

The complete mitochondrial genome of the photosymbiotic sea slug *Berghia stephanieae* (Valdés, 2005) (Gastropoda, Nudibranchia)

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ABSTRACT

Berghia stephanieae (Nudibranchia, Cladobranchia) is a photosymbiotic sea slug that feeds exclusively on sea anemones from the genus *Exaiptasia*. It then specifically incorporates dinoflagellates belonging to the Symbiodiniaceae obtained from their prey. Here, we present the complete mitochondrial genome sequence of *B. stephanieae* combining Oxford Nanopore long read and Illumina short-read sequencing data. The mitochondrial genome has a total length of 14,786 bp, it contains the 13 protein-encoding genes, 23 tRNAs, and two rRNAs and is similar to other nudibranchs except for the presence of a duplicated *tRNA-Ser 1*.

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The aeolid nudibranch *Berghia stephanieae* (Valdés 2005) (Nudibranchia, Cladobranchia) is a stenophagous species that preferentially preys on the photosymbiotic sea anemone *Exaiptasia diaphana* (Rapp 1829). The slug digests all the anemones' tissue and incorporates the dinoflagellate symbionts Symbiodiniaceae Fensome et al. 1993, in epithelial cells of the digestive gland system (Valdés 2005; Carmona et al. 2014). Once ingested, the symbionts are retained photosynthetically active for about 10 days (Mies et al. 2017), but the slugs are even able to overcome prolonged starvation periods up to 48 days apo-symbiotically (symbiont-free) (Bleidißel 2010). Further, because apo-symbiotic adults lose their biomass in the same manner as photosymbiotic ones, the photosymbiotic relationship of *B. stephanieae* and Symbiodiniaceae is rather considered as non-mutalistic than a stable one (Mies et al. 2017; Monteiro et al. 2019). Nevertheless, *B. stephanieae* is an important species to understand the evolution of photosymbiosis in Cladobranchia, because the species seems to be in a transitional state between non-photosymbiotic and photosymbiotic. To better understand the genomic adaptations needed to evolve a stable photosymbiosis (Melo Clavijo et al. 2018), the metabolism of the mitochondrial genome can give valuable insights into a potential connectivity of the host and the symbiont (Rauch et al. 2017). As a first step toward more comprehensive studies, we sequenced the mitochondrial genome of *B.*

stephanieae using a combination of Oxford Nanopore long-read and Illumina short-read sequencing.

Specimens of *B. stephanieae* were purchased from a local provider (Seepferdchen24 Meeresaquaristik GmbH, Posthausen) in February 2019 and cultivated in our lab at 25 °C, at a day/night cycle of 12 h/12 h. Voucher material was preserved in 96% ethanol and stored in the Biobank at the Zoological Research Museum Alexander Koenig (Bonn, Germany, voucher no. ZFMK-TIS-53240, biobank@leibniz-zfmk.de). Seven specimens of *B. stephanieae* were frozen in liquid nitrogen and total DNA was extracted using a modified protocol based on the E.Z.N.A.[®] Mollusc DNA Kit, Omega (Georgia, USA) and after Schalamun et al. (2019) (Supplementary material S1). The genomic library preparation was performed using the 1D Ligation Sequencing Kit SQK-LSK109, Oxford Nanopore Technologies (Oxford, UK) for long-read sequencing on a MinION device, using a modified manufacturer's protocol (Supplementary material S1) generating about 13 GB of long reads. An additional library (insert size 100 bp, single end) was prepared using the Nextera DNA Library Prep Kit (California, USA) for Illumina sequencing on a HiSeq2500 platform resulting in approximately 5.5 GB of data. A hybrid assembly was done using SPAdes V3.14.1 (Nurk et al. 2013; Antipov et al. 2016), the assembled genome was annotated using the MITOS2 webserver (Bernt et al. 2013; Donath et al. 2019), and annotations were manually edited

