Wei et al. *Zool. Res.* 2024, 45(1): 215−225
https://doi.org/10.24272/j.issn.2095-8137.2023.039

ZR | Zoological Research

Article Open Access

Molecular phylogenetic relationships based on mitochondrial genomes of novel deep-sea corals (Octocorallia: Alcyonacea): Insights into slow evolution and adaptation to extreme deep-sea environments

Zhan-Fei Wei[1,2,#], Kai-Wen Ta[3,#], Nan-Nan Zhang[2,#], Shan-Shan Liu[2], Liang Meng[2], Kai-Qiang Liu[1], Chong-Yang Cai[2], Xiao-Tong Peng[3,*], Chang-Wei Shao[1,*]

[1] *National Key Laboratory of Mariculture Biobreeding and Sustainable Goods*, *Yellow Sea Fisheries Research Institute*, *Chinese Academy of Fishery Sciences*, *Qingdao*, *Shandong* 266071, *China*

[2] *BGI-Qingdao*, *BGI-Shenzhen*, *Qingdao*, *Shandong* 266555, *China*

[3] *Institute of Deep-Sea Science and Engineering*, *Chinese Academy of Sciences*, *Sanya*, *Hainan* 572000, *China*

ABSTRACT

A total of 10 specimens of Alcyonacea corals were collected at depths ranging from 905 m to 1 633 m by the manned submersible Shenhai Yongshi during two cruises in the South China Sea (SCS). Based on mitochondrial genomic characteristics, morphological examination, and sclerite scanning electron microscopy, the samples were categorized into four suborders (Calcaxonia, Holaxonia, Scleraxonia, and Stolonifera), and identified as 9 possible new cold-water coral species. Assessments of GC-skew dissimilarity, phylogenetic distance, and average nucleotide identity (ANI) revealed a slow evolutionary rate for the octocoral mitochondrial sequences. The nonsynonymous (*Ka*) to synonymous (*Ks*) substitution ratio (*Ka*/*Ks*) suggested that the 14 protein-coding genes (PCGs) were under purifying selection, likely due to specific deep-sea environmental pressures. Correlation analysis of the median *Ka*/*Ks* values of five gene families and environmental factors indicated that the genes encoding cytochrome *b* (cyt *b*) and DNA mismatch repair protein (*mutS*) may be influenced by environmental factors in the context of deep-sea species formation. This study highlights the slow evolutionary pace and adaptive mechanisms of deep-sea corals.

Keywords: Mitochondrial genome; Alcyonacea; *Ka*/*Ks* evolution; Environmental factors

INTRODUCTION

The Octocorallia subclass of anthozoans, commonly referred to as sea fans, sea whips, sea pens, or soft corals, provides critical habitat for many aquatic organisms and microorganisms. These corals are widely distributed across a range of water environments, from shallow tropical regions to the deep sea (Baillon et al., 2012; Fryer et al., 2020; Roberts et al., 2006; Van De Water et al., 2018). The Octocorallia subclass comprises three orders, with more than 3 500 species in 378 genera and 55 families (McFadden et al., 2010). Within the Octocorallia subclass, the Alcyonacea order has undergone significant taxonomic revision since the late 20th century. This order encompasses all soft corals and provides habitat for biota in deep-sea ecosystems (McFadden et al., 2010; Zhou et al., 2021). Corals of the Alcyonacea order are well-adapted to the harsh conditions present in deep oceans, such as darkness, low temperatures, and high hydrostatic pressure. These cold-water corals act as ecosystem engineers in extreme deep-sea environments (Hebbeln et al., 2020; Roberts et al., 2006) and are noted for their long lifespans and slow growth rates (Sun et al., 2010; Thierens et al., 2013). Cold-water coral forests consisting of bamboo corals and gorgonian corals from the Isididae and Gorgoniidae families, respectively, have been documented in the South China Sea (SCS) at depths ranging from 1 200 to 1 380 m (Li & Wang, 2019).

Mitochondria are essential for cellular energy generation and play critical roles in the differentiation, apoptosis, growth,

Received: 27 March 2023; Accepted: 11 September 2023; Online: 12 September 2023

Foundation items: This study was supported by the Marine S&T Fund of Shandong Province for Pilot National Laboratory for Marine Science and Technology (Qingdao) (No. 2022QNLM030004), Hainan Science and Technology Department (ZDKJ2019011), Open Project Fund of Key Laboratory of Sustainable Development of Polar Fisheries, Ministry of Agriculture and Rural Affairs of PRC (2022OPF02), State Key R&D Project (2021YFF0502500), and Qingdao Postdoctoral Applied Research Project (JZ2223j06100)

[#]Authors contributed equally to this work

*Corresponding authors, E-mail: xtpeng@idsse.ac.cn; shaocw@ysfri.ac.cn

and division of eukaryotic cells (Kroemer & Reed, 2000; Taanman, 1999). Mitochondrial genomes, characterized by their small size, maternal inheritance, compact gene arrangement, high conservation, and simplified structure, serve as powerful molecular markers in studies exploring phylogenetic relationships and biomolecular evolution (Li et al., 2021; McFadden et al., 2010; Taanman, 1999; Yan et al., 2019). Within the Cnidaria phylum, which includes the anthozoans, the nucleotide sequences of mitochondrial genes exhibit relatively low mutation rates and high stability (Hellberg, 2006; Shearer et al., 2002). Mitochondrial genomes are typically circular and consist of protein-coding genes (PCGs), ribosomal RNA (rRNA) genes, and transfer RNA (tRNA) genes (Boore, 1999). Interestingly, the DNA mismatch repair protein (*mutS*) homolog, which is implicated in DNA mismatch repair and intramolecular recombination, is present in the mitochondria of all octocorals, with studies suggesting it may be horizontally transferred from the epsilonproteobacteria or DNA viruses (Bilewitch & Degnan, 2011; Brugler & France, 2008; Ogata et al., 2011). The presence of *mutS* in octocoral mitochondrial genomes may contribute to the slower nucleotide substitution rates and participate in gene rearrangement events during evolution (Brockman & McFadden, 2012; Muthye et al., 2022). Species-specific conserved DNA fragments, such as *mutS* and cytochrome c oxidase subunit 1 (*cox1*), are often employed for the identification of coral taxon (Xu et al., 2021).

As key organelles involved in energy and redox homeostasis, mitochondria are sensitive to environmental changes in animals (Sokolova, 2018). Variations in mitochondrial nucleotide sequences can improve the fitness and metabolism of organisms within extreme environments (Carapelli et al., 2019). For example, certain mitochondrial genes in alvinocaridid shrimps have undergone positive selection to facilitate adaptation to the harsh conditions of hydrothermal vents (Wang et al., 2017). Similarly, mitochondrial genes under positive selection pressure may contribute to the survival of anemones in the deep-sea environment (Zhang et al., 2017). Natural selection not only acts on phenotypic traits but also reduces mitochondrial dysfunction by inhibiting deleterious mutations in mitochondrial genes (Shtolz & Mishmar, 2019). Environmental factors, acting as selective forces, can also induce gene mutations that influence evolutionary processes, potentially manifesting as variations in mitochondrial genes.

In the present study, morphological and mitochondrial genome analyses led to the identification of 9 novel cold-water coral species belonging to the Alcyonacea order. The characteristics of the mitochondrial genomes were analyzed using various bioinformatics approaches. Furthermore, based on a comparative assessment of mitochondrial genomes, variation and conservation across the Octocorallia subclasses were evaluated. The contributions of environmental factors to the evolution of mitochondrial genes were also assessed, providing potential evidence for the adaptation of cold-water corals to extreme deep-sea environments. Our study delineated the characteristics of the mitochondrial genomes of deep-sea corals and the impact of environmental factors on the evolution of these genes.

MATERIALS AND METHODS

Ethics statement

Ethics approval for all deep-sea coral experiments was granted by the Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences following the recommendations of the Regulations for the Administration of Affairs Concerning Experimental Animals of China (Qingdao, China) (Approval No.: YSFRI-2022016).

Sampling, DNA extraction, and sequencing

A total of 10 deep-sea coral colonies were collected from the SCS during two cruises between August and November 2020 (Figure 1; Table 1). The samples were captured by a manned submersible Shenhai Yongshi equipped with mechanical arms, then stored in a bio-box. Once the manned submersible returned, the coral samples were immediately rinsed twice with deionized water, then stored at −80 °C in alcohol for subsequent experiments in the shipboard laboratory. A conductivity, temperature, and depth (CTD) sensor (Sea-Bird, USA) on the manned submersible was employed to monitor variations in deep-sea environmental factors during sampling, including temperature, salinity, and density. Sampling site information (i.e., longitude and latitude) was also collected by the manned submersible.

