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The first complete mitochondrial genome of *Synandwakia multitentaculata* (Actiniaria, Andvakiidae)

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ABSTRACT

The complete mitochondrial genome of *Synandwakia multitentaculata*, a marine protected species endemic to the western coast of Korea, was sequenced and analyzed. The circular mitogenome is 18,350 bp with AT-rich composition (G + C content: 34.3%) and contains 17 genes: 13 protein-coding genes (PCGs), two ribosomal RNAs, and two tRNAs. All PCGs are encoded on the heavy strand and initiate with ATG. The mitogenome shows characteristic Actiniaria split-gene architecture in *COX1* and *ND5*, with *ND1* and *ND3* located within the *ND5* intron. Phylogenetic analysis of 13 PCGs placed *S. multitentaculata* with *Diadumene lineata* within Metridioidea, providing the first mitochondrial genomic resource for *Synandwakia* and the family Andvakiidae.

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Synandwakia multitentaculata; Actiniaria; marine protected species; complete mitogenome; phylogenetic analysis

Introduction

Actiniaria, commonly referred to as sea anemones, comprises approximately 1,170 species worldwide, but only 2.1% have available mitogenome data (NCBI 2025; WoRMS Editorial Board 2025). The family Andvakiidae, classified within the suborder Enthemonae, includes species characterized by cylindrical bodies, wrinkled lips, and physa-based substrate attachment (Rodríguez et al. 2025). The genus *Synandwakia* comprises two recognized species: *S. hozawai* from Mutsu Bay, Japan (Uchida 1932), and *S. multitentaculata* from the western coast of Korea (Song 2003).

Synandwakia multitentaculata Song, 2003 was described as a new species with specimens collected from Yeongjongdo, Incheon. After its initial report, the species remained unobserved until 2021 and was designated a marine protected species in 2016. Currently, it occurs at three sites in western Korea: Wangsan Beach and Eulwangri Beach on Yeongjongdo Island (Incheon), and Gosapo Beach in Byeonsan. However, the western coast of Korea faces significant threats from extensive reclamation projects and rising seawater temperatures, which have resulted in habitat loss and pose risks to intertidal species including reduced reproductive capacity and inhibited growth (Jeong et al. 2003; Seong 2010; Ross and Adam 2013; Choi 2014, 2023; Yao and Somero 2014; Kim and Chun 2021). These pressures underscore the urgent need for genetic resources to support effective conservation management.

No mitogenome data currently exist for Andvakiidae or *Synandwakia*. In this study, we present the complete mitogenome sequence of *S. multitentaculata*, providing essential information for the conservation management and phylogenetic studies of this marine protected species.

Materials and methods

One specimen was obtained from Wangsan Beach's intertidal zone in Incheon, Korea (37°27'21"N, 126°22'01"E; Figure 1). The specimen was deposited at the Cnidaria Bioresources Bank of Korea, Woosuk University (voucher: MBCL23CnAnI019 (=CBB23CnAnI025); contact: Sung-Jin Hwang (identifier), buteo2@woosuk.ac.kr). Species identification was confirmed by morphological examination following Song (2003).

Genomic DNA was isolated from tentacle and column tissues using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-tek, Norcross, GA, USA). Mitochondrial DNA was enriched using Exonuclease-Resistant Random Primers (Thermo Fisher Scientific, Waltham, MA, USA), which are broadly applicable to marine invertebrates, instead of the human-specific primers supplied with the REPLI-g Mitochondrial DNA Kit (Qiagen, Venlo, Netherlands) (Kim and Shin 2021; Lee et al. 2025). Prior to sequencing, library preparation was performed by mechanically shearing genomic DNA, purifying the fragments, and constructing indexed libraries with an Illumina-compatible preparation kit. The enriched DNA was subsequently sequenced on the Illumina NovaSeq 6000 platform at

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Figure 1. *Synandwackia multitentaculata*. (A) Living individual in its natural habitat of Wangsan Beach, Republic of Korea. (B) Voucher specimen (MBCL23CnAnI019) used for mitogenome sequencing. Photographs were taken by Seung-Joon Lee.

the National Instrumentation Center for Environmental Management (Seoul, South Korea), generating 150 bp paired-end reads with approximately $56.81\times$ coverage.