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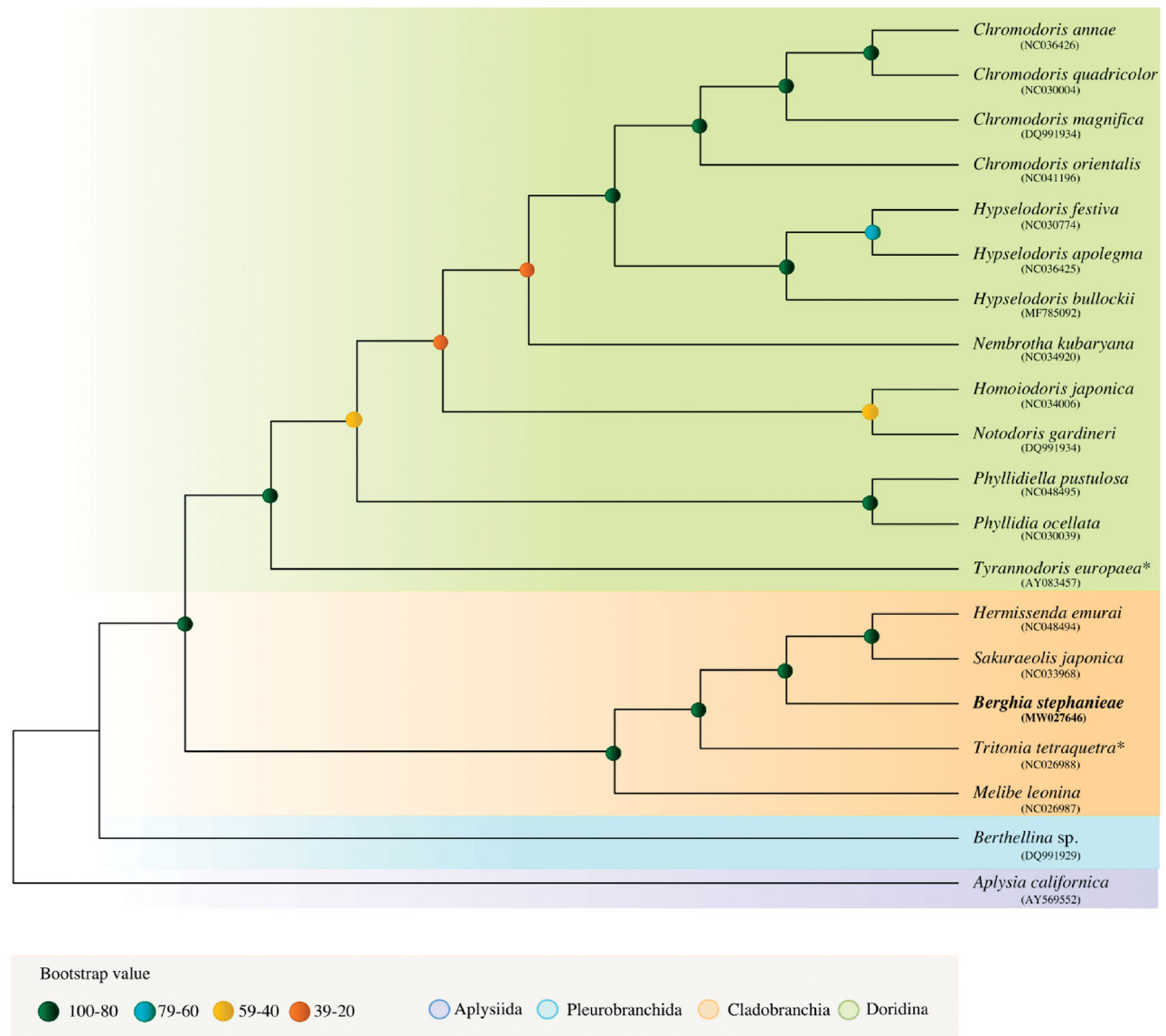


Figure 1. The molecular phylogeny of *Berghia stephanieae* and other nudibranchs based on the whole mitochondrial genome. The phylogenetic tree was calculated under the maximum-likelihood optimality criterion and 1,000 bootstrap replicates using *Aplysia californica* as outgroup. The accepted names (WoRMS Editorial Board, 2020) for *Tyrannodoris europaea* (synonym *Roboastrea europaea*) and *Tritonia tetraquetra* (synonym *Tritonia diomedea*) were used (*).

using Geneious 9.1.5. (<https://www.geneious.com>). Duplicated tRNAs were further confirmed with ARWEN v.1.2 (Laslett and Canback 2008).