The coral samples were soaked in liquid nitrogen, crushed using sterilized mortars, and then subjected to total DNA extraction using phenol-chloroform with some modification. CTAB lysis buffer (3 mL) containing 5 μL of β-mercaptoethanol and 20 μL of proteinase (20 mg/mL) was added to the crushed sample, followed by incubation at 55 °C for 1 h to disrupt the cells. An equal volume of phenol/chloroform/isopentyl alcohol (25:24:1) was added and fully mixed. The DNA in the supernatant was extracted using an equal volume of chloroform/isopentyl alcohol (24:1). DNA was precipitated using a 0.7×volume of isopropyl alcohol and diluted with 50 μL of deionized water.

The RNA fragments were removed with a final concentration of 1 mg/μL RNaseA (Takara, Dalian, China) at 37°C for 10 min. The quality and quantity of the extracted DNA were checked using gel electrophoresis and a Qubit 2.0 Fluorometer (Life, USA). Approximately 1 μg of DNA was fragmented into 500 bp fragments by ultrasonication to prepare paired-end short-read libraries. The DNA libraries were constructed using a MGIEasy Universal DNA Library Prep Kit (MGI, China) and sequenced on the MGISEQ-2000 platform with 100 bp paired-end reads to obtain approximately 6 Gb per sample.

Morphological identification

To identify the taxa of the 10 deep-sea coral samples, their general morphology and anatomy were studied according to previous studies, with some modification (Li et al., 2020). Parts of individuals, including tentacles, were incubated in sodium hypochlorite for 15 min, then centrifuged at 3 000 r/min for 2 min at room temperature. The precipitates were subsequently washed with distilled water and 70% ethanol to collect the sclerites, which were air-dried, mounted on carbon double adhesive tape, and coated for scanning electron microscopy (SEM, FEI Apreo, USA) with a voltage of 2 kV and working distance of 10 mm.

Assembly and annotation of mitochondrial genomes

The raw data were confirmed by Fastqc (v.0.11.9) with default settings (Andrews, 2014). Low-quality reads and adaptors were removed using Fastp (v.0.22.0) ("-f 5 -F 5 -w 24 -c -q 20 -g -W 5 -3 -l 50") (Chen et al., 2018). MEGAHIT (v.1.1.2) was used to assemble the quality-filtered reads to contigs with

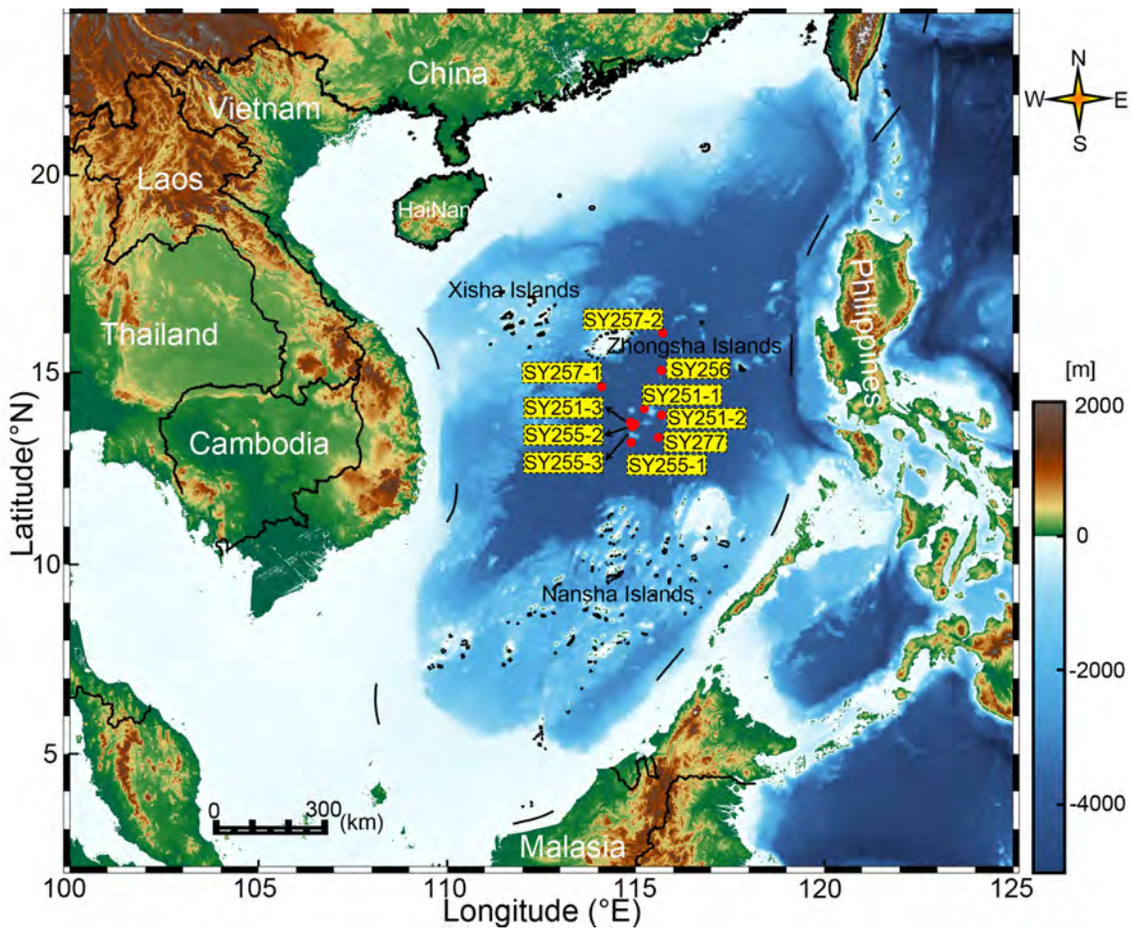

Figure 1 Sites of cold-water coral collection in SCS

Red circles denote samples collected at different depths during two cruises. Detailed information is shown in Table 1.

Table 1 Information on 10 mitochondrial genomes from this study

Sample ID	Species	Genome size (bp)	GC content	No. PCGs	No. tRNA	No. rRNA	Temperature (°C)	Salinity (ppt)	Density (kg/m³)	Longitude (°E)	Latitude (°N)	Depth (m)
SY251-1	*Sibogagorgia dennisgordoni* **sp. nov.**	19 530	0.36	14	1	2	2.75	34.53	1 034.91	115.22	14.16	1 623
SY251-2	*Victorgorgia eminens* **sp. nov.**	18 663	0.37	14	1	2	3.31	34.51	1 033.59	115.67	13.99	1 329
SY251-3	*Acanthogorgia hirsuta* **sp. nov.**	19 044	0.37	14	1	2	3.07	34.52	1 034.13	114.85	13.81	1 440
SY255-1	*Candidella helminthophora* **sp. nov.**	18 872	0.38	14	1	2	4.99	34.44	1 031.4	114.88	13.3	905
SY255-2	*Hemicorallium guttatum* **sp. nov.**	19 104	0.39	14	1	2	4.86	34.45	1 031.5	114.97	13.77	925
SY255-3	*Isidella* **sp. nov.**	19 481	0.38	14	1	2	3.14	34.51	1 033.74	114.91	13.7	1 356
SY256	*Calyptrophora wyvillei* **sp. nov.**	19 270	0.38	14	1	2	2.75	34.53	1 035.07	115.66	15.13	1 633
SY257-1	*Hemicorallium guttatum* **sp. nov.**	19 049	0.39	14	1	2	3.19	34.51	1 033.57	114.1	14.72	1 320
SY257-2	*Lepidisis* **sp. nov.**	19 223	0.38	14	1	2	2.95	34.52	1 034.66	115.7	16.08	1 552
SY277	*Victorgorgia macrocalyx* **sp. nov.**	13 067	0.37	9	0	2	4.54	34.46	1 031.73	115.59	13.42	964

the parameter "--min-contig-len 1000 --k-list 21,33,55,77,99,127" (Li et al., 2014). Additionally, 134 reference octocoral mitochondrial genomes (number of PCGs >7 and length >10 kbp) from the NCBI database were used to identify potential mitochondrial contigs using BLASTn with an e-value of 1e-5 (Supplementary Table S1). The quality-filtered data were compared with the potential mitochondrial contigs using bowtie2 (v2.4.4) ("--very-fast-local") to extract potential mitochondrial reads (Langmead & Salzberg, 2012). Subsequently, the reads were reassembled into mitochondrial genomes using SPAdes (v.3.6.2) with "--careful -k 21,33,55,77,99" (Nurk et al., 2013).

