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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of *Batocera rubus* Linnaeus, 1785 (Coleoptera: Cerambycidae)

Rong Deng[a,b], Bowei Zhou[c,b], Yiqi Lin[a,b], Yunzhu Sun[a,b], Xianyun Lin[d] and Songqing Wu[a,b]

[a]College of Forestry, Fujian Agriculture and Forestry University, Fuzhou, China; [b]Key Laboratory of Integrated Pest Management in Ecological Forests, Fujian Province University, Fujian Agriculture and Forestry University, Fuzhou, China; [c]College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, China; [d]Shunchang County Forestry Bureau, Nanping City, China

ABSTRACT

Batocera rubus severely impacts on the health of banyan trees. In this study, the whole mitochondrial genome for *B. rubus* was found to be 16,158 bp with a GC content of 23.9%, including 39.1% A, 37.0% T, 14.8% C, and 9.1% G. This genome contains 13 protein-coding genes, 22 tRNAs, and two rRNAs. Phylogenetic analysis revealed that *B. rubus* is close to *Batocera celebiana*. This study provides valuable information that can help improve the classification and phylogeny of *B. rubus* and facilitate further evolutionary studies.

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Introduction

Batocera rubus Linnaeus 1785 (Coleoptera: Cerambycidae) is widely distributed beetle species found in China, India, South Korea, and many other countries (Liu et al. 2003). Due to their large size and long life cycle, the damage caused by *Batocera rubus* to trees is often relatively large. The larvae feed on the phloem and xylem of plants affecting their growth and leading to the loss of the affected branches, stems, and even the entire plant (Liu et al. 2003). *B. rubus* has a severe economic impact in forestry industry; however, there is a paucity of research on *B. rubus* that limits our understanding as well as the prevention and control of this pest. To better understand and reveal the genetic and evolutionary familiarity of *B. rubus*, the whole mitochondrial genome of *B. rubus* was sequenced and assembled. The genome of *B. rubus* has been published and can be retrieved from GenBank (BioProject: PRJNA796453; BioSample: SAMN24860787; SRA: SRR17574369).

Materials and methods

Batocera rubus adults were trapped by using the trapping method in Minhou, Fujian Province, China (118°57′21″E, 26°9′19″N). Adults are medium-sized yellow-brown beetles (Hemadri and Reddy 2019). *Batocera rubus* has a kidney-shaped spot marking on their pronotum as well as white or rose-red colors (Figure 1). There are four white spots on each elytron; the fourth spot is the smallest and the second spot is the largest. There are often one or two small spots that are higher than the second spot and are sometimes coupled to the second spot. The spots on the elytra are arranged in a longitudinal line along the midline. The bottom of the elytra features a small and dense granular protuberance that occupies approximately 1/4 of the elytra. The insect samples were kept with a TN-202101 voucher at the Key Laboratory of

Figure 1. Female of *Batocera rubus* Linnaeus, 1785, dorsal view. Scale = 1 cm. This is an original image by the authors.

CONTACT Songqing Wu dabinyang@126.com College of Forestry, Fujian Agriculture and Forestry University, Fuzhou 350000, China; Key Laboratory of Integrated Pest Management in Ecological Forests, Fujian Province University, Fujian Agriculture and Forestry University, Fuzhou 350000, China

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Integrated Pest Management in Ecological Forests, Fujian Agriculture and Forestry University.

Total genomic DNA from a single adult legs was extracted by using the TruSeq DNA Sample Preparation kit (Vanzyme, Nanjing, China), and the DNA concentration was determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). A DNA library was constructed using the transposase method and the library was prepared with randomly interrupted 300-bp fragments. The constructed library was sequenced with 150-bp pair-end reads on the Illumina Hiseq2500 platform (Illumina, San Diego, CA). Clean reads were assembled using MitoZ and metaSPAdes (Nurk et al. 2017). Annotation was performed using GeSeq (Kearse et al. 2012; Guyeux et al. 2019) and plotted on a gene map using the ogdraw software (Greiner et al. 2019). The nucleotide sequences of different insect species were downloaded from the NCBI website (https://www.ncbi.nlm.nih.gov/) and used for phylogenetic analysis. An evolutionary tree containing the COI gene of 17 different species via MEGA 7.0 was constructed (Górecki et al. 2011; Kumar et al. 2016) using the maximum-likelihood statistical method and the Kimura 2-parameter model with 1000 bootstrap replicates.