The mitochondrial genome of *B. stephanieae* (GenBank accession number: MW027646) has a total length of 14,786 bp and consists of 13 protein-coding genes, two ribosomal RNA (rRNA) genes, and 23 tRNA genes. The base composition of the mitogenome is 26% A, 15% C, 21% G, and 38% T. The gene order is as follows: *tRNA-Lys (aaa)*, *cox1*, *tRNA-Val (gta)*, the large-subunit rRNA (*rrnL*), *tRNA-Leu (cta) 1*, *tRNA-Ala (gca)*, *tRNA-Pro (cca)*, *nad6*, *nad5*, *nad1*, *tRNA-Tyr (tac)*, *tRNA-Trp (tga)*, *nad4L*, *cob*, *tRNA-Asp (gac)*, *tRNA-Phe (ttc)*, *cox2*, *tRNA-Gly (gga)*, *tRNA-His (cac)*, *tRNA-Cys (tgc)*, *-tRNA-Gln (caa)*, *-tRNA-Leu (tta) 2*, *-atp8*, *-tRNA-Asn (aac)*, *-atp6*, *-tRNA-Arg (cga)*, *-tRNA-Glu (gaa)*, *-the small-subunit rRNA (rrnS)*, *-tRNA-Met (atg)*, *-nad3*, *-tRNA-Ser (tca) 2*, *tRNA-Ser (agc) 1*, *tRNA-Ser (aga) 1*, *nad4*, *-tRNA-Thr (aca)*, *-cox3*, *tRNA-Ile (atc)*, *nad2*. The mitogenome of *B. stephanieae* is similar in size, base composition, has the same coding regions and

gene arrangement compared to all publicly available nudibranch mitochondrial genomes (Sevigny et al. 2015; Karagozlu, Sung, Lee, Kim, et al. 2016; Karagozlu, Sung, Lee, Kwak, et al. 2016; Xiang, Lin, Wang, et al. 2016; Xiang, Lin, Zhao, et al. 2016; Lin et al. 2017; Yu et al. 2018; Dinh Do, Choi, et al. 2019; Dinh Do, Kim, et al. 2019) and only differs in the presence of a duplicated *tRNA-Ser 1*.

Full-length mitochondrial genome sequences of 20 Nudipleura species were downloaded from NCBI and aligned using MAFFT (Auto mode) V7.222 (Katoh and Stanley 2013). A phylogenetic tree was built based on the maximum likelihood criterion using IQ-TREE version 2.0.5 (Minh et al. 2020) with the Model Finder Plus option (-m TEST), 1000 bootstrap replicates, and *Aplysia californica* J. G. Cooper 1863 set as outgroup. *Berghia stephanieae* clustered with the other Cladobranchia species, and forms a monophyletic clade with *Sakuraeolis japonica* (Baba 1937) and *Hermissenda emurai* (Baba 1937), that corresponds to the superfamily Aeolidioidea (Figure 1).

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Disclosure statement

The authors declare there are no relevant financial or non-financial competing interests to report.

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Data availability statement

The data that support the findings of this study are openly available in Figshare (www.figshare.com) at <http://doi.org/10.6084/m9.figshare.12994064>

