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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of *Brachyrhynchus triangulus* (Hemiptera: Aradoidea: Aradidae)

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ABSTRACT

The complete mitochondrial genome of *Brachyrhynchus triangulus* Bergroth, 1889 was sequenced and annotated in the present study. It was a typical circular DNA molecule of 15,170 bp, with 37 genes and a control region. The percentages of A, C, G, and T nucleotides in the genome of *B. triangulus* were 41.1%, 17.4%, 11.9%, and 29.4%, respectively. Thirteen protein-coding genes (PCGs) start with a ATN codon or a TTG codon and terminate with a TAA codon or a TAG codon or a single T residue. With the exception of $tRNA^{Ser(AGN)}$, each of the 22 tRNA genes had a clover-leaf structure and ranged in length from 62 to 69 bp. The length of *lrRNA* and *srRNA* was 1241 bp and 828 bp, respectively. The control region had a length of 708 bp and an A + T content of 74.6%. The sister relationship between *B. triangulus* and *Brachyrhynchus hsiaoi* is supported by the phylogenetic tree. Additionally, it proved the sister relationship between Mezirinae and Aneurinae, supporting the classical taxonomy of the Aradidae.

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Brachyrhynchus triangulus (Hemiptera: Aradidae) was discovered in Burma by Bergroth (Bergroth 1889). Aradids were known as flat bugs because of their strongly flattened dorsoventrals and bark bugs because they live under the bark of rotting trees. Flat bugs are brown to black in color (Bhagat 2015). They are mysterious and their economic functions have not been discovered (Chandra and Kushwaha 2015). In this study, we first present the complete mitogenome of *B. triangulus* and provide valuable genetic information for further research on the classification and genomics of Aradidae.

The specimens used in this experiment were obtained on 26 March 2020, from the Tongbiguan Nature Reserve, Yingjiang, Yunnan province (24.70579N, 97.93179E), and were identified by Professor Xiaoshuan Bai from Inner Mongolia Normal University. The voucher specimen was deposited with the voucher number BtYj-1 in absolute ethanol at −20 °C in the molecular laboratory of Inner Mongolia Normal University (Xiaoshuan Bai: baixs2007@aliyun.com). Since *Brachyrhynchus triangulus* is neither a vertebrate or a protected invertebrate, the reserve gave its consent for the field study to be carried out. High-throughput sequencing techniques were used to extract the mitochondrial genome of *B. triangulus*, which was then submitted to the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/, accession number: OK323200).

Total DNA was extracted from the feet of *B. triangulus* using the TIANamp Genomic DNA Kit and sent to BerryGenomics (Beijing, China) for sequencing. Total genomic DNA was extracted using the Illumina NovaSeq 2500 platform. A total of 6 Gb clean data were returned and assembled by IDBA-UD v1.0 (Peng et al. 2012), and mitochondrial genome sequence was obtained using a Python software package. The annotation of *B. triangulus* was done by MITOS (http://mitos.bioinf.uni-leipzig.de/index.py). The secondary structure of tRNA genes was predicted by tRNA scan-SE 2.0 (Lowe and Eddy 1997) (http://lowelab.ucsc.edu/tRNAscan-SE/). The 13 PCGs were annotated using a homology alignment method, and we downloaded the nucleotide sequences of 13 PCGs genes of seven species of Aradidae from NCBI. The locations of the 13 PCGs were determined by manual alignment using MEGA7 (Sudhir et al. 2016).

The mitochondrial genome of *B. triangulus* is 15,170 bp and consists of 37 genes. These genes encode 13 protein-coding genes (PCGs), 22 tRNA-coding genes, two ribosomal RNAs, and a control region. Among them, 23 genes encode in the J-strand and 14 genes encode in the N-strand. Except for the rearrangements between $tRNA^{Ile}$ and $tRNA^{Gln}$, the sequences and orientations of these genes are identical to the putative ancestral arrangement of *Drosophila yakuba* (Clary and Wolstenholme 1985). This rearrangement is found in seven other sequenced flat bugs, which may be considered a unique feature of the Aradidae mt-genome (Shi et al. 2012; Li et al. 2016; Song et al. 2016). The nucleotide compositions are 41.1% A, 17.4% C, 11.9% G, and 29.4% T, respectively, with a strong AT bias.