De novo assembly was performed using NOVOPlasty v3.3.4 (Dierckxsens et al. 2017) with default parameters (default k-mer = 38), using a previously obtained partial mtDNA *COX1* sequence of *S. multitentaculata* as the seed/reference. Mitochondrial genome annotation was subsequently carried out using the MITOS2 pipeline on the Galaxy web server. Geneious Prime (Biomatters Ltd, Auckland, New Zealand) was used specifically for visualization and final curation of the annotated mitogenome, including positioning *COX1* at the first locus to generate the complete circular mitogenome map. Exon–intron arrangements of *COX1* and *ND5*, both known to undergo cis-splicing—a characteristic feature distinguishing Actiniaria from other cnidarians (Emblem et al. 2014; Johansen and Emblem 2020)—were visualized using PMGmap software (Zhang et al. 2024) (Figures S2 and S3). Ribosomal RNA genes were verified through homology comparisons with the closely related *Diadumene lineata*, and transfer RNA genes were manually inspected to confirm their predicted secondary structures. For phylogenetic analysis, mitogenome sequences from 23 Actiniaria species, including *S. multitentaculata*, and one Corallimorpharia outgroup (*Corynactis californica*) were used. Nucleotide sequences of the 13 PCGs, excluding termination codons, were concatenated and aligned using MAFFT v.7 (Katoh and Standley 2013). Maximum-likelihood phylogenetic reconstruction was conducted using raxmlGUI 2.0 (Edler et al. 2021) with the GTR + G + I substitution model. Branch support was evaluated through 10,000 bootstrap replicates. The manuscript text was reviewed for English grammar and clarity using generative AI (Claude, Anthropic). All scientific content and interpretations were prepared by the authors.

Results

The complete mitochondrial genome of *Synandwackia multitentaculata* is 18,350 bp and contains 17 genes: 13 protein-coding genes (PCGs), two ribosomal RNA genes (*rnrS*, *rnrL*), and two transfer RNA genes (*trnM*, *trnW*) (Figure 2). The circular

configuration of the mitogenome was verified by detecting overlapping terminal regions in the *de novo* assembly and by confirming uniform sequencing coverage (average $56.81\times$) across the entire genome (Figure S1). All PCGs are encoded on the heavy strand, initiating with ATG and terminating with TAA (11 genes: *ATP6*, *ATP8*, *COX1*, *COX3*, *COB*, *ND1*, *ND3*, *ND4*, *ND4L*, *ND5*, and *ND6*) or TAG (two genes: *COX2* and *ND2*).

The mitogenome shows AT richness, with nucleotide composition of A: 29.9%, C: 15.3%, G: 19.0%, and T: 35.9% (G + C: 34.3%). Analysis of different genomic regions revealed similar patterns: PCGs (11,712 bp in total) exhibit G + C: 37.0%; ribosomal RNA genes (3,073 bp in total) show G + C: 41.2%; transfer RNA genes (144 bp in total) display G + C: 47.9%.

The *COX1* and *ND5* genes exhibit a characteristic split-gene architecture with two exons separated by a single intron (Figures S2 and S3). The *COX1* gene consists of exons of 915 bp and 699 bp, separated by an 834 bp intron, generating a mature mRNA of 1,614 bp. The *ND5* gene contains exons of 716 bp and 1,117 bp, separated by a 1,681 bp intron, producing a mature mRNA of 1,833 bp. Both genes undergo cis-splicing to generate functional proteins. The *ND1* (984 bp) and *ND3* (356 bp) genes are located within the *ND5* intronic region, a characteristic organizational feature of Actiniaria mitogenomes.

Maximum-likelihood phylogenetic analysis revealed that *Synandwackia multitentaculata* forms a well-supported clade with *Diadumene lineata* (bootstrap support: 100%), clustering with *Metridium senile* within Metridioidea (Figure 3). The phylogenetic tree resolved three major superfamily-level clades: Metridioidea, Actinioidea, and Actinostoloidea. Within Actinioidea, multiple lineages formed distinct subclades with varying levels of support. The reconstruction further indicates that Actiniidae is paraphyletic, with member species distributed across multiple clades. The base of the Actinioidea clade shows moderate bootstrap support (72%), suggesting some uncertainty in the evolutionary relationships.

