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MITOGENOME REPORT

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Complete mitochondrial genome of *Acanthochitona defilippii* (Polyplacophora: Chitonida) from South Korea

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ABSTRACT

The chiton (Polyplacophora) occupies a significant position in molluscan evolutionary history as one of the most primitive groups within the phylum Mollusca. *Acanthochitona defilippii* (Tapparone-Canefri 1874) (Chitonida: Acanthochitonidae) is a commonly found intertidal chiton species in South Korea. In this study, we characterized the complete mitochondrial genome of *A. defilippii* (14,999 bp long), comprising 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNA genes, and an A + T rich region (166 bp). The base composition is as follows: 31.82% for A, 11.63% for C, 16.69% for G, and 39.86% for T. We reconstructed a maximum likelihood (ML) tree to elucidate phylogenetic relationships among the eight chitonid families using the nucleotide sequences of all PCGs. The ML tree revealed that *A. defilippii* clustered with *Acanthochitona avicula* (BP 100) within the family Acanthochitonidae. Acanthochitonidae formed a sister group with Mopaliidae. The results could provide a valuable understanding the phylogenetic relationships of chitonid species.

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Introduction

The genus *Acanthochitona* (Polyplacophora: Chitonida: Acanthochitonidae) consists of 83 species and is widely distributed in various regions, including the Southern Hemisphere and the northeastern Atlantic. Among them, the chiton *Acanthochitona defilippii* (Tapparone-Canefri 1874) is widely distributed in the intertidal zone across Japan, China, and the Indo-Pacific region, and it is among the most common intertidal species in South Korea (Hong and Van Belle 1990). They are small to medium-sized creatures with elongated oval bodies covered in eight overlapping, articulated plates. Their palates are small, and they have a wide, fleshy girdle with nine pairs of suture bundles and various short spicules, featuring spines that range in color from dark brown to green and white (Yeh et al. 2005) (Figure 1). *A. defilippii* is known for maintaining its past life history and ecological characteristics for approximately 300 myr, rendering chitons as living fossils and holding a significant position in molluscan evolutionary history as the most primitive group (Scherholz et al. 2013). Despite their importance in the evolutionary aspect, few studies reported with respect to the phylogenetic relationships of the species. In recent years, mitochondrial genome (mitogenome) data have emerged as a powerful tool across various scientific disciplines, including molecular phylogenetics and population genetics (Lee et al. 2012; Kim et al. 2019; Choi, Choi, et al. 2021; Choi and Hwang 2021; Choi, Yeo, et al. 2021; Park et al. 2021; Akintola et al. 2022; Choi and Hwang 2023). Until now, in the genus *Acanthochitona*, there have been published only two complete mitogenomes from *Acanthochiona rubrolineata* and *Acanthochitona aviula*. In this study, we aimed to provide a foundational basis for the molecular investigation of *A. defilippii* by characterizing a complete mitochondrial genome and elucidating its phylogenetic position. It is the third *Acanthochitona* mitogenome to be completely characterized.

Materials and methods

The specimen of *A. defilippii* was collected from Seogwipo, Jeju Island, South Korea (33°14′24″N, 126°32′44″E) and preserved in absolute ethanol. It was deposited under the voucher number LEGOM040550 at Kyungpook National University, Daegu, South Korea (Prof. Ui Wook Hwang,

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Figure 1. A Photograph of *Acanthochitona defilippii* attached on a rock surface. The photo was taken by the author (I Hyang Kim).