Data accessibility statement

The mitogenome sequences of *Chromodoris annae* Bergh 1877 (NC_036426) (Lin et al. 2017), *Chromodoris quadricolor* (Rüppell and Leuckart 1830) (NC_030004) (Xiang, Lin, Zhao, et al. 2016), *Chromodoris magnifica* (Quoy and Gaimard 1832) (DQ991931) (Medina et al. 2011), *Chromodoris orientalis* Rudman 1983 (NC_041196) (Yu et al. 2018), *Hypselodoris festiva* (Adams 1861) (NC_030774) (Karagozlu, Sung, Lee, Kwak, et al. 2016), *Hypselodoris apolegma* (Yonow 2001) (NC_036425) (Lin et al. 2019), *Hypselodoris bullockii* (Collingwood 1881) (MF785092) (Lin et al. 2019), *Nembrotha kubaryana* Bergh 1877 (NC_034920) (Xiang et al. 2017), *Homoiodoris japonica* Bergh 1882 (NC_034006) (Liu et al. 2016), *Notodoris gardineri* Eliot 1906 (DQ991934) (Medina et al. 2011), *Phyllidiella pustulosa* (Cuvier 1804) (NC_048495) (Dinh Do, Choi, et al. 2019), *Phyllidia ocellata* Cuvier 1804 (NC_030039) (Xiang, Lin, Wang, et al. 2016), *Tyrannodoris europaea* (García-Gómez 1985) (registered as its synonym *Roboastra europaea* García-Gómez 1985 AY083457) (Grande et al. 2002), *Hermisenda emurai* (NC_048494) (Dinh Do, Kim, et al. 2019), *Sakuraeolis japonica* (NC_033968) (Karagozlu, Sung, Lee, Kim, et al. 2016), *Tritonia tetraquetra* (Pallas 1788) (registered as its synonym *Tritonia diomedea* Bergh 1894 NC_026988) (Sevigny et al. 2015), *Melibe leonina* (Gould 1852) (NC_026987) (Sevigny et al. 2015), *Berthellina* sp. Gardiner 1936 TLT-2006 (DQ991929) (Medina et al. 2011), and *Aplysia californica* (AY569552) (Knudsen et al. 2006) are accessible via download from GenBank. Mitogenome data supporting this study are openly available in GenBank at: <https://www.ncbi.nlm.nih.gov/nuccore/MW027646>. Associated BioProject, SRA, and BioSample accession numbers are <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA684024>, SRR13242883, SRR13242884, SRR13242885, and SAMN17050363, respectively.

References

Adams A. 1861. On some new species of Mollusca from the north of China and Japan. *Ann Mag Nat Hist.* 3(8):135–142.
 Antipov D, Korobeynikov A, McLean JS, Pevzner PA. 2016. HybridSPAdes: an algorithm for hybrid assembly of short and long reads. *Bioinformatics.* 32(7):1009–1015.
 Bergh LSR. 1877. Malacologische Untersuchungen. Zweiter Theil, Wissenschaftliche Resultate. 2(11):429–494.

