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MITOGENOME REPORT

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Complete mitochondrial genome of *Saldoida armata* Horváth, 1911 (Heteroptera: Saldidae) and phylogenetic analysis

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ABSTRACT

The complete mitochondrial genome of *Saldoida armata* (Heteroptera: Saldidae) is 16,049 bp in length, comprising 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs) and a control region. All the PCGs are initially encoded by ATN, TTG or GTG, and terminated coding with TAA or a single T. With the exception of *trnS(AGN)*, all tRNAs exhibit a typical cloverleaf secondary structure. Phylogenetic analysis reveals the sister relationship of *S. armata* with other Saldidae members. The complete mitogenome of *S. armata* will provide useful genetic information for species identification, phylogenetic analysis and conservation of this species.

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Introduction

Saldidae (Heteroptera: Leptopodomorpha) insects are also called shore bugs, which typically small, ranging from 2 to 8 mm in length, and usually with large eyes (Panizzi and Grazia 2015). Two subfamilies comprising 29 genera and 335 species were described in this family (Henry 2017). All species in this group are predatory, and the majority inhabit damp areas adjacent to fresh water (Schuh and Weirauch 2020). *Saldoida armata* Horváth, 1911 belongs to Saldidae, mainly distributed in China, Southeast Asia, South Asia and Australia (Polhemus and Polhemus 2012). The morphology of *S. armata* mimicry the ants, setting it apart from most other shore bugs. The body is general coloration rusty brown, sparingly marked with black and white. In the center of the thorax, bearing a pair of backward curving horns (Figure 1).

Only four mitochondrial genomes (mitogenomes) of Saldidae have been reported in GenBank so far, and to fill in the gaps in the genetic data of the genus *Saldoida*, we sequenced and annotated the complete mitogenome of *S. armata*. The results would be able to furnish molecular insights for future investigations into this species and its related lineages.

Materials and methods

The specimen of *S. armata* was collected in July 2021 from Zhaotong City, Yunnan Province, China (28.590°N, 104.239°E), which lived on the bank of a small water hole. The whole body was used to extract the total genome followed the operating manual of DNeasy© Tissue Kit (Qiagen, Hilden, Germany). Total genomic DNA was eluted in 100 µl double distilled water (ddH_2O). The remaining genomic DNA was deposited at −20 °C in Animal Collection of Chuzhou University (no. HEM337, Yan Dong, dongyan_bio@126.com).

Genomic DNA was sequenced by the next-generation sequencing method using the Illumina Hiseq 2500 platform with 150 bp paired-end reads and 350 bp insert size. The sequence data was assembled using Geneious prime 2023.2.1 (www.geneious.com) and using the uploaded *COX2* sequence (GenBank: OP394045) as the reference. The clean data was map to the reference with custom sensitivity method. The minimum overlap was 50 bp and the minimum overlap identity was 98%. The mitogenome was annotated by MitoZ 2.4 (Meng et al. 2019) with confirmation achieved through alignment with homologous genes from closely related species using Geneious software.

For phylogenetic analyses, seven complete mitogenomes of Leptopodomorpha available in GenBank were utilized as the ingroup. Among these species, two mitogenomes were assembled from the SRA data (*Valleriola* sp. SRR5137182 and *Calacanthia angulosa* SRR13843814). Additionally, three species from closely related infraorders (Pentatomomorpha: *Eurydema qinlingensis* MG584836; Cimicomorpha: *Reduvius gregoryi* KY069969 and *Triatoma migrans* MK770624) were used as outgroup. Maximum-likelihood (ML) tree was constructed by IQ-TREE 2.1.3 (Minh et al. 2020) under the GTR+I + G model and Bayesian inference (BI) tree was constructed by PhyloBayes MPI 1.8c (Lartillot et al. 2013), using sequences of 13 PCGs and 2 rRNAs. Each gene was aligned

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using MAFFT 7 (Katoh and Standley 2013), and trimed using trimAl 1.4.1 (Capella-Gutiérrez et al. 2009) by using a heuristic selection of the automatic method based on similarity statistics. rRNA data were aligned and trimed based on nucleotide data. But for PCG dataset, we used the coding sequences file to get the back-translation for a given AA alignment and ignored stop codons in the input coding sequences.

Figure 1. Female adult of *Saldoida armata*. The ecological photo was taken using Canon 70D digital camera with 100 mm macro lens and processed by Yun-Fei Wu.

Results

In this research, 300 Mb clean data was obtained by a single library and produced a final mitogenome for *S. armata* with an average sequencing depth of 896× (Figure S1). The complete mitogenome is 16,049 bp long and comprises 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs) and a control region (Figure 2). Notably, this mitogenome stands out as the largest among those species within the Saldidae family, even surpassing counterparts in the infraorder Leptopodomorpha. Gene order is consistent with other published Leptopodomorpha insects. Regarding nucleotide composition, the mitogenome of S. armata exhibits the following distribution: A (44.6%), T (28.7%), G (10.7%), C (16.0%), and A + T content (73.3%). Among the 13 PCGs, nine PCGs are initiated by typical ATN codons, but *ND2* use the GTG, and *COX1*, *ATP8*, *ND1* use the TTG as the start codon. Most of PCGs are terminated with TAA stop codon, while *ND3*, *ND5* and *CYTB* are terminated with TAG, *COX1*, *COX2*, *COX3* are terminated with a single T.