uwhwang@knu.ac.kr). Genomic DNA was extracted from the foot of the specimen using a DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). The quality and quantity of DNA were checked using Nanodrop4000 (Thermo Fisher Scientific, United States). For genome sequencing, Illumina TruSeq library was constructed with an average insert size of 350 bp using TruSeq DNA Nano 350 bp kit (Illumina, United states). After library preparation, the sequencing was performed using the Illumina NovaSeq 6000 platform with the production of paired-end reads length of 151 bp in DNA Link Inc. (Seoul, South Korea). The detailed information of depth and coverage for sequencing is displayed as a plot in Supplementary Figure 1. The sequences were assembled using NOVOPlasty 4.3.5. (Dierckxsens et al. 2017) with the mitogenome of *Acanthochitona avicula* (Irisarri et al. 2020) as a reference genome. 22 tRNA genes were predicted using tRNAscan-SE (Chan and Lowe 2019) and ARWEN (Laslett and Canbäck 2008). The 13 protein-coding genes (PCGs) and two rRNA genes were searched using EMBOSS Transeq (Madeira et al. 2022) and the MITOS web server (http://mitos.bioinf.uni-leipzig.de/) (Bernt et al. 2013). After annotation, the circular mitogenome of *A. defilippii* was visualized using Proksee (Grant et al. 2023). The phylogenetic tree was reconstructed using the maximum likelihood (ML) method through the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) (Trifinopoulos et al. 2016). For the sequence alignment set, mitogenome data from 22 polyplacophoran specimens were retrieved from the NCBI GenBank database. *Scutopus*

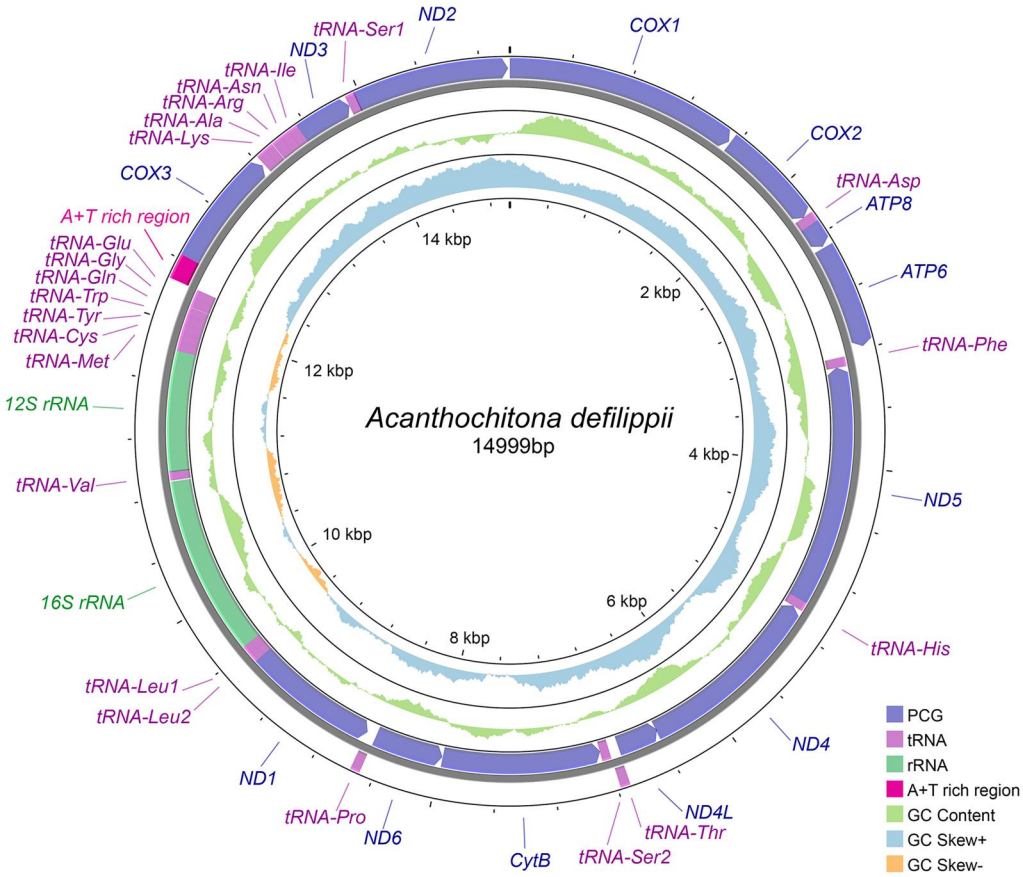

Figure 2. Circular map of the complete mitochondrial genome of *acanthochitona defilippii*. The total length of the complete mitochondrial genome is 14,999 bp. Genes located on the outer side (*ATP6*, *ATP8*, *COX1–3*, *ND2*, and *ND3*) are on the heavy strand, while those on the inner side (*CytB*, *ND1*, *ND4*, *ND4L*, and *ND6*) are on the light strand. The inner circle illustrates the GC-skew, representing the variation from the overall average GC content across the entire sequence. The complete mitochondrial genome map was visualized using the Proksee program (Grant et al. 2023).