The Mitos2 and Ge-SEQ webservers (http://mitos2.bioinf.uni-leipzig.de/index.py and https://chlorobox.mpimp-golm.mpg.de/geseq.html) were used to annotate the mitochondrial assemblies with a gene identity of 85% (Donath et al., 2019; Tillich et al., 2017), while the NR database was also used to verify the PCGs. Online tRNAscan-SE2.0

software (http://lowelab.ucsc.edu/tRNAscan-SE) was used to identify the tRNA genes (Lowe & Chan, 2016). The output GenBank format files were manually confirmed and modified based on the annotation results, which were used to draw the circular map using Organellar Genome DRAW (OGDRAW) on the MPI-MP CHLOROBX website (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Greiner et al., 2019).

Mitochondrial assembly structural analysis

Genome size and GC content of the mitochondrial genomes were calculated using in-house scripts. Average nucleotide identity (ANI) values of the 134 reference octocoral genomes and our 10 mitochondrial genomes were determined through pairwise comparisons using fastANI with the parameter "--minFraction 0.8" (Table 1; Supplementary Table S1) (Richter et al., 2016). Tandem repeats were identified using Tandem Repeats Finder (v.4.09) with default settings (https://tandem.bu.edu/trf/trf.html) (Benson, 1999). Composition skewness of each PCG in the mitochondrial genomes was calculated using the following formulas: AT-skew=(A−T)/(G+C), GC-skew=(G−C)/(G+C). Additionally, 125 of the 134 reference octocoral genomes and nine of our mitochondrial genomes were complete and contained adenosine triphosphate (ATP) synthase F0 subunit 6 (*atp6*), ATP synthase F0 subunit 8 (*atp8*), cytochrome *b* (cyt *b*), *cox1* , cytochrome c oxidase subunit 2 (*cox2*), cytochrome c oxidase subunit 3 (*cox3*), NADH dehydrogenase subunit 1 (*nad1*), NADH dehydrogenase subunit 2 (*nad2*), NADH dehydrogenase subunit 3 (*nad3*), NADH dehydrogenase subunit 4 (*nad4*), NADH dehydrogenase subunit 4L (*nad4l*), NADH dehydrogenase subunit 5 (*nad5*), NADH dehydrogenase subunit 6 (*nad6*), and *mutS* . These genes were selected to calculate dissimilarity of GC-skew (method=Euclidean) with the vegdist function and principal coordinates analysis (PCoA) in R (v.4.3.1).

Phylogenetic analysis

A phylogenetic tree was constructed using the 134 reference octocoral genomes, three reference hexacoral genomes, and 10 mitochondrial genomes from this study (Table 1; Supplementary Table S1). Within the mitochondrial genomes, 14 conserved PCGs were extracted using an in-house script and aligned using MAFFT L-INS-i (v7.294b), respectively (Katoh & Toh, 2010). The aligned protein sequences for each PCG were trimmed using trimAl (v.1.4) with the parameter "-automated1" to remove poorly aligned regions (Capella-Gutiérrez et al., 2009). Subsequently, the trimmed protein sequences were concatenated using an in-house script. A maximum-likelihood phylogenetic tree was then generated using iq-tree (v.1.6.12) with "MFP" to select the optimal model ("-m MFP -bb 1000 -alrt 1000") (Nguyen et al., 2015). Three *Hexacorallia* reference genomes were designated as the outgroup for analysis. Additionally, the resultant treefile, after removal of the outgroup, was used to compute phylogenetic distances with the ape package in R (v.4.3.1).

***Ka*/*Ks* ratio analysis**

To elucidate the selective pressure on the mitochondrial genes of cold-water corals, the complete mitochondrial genomes were also selected to analyze the *Ka*/*Ks* ratio using KaKs_Calculator2 with the averaging model (MA) and coelenterate mitochondrial genetic code (Table 1; Supplementary Table S1) (Wang et al., 2010). In addition, the median *Ka*/*Ks* values of the 14 PCGs were used to illustrate the genetic evolutionary heterogeneity among our nine complete mitochondrial genomes using the vegdist function (method=Euclidean) in R (v.4.3.1). Additionally, the median *Ka*/*Ks* values of the *atp* , cyt *b*, *mutS*, *cox* , and *nad* gene families were co-analyzed with environmental factors to assess the importance of environmental factors in molecular evolution using the Mantel test in R (v.4.3.1). The relationship between environmental factors was determined using Spearman correlation analysis with the corrplot package in R (v.4.3.1). Pairwise correlation analyses of GC-skew dissimilarity, phylogenetic distance, and ANI values from the complete mitochondrial genomes described above were conducted using MRM in R (v.4.3.1).

RESULTS

Genomic structure

Ten deep-sea coral samples were collected at depths ranging from 905 m to 1 633 m by a manned submersible (Shenhai Yongshi, China) during two cruises in the SCS (Figure 1; Table 1). The count of paired-end reads for potential mitochondrial genomes was 211 303 (Supplementary Table S2). Ten mitochondrial genomes were assembled, with genome sizes ranging from 13 067 bp to 19 530 bp and GC content ranging from 0.36 to 0.39 (Table 1). Circular structure maps with 14 PCGs were obtained for all mitochondrial genomes, except for SY277, which only contained nine PCGs (Table 1). Moreover, two rRNA genes (*rrnS* and *rrnL*) and one tRNA (tRNA-Met) were present in the nine complete mitochondrial genomes. The *mutS* gene was identified in all 10 mitochondrial genomes (Figure 2).

The complete mitochondrial genomes exhibited different gene orders and arrangements (Figure 2; Supplementary Table S3). Among the complete mitochondrial genomes, the genomic structures of SY256, SY255-1, SY251-3, and SY251-2 exhibited identical genomic structures. In these genomes, *cox1*, *rrnS*, *nad1*, cyt *b*, *nad6*, *nad3*, *nad4l*, *mutS*, *rrnL*, *nad2*, *nad5*, and *nad4* were on the heavy strand, and *trnM*, *cox3*, *atp6*, *atp8*, and *cox2* were on the light strand (Figure 2). The genomic structures of SY257-2 and SY255-3 were found to be identical, in which *cox1*, *rrnS*, *nad1* , cyt *b*, *nad6*, *nad3* , and *nad4l* were on the heavy strand, and *nad4*, *nad5*, *nad2*, *rrnL*, *mutS*, *trnM*, *cox3*, *atp6*, *atp8* , and *cox2* were on the light strand (Figure 2). The SY257-1 and SY255-2 were same species, with *nad6*, *nad3*, *nad4l*, *mutS*, *rrnL*, *nad2*, *nad5*, and *nad4* on the heavy strand, and *trnM*, *cox3*, *atp6*, *atp8*, *cox2*, cyt *b*, *nad1*, *rrnS*, and *cox1* on the light strand (Figure 2). The SY251-1 mitochondrial genome had a unique genomic structure, with *cox1*, *rrnS*, *nad1*, cyt *b*, *mutS*, *rrnL*, *nad2*, *nad5*, and *nad4* on the heavy strand, and *trnM*, *cox3*, *atp6*, *atp8*, *cox2*, *nad4l*, *nad3* , and *nad6* on the light strand (Figure 2). The genomic structure of the partial SY277 genome may be the same as that of SY256, SY255-1, SY251-3, and SY251-2 (Figure 2).

Except for *nad4l* , which utilized GTG as its start codon in SY251-1, ATG was the conserved start codon for all 14 PCGs across the mitochondrial genomes in this study. Furthermore, TAA (31.29%) and TAG (68.71%) were the primary termination codons for the 14 PCGs across the 10 mitochondrial genomes (Supplementary Figure S1). Tandem repeats were only identified in three mitochondrial genomes. One tandem repeat was found in SY251-1 with 94% sequence identity and a length of 35 bp. Two tandem repeats were

