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MITOGENOME REPORT

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Complete mitochondrial genome of the giant triton snail *Charonia tritonis* (Tonnoidea: Charoniidae)

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ABSTRACT

The giant triton snail, *Charonia tritonis* (Linnaeus, 1758), crucial for coral reef ecosystems as a primary predator of the crown-of-thorns sea star, is experiencing a significant decline due to overfishing for its ornamental shell, underscoring the urgent need for conservation and deeper understanding of its role within marine biodiversity. This study presents the first complete mitogenome sequence of *C. tritonis*. Spanning 15,346 bp, the *C. tritonis* mitogenome comprises 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes. Phylogenetic analysis of 88 Littorinimorpha mitogenomes confirms *C. tritonis* and *C. lampas* are grouped together within the family Charoniidae as a sister group to the remaining Tonnoidea families. This research not only enhances the taxonomic classification and conservation efforts for marine gastropods but also serves as a vital reference for future evolutionary and genetic studies within the Caenogastropoda.

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Introduction

The Tonnoidea are a moderately diverse group of large, predatory gastropods, comprising approximately 360 valid species. They play a numerically significant role as key predators in shallow, tropical marine environments, where they primarily prey on echinoderms. The classification of superfamily Tonnoidea has been updated by Strong et al. (2019), based on phylogenetic analyses of mitochondrial genes (*COX1*, *16S*, *12S*) and a nuclear gene (*28S*). This proposed revision encompasses nine families under Tonnoidea: Bursidae, Cassidae, Charoniidae, Cymatiidae, Laubierinidae, Personidae, Ranellidae, Thalassocyonidae, and Tonnidae. Notably, the taxonomy of the giant triton snail, *Charonia tritonis* (Linnaeus, 1758), has been revised, moving it from the family Ranellidae to Charoniidae. Inhabiting the shallow waters of both tropical and temperate seas, the giant triton snail is found across the South China Sea, the Indian Ocean, New Zealand, Japan, and other Indo-Pacific regions. As one of the largest marine benthic carnivores, it plays a pivotal role as the chief predator of the crown-of-thorns sea star (*Acanthaster planci*), thereby significantly contributing to the regulation of *A. planci* populations and the maintenance of coral reef ecosystem health. Unfortunately, overfishing for its ornate shell has significantly reduced the *C. tritonis* population (Hoey and Chin 2004). Despite its ecological importance, *C. tritonis* has not yet been evaluated for the IUCN Red List or included in the CITES species checklist. This study presents the first report of the complete mitogenome sequence for *C. tritonis*, offering valuable molecular insights for its conservation and enhancing our understanding of the phylogenetic relationships within Caenogastropoda.

Figure 1. The specimen of *Charonia tritonis* (male). This photo was taken by Xiang Zhang.

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Table 1. Mitogenomes used in the phylogenetic analysis as shown in Figure 3.

Scientific name	Superfamily	Family	GenBank ID	Length (bp)	Reference
Ficus subintermedia	Ficoidea	Ficidae	OR522697	16,255	Direct submission
Ficus variegata	Ficoidea	Ficidae	MW376482	15,736	Direct submission
Bufonaria rana	Tonnoidea	Bursidae	MT408027	15,510	Zhong et al. (2020)
Lampasopsis thomae	Tonnoidea	Bursidae	MW316791	15,393	Sanders et al. (2021)
Lampasopsis thomae	Tonnoidea	Bursidae	MW316792	15,392	Sanders et al. (2021)
Tutufa rubeta	Tonnoidea	Bursidae	MW316790	15,397	Sanders et al. (2021)
Galeodea echinophora	Tonnoidea	Cassidae	KP716635	15,388	Osca et al. (2015)
Monoplex parthenopeus	Tonnoidea	Cymatiidae	EU827200	15,270	Cunha et al. (2009)
Charonia lampas	Tonnoidea	Charoniidae	KU237290	15,330	Choi and Hwang (2021)
Charonia lampas	Tonnoidea	Charoniidae	MG181942	15,405	Cho et al. (2017)
Charonia tritonis	Tonnoidea	Charoniidae	MT043269	15,346	This study
Charonia tritonis	Tonnoidea	Charoniidae	OR251120.2	15,346	This study
Tonna galea	Tonnoidea	Tonnidae	OR282483	17,504	Direct submission
Tonna galea	Tonnoidea	Tonnidae	OR506572	15,946	Direct submission

