MITOCHONDRIAL DNA PART B: RESOURCES
2024, VOL. 9, NO. 2, 257–261
https://doi.org/10.1080/23802359.2024.2311745

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MITOGENOME REPORT

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The complete mitochondrial genome of *Aphidius colemani* (Hymenoptera: Braconidae: Aphidiinae)

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ABSTRACT

The genome-level features are crucial genetic resources for species identification and phylogenetic analysis. Here, the complete mitochondrial genome of *Aphidius colemani* Viereck 1912 (Hymenoptera: Braconidae: Aphidiinae) was sequenced, determined and analyzed. The circular genome is 16,372 bp in length with an overall base composition of 38.9% for A, 46.2% for T, 6.7% for C, and 8.2% for G. The mitochondrial genome of *A. colemani* contained 13 protein-coding genes that initiated by the ATN codon, 22 transfer RNA genes, two ribosomal RNA genes (rRNAs), and a control region (CR). It shared the same gene arrangement patterns that occurred in two tRNA clusters of *trnI-trnQ-trnM* and *trnW-trnC-trnY* with *Aphidius gifuensis*. Phylogenetic analyses using Bayesian inference and Maximum-likelihood methods supported that the two species of Aphidiinae formed a clade and sister to other subfamilies of Braconidae.

ARTICLE HISTORY
Received 13 September 2023
Accepted 24 January 2024

KEYWORDS
Mitochondrial genome; braconidae; *aphidius colemani*

Introduction

Hymenopteran mitochondrial genomes typically show high A + T content (Cha et al. 2007), strong base composition bias (Dowton & Austin et al. 1997), and extensive gene rearrangements (Dowton et al. 2003). Therefore, Hymenoptera is an important group for studying mitochondrial genomes in insects. Within the species-rich family Braconidae, patterns of mitochondrial gene rearrangement are used commonly for recovering phylogenetic relationships (Wei et al. 2010; Li et al. 2016; Feng et al. 2020). The subfamily Aphidiinae (Hymenoptera: Braconidae) are tiny endoparasitic wasps which exclusively utilize aphids as hosts, and some species of Aphidiinae are widely used as biological control agents of aphids (De Conti et al. 2008; Gadallah et al. 2022). This subfamily currently includes about 657 species classified into 63 genera worldwide (Gadallah et al. 2022). However, only two complete mitochondrial genomes (*Aphidius gifuensis* and *Binodoxys acalephae*) have been sequenced and annotated from the Aphidiinae (Feng et al. 2020; Xu et al. 2023). The parasitic wasp *A. colemani* Viereck 1912 is an important natural enemy insect that can be used to suppress the population of many aphid pests (Woolley et al. 2022; Stara et al. 2011; Figure 1). Here, we sequenced the complete mitochondrial genome of *A. colemani* and inferred the phylogenetic relationships among main subfamilies of Braconidae. The

Figure 1. Adult image of *Aphidius colemani* Viereck 1912 (Hymenoptera: Braconidae: Aphidiinae). The main distinguishing morphological characters including: filiform antennae with 15 or 16 segments and 0–2 longitudinal placodes; maxillary palpi 4-segmented and labial palpi 2-seg- mented; forewing venation incomplete, with R1 longer than stigma length; r&Rs same length than stigma but shorter than R1 and stigma with elongate triangular shape. The photo of species was taken by the first author Jia-Yu Lin in China Agricultural University, Beijing, China.

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2024.2311745.

results will contribute to our understanding of the mitochondrial genomic evolution and phylogeny of Aphidiinae (Wei et al. 2010; Tang et al. 2018).

Materials and methods

The specimens of *A. colemani* were collected from Dahe Township, Wuzhong city, Ningxia province (37.39°N, 105.90°E) and identified by Jiayu Lin and Shujun Wei. A voucher specimen is stored at the Entomological Museum of China Agricultural University (No. HYM013, Jia-Yu Lin, esther@cau.edu.cn). Total genomic DNA extraction was performed using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The Illumina TruSeq library was prepared with an average insert size of 350 bp and sequenced with the paired-end reads length of 150 bp on Illumina NovaSeq 6000 platform (Berry Genomic, Beijing, China). A total of 10.5 Gb raw data was obtained. We used Prinseq (Schmieder and Edwards 2011) to remove short and low-quality reads with the poly-Ns $>$ 15 bp, or $>$ 75 bp bases with a quality score $<$ 30. The remaining reads were *de novo* assembled using IDBA-UD version 1.0 (Peng et al. 2012), with minimum and maximum *k* values of 45 and 145 bp, respectively. Genes were annotated using the Geneious version 10.3 (Kearse et al. 2012) and MITOS Web Server (http://mitos2.bioinf.uni-leipzig.de/index.py; Bernt et al. 2013).

