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MITOGENOME REPORT

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The complete mitochondrial genome of a jujube geometrid, *Sucra jujuba* (Lepidoptera: Geometridae) and its phylogenetic analysis

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ABSTRACT

Sucra jujuba Chu, 1979 (Lepidoptera: Geometridae) is a major insect pest in jujube plantation. In this study, we have sequenced the complete mitochondrial genome of *S. jujuba*. The circular genome was 15,557 bp in length and contained 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and one AT-rich region (GenBank accession no. MZ507574). The nucleotide composition was significantly biased (A, T, C, and G were 41.85%, 39.65%, 10.97%, and 7.53%, respectively) with A + T contents of 81.50%. The Bayesian phylogenetic analysis of the concatenated nucleotide sequences of 13 PCGs from 30 species in the subfamily Ennominae and two outgroup species was performed. The results indicated that *S. jujuba* was closely related to *Amraica recursaria* in the subfamily Ennominae.

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Ennominae; *Sucra jujuba*; coverage depth; Bayesian posterior probability

Introduction

The jujube geometrid, *Sucra jujuba* Chu, 1979 is native to northern regions of China where it has become a major leaf-feeding pest of Chinese jujube trees (*Ziziphus jujuba* Mill.) (Chu 1979). The male moth has pectinate antennae and a gray-brown body, and its body length is 12–13 mm. It has a wing span of about 35 mm and is characterized by a noticeable black corrugated line in the middle of hindwings, while the female moth has filiform antennae, a body length of about 15 mm and is wingless (Figure 1) (Li et al. 1981; Li 1992). The larvae of *S. jujuba* feed solely on the leaves of jujube trees and seriously affect the yield and quality of jujube. It is widely distributed in Northern China (Henan, Hebei, Shandong, Shanxi, Shaanxi, and Ningxia Provinces) and has caused considerable economic losses to jujube industry (Hou et al. 2002; Li et al. 2016). In this study, we sequenced and assembled the complete mitogenome of *Sucra jujuba*, and performed the phylogenetic analysis among the species in the subfamily Ennominae, which will contribute to get a better understanding of the phylogenetic relationships of *Sucra jujuba* and provide new ideas for jujube pest control.

Materials and methods

Sample collection and genomic DNA extraction

The female specimen of *S. jujuba* was collected from a jujube orchard in Shenmu County, Yulin City, Shaanxi Province, China (110.5663° E, 38.2885° N, altitude 716.1 m). The genomic DNA was extracted from the muscle tissues of the specimen's thorax using TIANamp Genomic DNA Kit (Tiangen, Beijing, China). The quality and quantity of the DNA were assessed by 1.0% agarose gel electrophoresis and SimpliNano spectrophotometer (GE Healthcare, Piscataway, NJ). The specimen was deposited in the Laboratory of Pest Monitoring and Control Center, Bio-Agriculture Institute of Shaanxi, Xi'an, Shaanxi, China (http://swny.ac.cn/, contact person and email: Bo Hong, hongbo82@xab.ac.cn) under the voucher number SN2020ZCH01.

Mitochondrial genome sequencing, assembly, and annotation

The complete mitochondrial genome sequencing was performed on Illumina NovaSeq 6000 platform with 150 bp paired-end reads at Biomarker Technologies Co., Ltd. (Beijing, China). The mitogenome sequence was assembled using MITObim v1.9.1 (Hahn et al. 2013) with the species *Ectropis grisescens* (GenBank accession no. MW337302) (Song et al. 2021) as the reference genome. The average coverage depth for the mitochondrial genome sequence was calculated using Bowtie2 v 2.3.4 (Langmead and Salzberg 2012) and SAMtools v1.7 (https://github.com/samtools/samtools; Danecek et al. 2021) to confirm the assembly correctness. The rough boundaries of each gene were first identified using the MITOS2 Web Server (http://mitos2.bioinf.uni-leipzig.de/index.py; Bernt