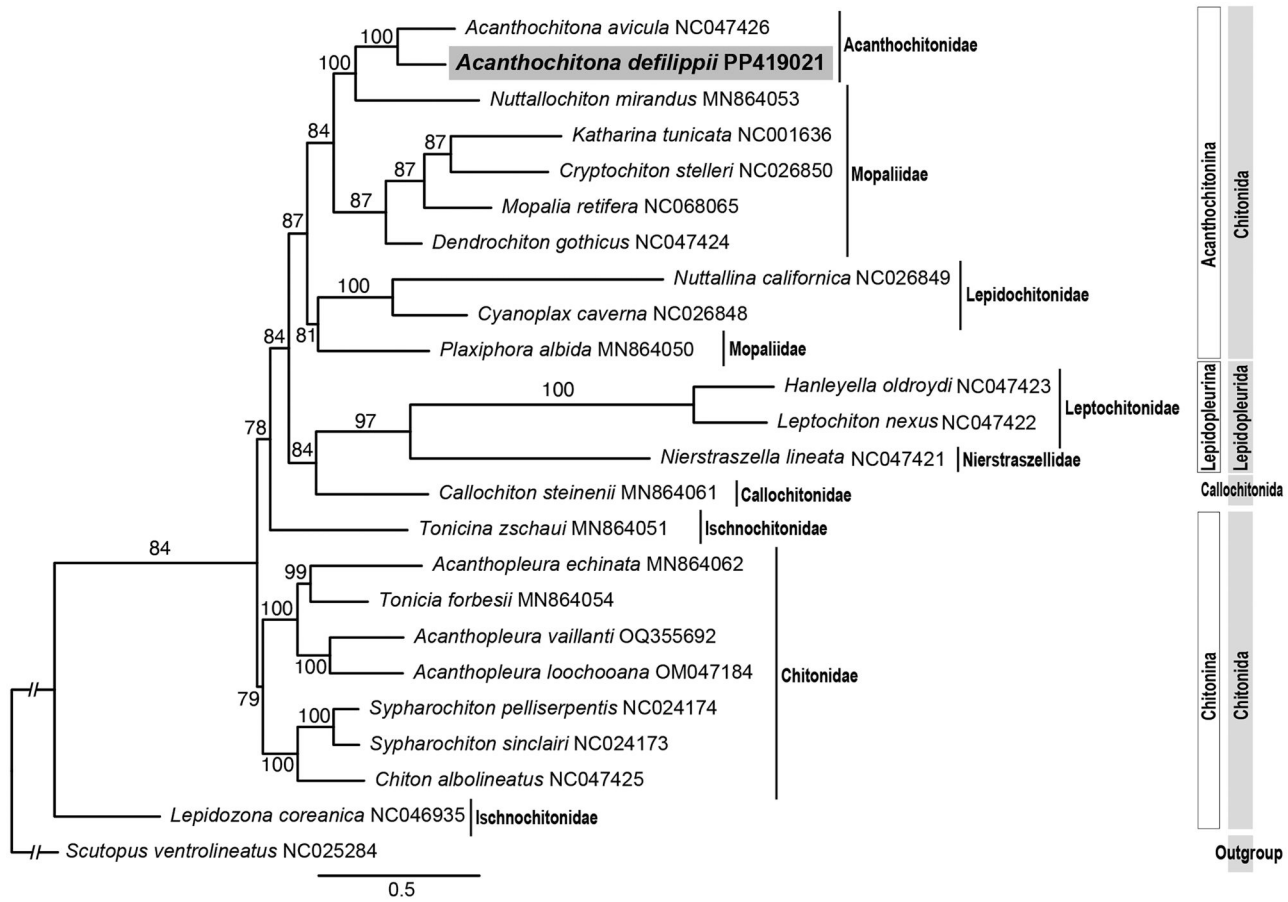

Figure 3. A Maximum-likelihood tree reconstructed with nucleotide sequences of 13 mitochondrial PCGs from 23 chitonid species. The phylogenetic dataset consists of Chitonida (19 species), Callochitonida (1 species), and Lepidopleurida (3 species). The gray square box represents the examined species, *A. defilippii* in this strand. *Scutopus ventrolineatus* (caudofoveata) was set as an outgroup species. The number next to or below the species name indicated the accession number in GenBank. The numbers above each branch indicate the bootstrap support value. The following sequences were used: *Acanthochitona avicula* NC047426 (Irisarri et al. 2020), *Acanthopleura echinate* MN864062 (Irisarri et al. 2020), *Acanthopleura loochooana* OM047184 (unpublished), *Acanthopleura vaillanti* OQ355692 (Alnashiri et al. 2024), *Callochiton steinenii* MN864061 (Irisarri et al. 2020), *Chiton albolineatus* NC047425 (Irisarri et al. 2020), *Cryptochiton stelleri* NC026850 (Irisarri et al. 2014), *Cyanoplax caverna* NC026848 (Irisarri et al. 2014), *Dendrochiton gothicus* NC047424 (Irisarri et al. 2020), *Hanleyella oldroydi* NC047423 (Irisarri et al. 2020), *Katharina tunicata* NC001636 (Boore and Brown 1994), *Lepidozona coreanica* NC046935 (Sun et al. 2023), *Leptochiton nexus* NC047422 (Irisarri et al. 2020), *Mopalia retifera* NC068065 (unpublished), *Nierstraszella lineata* NC047421 (Irisarri et al. 2020), *Nuttallina californica* NC026849 (Irisarri et al. 2014), *Nuttallochiton mirandus* MN864053 (Irisarri et al. 2020), *Plaxiphora albida* MN864050 (Irisarri et al. 2020), *Scutopus ventrolineatus* NC025284 (Osca et al. 2014), *Sypharochiton pelliserpentis* NC024174 (Veale et al. 2016), *Sypharochiton sinclairi* NC024173 (Veale et al. 2016), *Tonicia forbesii* MN864054 (Irisarri et al. 2020) and *Tonicina zschaui* MN864051 (Irisarri et al. 2020).

