

The complete mitochondrial genome of *Anidiocerus bimaculatus* Zhang, Li & Qi, 2008 (Hemiptera: Cicadellidae: Eurymelinae: Idiocerini)

Ya-Dong Wang, Jun Du, Bing-Zhen Pan and Jia-Jia Wang

College of Biology and Food Engineering, Chuzhou University, Chuzhou, China

ABSTRACT

In this study, the complete mitochondrial genome of *Anidiocerus bimaculatus* was sequenced and annotated for the first time, which belongs to the subfamily Eurymelinae. The mitogenome of *A. bimaculatus* was 15,267 bp in length and contained 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and one non-coding control region. In this mitogenome, all the PCGs are initially encoded by ATT, ATA, ATG, or TTG, and terminated by TAA, or single T. The overall base composition of *A. bimaculatus* is 43.6% adenines, 36.0% thymines, 9.1% guanines, and 11.3% cytosines. ML phylogenetic analyses confirmed that Idiocerini forms a monophyletic clade and the newly sequenced *A. bimaculatus* clustered within the Idiocerini clade based on 13 protein-coding genes and two rRNA genes.

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Introduction

Eurymelinae stands out as one of the largest subfamilies within the family Cicadellidae (Tian et al. 2022). Species in this group are small, with short and wide head capsules, often wedge-shaped. They exhibit a diverse feeding behavior on various trees and shrubs, including some of economic importance, with certain members being recognized as agricultural pests (Gnanesan et al. 2007; Fletcher and Dangerfield 2002). Widely distributed across the globe, the Eurymelinae subfamily encompasses over 1,400 species, organized into 182 genera across 11 tribes (Tian et al. 2022). Notably, the tribe Idiocerini, initially classified as the subfamily Idiocerninae of the family Cicadellidae, has now been confirmed as one of the tribes within the subfamily Eurymelinae (Xue et al. 2020). At present, the Idiocerini tribe presently includes around 70 genera and 500 species. The endemic Chinese Idiocerini genus, *Anidiocerus* Maldonado-Capriles (1976), gained recognition with the description of its type species, *A. variabilis* Maldonado-Capriles, identified in Taiwan (Maldonado-Capriles 1976). Subsequent contributions by Cai and Shen introduced *A. flavidus* from Henan (Cai and Shen 1998), Zhang and Li described *A. fanjingensis* from Guizhou (Zhang and Li 2005), Zhang et al. added *A. bimaculatus* from Hainan, and *A. curvatus* from Guizhou (Zhang et al. 2008), while Xue et al. contributed *A. longimus* from Zhejiang and *A. brevispinus* from Shaanxi (Xue et al. 2013). In 2017, Xue et al. expanded the genus by including *A. fasciatus* from Yunnan (Xue et al. 2017). Currently, there are eight

recognized species in this genus, though mitochondrial genome data for these species has yet to be reported.


In this study, we contribute to the understanding of this genus by reporting the complete mitochondrial genome of *A. bimaculatus*. This genetic information serves as a valuable resource for future studies across various scientific disciplines.

Materials and methods

A. bimaculatus specimen was collected from Jianfeng Town, Ledong Li Autonomous County, Hainan Province, China (18° 69' 52"N, 108° 79' 14"E) in June 2022. A species reference image for the species is shown in Figure 1. The identification characteristics of *A. bimaculatus* are pygofer with ventral inner process fork-shaped, aedeagus without process or process very short tooth-like, and pronotum with large black rounded marking on lateral margin (Xue et al. 2017). Total genomic DNA was extracted from the specimen using a QIAamp® DNA Micro Kit (Qiagen, Germany). The specimen and genome DNA was deposited at -80°C in the Animal Collection of Chuzhou University with the voucher number GZCI23 (Yan Dong, dongyan_bio@126.com).