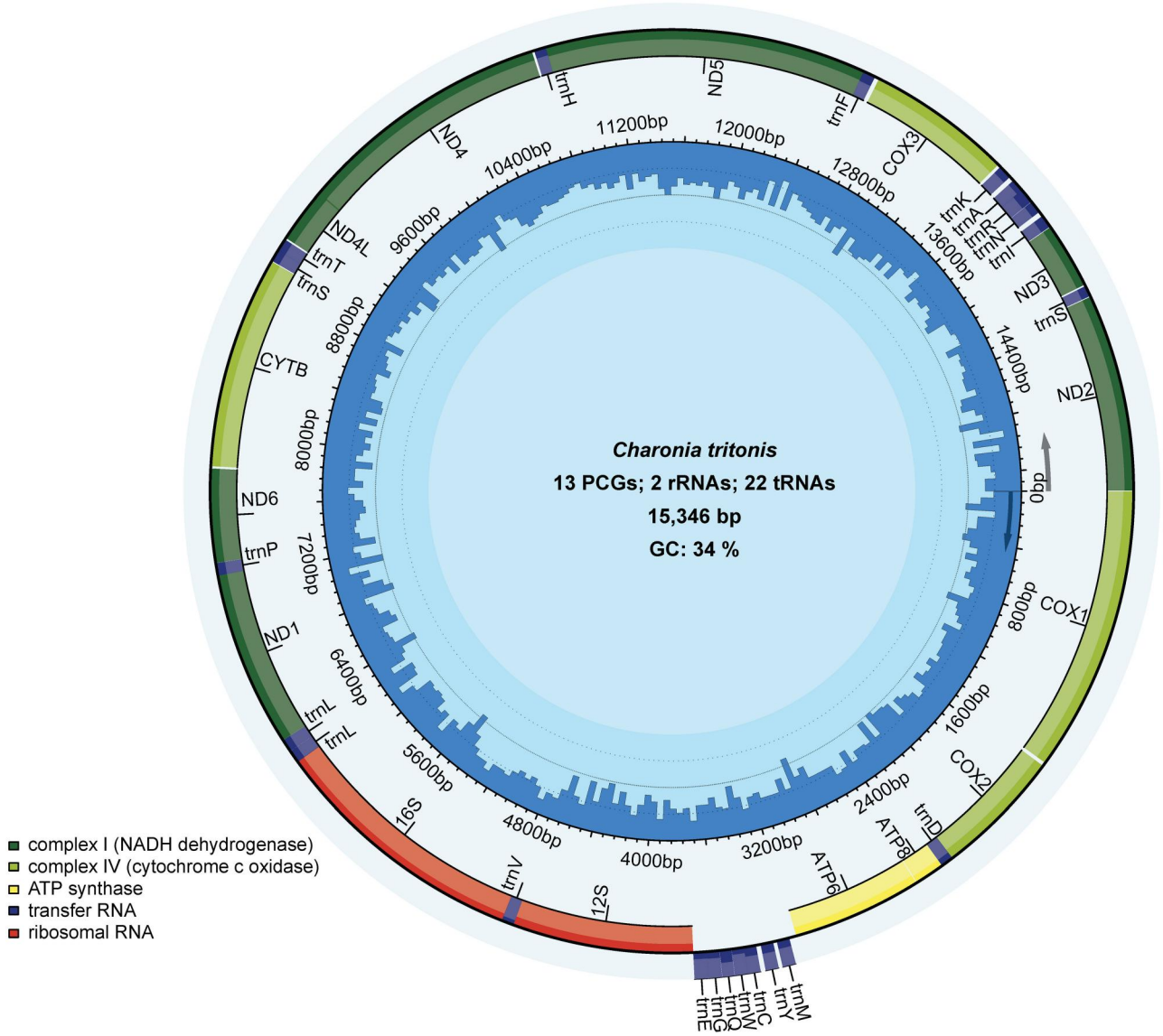

Figure 2. Mitochondrial genome map of *Charonia tritonis* (GenBank accession number MT043269). In the inner circle, the shaded parts indicate the GC content. Represented with arrows, the transcription directions for the inner and outer genes are listed clockwise and anticlockwise, respectively.

