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MITOGENOME REPORT

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The complete mitochondrial genome of the pulmonate snail *Melampus sincaporensis* (Gastropoda: ellobiidae) from South Korea

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ABSTRACT
The pulmonate snail *Melampus sincaporensis* L. Pfeiffer, 1855 (Gastropoda: Ellobiidae) lives in extreme environments such as salt marshes with limited oxygen. Here, we characterized the complete mitochondrial genome of *M. sincaporensis* from South Korea. It is 14,962 bp in length and includes 13 protein-coding genes (PCGs), two genes, 21 tRNA genes lacking one *tRNA-Ser*, and two A + T rich regions. Among these 36 genes, 24 were encoded on the heavy strand and 12 on the light strand. A maximum likelihood tree constructed using nucleotide sequences of the 13 PCGs did not support the monophyly of Ellobiidae. This study could provide useful information for exploring phylogenetic relationships among ellobiids and their related species.

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Melampus sincaporensis; mitochondrial genome; panpulmonata; Ellobiidae

Introduction

Melampus sincaporensis L. Pfeiffer, 1855 (Gastropoda: Ellobiidae) is a small, air-breathing salt marsh snail. Ellobiidae lives above the high-tide mark in mangrove regions, salt marshes, and rolled stone shores globally (Martins 1996). They are of interest for studies investigating habitat transitions from intertidal to terrestrial realms (Romero et al. 2016a). Ellobiidae consists of approximately 250 species within 24 genera (White et al. 2011), although its monophyly has been questioned (White et al. 2011; Yi et al. 2017). Recently, mitochondrial genome data have been proven useful in research fields such as molecular phylogeny, population genetics, and studies of metabolic processes (Kim et al. 2011; Lee et al. 2012; Baek et al. 2014; Baek et al. 2020; Choi et al. 2021; Park et al. 2021; Shin et al. 2021; Park and Hwang 2022; Choi and Hwang 2023; Kim and Hwang 2023). Herein, we sought to characterize the complete mitochondrial genome of *M. sincaporensis* and to examine its phylogenetic position of within the family Ellobiidae.

Materials and methods

A specimen of *M. sincaporensis* was collected from the brackish water area in Daejin-ri, Sacheon-si, Gyeongsangnam-do, South Korea (35°02′21.9"N 127°58′30.3"E) (Figure 1). Its identification was performed based on morphological characters such as shell shape, size, color, striation, and teeth with Raven and Vermeulen (2007). Genomic DNA was extracted from the foot of the specimen using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The specimen was deposited under the voucher number LEGOM030626 at the Animal Molecular Phylogenetics Lab., Kyungpook National University (UWH, uwhwang@knu.ac.kr). DNA concentration and quality were checked using NanoDrop (Thermo Fisher Scientific, USA).

The mitochondrial genome fragments were amplified by polymerase chain reaction (PCR) using universal and specific primers (Table 1). Amplified PCR products were identified on a 1.0% agarose gel (Figure S1). Then, they were purified using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and APrep^{TM} Gel DNA Kit (AP Biotech, Korea). Partial sequences were obtained with the ABI Prism 3730 DNA sequencer (PerkinElmer, USA) using BigDye Termination Sequencing Kit (PerkinElmer, USA) by the Sanger sequence method. The read coverage plot is not included as the data was obtained through Sanger sequencing.

Sequenced fragments were assembled using BioEdit version 7.2.5 (Hall 1999). Mitochondrial PCGs as well as rRNA and tRNA genes were predicted and annotated using MITOS web server (Bernt et al. 2013), tRNAscan-SE (Chan and Lowe

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Figure 1. The shell of *Melampus sincaporensis* (scale bar = 2 mm). From the left is a ventral, lateral, and dorsal view, respectively (photographed by HY).

Table 1. PCR primers used in the present study.

