

# Complete mitochondrial genome of the worm snail *Thylacodes adamsii* (Littorinimorpha: Vermetidae) from South Korea

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## ABSTRACT

The worm snail *Thylacodes adamsii* (Mörch, 1859) (Littorinimorpha: Vermetidae) is a sessile gastropod that mainly inhabits rocky shores along the warm temperate to tropical ocean. Herein, the complete mitochondrial genome (mitogenome) of *T. adamsii* from South Korea was characterized. The genome is 14,913 bp in length and contains 13 protein-coding genes (PCGs), 22 tRNA genes, and 2 rRNA genes. The genome organization and base composition of *T. adamsii* are similar to those of other vermetids. A phylogenetic tree was reconstructed using maximum likelihood based on the nucleotide sequences of the 13 PCGs; this tree supported the monophyly of Vermetidae. The complete mitogenome of *T. adamsii* can assist with molecular species identification and vermetid phylogenetic research in the future.

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## Introduction

The worm snail family Vermetidae (order Littorinimorpha) is a clade of sessile gastropod that is characterized by tube-like shells without the regular shell coiling. Vermetids mainly inhabit rocky shores along the warm temperate to tropical oceans worldwide (Rawlings et al. 2010). Vermetidae is a relatively poorly studied taxon among mollusks. Within the order Littorinimorpha, the phylogenetic position of Vermetidae of this family has been controversial (Colgan et al. 2007; Rawlings et al. 2010). The mitochondrial genome (mitogenome) has often been used as a phylogenetic marker in mollusks (Lee et al. 2012; Cha et al. 2018; Jiang et al. 2019; Kim et al. 2019; Choi et al. 2020; 2021a; 2021b; Choi and Hwang 2021; Shin et al. 2021; Hong et al. 2023; Kim & Hwang 2023). However, only four mitogenomes of vermetid species have been reported to date. In this study, we characterized the complete mitogenome of the vermetid species *Thylacodes adamsii* (Mörch, 1859) and attempted to infer the phylogenetic relationships among the members of Vermetidae and other littorinimorph families based on the nucleotide sequences of 13 protein-coding genes (PCGs). Thus, the complete mitogenome of *T. adamsii* may be useful for identifying molecular species and understanding vermetid phylogeny.

## Materials and methods

A *T. adamsii* specimen was collected from Hajeong-ri, Guryongpo-eup, Nam-gu, Pohang-si, South Korea (35°96'94.5"N, 129°55'46.4"E) and stored in 95% ethanol (Figure 1). It has been deposited under voucher number LEGOM030627 at the Animal Molecular Phylogenetics Lab., Kyungpook National University (UWH, [uwhwang@knu.ac.kr](mailto:uwhwang@knu.ac.kr)). Genomic DNA was extracted from muscle tissue using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). The quality and quantity of DNA were checked using using Nanodrop2000 (Thermo Fisher Scientific, United States). For genome sequencing, Illumina QIAseq library was constructed with an average insert size of 150 bp using QIAseq FX Single cell DNA Library Kit (QIAGEN, Germany). Sequencing was performed from an Illumina paired-end library, and 150 bp paired-end reads were generated through the Illumina HiSeq 4000 platform, producing 38,890,026 reads (not published) (Figure S1). The mitochondrial genome assembly of *T. adamsii* was conducted using Novoplasty 4.3.3 (Dierckxsens et al. 2020). After manual curation, mitochondrial genes were annotated as PCGs, tRNAs, and rRNAs was carried out using the MitoFinder v.1.4.1 (Allio et al. 2020). Thereafter, the circular mitochondrial genome of *T. adamsii* was visualized using Proksee (Grant et al. 2023). The complete mitochondrial

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**Figure 1.** Reference image of *Thylacodes adamsii*, photographed by Eun Hwa Choi.

genome sequence of *T. adamsii* has been submitted to NCBI GenBank.

To investigate the phylogenetic placement of *T. adamsii* based on 13 mitochondrial PCGs, we examined the phylogenetic relationships among 29 caenogastropod species and one vetigastropod species. Each PCG was aligned using ClustalW (Thompson et al. 1994), and poorly aligned sites were removed by using Gblock 0.91b (Castresana 2000). The vetigastropod species *Haliotis rubra* was used as the outgroup. A maximum-likelihood (ML) tree was inferred using the IQ-TREE online webserver (Trifinopoulos et al. 2016) for phylogenetic analysis.