Materials and methods

Two specimens of *C. tritonis* were collected in a dead state from the waters of the Xisha Archipelago in the South China Sea (111°39′10″E, 16°15′28″N) (Figure 1). Species identification was based on features such as smooth, broad, and flattened spiral ribs with wavy, puckered edges, and a broad, short siphonal canal with thin folds along the columellar wall (Motti et al. 2022). The specimens have been deposited at the Specimen Museum of the School of Marine Biology and Fisheries at Hainan University (http://en.hainanu.edu.cn/,

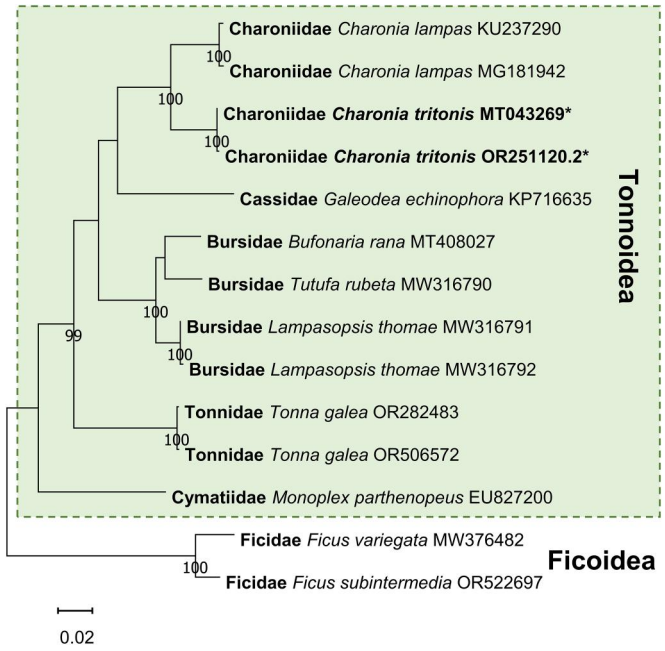

Figure 3. A simplified maximum-likelihood (ML) tree was constructed based on the amino acid sequence alignment of 13 protein-coding genes (PCGs) from 12 mitogenomes of Tonnoidea. The two newly sequences of *Charonia tritonis* are highlighted by an asterisk and bold. The best-fitting model, mtMet + F + R10, selected based on the Bayesian information criterion, was implemented for the ML analysis. Ficoidea was used as outgroup. Branch support values were inferred using the ultrafast bootstrap method via the IQ-TREE web server. The NCBI GenBank accession numbers of all mitogenomes and their citations are shown in the figure and Table 1, respectively. The following mitogenomes were used: OR522697 (direct submission), MW376482 (direct submission), MT408027 (Zhong et al. 2020), MW316790–MW316792 (Sanders et al. 2021), KP716635 (Osca et al. 2015), EU827200 (Cunha et al. 2009), KU237290 (Choi and Hwang 2021), MG181942 (Cho et al. 2017), MT043269 (this study), OR251120.2 (this study), OR282483 (direct submission), and OR506572 (direct submission).

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Total genomic DNA was extracted from the foot muscle using the FastPure Cell/Tissue DNA Isolation Mini Kit (Vazyme, Nanjing, China). The genomic DNA of specimen ctri20181201 was sequenced using PE150 chemistry on an Illumina HiSeq 2500 (BGI, Shenzhen, China). The quality of the raw sequencing data was assessed with FastQC (Andrews 2010) and trimmed using Trimmomatic (Bolger et al. 2014) prior to assembly with the MitoZ pipeline (Meng et al. 2019), yielding the mitogenome of *C. tritonis* (GenBank accession number MT043269). To confirm the accuracy of the mitogenome assembly, 23 consensus PCR primer pairs (Supplementary Table S1), designed based on the mitogenome (MT043269) using Primer-BLAST (Ye et al. 2012), were employed to sequence the second specimen (ctri20181202) through Sanger sequencing. These primer pairs were synthesized by BGI (Shenzhen, China). On an ABI 2700 Thermo Cycler (Foster City, CA), PCR amplifications were performed in 25 μL reaction volumes containing PrimeSTAR GXL Premix (TAKARA, Beijing, China), each primer at 0.6 μM and template DNA over 4 ng/μL. The PCR protocol included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were verified on 2.0% agarose gels (Supplementary Figure S1) and sequenced on an ABI3730XL DNA Analyzer (BGI, Shenzhen, China) after purification using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). DNA sequences were assembled with SeqMan (Lasergene) to finalize the mitogenome (OR251120.2). The mitogenomes were annotated using GeSeq (Tillich et al. 2017), and the mitogenome map for MT043269 was created with Chloroplot (Zheng et al. 2020).

The percentage of nucleotide similarity between the mitogenomes was calculated by BioEdit v7.7.1.0 (Hall 1999).

