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MITOGENOME REPORT

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The complete mitochondrial genome of the bubble-gum coral *Paragorgia papillata* (Octocorallia: Coralliidae) from the seamount in the tropical Western Pacific

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ABSTRACT

The complete mitochondrial genome of *Paragorgia papillata* Li et al. 2021, a deep-sea gorgonian inhabiting at 858 m in Caroline Ridge, was obtained in this study. The length of the mitochondrial genome is 19,018 bp with 14 protein coding genes, one transfer RNA (tRNA-Met) and two ribosomal RNA genes contained in this circular molecule. Phylogenetic analysis indicated that *P. papillata* and *P. coralloides* Bayer, 1993 were two closely related species, and a total of 26 mutational sites (four nonsynonymous mutations included) can be detected between their mitochondrial genomes. This exhibits a case that mitochondrial genomes can be applied to differentiate closely related species in gorgonians. The phylogenetic tree constructed with mitochondrial genomes showed that the families in Octocorallia are reciprocally monophyletic, provided that the family names were revised according to the systematic revision of Octocorallia guided by phylogenomics. However, the relationships of the families within each order were different between the previous phylogenomic work and ours. Integrating mitochondrial genomes from a wider array of Octocorallia families is essential for a more accurate comparison of phylogenies derived from nuclear and mitochondrial sequences in future study.

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1. Introduction

The gorgonians have been considered as the foundation species of seamounts and continental margins in deep-sea ecosystems (Roberts et al. 2006). As one of the dominant megafaunal taxa in hard bottom environments like continental shelves and seamounts, they serve as substrate for the benthic faunas and provide refuge for the epifaunal fish to escape from the predators, thus playing a fundamental ecological role in deep-sea benthic environments (Li et al. 2017). *Paragorgia papillata* Li et al. 2021 is one of the gorgonians inhabiting the tropical Western Pacific (Figure 1). Their identification as a new species that differentiated from the closely related species *P. coralloides* has been formally established only recently (Li et al. 2021). Up to now, there are five species in *Paragorgia* discovered in the Western Pacific deep-sea ecosystem including *P. splendens* Thomson and Henderson, 1906, *P. sibogae* Bayer, 1993, *P. rubra* Li et al. 2017, *P. coralloides* Bayer, 1993 and *P. papillata*. However, only the mitochondrial genome of *P. coralloides* has been public (Brockman and McFadden 2012). In this study, the complete mitochondrial genome of another species in *Paragorgia* (*P. papillata*) was sequenced and it is a good chance to inspect the potency of the usage of whole mitochondrial genomes to discriminate two closely related species in

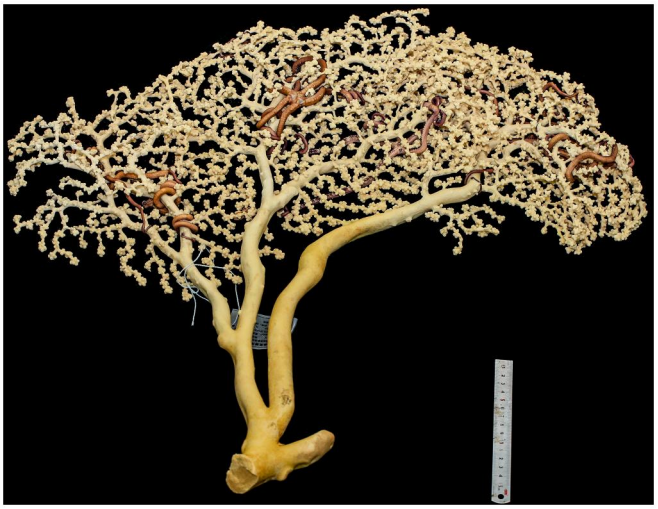

Figure 1. A picture of a colony of *Paragorgia papillata* after soaked in alcohol, with brittle stars intertwining on its surface. The specimen was photographed by Yang Li.