Results

A total of 46,027,082 clean reads were obtained out of 47,766,884 raw reads. The complete mitogenome of *B. rubus* is sequenced to be 16,158 bp in length with an average depth of 3073.49X (Supplementary Figure 1). In addition, the Genebook of *B. rubus* revealed that the overall mitochondrial genome of *B. rubus* contains 13 PCGs (Figure 2), which encode 3677 amino acids. Nine PCGs (ATP6, ATP8, COX1, COX2, COX3, CYTB, ND2, ND3, and ND6) were coded clockwise, and four PCGs (ND1, ND4, ND4L, and ND5) were coded counterclockwise. The gene length of rrnS was 780 bp, and that of rrnL was 1294 bp. The genome-wide GC content was 23.90%, of which 39.1% was A, 14.8% C, 9.1% G, and 37.0% T.

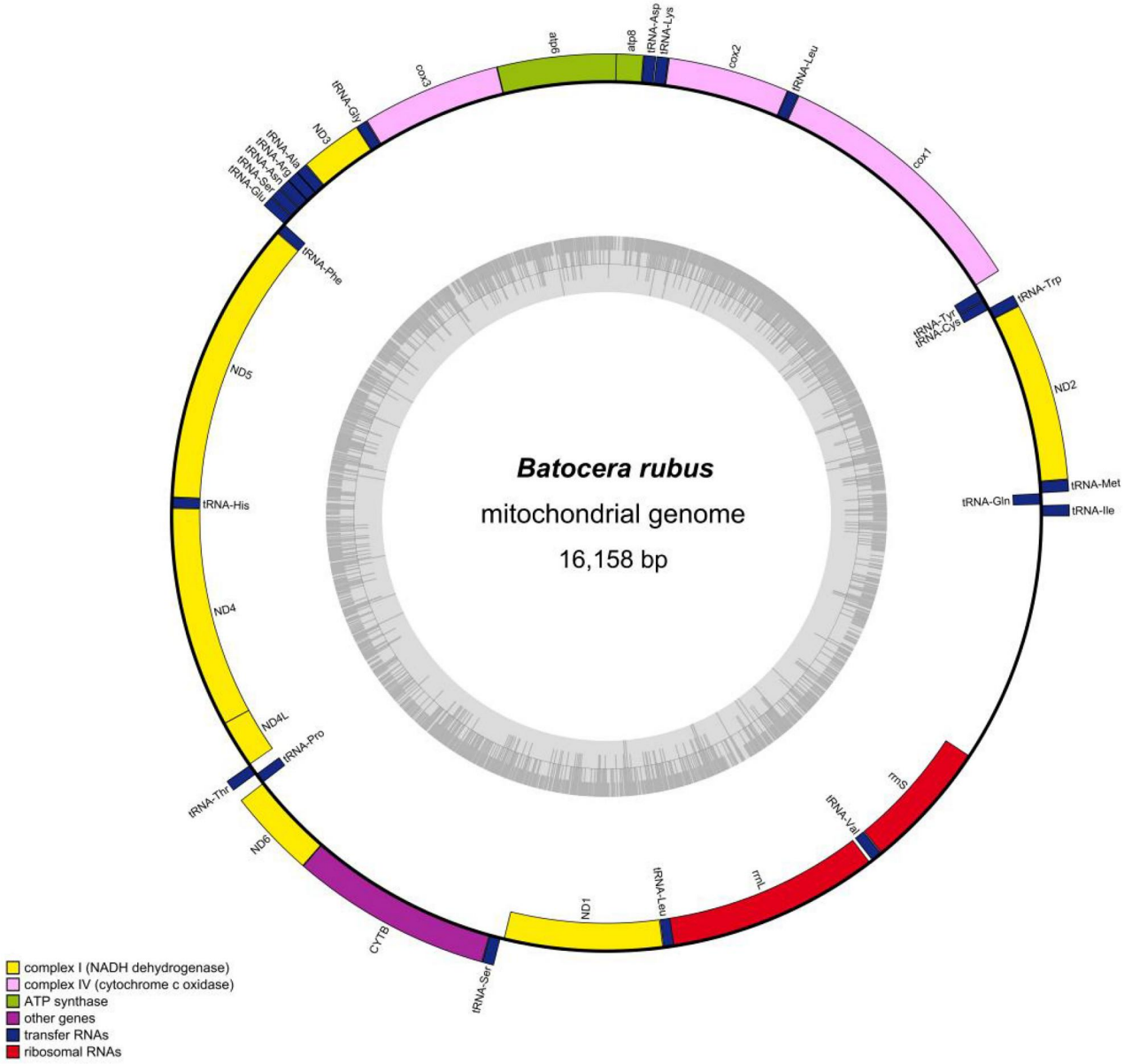

Figure 2. Complete mitochondrial genome map of *Batocera rubus*. The arrangement of 37 genes is represented in the map, including 13 protein coding genes, 22 tRNA genes, and two rRNA genes.

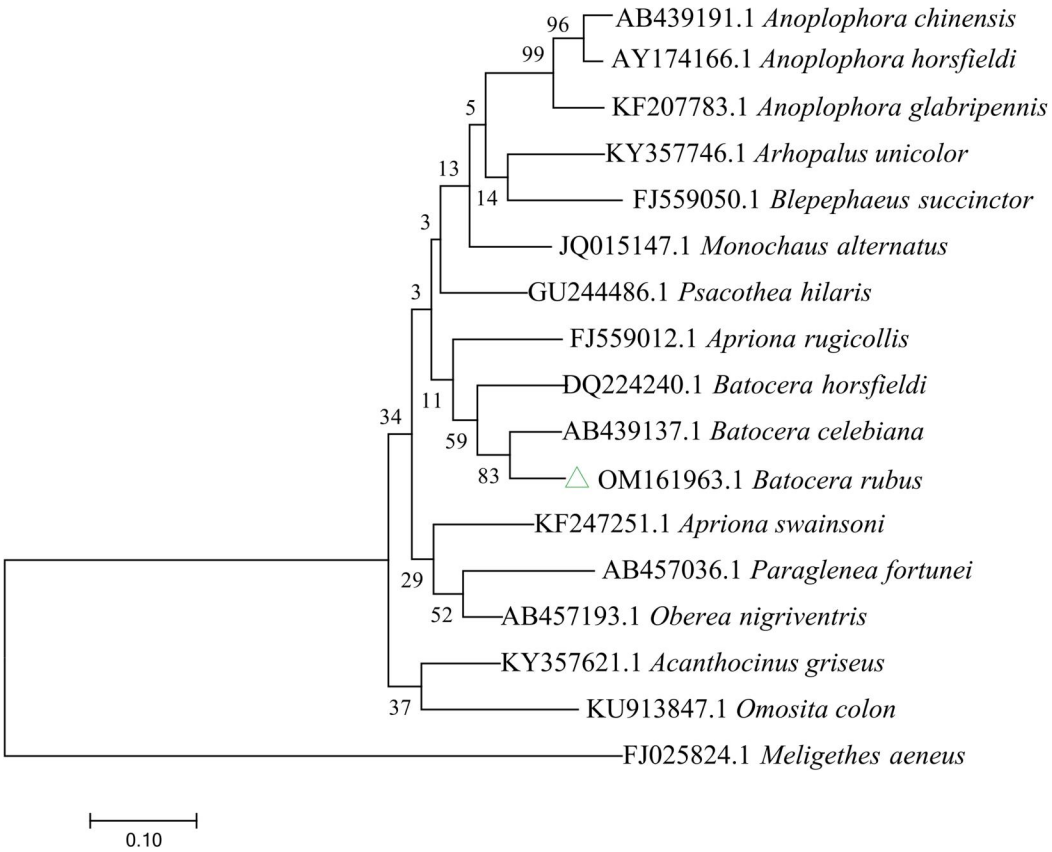

Figure 3. Maximum-likelihood tree of *Batocera rubus* related to 16 different species of Coleoptera based on the COI gene. Bootstrap support values are labeled near the branch.

