The complete mitochondrial genome of sea slug *Phyllidia elegans* Bergh, 1869 (nudibranchia, phyllidiidae) from the South China Sea

Zhehao Li\(^{a,b}\), Xiaoji Zeng\(^{a,b}\) and Gang Ni\(^{a}\)

\(^{a}\)Ministry of Education Key Laboratory of Mariculture, Ocean University of China, Qingdao, China; \(^{b}\)Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao, China

**ABSTRACT**

In this study, the complete mitochondrial genome (mitogenome) sequence of sea slug, *Phyllidia elegans* Bergh, 1869 (Nudibranchia, Phyllidiidae), was sequenced and characterized. The assembled mitogenome was 14618 bp in length, including 13 protein-coding genes (PCGs), two ribosomal RNA genes, and 22 transfer RNA genes. The overall base composition of *P. elegans* mitogenome is 32.1% for A, 13.5% for C, 15.7% for G, and 38.7% for T. The gene order was identical to other Phyllidiid species. Phylogenetic analysis placed *P. elegans* and *Phyllidia ocellata* in one clade.

Nudibranchs, also known as sea slugs, are shell-less and brightly colored marine gastropod mollusks (Penney et al. 2020). They occur throughout the world’s oceans, and their richness can reflect the diversity and health of coral reefs (Xiang et al. 2017; Undap et al. 2019). *Phyllidia elegans* Bergh, 1869 is a species of Nudibranchia, Phyllidiidae, with major distribution in the tropical Indo-Pacific region (Brunkhorst 1993). It has cream-colored tubercles on the dorsum with black longitudinal stripes on the foot sole (Brunkhorst 1993; Dominguez et al. 2007). In this study, we reported the complete mitochondrial genome (mitogenome) of *P. elegans* and examined its phylogenetic position.

The *P. elegans* specimen was collected from the South China Sea (16°12′9.4″ N, 111°40′43.5″ E) through Scuba diving and deposited in Fisheries College, Ocean University of China (https://scxy.ouc.edu.cn/main.htm, contact person: Zeng Xiaqi, email: zengxq@ouc.edu.cn) under the voucher number HN-XS2108003. Since *P. elegans* is neither an endangered nor a protected species in China, no specific permissions or licenses are required for collection. Total genomic DNA was extracted from foot tissue using Tsingke DNA extraction Kit. The library was constructed and sequenced by the Illumina HiSeq X Ten platform to obtain paired-end reads (150 bp). Clean data was assembled by NOVOPlasty 4.2 software (Dierckxsens et al. 2017) using cox1 gene fragment of *Phyllidiella pustulosa* as a seed sequence. The mitogenome was annotated using MITOS (Bernt et al. 2013) and tRNAscan-SE (Lowe and Chan 2016).

The complete mitogenome of *P. elegans* was 14618 bp in length and encoded 37 genes including 13 protein-coding genes (PCGs), two ribosomal RNA (rRNA), and 22 transfer RNA (tRNA). The gene order was identical to other reported mitogenomes of Phyllidiid species as follows (e.g., Xiang et al. 2016; Do et al. 2019): cox1, tRNA-Val, rrnL, tRNA-Leu, tRNA-Ala, tRNA-Pro, nad6, nad5, nad1, tRNA-Tyr, tRNA-Arg, nad4I, cob, tRNA-Asp, tRNA-Phe, cox2, tRNA-Gly, tRNA-His, tRNA-Cys, tRNA-Gln, tRNA-Leu, atp8, tRNA-Asn, atp6, tRNA-Arg, tRNA-Glu, rrnS, tRNA-Met, nad3, tRNA-Ser, tRNA-Thr, nad4, tRNA-Thr, cox3, tRNA-Ile, nad2, and tRNA-Lys. Within these genes, thirteen genes (atp8, atp6, nad3, cox3, rrn5, tRNA-Gln, tRNA-Leu, tRNA-Asn, tRNA-Arg, tRNA-Glu, tRNA-Met, tRNA-Ser, and tRNA-Thr) were located on the light strand, while all the others were located on the heavy strand. The base composition of this mitogenome was A = 32.1%, C = 13.5%, G = 15.7%, and T = 38.7%. Most PCGs (cox1, nad5, nad1, cox2, atp8, atp6, nad3, nad4, cox3, and nad2) started with ATG as the start codon, and three genes (nad6, nad4I, and cob) with ATA codon. All PCGs used the conventional stop codons TAA and TAG except one gene nad2 using an incomplete stop codon T.