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gorgonians. This evaluation is relevant because the fragmented barcodes in mitochondrial genomes are always notoriously incompetent in constructing a confident phylogenetic relationship for Octocorallia at species or population level due to their slow evolutionary rates (McFadden et al. 2011). The result of this study will lay foundations for future studies on the phylogeny and genetic resource conservation of *Paragorgia*, an ecologically pivotal genus of deep-sea corals.

2. Materials and methods

The sample of *P. papillata* was collected from 858 m depth at Caroline Ridge (140°14′32″E, 10°06′47″N) by the submersible remotely operated vehicle (ROV) *Faxian* during the cruise of the R/V *Kexue* to the tropical Western Pacific in 2019. The sample was deposited in specimen room in the laboratory of marine organism taxonomy and phylogeny of the Institute of Oceanology, Chinese Academy of Sciences (http://www.qdio.ac.cn/motp/; Yang Li, liyang@qdio.ac.cn) under accession no. M6106. DNA extraction, high-throughput sequencing, sequence assembly, and gene annotation were performed according to our previous procedures (Li et al. 2020). Briefly, a paired-end library with an insert size of 300 bp was prepared with total genomic DNA using the TruSeq DNA Sample Prep Kit (Illumina, USA). The above library was sequenced by an Illumina HiSeq Xten system (2 × 150bp paired-end reads) (Illumina, USA) at Novogene Bioinformatics Technology Co., Ltd. (TianJin, China). Adapters and parts with a quality score below 15 were removed from raw reads by Trimmomatic 0.36 (Bolger et al. 2014). The clean reads were assembled using MitoFlex 0.2.9 assembler (Li et al. 2021) with default parameters. The read coverage depth of the produced mitochondrial genome was calculated with the Draw_SequencingDepth.py script (Ni et al. 2023). The obtained mitochondrial genome was aligned with that of the closely related species *P. coralloides* to detect their difference. The phylogenetic tree was constructed using the PhyloSuite1.1.15 pipeline (Zhang et al. 2019) based on the concatenated 14 protein coding genes of *P. papillata* and other 35 octocorals (Brockman and McFadden 2012; Figueroa and Baco 2014; Gastineau et al. 2023). The final alignment was 15,024 bp long. The maximum-likelihood phylogeny was deduced using IQ-TREE 1.6.8 (Nguyen et al. 2014). with taxa in the order of Malacalcyonacea set as outgroup. The TVM + F + R4 model was selected based on the Bayesian Information Criterion recommended by the built-in ModelFinder module in IQ-tree (Chernomor et al. 2016).

3. Results and discussion

A total of 2.6 Gb data was yielded by Illumina sequencer. After assembled, the complete mitochondrial genome of *P. papillata* was obtained. This mitochondrial genome was at the length of 19,018 bp with an average sequencing depth of 269 × (Figure S1). Among the 19,018 bp, the nucleotides A, C, G and T were 5,713 bp (30.04%), 3,472 bp (18.26%), 3,811 bp (20.04%) and 6,022 bp (31.67%), respectively. The GC content was 38.29%, slightly higher than that of the closely related species *P. coralloides* (38.28%). These GC content values were normal, because the GC contents of mitochondrial genomes in Octocorallia can range approximately from 34% (*Acrossota amboinensis* NC_061991[14]) to 45% (*Tenerodus fallax* OL616286 (Muthye et al. 2022)). There were 14 protein coding genes, 1 transfer RNA gene (tRNA-Met) and 2 ribosomal RNA genes in the mitochondrial genome of *P. papillata* with tRNA-Met, COX3, ATP6, ATP8, COX2, NAD4L, NAD3, NAD6 encoded on the light strand, and the remaining were encoded on the heavy strand (Figure 2). Both the start codons and stop codons in the 14 protein coding genes