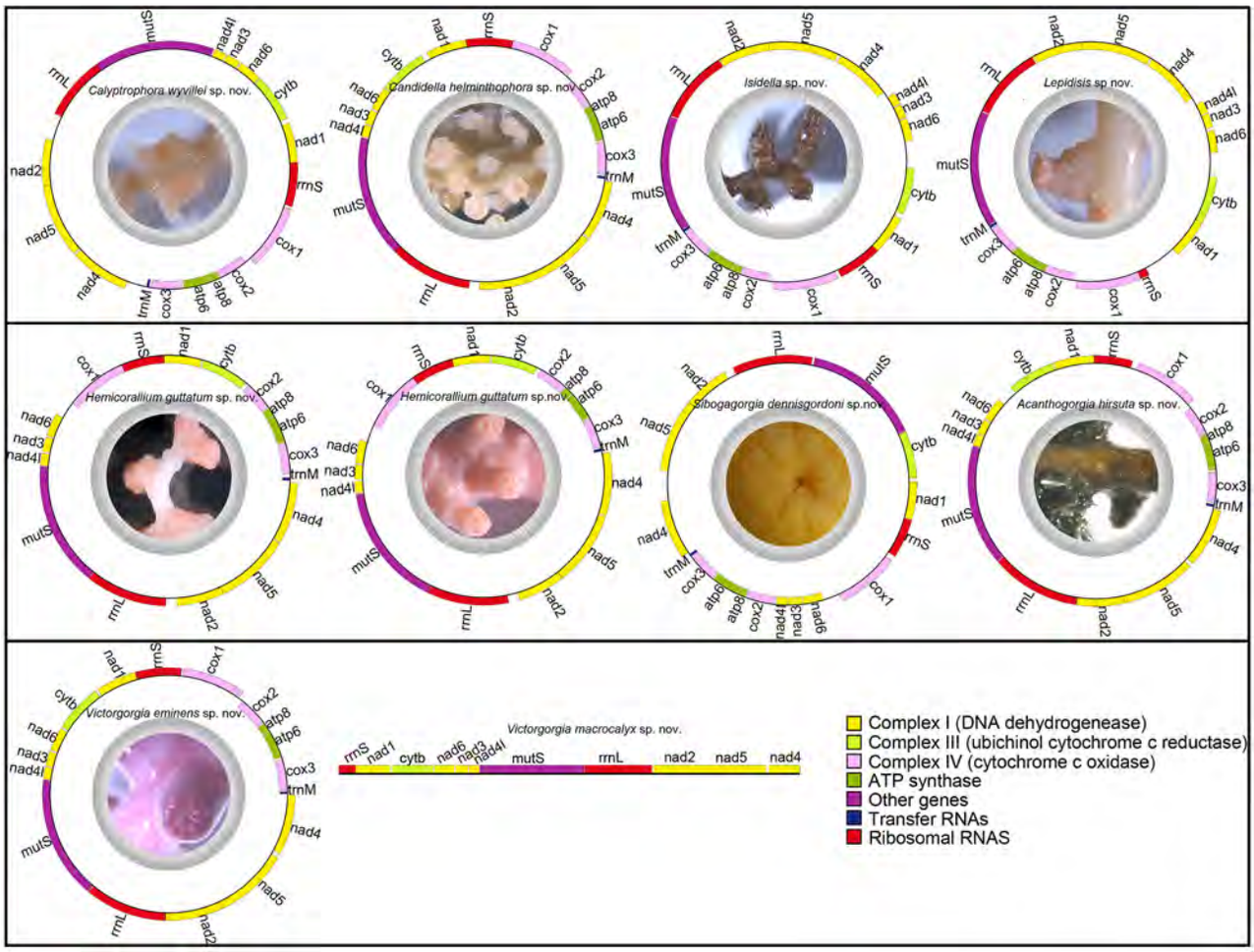

Figure 2 Circular maps of 10 cold-water coral samples

Gene map showing arrangement of 14 PCGs and one tRNA gene, and their positions on heavy or light strands. Gene arrangements are shown in Supplementary Table S3.

identified in SY255-3 with sequence identities of 88% and 95%, and lengths of 41 bp and 34 bp. Three tandem repeats were found in SY257-2 with sequence identities ranging from 89% to 100%, and lengths ranging from 30 bp to 61 bp.

Phylogenetic tree and morphological identification

The 10 mitochondrial genomes were assigned into four suborders and represented 9 new deep-sea species based on phylogenetic and morphological analysis (Figure 3; Supplementary Figures S2–S7). Among the genomes, SY257-2 and SY255-3 were clustered in the Isididae family with articulated skeletons (Figure 3; Supplementary Figure S2). Based on its whip-shaped unbranched colony and sclerite morphology, SY257-2 may represent a new species within the *Lepidisis* genus (*Lepidisis* **sp. nov.**) (Supplementary Figure S2A, B) (Heestand Saucier et al., 2021). While SY255-3 had similar sclerites and microcrystal ultra-structure to the *Lepidisis* genus, differences in morphology suggested that it may represent a novel species within the *Isidella* genus (*Isidella* **sp. nov.**) (Supplementary Figure S2C , D) (Heestand Saucier et al., 2021). Both SY256 and SY255-1 were clustered within the Primnoidae family and displayed different sclerite morphologies, potentially representing two new species in the *Calyptrophora* genus (*Calyptrophora wyvillei* **sp. nov.**) (Supplementary Figure S3A, B) and *Candidella* genus (*Candidella helminthophora* **sp. nov.**) (Supplementary Figure S3C, D) (Cairns, 2009, 2012). All four of the above deep-sea species belonged to the Calcaxonia suborder. Both SY257-1 and SY255-2 showed similar appearances and were placed in the *Hemicorallium* genus of the Coralliidae family (Figure 3; Supplementary Figure S4). Given their yellowish-white color, pale vermilion red colonies, and multiple sclerites, they may represent the same new species of *Hemicorallium guttatum* **sp. nov.** (Supplementary Figure S4) (Tu et al., 2016). SY251-1 was clustered in the Paragorgiidae family with an independent branch (Figure 3), and exhibited similar morphology and sclerite characteristics as those in the *Sibogagorgia* genus, inferring it may represent a novel species within that genus (*Sibogagorgia dennisgordoni* **sp. nov.**) (Supplementary Figure S5) (Sánchez, 2005). SY277 and SY251-2 were grouped together within the Clavulariidae family (Figure 3). Given their respective purple to pink and black polyps and coenenchyma and straight to slightly curved tuberculate spindles on their sclerites, they may represent two novel species within the *Victorgorgia* genus (*Victorgorgia macrocalyx* **sp. nov.** and *Victorgorgia eminens* **sp. nov.**) (Supplementary Figure S6) (Li et al., 2020; Moore et al., 2017). Although SY251-3 exhibited characteristics similar to those of the *Echinogorgia* genus in the Plexauridae family (Figure 3), its morphology and sclerites were more similar to the *Acanthogorgia* genus, suggesting that it may represent a novel species within that genus (*Acanthogorgia hirsuta* **sp. nov.**) (Supplementary Figure S7) (Horvath, 2019). Moreover,

