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Research paper

# The complete mitochondrial genome of *Thitarodes sejilaensis* (Lepidoptera: Hepialidae), a host insect of *Ophiocordyceps sinensis* and its implication in taxonomic revision of *Hepialus* adopted in China

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ABSTRACT

The mitochondrial genome is widely used for phylogenetic analyses and evolutionary biology. The complete mitochondrial genome of *Thitarodes sejilaensis* (Lepidoptera: Hepialidae) was sequenced and analyzed in this study. This mitogenome is a typical circular molecule of 15,290 bp, with the gene content, orientation and order identical to other insects in the family Hepialidae. The genome nucleotide composition is heavily biased towards As and Ts, accounting for 80.87% of total nucleotide content. The major strand shows a positive AT-skew and negative GC-skew. All 13 protein-coding genes (PCG) are initiated by the canonical putative start codons ATN, except for COI and ND1 that use the initiation codons CGA and TTG, respectively. Nine PCGs share the complete termination codons TAA, while the remaining PCGs use an incomplete termination codon T. Additionally, the codon distribution and Relative Synonymous Codon Usage of all PCGs in the *T. sejilaensis* mitogenome are consistent with other Hepialidae mitogenomes. Among 22 transfer RNAs, 21 have the typical clover-leaf structure, while *tRNASer*(AGN) does not possess the dihydrouridine (DHU) arm and could not form a stable stem-loop structure. The secondary structures of 2 ribosomal RNA genes broadly conform to the proposed models of these genes documented in other lepidopteran insects. *T. sejilaensis* AT-rich region exhibits three repetitive sequences of 118 bp. Other regions contain 22-bp overlapping nucleotides and 72-bp intergenic nucleotides. The phylogenetic relationships were constructed by two datasets, the amino acid sequence derived from protein-coding genes and the nucleotide sequence of 13 PCGs and 2 rRNAs. Using Maximum Likelihood (ML), we reconstructed a phylogenetic tree which supported a more primitive taxa of Hepialoidea within Lepidoptera. Moreover, according to comparisons based on the CytB sequences and morphological characteristic, *Hepialus* species reported in China should be revised. Our taxonomic recommendations include assigning these species to the following genera: *Thitarodes*, *Ahamus*, *Hepialus* and *Parahepialus*.



## 1. Introduction

Mitochondrial genomes (mitogenomes) have been widely studied in insects. They are generally 14–20 kb and have a unique translation system of 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes used for synthesis of the 13 inclusive protein-coding genes (PCGs), and an AT-rich control region of highly variable length that are involved in the initiation of transcription and replication of the genome (Simon et al., 1994; Simon et al., 2006). Mitogenomes are used across many disciplines including animal health, comparative and evolutionary genomics, molecular evolution, phylogenetics and population genetics (Salvato et al., 2008).

Traditional taxonomy classifies organisms according to their morphological characteristics and physiological behavior. Because morphological plasticity and convergent evolution can influence traditional taxonomy, molecular taxonomy that complements traditional taxonomy has practical significance. Because of the special characteristics of small size, lack of intermolecular recombination, maternal inheritance and relatively rapid frequency of evolution, the mitogenome has been broadly used for phylogenetic reconstruction and rapid species identification (Chai et al., 2012; Hebert et al., 2003). Analysis of family-level evolutionary relationships within Noctuoidea based on nucleotide sequences of 13 PCGs, have been consistent with traditional relationships based on morphological data (Dong et al., 2016). Based on CO1 gene alone, the *Astraptes fulgerator* complex was reclassified into ten distinct species (Hebert et al., 2004) and *Philaenus spumarius* was accurately classificated by DNA barcoding (Seabra et al., 2010). However, the selection of marker genes, the definition of species and the potential for interference by other genes limit the utilization of DNA barcoding by

*Abbreviations:* PCG, protein-coding genes; DHU, dihydrouridine; ML, Maximum Likelihood; rRNA, ribosomal RNA; tRNA, transfer RNA.

