MITOCHONDRIAL DNA PART B: RESOURCES
2023, VOL. 8, NO. 10, 1059–1062
https://doi.org/10.1080/23802359.2023.2263198

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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of soft coral, *Eleutherobia rubra* (Brundin, 1896) (Cnidaria; Anthozoa; Malacalcyonacea; Alcyoniidae)

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ABSTRACT

The mitogenome of a soft coral, *Eleutherobia rubra* (Brundin, 1896), was completely sequenced for the first time. The total mitogenome length of *E. rubra* is 18,724 bp with 14 protein-coding genes, two ribosomal RNA genes, one transfer RNA gene (*tRNA–Met*), and one non-coding region (NCR). The gene order is also consistent with other Alcyoniidae species. The base composition is 30.1% A, 16.7% C, 19.5% G, and 33.7% T, with a G–C content of 36.2%. This is the first record of the complete mitogenome sequence of the genus *Eleutherobia*.

ARTICLE HISTORY
Received 25 May 2023
Accepted 20 September 2023

KEYWORDS
Eleutherobia rubra; soft coral; Alcyoniidae; complete mitogenome; phylogenetic analysis

Introduction

Eleutherobia rubra (Brundin, 1896) belonging to Alcyoniidae is a finger-shaped azooxanthellate soft coral. Since *Eleutherobia* was first reported by Pütter in 1900, 13 species are known to date. *Eleutherobia rubra* inhabits the temperate waters around Korea, Japan, the USA (California), and Australia, at depths of 20–182 m (Song 1976; Verseveldt and Bayer 1988; Imahara et al. 2014). In Korea, this species occurs in a wide area around the western, southern, and eastern coasts, and forms several populations with high density, particularly in the coastal sea off the south coast of Korea (Figure 1). However, these populations are threatened by global warming. The Kuroshio, which transports excess heat from tropical ocean to the north in the western North Pacific, has warmed twice to three times faster than the global average (Wang et al. 2016; Lam et al. 2021; Sasaki and Umeda 2021; Wan et al. 2023). Consequently, the East Asian marginal seas including Korean peninsula, which are strongly affected by the Kuroshio Current, have become rapidly warming regions (Wang and Wu 2022; Lee et al. 2023). Recently, loss of these populations and changes in transcription and symbiotic bacterial composition have been observed due to thermal stress (Lee et al. 2020, 2023). Coral bleaching and mass mortality due to heatwaves have also been observed worldwide, including in the Mediterranean and the Great Barrier Reefs (Hughes et al. 2017; Garrabou et al. 2022; McGowan and Theobald 2023).

Populations with rapidly decreasing population sizes are easily at risk of extinction due to loss of genetic diversity (Kliman et al. 2008). Genetic management of species at risk of potentially endangered and resolving taxonomic uncertainties using genetic markers are important factors in conservation genetics (Frankham 2019; Hoban et al. 2023). Therefore, as the beginning of efforts to conserve the *E. rubra* population by identifying and managing genetic diversity using mitochondrial markers, the *E. rubra* mitogenome was analyzed. Approximately, 3,290 species of octocorals are known worldwide, but mitogenome data have been reported from only 6.7% (about 221 species) species so far (NCBI 2023; WoRMS Editorial Board 2023). This is the first report of the complete mitogenome in *Eleutherobia*. In addition, these data will also provide valuable information for further studies on the molecular taxonomy and phylogeny of octocorals, which are problematic in their taxonomy due to limited range of morphological characters, and insufficient and inadequate descriptions (Kessel et al. 2023).

Materials and methods

One specimen of *E. rubra* was collected from the subtidal zone of Eoyu Island in the coastal sea off the south coast of Korea (34°39′34.58″ N, 128°34′31.43″ E), and deposited at the Cnidaria Bioresources Bank of Korea, Woosuk University, Jincheon, South Korea (voucher number: CBB17CnAnE226, Prof. Sung-Jin Hwang, buteo2@woosuk.ac.kr). For identification,

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2023.2263198.