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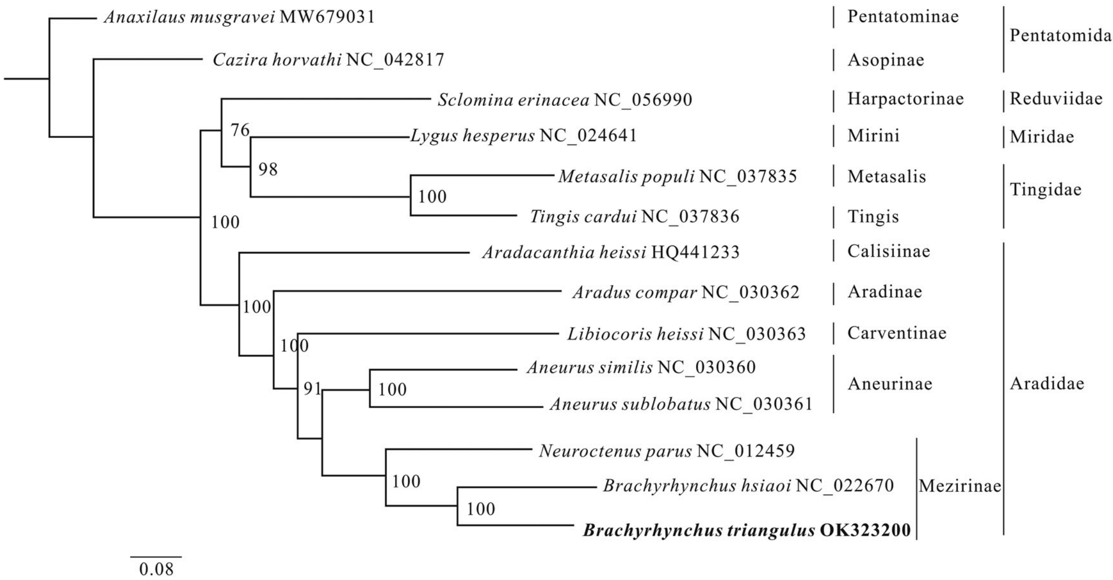

Figure 1. The phylogenetic relationships of the 14 species are derived from analysis of the combined 13 protein-coding sequence data based on the maximum-likelihood (ML) method. The best model is selected using ModelFinder (Kalyaanamoorthy et al. 2017) as GTR + F+I + G4, and later the ML tree is inferred from IQ-Tree v.1.6.8 (Lam-Tung et al. 2015). The node values are obtained with 1000 repetitions of bootstrap percentages.

The 13 PCGs have a total length of 10,945 bp and can encode a total of 3638 amino acids. In general, the start codon of the mitochondrial gene in invertebrates is commonly ATN, while TTG or GTG has also been found to be the start codon for several insects (Clary and Wolstenholme 1985). The complete stop codon is TAA and TAG, and insect mitochondria also have incomplete stop codons, such as TA and T. The incomplete stop codon is usually found between PCG and its adjacent tRNA genes. Ten PCGs are initiated with ATN (six for ATG, three for ATA, and one for ATT) as the start codon, and the others (*COI*, *COII*, and *ND1*) are initiated with TTG. Most PCGs terminate with TAA or TAG as a stop codon, and the others (*COII*, *COIII*, *ND4*, and *ND5*) terminate with a single T residue.

Except for the dihydrouridine (DHU) arm of $tRNA^{Ser(AGN)}$, which forms a simple loop, all 22 of the tRNA genes, ranging in length from 62 to 69 bp, have a clover-leaf structure. The overlap between $tRNA^{Trp}$ and $tRNA^{Cys}$ is the largest (8 bp). The length of *lrRNA* is 1241 bp with 74.3% A + T content, and the length of *srRNA* is 828 bp with 66.7% A + T content. The 708 bp control region is located between *srRNA* and $tRNA^{Gln}$, with 74.6% A + T content.

We constructed a phylogenetic tree of *B. triangulus* using the maximum-likelihood (ML) method to resolve the phylogenetic position of *B. triangulus* (Figure 1). The findings indicated a tight relationship between *Brachyrhynchus triangulus* and *Brachyrhynchus hsiaoi*. It also demonstrated a sisterly relationship between Mezirinae and Aneurinae, supporting the classical taxonomy of the Aradidae (Heiss and Grebennikov 2014; Song et al. 2016).

Author contributions

Xiaojun Zhu: conceptualization, methodology, formal analysis, resources, investigation, writing – original draft, writing – review and editing, and visualization. Hongge Qian: investigation, writing – review and editing. Xiaoshuan Bai: resources, formal analysis, investigation, writing – review and editing, visualization, supervision, project administration, and funding acquisition.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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. OK323200. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA838196, SRR19226807, and SAMN28408660, respectively.

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