The genome was sequenced by the next-generation sequencing method with the Illumina HiSeq 2500 platform with 150 bp paired-end reads and 350 bp insert size. The mitogenome was assembled using Geneious prime 2023.2.1 (www.geneious.com). The reference sequence for the raw data assembly of the *A. bimaculatus* mitogenome is *Idioscopus clypealis* (MF784430, Dai et al. 2018). The mitogenome was

CONTACT Jia-Jia Wang  wjchuzhou01@126.com  College of Biology and Food Engineering, Chuzhou University, Chuzhou, China.

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annotated by MitoZ 2.4 (Meng et al. 2019) and then confirmed by alignment with homologous genes from closely related species in Geneious software. Additionally, Inverted Repeats Finder (<https://tandem.bu.edu/irf/home>) was used to calculate repeats with default parameters (Warburton et al. 2004).

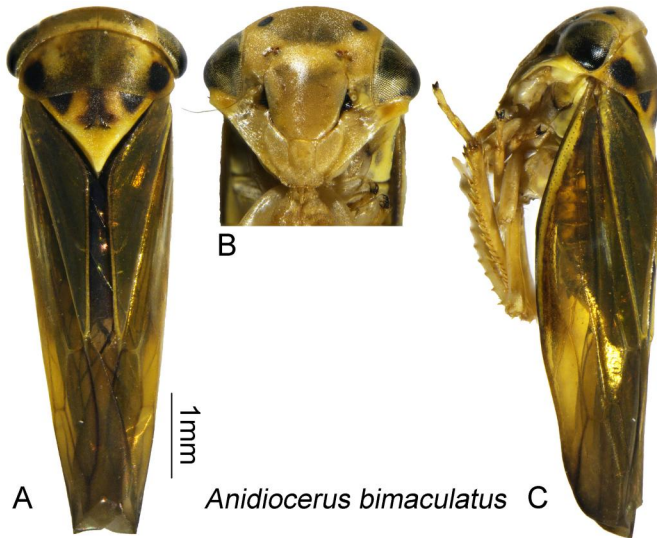


Figure 1. *Anidiocerus bimaculatus* Zhang et al. (2008) image. Photographed and processed by Ya-Dong Wang at Chuzhou University. (A) dorsal view; (B) face; (C) lateral view. Scale bar = 1.0 mm.

As of April 2024, among the 11 tribes within the Eurymelinae subfamily, only the Idiocerini and Macropsini tribes have published complete mitogenome data. In the phylogenetic analyses, 28 currently available complete mitogenome species of the Eurymelinae retrieved from GenBank were used as ingroups. Two species from closely related families *Alobaldia tobae* (KY039116) and *Yanocephalus yanonis* (KY039113, Song et al. 2017) were used as outgroups. The phylogenetic tree was reconstructed with Maximum Likelihood (ML) methods based on the 13 PCGs and 2 rRNAs by IQ-TREE v2.1.3 (Minh et al. 2020). Each gene was aligned using MAFFT 7 (Kato and Standley 2013) and trimmed with trimAl 1.4.1 (Capella-Gutiérrez et al. 2009), then concatenated individual genes using MEGA X (Kumar et al. 2018).

Results

In this research, 4.9 Gb clean data was obtained by a single library and produced a final mitogenome for *A. bimaculatus* with an average sequencing depth of 147.6× (Figure S1). The assembled mitogenome of *A. bimaculatus* (GenBank accession No: OR900075) exhibited a length of 15,267 bp. It comprises 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and a non-coding control region (Figure 2). The nucleotide composition of *A. bimaculatus* mitogenome is 43.6% adenines, 36.0% thymines, 9.1% guanines, and 11.3% cytosines, with a G + C content of 20.4%. The total length of the 13 PCGs is 10,938 bp, encoding 3,635 amino acids, constituting 71.6% of

