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MITOGENOME REPORT

Complete mitochondrial genome of *Laeocathaica amdoana* Möllendorff, 1899 and phylogenetic analysis of Camaenidae (Gastropoda: Stylommatophora: Helicoidea)

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ABSTRACT

The first complete mitochondrial genome of the dart sac-bearing camaenid *Laeocathaica* Möllendorff, 1899 was sequenced and analyzed in this study. The whole mitogenome of *Laeocathaica amdoana* Möllendorff, 1899 was 14,660 bp in length and its nucleotide composition showed high AT-content of 67.45%. It had 37 genes, including 13 protein-coding genes, two ribosomal RNA genes, and 22 transfer RNA genes. The phylogeny yielded by both Bayesian inference and maximum-likelihood method suggested that *Laeocathaica* was closely related to the other dart sac-bearing camaenids with known complete mitochondrial genome. These genetic data are expected to provide fundamental resources for further genetic studies on the camaenids.

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1. Introduction

Laeocathaica amdoana Möllendorff, 1899 belongs to the genus *Laeocathaica* Möllendorff, 1899, a group of dart sac-bearing camaenids (Möllendorff 1899). *L. amdoana* is characterized by a typical *Laeocathaica* sinistral shell and anatomically, two tiny proximal accessory sacs and each has an opening leading to the dart sac chamber. This species is endemic to NW Sichuan and S Gansu, China (Wu et al. 2023).

At present, the public nucleotide database has no information pertaining to the mitogenome of this genus. For the first time, we sequenced, annotated, and analyzed the complete mitochondrial genome of *L. amdoana*, which might provide new data for the reconstruction of the camaenid phylogeny.

2. Materials

The specimen (voucher no. HBUMM08456, Figure 1) was collected from Shichuanba, Wenxian County, Gansu Province (33.17534°N, 105.019362°E) and deposited in Hebei University (contact Min Wu: minwu1969@aliyun.com). The reference images were prepared using a Canon camera. Species identification was based on shell morphology (Figure 1(A)) and genital anatomy according to the diagnostic characteristics proposed by Wu et al. (2023). The elongated part between atrium and dart sac apparatus and two tiny proximal accessory sacs (Figure 1(B)) make this species clearly different from those species with similar shells in the genus *Laeocathaica*, i.e. *L. distinguenda* Möllendorff, 1899 (in which the part between atrium and dart sac is not elongated) and *L. tropidorhaphe* Möllendorff, 1899 (in which the proximal accessory sac is absent) and is obviously *L. amdoana*.

3. Methods

3.1. DNA extraction, sequencing, and assembly

We sequenced the mitogenome of *L. amdoana* using the next-generation sequencing (NGS) techniques. Genomic DNA was extracted using CTAB method. Raw data were generated using Illumina Novaseq6000 platform. The lengths of reads and inserted sequence are 2 × 150 bp and 400 bp, respectively. Software fastp v0.36 (Chen et al. 2018) was applied to filter raw data. After quality control (QC), the clean reads were assembled via SPAdes v3.15 (Bankevich et al. 2012). Then, we used blastn (Altschul et al. 1997) to compare scaffolds with those existing sequences in the NT database (NCBI) to investigate sequence similarity. GapFiller v1.11 (Boetzer and Pirovano 2012) was used to supplement gaps. The sequences were checked and corrected using PrInSeS-G (Massouras et al. 2010) before the complete circular mitogenome sequence was obtained.

3.2. Annotation of mitogenome

The limits of protein-coding genes (PCGs) were adjusted with aids of genewise (Birney et al. 2004) and tblastn (Altschul