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et al. 2013) with the invertebrate genetic code. The nucleotide sequences of 13 PCGs and two rRNA genes were further confirmed by the published lepidopteran mitogenomes using MEGA 11 (Tamura et al. 2021). The positions of tRNA genes were determined using the tRNAscan-SE 2.0 Search Server (http://lowelab.ucsc.edu/tRNAscan-SE/; Chan et al. 2021). Finally, the circular genome visualization was generated using Proksee (https://proksee.ca/; Grant et al. 2023). To better understand the base usage bias of the mitogenome, we also calculated the nucleotide skew values using the formulae AT-skew = (A − T)/(A + T) and GC-skew = (G − C)/(G + C) (Perna and Kocher 1995).

Figure 1. Female specimen of *Sucra jujuba* Chu, 1979 was collected from a jujube orchard in Shenmu County, Yulin City, China. Photographed by Bo Hong on 17 June 2020.

Phylogenetic analysis

Phylogenetic analysis was conducted using the mitogenome sequences of 30 species (including *S. jujuba*) in the subfamily Ennominae and two outgroup species in the family Geometridae, *Idaea effusaria* Christoph, 1881 (Sterrhinae) and *Pasiphila chloerata* Mabille, 1870 (Larentiinae). The sequences were downloaded from the GenBank database (Wang et al. 2017; Chen et al. 2019; Du et al. 2019; Song et al. 2019; Xie 2020; Huang et al. 2021; Song et al. 2021; Sun et al. 2021; Chen et al. 2022; Lu et al. 2023; Zou et al. 2023). All nucleotide sequences of 13 protein-coding genes (PCGs) for these species