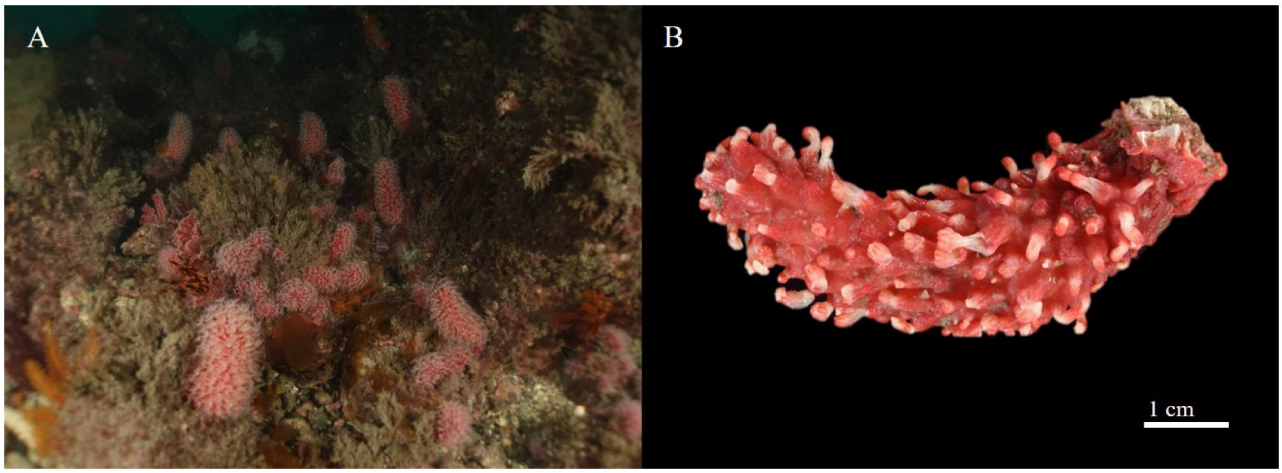

Figure 1. *Eleutherobia rubra*. (A) Population in the coastal sea off the south coast of Korea. (B) Voucher specimen used in this study. Photographs of habitat and voucher were taken by Seung-Hwan Park (underwater photographer at H Dive) and Chi-Hyeon Kim, respectively, and copyright license agreements were obtained.

detailed morphological characteristics of the sclerites were confirmed following Song (1976).

Total genomic DNA was extracted from the polyp tissue of the voucher specimen using the phenol–chloroform method using a lysis buffer containing high concentration of urea (10 mM Tris–HCl, pH 8.0; 125 mM NaCl; 10 mM EDTA, pH 8.0; 1% SDS; 8 M urea) developed by Asahida et al. (1996). The complete mitogenome sequence was amplified using long-range PCR (LR-PCR), and then three LR-PCR products covering whole mitochondrial genome were sequenced by the primer walking method with 26 primers newly designed in this study (Table S1 for primers and Figure S1 for PCR gel image). The PCR reaction solutions were made using AccuPower ProFi Taq PCR PreMix (Bioneer, Daejeon, South Korea), and PCR amplification was performed according to the user manual of the ProFlex PCR System (Life Technologies, Carlsbad, CA). LR-PCR products were directly sequenced using 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

The complete circular mitogenome sequence of *E. rubra* was assembled starting with COX1 partial sequences as the first location in the gene map, the circular form was determined by identifying overlapped sequences between the first and the last partial sequences and compared the completed sequence with other mitogenome sequences in Alcyoniidae species. All LR-PCR sequences were checked and corrected during the assembly process using Geneious 9.1.8 (Biomatters, Auckland, New Zealand) (Kearse et al. 2012). The 14 protein-coding genes (PCGs) were annotated by identifying their open reading frames (ORFs), and by comparing them with other reported mitogenomes of Alcyoniidae species using MITOS web server (Bernt et al. 2013). The two ribosomal RNA genes (rRNAs) and one transfer RNA gene were determined by comparison with homologous gene sequences of other Alcyoniidae mitochondrial genomes.

The phylogenetic analysis was performed using mitogenomes sequences of eight previously published Alcyoniidae (Muthye et al. 2022) and the *E. rubra* in this study. Two Xeniidae mitogenomes were used as outgroup. Mitogenome sequences (14 PCGs nucleotides, excluding stop codons) of a total 11 species including *E. rubra* were concatenated and aligned using the multiple sequence alignment program, MAFFT v.7 (Katoh and Standley 2013) for phylogenetic analysis. A phylogenetic tree was reconstructed based on the concatenated dataset using the maximum-likelihood (ML) method with the GTR + G + I model in raxmlGUI 2.0 (Edler et al. 2021), with the bootstrap values being calculated from 10,000 replicates.