Bergh LSR. 1882. Beitrage zur kenntniss der japonischen Nudibranchien. *Verh Zool Bot Ges Wien.* 31:219–250.
 Bergh R. 1894. Reports on the dredging operations off the West Coast of Central America to the Galapagos, to the West Coast of Mexico, and in the Gulf of California, in charge of Alexander Agassiz, carried on by the U.S. Fish Commission Steamer "Albatross", during 1891, Lieut. Commander Z.L.Tanner, U.S.N., Commanding. XIII. Die Opisthobranchien. *Bull Mus Comp Zool.* 25(10):125–233.
 Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsich G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2): 313–319.
 Bleidissel S. 2010. Molekulare Untersuchungen zur Evolution der Aeolidida (Mollusca, Gastropoda, Nudibranchia, Cladobranchia) und zur Evolution einer sekundären Symbiose mit Symbiodinium (Dinoflagellata) in den Aeolidida. Wuppertal: Bergische University of Wuppertal.
 Carmona L, Pola M, Gosliner TM, Cervera JL. 2014. The Atlantic-Mediterranean genus *Berghia* Trinchese, 1877 (Nudibranchia: Aeolidiidae): taxonomic review and phylogenetic analysis. *J Mollus Stud.* 80(5):482–498.
 Collingwood C. 1881. II. On some new species of nudibranchiate mollusca from the eastern seas. *Trans Linn Soc London Zoo.* 2(2):123–140.
 Cooper JG. 1863. On new or rare Mollusca inhabiting the coast of California. No. II. *Proc Calif Acad Sci.* 1(3):56–60.
 Cuvier G. 1804. Mémoire sur la phyllidie et le pleurobranche. *Ann Mus Hist Nat Paris.* 5:266–276.
 Dinh Do T, Choi TJ, Jung DW, Kim JI, Karagozlu MZ, Kim CB. 2019. The complete mitochondrial genome of *Phyllidiella pustulosa* (Cuvier, 1804) (Nudibranchia, Phyllidiidae). *Mitochondrial DNA B.* 4(1):771–772.
 Dinh Do T, Kim JI, Jung DW, Choi TJ, Karagozlu MZ, Kim CB. 2019. Characterization of the complete mitochondrial genome of *Hermisenda emurai* (Baba, 1937) (Nudibranchia, Facelinidae). *Mitochondrial DNA B.* 4(1):860–861.
 Donath A, Jühling F, Al-Arab M, Bernhart SH, Reinhardt F, Stadler PF, Middendorf M, Bernt M. 2019. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Res.* 47(20):10543–10552.
 Eliot CNE. 1906. Nudibranchiata, with some remarks on the families and genera and description of a new genus, *Doridomorpha*. In: Gardiner JS, editor. The fauna and geography of the Maldive and Laccadive Archipelagoes. Cambridge: Cambridge University Press. 2(1):540–573.
 Fensome RA, Taylor FJR, Norris G, Sarjeant WAS, Wharton DI, Williams GL. 1993. A classification of fossil and living dinoflagellates. *Micropaleontology.* 7:1–351.
 García-Gómez JC. 1985. A new species of *Roboastra* from the Gibraltar strait (southern Spain). *J Molluscan Stud.* 51:169–176.
 Gardiner AP. 1936. Engel's paper on "The English species of the family Pleurobranchidae". *J Conchol.* 20:195–198.
 Gould AA. 1852. Mollusca and shells. In: Wilkes C, editor. United States Exploring Expedition during the years 1838, 1839, 1840, 1841, 1842 under the command of Charles Wilkes. Boston: Little, Brown. Vol. 12; p. 1–510.
 Grande C, Templado J, Lucas Cervera J, Zardoya R. 2002. The complete mitochondrial genome of the nudibranch *Roboastra europaea* (Mollusca: Gastropoda) supports the monophyly of opisthobranchs. *Mol Biol Evol.* 19(10):1672–1685.
 Karagozlu MZ, Sung JM, Lee J, Kwak W, Kim CB. 2016. Complete sequences of mitochondrial genome of *Hypselodoris festiva* (A. Adams, 1861) (Mollusca, Gastropoda, Nudibranchia). *Mitochondrial DNA B Resour.* 1(1):266–267.
 Karagozlu MZ, Sung JM, Lee J, Kim SG, Kim CB. 2016. Complete mitochondrial genome analysis of *Sakuraeolis japonica* (Baba, 1937) (Mollusca, Gastropoda, Nudibranchia). *Mitochondrial DNA B Resour.* 1(1):720–721.
 Knudsen B, Kohn AB, Nahir B, McFadden CS, Moroz LL. 2006. Complete DNA sequence of the mitochondrial genome of the sea-slug, *Aplysia californica*: conservation of the gene order in Euthyneura. *Mol Phylogenet Evol.* 38(2):459–469.