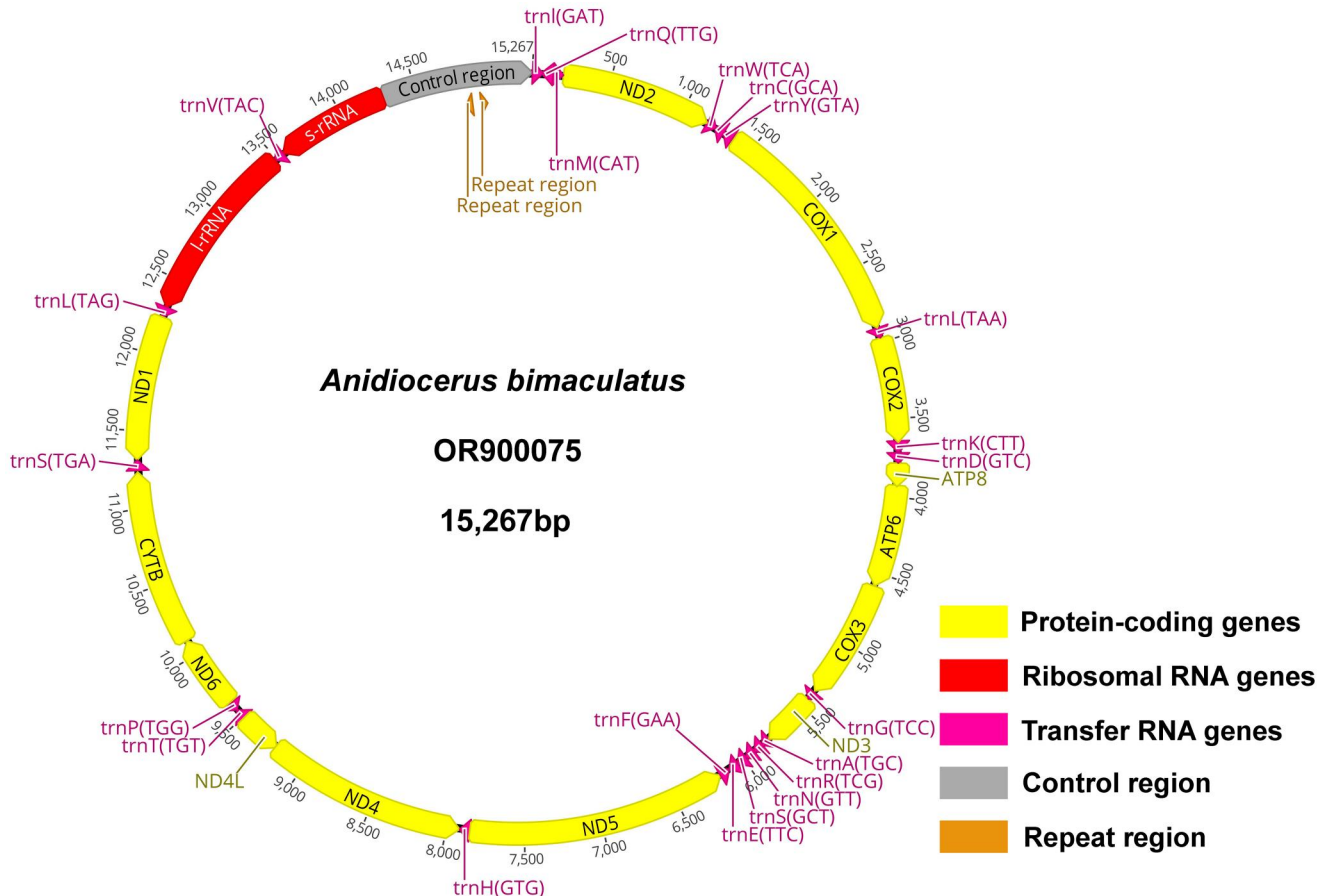


Figure 2. Mitochondrial genome map of the *Anidiocerus bimaculatus*. A circular mitochondrial genome map was drawn using Geneious prime 2023.2.1.