The mitogenome of *P. elegans* and 16 related species with available mitogenomes on NCBI were used to infer the phylogenetic position of *P. elegans*. Two species *Pleurabronchaea novaezealandiae* and *Berthellina* sp. in the Pleurobranchia were chosen as outgroups. The sequences of each PCG were individually aligned in Mafft v.7 (Katoh and Standley 2013) with default settings, and ambiguously aligned regions were eliminated using Gblocks v.0.91b (Talavera and Castresana 2007). ModelFinder (Kalyaanamoorthy et al. 2017) selected the following models as the best-fit substitution model under the Bayesian Information Criterion: TVM + F + I + G4 for atp6, atp8, nad2, nad3, nad4, nad4I, nad5, and nad6; GTR + F + I + G4 for...
cob, nad1, and cox2; TIM + F + I + G4 for cox1 and cox3. A maximum-likelihood (ML) tree based on the 13 PCGs was constructed using IQ-TREE 1.6.8 (Guindon et al. 2010, Nguyen et al. 2015, Chernomor et al. 2016) with 1000 ultrafast bootstrap replicates (Minh et al. 2013). The phylogenetic result showed that P. elegans was clustered with Phyllidia ocellata in the Phyllidiidae with maximum support of 100% (Figure 1). The mitogenome of P. elegans could be useful in further phylogenetic analysis of Phyllidiidae within Nudibranchia.

Ethical approval

This study was reviewed and approved by the Institutional Review Board of Ocean University of China. This article does not contain any studies with human participants or animals requiring ethical approval.

Author contributions

Xiaoqi Zeng and Gang Ni conceived this study, and reviewed and edited the drafting of the paper. Zhehao Li performed the experiment, analyzed and interpreted the data, and prepared the original draft. All authors agreed to be accountable for all aspects of the work and approved the final draft to be published.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the grants from National Programme on Global Change and Air-Sea Interaction [No. GASI-02-SCS-YDsum] and the Young Talent Program of Ocean University of China [No. 862201013143].

Data availability statement

The genome sequence that supports the findings of this study was openly available in GenBank of NCBI under accession No. OM273027 (https://www.ncbi.nlm.nih.gov/search/all/?term=OM273027). BioProject no. PRJNA832552; Biosample: SAMN27914503; SRA no.: SRR18959687.

References

Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsch G, Putz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2): 313–319.

Brunckhorst DJ. 1993. The systematics and phylogeny of Phyllidiid Nudibranchs (Doridoidea). Rec Aust Mus. 16:1–107.

Chernomor O, Von Haeseler A. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol. 65(6): 997–1008.

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

Do TD, Choi TJ, Jung DW, Kim JI, Karagozlu MZ, Kim CB. 2019. The complete mitochondrial genome of Phyllidiella pustulosa (Cuvier, 1804) (Nudibranchia, Phyllidiidae). Mitochondrial DNA Part B-Resources. 4(1): 771–772.

Dominguez M, Quintas P, Troncoso JS. 2007. Phyllidiidae (Opisthobranchia: Nudibranchia) from Papua New Guinea with the description of a new species of Phyllidiella. American Malacological Bulletin. 22(1): 89–117.

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59(3): 307–321.

Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 14(6): 587–589.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4): 772–780.

Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res. 44(W1): W54–W57.

Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol. 30(5): 1188–1195.

Figure 1. The ML phylogenetic tree based on 13 PCGs of 17 nudibranch species. Two species Pleurobranchaea novaeseelandiae and Berthella sp. belonging to Pleurobranchia were used as outgroups. Numbers near the nodes represent ML bootstrap value.
Nguyen L-T, Schmidt HA, Von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32(1):268–274.

Penney BK, Ehresmann KR, Jordan KJ, Rufo G. 2020. Micro-computed tomography of spicule networks in three genera of dorid sea-slugs (Gastropoda: Nudipleura: Doridina) shows patterns of phylogenetic significance. Acta Zool. 101(1):5–23.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 56(4):564–577.

Undap N, Papu A, Schillo D, Ijong FG, Kaligis F, Lepar M, Hertzer C, Bohringer N, König GM, Schaberle TF, et al. 2019. First survey of heterobranch sea slugs (Mollusca, Gastropoda) from the Island Sangihe, North Sulawesi, Indonesia. Diversity-Basel. 11(9):170.

Xiang P, Lin M, Wang Y, Audira G, Liang ST, Hsiao CD. 2017. The complete mitogenome of sea slug, *Nembrotha kubaryana* (Mollusca: Polyceridae). Conservation Genet Resour. 9(2):245–247.

Xiang P, Lin M, Wang Y, Shen KN, Hsiao CD. 2016. The complete mitogenome of sea slug, *Phyllidia ocellata* (Mollusca: Phyllidiidae). Mitochondrial DNA B Resour. 1(1):96–97.