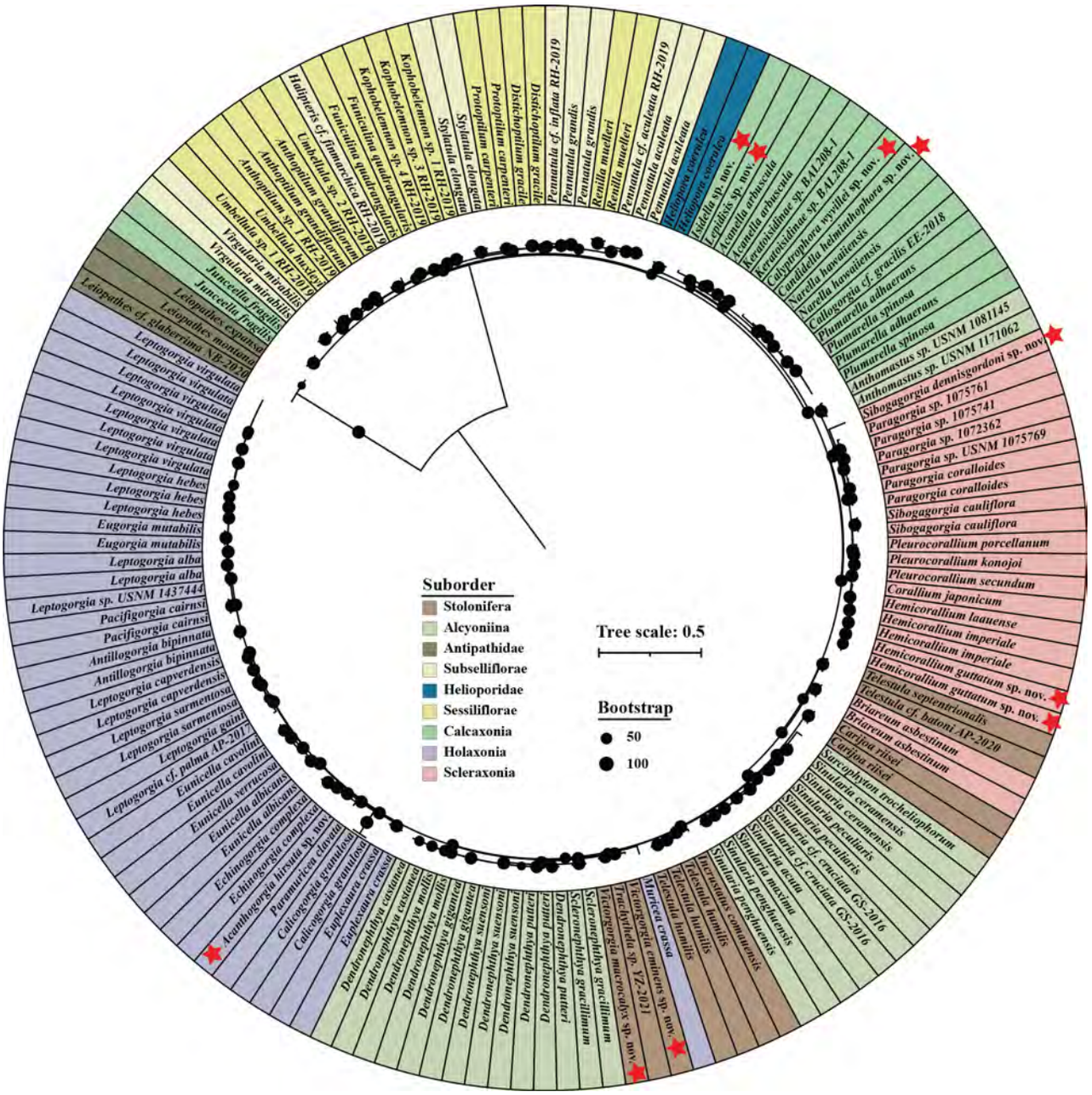

Figure 3 Maximum-likelihood phylogenetic tree of octocoral mitochondrial genomes

Tree was generated based on concatenated proteins of 14 PCGs by iq-tree. Three hexacoral mitochondrial genomes were used as the outgroup. Black dots on branches denote bootstrap support, with only those >50% depicted. Mitochondrial genomes in our study are marked with red stars. Reference mitochondrial genomes were collected from the NCBI database and are shown in Supplementary Table S1.

it was noteworthy that the species within the same family exhibited closer phylogenetic distances, and closer phylogenetic distances were also observed between those from different families in different suborders (Supplementary Figure S8).

ANI analysis

ANI pairwise comparisons for all octocoral mitochondrial genomes further demonstrated that our mitochondrial genomes represented 9 novel species assigned to four suborders (Calcaxonia, Holaxonia, Scleraxonia, and Stolonifera) (Figure 4A). Results showed that *Hemicorallium guttatum* **sp. nov.** was closely related to *Hemicorallium imperiale* (KC782352.1), with 99.66% and 99.74% sequence similarities, respectively (Figure 4Ba). *Acanthogorgia hirsuta* **sp. nov.** shared 97.43% sequence identity with *Echinogorgia complexa* (NC_020457.1 and HQ694727.1) (Figure 4Bb). *Sibogagorgia dennisgordoni* **sp. nov.** displayed 93.40% sequence similarity with *Sibogagorgia cauliflora* (NC_026193.1 and KM015354.1) (Figure 4Bc). *Candidella helminthophora* **sp. nov.** and *Calyptrophora wyvillei* **sp. nov.** shared 99.31% and 98.05% sequence identity with *Narella hawaiiensis* (NC_026192.1 and KM015351.1), respectively, and exhibited a sequence similarity of 98.34% to each other (Figure 4Bd). *Victorgorgia eminens* **sp. nov.** and *Victorgorgia macrocalyx* **sp. nov.** showed 98.40% and 98.59% sequence identities, respectively, with *Trachythela sp. YZ-2021* (MW238423.1) from the *Trachythela* genus, and 98.62% identity with each other (Figure 4Be). In addition, *Isidella* **sp. nov.** and *Lepidisis* **sp. nov.** showed 98.33% and 98.30%

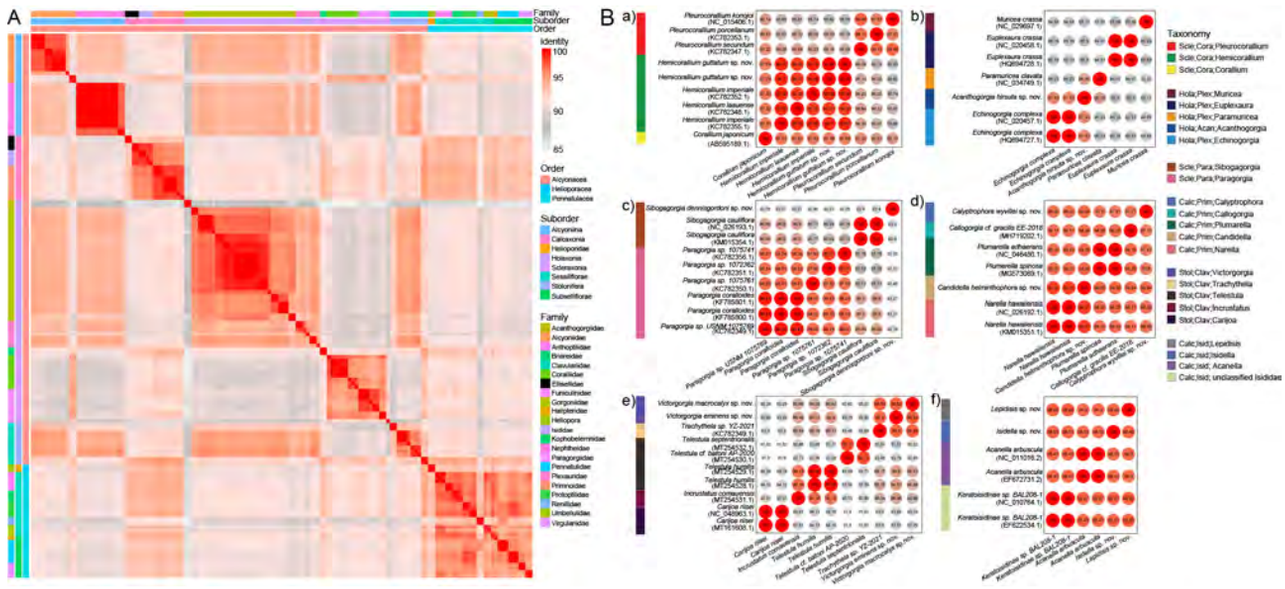

Figure 4 ANI plots of octocoral mitochondrial genomes

Octocoral mitochondrial genomes were used to calculate ANI values through pairwise comparison. A: ANI values of all mitochondrial genomes were sorted by taxonomy based on phylogenetic tree. B: ANI values of our 10 mitochondrial genomes. Reference mitochondrial genomes were collected from NCBI database and are shown in Supplementary Table S1. Suborder: Scle: Scleraxonia; Hola: Holaxonia; Clac: Calcaxonia; Stol: Stolonifera. Family: Cora: Coralliidae; Plex: Plexauridae; Acan: Acanthogorgiidae; Prim: Primnoidae; Clav: Clavulariidae; Isid: Isididae.

sequence similarities, respectively, with *Acanella arbuscula* (NC_011016.2 and EF672731.2) from the *Acanella* genus, and 98.46% sequence similarity to each other (Figure 4Bf). Interestingly, higher ANI values were observed in corals from different families, which may help in deep-sea species identification.

Positive selection analysis

All *Ka*/*Ks* ratios calculated from the 14 PCGs were examined to evaluate selective pressure on mitochondrial genes (Figure 5). However, all *Ka*/*Ks* ratios of the 14 PCGs from nine of our mitochondrial genomes were less than 1. Notably, *atp8* had the highest median *Ka*/*K* s value in *Sibogagorgia dennisgordoni* **sp. nov.**, *Candidella helminthophora* **sp. nov.**, *Hemicorallium guttatum* **sp. nov.**, and *Calyptrophora wyvillei* **sp. nov.** (Kruskal- Wallis test, P <0.01), while *mutS* had the highest median *Ka*/*Ks* value in *Victorgorgia eminens* **sp. nov.**, *Acanthogorgia hirsuta* **sp. nov.**, *Isidella* **sp. nov.**, and *Lepidisis* **sp. nov.** (Kruskal-Wallis test, P<0.01). Conversely, *nad4l* had the lowest median *Ka*/*Ks* value (Kruskal-Wallis test, P<0.01), except for *Sibogagorgia dennisgordoni* **sp. nov.**, in which *cox1* was the lowest (Figure 5).

In addition, species with closer phylogenetic relationships, closer phylogenetic distances, and higher ANI values exhibited lower dissimilarities in median *Ka*/*Ks* values across the 14 PCGs (Supplementary Figure S9). Specifically, *Isidella* **sp. nov.** and *Lepidisis* **sp. nov.** displayed reduced mitochondrial evolutionary heterogeneity among the nine complete mitochondrial genomes based on median *Ka*/*Ks* values. Low genetic evolutionary heterogeneity was also observed between *Candidella helminthophora* **sp. nov.** and *Calyptrophora wyvillei* **sp. nov.** Furthermore, the dissimilarities among the remaining three novel species were substantial, consistent with the phylogenetic tree and ANI analyses.