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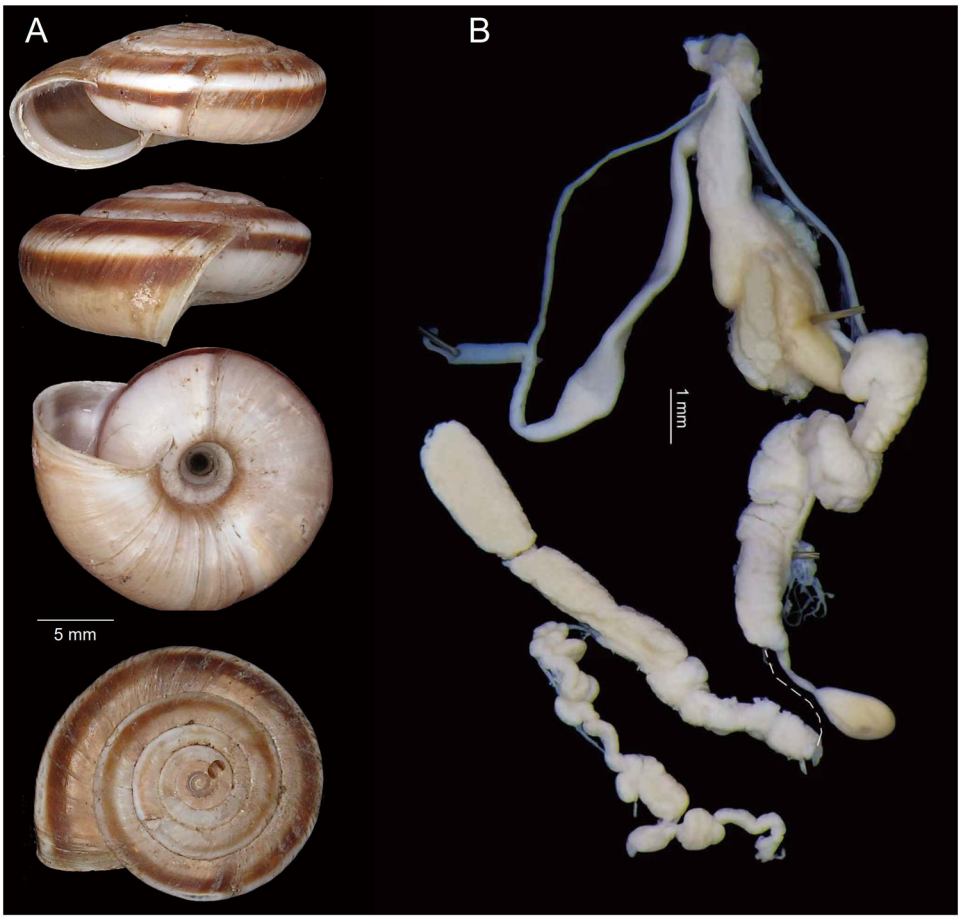

Figure 1. Species reference images of *Laeocathaica amdoana* Möllendorff, 1899 (voucher no. HBUMM08456).

et al. 1997) against the mitogenomic sequences of other assumed related species. Ribosomal RNA genes (rRNAs) were predicted using cmsearch (Nawrocki and Eddy 2013) based on the CM models constructed by mitos (Bernt et al. 2013). Transfer RNA genes (tRNAs) were identified with MiTFi (Li et al. 2013). After predicting the gene positions by softwares, we manually checked and adjusted the boundaries of every gene according to the criteria mentioned by both Fourdrilis et al. (2018) and Ghiselli et al. (2021). We used a circos plot (Figure 2) to display the annotation results.

3.3. Phylogenetic analysis

Complete mitochondrial genomes of two species of Helicidae and 11 camaenids including that sequenced by this work, all known so far, were obtained from NCBI for reconstructing the phylogeny of Camaenidae. We use helicids *Helix pomatia* Linnaeus, 1758 and *Cylindrus obtusus* Draparnaud, 1805 as the outgroups, considering that phylogenetically Helicidae and Bradybaenidae (sensu Wade et al. 2001) (=Bradybaeninae) are almost the nearest relatives based on *5.8S* gene, the complete internal transcribed spacer 2 (*ITS2*) region, and approximately 840 nucleotides of the large subunit (*28S*) gene (Wade et al. 2001, 2007). In addition, the mitochondrial genome of camaenid *Euhadra herklotsi* was sequenced in 1997, and was submitted to GenBank in nine separate parts (see Figure 3), instead of a complete circular genome, so we synthesized these nine records to obtain 37 genes (for details of the complete mitogenome that we organized, see supplementary material 1).