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**Table 1**
Regions and primers in present paper.

| Fragment | (Region) | Primer (F/R) | Primer sequence (F/R) 5′ → 3′ |
|---|---|---|---|
| F1 | nad2-trnM | J-185/N-963 | GCTTTTGGGCTCATACTTC/GGTAAGCCACCTAATGATAGTAA |
| F2 | nad2-cox1 | J-728/N-1852 | GGGATTAAACCAAACCTCAT/CTACAGATGCTCCTGAATGG |
| F3 | cox1 | J-1464/N-2149 | GGTCAACAAATCATAAAGATATTG/TAAACTTCAGGGTGACCAAAAAAT |
| F4 | cox1-cox3 | J-2027/N-5308 | TTATTATCATTACCCGTACTGGC/CGTGAAGACCGTGGAAACC |
| F5 | cox3-nad3 | J-4483/N-5674 | TCGACCTGGAACTTTAGC/TGGATCAAATCCACATTCA |
| F6 | cox3-nad5 | J-5309/N-7406 | GTTTCCACGGTCTTCACG/GCTCCTACTCCTGTTTCTGC |
| F7 | nad5-nad4 | J-7383/N-8823 | CTAAAGTTGATGAATGAACTAAAG/GCTCATGTTGAAGCTCC |
| F8 | nad5-cob | J-7695/N-10943 | AACCTAAACCATCCCAACCT/GCATAAAATCGTGTTAGTGTAGC |
| F9 | cob | J-10806/N-11276 | TATGTACTACCATGAGGACAAATATC/ATTACACCTCCTAATTTATTAGGAAT |
| F10 | cob-rrnL | J-10932/N-13806 | CAGTAGATAATGCTACACTAACACGAT/TATTGTATCTTGTGTATCAGAGTTTA |
| F11 | rrnL-rrnS | J-13228/N-14495 | CCTTTGCACAGTCAAAATAC/AAACTAGGATTAGATACCC |
| F12 | rrnS-nad2 | J-14219/N-412 | TATTGTATAACCGCAACTGCTG/TAAGTTTGAGGAAGTTAATGCTTG |

itself (Lorenzo, 2005; Goetze, 2011; Smith et al., 2012). Further sequence analysis and morphological classification are usually required.

Larvae of *Thitarodes* in South East Asia are the host for the fungus *Ophiocordyceps sinensis* (Berkeley) Saccardo (Dong Chong Xia Cao in Chinese) (Winkler, 2009), which is one of the most valuable resources for traditional Chinese medicine (Zhu et al., 1998; Buenz et al., 2005; Yi et al., 2016). In 1968, the genus *Thitarodes* was established from the original *Hepialus* based on the presence of an acute process from the base of the valve on male genitalia (Viette, 1968). However, the genus *Thitarodes* was not adopted by Chinese scientists until 2010 (Chu and Wang, 1985; Chu et al., 2004), so all the new species of *Hepialus* reported in China were still assigned to the genus *Hepialus*. In 1996, Ueda pointed out that the morphological difference in male genitalia described by Chu and Wang (1985) was insignificant as the key character for only one genus *Hepialus*, and more genera from the current *Hepialus* in China would be established according to this difference (Ueda, 1996). As a result many species of "*Hepialus*" described by Chinese scientists were transferred to *Thitarodes* (Nielsen et al., 2000). Based on morphological characteristic of the valve in male genitalia and the fragment of the CytB gene, Zou et al. (2010) suggested dividing Chinese *Hepialus* into four genera, *Parahepialus*, *Ahamus*, *Hepialus* and *Thitarodes*. Chinese researches have widely adopted this taxonomic system (Cao et al., 2012).