All 13 protein coding genes (PCGs) for 14 braconid species and two outgroup species (Ichneumonidae) were aligned and concatenated using MUSCLE by codon (Edgar 2004) to infer the phylogenetic relationships among subfamilies of Braconidae. The best fit substitute models of the partition schemes was determined by the program PartitionFinder2 with BIC scoring criteria (Lanfear et al. 2017). Phylogenetic trees were inferred with Bayesian inference (BI) implemented in MrBayes version 3.2.6 and maximum likelihood (ML) implemented in IQ-tree version 2.0.6 (Ronquist et al. 2012; Trifinopoulos et al. 2016).

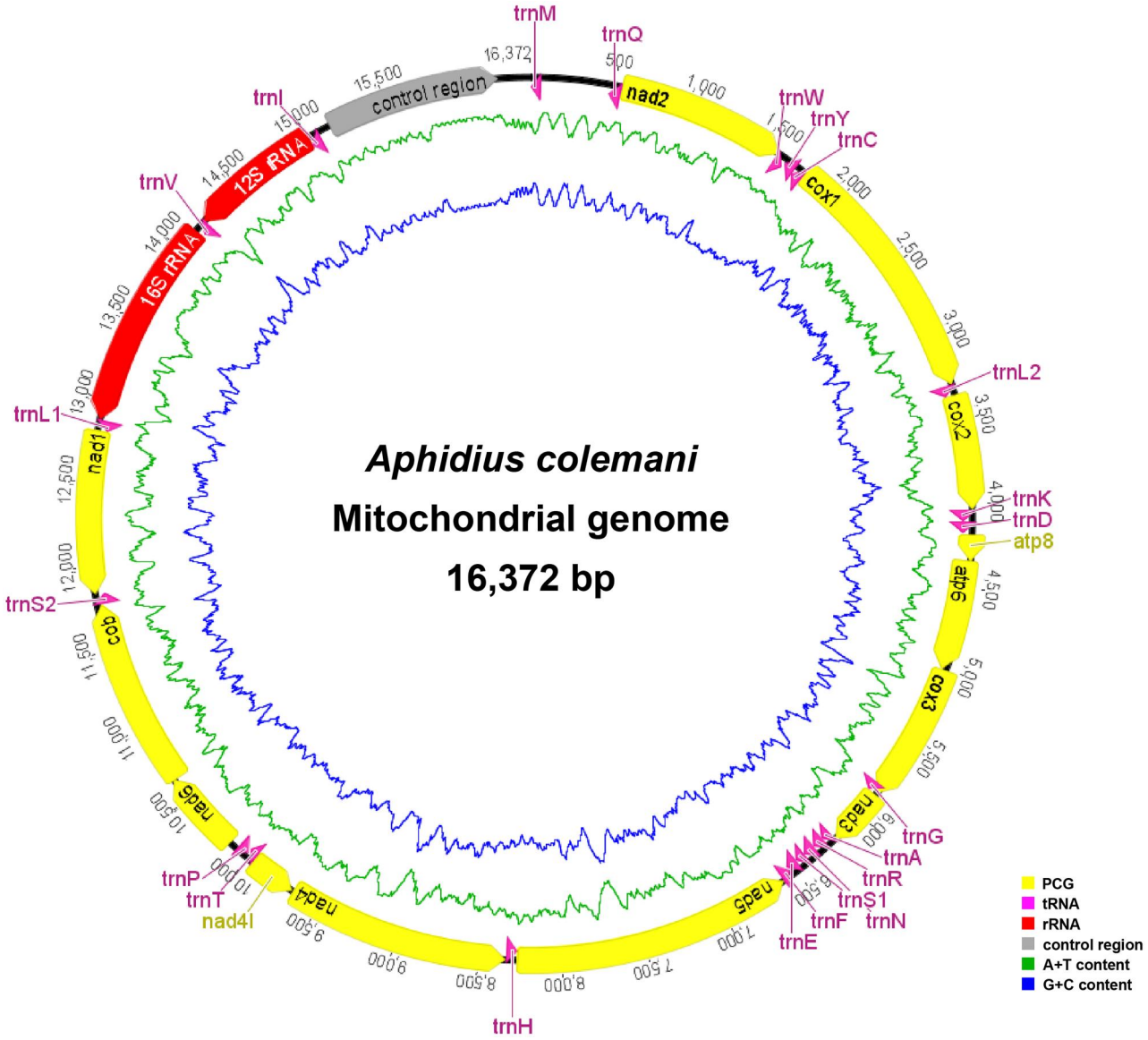

Figure 2. The mitochondrial genome map of *Aphidius colemani* Viereck 1912. The green line in the circle shows the a + T content, and the blue shows the G + C content. Protein-coding genes (PCGs) are shown as yellow arrows, transfer RNA (tRNA) genes as pink arrows, ribosomal RNA (rRNA) genes as red arrows, and control region as grey arrow. Arrows indicate the orientation of gene transcription.