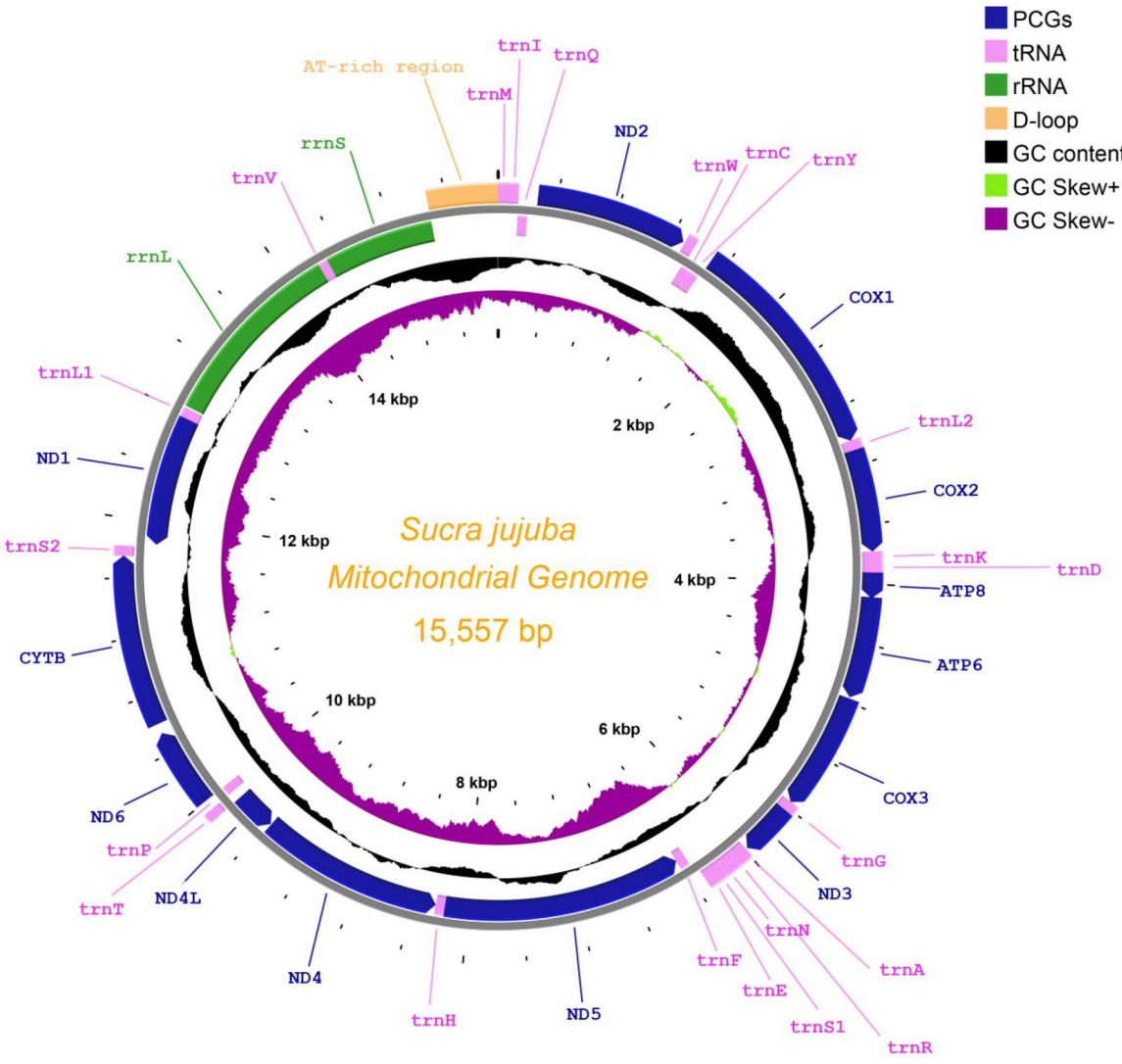

Figure 2. The mitochondrial genome map of *Sucra jujuba* Chu, 1979 with 13 PCGs, 22 tRNAs, two rRNAs, and one at-rich region.

were aligned using MAFFT7.037 with L-INS-i strategy (Katoh et al. 2002) and concatenated using DAMBE 6.4.81 (Xia 2017). The Bayesian inference (BI) phylogenetic tree was constructed using MrBayes3.2.6 (Ronquist and Huelsenbeck 2003). Two independent runs with four Markov chains were performed for 1,000,000 generations and sampled every 1000 generations. Finally, the phylogenetic tree was visualized and edited with FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Characteristics of mitochondrial genome

The complete mitochondrial genome of *Sucra jujuba* (GenBank accession no. MZ507574) is a circular molecule of 15,557 bp in length, and consists of 37 genes, including 13 PCGs, 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and one control region (non-coding AT-rich region) (Figure 2). The average coverage depth of the mitochondrial genome is 2762.45× (Supplementary Figure S1). The nucleotide composition is significantly biased (A, T, C, and G are 41.85%, 39.65%, 10.97%, and 7.53%, respectively) with A + T contents of 81.50%. AT-skew and GC-skew are 0.027 and −0.186, respectively. There are 17 intergenic spacer regions ranging in size from 1 to 66 bp (255 bp in total) and five overlapping regions (24 bp in total) throughout the whole genome.

Among 13 PCGs, except for *cox1* which uses CGA as an initiation codon, all other PCGs are initiated with typical ATN codons (*nad2*, *nad3*, and *nad5* with ATT, *nad6* and *nad1* with