Genetic distance analysis

The dissimilarities among the eight suborders were assessed using GC-skew calculations of the 14 PCGs (Supplementary Figure S10). Although the suborders explained 34.4% of the variance, the PCoA results did not reveal clear separations based on suborder classifications. This observation suggests that corals within the same suborder may not necessarily possess close genetic distances and may intermix with other suborders. The GC-skew-based dissimilarities aligned with both the phylogenetic distance and ANI findings. Furthermore, pairwise correlation analyses demonstrated a positive correlation between GC-skew dissimilarity and phylogenetic distance (R^2=0.37, P<0.001), a negative correlation between GC-skew dissimilarity and ANI (R^2=0.37, P <0.001), and a negative correlation between ANI and phylogenetic distance (R^2=0.88, P<0.001) (Supplementary Figure S11).

Environmental factor analysis

Various environmental factors, including temperature, salinity, density, depth, longitude, and latitude, were collected during sampling (Table 1). The water temperature ranged from 2.75°C to 4.99°C in the deep-sea area. Both salinity and density were stable during sampling, with an average of 34.50±0.03 ppt and 1 033.43±1.33 kg/m³, respectively. Correlation analysis of environmental factors showed that temperature was strongly negatively correlated with depth, salinity, and density (R<−0.97) (Supplementary Figure S12). Additionally, a strong positive correlation was observed between salinity and density (R=0.98) (Supplementary Figure S12). Furthermore, an increase in salinity and density was observed with increasing depth (R>0.97) (Supplementary Figure S12). The correlation between latitude and other environmental factors was higher than that between longitude and other environmental factors. Co-analysis of environmental factors and median *Ka*/*K* s values of five gene families revealed that the cyt *b* gene was positively correlated with all environmental factors, except for latitude, longitude, and salinity, while *mutS* was correlated with all environmental factors, except for latitude and longitude. No significant correlations between other genes and environmental factors

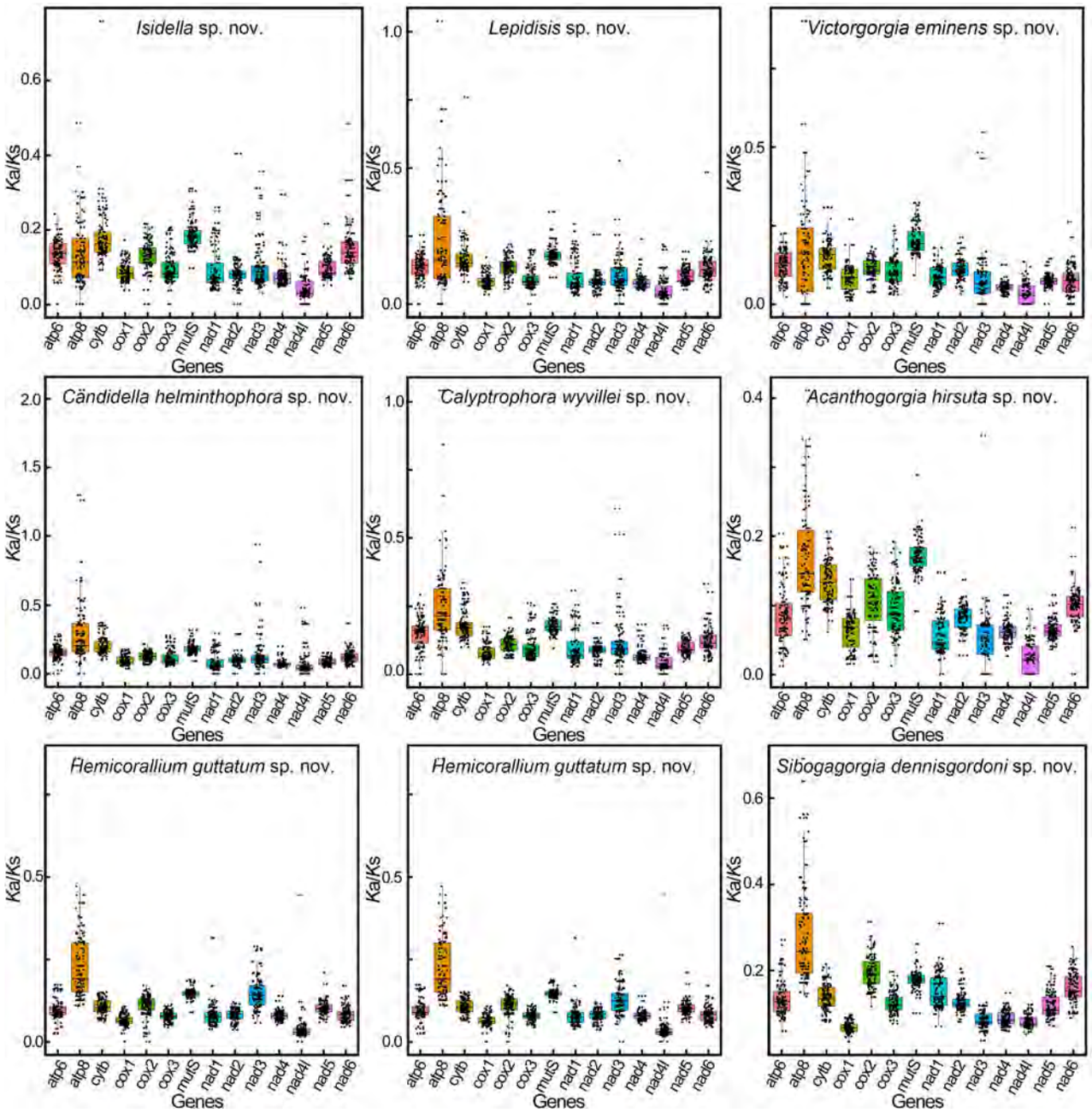

Figure 5 Boxplots of *Ka*/*Ks* ratios of 14 PCGs from nine complete mitochondrial genomes in this study

Complete octocoral mitochondrial genomes were used to calculate *Ka*/*Ks* ratios, as shown in Supplementary Table S1.

were found.

DISCUSSION

In the present study, 10 cold-water coral samples were collected at depths ranging from 905 m to 1 633 m in the SCS. Based on morphological identification and mitochondrial genome structure, the samples were classified within the Alcyonacea order of the Octocorallia subclass, and subsequently divided into four suborders (Calcaxonia, Holaxonia, Scleraxonia, and Stolonifera) and identified as 9 possible novel cold-water coral species. Phylogenetic analysis of all publicly available mitochondrial genomes from the Octocorallia subclass revealed that different suborders may have overlapping positions on the phylogenetic tree. ANI values within the same family were lower than those between families. Conversely, analysis of phylogenetic distance yielded the opposite results, while GC-skew-based dissimilarities did not demonstrate clear separation by suborder. These results support the hypothesis that mitochondrial genomes in the Octocorallia subclass exhibit a lower evolutionary rate compared to that of other animal species (Shearer et al., 2002).

The GC content and genome size of our cold-water samples, which ranged from 0.36–0.39 and 13 067–19 530 bp, respectively, were the same as those in the Alcyonacea order. No obvious genome expansions or contractions were found in any of the 10 mitochondrial genomes, indicating minimal gene loss or acquisition events during the diversification process in deep-sea corals. The stable genomic architecture in terms of both size and GC content within the Alcyonacea order may be associated with the slow rates of nucleotide substitution in anthozoans (Hellberg, 2006; Shearer et al., 2002).

Although gene number was highly conserved in the Alcyonacea order, significant differences in gene order and position on heavy and light strands were observed in the different families. Five different gene arrangements have been described in the Octocorallia subclass (Beaton et al., 1998; Brockman & McFadden, 2012; Brugler & France, 2008; Park et al., 2012; Uda et al., 2011). In the current study, we observed four different gene arrangements, among which *Calyptrophora wyvillei* **sp. nov.**, *Candidella helminthophora* **sp. nov.**, *Acanthogorgia hirsuta* **sp. nov.**, and *Victorgorgia eminens* **sp. nov.** exhibited ancestral arrangements in octocorals (McFadden et al., 2006; Uda et al., 2011). *Sibogagorgia dennisgordoni* **sp. nov.** displayed the "konojoi" arrangement discovered in *Corallium konojoi* (Uda et al., 2011). The *Hemicorallium guttatum* **sp. nov.** showed the "japonicum" arrangement consistent with *Paracorallium japonicum* (Uda et al., 2011). Additionally, the gene arrangements of *Isidella* **sp. nov.** and *Lepidisis* **sp. nov.** were the same as that of Keratoisidinae (Van Der Ham et al., 2009). Based on mechanisms such as tandem duplication via slipped-strand mispairing, followed by random deletion of genes and gene inversion via intramitochondrial recombination (Brugler & France, 2008; Mueller & Boore, 2005), we hypothesized that at least three gene rearrangement events may have occurred during cold-water coral evolution in the deep-sea environment, with one gene rearrangement event occurring during the evolution of the Calcaxonia suborder and two gene rearrangement events occurring in the Scleraxonia suborder.

