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The complete mitochondrial genome of *Attacus atlas formosanus* Villiard, 1969 (Lepidoptera: Saturniidae)

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ABSTRACT

The complete mitochondrial genome (mitogenome) of *Attacus atlas formosanus* (Villiard, 1969) is 15,280 bp in length, with the typical gene content and arrangement usually observed in Insecta. It contains 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and one AT-rich region. The overall nucleotide composition of the mitogenome was 39.8% A, 12.9% C, 7.7% G, and 39.6% T, with an A + T bias of 79.4%. Phylogenetic analyses of 23 species in Saturniidae and 3 species in Bombycidae by Bayesian inference showed that *A. atlas formosanus* belonged to the Tribe *Attacini*, closely related to Tribe *Saturniini*. Besides, *A. atlas formosanus* is closely related to *A. atlas* with 99% sequence identity. This result well supported the taxonomic position of Saturniidae and their close relationship with the family Bombycidae.

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The Atlas moth, *Attacus atlas* (Linnaeus, 1758) (Lepidoptera: Saturniidae), is one of the largest lepidopterans with a wingspan measuring up to 24 cm and a wing surface area of about 160 cm² (Holloway 1987). Moreover, the silks which were produced by *A. atlas* larvae, were alternatively severed for commercial silk production (Reddy et al. 2013). The *Attacus atlas* was first described by Carl Linnaeus under genus *Phalaena* in 1758 and the genus *Attacus* was then established by Linnaeus in 1767. Under the genus *Attacus*, 12 species were listed as valid (Peigler 1983). The population of *A. atlas* (Linnaeus, 1758) was distributed in tropical and subtropical forests located in Java (six subspecies), India (five subspecies), Sumatra (four subspecies), southern China (three subspecies), Sri Lanka (one subspecies), Malaya (one subspecies), Andamans (one subspecies), Simalur (one subspecies), Borneo (one subspecies), Sulawesi (one subspecies), Bali (one subspecies), and four subspecies in Myanmar, Thailand, Laos, and Vietnam (Holloway 1987; Peigler 1983; Roepke 1953). *A. atlas formosanus* (Villiard, 1969) is the largest moth and the endemic subspecies of Taiwan. However, the complete mitochondrial genome (mitogenome) of *A. atlas formosanus* is still being unraveled. Total genomic DNA was extracted from a mixture of nucleopolyhedrovirus (NPV) infected larvae and conducted to DNA sequencing by following the previously published methods (Huang et al. 2019). Reference-assisted *de novo* assembly approach was utilized by using MIRA assembler (Chevreux et al. 2004) with the mitogenome of *A. atlas* isolate SYAU01 (GenBank: NC_021770.1) as the reference genome. Circularity inference was also confirmed as mentioned

in previous research (Huang et al. 2015; Jacob Machado et al. 2018) to obtain the complete circular mitogenome. The complete mitogenome of *A. atlas formosanus* is 15,280 bp in size (GenBank: MZ098706). It consisted of 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and one AT-rich region. The overall nucleotide composition of the mitogenome was 39.8% A, 12.9% C, 7.7% G, and 39.6% T, with an A + T bias of 79.4%. Phylogenetic analyses for 23 species in Saturniidae were performed by MrBayes 3.2 (Ronquist et al. 2012), following the analysis procedure in our previous study (Huang et al. 2015). *Papilio maraho*, *Thitarodes renzhiensis* and 3 species in Bombycidae were used as outgroups. The phylogenetic tree showed the relative position that the *A. atlas formosanus* of insular origin is closely related to *A. atlas* of continental origin, and belongs to the Tribe *Attacini* (Figure 1(A)). When these two atlas moths from different geographic origins were compared, it was found that they shared 99% sequence identity with a higher A + T bias (Figure 1(B)). The mitochondrial gene COX1 was used as DNA barcode target for species identification (Chen et al. 2012; Jin et al. 2018; Li et al. 2011; Xu et al. 2019). The start codons for COX1 gene of the lepidopteran insects are not uniform (Hao et al. 2012), which has been extensively discussed and has long remained a matter of some controversy (Kim et al. 2009). *A. atlas formosanus* uses TTG as the start codon while *A. atlas* uses CGA (Chen et al. 2014; Lee et al. 2021; McCullagh et al. 2020). Moreover, a 4-bp TTAG putative initiator of COX1 was observed (Kim et al. 2006; Yukuhiro et al. 2002) but not identified in *A. atlas*. Furthermore,