All of the tRNAs were identified by tRNAscan-SE 2.0.2 online software (Lowe and Chan 2016), all tRNAs can be folded into a typical cloverleaf structure, except for *trnS(AGN)* lacking the dihydrouridine (DHU) arm, which was determined by comparison with other shore bugs (Hua et al. 2008). The lengths of the tRNAs vary, ranging from 61 bp (*trnA*) to 70 bp

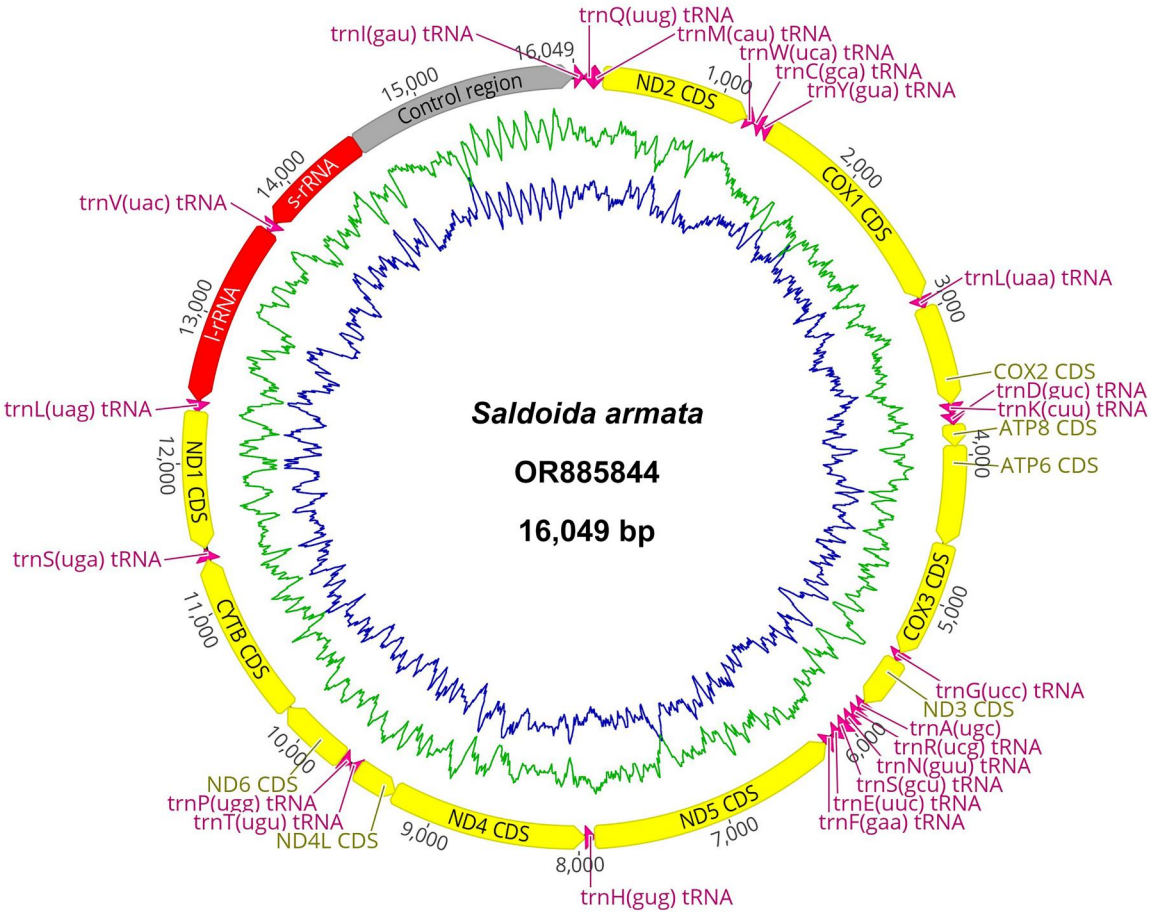

Figure 2. Circular map of the *Saldoida armata* mitochondrial genome and graphic representation of at (green) and GC (blue) content and their changes throughout the genome.

