The complete mitochondrial genome of *Spurilla braziliana* MacFarland 1909 (Nudibranchia, Aeolidiidae).

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Abstract

Spurilla braziliana MacFarland 1909 is a morphologically diverse nudibranch found in the Pacific and Western Atlantic. The complete mitochondrial genome of *S. braziliana* has been constructed using next-generation sequencing technology. The mitochondrial genome is 14,291 bp and contains 13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes. Molecular phylogenetic analysis using the maximum likelihood method revealed that *S. braziliana* is included in the superfamily Aeolidioidea and forms a monophyletic group with *Berghia stephanieae*, a nudibranch of the family Aeolidiidae. This study reinforces existing taxonomic insights and provides a basis for further molecular phylogenetic analysis.

Introduction

Members of the genus *Spurilla* are unique nudibranchs capable of storing and utilizing cnidarian nematocysts in their bodies (Greenwood and Mariscal 1984). These sea slugs are widely distributed in the world's oceans and attract attention from a taxonomic perspective due to their diverse coloration and morphological characteristics (Carmona et al. 2014). Previous research by Carmona et al. (Carmona et al. 2013) has shown that only one species of *Spurilla*, *S. braziliana*, is found in the Pacific Ocean. However, there is significant morphological diversity within *S. braziliana* (Carmona et al. 2014) suggesting that further molecular phylogenetic analysis may be required to distinguish the different morphotypes. This study reports the complete mitochondrial genome sequence of one morphotype of *S. braziliana* from Japan.

Materials and methods

A sample of *Spurilla braziliana* was collected on June 10, 2021 from the intertidal area of Miura City (35°16'18.8"N 139°34'04.7"E), Kanagawa Prefecture, Japan (Figure 1) and preserved in NucleoProtect RNA (MACHEREY-NAGEL) for 5.5 months at -80 °C before DNA extraction. The sample was then transferred to 99% ethanol and for deposition to the University Museum of the University of Tokyo (http://www.um.u-tokyo.ac.jp/, Assoc. Prof. Takenori Sasaki, sasaki@um.u-tokyo.ac.jp) under voucher number RM34044. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's instructions.

A sequencing library was prepared from the DNA sample with MGIEasy FS DNA Library Prep Set (MGI) and sequenced with DNBSEQ-T7 (MGI) at Genome-Lead Corporation, Japan. Sequencing resulted in 63.6 Gbp of data and the reads were assembled with CLC Genomics Workbench version 8.5 (QIAGEN Aarhus A/S) to construct a primary assembly. The primary assembly was polished using bwa-mem (Li 2013), samtools (Danecek et al. 2021), and pilon (Walker et al. 2014). In order to improve the polished primary assembly, the sequence was circularized by the ends, cut at a site differing from the circularization point, and polished once more. The polished mitochondrial genome

was annotated with MITOS (Donath et al. 2019) and manually corrected on Geneious Prime Java version 11.0.14.1+1 (Biomatters Ltd.).

The mitochondrial genome was aligned with 21 known Nudibranchia mitochondrial genomes using the concatenated nucleotide sequences of the 13 annotated protein-coding genes with MAFFT v7.508 (Katoh and Standley 2013). Based on the alignment, the molecular phylogenetic tree was generated with the maximum likelihood method applying the bootstrap method of 1000 times in MEGA version 10.1.8 (Kumar et al. 2018).

Figure 1. The individual of *S. braziliana* used in this study. Its morphotype is similar to the one shown in Fig. 4E in the previous taxonomic study (Carmona et al. 2014). The picture was taken by Hideaki Mizobata.

Results

The mitochondrial genome of *S. braziliana* (GenBank accession ID: LC759638) is 14,291 bp and has a GC content of 33.94%. It consists of 13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes (Figure 2). The gene order is identical to that of *Sakuraeolis japonica* (Karagozlu et al. 2016) and *Hermissenda emurai* (Do et al. 2019), both of which belong to the superfamily Aeolidioidea, the same as *S. braziliana*. *Berghia stephanieae*, which belongs to the same family, Aeolidiidae, as *S. braziliana*, has been reported to exhibit *tRNA-Ser 1* gene duplication (Clavijo et al. 2021). However, this feature was not observed in *S. braziliana*.

