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The complete mitochondrial genome of *Choristoneura metasequoiacola* Liu,1983 (Lepidoptera: Tortricidae)

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ABSTRACT

Choristoneura metasequoiacola Liu, 1983 is an important caterpillar species that specifically infests the leaves and branches of *Metasequoia glyptostroboides* Hu & W. C. Cheng 1948 with short larval infestations, long-term dormancy, and has a limited distribution in Lichuan, Hubei, China. The complete mitochondria genome of *C. metasequoiacola* was determined by using Illumina NovaSeq, and analyzed based on previously annotated sibling species. In total, we obtained mitochondria genome with 15,128 bp in length, circular in shape with a double-stranded closed ring structure, including 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes, and an AT-rich region. Of which the nucleotide composition was highly A + T biased, accounting for 81.98% of the whole mitogenome. Thirteen protein-coding genes (PCGs) were 11,142 bp; Twenty-two tRNA genes and AT-rich region were 1,472 and 199 bp, respectively. Phylogenetically, the relationship between *Choristoneura* spp. (containing *C. metasequoiacola*) and *Adoxophyes* spp. was closer than any other two genera from Tortricidae, and the relationship between *C. metasequoiacola* and *C.murinana* was the closest among nine sibling species from that genus, which helps to explain species evolution within the family Tortricidae.

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Choristoneura metasequoiacola Liu, 1983 (Lepidoptera: Tortricidae) is an important caterpillar species that specifically infests the leaves and tender branches of dawn redwoods, *Metasequoia glyptostroboides* Hu & W. C. Cheng 1948 in the seed tree orchard in Lichuan, Hubei, China (Wang et al. 2004). Notably, the dawn redwoods is a unique endangered relict species in China, which fossils have been found from the late Cretaceous period through the Pliocene (McNamara 2001). Obviously, these caterpillars are a potential threat to the seed trees due to their limited natural distribution, rapid infestation during larval stage, and long dormant period (Wang et al. 2003). Previous research has revealed that *C. metasequoiacola* has only one generation per year, with the larvae active from the beginning of April to the end of June, feeding on young leaves and branches, which leads to the reduction of cone maturation, and possible death of young trees (Liu 1983; Xu 1983; Xu and Liao 1985). To date, approximately sixty species of *Choristoneura* spp. were reported to infest different plant species, of which *C. metasequoiacola*, *C. diversana*, *C. luticostana*, *C. lafauriana and C. murinana* were recorded in China (Cui et al. 2012). However, except the mitochondrial genome of *C. murinana* (15,540 bp, NC_037396) and COX1 gene of *C. diversana* (1528 bp, MT711513) and *C. luticostana* (1528 bp, MT711514), little is known of mitochondrial genome of *C. metasequoiacola*. Therefore, the complete mitochondria genome of *C. metasequoiacola* was determined by using Illumina NovaSeq, and then analyzed with a previously annotated sibling species to reveal the structure and function of its mitochondrial genome and their phylogenetic relationship.

Materials and methods

The samples of *C. metasequoiacola* were collected from Lichuan, Hubei, China (30°4′N, 108°36′E), and the specimens (Figure 1) were deposited in Key Laboratory of Integrated Pest Management in Ecological Forests, Fujian Agriculture and Forestry University, Fuzhou, China (www.fafu.edu.cn, voucher no. ER-202206, in charge of the samples: Yun Liang, 1200429003@fafu.edu.cn).

Total genomic DNA of muscle tissue from legs and abdominal was extracted using EZNA Insect kit (Omega Bio-Tek, USA) following the manufacturer's instructions. After DNA extraction, 1 μg of purified DNA was fragmented to construct short-insert libraries (insert size of 430 bp) according to the manufacturer's instructions (TruSeq™ Nano DNA Sample Prep Kit, Illumina, San Diego, CA, USA). Subsequently, the

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Figure 1. Morphological traits of the adults of *Choristoneura metasequoiacola* Liu, 1983 (all pictures were photoed by Lingzhi Zheng and Hongmin Wu).

libraries were sequenced using the Illumina NovaSeq (Ilumina, San Diego, CA, USA).