The extraction and alignment of PCGs and rRNA genes were performed with PhyloSuite v1.2.3 (Xiang et al. 2023). Ambiguously aligned fragments of 13 PCG alignments were removed in batches using Gblocks 0.91b (Talavera and Castresana 2007) with default parameter settings, and gap sites of rRNA genes were removed with trimAl v1.2rev57 (Capella-Gutiérrez et al. 2009) using '-automated1' command. DAMBE v7.3.32 (Xia 2018) was employed to make the saturation test for every gene of PCGs and rRNAs. Unsaturated sequences were concatenated in the same order for subsequent analyses. Best-fit partition model (Edge-linked, Table 1) was selected under BIC criterion using ModelFinder (Kalyaanamoorthy et al. 2017). Bayesian inference phylogenies were inferred using MrBayes 3.2.7a (Ronquist et al. 2012) under partition model (two parallel runs, 530,000 generations), in which the initial 25% of the sampled data were discarded as burn-in. Maximum-likelihood phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under Edge-linked partition model for 5000 ultrafast (Minh et al. 2013) bootstraps.

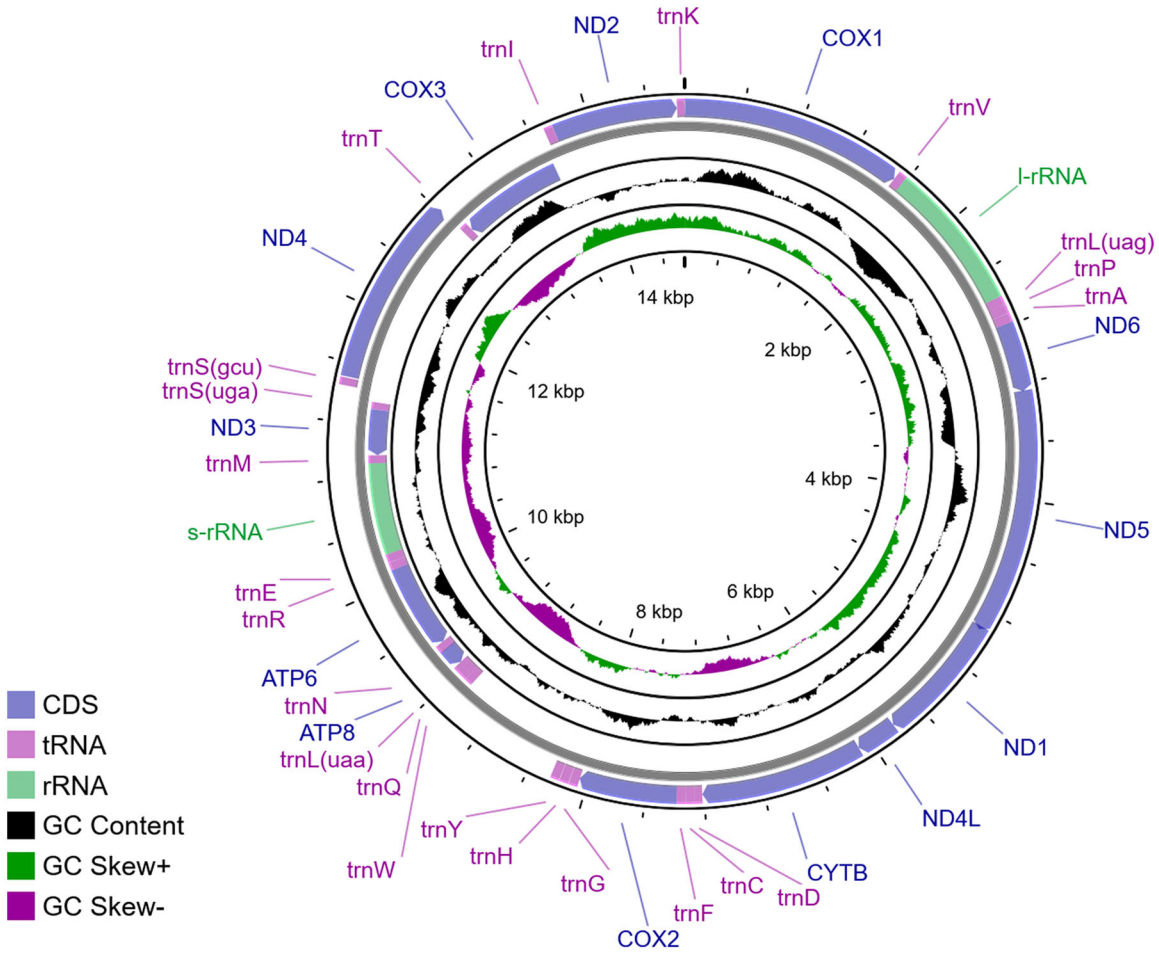

Figure 2. The mitochondrial genome of *Laeocathaica amdoana* Möllendorff, 1899, HBUMM08456 (OP866270). Arrows indicate the directions of transcription.