For the phylogenetic analyses, first, all available mitogenomes of the order Littorinimorpha (as of 21 February 2024) were retrieved from the NCBI GenBank database, excluding records marked as 'unverified' and those species names identified with 'sp.' (i.e. 151 mitogenomes, Supplementary Table S2). These 151 mitogenomes were then clustered at a 0.95 identity threshold using CD-HIT-EST v4.6.1 (Fu et al. 2012) to reduce redundancy, resulting in 88 unique Littorinimorpha mitogenomes. Second, the amino acid sequences of each of the 13 protein-coding genes (PCGs) from these 88 unique mitogenomes, along with two outgroup species, *Haliotis discus hannai* (KF724723; Yang et al. 2015) and *Haliotis rubra* (AY588938; Maynard et al. 2005) from the order Lepetellida, were aligned separately using Clustal X v2.1 (Larkin et al. 2007), applying code constraints. Third, the 13 aligned amino acid sequences were concatenated from A to Z based on gene names. Fourth, a maximum-likelihood (ML) phylogenetic tree, utilizing the best-fitting model mtMet + F + R10 based on the Bayesian information criterion, was generated by the IQ-TREE web server (Trifinopoulos et al. 2016) with 1000 ultrafast bootstrap replicates (Supplementary Figure S2). Lastly, based on the ML tree resulting from the concatenated amino acid sequences of 90 unique mitogenomes, all available mitogenomes of Tonnoidea were designated as the ingroup, while Ficoidea served as the outgroup (Table 1). This dataset was utilized to reconstruct the simplified ML tree using the same methodology as described above.

Results

The lengths of the two mitogenomes of *C. tritonis* obtained in this study are the same (15,346 bp). The gene content and arrangement of the two mitogenomes are consistent, featuring 13 PCGs, 22 tRNA genes, and two rRNA genes (Figure 2). Intergenic nucleotides range from seven nucleotide overlaps (*NAD4L* and *NAD4*) to 39 nucleotide spaces (*ATP6* and *trnM*) between PCGs, rRNA genes and/or tRNA genes. All 13 PCGs are transcribed on the heavy strand and begin with an ATG start codon. Except for *COX3* and *NAD4L*, which are terminated by the TAG codon, the remaining genes are terminated by the TAA codon. The 22 tRNAs vary in length from 62 bp (*trnQ*) to 79 bp (*trnR*). The *16S* and the *12S* rRNAs gene are 1386 bp and 969 bp, respectively. The overall nucleotide composition of the mitogenome (MT043269) of *C. tritonis* is 29.85% A, 35.76% T, 16.69% G, and 17.70% C. The mitogenome (OR251120.2) shows a 99.75% nucleotide similarity to MT043269, with 29.83% A, 35.76% T, 16.72% G, and 17.69% C. Both mitogenomes display an A + T bias in their

nucleotide composition. The mitogenome of *C. tritonis* (MT043269) exhibits 85.63% and 85.84% nucleotide similarity with *Charonia lamps* (KU237290; Choi and Hwang 2021) and *C. lampas* (MG181942; Cho et al. 2017), respectively.

The alignment of the concatenated sequences of 12 mitogenomes of Tonnoidea derived from 13 PCGs spans 3740 amino acids. The phylogenetic analysis results show that the *C. tritonis* is the sister to *C. lampas* (Figure 3).

Discussion and conclusions

The gene content and arrangement of *C. tritonis* align with those of the previously reported mitogenomes from all species within the superfamily Tonnoidea (Choi and Hwang 2021). Unlike typical animal mitogenomes, it lacks a control region (Li et al. 2015). *Charonia tritonis* and *C. lampas* are grouped together within Charoniidae, forming a sister group to the other Tonnoidea families (Bursidae, Cassidae, Cymatiidae, and Tonnidae) (Supplementary Figure S2), which supports the proposed relationship as reported by previous studies (Cho et al. 2017; Strong et al. 2019; Choi and Hwang 2021). These data provide valuable genomic resources for the conservation of *C. tritonis* and serve as a reference for species delimitation and evolutionary research within the Caenogastropoda.

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Author contributions

SW, XZ, and YZ conceived and designed the study; XZ and CH wrote the draft of the manuscript; HL, JL, and CH conducted laboratory work and performed analyses; and that all authors agree to be accountable for all aspects of the work.

Ethical approval

The sample collection was approved by the Animal Care and Use Committee of Hainan University (no. HNU170201, approval date: 1 February 2017, Hainan 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 of this study are publicly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession numbers MT043269.1 and OR251120.2. Additionally, the Sanger sequencing results of PCR products for OR251120.2 and the alignment of 13 protein-coding genes from 91 mitogenomes are accessible at https://doi.org/10.6084/m9.figshare.23506914.v4.

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