The raw reads were filtered to obtain clean reads by using Trimmomatic v0.39 (Bolger et al. 2014), that included removing the adapter sequence in reads and the bases containing non-AGCT in the 5′ end before shearing, pruning the reads terminal with lower sequencing quality (the sequencing quality value was less than Q20).Then small segments (less than 75 bp) were discarded after removing 10% of reads containing N. De novo assembly by using GetOrganelle v1.6.4 (Jin et al. 2020) based on the mitochondrial genome of a closely related species that generated contigs of its mitochondrial genome, which were optimized by the scaffold results from SPAdes-3.13.0 (Bankevich et al. 2012). The assembled sequences were reordered and oriented according to the reference mitochondrial genomes, which generated the final mitochondrion genomic sequence. The mitochondrial genome length was divided by the length used for assembly to get the depth. The mitochondrion genes were annotated using the MITOS (http://mitos2.bioinf.uni-leipzig.de/index.py), then the circular mitochondrial genome map of *C. metasequoiacola* was created by using the CGview (https://cgview.ca).

A phylogenetic tree was constructed by using the Maximum Likelihood (ML) method with 1000 bootstrap replicates through MEGA11.0 (Tamura et al. 2021) based on the complete mitogenome data of *C. metasequoiacola* together with the other 13 species of Tortricidae from GenBank: MG948541, NC037394, MG932647, MG944242, MG948544, NC03942, MG948543, HQ452340, JX872403, DQ073916, MT165691, HQ392511, NC024582, and NC002355 from *Bombyx mori* was chosen as the outgroup. The genetic distance was calculated by using the K2P mode.

Results

In total, 21,517.8 MB of clean data was obtained from the 21,558.3 MB raw reads, and 2,386.87 MB from the assembled sequence. The complete mitogenome of *C. metasequoiacola* was circular in shape with 15,128bp in length. The entire depth of mitogenome was 1,577.78 with each sequencing depth ranged from 585.06 to 6,277.11. The overall base composition of mitogenome was 40.32% A, 40.66% T, 11.07% C, 7.95% G, respectively, showing obvious AT bias. The AT-skew of the whole mitochondrial genome sequence was −0.0042, with the GC-skew −0.16. The complete mitogenome comprised 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNA), 2 ribosomal RNA genes (rRNA), and an AT-rich region (Figure 2). The total length of 13 PCGs was 11,142 bp, including 1 cytochrome B gene (Cyt B), 2 ATP synthetase genes (*ATP6, ATP8*), 7 NAD oxidase subunit genes (*NAD1-6, NAD4L*), and 3 cytochrome C oxidase subunit genes (*COX1-3*). All of 13 PCGs start with a typical ATN codon (ATA for *ATP6*; ATC for *ATP8*; ATG for *CytB, ATP6, NAD1, NAD4, NAD4L, COX1, COX2, COX3*; ATT for *NAD2, NAD3, NAD5*). 10 PCGs had the common stop codon TAA (*CytB, ATP6, ATP8, NAD1, NAD2, NAD3, NAD4L, NAD6, COX1, COX3*), 3 PCGs (*NAD4, NAD5,*

