

Complete mitogenome of *Antheraea formosana* Sonan, 1937 (Lepidoptera: Saturniidae): an endemic silkmoth in Taiwan

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ABSTRACT

The complete mitogenome of an endemic silkmoth in Taiwan, *Antheraea formosana*, was determined using Illumina next-generation sequencing. The mitogenome is 15,318 bp in length and consists of 13 protein-coding genes (PCGs), two rRNAs, 22 tRNAs, and one non-coding control region. The overall base composition of the mitogenome showed a high A + T bias, and the A + T content (80.2%) was significantly higher than the G + C content (19.8%). All PCGs use the typical ATN as the initiation codon, with the exception of *cox2*, which begins with GTG, respectively. The complete mitogenome was used to reconstruct a phylogenetic tree, indicating that *A. formosana* is more closely related to *Antheraea assamensis* than other *Antheraea* species, with 93.19% nucleotide similarity.

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The tasar silkmoth (*Antheraea* Hübner, 1819) belongs to the Lepidoptera family Saturniidae and is of considerable economic importance worldwide (Kitching et al. 2018). *Antheraea* is the largest genus used for silk production and contains more than 35 described species that are widely distributed throughout Asia (Liu et al. 2008). Currently, the utilization of silkmoths, including *Antheraea pernyi* and *Antheraea assamensis*, for tussah production is greatly prevalent in China, India, and Korea (Peigler 1993; Liu et al. 2010; Li et al. 2017). *Antheraea formosana* Sonan, 1937, a silk-producing Lepidoptera and an endemic species in Taiwan, is a medium to large-sized silkmoth with a wing span of 110–160 mm, which is distributed in low- and middle-altitude mountainous areas (Chowdhury 2004; Wu et al. 2020). Because *A. formosana* behaves as a multivoltine (more than two generations per year), it is a potential alternative candidate for future tussah production in Taiwan. However, there have been no genomic studies on *A. formosana*. In the present study, we report the whole mitogenome sequence of *A. formosana* and reconstruct its phylogeny with other *Antheraea* species.

One *A. formosana* was collected from Taoyuan District, Kaohsiung (23°09' N, 120°45' E) in Taiwan. The collection location in this study is not a privately-owned or protected area, and it is not an endangered or protected species in Taiwan. No permits were required for this study. About an abdominal half of a moth was used to extract total genomic DNA by the use of proteinase-K and phenol-chloroform

method (Henry et al. 1990). The DNA sample was preserved at the Graduate Institute of Bioresources, National Pingtung University of Science and Technology (Kuo-Hsiang Hung, khung424@npust.edu.tw), under the voucher number 2021-AF1. The genomic DNA was used for Illumina library preparation, and subsequently, paired-end reads were sequenced using the NovaSeq 6000 platform (Illumina, San Diego, CA, USA). The raw sequences went through a filtering process to obtain the qualified reads by FASTP v.0.20 (Chen et al. 2018), and FLASH v.1.2 was used to merge paired-end reads (Magoč and Salzberg 2011). The complete circular mitogenome of *A. formosana* was assembled de novo using MitoFinder v.1.3 (Allio et al. 2020) from a randomly sampled subset of total genomic reads (23,690,470 reads). The assembled mitogenome was annotated using the MITOS2 web server to predict the location of protein-coding regions/genes, tRNAs, and rRNAs (<http://mitos2.bioinf.uni-leipzig.de/index.py>) (Donath et al. 2019). The sequence with annotated features was deposited in GenBank (Accession Number OK078922).

We also inferred phylogenetic relationships based on multiple sequence alignments of the other four *Antheraea* species mitogenomes, and *Bombyx mori* was used as an outgroup. The MAFFT online server (Katoh et al. 2019) was used to align the mitochondrial sequences, and a maximum-likelihood tree was constructed based on full mitogenome sequences using MEGA X with 1000 bootstrap replicates (Kumar et al. 2018).

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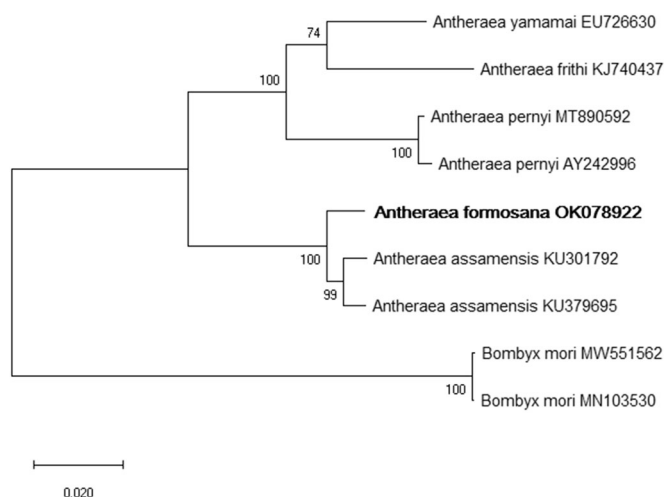


Figure 1. The maximum likelihood (ML) phylogenetic tree indicates the relationship between *Antheraea formosana* and four other *Antheraea* species. *Bombyx mori* was used as an outgroup. GenBank accession numbers of each species are listed in the tree. The numbers on the branch lengths are bootstrap values.

The mitogenome of *A. formosana* was 15,318 bp long. It contains 37 genes, comprising 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes. The frequencies of adenine, cytosine, guanine, and thymine were 39.3, 12.0, 7.8, and 40.9%, respectively. Therefore, the A+T and G+C contents were 80.2 and 19.8%, respectively. This high A+T bias is similar to that present in other lepidopterans. Twelve PCGs started with a typical ATN codon: four (*nad2*, *cox1*, *atp8*, *nad5*) with ATT, three (*nad3*, *nad6*, *cob*) with ATA, and five (*cox3*, *atp6*, *nad4*, *nad4L*, *nad1*) with ATG. However, *cox2* is associated with GTG. This pattern is similar to that of other *Antheraea* species, except for *cox1*, in which ATT was the start codon. The *cox1* was reported to have CGA as start codons in *Antheraea* species (Singh et al. 2017).

The phylogenetic trees indicated that the *Antheraea* species were separated into two distinct monophyletic clades. *Antheraea formosana* is closely related to *A. assamensis*, with 93.19% nucleotide similarity within the same clade, and other *Antheraea* species clustered in another clade (Figure 1). In conclusion, this research provides useful information for further phylogenetic and evolutionary analyses, as well as enriches the mitogenome database of *Antheraea* species.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Author contributions

An-Ping Cheng, Chi-Chun Huang, Chih-Chiang Wang, I-Ling Lai, and Kuo-Hsiang Hung were involved in the conception and design. An-Ping Cheng and Yu-Tzu Cheng were involved in the collection of materials. Chi-Chun Huang, Yu-Tzu Cheng, Yu-Wei Tseng, Chih-Chiang Wang, and I-Ling Lai were involved in the analysis and interpretation of the data. An-Ping Cheng, Chi-Chun Huang, and Kuo-Hsiang Hung were involved in the drafting of the paper. All authors agreed to be accountable for all aspects of this work.

Data availability statement

The genome sequence data are available in GenBank (<https://www.ncbi.nlm.nih.gov/>) under accession no. OK078922. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA766508, SRR16093533, and SAMN21849811, respectively.

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