ventrolineatus was used as an outgroup in the phylogenetic analysis.

Results

We successfully sequenced the complete mitogenome of *A. defilippii*, which is 14,999 bp in length (Figure 2). It has been deposited in the GenBank database (accession number: PP419021). The overall base composition of the mitogenome was 31.82% for A, 11.63% for C, 16.69% for G, and 39.86% for T, with a GC content of 28.33%. Gene annotation revealed that it encodes 13 PCGs, 22 tRNAs, and two rRNAs. All PCGs start with a typical 'ATG' codon. As for the stop codon, four PCGs (*ND1, ND2, ND4,* and *ND5*) are used a 'TAG' stop codon and eight PCGs (*ATP6, ATP8, COX1, COX2, COX3, CytB, ND3, ND4L*) end with a 'TAA' stop codon. The remaining one, *ND6*, has a stop codon that ends with T–. The A + T rich region is 166 bp in length, located between *tRNA-Glu* and *COX3*. The 12S rRNA and the 16S rRNA genes are 854 bp and 1273 bp long, respectively, and separated by *tRNA-Val*. All tRNAs, except for *tRNA-Ser2*, have the typical cloverleaf structure ranging from 58–68 bp in length.

Phylogenetic relationships among 23 polyplacophoran species were investigated, including 19 species of Chitonida, 1 of Callochitonida, and 3 of Lepidopleurida (Figure 3). Most of the clades in the ML tree were supported with high bootstrapping values ranging from BP 70 to BP 100. Chitonida is largely divided into two groups, and within that group, Acanthochitonina and Chitonina belongs to each suborder. *A. defilippii* clustered with *A. avicula* within the family Acanthochitonidae with a high confidence value (BP 100) within the suborder Acanthochitonina. *Nuttallochiton mirandus*, belonging to the Mopaliidae, appeared as a sister taxon of the two *Acanthochitona* species (BP 100). Interestingly, the family Mopaliidae, belonging to the suborder Acanthochitonina, was not supported as monophyletic, which was divided into three different genetic lineages.