Primers (F)	Sequences (5′–3′)	References
Molluscox3R	TCS ACG AAR TGT CAR TAT CAR G	Irisarri et al. (2020)
UCYTB151F	TGT GGR GCN ACY GTW ATY ACT AA	Merrit et al. (1998)
MS_F10299*	GAG CCC TGC CCA ATG AGT AA	This study
Primers (R)	Sequences (5′–3′)	References
16Sbr	CCG GTT TGA ACT CAG ATC ATG T	Palumbi (1996)
HCO700dy2	TCA GGG TGA CCA AAA AAY CA	Walker et al. (2007)
UCYTB270R	AAN AGG AAR TAY CAY TCN GGY TG	Merrit et al. (1998)

*Primer designed specifically for *Melampus sincaporensis* in this study.

2019), ARWEN (Laslett and Canbäck 2008), and EMBOSS Transeq (Madeira et al. 2022). The circular mitochondrial genome of *M. sincaporensis* was then visualized using Proksee (Grant et al. 2023).

For the phylogenetic analysis, the mitochondrial genome sequence data of 13 panpulmonate species were retrieved from the NCBI GenBank, including *Salinator rhamphidia* (Gastropoda: Amphibolidae) set as an outgroup. The nucleotide sequences of the 13 mitochondrial PCGs were aligned with the ClustalW method (Thompson et al. 1994), followed by the removal of poorly aligned sites using the Gblock 0.91b (Castresana 2000). As a result, the total length of 10,360 bp was used for the phylogenetic analysis. The TVM+F + I + G4 substitution model was selected as the best-fit model by the IQ-TREE web server (Trifinopoulos et al. 2016). Under the selected substitution model, the maximum likelihood (ML) tree was reconstructed with 1000 ultrafast bootstrap replicates.

Results

The complete mitochondrial genome of *M. sincaporensis* (GenBank accession number OR777056) (Figure 2) was found to be 14,962 bp in length and to contain 13 PCGs, 2 rRNA genes, and 21 tRNA genes. Twenty-four genes were encoded on its heavy strand, while four PCGs (*ATP6*, *ATP8*, *ND3*, and *COX3*), seven tRNA genes (*tRNA-Gln*, *tRNA-Leu* (TAA), *tRNA-Asn*, *tRNA-Arg*, *tRNA-Glu*, *tRNA-Met*, and *tRNA-Thr*), and the 12S rRNA gene located on its light strand. Interestingly, it contained only one *tRNA-Ser* (GCT). It possessed two A + T rich regions (113 bp and 186 bp) located between *ND3* and *tRNA-Thr* and between *COX3* and *tRNA-Ser*, respectively. There were seven overlapping regions in the mitochondrial genome with 1 to 17 bp length. The TTG start codon was used for seven PCGs (*COX1*, *COX3*, *ND3-6*, and *CYTB*), GTG for *ND1* and *ND2*, ATG for *COX2* and *ND4L*, and ATA for *ATP6* and *ATP8*. All PCGs had one of the complete start codons TAA or TAG except for *ND2* and *COX3*, which had an incomplete stop codon T.

In the ML tree (Figure 3), Ellobiidae was not shown to be monophyletic by Trimusculidae nesting within the clade. Within Ellobiidae, the subfamily Ellobiinae was monophyletic (BP 100), whereas Pythiinae was polyphyletic. *M. sincaporensis* was identified as a sister group of *Trimusculus reticulatus* and *Carychium tridentatum*.

Discussion and conclusion

Herein, we characterized and presented the first complete mitochondrial genome of *M. sincaporensis* and explored phylogenetic relationships among the 13 panpulmonate species.

When comparing the gene composition in the family Ellobiidae, *M. sincaporensis* was found to have only one *tRNA-Ser*, similar to *Ellobium chinensis*, whereas the remaining six ellobiid species (*Auriculinella bidentata*, *Auriculastra duplicata*, *Ovatella vulcani*, *Carychium tridentatum*, *Pedipes pedipes*, and *Myosotella myosotis*) had two *tRNA-Ser* (Jun et al. 2016). In the case of mitochondrial gene arrangement, *M. sincaporensis* was identical to *E. chinense*, except for the positions of *tRNA-Thr*, *COX3*, *tRNA-Ser*, and *ND4*.