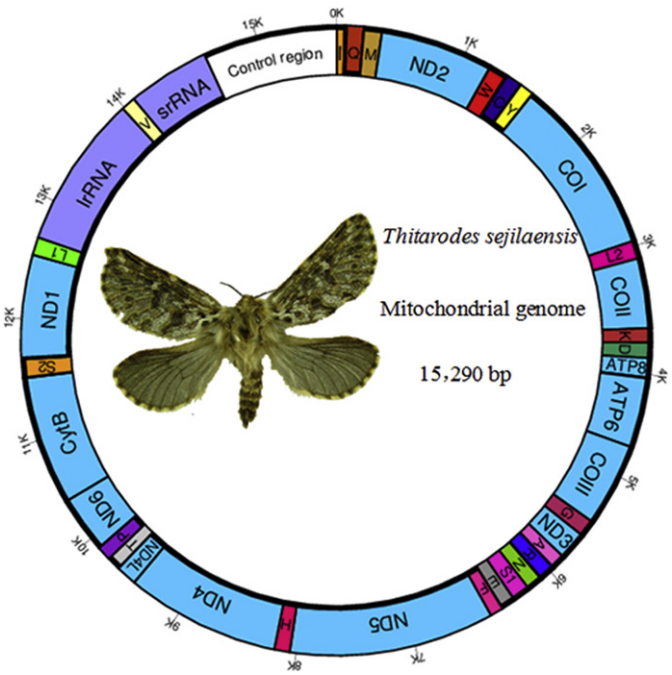


**Fig. 1.** Circular map of the mitochondrial genome of *Thitarodes sejilaensis*.

*T. sejilaensis* is a recently described species found between the altitudes of 3600 to 4800 m in the Nyainqentanglha Range and Heng Duan Mountains of the Tibetan Plateau (Zou et al., 2011). The complete mitogenome of *T. sejilaensis* was sequenced and analyzed in this study. Furthermore, its mitogenome was compared with six other Hepialidae species. Results should help inform phylogenetic hypotheses about the position of Hepialidae within the Lepidoptera and provide additional support for our revision of the *Hepialus* species reported in China.

## 2. Materials and methods

### 2.1. Sample and DNA extraction

*T. sejilaensis* adults were collected on Mt. Sejila (29° 37′N, 94° 37′E), Tibetan Plateau, China, in August, 2011. Samples were preserved in 100% ethanol and stored at −40 °C until DNA extraction. Whole genomic DNA was extracted from a single sample using the protocol of Organization Genomic DNA Extraction Kit (GENEray, Shanghai, China). The quality of PCR products was assessed through electrophoresis in a 1% agarose gel and stained with Gold View.

### 2.2. PCR amplification and sequencing

In order to get the whole genome, the initial PCR primers were designed using Primer Premier 5.0 software based on universal primers of insect mitochondrial DNA (Simon et al., 1994) and six other Hepialidae mitochondrial DNA sequences (*Ahamusyunnanensis*, NC_018095.1; *Hepialus xiaojinensis*, NC_028348.1; *Napialus hunanensis*, NC_024424.1; *Thitarodes gonggaensis*, NC_026903.1; *Thitarodes pui*, NC_023530.1; *Thitarodes renzhiensis*; NC_018094.1) that are available in GenBank. Then, based on the known sequences, six specifically designed primer pairs as listed in Table 1, were used for the remaining PCRs. PCR reaction was performed in a 25 μl volume with 0.25 μl of LA Taq (TaKaRa Co., Dalian, China), 1 μl (about 50 ng) of DNA, 2.5 μl 10× LA Taq buffer ($Mg^{2+}$ plus), 4 μl dNTPs and 10 pmol each primer. PCR amplification was performed under the following procedure: primary denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 45 s at 45–55 °C, elongation for 1–3 min (depending on putative length of the fragments) at 68 °C, and a final extension step of 72 °C for 10 min. The PCR products were resolved by electrophoresis in 1% agarose gel. After purification with TIANgel Midi Purification Kit (TIANGEN, Beijing, China), the PCR fragments were sent to Shanghai Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China) for sequencing.

### 2.3. Sequence analysis

The complete mitogenome of *T. sejilaensis* was obtained by the assembly of twelve overlapping sequences via the alignment of neighboring fragments using SeqMan in the DNASTAR 7.1. All 37 mitogenome sequences were annotated by Mitos Web Server (http://mitos.bioinf.