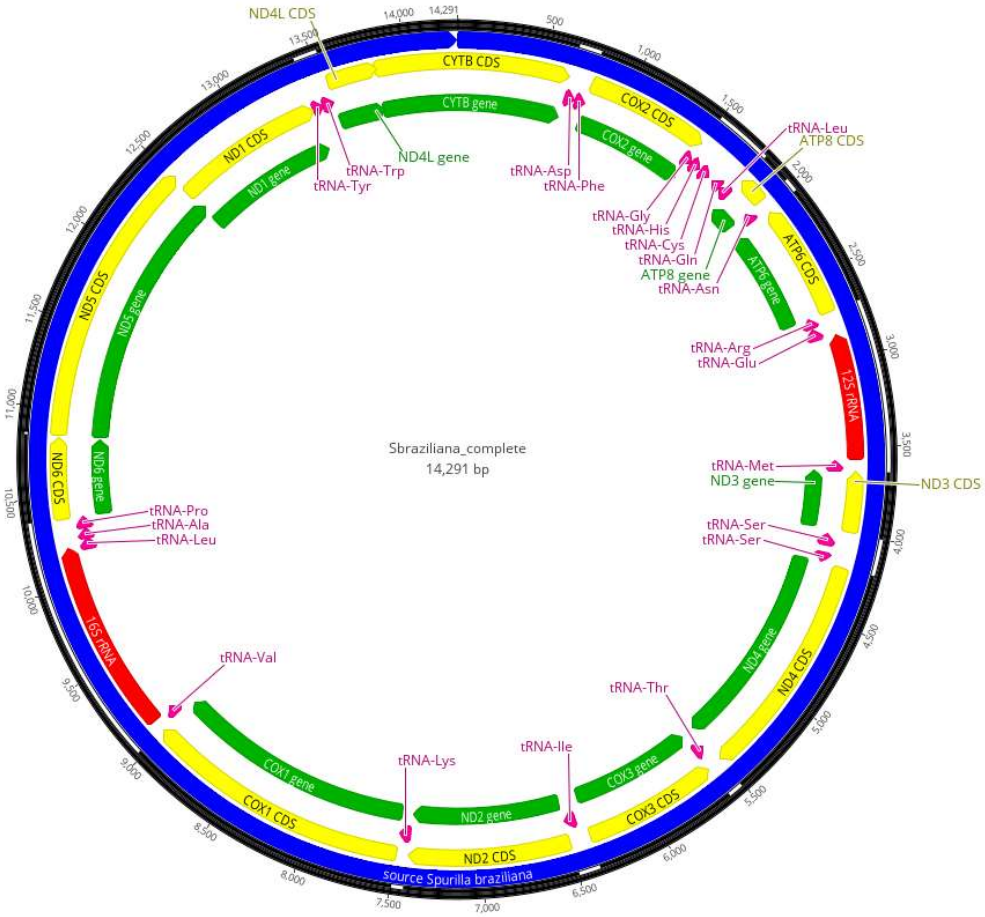

Figure 2. Circular map of the complete mitochondrial genome annotated to show the locations and orientation of its 13 protein-coding genes, 2 rRNA genes, and 22 tRNA genes.

As shown in the molecular phylogenetic tree (Figure 3), *S. braziliana* forms a monophyletic group with members of the suborder Cladobranchia and the superfamily Aeolidioidea. It also forms a monophyletic group with *B. stephanieae*, a member of the same family, Aeolidiidae.