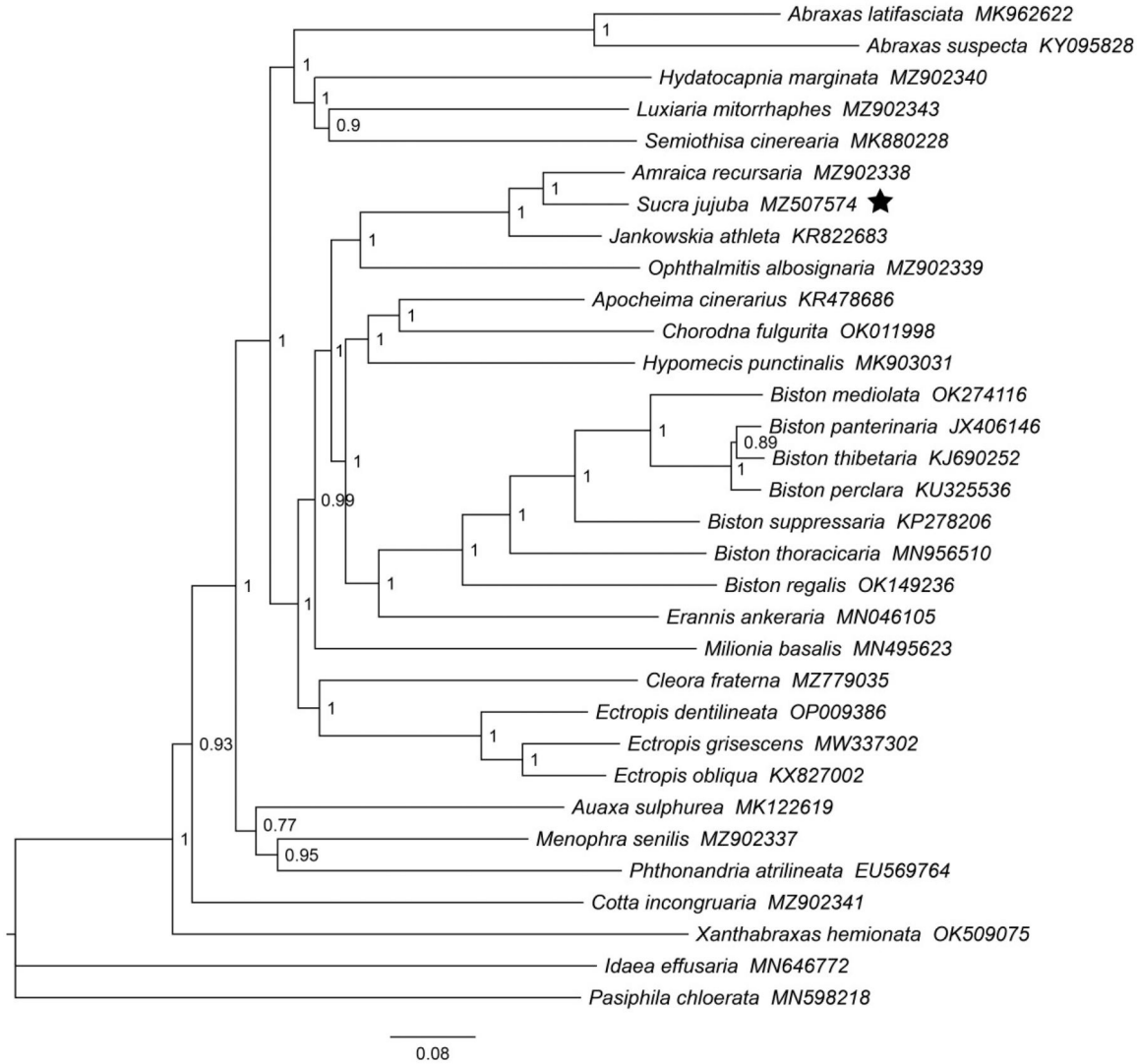

Figure 3. The Bayesian phylogenetic tree of *S. jujuba* with 29 species in the subfamily Ennominae based on the concatenated nucleotide sequences of 13 PCGs. The black pentagram represented the branch of *S. jujuba*. The sequences used to construct the tree were as follows: *Abraxas latifasciata* MK962622, *Abraxas suspecta* KY095828 (Sun et al. 2017), *Hydatocapnia marginata* MZ902340, *Luxiaria mitorrhaphes* MZ902343, *Semiothisa cinerearia* MK880228 (Zou et al. 2023), *Amraica recursaria* MZ902338, *Sucra jujuba* MZ507574, *Jankowskia athlete* KR822683 (Xu et al. 2016), *Ophthalmitis albosignaria* MZ902339 (Zheng et al. 2022), *Apocheima cinerarius* KR478686 (Liu et al. 2014), *Chorodna fulgurita* OK011998, *Hypomecis punctinalis* MK903031 (Sun et al. 2021), *Biston mediolata* OK274116, *Biston panterinaria* JX406146 (Yang et al. 2013), *Biston thibetaria* KJ690252 (Chen et al. 2017), *Biston perclara* KU325536 (Chen et al. 2017), *Biston suppressaria* KP278206 (Chen et al. 2016), *Biston thoracicaria* MN956510 (Huang et al. 2021), *Biston regalis* OK149236, *Erannis ankeraria* MN046105 (Chen et al. 2019), *Milionia basalis* MN495623 (Du et al. 2019), *Cleora fraternal* MZ779035, *Ectropis dentilineata* OP009386 (Lu et al. 2023), *Ectropis grisescens* MW337302 (Song et al. 2021), *Ectropis obliqua* KX827002 (Wang et al. 2017), *Auaxa sulphurea* MK122619, *Menophra senilis* MZ902337, *Phthonandria atrilineata* EU569764 (Yang et al. 2009), *Cotta incongruaria* MZ902341, *Xanthabraxas hemionata* OK509075 (Chen et al. 2022), *Idaea effusaria* MN646772 (Xie 2020), and *Pasiphila chloerata* MN598218 (Song et al. 2019). Bayesian posterior probabilities were shown for each node.

ATA, *cox2*, *atp6*, *cox3*, *nad4*, *nad4l*, and *cytb* with ATG, and *atp8* with ATC). All the PCGs have a typical TAN stop codon; nine PCGs (*nad2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad4l*, *nad6*, *cytb*, and *nad1*) are terminated with TAA, and four PCGs (*cox1*, *cox2*, *nad4*, and *nad5*) are terminated with an incomplete stop codon T. All 22 tRNAs range from 65 to 71 bp, and are predicted to contain typical cloverleaf secondary structures except the gene *trnS1*, whose DHU arm is replaced by a simple loop. The two rRNA genes are encoded on the N-strand of the mitochondrial genome. *rrnL* (*16S rRNA*) is 1377 bp in length with A + T contents of 84.75%, and *rrnS* (*12S rRNA*) is 784 bp in length with A + T contents of 85.71%. The AT-rich region is 473 bp in length with A + T contents of 95.98%, and is located between *trnM* and *rrnS* (Figure 2).