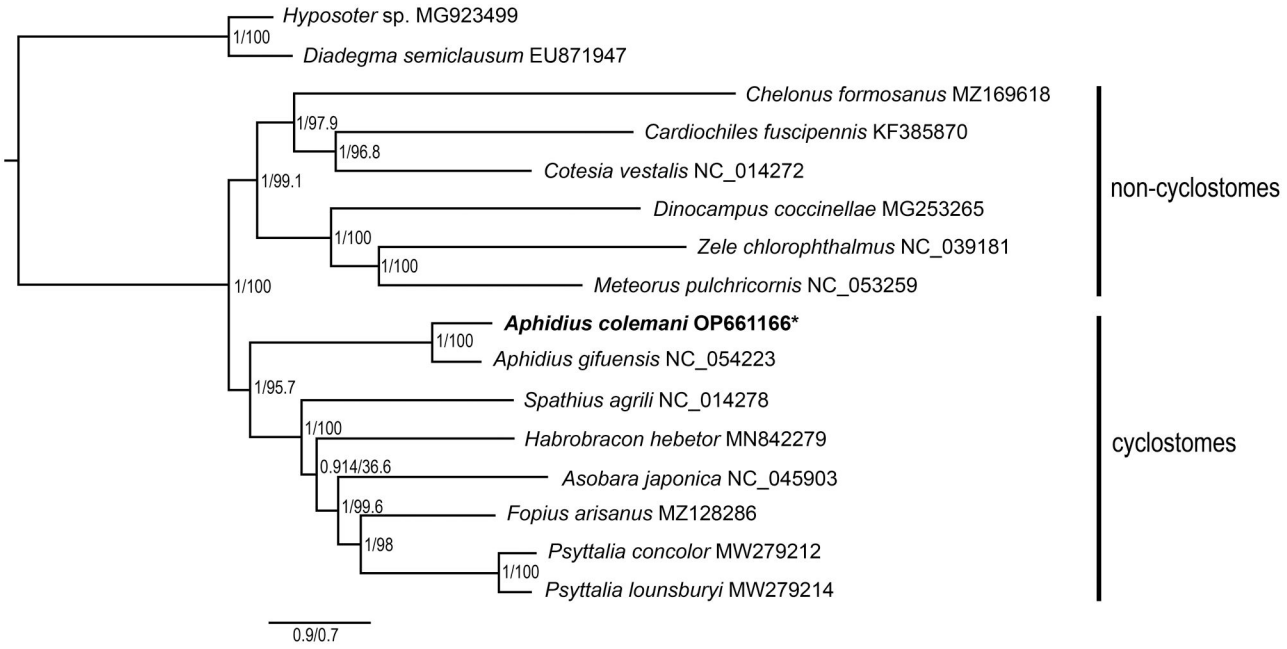

Figure 3. Phylogenetic relationships among subfamilies of Braconidae inferred from Bayesian and maximum-likelihood methods based on nucleotide sequences of 13 protein-coding genes. Values on the nodes represent the Bayesian posterior probabilities (left) and maximum-likelihood bootstrap support (right). * represents the newly sequenced mitochondrion genome in the present study. The following sequences were used to infer the tree: *Hyposoter* sp. MG923499 (Tang et al. 2018), *Diadegma semiclausum* EU871947 (Wei et al. 2009), *Chelonus formosanus* MZ169618, *Cardiochiles fuscipennis* KF385870, *Cotesia vestalis* NC_014272 (Wei et al. 2010), *Dinocampus coccinellae* MG253265, *Zele chlorophthalmus* NC_039181, *Meteorus pulchricornis* NC_053259 (Park et al. 2019), *Aphidius colemani* OP661166, *Aphidius gifuensis* NC_054223 (Feng et al. 2020), *Spathius agrili* NC_014278 (Wei et al. 2010), *Habrobracon hebetor* MN842279 (Huang et al. 2020), *Asobara japonica* NC_045903 (Zhang et al. 2020), *Fopius arisanus* MZ128286, *Psyttalia concolor* MW279212 (Powell et al. 2020), *Psyttalia lounsburyi* MW279214 (Powell et al. 2020).

Results

In total, 10,476.08 MB of clean data was obtained from the 10.5GB raw data after quality filtering. The complete mitochondrial genome of *A. colemani* (GenBank: OP661166) was 16,372 bp in length and encodes the typical 37 coding genes and a control region of insects (Figure 2). The average coverage depth of the mitochondrial genome was 2418× (Supplementary Figure S1). Base composition analyses indicated that the entire mitochondrial genome is significantly biased toward adenine (A) and thymine (T) with an A + T content of 85.1%. All 13 protein-coding genes (PCGs) utilize ATN as the start codon. Three start codons for PCGs were used: ATA (*nad2*, *nad1*, *atp6*, *atp8* and *cox3*), ATT (*cox2*, *nad3*, *nad4*, *nad5* and *nad6*), ATG (*cox1* and *cytb*), ATC (*nad4l*). The complete termination codon TAA or TAG was utilized by most of the PCGs, while the incomplete stop codon T or TA was employed only by *nad5* and *atp6*.

The tree topologies reconstructed by BI and ML analyses all supported the close relationships of *A. colemani* and *A. gifuensis* (Figure 3). The monophyly of the subfamily Aphidiinae was also recovered with high Bayesian posterior probabilities (BPP = 1) and ML bootstrap values (BSV = 100).

Discussion and conclusion