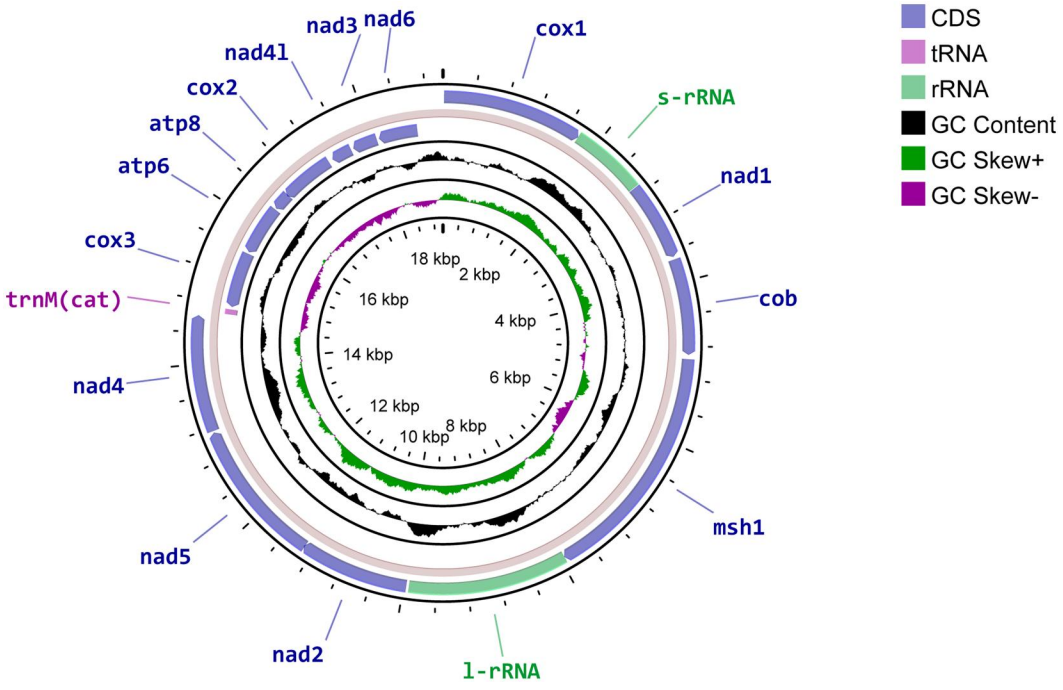

Figure 2. A circular genomic map of the mitochondrial genome of *Paragorgia papillata*, with 14 protein coding genes, one ORF, 1 tRNA, and 2 rRNAs.

were canonical (ATG as start codons and TAG or TAA as stop codons). The gene arrangements of *P. papillata* and *P. coralloides* were identical. This gene order was also the same with those in genus *Pleurocorallium*, which has been named as 'konojoi' type gene order (Figueroa and Baco 2014).

The alignment (19,018 bp in length) between the mitochondrial genomes of *P. papillata* and *P. coralloides* showed 26 mutational sites which included 24 point mutations and 2 indels. Among the 24 point mutations, two mutational sites led to two amino acid changes in the gene of MutS (the commonly used barcode in octocorals), one nonsynonymously mutational site was located in the nad5 gene, one nonsynonymous point mutation was in the nad6 gene and the remaining 20 point mutations were situated within the noncoding regions. The phylogenetic tree indicated that *P. papillata* clustered with *P. coralloides* and the branch lengths for these two gorgonians were short (Figure 3). The result here implied the potentially effective utility of the whole mitochondrial genomes to differentiate two closely related taxa at the interspecific level of the gorgonians, however, there could be an impediment for its application in the studies of population genetics or phylogeography at the intraspecific level, considering the limited informatively mutational sites between the two species observed in this study.