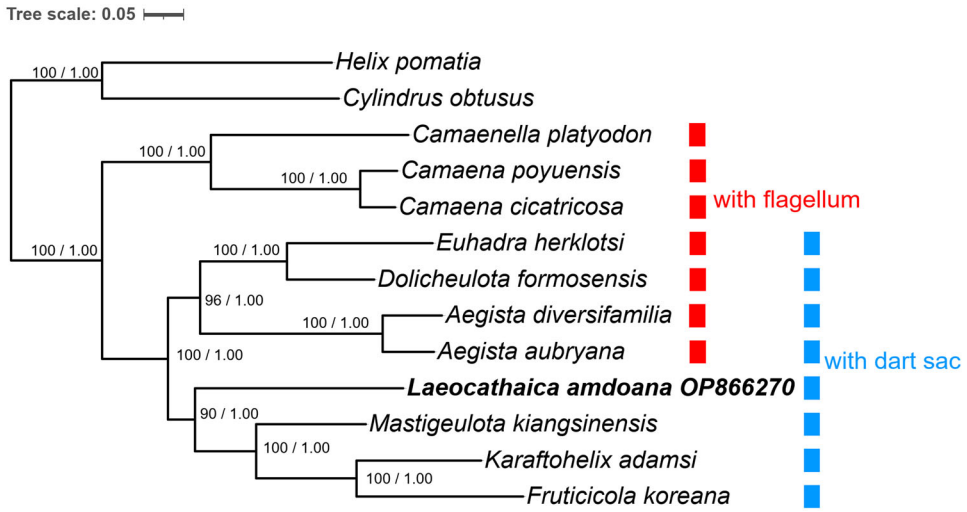

Figure 3. Bayesian phylogram based on the 1st and 2nd codons of eight unsaturated PCGs and two rRNA genes, with Helicidae as outgroup. Numbers near nodes indicate Bayesian posterior probabilities (BPP) and maximum-likelihood bootstrap (BP) values, given as BP/BPP. The mitogenome of *L. amdoana* determined in this study is highlighted in bold. Scale bar is for substitutions per site. The following sequences were used: *Aegista diversifamilia* KR002567 and *Dolicheulota formosensis* KR338956 (Huang et al. 2016), *Camaena poyuensis* KT001074 (Lin et al. 2016), *Fruticicola koreana* KU237291 (unpublished), *Karaftohelix adamsi* KY230382 (unpublished), *Camaenella platyodon* MH362759 (unpublished), *Cylindrus obtusus* JN107636 (Groenenberg et al. 2012), *Mastigeulota kiangsinensis* KM083123 (Deng et al. 2016), *Camaena cicatricosa* KM365408 (Wang et al. 2014), *Aegista aubryana* KT192071 (Yang et al. 2016), *Helix pomatia* MK488031 (Groenenberg and Duijm 2019), and *Euhadra herklotsi* Z71693–Z71701 (Yamazaki et al. 1997).

Table 1. The best-fitting model of each partition selected for MrBayes and IQ-tree.

Partition	MrBayes	IQ-tree
cytb 1st + cox2 1st + cox3 1st	GTR + F + I + G4	TIM2 + F + I + I + R2
cytb 2nd + cox2 2nd + cox3 2nd	GTR + F + G4	TVM + F + G4
atp6 1st + nad1 1st + nad4 1st + nad5 1st	GTR + F + I + G4	TIM2 + F + I + G4
atp6 2nd + nad4 2nd + nad5 2nd	GTR + F + I + G4	GTR + F + I + G4
cox1 1st	GTR + F + I + G4	GTR + F + I + G4
cox1 2nd	GTR + F + I + G4	GTR + F + I + G4
nad1 2nd	GTR + F + I + G4	GTR + F + I + G4
rrnL + rrnS	GTR + F + I + G4	GTR + F + I + G4