The mitochondrial genome of *A. colemani* was highly conserved in terms of gene content, gene size, base composition and PCG codon usage within the subfamily Aphidiinae (Xu et al. 2023). The nucleotide composition of the *A. colemani* was as follows: T (46.2%), A (38.9%), C (6.7%), and G (8.2%). The A + T content was 85.1% which is consistent with the characteristic of a strong A + T bias in insect mitochondrial genomes (Guo et al. 2023; Huang et al. 2023). It presented the same gene arrangement as another species, *A. gifuensis,* from the same subfamily, both of which include gene rearrangement events in two tRNA clusters of *trnI-trnQ-trnM* and *trnW-trnC-trnY* (Feng et al. 2020).

Both topologies reconstructed by BI and ML approaches supported that all species of Braconidae were clustered as a monophyletic group, in line with previous studies (Li et al. 2016; Feng et al. 2020; Xu et al. 2023). Previous studies recovered the monophyly of three main groupings of subfamilies in Braconidae (aphidioid complex, cyclostomes, and non-cyclostomes), as well as the sister relationship of the aphidioid complex with other cyclostomes sensu stricto (Chen and van Achterberg 2019; Jasso-Martínez et al. 2022a, 2022b). Our phylogenetic results also supported that Aphidiinae was the earliest branching lineage of cyclostomes, forming sister clades with the remaining subfamilies in cyclostomes.

Acknowledgments

We thank Dr. Bingyan Li and Aimin Shi for their assistance in this study.

Ethical approval

The specimen collection protocol was approved by the Ethics Committee of China Agricultural University. The studies did not involve endangered or protected species.

Authors' contributions

J.Y.L., S.J.W., and F.S. contributed to the conception and design of the research. J.Y.L., J.H., L.J.M., H.L.Y and S.J.W. performed experiments and analyzed the data. J.Y.L drafted the manuscript, and S.J.W. and F.S. revised the manuscript. All authors contributed to data interpretation and all authors approved the final version of the manuscript.

Disclosure statement

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

Funding

This research was supported by the China National Tobacco Corporation of Science and Technology Major Projects [No. 110202001036[LS-05]]and the Research and Development Program of Ningxia Hui Autonomous Region [2021BEF03002].

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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. OP661166. The associated BioProject, Bio-Sample and SRA numbers are PRJNA898984, SAMN31635309 and SRR22213786 respectively.

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