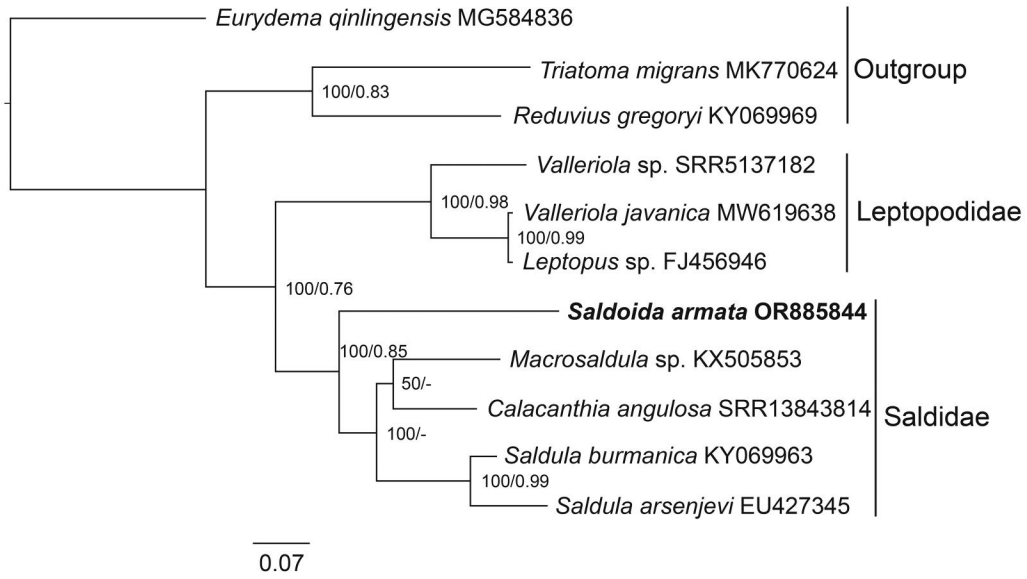

Figure 3. Phylogenetic tree of *Saldoida armata* and other related species based on 13 protein-coding genes and 2 rRNA genes. The numbers at the nodes separated by '/' indicate analyses based on either the ML (left) or BI (right) methods, respectively. '-' are lack of Bayesian posterior probabilities indicates that the nodes were not recovered in the BI analysis. GenBank accession numbers of each mitogenome are given after the species name, and the bootstrap value based on 1000 replicates is represented on each node. The bold font indicates the species sequenced in this study. The following sequences were used: *Eurydema qinlingensis* MG584836 (Zhao et al. 2019a); *Triatoma migrans* MK770624 (Zhao et al. 2019b); *Reduvius gregoryi* KY069969 (Liu et al. 2019); *Valleriola* sp. SRR5137182 (Wang et al. 2019); *Valleriola javanica* MW619638 (Ye et al. 2022); *Leptopus* sp. FJ456946 (Hua et al. 2009); *Macrosaldula* sp. KX505853; *Calacanthia angulosa* SRR13843814 (Ye et al. 2022); *Saldula burmanica* KY069963; *Saldula arsenjevi* EU427345 (Hua et al. 2008).

(trnK). Identification of the rRNA genes was accomplished through alignment with closely related species. The large (*l-rRNA*) and small (*s-rRNA*) ribosomal RNA genes span 1,249 bp and 766 bp, respectively. The control region is located between *s-rRNA* and *trnI* with 1,544 bp, and up to now, this size is the largest record in Saldidae.

Both phylogenetic trees exhibit the monophyly of Saldidae and Leptopodidae and the sister relationship of these two families (Figure 3), which is consistent with the previously study (Wang et al. 2020). However, The genus *Valleriola* is paraphyletic, with the sister relationship of *Valleriola javanica* and *Leptopus* sp. Furthermore, the systematic placement of *S. armata* was analyzed for the first time, suggesting a grouping with other shore bugs. The phylogenetic relationships of the three genera of Salicoridae were inconsistent between the two methods, with the topology displaying ((*Calacanthia* + *Macrosaldula*) + *Saldula*) by ML method and (*Calacanthia* + (*Macrosaldula* + *Saldula*)) by BI method.

Discussion and conclusion

The mitogenome of *S. armata* is 16,049 bp long, which is the largest in Saldidae so far, and this is mainly due to the large control region. The composition and the phylogenetic tree were predicted and analyzed in this research. Our findings provide basic data for future studies focusing on identification, population genetics and phylogeny in shore bugs.

Author contributions

Yunfei Wu: Conceptualization, analysis and interpretation of the data, writing original draft; Xu Liu and Fan Zhang: DNA extraction and sequence assembly, annotation and analysis; Jiajia Wang: Funding acquisition, revised the manuscript critically for intellectual content, phylogenetic tree constructing and analysis. All authors agree to be accountable for all aspects of the work.

Ethical approval

Experiments were performed in accordance with the recommendations of the Ethics Committee of College of Biology and Food Engineering, Chuzhou University. These policies were enacted according to the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The mitochondrion genome sequence data that support the findings of this study are openly available in GenBank of the National Center for Biotechnology Information (NCBI) at https://www.ncbi.nlm.nih.gov/ under the accession no. OR885844. The associated BioProject, Bio-Sample numbers, and SRA are PRJNA1047902, SAMN38599069, and SRR27027481, respectively.

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