Typically, either TAG or TAA serves as the termination codon formed by post-transcriptional polyadenylation, a common feature in many metazoan mitochondrial genomes (Brugler & France, 2008). Consistent with other octocoral mitochondrial genomes, only a single tRNA was identified, suggesting extensive loss of tRNA genes during evolution, as also observed in protozoans and angiosperms (Small et al., 1992). Tandem repeats are widely found in eukaryotic genomes as well as in some prokaryotes (Gao & Kong, 2005). The presence of tandem repeats in three of the mitochondrial genomes may be indicative of gene rearrangements during evolutionary adaptation to deep-sea environments.

The *Ka*/*K* s ratio is a common indicator used to assess selective pressure and evolutionary relationships among species in molecular studies (Zhu et al., 2017). Here, we analyzed the *Ka*/*K*s ratios of the 14 PCGs in the mitochondrial genomes to provide a comprehensive understanding of cold-water coral evolution in the deep-sea environment. Strong purifying selection may be a major driving force influencing mitochondrial genome diversity in deep-sea corals (Noll et al., 2022). Environmental conditions contribute substantially to the basic patterns of genome evolution driven by random genetic drift and mutation pressure (Schaack et al., 2020). Moreover, environmental factors can regulate the expression levels of PCGs, thereby influencing energy regulation, reproduction, and immune behaviors (Yan et al., 2019). Therefore, a species may undergo positive selection during adaptation to a new environmental niche, leading to the formation of novel functions (Nielsen, 2005). The purifying selection of cold-water corals indicated that the deep-sea environment variation might be insufficient to induce alterations in genetic functionality. In addition, the *atp8* gene, which plays an important role in ATP synthesis, exhibited the highest median *Ka*/*K* s value in selected cold-water coral species, consistent with previous studies in other organisms (Kumar et al., 2020; Lv et al., 2018; Niu et al., 2020). Based on their higher *Ka*/*K* s ratios and increased amino acid changes, the *atp8* and *mutS* genes may have evolved more rapidly, experiencing more relaxed selective constraints and accumulating more mutations.

In this study, environmental factors were initially co-analyzed with gene selective pressure. Significant positive relationships were observed between environmental factors and the evolution of the cyt *b* and *mutS* genes. The cyt *b* gene encodes cytochrome *b* and participates in the respiratory chain to produce electrochemical potential coupled with ATP synthesis, while the *mutS* gene participates in DNA mismatch repair and mediation of intramolecular recombination (Hsieh et al., 2001; Muthye et al., 2022). These results suggest that environmental factors may have influenced the evolutionary trajectory of deep-sea corals by affecting mutations in genes related to energy metabolism.

CONCLUSIONS

Cold-water corals provide both organic food and benthic habitats for many organisms, thereby contributing to stable ecosystems in deep-ocean regions characterized by darkness, low temperature, oligotrophy, and high hydrostatic pressure. As such, exploring the origins and evolutionary pathways of these deep-sea corals is of considerable importance. In this study, we conducted a systematic analysis of mitochondrial genomes from cold-water corals, classified into four suborders belonging to the Alcyonacea order and Octocorallia subclass. Our analysis contributes to a better understanding of the taxonomic status, species diversity, distribution, mitochondrial characteristics, and genetic plasticity of these deep-sea corals. Notably, our findings suggest that extreme deep-sea environmental factors may drive coral evolution by inducing mutations in genes related to energy metabolism, which warrants further validation through the collection of more environmental data in future studies.

DATA AVAILABILITY

The raw data and mitochondrial genomes in this study were submitted to NCBI (PRJNA951866), Science Data Bank (10.57760/sciencedb.j00139.00051), and GSA (PRJCA016049).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The project was conceived by S.S.L., X.T.P., and C.W.S.. Sample collection, DNA extraction, and DNA library preparation were carried out by N.N.Z., C.Y.C. and L.M. Morphological examination and sclerite scanning were carried out by K.W.T. Pipeline assembly, including data cleaning, genome assembly, annotation, and all downstream analyses, was carried out by Z.F.W. and K.Q.L. The manuscript was written by Z.F.W. with contributions from all authors. All authors edited the manuscript before submission. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We are grateful to the crew of the scientific research vessel *Tansuo Yihao* for sample collection and to the China National GeneBank for their technical support in library construction and sequencing.

REFERENCES

Andrews S. 2014. FastQC a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Baillon S, Hamel JF, Wareham Hayes V, et al. 2012. Deep cold-water corals as nurseries for fish larvae. *Frontiers in Ecology and the Environment*, **10**(7): 351−356.

Beaton MJ, Roger AJ, Cavalier-Smith T. 1998. Sequence analysis of the mitochondrial genome of *Sarcophyton glaucum*: conserved gene order among octocorals. *Journal of Molecular Evolution*, **47**(6): 697−708.

Benson G. 1999. Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Research*, **27**(2): 573−580.

Bilewitch JP, Degnan SM. 2011. A unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual self-contained function. *BMC Evolutionary Biology*, **11**(1): 228.

Boore JL. 1999. Animal mitochondrial genomes. *Nucleic Acids Research*, **27**(8): 1767−1780.

Brockman S, McFadden C. 2012. The mitochondrial genome of *Paraminabea aldersladei* (Cnidaria: Anthozoa: Octocorallia) supports intramolecular recombination as the primary mechanism of gene rearrangement in octocoral mitochondrial genomes. *Genome Biology and Evolution*, **4**(9): 994−1006.

Brugler MR, France SC. 2008. The mitochondrial genome of a deep-sea bamboo coral (Cnidaria, Anthozoa, Octocorallia, Isididae): genome structure and putative origins of replication are not conserved among octocorals. *Journal of Molecular Evolution*, **67**(2): 125−136.

Cairns SD. 2009. Review of octocorallia (Cnidaria: Anthozoa) from Hawai'i and adjacent seamounts. Part 2: Genera *Paracalyptrophora*; *Candidella*; and *Calyptrophora*. *Pacific Science*, **63**(3): 413–448.

Cairns SD. 2012. The Marine Fauna of New Zealand: New Zealand Primnoidae (Anthozoa, Alcyonacea) - Part 1. Genera *Narella, Narelloides, Metanarella, Calyptrophora*, and *Helicoprimnoa*. Wellington: NIWA.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, **25**(15): 1972−1973.

Carapelli A, Fanciulli P P, Frati F, et al. 2019. Mitogenomic data to study the taxonomy of Antarctic springtail species (Hexapoda: Collembola) and their adaptation to extreme environments. *Polar Biology*, **42**(4): 715−732.

Chen SF, Zhou YQ, Chen Y, et al. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, **34**(17): i884−i890.

Donath A, Jühling F, Al-Arab M, et al. 2019. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Research*, **47**(20): 10543−10552.

Fryer P, Wheat CG, Williams T, et al. 2020. Mariana serpentinite mud volcanism exhumes subducted seamount materials: implications for the origin of life. *Philosophical Transactions of the Royal Society A:Mathematical, Physical and Engineering Sciences*, **378**(2165): 20180425.

Gao H, Kong J. 2005. Distribution characteristics and biological function of tandem repeat sequences in the genomes of different organisms. *Zoological Research*, **26**(5): 555−564. (in Chinese)

Greiner S, Lehwark P, Bock R. 2019. OrganellarGenomeDRAW (OGDRAW) version 1.3. 1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Research*, **47**(W1): W59−W64.

Hebbeln D, Wienberg C, Dullo WC, et al. 2020. Cold-water coral reefs thriving under hypoxia. *Coral Reefs*, **39**(4): 853−859.

Heestand Saucier E, France SC, Watling L. 2021. Toward a revision of the bamboo corals: part 3, deconstructing the Family Isididae. *Zootaxa*, **5047**(3): 247−272.

Hellberg ME. 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evolutionary Biology*, **6**: 24.

Horvath EA. 2019. A review of gorgonian coral species (Cnidaria, Octocorallia, Alcyonacea) held in the Santa Barbara Museum of Natural History research collection: focus on species from Scleraxonia, Holaxonia, and Calcaxonia - Part I: introduction, species of Scleraxonia and Holaxonia (Family Acanthogorgiidae). *ZooKeys*, **860**: 1−66.

Hsieh HM, Chiang HL, Tsai LC, et al. 2001. Cytochrome b gene for species identification of the conservation animals. *Forensic Science International*, **122**(1): 7−18.

Katoh K, Toh H. 2010. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics*, **26**(15): 1899−1900.

