

The complete mitochondrial genome of *Exoristobia philippinensis* (Hymenoptera: Chalcidoidea: Encyrtidae) and phylogenetic analysis

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ABSTRACT

Exoristobia philippinensis (Hymenoptera: Encyrtidae) is a worldwide parasitic wasp. This work presents the mitochondrial genome (mitogenome) of *E. philippinensis* for the first time. The complete mitochondrial genome of *E. philippinensis* was sequenced and annotated, which was 15,751 bp in length, and encoded 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and two ribosomal RNA genes (rRNAs). All 13 PCGs were initiated by the ATN (ATG, ATT, and ATA) codon, terminated with the stop codon TAA except for *ND1* which ends with TAG. Phylogenetic analysis showed that *E. philippinensis* has a sister relationship with the genus *Lamennaisia*.

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Introduction

Exoristobia, a genus of Hymenoptera parasitic wasps, was established by Ashmead (1904) based on the type species *Exoristobia philippinensis* (Figure 1). *E. philippinensis* has a wide distribution in the world, and it is mainly distributed in Philippines, Cuba, India, the United States, and China (Noyes 2019). As a genus of parasitic wasps, *Exoristobia* mainly parasitizes the pupae of flies: Tachinidae, Sarcophagidae, Calliphoridae, Muscidae, Anthomyiidae, and Phoridae (Zu et al. 2021). Up to now, 13 species of *Exoristobia* have been reported worldwide (Zhang et al. 2022), but there are no reports on their complete mitochondrial genome. The present study undertook the first sequencing, annotation, and characterization of the mitogenome of *E. philippinensis*. This research provides a reference value for further study of Encyrtidae.

Materials and methods

Specimens of *E. philippinensis* were reared from *Lucilia sericata* (Diptera: Calliphoridae) in this study. *L. sericata* were collected at Haikou, Hainan Province, China (19.939 N, 110.172 E) in April 2023. The specimens of *E. philippinensis* were dissected and prepared for identification using a stereoscopic microscope, and then were identified as *E. philippinensis* by Dr. Guo-Hao Zu based on the morphological features. All specimens used for molecular experiments were stored in absolute ethanol and stored in a -40°C refrigerator. Voucher specimens of this study were deposited in the insect collections of Tianjin Agricultural University (Dr. Guo-Hao Zu,


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After morphological identification, total genomic DNA was extracted from tissues using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The qualified libraries were pooled and sequenced on Illumina platforms with PE150 strategy in Novogene Bioinformatics Technology Co., Ltd. (Beijing, China), according to effective library concentration and data amount required. Fastp 0.23.4 (Chen et al. 2018) was used to quality trim and retain high-quality reads with quality value >20 . 2.2 Gb clean reads were obtained and assembled using MitoZ v3.6 (Meng et al. 2019). We mapped the clean data onto the mitochondrial genome to obtain the sequencing coverage depth (Figure S1). By comparison with the homologous sequences of other Chalcidoidea species from GenBank, the mitochondrial genome of *E. philippinensis* was annotated using MITOS WebServer (<http://mitos2.bioinf.uni-leipzig.de/index.py>) under the invertebrate mitochondrial code (Donath et al. 2019).

All 13 PCGs were extracted from the complete mitochondrial DNA sequences of 15 closely related taxa of Encyrtidae. One species from Chalcididae, *Haltichella nipponensis* (GenBank accession number NC_060544), was used as an outgroup (Zhao et al. 2021) (Table S1). To investigate the phylogeny of *Exoristobia* in Encyrtidae, the family-level relationships within Chalcidoidea were reconstructed by using 13 PCGs with two inference methods (BI and ML).