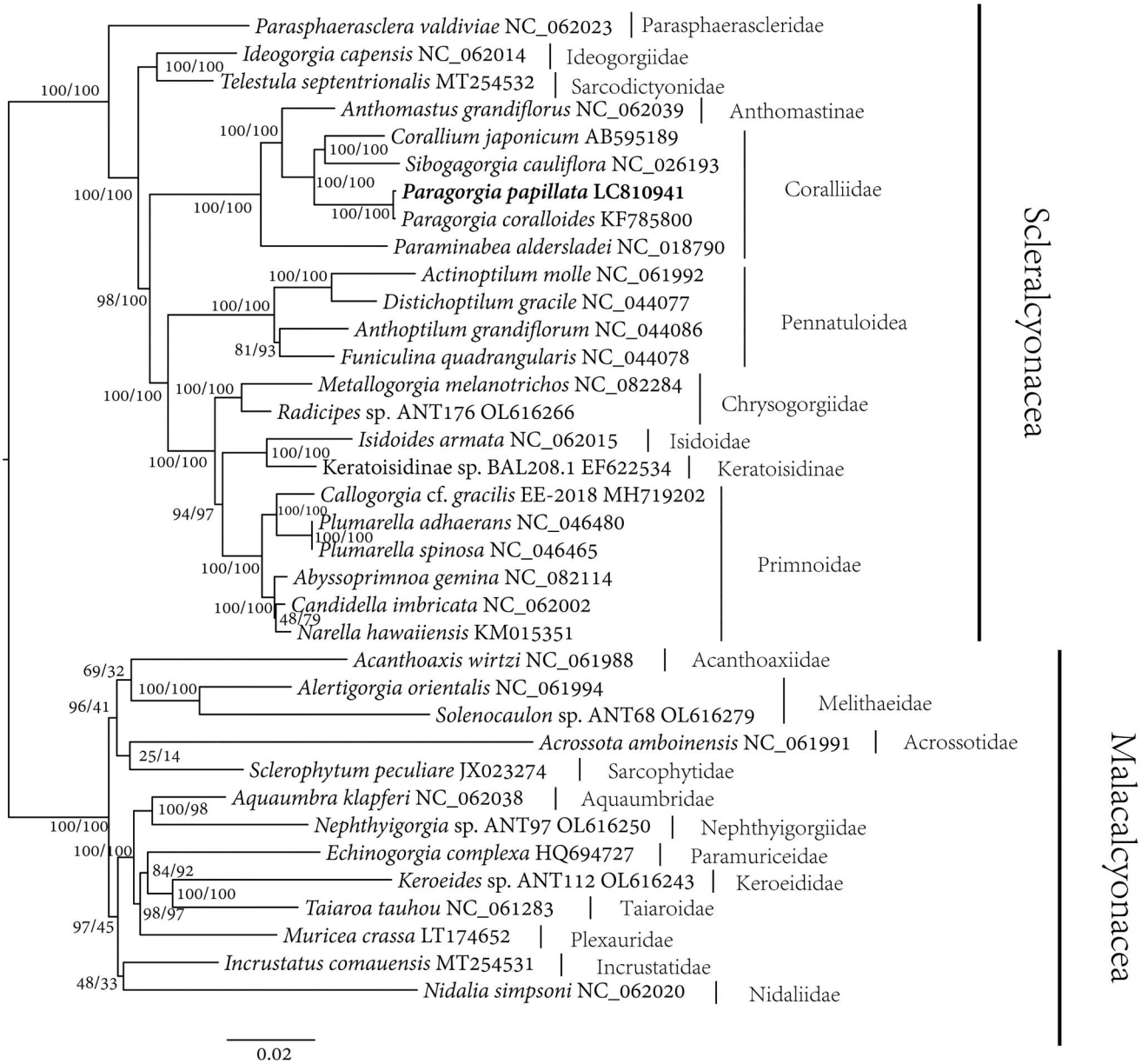

Figure 3. The phylogenetic tree of *Paragorgia papillata* and other 29 gorgonians based on 14 protein coding genes of the whole mitochondrial genome. The sequences used in the tree are listed as follows: NC_018790, JX508792 (Brockman and McFadden 2012); NC_026193, KM015351, KM015354 (Figueroa and Baco 2014); AB595189 (Uda et al. 2011); MT254531, MT254532 (Angelo et al. 2021); NC_062002, NC_062039, NC_061283, NC_061988, NC_061991, NC_061992, NC_061994, NC_062014, NC_062015, NC_062020, NC_062023, NC_062038, OL616243, OL616250, OL616266, OL616279 (Muthye et al. 2022); LT174652 (Angelo et al. 2016); HQ694727 (Park et al. 2012); NC_046465 (Choi et al. 2020); EF622534 (Brugler and France 2008); NC_044078, NC_044086, NC_044077 (Hogan et al. 2019); JX023274 (Shen et al. 2021); MH719202 (Easton and Hicks 2019); NC_082114 (Gastineau et al. 2023); NC_046480, NC_082284, KF785800 (unpublished) and LC810941 (in this study). Numbers near the nodes indicate SH-aLRT and ultrafast bootstrap support values from 20,000 replicates. The (super)families each species belongs to are shown besides each species name. The species names and the corresponding (super)family names are adopted according to the WoRMS database (https://www.marinespecies.org/).

Certainly, more genetic information from other intraspecific and interspecific samples will present more conclusive evidence in future study.

The revision of Octocoralline systematics has been performed in 2022 based on the phylogenomic tree built from ultraconserved and exon loci (McFadden et al. 2022). The major revisions in that work involved the reorganization of three previously accepted orders in Octocorallia (Alcyonacea, Pennatulacea and Helioporacea) into two new orders (Scleralcyonacea and Malacalcyonacea). Moreover, a proportion of families in Octocorallia have been revised so that the poly- or paraphyletic families deduced from phylogenomic analysis become reciprocally monophyletic clades. In this work, the phylogeny inferred from mitochondrial genomes was consistent with the revision work in terms of the order level adjustment (the affiliations of the families in these two works were consistent) and family names revision (the families after revision in the present tree were all monophyletic (Figure 3)). Nevertheless, the topological relationships of the families within each order in this study were not agreeable with the phylogenomic tree. For example, the family Coralliidae that *P. papillata* belonged to was sister to