Discussion and conclusions

This study reports the first complete mitogenome of *Synandwackia multitentaculata*, representing the first molecular

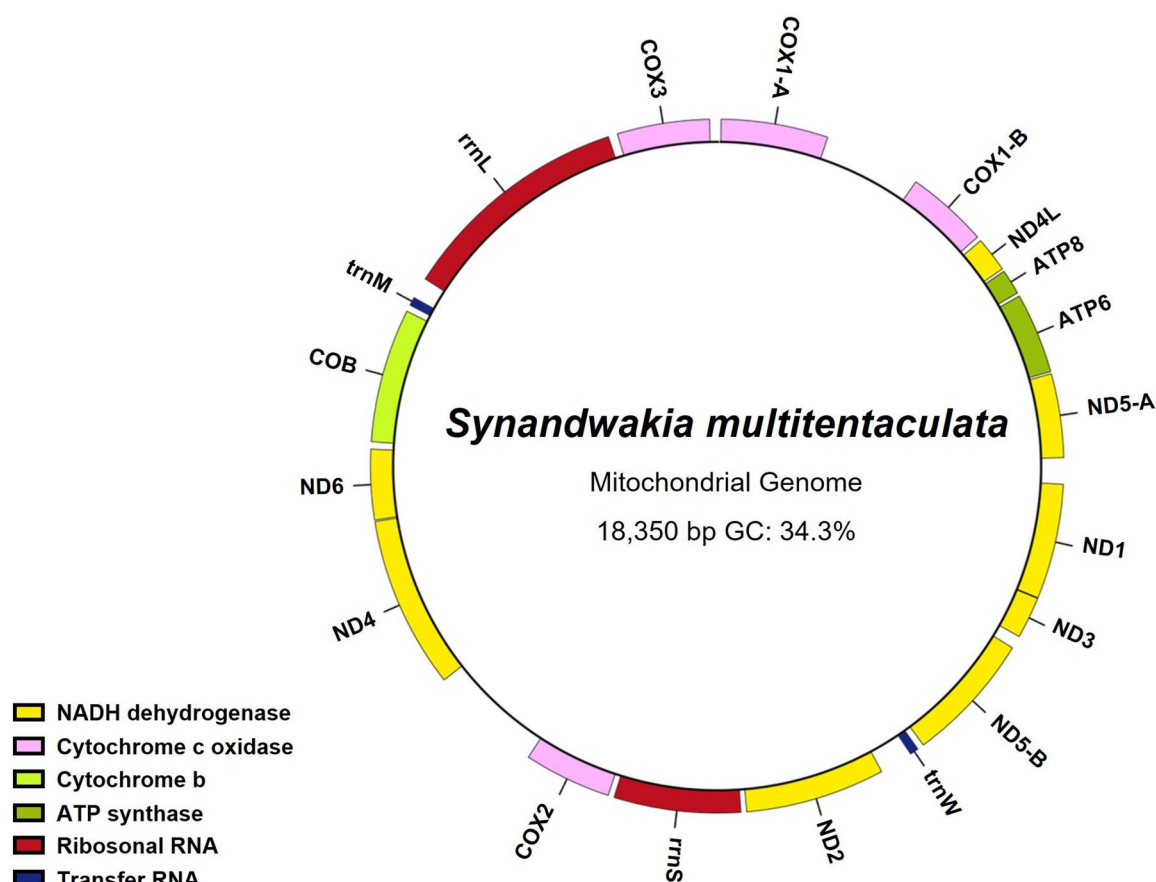


Figure 2. Circular representation of the complete mitogenome for *Synandwakia multitentaculata* (18,350 bp; G + C content: 34.3%). Gene annotations are color-coded according to functional groups: yellow (NADH dehydrogenase), pink (cytochrome c oxidase), green (cytochrome b), light green (ATP synthase), red (ribosomal RNA), and blue (transfer RNA).

characterization of genus *Synandwakia* and providing essential genetic resources for conservation. The mitogenome (18,350 bp) is comparable to other Actiniaria species (18–20 kb) (Li et al. 2023), and shows conserved gene content observed in sea anemone mitogenomes.