First, gene alignments were refined using the codon-aware program MACSE v2.06 (Ranwez et al. 2018). Ambiguously aligned fragments of 13 alignments were removed in batches using Gblocks 0.91b (Talavera and Castresana 2007). Gene

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alignments were concatenated using PhyloSuite v.1.2.3 (Zhang et al. 2020; Xiang et al. 2023). The best-fit model GTR+R4+F was selected by using the Bayesian information criterion (BIC) in ModelFinder v2.2.0 (Kalyaanamoorthy et al. 2017). Maximum-likelihood (ML) phylogenies were inferred using IQ-TREE v2.2.0 (Nguyen et al. 2015) under the GTR+R4+F model for 1000 standard bootstraps to estimate node reliability. The Bayesian inference phylogenies were inferred using MrBayes v3.2.7a (Ronquist et al. 2012) with two parallel runs, four Markov chains, and 2,000,000

generations. The first 25% of trees were discarded as burn-in. After the average standard deviation of split frequencies fell below 0.01, stationarity was assumed.

Results

The complete mitochondrial genome of *E. philippinensis* is 15,751 bp (GenBank accession number OR575036). It consists of 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and a control region (CR) (Figure 2). The nucleotide composition of the mitochondrial genome is biased toward A and T, with 83.6% of A + T content (45.5% for A, 10.4% for C, 6% for G, and 38.1% for T). Among 13 PCGs, ATT as a start codon is used for ND1, ND2, ND3, ND4L, ND5, ATP8, COII, ATG for ND4, ND6, ATP6, COIII, CYTB, and ATA for COI; as the stop codons, TAA for all PCGs except ND1, TAG for ND1. The 22 tRNA genes vary from 62 bp (*trnS1*) to 72 bp (*trnK*). Two rRNA genes (*rnl* and *rns*) are located at *trnL1/trnA* and *trnA/trnV* regions, respectively. The lengths of *rnl* and *rns* in *E. philippinensis* are 1309 and 822 bp, with different AT contents of 88.3% and 88.7%. The entire complete sequence contains eight overlapping gene regions, with the largest region spanning 10 bp (*trnS2/ND1*) and the shortest region spanning only 1 bp (*trnY/ND3*). The intergenic regions consist of 22 segments, ranging from 1 bp (*trnH/ND4*, *ND6/CYTB*, *trnA/rns*) to 69 bp (*trnG/COIII*) in length (Table S2).

In the selection of the control region, we assumed that the sequence with a length of 797 bp between *trnW* and



Figure 1. The morphological characteristics of *Exoristobia philippinensis*. This picture was taken by Zhi-Peng Chen at Xiqing District, Tianjin, China.