Discussion and conclusion

We characterized the complete mitogenome of *A. defilippii* and the arrangement of the 13 PCGs in order and strand position was identical to that of *A. avicula*. The ML phylogeny based on the 13 PCGs in this study (Figure 3) places *A. defilippii* within the family of Acanthochitonidae (BP 100). Acanthochitonidae was clustered with Mopaliidae, but the monophyly of Mopaliidae was not supported, which the taxon was divided into three different groups. The phylogenetic relationship was also reported in Irisarri et al. (2020). There requires further examination for the phylogenetic relationship of the family Mopaliidae with a reexamination of taxonomic status based on an integrative approach based in morphological and molecular characters. The mitogenome information of *A. defilippii* reported here could contribute to a comprehensive understanding of acanthochitonid phylogeny and chitonid evolutionary history.

Authors contributions

UWH and EHC designed the study. IHK and UWH wrote the manuscript. IHK, CRS, GK, BP and KBK 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.

Ethical approval

The material involved in this article does not involve any ethical conflicts. This species is not endangered according to the CITES catalog 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 PP419021. The associated BioProject, Bio-Sample, and SRA numbers are PRJNA1085534, SAMN40304573, SRR28268872 respectively.

Disclosure statement

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

References

Akintola AA, Park B, Choi EH, Hwang UW. 2022. Complete mitochondrial genome of a malaria vector mosquito *Anopheles sinensis* from South Korea. Mitochondrial DNA B Resour. 7(5):881–883. doi:10.1080/23802359.2022.2077665.

Alnashiri H, Thomas L, Philip S, Thaikkottathil M, Sureshkumar S, Kutty R. 2024. Complete mitochondrial genome and phylogenetic relationships of the red sea Chiton *Acanthopleura vaillantii* Rochebrune, 1882 (Polyplacophora: chitonida). Thalassas. 40(1):51–58. doi:10.1007/s41208-023-00648-0.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319. doi:10.1016/j.ympev.2012.08.023.

Boore JL, Brown WM. 1994. Complete DNA sequence of the mitochondrial genome of the black chiton, *Katharina tunicata*. Genetics. 138(2):423–443. doi:10.1093/genetics/138.2.423.

Chan PP, Lowe TM. 2019. tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol Biol. 1962:1–14.

Choi EH, Choi NR, Hwang UW. 2021. The mitochondrial genome of an endangered freshwater snail *Koreoleptoxis nodifila* (Caenogastropoda: Semisulcospiridae) from South Korea. Mitochondrial DNA B Resour. 6(3):1120–1123. doi:10.1080/23802359.2021.1901626.

Choi EH, Hwang UW. 2021. The complete mitochondrial genome of an endangered triton snail *Charonia lampas* (Littorinimorpha: Charoniidae) from South Korea. Mitochondrial DNA B Resour. 6(3):956–958. doi:10.1080/23802359.2021.1889416.

Choi EH, Hwang UW. 2023. Complete mitochondrial genome of a golden orb-web spider *Trichonephila clavata* (Chelicerata, Arachnida) from South Korea. Mitochondrial DNA B Resour. 8(7):723–725. doi:10.1080/23802359.2021.1955633.

Choi EH, Yeo MY, Kim G, Park B, Shin CR, Baek SY, Hwang UW. 2021. *Liolophura* species discrimination with geographical distribution patterns and their divergence and expansion history on the northwestern Pacific coast. Sci Rep. 11(1):17602. doi:10.1038/s41598-021-96823-5.

Dierckxsens N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45(4):e18. doi:10.1093/nar/gkw955.