The phylogenetic analysis based on the 13 PCGs of the mitochondrial genome among ellobiid species showed that *M. sincaporensis* was closely related to *T. reticulatus*.

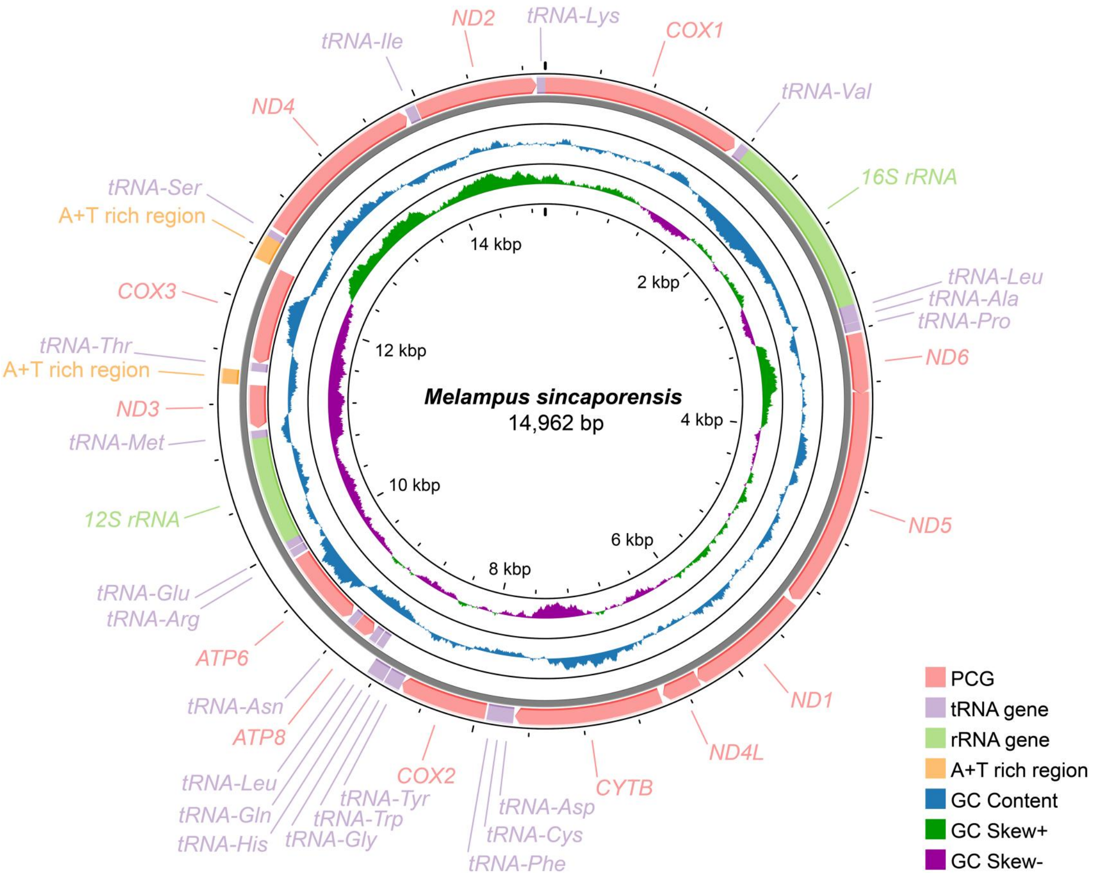

Figure 2. Circular map of the complete mitochondrial genome of *Melampus sincaporensis*. The complete mitochondrial genome is 14,962 bp in length. Genes are shown with standard abbreviations. The outer circle indicates the heavy strand (outer line) and the light strand (inner line). The inner circle indicates the GC skew, which is the deviation from the average GC content of the entire sequence.