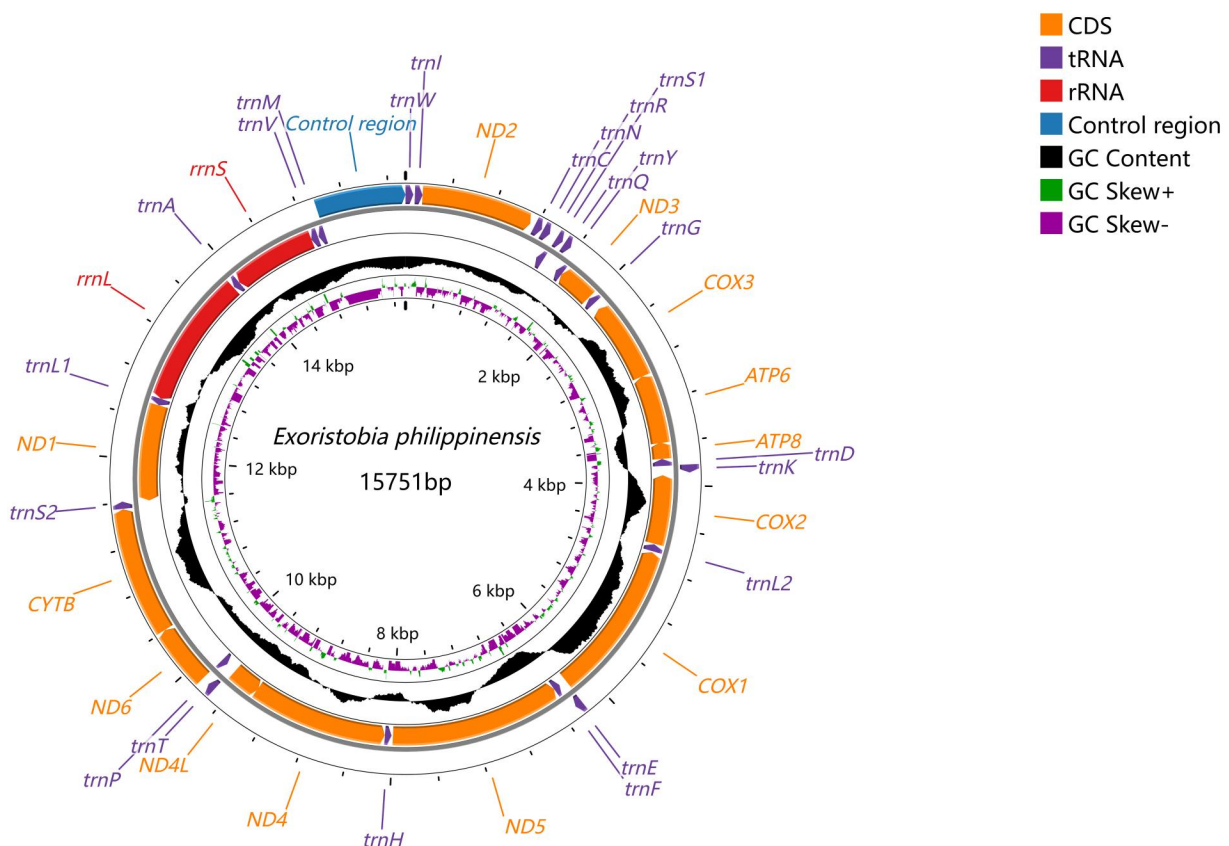


Figure 2. The circular-mapping mitochondrial genome of *Exoristobia philippinensis*. Gene names on the outside line side indicated that these genes were located on the H-strand, whereas the others were located on the L-strand. Color codes for different genes are listed on the map.