Grant JR, Enns E, Marinier E, Mandal A, Herman EK, Chen C, Graham M, Van Domselaar G, Stothard P. 2023. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Res. 51(W1):W484–W492. doi:10.1093/nar/gkad326.

Hong JS, Van Belle RA. 1990. The Chiton Fauna (Mollusca: polyplacophora) of Korea. Part II: suborder Acanthochitonina. The Korean Journal of Zoology. 33(4):373–395.

Irisarri I, Eernisse DJ, Zardoya R. 2014. Molecular phylogeny of Acanthochitonina (Mollusca: polyplacophora: chitonida): three new mitochondrial genomes, rearranged gene orders and systematics. J Nat Hist. 48(45-48):2825–2853. doi:10.1080/00222933.2014.963721.

Irisarri I, Uribe JE, Eernisse DJ, Zardoya R. 2020. A mitogenomic phylogeny of chitons (Mollusca: Polyplacophora). BMC Evol Biol. 20(1):22. doi:10.1186/s12862-019-1573-2.

Kim G, Jeong KC, Choi EH, Ryu SH, Lim YJ, Jun J, Lee YS, Hwang UW. 2019. The complete mitochondrial genome of an Asian crested ibis *Nipponia nippon* (Pelecaniformes, Threskiornithidae) from South Korea. Mitochondrial DNA B Resour. 4(2):3707–3708. doi:10.1080/23802359.2019.1680321.

Laslett D, CANBäCK B. 2008. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 24(2):172–175. doi:10.1093/bioinformatics/btm573.

Lee JH, Choi EH, Kim SK, Ryu SH, Hwang UW. 2012. Mitochondrial genome of the cockscomb pearl mussel *Cristaria plicata* (Bivalvia, Unionoida, Unionidae). Mitochondrial DNA. 23(1):39–41. doi:10.3109/19401736.2011.643882.

Madeira F, Pearce M, Tivey ARN, Basutkar P, Lee J, Edbali O, Madhusoodanan N, Kolesnikov A, Lopez R. 2022. Search and sequence analysis tools services from EMBL-EBI in 2022. Nucleic Acids Res. 50(W1):W276–W279. doi:10.1093/nar/gkac240.

Osca D, Irisarri I, Todt C, Grande C, Zardoya R. 2014. The complete mitochondrial genome of *Scutopus ventrolineatus* (Mollusca: Chaetodermomorpha) supports the Aculifera hypothesis. BMC Evol Biol. 14:197. doi:10.1186/s12862-014-0197-9.

Park B, Choi EH, Kim G, Shin CR, Hwang J, Baek SY, Hwang UW. 2021. The complete mitochondrial genome of the two-spotted cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae) from South Korea. Mitochondrial DNA B Resour. 6(3):1144–1146. doi:10.1080/23802359.2021.1901617.

Scherholz M, Redl E, Wollesen T, Todt C, Wanninger A. 2013. Aplacophoran mollusks evolved from ancestors with polyplacophoran-like features. Curr Biol. 23(21):2130–2134. doi:10.1016/j.cub.2013.08.056.

Sun D, Lin Z, Teng X, Xu L, Qian L, Yu X, Wu H, Wang Z, Jin L, Liu X, et al. 2023. The complete mitochondrial genome and phylogenetic analysis of *Lepidozona coreanica* (Reeve, 1847). Mitochondrial DNA B Resour. 8(5):629–633. doi:10.1080/23802359.2023.2183723.

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44(W1):W232–W235. doi:10.1093/nar/gkw256.

Veale AJ, Williams L, Tsai P, Thakur V, Lavery S. 2016. The complete mitochondrial genomes of two chiton species (*Sypharochiton pelliserpentis* and *Sypharochiton sinclairi*) obtained using Illumina next generation sequencing. Mitochondrial DNA A DNA Mapp Seq Anal. 27(1):537–538. doi:10.3109/19401736.2014.905846.

Yeh TY, Cheng YT, Hsueh PW. 2005. On a new record of an intertidal chiton *Acanthochitona defilippii* (Tapparone-Canefri, 1874) (Mollusca: polyplacophora) from Taiwan. Collection and Research. 18: 65–68.