- Laslett D, Canback B. 2008. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. *Bioinformatics*. 24 (2): 172–175.
- Lin GM, Xiang P, Audira G, Hsiao CD. 2019. Low coverage whole genome sequencing yields the complete mitogenome of *Hypselodoris bullocki* and *Hypselodoris apolegma* (Mollusca: Chromodorididae). *J Coast Res*. 97(sp1):23–28.
- Lin GM, Xiang P, Sampurna BP, Hsiao CD. 2017. Genome skimming yields the complete mitogenome of *Chromodoris annae* (Mollusca: Chromodorididae). *Mitochondrial DNA B Resour*. 2(2):609–610.
- Liu C, Wu X, Shen HD. 2016. Complete mitochondrial genome of *Vaginulus alte* and *Homoiodoris japonica*. *Mitochondrial DNA A DNA Mapp Seq Anal*. 27(5):3454–3457.
- Medina M, Lal S, Vallès Y, Takaoka TL, Dayrat BA, Boore JL, Gosliner T. 2011. Crawling through time: transition of snails to slugs dating back to the Paleozoic, based on mitochondrial phylogenomics. *Mar Genomics*. 4(1):51–59.
- Melo Clavijo J, Donath A, Serôdio J, Christa G. 2018. Polymorphic adaptations in metazoans to establish and maintain photosymbioses. *Biol Rev Camb Philos Soc*. 93(4):2006–2020.
- Mies M, Voolstra CR, Castro CB, Pires DO, Calderon EN, Sumida PYG. 2017. Expression of a symbiosis-specific gene in *Symbiodinium* type A1 associated with coral, nudibranch and giant clam larvae. *R Soc Open Sci*. 4(5):170253.
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol*. 37(5):1530–1534.
- Monteiro EA, Güth AZ, Banha TN, Sumida PY, Mies M. 2019. Evidence against mutualism in an aeolid nudibranch associated with Symbiodiniaceae dinoflagellates. *Symbiosis*. 79(2):183–189.
- Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Pribelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, et al. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol*. 20(10):714–737.
- Pallas PS. 1788. *Marina varia nova et rariora*. *Novi Comment Acad Sci Imp Petropol*. 2:229–249.
- Quoy JRC, Gaimard JP. 1832. *Voyage de decouvertes de l’Astrolabependant les annees 1826-1829 sous le commandement de M. 3. Durnond d’Illruille*. *Zoologic*. 2:1–686.
- Rapp W. 1829. *Über die Polyphen im Allgemeinen und die Actinien*. Grolsherzogt Sdch Weimar. :62.
- Rauch C, Christa G, de Vries J, Woehle C, Gould SB. 2017. Mitochondrial genome assemblies of *Elysia timida* and *Elysia cornigera* and the response of mitochondrion-associated metabolism during starvation. *Genome Biol Evol*. 9(7):1873–1879.
- Rudman WB. 1983. The Chromodorididae (Opisthobranchia: Mollusca) of the Indo-West Pacific: *Chromodoris splendida*, *C. aspersa* and *Hypselodoris placida* colour groups. *Zool J Linnean Soc*. 78(2):105–173.
- Rüppell E, Leuckart FS. 1830/1831. *Neue wirbellose Thiere des Rothen Meeres*. Atlas zu der Reise im nordlichen Afrika von Eduard Rüppell. Frankfurt: H.L. Brönnner; p. 15–47.
- Schalamun M, Nagar R, Kainer D, Beavan E, Eccles D, Rathjen JP, Lanfear R, Schwesinger B. 2019. Harnessing the MinION: An example of how to establish long-read sequencing in a laboratory using challenging plant tissue from *Eucalyptus pauciflora*. *Mol Ecol Resour*. 19(1):77–89.
- Sevigny JL, Kirouac LE, Thomas WK, Ramsdell JS, Lawlor KE, Sharifi O, Grewal S, Baysdorfer C, Curr K, Naimie AA, et al. 2015. The mitochondrial genomes of the nudibranch mollusks, *Melibe leonina* and *Tritonia diomedea*, and their impact on gastropod phylogeny. *PLoS One*. 10(5): e0127519. pmid:25996944
- Valdés A. 2005. A new species of *Aeolidiella* Bergh, 1867 (Mollusca: Nudibranchia: Aeolidiidae) from the Florida keys. *USA Veliger*. 47(3): 218–223.
- 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.
- Xiang P, Lin M, Zhao L, Shen KN, Hsiao CD. 2016. Low-coverage genome sequencing yields the complete mitogenome of Pyjama Slug, *Chromodoris quadricolor* (Mollusca: Chromodorididae). *Mitochondrial DNA B Resour*. 1(1):94–95.
- Yonow N. 2001. Results of the Rumphius Biohistorical Expedition to Ambon (1990). Part 11. Doridacea of the families Chromodorididae and Hexabranthidae (Mollusca, Gastropoda, Opisthobranchia, Nudibranchia), including additional Moluccan material. *Zool Meded*. 75:1–50.
- Yu C, Kim H, Kim HJ, Jung YH. 2018. The complete mitochondrial genome of the Oriental sea slug: *Chromodoris orientalis* (Nudibranchia, Chromodorididae). *Mitochondrial DNA B Resour*. 3(2):1017–1018.

Appendix 1.

DNA extraction protocol and library preparation 1 D SQK-LSK109.