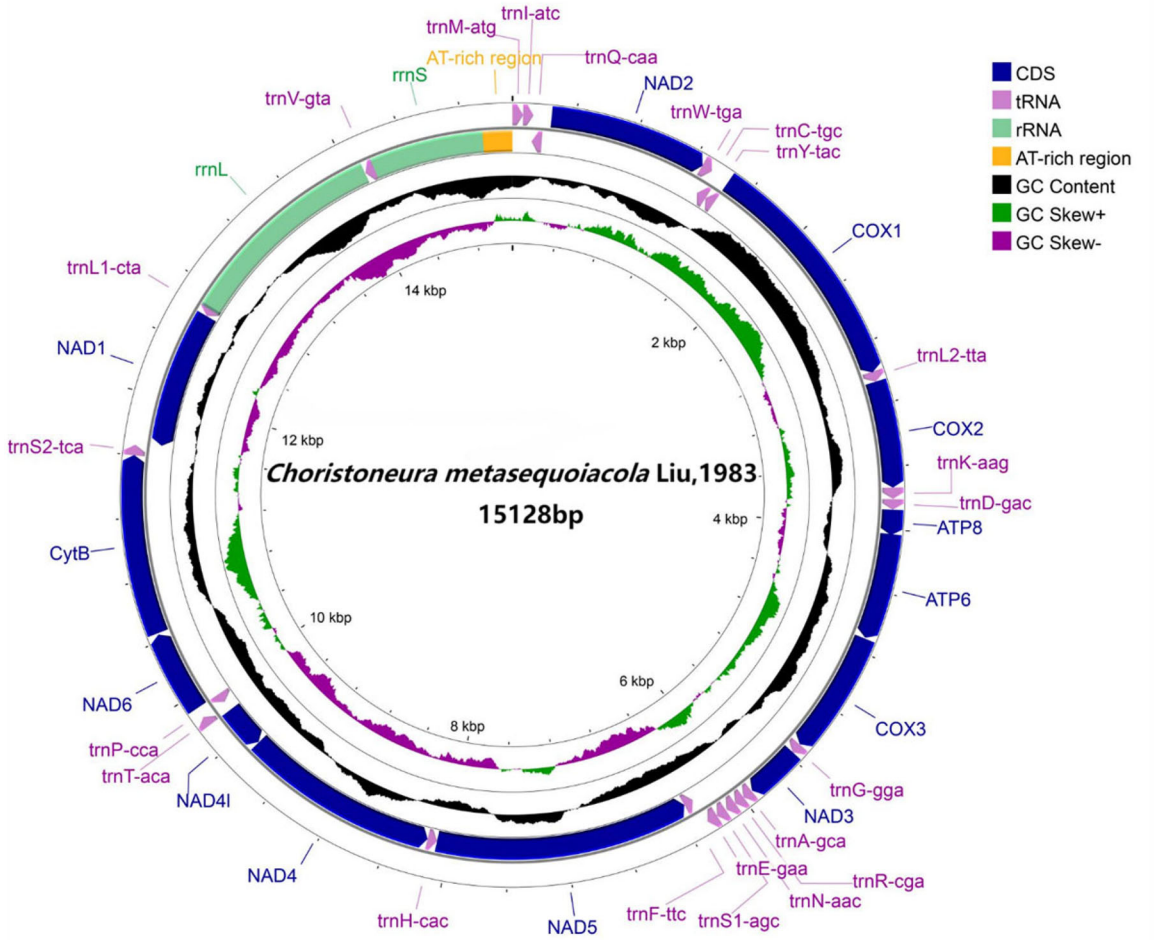

Figure 2. The mitochondrial genome structure of *Choristoneura metasequoiacola* Liu, 1983.

COX2) had an incomplete stop codons ended with T. The total length of 22 tRNA genes was 1,472bp, the 22 tRNA genes ranged from 65 to 71 nucleotides. The total length of two rRNA genes was 2,189bp, with 85.43% AT content. The rrnS, rrnL length was 765 , 1424 bp, respectively. The AT-rich region was 199 bp in length located between rrnS and trnM-atg, with highly 94.97% AT content.

The phylogenetic tree created by Maximum Likelihood showed a close relationship of the genera Choristoneura and Adoxophyes (*Choristoneura* + *Adoxophyes*) + *Grapholita*) indicating that the relationship between *Choristoneura* spp. (containing *C. metasequoiacola*) and *Adoxophyes* spp.) was closer than any other two species from Tortricidae. The relationship between *C. metasequoiacola* and the other eight sibling species was ((((((*C. occidentalis* + *C. biennis)* + *C. fumiferana)* + *C. pinus)* + *C. rosaceana)* + (*C. conflictana* + (*C. metasequoiacola* + *C. murinana*))) + *C. longicellana*), indicating that the relationship between *C. metasequoiacola* and *C. murinana* was the closest among nine sibling species from *Choristoneura* spp. (Figure 3). The genetic distance between *C. metasequoiacola* and *C. murinana* was the smallest (0.016) (Table 1) by using the K2P mode, which further indicated the closest relationship between these two species.

