html) (Lohse et al. 2007). The raw reads were mapped to the mitogenomic sequence using bwa version 0.7.17 (Li and Durbin 2009) and sorted using the samtools version 1.9 (Li et al. 2009), then the depth was calculated using bedtools version 2.27.1 (Maurano et al. 2012) and the figure was plotted by the barplot function in R version 3.4.3.

3.2. Phylogenetic analysis

A total of 24 species of the Elateroidea were chosen as ingroups, and another three species of Elateriformia were selected as outgroups (Table 1). The protein-coding sequences were aligned using MAFFT version 7.313 (Katoh and Standley 2013). After removing gaps and ambiguous sites using trimAl (Capella-Gutiérrez et al. 2009), we concatenated the sequences using PhyloSuite version 1.2.2 (Zhang et al. 2020). The optimal partition schemes and substitution models under codon mode were predicted using PartitionFinder2 (Lanfear et al. 2012) with the Bayesian information criterion (Tables S2 and S3).

Phylogenies were extrapolated by Bayesian inference (BI) and maximum-likelihood (ML) methods. The ML trees were constructed with ultrafast 5000 bootstrap replicates based on

partition schemes of the genes and codons position using IQ-Tree version 1.6.8 (Nguyen et al. 2015). The BI tree was produced with four parallel runs for 10 million Markov Chain Monte Carlo (MCMC) generations based on partition schemes of codons position by MrBayes version 3.2.6 (Ronquist and Huelsenbeck 2003). Phylogenetic trees were visualized and annotated using the Interactive Tree of Life (ITOL) (https://itol.embl.de/itol.cgi) (Letunic and Bork 2021).

4. Results

The complete mitochondrial genome of the *Themus foveicollis* was 16,469 bp in size with relatively high sequencing depth (Figure S1), which was composed of 14 genes (8 tRNAs, 4 PCGs, and 2 rRNAs) transcribed on the minority strand (N-strand), while the rest (14 tRNAs and 9PCGs) on the majority strand (J-strand) (Figure 2). The composition of nucleotide bases for this mitogenome is as follows: A: 41.1%, T: 37.5%, G: 8.6%, C: 12.9%. All PCGs in mitogenome were initiated with the standard ATN codon and terminated with TAR (TAG/TAA) (Table S4).

The topologies constructed by ML and BI analyses based on the PCGs dataset were slightly different (Figures 3 and S2). But *T. foveicollis* was consistently grouped with the remaining genera of Cantharidae (represented by *Cantharis*, *Lycocerus*, *Rhagonycha*, and *Chauliognathus*), which was robust and well-supported (BS = 96/95, PP = 1). Further *Chauliognathus* (as the sole representative of Chauliognathinae) was recovered sister to the others with high supporting values (BS = 96/95, PP = 1), which all belonged to the subfamily Cantharinae. However, within Cantharinae, *Themus* was recovered sister either to all others of Cantharinae (BS = 51, PP = 0.96) or to *Rhagonycha* with relatively low support values (BS = 72).

5. Discussion and conclusion

In this study, we successfully sequenced and annotated the first complete mitochondrial genome of the cantharid genus *Themus*, with *T. foveicollis* as the representative species. The newly sequenced mitogenome contains 37 genes and a non-coding region, which are arranged in the same way as the hypothetical ancestral insect (Clary and Wolstenholme 1985), and showing a typical high A + T bias (A + T: 78.6%, G + C: 21.4%) and a higher proportion of bases A and C than T and G, as other insects (Raupach et al. 2022). The inferred phylogeny indicated that *Themus* was consistently grouped with the remaining genera of Cantharidae and placed in a monophyletic clade of Cantharinae in high supporting values, which are congruent with the morphological classification (Kazantsev and Branucci 2007). However, within Cantharinae, the phylogenetic relationship between *Themus* and others remain unclear due to inconsistent topologies by different cladistic methods, which require more sampling data to clarify this aspect in future. Nevertheless, the newly sequenced mitochondrial genome in this study will facilitate further investigations into the phylogenetic relationship of the Cantharinae or Cantharidae, even Elateroidea.

Ethical approval

No specific permits were required for the insect specimens collected for this study. The field studies did not involve endangered or protected species. The insect species sequenced is a common Cantharidae species in China and is not included in the 'List of Protected Animals in China'.

Author contributions

Conceptualization: Xiaoxiao Wang, Ya Kang, Guanglin Xie, and Yuxia Yang; methodology: Xiaoxiao Wang and Ya Kang; formal analysis: Xiaoxiao Wang and Ya Kang; investigation: Ya Kang, Yuxia Yang; writing – original draft preparation: Xiaoxiao Wang, Ya Kang, Yuxia Yang; writing – review and editing: Xiaoxiao Wang, Ya Kang, and Yuxia Yang; All authors have read and agreed to the published version of the manuscript.

Disclosure statement

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

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Data availability statement

The genome sequence data that support the finding of this study is openly available in GenBank of NCBI at https://www.ncbi.nih.gov under the accession no. OQ621596. The associated BioProject, SRA, and BioSample numbers are PRJNA941014, SRR23926838, and SAMN33775840, respectively.

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