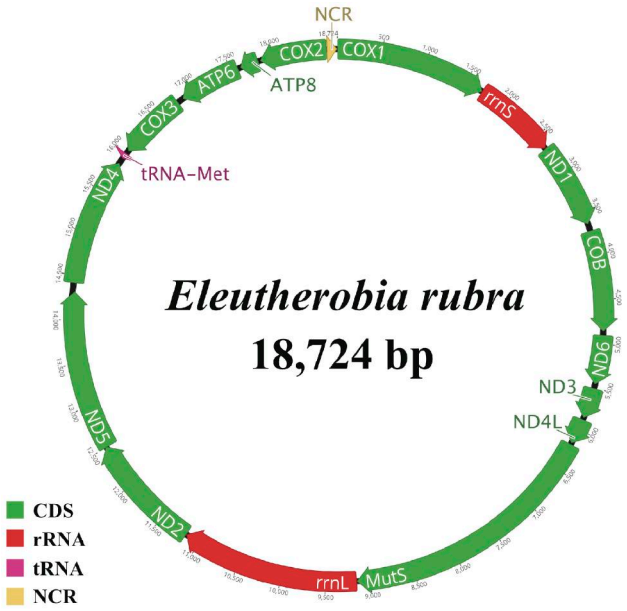

Figure 2. Circular representation of the complete mitogenome for *E. rubra*. The genes were colored based on their functional groups. Arrows show the directions of transcription.

Results

The complete circular mitogenome of *E. rubra* was 18,724 bp in length (GenBank accession no. ON814482) (Figure 2), which was within published mitogenome lengths for Alcyoniids (18–19 kb). MutS gene, which is involved in DNA repair in octocoral mitochondria, was found in *E. rubra* mitogenome. Recently, the gene was utilized as a highly effective molecular marker for phylogenetic analysis in the octocoral