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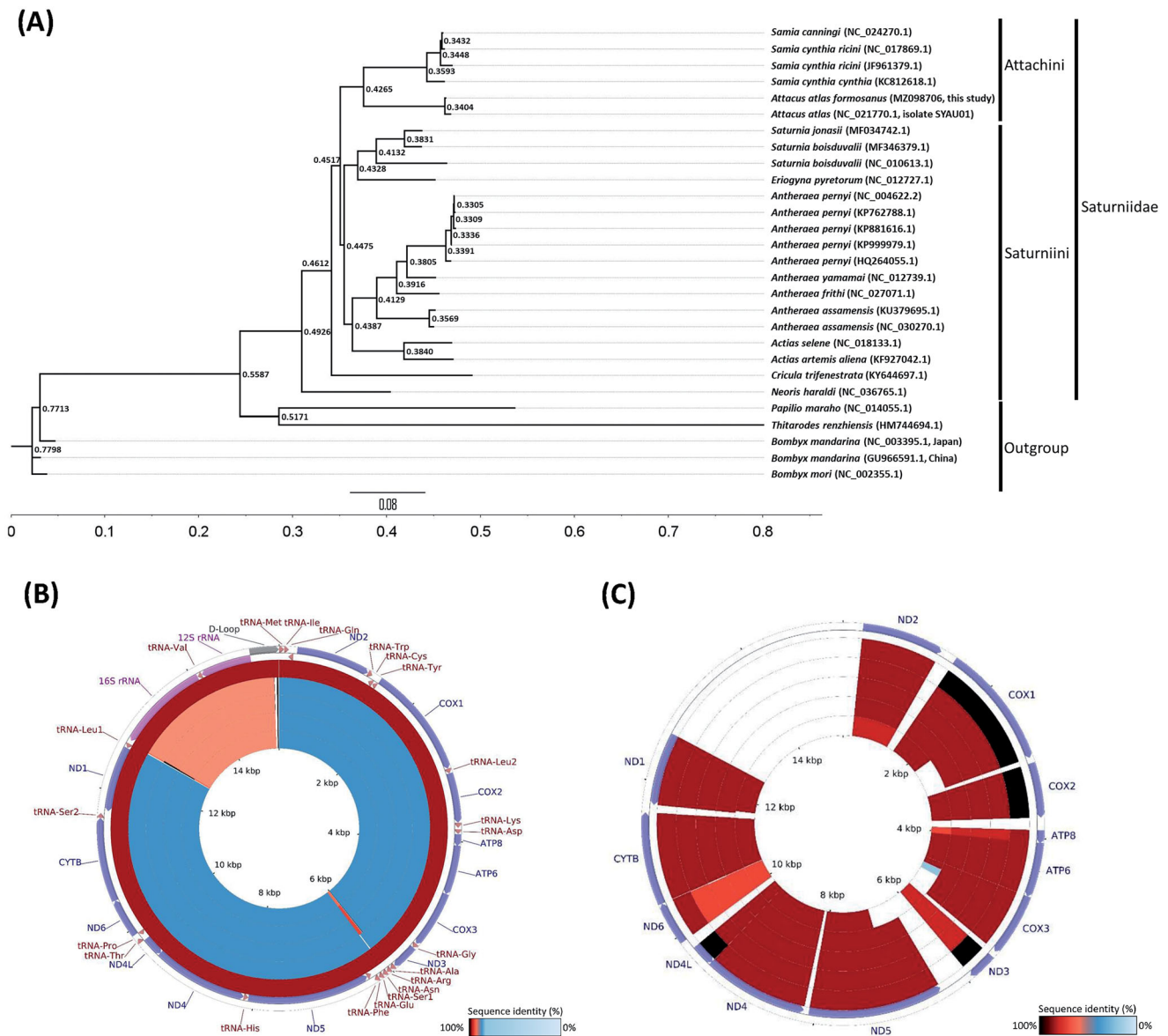


Figure 1. The phylogenetic and comprehensive mitogenomic analysis of mitogenomes from 23 species in Saturniidae. (A) Phylogenetic analysis based on whole mitogenomes from 23 species in Saturniidae; *Papilio maraho*, *Thitarodes renzhiensis* and 3 species in Bombycidae are the outgroup; (B) Whole mitogenome comparison; From outer to inner: *Attacus atlas*, *Samia cynthia cynthia*, *Samia canningi*, *Samia cynthia ricini*, and *Samia cynthia ricini*, and (C) Coding gene sequences comparison of 6 closely related species; From outer to inner: *Attacus atlas*, *Samia canningi*, *Samia cynthia cynthia*, *Samia cynthia ricini*, and *Samia cynthia ricini*.

tetranucleotides (Flook et al. 1995) and hexanucleotides (Beard et al. 1993; Mitchell et al. 1993) were also proposed as the start codon for this gene. In addition, the ND4 gene uses ATG as the start codon in *A. atlas* but ATT in *A. atlas formosanus*. The CYTB gene uses ATA as the start codon *A. atlas* but ATG in *A. atlas formosanus*. Though the high mitogenome identity was found between *A. atlas* of continental origin and *A. atlas formosanus* of insular origin, 9 coding gene sequences of *A. atlas formosanus* mitogenome displayed difference from those of *A. atlas* (Figure 1C), presumably because of the geographic evolutionary force that the area between the habitats of *A. atlas* and *A. atlas formosanus* may harbor.

Disclosure statement

The authors declare that there is no conflict of interest involved in this work.

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Data availability statement

The mitogenome data is available in NCBI at (<https://www.ncbi.nlm.nih.gov>), reference number (GenBank: MZ098706).

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