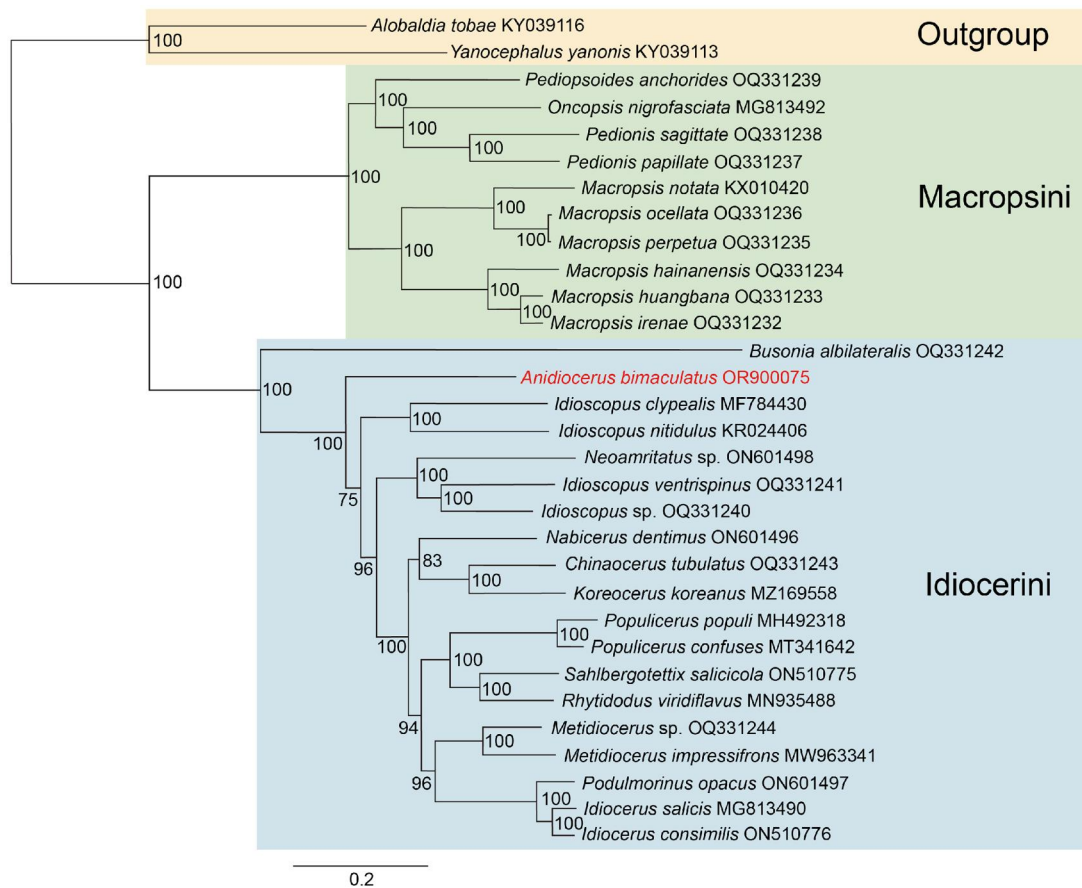


Figure 3. Phylogenetic tree of *Anidiocerus bimaculatus* and other related species based on 13 PCGs and 2 rRNAs. Bootstrap value based on 1000 replicates is represented on each node. GenBank accession numbers of each mitogenome are given after the species name, with the species sequenced in this study indicated in red. The following sequences were used: *Idioscopus nitidulus* KR024406 (Choudhary et al. 2018); *Populicerus populi* MH492318; *Idiocerus salicis* MG813490 (Wang et al. 2018); *Metidiocerus impressifrons* MW963341 (Ma et al. 2022); *Idioscopus clypealis* MF784430 (Dai et al. 2018); *Populicerus confuses* MT341642 (Shan et al. 2020); *Alobaldia tobae* KY039116; *Yanocephalus yanonis* KY039113 (Song et al. 2017); *Podulmorinus opacus* ON601497; *Neoamritatus* sp. ON601498; *Idiocerus consimilis* ON510776; *Nabicerus dentimus* ON601496; *Sahlbergotettix salicicola* ON510775 (Tian et al. 2022); *Macropsis notata* KX010420; *Oncopsis nigrofasciata* MG813492 (Wang et al. 2020); *Rhytidodus viridiflavus* MN935488 (Di et al. 2020); *Koreocerus koreanus* MZ169558 (Yu and Li 2021); *Idioscopus ventrispinus* OQ331241; *Pediopsoides anchorides* OQ331239; *chinaocerus tubulatus* OQ331243; *Macropsis hainanensis* OQ331234; *Busonia albilateralis* OQ331242; *Pedionis sagittate* OQ331238; *Macropsis irenae* OQ331232; *Macropsis huangbana* OQ331233; *Macropsis ocellata* OQ331236; *Macropsis perpetua* OQ331235; *Idioscopus* sp. OQ331240; *Metidiocerus* sp. OQ331244; *Pedionis papillate* OQ331237 (Yu 2023).

the complete mitogenome. Among these, four PCGs (*ND5*, *ND4L*, *ND4*, and *ND1*) were encoded by the minority strand (N-strand), while the other nine were located on the majority strand (J-strand). Among the 13 PCGs, 11 PCGs are initiated by ATT, ATA, or ATG codons, *ATP8* and *ND5* use the TTG as the start codon. Termination codons TAA were observed in all PCGs, except for *ND4*, *ND5*, and *COX2* which concluded with a single T.