Discussion and conclusion

The mitochondrial genome of *C. metasequoiacola* composition and structure was determined in this paper, which was similar to some other Lepidoptera species previously reported, indicating that their mitochondrial genes of Lepidopteran insects have a certain conservation in evolution, which would enrich the mitochondrial gene database of Lepidopteran insects, and is helpful to understand the Tortricidae evolution and phylogeny of Lepidopteran (Wu et al. 2016; Peng et al. 2017; Liu et al. 2020; Liu et al. 2021; Li et al. 2021).

To date, mitochondrial genomes of 32 species from the family of Tortricidae have been identified, of which 14 species were from *Choristoneura* genus (NCBI, https://www.ncbi.nlm.nih.gov/). We have revealed that *C. metasequoiacola* was the closest sibling species to *C. murinana* by using phylogenetic tree, which was consistent with the genetic distance, these two species had very high similarity in the mitochondrial gene structure and gene length after further comparison. Consequently, we assumed that they might have evolved from the same ancestor millions of years ago. However, *C. murinana* is widely distributed in Heilongjiang, Gansu, Qinghai, Taiwan and other provinces across China (Liu

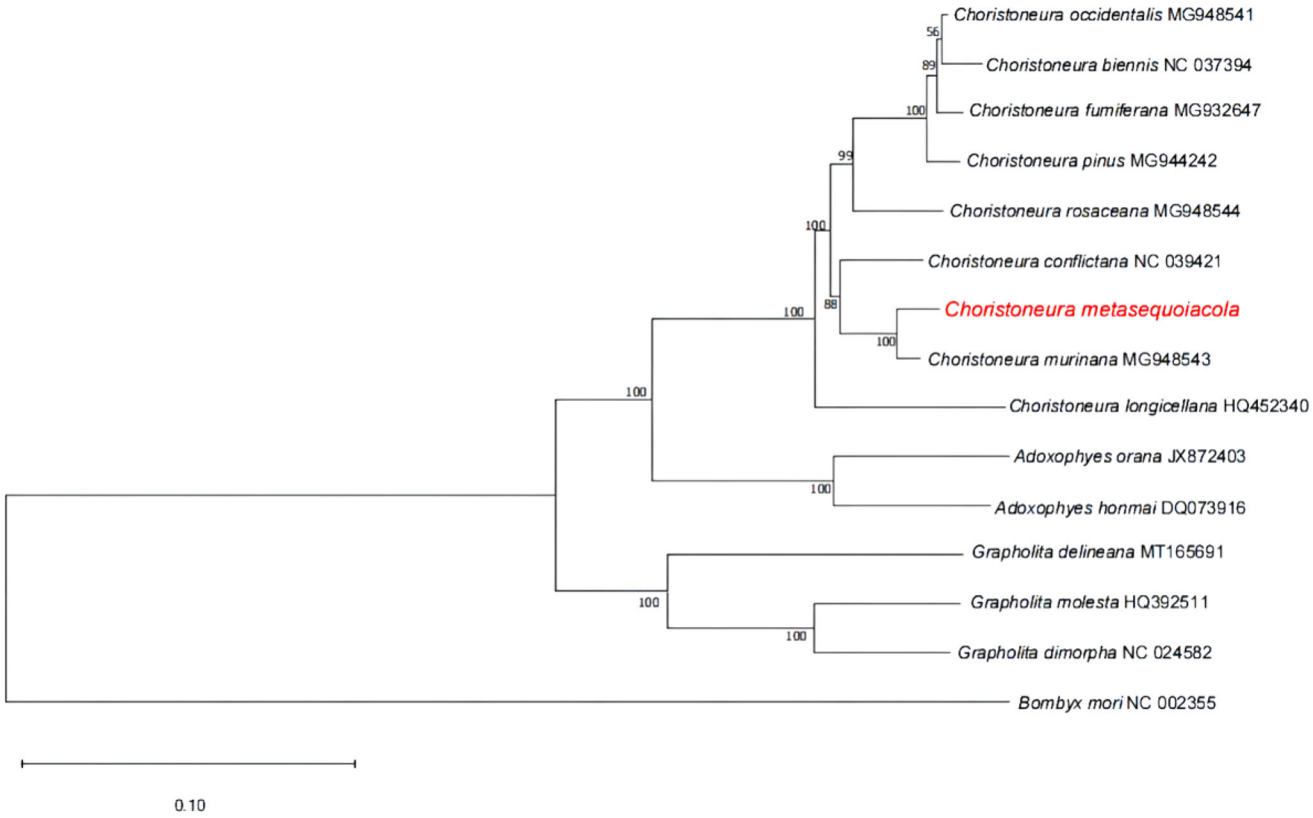

Figure 3. Maximum likelihood tree based on mitogenome of 14 Tortricidae by using MEGA 11.0 (bootstrap values based on 1000 replicates).

Table 1. The genetic distance among *Choristoneura* spp.

	C. longicellana	*C. metasequoiacola*	*C. fumiferana*	*C. pinus*	*C. occidentalis*	*C. murinana*	*C. rosaceana*	*C. biennis*	*C. conflictana*	*B. mori*
C. longicellana										
C. metasequoiacola	0.055									
C. fumiferana	0.066	0.049								
C. pinus	0.065	0.049	0.018							
C. occidentalis	0.060	0.043	0.008	0.013						
C. murinana	0.057	0.016	0.042	0.044	0.036					
C. rosaceana	0.060	0.043	0.042	0.042	0.036	0.039				
C. biennis	0.067	0.050	0.019	0.020	0.011	0.046	0.044			
C. conflictana	0.059	0.039	0.043	0.044	0.037	0.035	0.040	0.046		
B. mori	0.175	0.166	0.174	0.173	0.168	0.168	0.168	0.173	0.169	

