Characterization of the complete mitochondrial genome of *Pseudorhaetus sinicus* Boileau, 1899 (Coleoptera: Lucanidae)

Zhibile Zhao\(^\ast\)\(^\ast\)
Long Wu\(^b\)
Yu Bai\(^a\)\(^c\)
Chun Wang\(^a\)
Guiling Qi\(^a\)
Can Li\(^a\)
Yu Cao\(^a\)

\(^a\)Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guizhou Provincial Engineering Research Center for Biological Resources Protection and Efficient Utilization of the Mountainous Region, Guiyang University, Guiyang, China; \(^b\)College of The Environment & Ecology, Museum of Biology, Xiamen University, Xiamen, China

**ABSTRACT**

*Pseudorhaetus sinicus* is a stag beetle common to China and Vietnam, but whose distribution is limited within China. Little is known about the molecular biological characteristics of this species, so we characterized its complete mitochondrial genome (GenBank accession number MZ504793.1). The mitogenome consists of a circular DNA molecule of 18,126 bp, with 67.693% AT content. It contains 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes. The PCGs have typical ATN (Met) start codons and TAN stop codons. Phylogenetic analysis suggests that *P. sinicus* is closely related to *Prosopocilus confucius*. This newly described mitochondrial genome provides a valuable resource for the phylogenetic analysis of Lucanidae beetles.

*Pseudorhaetus sinicus*, which belongs to order Coleoptera, family Lucanidae, is distributed mainly in Vietnam and China. It is commonly found in Fujian, Zhejiang, Jiangxi, and Guizhou Provinces, China. The partial mitochondrial genome (KP987575.1) of *P. sinicus* from Daming Mountain in Guangxi Province, China, has previously been sequenced (Wu et al. 2016). Here, we have characterized the complete mitogenome of *P. sinicus* from Yong’an City, Fujian Province, China, to better understand the molecular evolution and taxonomic classification of *P. sinicus*.

A specimen of adult *P. sinicus* was collected in Longtou Valley (117.36517°N, 25.94136°E), Fujian Tianbaoyan National Nature Reserve, Yong’an City, Sanming City, China, on 24 September 2020, and deposited by Yu Cao (Email: yucaosuccess@126.com) in the animal specimen room of Guiyang University (specimen accession number: GYU-20200924-001). Total genomic DNA was isolated using an Aidlab Genomic DNA Extraction Kit (Aidlab Co., Beijing, China). Universal primers were designed (Supplementary Table 1) to match generally conserved regions to amplify short fragments from 12S and 16S rRNA, cox1, cox2, nad1, nad2, and nad5. PCR products were cloned into a pMD18-T vector (Takara Bio, Kusatsu, Japan) and then sequenced, or sequenced directly by the dye-deoxy nucleotide procedure, using an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA). Thirty-six short sequences were obtained which range in length from 253 bp to 1081 bp.

The complete mitogenome of *P. sinicus* (GenBank accession number: MZ504793.1) was assembled manually. It is a circular DNA molecule that is 18,126 bp long (nucleotide composition: 36.2% A, 31.5% T, 21.2% C, and 11.2% G; 67.7% AT content). Using Perna and Kocher’s formula (Perna and Kocher 1995), the AT- and GC-skews of the major strand of the mitogenome were calculated to be 0.070 and −0.309, respectively. The AT-rich region of the mitogenome is 3549 bp long with 70.4% AT content, and is located between the genes that encode small regulatory RNA (sRNA) and tRNA-Ile.

The *P. sinicus* mitogenome contains 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes, which were annotated using the MITOS web server (http://mitos.bioinf.uni-leipzig.de/) (Bernt et al. 2013). The transcriptional start and stop sites of the PCGs were manually corrected using *P. sinicus* (KP987575.1) from Daming Mountain, *Tenebrio obscurus* (Bai et al. 2018), *Zophobas atratus* (Bai et al. 2019), and *Blaps rhynchoptera* (Yang et al. 2019) genomic sequences as references. All 13 PCGs had typical ATN (Met) start codons. Three genes (*atp6, nad3*, and *nad1*) had ATA start codons, one (*nad2*) had an ATC start codon, five (*cox3, nad4, nad4l, nad6*, and *cob*) had ATG start codons, and four (*cox1, cox2, atp8*, and *nad5*) had ATT start codons. All 13

*CONTACT* Yu Cao (yucaosuccess@126.com) Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guizhou Provincial Engineering Research Center for Biological Resources Protection and Efficient Utilization of the Mountainous Region, Guiyang University, Guiyang, China

*Both authors contributed equally to this work.

**ARTICLE HISTORY**

Received 24 June 2021
Accepted 21 October 2021

**KEYWORDS** *Pseudorhaetus sinicus*; Lucanidae; mitochondrial genome; stag beetle; mitogenome

**CONTACT** Yu Cao (yucaosuccess@126.com) Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guizhou Provincial Engineering Research Center for Biological Resources Protection and Efficient Utilization of the Mountainous Region, Guiyang University, Guiyang, China

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PCGs had typical TAN stop codons. Four genes (atp8, atp6, nad4l, and nad6) had TAA stop codons, four (nad2, nad3, nad1, and cob) had TAG stop codons, and five (cox1, cox2, cox3, nad4, and nad5) had incomplete stop codons that were completed by the addition of A nucleotides at the 3' ends of the encoded mRNAs. The 22 tRNA-encoding genes were interspersed throughout the coding region and ranged from 61 bp (tRNA-Cys) to 71 bp (tRNA-Lys) long. The genes encoding large rRNA and srRNA were 1269 bp and 760 bp long, respectively.

To validate the phylogenetic position of P. sinicus, its mitochondrial PCGs and those of 16 other species in class Insecta were used to construct a maximum-likelihood phylogenetic tree with 1000 replicates using MEGA X software (Kumar et al. 2018) (Figure 1). The mitogenomes of T. obscurus (Bai et al. 2018) and Z. atratus (Bai et al. 2019) were selected as the outgroup. P. sinicus is closely related to Prosopocoilus confucius (Lin et al. 2017) and our tree is consistent with this. Overall, this study provides insights into the mitogenome of P. sinicus, and also provides essential genetic and molecular data for further phylogenetic and evolutionary analyses of family Lucanidae.

Disclosure statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Funding
This work was financially supported by the Natural Science Foundation from Guizhou Education Department [(2020)057], the First-class Discipline Construction of Guizhou Province [XKTJ(2020)14], the Program for Academician Workstation in Guiyang University [20195605], the Training Project for High-Level Innovative Talents in Guizhou Province [No. 2016 [4020]], and the Research Fund Projects for Postgraduates of Guiyang University [GYU-YJS [2019]-14].

Figure 1. Maximum-likelihood phylogenetic tree of Pseudorhaetus sinicus and 16 other species in class Insecta based on the sequences of 13 protein-coding regions in their mitogenomes.

Figure 1. Maximum-likelihood phylogenetic tree of Pseudorhaetus sinicus and 16 other species in class Insecta based on the sequences of 13 protein-coding regions in their mitogenomes.

ORCID
Yu Bai http://orcid.org/0000-0002-8237-9514

Data availability statement
The genome sequence data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov under accession no. MZ504793.1.

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