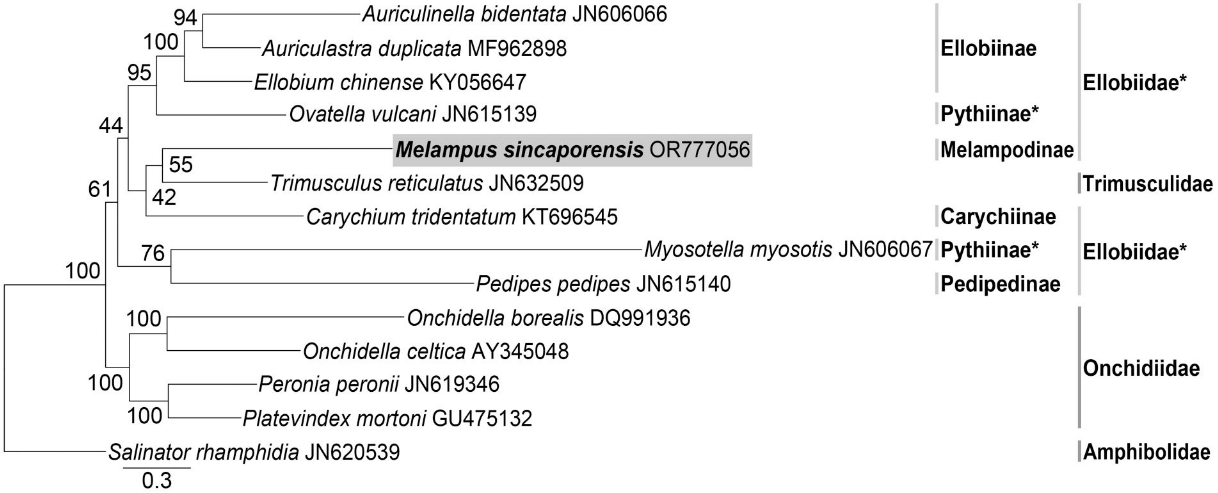

Figure 3. The maximum likelihood tree of *Melampus sincaporensis* and other panpulmonate species based on the nucleotide sequences of the mitochondrial 13 PCGs. *Salinator rhamphidia* was used as an outgroup. The species in bold is the studied species herein. Ellobiidae are presented with their respective subfamilies. Groups marked with an asterisk indicate that they are not monophyletic. The numbers above the nodes indicate maximum likelihood support values. The NCBI GenBank accession numbers of each species are indicated in parentheses. The following sequences were used: *Auriculinella bidentata* JN606066 (White et al. 2011), *Trimusculus reticulatus* JN632509 (White et al. 2011), *Ovatella vulcani* JN615139 (White et al. 2011), *Myosotella myosotis* JN606067 (White et al. 2011), *Pedipes pedipes* JN615140 (White et al. 2011), *peronia peronii* JN619346 (White et al. 2011), *Salinator rhamphidia* JN620539 (White et al. 2011), *Auriculastra duplicate* MF962898 (Yi et al. 2017), *Ellobium chinense* KY056647 (Jun et al. 2016), *Carychium tridentatum* KT696545 (Romero et al. 2016b), *Onchidella borealis* DQ991936 (Medina et al. 2011), *Onchidella celtica* AY345048 (Grande et al.2004), *platevindex mortoni* GU475132, and *Melampus sincaporensis* OR777056 (this study).

Interestingly, Ellobiidae was not shown to be monophyletic in our analysis due to Trimusculidae as mentioned in White et al. (2011). Future studies could focus on accumulating whole genome data as well as mitochondrial genome data from more ellobiid species to understand the phylogenetic relationships of the family better. The current study provides a useful resource for conservation, population genetics, and phylogenetic studies of ellobiid species.

Author contributions

UWH and EHC designed the study. HY and UWH wrote the manuscript. HY, CRS, GK, and BP carried out the sampling, molecular experiments, and data analyses. All authors revised the manuscript and agreed to be responsible for all aspects of the work.

Disclosure statement

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

Ethical approval

The material involved in this article does not involve any ethical conflicts. This species is not endangered according to the CITES catalogue or IUCN Red List, and the sample was not collected from a natural reserve, so the collection did not require any specific permissions or licenses.

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Data availability statement

The 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 number OR777056. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1068672, SRR28201758, and SAMN39600027, respectively.

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