2002), while the *C. metasequoia*'s natural habitat is limited to Lichuan, Hubei province, China (Liu 1983; Xu and Liao 1985). Obviously, there is distinctly geographical isolation between them. *C.murinana* mainly infests *Abies fabri* (Mast.) Craib forests (Liu 2002), while *C. metasequoiacola* specifically feed on leaves of dawn redwoods (Xu 1983; Xu and Liao 1985), and their feeding habits to hosts were gradually specialized. Interestingly, the male genitalia of *C. metasequoiacola* was claw-shaped and club-like, while the male genitalia of *C. murinana* was claw-shaped and elongated (Liu 2002), indicating they have evolved significantly different reproductive structure. Therefore, these results can help us infer that these two species may be derived from a common ancestor, but the differentiation of reproductive structure and the variation at the gene level were caused by the long-term geographical isolation and host specialization. On the contrary, these traits may be jointly regulated by gene expression at the molecular level that have now evolved into very separate directions.

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Ethical approval

All samples of *Choristoneura metasequoiacola* Liu, 1983 were serious defoliator pest species which collected from the infested leaves and branches of dawn redwoods, *Metasequoia glyptostroboides* Hu & W. C. Cheng (1948) in the natural forest. No ethical permissions were required since *C. metasequoiacola* is a native forest pest species.

Author contributions

Conception and Design: Yun Liang, Xunru Ai, Guanghong Liang. Sample collection: Yun Liang, Zewei He, Zhongwu Xiong and Jianfeng Hong. The experimental operation:Yun Liang, Xingyuan Fang, Guanghong Liang. Data processing and analysis: Yun Liang, Lingzhi Zheng, Hongmin Wu.

The original manuscript writing, review and editing: Yun Liang, Guanghong Liang. Funding acquisition: Guanghong Liang. All authors read the final manuscript and agreed to be accountable for their own aspect(s).

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 findings of this study are publically available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under accession numbers OP747297. The associated BioProject, BioSample and SRA, numbers are PRJNA897732, SAMN31580727, and SRR22242676, respectively.

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