The mitogenome of *S. multitentaculata* exhibits distinctive Actiniaria structural features. Most notably, both *COX1* and *ND5* genes display split-gene architecture with two exons separated by a single intron undergoing cis-splicing (Emblem et al. 2014; Johansen and Emblem 2020). This unusual gene structure, distinguishing Actiniaria from other cnidarian orders, may have regulatory functions in mitochondrial gene expression (Johansen and Emblem 2020). The *ND1* and *ND3* genes within the *ND5* intronic region exemplify Actiniaria genomic organization. The AT-rich composition (G + C content: 34.3%) is consistent with cnidarian mitochondrial genomes, including Actiniaria (30–40%) and other anthozoans (33–39%) (Niu et al. 2018; Terraneo et al. 2022; Li et al. 2023; Yang et al. 2023; Wei et al. 2024; Lee et al. 2025). Heavy strand encoding of all PCGs is a conserved Actiniaria organizational feature (Emblem et al. 2014; Johansen et al. 2021).

Phylogenetic analysis places *S. multitentaculata* within Metridioidea, clustering with *Diadumene lineata* (bootstrap: 100%) and *Metridium senile*. The close relationship between *S. multitentaculata* (Andvakiidae) and *D. lineata*

(Diadumenidae) differs from previous placements of Andvakiidae closer to Hormathiidae (Rodríguez et al. 2014). Broader taxonomic sampling across Metridioidea families is needed to accurately resolve interfamilial phylogenetic relationships. Three superfamily clades (Metridioidea, Actinioidea, and Actinostoloidea) support previous evidence of polyphyletic Actiniaria families (Rodríguez et al. 2014; Daly et al. 2017). Limited mitogenome representation in Metridioidea restricts robust testing of family-level monophyly.

The complete mitogenome provides critical conservation tools for *S. multitentaculata*, including molecular markers for assessing genetic diversity across the three known populations and eDNA-based monitoring protocols. Given the species' restricted distribution and threats from coastal reclamation and climate change (Seong et al. 2010; Ross and Adam 2013; Choi 2014, 2023), these molecular resources are essential for effective conservation strategies.

Ethical approval

This study was conducted in compliance with all ethical and legal requirements of Republic of Korea. The collection of the marine protected species, *Synandwakia multitentaculata*, was specifically authorized by the Ministry of Oceans and Fisheries under official permit (No. 2023-01) from the relevant authorities. The specimen was collected at Wangsan Beach, Incheon, in full compliance with conservation regulations.