## Results

The complete mitochondrial genome of *T. adamsii* (GenBank accession number: OR757106) (Figure 2) was 14,913 bp in length and comprised 13 PCGs, 22 tRNA genes, and two rRNA genes. The base composition for A, G, C and T was 27.33%, 19.11%, 15.63%, and 37.93%, respectively. All PCGs started with an ATG codon except for *ND4*, which started with a TTG codon. In terms of stop codons, all PCGs stopped with a typical TAN codon except for *COXI*, which stopped with an incomplete termination codon T. The 12S and 16S rRNA genes were 938 bp and 1,222 bp in length, respectively.

The reconstructed ML tree (Figure 3) supported the monophyly of Neogastropoda, Cerithioidea, and Architaenioglossa within the subclass Caenogastropoda, although Littorinimorpha

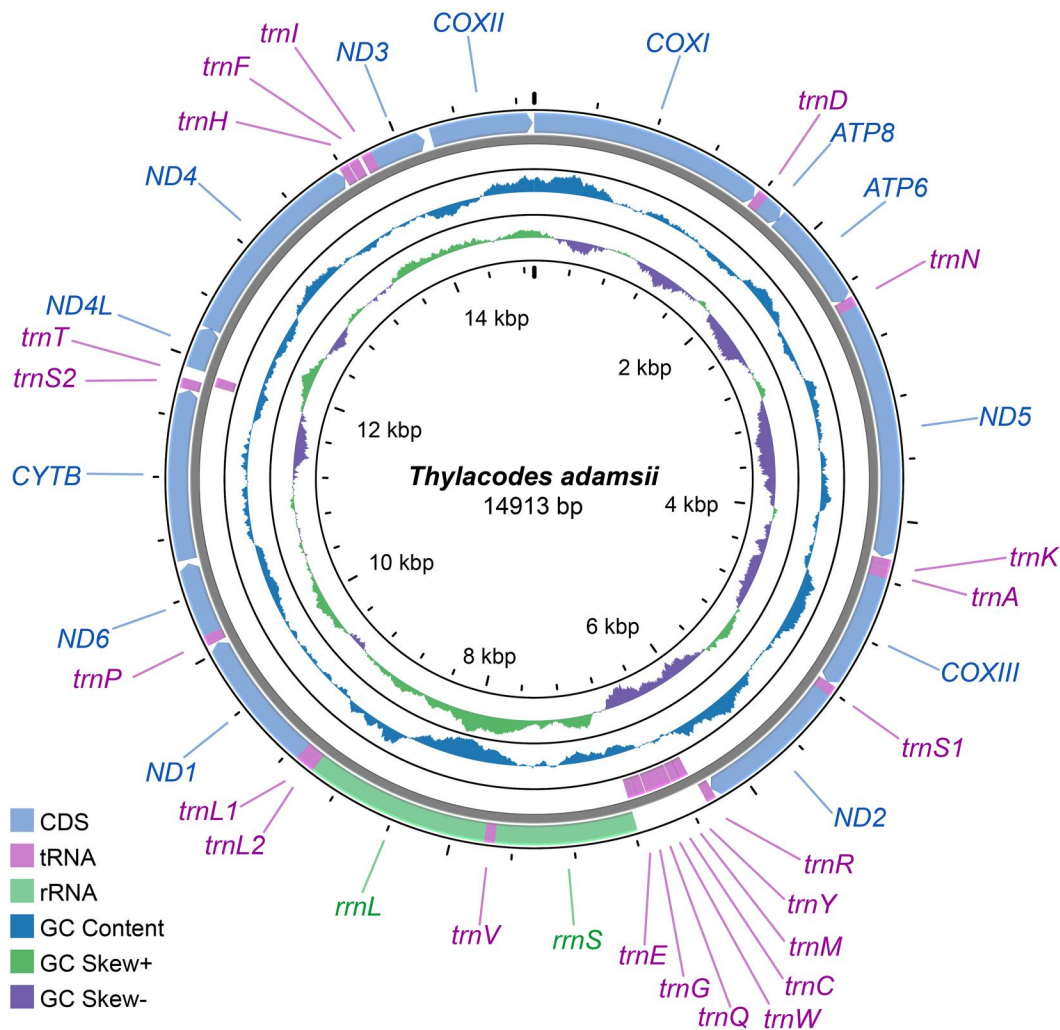
was polyphyletic. Littorinimorpha and Neogastropoda were grouped together (BP = 100), except for the superfamily Vermetoidea of Littorinimorpha. Vermetoidea was monophyletic and was placed at the basal position of Caenogastropoda. Within Vermetoidea, *T. adamsii* was closest to *T. squamigerus* (BP = 100).