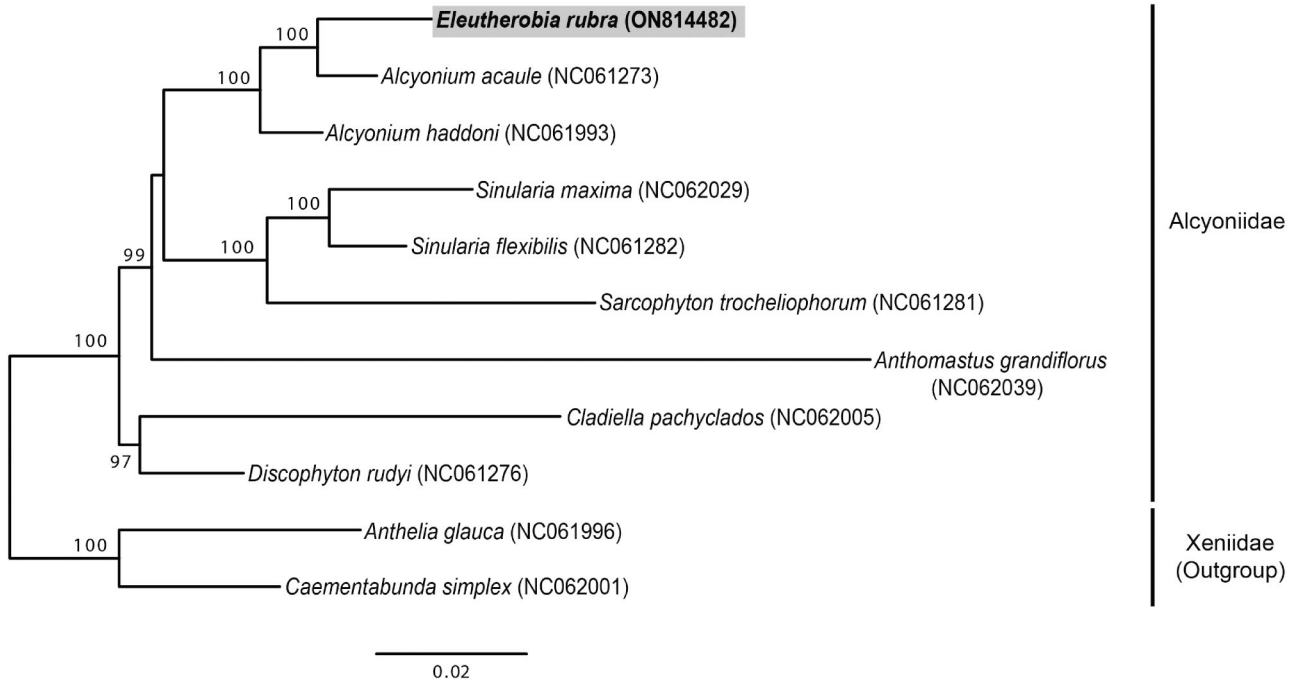

Figure 3. Maximum-likelihood tree based on concatenated 14 PCGs sequence dataset from 11 Malacalcyonacea species. Two Xeniidae species (*Anthelia glauca* and *Caementabunda simplex*) were used as outgroups. GenBank accession numbers of each sequence are marked behind their corresponding species names in the tree. Sequence obtained in this study is in bold.

group (McFadden et al. 2022; Muthye et al. 2022). *E. rubra* mitogenome has 17 genes (14 PCGs, two rRNAs, and one transfer RNA gene (*tRNA–Met*)) and one non-coding region (NCR) of 109 bp. The gene order shows an ancestral octocoral mt gene order, consistent with other Alcyoniidae species (Figueroa and Baco 2015). Regarding its nucleotide base composition, this mitogenome has A, C, G, and T contents of 30.1%, 16.7%, 19.5%, and 33.7%, respectively, with a G + C content of 36.2%. Two rRNAs (*rrnS* and *rrnL*) and 10 PCGs were encoded by the heavy strand. Four PCGs (*ATP6*, *ATP8*, *COX2*, and *COX3*) and *tRNA–Met* were encoded by the light strand. All PCGs began with ATG as a start codon. In addition, all PCGs used TAR as a stop codon. Ten genes (*ATP8*, *COX1–3*, *COB*, *ND1–3*, *ND5*, and *ND6*) ending with TAG and four genes (*ATP6*, *MutS*, *ND4*, and *ND4L*) ended with TAA. The two rRNA genes contained *rrnS* and *rrnL* between *COX1* and *ND1* or between *MutS* and *ND2*. NCR was located between *COX1* and *COX2*.

Phylogeny analysis indicated that this new mitogenome sequence of genus *Eleutherobia* clustered with *Alcyonium* species in family Alcyoniidae (Figure 3). The tree showed that *E. rubra* and *A. acaule* had a close relationship with a very high nodal support (100% BP in ML). *E. rubra* and *A. acaule* shared sequence similarities of 98% for the total length of mitogenome sequences (18,724 bp of *A. acaule*). According to a previous study (McFadden et al. 2022), *Eleutherobia* species and *A. acaule* belonged to a sister group in phylogenetic analysis using MutS gene, similar to results of this study.

Discussion and conclusions

Through this study, we report the first complete mitogenome sequencing with *E. rubra* for the genus *Eleutherobia*. The results of this study can be used for the population conservation of *E. rubra* by identifying and managing genetic diversity using mitochondrial genetic markers, and for comprehensive taxonomic studies of the Alcyoniidae including this species as a representative of the genus *Eleutherobia*.

Author contributions

Chi-Hyeon Kim: data analysis and manuscript writing. Sang-Hwa Lee: mapping and phylogenetic analysis. In-Young Cho: identification and manuscript revising. Min-Seop Kim: manuscript reviewing. Seonock Woo: manuscript reviewing. Keun-Yong Kim: data collection and analysis. Sung-Jin Hwang: designing this study, manuscript reviewing, and approval of final version to be published.

Ethical approval

This research does not involve ethical research. However, the sample collection area was designated and protected as Hallyeohaesang National Park, so the sample was collected with permission from the Korea National Park Service. *E. rubra* is widespread in South Korea, and is not listed as a threatened or endangered species.

Disclosure statement

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This work was supported by a grant from the National Marine Biodiversity Institute of Korea under Grant [2023M00300].

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Data availability statement

The data that support the findings of this study are openly available in the GenBank of NCBI at https://www.ncbi.nlm.nih.gov under the accession ON814482. The associated BioProject, SRA and Bio-sample numbers are PRJNA930047, SRR24682002, and SAMN29133432, respectively.

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