4. Results

4.1. Mitogenomic characterization

The average sequence coverage was 39.34×. The complete mitochondrial genome of *L. amdoana* was circular and 14,660 bp in length. The nucleotide composition was 28.68%, 38.77%, 18.18%, and 14.37% for A, T, G, and C, respectively. This mitogenome contained 37 genes, including 13 PCGs, two rRNA genes, and 22 tRNA genes. Among them, 23 genes were transcribed along the forward direction and the rest along the reverse direction. A few genes were overlapped with their neighboring genes. The length of gene overlaps in the whole mitochondrial genome was 40 bp. All PCGs used ATK as the start codon except *COX2* and *NAD3* starting with TTG, and *CYTB* starting with GTG. In addition, all PCGs use TAR as stop codon, except *COX2* ending with an abbreviated stop codon T, and *ATP6* and *ATP8* ending with TA. The length of tRNA genes ranged from 53 to 63 bp. Intergenic regions that contained 952 bp in total ranged between 1 and 816 bp, accounting for 6.50% of the whole mitogenome. The longest intergenic region, also an AT-rich region, was located between $trnW^{uca}$ and $trnY^{gua}$ with a 67.89% AT content.

4.2. Phylogenetic analysis

The saturation tests indicated that *ATP8*, *NAD2*, *NAD3*, *NAD4L*, and *NAD6* are saturated genes. The 1st and 2nd codons of the rest PCGs and two rRNA genes were therefore concatenated for phylogenetic analyses. The phylogenies reconstructed by Bayesian and ML methods were topologically identical (Figure 3). It indicated that *L. amdoana* was sister to the clade (*Mastigeulota kiangsinensis*, (*Karaftohelix adamsi*, *Fruticicola koreana*)). The clade of dart sac-bearing camaenids was divided into two groups based on the status of flagellum. The camaenids without dart sac apparatus are basal in the clade including all the examined camaenids.

5. Discussion and conclusions

The proposed phylogeny (Figure 3) coincides with that by the phylogeny based on the 13 PCGs on the positions of *Mastigeulota* Pilsbry, 1895, *Euhadra* Pilsbry, 1890 and *Camaena* Albers, 1850 (Wang et al. 2014). The phylogenetic result (Figure 3) generally agrees with the phylograms based on the sequence data of *16S* and *COX1* (Chen et al. 2021), where Camaeninae that has no dart sac apparatus and the dart sac-bearing Bradybaeninae, with exception of *Pseudostegodera* Wu et Chen, 2021, *Stegodera* Martens, 1876, and *Nesiohelix* Kuroda et Emura 1943, appeared as sister taxa. The topology where the monophyly *Dolicheulota* Pilsbry, 1901 + *Euhadra* (1.0 BPP and 100% BP) and *Aegista* Albers, 1850 are sister taxa (Figure 3), is consistent with the *16S* + *COX1*-based phylogeny proposed by Jirapatrasilp et al. (2022).

In *Laeocathaica*, the change of the gene order, in comparison to those in the other camaenid taxa, was observed (for details, see supplementary material 2). The gene order of *Laeocathaica* agrees with that of *Mastigeulota* on the same branch, and *Euhadra* and *Dolicheulota* on the sister branch. The present work confirms that the evolution of flagellum and dart sac apparatus might have played significant roles in the course of formation of camaenid diversity (Figure 3).

By this work, we reported the mitogenome of *L. amdoana* for the first time, which is congruent with the typical mitochondrial genome structure in metazoan (Saccone et al. 1999). The systematic position of *Laeocathaica* within Camaenidae was first inferred based on complete mitogenomes, and these results would provide fundamental resources for further studies on the evolution of this genus and the phylogenetics of Camaenidae.

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Author contributions

WS prepared figures, analyzed data, uploaded data to NCBI, and drafted the paper. MW designed this work, identified the species, drafted and approved the final draft. They both agree to be accountable for this work.

Ethical approval

This research did not involve ethical research because snails are exempt from ethical approval or permission.

Disclosure statement

No potential conflict of interest was reported by the authors, and the authors alone are responsible for the content and writing of the paper.

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Data availability statement

The GenBank ID of this genome sequence is OP866270. The BioProject, BioSample, and SRA numbers of metadata are PRJNA912872, SAMN32260481, and SRR22762582, respectively. The CM models for predicting rRNA genes can be downloaded on GitLab at https://gitlab.com/Bernt/MITOS/tree/master/data/modelle-v1.

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