Phylogenetic analysis

Phylogenetic analysis of *S. jujuba*, along with 29 species in the subfamily Ennominae, was performed using the BI method, with *I. effusaria* and *P. chloerata* being used as outgroup species. The phylogenetic tree showed that *Sucra jujuba* was more closely related to *Amraica recursaria* than to other species with high Bayesian posterior probability (BPP = 1) (Figure 3).

Discussion and conclusions

In this study, we assembled and described the characteristics of the mitochondrial genome of *S. jujuba*. The gene arrangement of the mitogenome of *S. jujuba* is identical to that of most of lepidopteran mitochondrial genomes with the '*trnM-trnI-trnQ*' cluster (Wang et al. 2017; Huang et al. 2021; Zou et al. 2023). The phylogenetic analysis indicated that *S. jujuba* was more closely related to *A. recursaria* than to other species in the subfamily Ennominae, which was consistent with the previous study by Zou et al. (2023). This study offers significant molecular data for advancing the evolutionary and phylogeographic analysis of *S. jujuba*, as well as establishing a foundation for further investigation into the phylogenetic relationships among species in the subfamily Ennominae.

Author contributions

X.L.W. and B.H. contributed to the conception and design of the research. Y.Y.Z., K.W., and B.H. collected the specimens and performed the experiments. X.L.W., Y.L., and B.H. analyzed the data. X.L.W. and Y.Y.Z. drafted the manuscript, Y.L. and B.H. revised the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Ethics statement

There is no ethical research involved in this study. The specimen collection protocol was approved by Shaanxi Academy of Sciences. The study did not involve endangered or protected species.

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 openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession no. MZ507574. The associated BioProject, BioSample, and SRA numbers are PRJNA758210, SAMN21016412, and SRR15650334, respectively.

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