## Discussion and conclusion

In this study, the complete mitogenome of *T. adamsii* was characterized. The arrangement and strand position of the genes coincided with those of *Ceraesignum maximum* (G. B. Sowerby I, 1825) (Littorinimorpha: Vermetidae) (Rawlings et al. 2010).

According to the ML tree (Figure 3), the phylogenetic relationships among the caenogastropod orders were almost identical to those reported in a recent phylogenetic study based on mitogenomes by Choi et al. (2021a). The monophyly of Littorinimorpha was not recovered because Vermetoidea formed an independent clade within Littorinimorpha. Phylogenetic relationships within Littorinimorpha were similar to those reported by Jiang et al. (2019), which was inferred from 13 mitochondrial PCGs. *T. adamsii* appeared in the clade of Vermetoidea (Vermetidae). Further research is warranted to determine whether Littorinimorpha is monophyletic or not. These results may help determine the position of Vermetidae within Littorinimorpha and infer the phylogenetic relationships of Littorinimorpha.





**Figure 2.** Circular map of the complete mitochondrial genome of *Thylacodes adamsii*.

The complete mitochondrial genome is 14,913 bp in length and contains standard gene components. Of the 37 typical mitochondrial genes, 29 were located in the heavy strand (H-strand; genes named outside the circle) and the remaining 8 were in the light strand (L-strand; inside). The inner circle indicates the GC skew, which is the deviation from the average GC content of the entire mitogenome sequences.

### Authors' contributions

UWH and EHC designed this study and involved in the sample collection. UWH and YL wrote the manuscript. YL, EHC, and KBK conducted the molecular work and data analyses. All authors revised the manuscript and agreed to be responsible for all aspects of the work.

### Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

### 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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### ORCID

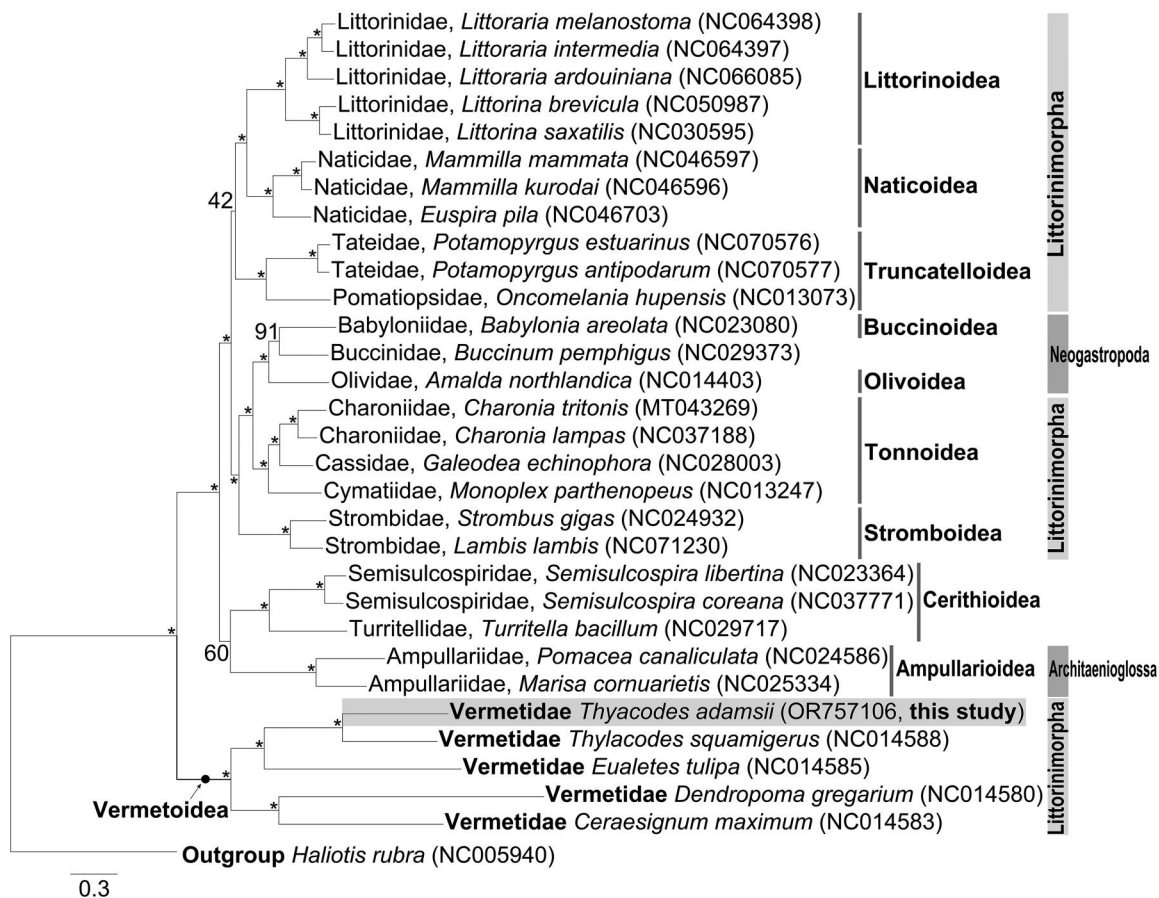
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### Data availability statement