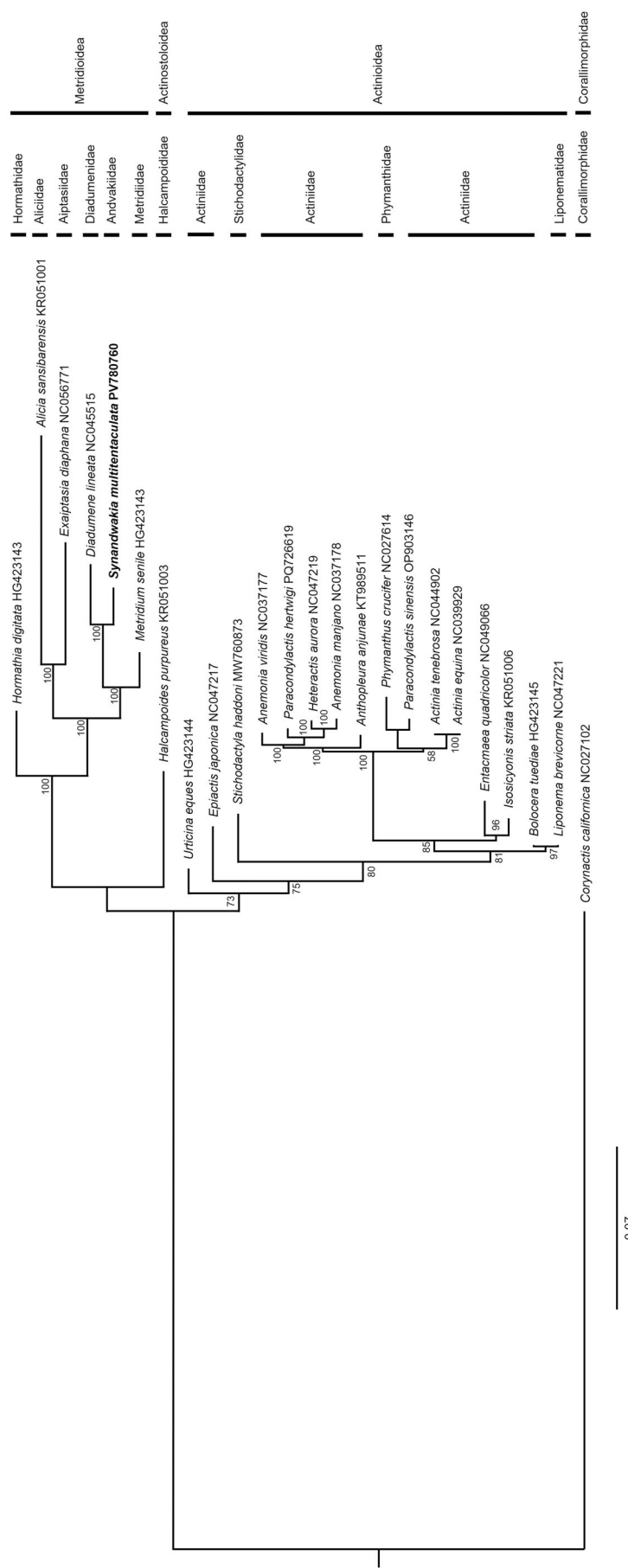


Figure 3. Maximum-likelihood tree based on concatenated sequences of 13 PCGs from 23 Actinaria species. *Corynactis californica* (Corallimorpharia) was used as the outgroup. Bootstrap values (10,000 replicates) are displayed at nodes. GenBank accession numbers follow corresponding species names in the tree. The target species (*Synandwakia multidentata*) is indicated in bold. Family and superfamily classifications are shown on the right. The tree topology reveals three superfamily-level lineages: Metridioidea, Actinioidea, and Actinostoloidea. The following sequences were used: *Anemonia manjano* NC037178 (Chi et al. 2018), *Heteractis aurora* NC047219 (unpublished), *Paracondylactis hertwigi* PQ726619 (Lee et al. 2025), *Anemonia viridis* NC037177 (Chi et al. 2018), *Anthopleura anjunae* KT989511 (Zhang and Zhu 2017), *Phymanthus crucifer* NC027614 (Fook et al. 2016), *Paracondylactis sinensis* OP903146 (Li et al. 2023), *Actinia tenebrosa* NC044902 (Surm et al. 2019), *Actinia equina* NC039929 (Wilding and Weedall 2019), *Entacmaea quadricolor* NC049066 (unpublished), *Isosicyonis striata* KR051006 (Fook et al. 2016), *Bolocera tuediae* HG423145 (unpublished), *Liponema brevicoorne* NC047221 (unpublished), *Stichodactyla haddoni* MW760873 (Johansen et al. 2021), *Epiactis japonica* NC047217 (unpublished), *Urticina eques* HG423144 (Emblem et al. 2014), *Alicia sansibarensis* KR051001 (Fook et al. 2016), *Exaiptasia diaphana* NC056771 (Emblem et al. 2014), *Diadumene lineata* NC045515 (unpublished), *Synandwakia multidentata* PV780760 (this study), *Metridium senile* HG423143 (Emblem et al. 2014), *Hormathia digitata* HG423146 (Emblem et al. 2014), *Halcampoides purpureus* KR051003 (Fook et al. 2016), and *Corynactis californica* NC027102 (Lin et al. 2014).

Author contributions

CRediT: **Seung-Joon Lee**: Investigation, Software, Writing – original draft; **Sang-Hwa Lee**: Data curation, Software; **In-Young Cho**: Resources; **Sung-Jin Hwang**: Conceptualization, Project administration, Supervision, Writing – review & editing.

Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in the GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> under the accession number PV780760. The associated BioProject, SRA and Bio-sample numbers are PRJNA1275495, SRR33945937, and SAMN49012214, respectively.

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