the clade composed of Parasphaerascleridae, Ideogorgiidae and Sarcodictyonidae based on the phylogenomic analysis (see Figure 1 in that study) (McFadden et al. 2022). In the present study, however, Coralliidae clustered with the clade comprising Pennatuloidea, Chrysogorgiidae, Isidoidae, Keratoisidinae and Primnoidae, whereas Parasphaerascleridae, Ideogorgiidae and Sarcodictyonidae, that clustered with Coralliidae in (McFadden et al. 2022), diverged early in Scleralcyonacea in this study (Figure 3). If based on the single mtMutS, Coralliidae seemed to be placed in a polytomy with other families of Scleralcyonacea (clades including all families in Scleralcyonacea except Cornulariidae) (see Figure 2 in (McFadden et al. 2022)). The difference may be brought about by the intrinsic drawbacks of mitochondrial genes that deduce deeper nodes with poor resolution for Octocorallia (McFadden et al. 2010; McFadden et al. 2006), but we should note that the families with mitochondrial genomes available in Octocorallia were not as comprehensive as those in the phylogenomic tree, thus a more reasonable comparison awaits more available mitochondrial genomes in future study.

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Author contributions

Junyuan Li: Conceptualization, Investigation, Methodology, Software, Validation, Formal analysis, Visualization, Data Curation, Writing - Original draft, Writing - Review and Editing, Funding Acquisition; Kuidong Xu: Supervision, Project administration, Funding Acquisition; Yang Li: Conceptualization, Validation, Data Curation, Supervision, Project administration, Funding Acquisition.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI (https://www.ncbi.nlm.nih.gov/) at under the accession no. LC810941. The associated BioProject, SRA, and Bio-Sample numbers are PRJDB17833, DRR541556, and SAMD00762597, respectively.

Disclosure statement and declaration of interest

No potential conflict of interest was reported by the author(s). The authors are responsible for the content and writing of this article. The authors confirm that our article should be considered in Mitochondrial DNA: Part B, and that it is not currently under consideration in any other journals.

Ethical statement

Samples were collected from Caroline Ridge (140°14′32″E, 10°06′47″N) in the tropical Western Pacific. Approval number for access to field sites and experiments is KFJBRP-017 of the Biological Resources Programme of Chinese Academy of Sciences.

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