Table 1. Information on the mitogenomes of the species used in this study

Order	Family	Species	GenBank no.	Articles cited
Coleoptera	Cerambycidae	*Acanthocinus griseus*	KY357621.1	Martikainen et al. 2002
		Anoplophora chinensis	AB439191.1	Wang et al. 2017
		Anoplophora horsfieldi	AY174166.1	Ohbayashi et al. 2018
		Anoplophora glabripennis	KF207783.1	McKenna et al. 2016
		Apriona rugicollis	FJ559012.1	Lin et al. 2021
		Apriona swainsoni	KF247251.1	Que et al. 2019
		Arhopalus unicolor	KY357746.1	Wen et al. 2021
		Batocera celebiana	AB439137.1	Ohbayashi et al. 2009
		Batocera horsfieldi	DQ224240.1	Yang et al, 2018
		Blepephaeus succinctor	FJ559050.1	Dai et al. 2020
		Monochaus alternatus	JQ015147.1	Zhou et al. 2018
		Oberea nigriventris	AB457193.1	Kusakabe et al. 2001
		Psacothea hilaris	GU244486.1	Saeb and Grewal 2014
		Paraglenea fortunei	AB457036.1	Tsubaki et al. 2014
	Nitidulidae	*Meligethes aeneus*	FJ025824.1	Smart and Blight 2000
		Omosita colon	KU913847.1	Wang et al. 2020

The evolutionary tree included 13 Cerambycidae (*Monochamus alternatus*, *Anoplophora chinensis*, *Anoplophora glabripennis*, *Psacothea hilaris*, *Paraglenea fortunei*, *Anoplophora horsfieldii*, *Blepephaeus succinctor*, *Apriona rugicollis*, *Batocera horsfieldi*, *Batocera celebiana*, *Apriona swainsoni*, *Oberea nigriventris*, and *Acanthocinus griseus*), two Nitidulidae (*Omosita colon* and *Meligethes aeneus*), and one Aseminae (*Arhopalus unicolor*) (Figure 3).

The phylogenetic tree revealed that *B. rubus* constituted a monophyletic group with 14 other Cerambycidae species using the entire mitochondrial genome of *Meligethes aeneus* as an outgroup. Additionally, phylogenetic tree analysis showed that *B. rubus* and *B. celebiana* formed a monophyletic clade with a high support value (BS = 83%), and these species showed a close relationship. In addition to providing genetic information that can be used for the genus *Batocera*, the entire mitochondrial genome of *B. rubus* will be helpful for studying the evolutionary traits of *Batocera* (Table 1).

Discussion

In recent decades, the unique features of the insect mitochondrial genome have led to a wide range of applications in phylogenetic and population genetic studies at different levels of population genetics, molecular evolution, comparative and evolutionary genomics, etc. (Galtier et al. 2009; Cameron 2014). In this study, the whole mitochondrial genome of *B. rubus* was successfully

sequenced and assembled, which assists in the further improvement of the taxonomy and phylogeny of *B. rubus*. Comparison of the mitochondrial genomes of *B. rubus* and *B. celebiana* revealed that the GC content of these species was essentially the same. In addition, the percentages of each nucleobase were the same, which may be related to the fact that they have the closest relatives in the evolutionary tree. The present results provide important information for studying the evolution of the recombinant mitochondrial genome of *B. rubus* and provide a basis for developing strategies for controlling *B. rubus*.

Author contributions

Conceived and designed the experiments: Songqing-Wu. Performed the experiments: Rong Deng, Bowei Zhou, Yiqi Lin, and Xianyun Lin. Analyzed the data: Yunzhu Sun. Wrote the paper: Rong Deng. All authors are involved: final approval of the version to be published. All authors agreed to take responsibility for all aspects of the work.

Ethical approval

The material involved in the article does not involve ethical conflicts. This study was permitted by the Key Laboratory of Integrated Pest Management in Ecological Forests, FAFU, China. All collection and sequencing work were strictly executed under local legislation and related laboratory regulations to protect wild resources.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The genome sequence data supporting this study's findings are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov under assessment number OM161963. The associated BioProject, Bio-Sample, and SRA numbers are PRJNA796453, SAMN24860787, and SRR17574369, respectively.

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