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 OR757106. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1070832, SRR27775017, and SAMN39662919, respectively.

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**Figure 3.** Maximum-likelihood tree inferred from nucleotide sequences of 13 PCGs of 30 caenogastropod species.

Maximum-likelihood tree showing the phylogenetic position of Vermetidae within Littorinimorpha. *Haliotis rubra* (Subclass Vetigastropoda) is used as an outgroup. The species examined in this study is presented in a gray box. The GenBank accession numbers of each species are indicated in parentheses. Numbers on the branches indicate nodal support bootstrapping values in percent. Branch supports are inferred from the ultrafast bootstrap method using IQ-TREE web server. The nodes exhibiting BP = 100 and mark an asterisk. The following sequences were used: *Littoraria melanostoma* NC064398 (Chen et al. 2023), *Littoraria intermedia* NC064397, *Littoraria arduiniana* NC066085 (Wang et al. 2024), *Littorina brevicula* NC050987 (Bai et al. 2020), *Littorina saxatilis* NC030595 (Marques et al. 2017), *Mammilla mammata* NC046597 (Liu et al. 2020), *Mammilla kurodai* NC046596 (Liu et al. 2020), *Euspira pila* NC046703 (Liu et al. 2020), *Potamopyrgus estuarinus* NC070576 (Sharbrough et al. 2023), *Potamopyrgus antipodarum* NC070577 (Sharbrough et al. 2023), *Oncomelania hupensis* NC013073, *Babylonia areolata* NC023080 (Xiong et al. 2014), *Buccinum pemphigus* NC029373 (Xu et al. 2016), *Amalda northlandica* NC014403 (McComish et al. 2010), *Charonia tritonis* MT043269, *Charonia lampas* NC037188 (Cho et al. 2017), *Galeodea echinophora* NC028003 (Osca et al. 2015), *Monoplex parthenopeus* NC013247 (Cunha et al. 2009), *Strombus gigas* NC024932 (Márquez et al. 2014), *Lambis lambis* NC071230 (Li et al. 2022), *Semisulcospira libertina* NC023364 (Zeng et al. 2014), *Semisulcospira coreana* NC037771 (Kim and Lee 2018), *Turritella bacillum* NC029717 (Zeng et al. 2016), *Pomacea canaliculata* NC024586 (Zhou et al. 2016), *Marisa cornuarietis* NC025334 (Wang and Qiu 2016), *thylacodes squamigerus* NC014588 (Rawlings et al. 2010), *Eualetes tulipa* NC014585 (Rawlings et al. 2010), *Dendropoma gregarium* NC014580 (Rawlings et al. 2010), *Ceraesignum maximum* NC014583 (Rawlings et al. 2010), *Haliotis rubra* NC005940 (Maynard et al. 2005), and *Thylacodes adamsii* OR757106.

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