The total length of the 22 tRNAs was 1,441 bp, with sizes ranging from 62 bp (*trnA*) to 74 bp (*trnK*), and A + T content varying from 71.2% *trnS* (GCT) to 89.1% *trnD*. Ribosomal RNA genes were identified through comparison with *Busonia albilateralis* OQ331242 (Yu 2023). The *l-rRNA* (1182 bp) was positioned between *trnL* (TAG) and *trnV*, while the *s-rRNA* (740 bp) was located between *trnV* and the control region, with A + T contents of 81.4% and 81.2%, respectively. The control region, spanning 971 bp, was situated between *s-rRNA* and *trnI*. The control region contains two repeat sequences, each 45 bp long.

The resulting phylogenetic tree supports the monophyly of the tribes Idiocerini and Macropsini within the subfamily

Eurymelinae (Figure 3), which is generally consistent with the previous study (Xue et al. 2020).

Discussion and conclusion

In this study, we conducted predictions and analyses on the composition and structure of the mitochondrial genome of *A. bimaculatus*, marking the first complete mitochondrial genome within the genus *Anidiocerus*. The mitochondrial genome length of *A. bimaculatus* (15,267bp) is close to that of other Idiocerini species, ranging from 14,733 bp (*Sahlbergotettix salicicola*, ON510775, Tian et al. 2022) to 16,842 bp (*Rhytidodus viridiflavus*, MN935488, Di et al. 2020). The observed phylogenetic tree topology in this study is mostly consistent with previous investigations on Idiocerini (Tian et al. 2022). The results indicate that *A. bimaculatus* is clustered within the Idiocerini clade. The information obtained holds valuable insights for understanding *A. bimaculatus* and future genomic research on this species. We anticipate that the findings presented here will catalyze subsequent investigations into the phylogenetic relationships within the Idiocerini.

Ethical approval

Experiments were performed in accordance with the recommendations of the Ethics Committee of the 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.

Author contributions

Y-D W: Conception and drafting of the work; JD and B-Z P: Acquisition, analysis, and interpretation of data for the work; J-J W: Final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

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 the findings of this study are openly available in the GenBank of NCBI at (<https://www.ncbi.nlm.nih.gov>) under accession no. OR900075. The associated Bio-Project, SRA, and Bio-Sample accession numbers are PRJNA1050006, SRR27213199, and SAMN38724029 respectively.

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