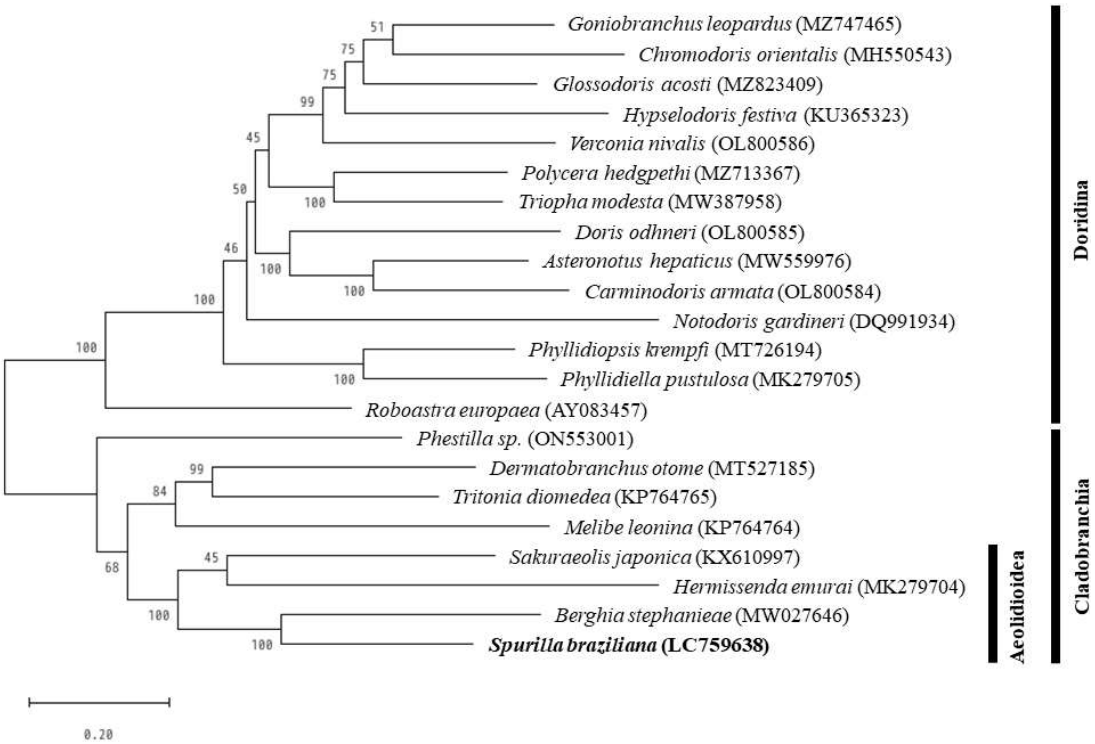

Figure 3. Maximum likelihood phylogenetic tree of *S. braziliana* and 21 other Nudibranchia species based on the concatenated nucleotide sequences of the 13 protein-coding genes in the mitochondrial genome. Bootstrap support values are shown for each node, based on 1000 replicates. The list of species and their GenBank accession IDs are as follows: *Goniobranchus leopardus* (MZ747465), *Chromodoris orientalis* (MH550543) (Yu et al. 2018), *Glossodoris acosti* (MZ823409), *Hypselodoris festiva* (KU365323) (Karagozlu et al. 2016), *Verconia nivalis* (OL800586) (Do et al. 2022), *Polycera hedgpethi* (MZ713367), *Triopha modesta* (MW387958) (Do et al. 2022), *Doris odhneri* (OL800585) (Do et al. 2022), *Asteronotus hepaticus* (MW559976), *Carminodoris armata* (OL800584) (Do et al. 2022), *Notodoris gardineri* (DQ991934) (Medina et al. 2011), *Phyllidiopsis krempfi* (MT726194) (Kim et al. 2021), *Phyllidiella pustulosa* (MK279705) (Do et al. 2019), *Roboastra europaea* (AY083457) (Grande et al. 2004), *Phestilla sp.* (ON553001), *Dermatobranchus otome* (MT527185) (Do et al. 2020), *Tritonia diomedea* (KP764765) (Sevigny et al. 2015), *Melibe leonina* (KP764764) (Sevigny et al. 2015), *Sakuraeolis japonica* (KX610997) (Karagozlu et al. 2016), *Hermissenda emurai* (MK279704) (Do et al. 2019), *Berghia stephanieae* (MW027646) (Clavijo et al. 2021).

Discussion

The previous molecular phylogenetic study of the superfamily Aeolidioidea using *COI*, *16S rRNA*, and *H3* genes showed that the monophyletic group containing the genera *Spurilla* and *Berghia* was a sister group to the group containing *Sakuraeolis* (Carmona et al. 2011). The phylogenetic tree generated using all the protein-coding genes of the mitochondrial genome corroborated these findings. The mitochondrial genome of *S. braziliana*, the first mitochondrial genome of its genus, will provide important data for future taxonomic studies on *Spurilla* nudibranchs, which are known for their highly variable morphology.

Declaration of interest

The authors have no financial or other conflicts of interest to disclose concerning the study.

Ethical approval

This article does not contain any animals requiring ethical approval.

Author contributions

Hideaki Mizobata, Kentaro Hayashi, and Ryo Yonezawa conducted the sample collection of *S. braziliana*. Hideaki Mizobata performed the experimental manipulation and analysis, excluding library preparation and next-generation sequencing. Shigeharu Kinoshita, Kazutoshi Yoshitake, and Shuichi Asakawa provided supervision throughout the study. Hideaki Mizobata and Andre Lanza wrote the manuscript. All authors reviewed and revised the manuscript draft and approved the final version of the manuscript to be published.

Data availability

The genome sequence 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 ID: LC759638. The associated BioProject, SRA, and Bio-sample numbers are PRJDB15401, DRR450785, and SAMD00585630, respectively.

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