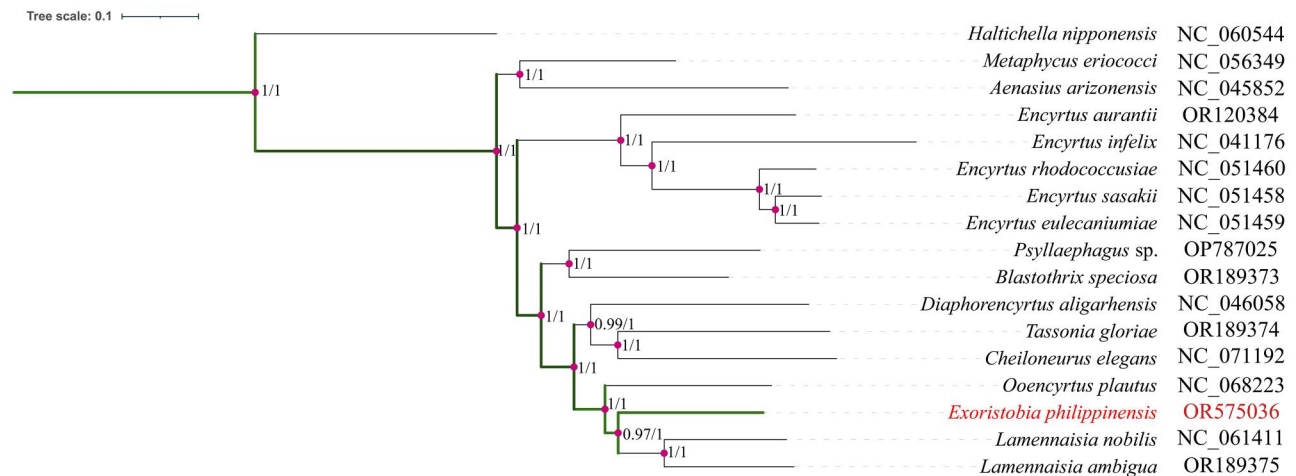


Figure 3. The phylogenetic tree was constructed by Bayesian's inference and maximum-likelihood methods. Each species involved in the tree has scientific name on the right side. The number at each node indicates the posterior probability and bootstrap values resulting from the analyses (BI on the left and ML on the right). Label annotations against each species name represent the accession number on GenBank. The following sequences were used: *Haltichella nipponensis* NC060544 (Zhao et al. 2021), *Metaphycus eriococci* NC_056349 (Zhou et al. 2021), *Aenasius arizonensis* NC_045852 (Ma et al. 2019), *Encyrtus infelix* NC_041176 (Xiong et al. 2019), *Encyrtus rhodococcisiae* NC_051460 (Rudoy et al. 2022), *Encyrtus sasakii* NC_051458 (Rudoy et al. 2022), *Encyrtus eulecaniumiae* NC_051459 (Rudoy et al. 2022), *Diaphorencyrtus aligarhensis* NC_046058 (Du et al. 2019), and *Ooencyrtus plautus* NC_068223 (Xing et al. 2022). *Blastothrix speciosa*, *Cheiloneurus elegans*, *Encyrtus aurantia*, *Lamennaisia ambigua*, *Lamennaisia nobilis*, and *Tassonia gloriae* were submitted by Tianjin Agricultural University and not published. *Psyllaephagus* sp. was submitted by Qin, Z and not published.

trnM as the control region. To validate the above conjecture, we examined the spacing and overlap patterns of the identified genes within the mitochondrial genome of Encyrtidae, and the results indicated that *E. philippinensis* is similar to other Encyrtidae members in relation to gene organization and composition (Zhang et al. 2019; Zhou et al. 2021). Based on the above findings, we compared the complete mitochondrial genome sequences of 11 other Encyrtidae on NCBI and found that the interval between tRNAs did not exceed 100bp, and their control regions were all located between *ND2* and *rrnS*. They were all close to *trnM* except for *Metaphycus eriococci*. This comparative analysis supported our initial finding, indicating that the control region is indeed located between *trnW* and *trnM*, and its length is 797 bp.

Discussion and conclusions

It can be obtained by phylogenetic analysis (Figure 3). The relative species of *E. philippinensis* is *Lamennaisia nobilis* and *Lamennaisia ambigua*, and the sister group of *Exoristobia* is *Lamennaisia*. From the perspective of the host, Coleoptera serves as the host for both *E. philippinensis* and *L. ambigua*. This host relationship provides further evidence to support the correctness of the phylogenetic analysis conducted. But from the perspective of traditional taxonomy, the genus of *Exoristobia* belongs to the tribe Cheiloneurini, and the genus of *Lamennaisia* does not have any recorded taxonomic system of tribe association. Based on the results of phylogenetic analysis, the phylogenetic relationship between *Lamennaisia* and Cheiloneurini in traditional taxonomy can be further elucidated.

After comparing and analyzing the complete mitochondrial genome of *E. philippinensis* with the complete mitochondrial genome of related species, its position in the known Encyrtidae is determined, which provides valuable insights into the evolutionary history of *Exoristobia* and its relatives,

enabling us to draw conclusions about their shared ancestry and divergence patterns.

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Author contributions

Zhi-Hao Chi and Cheng-Hui Zhang contributed significantly to analysis and manuscript preparation. Zhi-Hao Chi, Cheng-Hui Zhang, Wen-Yu Cui, and Hai-Yang Wang performed the experiments and data analyses. Zhi-Peng Chen selected species and took species photos. Guo-Hao Zu and Zhi-Hao Chi were involved in the revising paper critically for intellectual content. Guo-Hao Zu contributed to the conception and design of the study and the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

Ethics statement

The specimen *Lucilia sericata* used and the parasitic *Exoristobia philippinensis* used in this study comply with international ethical requirements. Our practices are in full compliance with Chinese legislation, as well as adhering to pertinent guidelines, policies, and regulations. Notably, the collection site is not designated as a nature reserve, and the acquisition of specimens was executed without causing harm to the local ecology. Furthermore, all experiments were carried out under the vigilant supervision and with the explicit permission of Tianjin Agricultural University.

Disclosure statement

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

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Data availability statement

The genome sequence data that support the findings are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/nuccore/2593598954> under the accession number OR575036. The associated BioProject, SRA, and BioSample accession numbers are PRJNA1053862, SRR27454293, and SAMN38877417, respectively.

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