Kroemer G, Reed JC. 2000. Mitochondrial control of cell death. *Nature Medicine*, **6**(5): 513−519.

Kumar V, Tyagi K, Chakraborty R, et al. 2020. The complete mitochondrial genome of endemic giant tarantula, *Lyrognathus crotalus* (Araneae: Theraphosidae) and comparative analysis. *Scientific Reports*, **10**(1): 74.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nature Methods*, **9**(4): 357−359.

Li DH, Liu CM, Luo RB, et al. 2014. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct *de Bruijn* graph. *Bioinformatics*, **31**(10): 1674−1676.

Li F, Lv YY, Wen ZY, et al. 2021. The complete mitochondrial genome of the intertidal spider (*Desis jiaxiangi*) provides novel insights into the adaptive evolution of the mitogenome and the evolution of spiders. *BMC Ecology and Evolution*, **21**(1): 72.

Li JR, Wang PX. 2019. Discovery of deep-water bamboo coral forest in the South China Sea. *Scientific Reports*, **9**(1): 15453.

Li Y, Zhan ZF, Xu KD. 2020. Morphology and molecular phylogenetic analysis of deep-sea purple gorgonians (Octocorallia: Victorgorgiidae) from seamounts in the Tropical Western Pacific, with description of three new species. *Frontiers in Marine Science*, **7**: 701.

Lowe TM, Chan PP. 2016. TRNAscan-SE on-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Research*, **44**(W1): W54−W57.

Lv YY, Li YP, Ruan ZQ, et al. 2018. The complete mitochondrial genome of *Glyptothorax macromaculatus* provides a well-resolved molecular phylogeny of the Chinese sisorid catfishes. *Genes*, **9**(6): 282.

McFadden CS, France SC, Sánchez JA, et al. 2006. A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences. *Molecular Phylogenetics and Evolution*, **41**(3): 513−527.

McFadden CS, Sánchez JA, France SC. 2010. Molecular phylogenetic insights into the evolution of octocorallia: a review. *Integrative and Comparative Biology*, **50**(3): 389−410.

Moore KM, Alderslade P, Miller KJ. 2017. A taxonomic revision of *Anthothela* (Octocorallia: Scleraxonia: Anthothelidae) and related genera, with the addition of new taxa, using morphological and molecular data. *Zootaxa*, **4304**(1): 1.

Mueller RL, Boore JL. 2005. Molecular mechanisms of extensive mitochondrial gene rearrangement in plethodontid salamanders. *Molecular Biology and Evolution*, **22**(10): 2104−2112.

Muthye V, Mackereth CD, Stewart JB, et al. 2022. Large dataset of octocoral mitochondrial genomes provides new insights into *mt-mutS* evolution and function. *DNA Repair*, **110**: 103273.

Nguyen LT, Schmidt HA, Von Haeseler A, et al. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**(1): 268−274.

Nielsen R. 2005. Molecular signatures of natural selection. *Annual Review of Genetics*, **39**: 197−218.

Niu WT, Xiao JG, Tian P, et al. 2020. Characterization of the complete mitochondrial genome sequences of three Merulinidae corals and novel insights into the phylogenetics. *PeerJ*, **8**: e8455.

Noll D, Leon F, Brandt D, et al. 2022. Positive selection over the mitochondrial genome and its role in the diversification of gentoo penguins in response to adaptation in isolation. *Scientific Reports*, **12**(1): 3767.

Nurk S, Bankevich A, Antipov D, et al. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *Journal of Computational Biology*, **20**(10): 714−737.

Ogata H, Ray J, Toyoda K, et al. 2011. Two new subfamilies of DNA mismatch repair proteins (MutS) specifically abundant in the marine environment. *The ISME Journal*, **5**(7): 1143−1151.

Park E, Hwang DS, Lee JS, et al. 2012. Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. *Molecular Phylogenetics and Evolution*, **62**(1): 329−345.

Richter M, Rosselló-Móra R, Oliver Glöckner F, et al. 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics*, **32**(6): 929−931.

Roberts JM, Wheeler AJ, Freiwald A. 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. *Science*, **312**(5773): 543−547.

Sánchez JA. 2005. Systematics of the bubblegum corals (Cnidaria: Octocorallia: Paragorgiidae) with description of new species from New Zealand and the Eastern Pacific. *Zootaxa*, **1014**(1): 1−72.

Schaack S, Ho EKH, Macrae F. 2020. Disentangling the intertwined roles of mutation, selection and drift in the mitochondrial genome. *Philosophical Transactions of the Royal Society B:Biological Sciences*, **375**(1790): 20190173.

Shearer TL, Van Oppen MJH, Romano S, L et al. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, **11**(12): 2475−2487.

Shtolz N, Mishmar D. 2019. The mitochondrial genome-on selective constraints and signatures at the organism, cell, and single mitochondrion levels. *Frontiers in Ecology and Evolution*, **7**: 342.

Small I, Maréchal-Drouard L, Masson J, et al. 1992. In vivo import of a normal or mutagenized heterologous transfer RNA into the mitochondria of transgenic plants: Towards novel ways of influencing mitochondrial gene expression?. *The EMBO Journal*, **11**(4): 1291−1296.

Sokolova I. 2018. Mitochondrial adaptations to variable environments and their role in Animals' stress tolerance. *Integrative and Comparative Biology*, **58**(3): 519−531.

Sun Z, Hamel JF, Edinger E, et al. 2010. Reproductive biology of the deep-sea octocoral *Drifa glomerata* in the Northwest Atlantic. *Marine Biology*, **157**(4): 863−873.

Taanman JW. 1999. The mitochondrial genome: structure, transcription, translation and replication. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, **1410**(2): 103−123.

Thierens M, Browning E, Pirlet H, et al. 2013. Cold-water coral carbonate mounds as unique palaeo-archives: the plio-pleistocene challenger Mound record (NE Atlantic). *Quaternary Science Reviews*, **73**: 14−30.

Tillich M, Lehwark P, Pellizzer T, et al. 2017. GeSeq-Versatile and accurate annotation of organelle genomes. *Nucleic Acids Research*, **45**(W1): W6−W11.

Tu TH, Dai CF, Jeng MS. 2016. Taxonomic revision of Coralliidae with descriptions of new species from New Caledonia and the Hawaiian Archipelago. *Marine Biology Research*, **12**(10): 1003−1038.

Uda K, Komeda Y, Koyama H, et al. 2011. Complete mitochondrial genomes of two Japanese precious corals, *Paracorallium japonicum* and *Corallium konojoi* (Cnidaria, Octocorallia, Coralliidae): Notable differences in gene arrangement. *Gene*, **476**(1-2): 27−37.

Van De Water JAJM, Allemand D, FerrierPagès C. 2018. Host-microbe interactions in octocoral holobionts - recent advances and perspectives. *Microbiome*, **6**(1): 64.

Van Der Ham JL, Brugler MR, France SC. 2009. Exploring the utility of an indel-rich, mitochondrial intergenic region as a molecular barcode for bamboo corals (Octocorallia: Isididae). *Marine Genomics*, **2**(3-4): 183−192.

Wang DP, Zhang YB, Zhang Z, et al. 2010. KaKs_Calculator 2.0: a toolkit incorporating Gamma-series methods and sliding window strategies. *Genomics, Proteomics & Bioinformatics*, **8**(1): 77−80.

Wang ZF, Shi XJ, Sun LX, et al. 2017. Evolution of mitochondrial energy metabolism genes associated with hydrothermal vent adaption of Alvinocaridid shrimps. *Genes & Genomics*, **39**(12): 1367−1376.

Xu Y, Zhan ZF, Xu KD. 2021. Morphological and molecular characterization of five species including three new species of golden gorgonians (Cnidaria: Octocorallia) from seamounts in the Western Pacific. *Biology*, **10**(7): 588.

Yan CJ, Duanmu XY, Zeng X, et al. 2019. Mitochondrial DNA: distribution, mutations, and elimination. *Cells*, **8**(4): 379.

Zhang B, Zhang YH, Wang X, et al. 2017. The mitochondrial genome of a sea anemone *Bolocera* sp. exhibits novel genetic structures potentially involved in adaptation to the deep-sea environment. *Ecology and Evolution*, **7**(13): 4951−4962.

Zhou Y, Feng CG, Pu YJ, et al. 2021. The first draft genome of a cold-water coral *Trachythela* sp. (Alcyonacea: Stolonifera: Clavulariidae). *Genome Biology and Evolution*, **13**(2): evaa265.

Zhu KC, Liang YY, Wu N, et al. 2017. Sequencing and characterization of the complete mitochondrial genome of Japanese Swellshark (*Cephalloscyllium umbratile*). *Scientific Reports*, **7**(1): 15299.