Complete mitochondrial genome sequences of *Physogyra lichtensteini* (Milne Edwards & Haime, 1851) and *Plerogyra sinuosa* (Dana, 1846) (Scleractinia, Plerogyridae): characterisation and phylogenetic analysis

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Abstract

In this study, the whole mitochondrial genomes of *Physogyra lichtensteini* and *Plerogyra sinuosa* have been sequenced for the first time. The length of their assembled mitogenome sequences were 17,286 bp and 17,586 bp, respectively, both including 13 protein-coding genes, two tRNAs, and two rRNAs. Their mitogenomes offered no distinct structure and their gene order were the same as other typical scleractinians. Based on 13 protein-coding genes, a maximum likelihood phylogenetic analysis showed that *Physogyra lichtensteini* and *Plerogyra sinuosa* are clustered in the family Plerogyridae, which belongs to the “Robust” clade. The 13 tandem mitogenome PCG sequences used in this research can provide important molecular information to clarify the evolutionary relationships amongst stony corals, especially at the family level. On the other hand, more advanced markers and more species need to be used in the future to confirm the evolutionary relationships of all the scleractinians.

Keywords

Evolutionary relationships, mitogenome data, Plerogyridae, “Robust” clade
Introduction

The order Scleractinia (Cnidaria, Anthozoa), including numerous reef-building coral species, is important as the constructors of coral reefs as an ecosystem. The mitogenome data of cnidarians contain important phylogenetic information for understanding their evolutionary history (Kayal et al. 2013). Single- or multiple-gene analysis of mitochondrial genes have already been used to infer phylogenetic relationships amongst scleractinians (Kitahara et al. 2016; Arrigoni et al. 2020).

In Scleractinia, three main clades have been defined based on molecular analyses, “Complex”, “Robust”, and “Basal” (Romano and Palumbi 1996; Kitahara et al. 2010; Stolarski et al. 2011). Plerogyridae Rowlett, 2020 is a small family of the “Robust” clade of corals containing four genera (Plerogyra Milne Edwards & Haime, 1848, Physogyra Quelch, 1884, Blastomussa Wells, 1968, and Nemenzophyllia Hodgson & Ross, 1982) (see Hoeksema and Cairns 2022), all from the Indo-West Pacific. Previously, the genera Plerogyra and Physogyra were placed in the Euphylliidae of the “Complex” group (Fukami et al. 2008), the family Plesiastreidae of the the “Robust” group (Dai and Horng 2009), and in Scleractinia incertae sedis (Budd et al. 2012; Benzoni et al. 2014; Waheed et al. 2015), but recently Rowlett (2020) placed them in the family Plerogyridae. Physogyra has one recently accepted species and four uncertain species, whereas Plerogyra has seven accepted species (Hoeksema and Cairns 2022). Through molecular analyses of two mitochondrial genes, Fukami et al. (2008) found that Plerogyra and Physogyra do not belong to the “Complex” clade of Scleractinia but to the “Robust” clade. Morphologically, plerogyrid species are characterised by mantle vesicles that are diurnally visible when the tentacles are partially retracted (Benzoni et al. 2014).

Physogyra lichtensteini (Milne Edwards & Haime, 1851) and Plerogyra sinuosa (Dana, 1846) are covered by round to irregularly bifurcating vesicles during the day and active, open polyps at night (Veron 2000; Benzoni et al. 2014). Physogyra lichtensteini is common in lagoons and reef slopes to deeper than 38 m (De Palmas et al. 2021). Colonies of Physogyra lichtensteini are generally massive. They are meandroid, with short, widely separated valleys interconnected with a light, blisterly coenosteum. Septa are large, solid, smooth-edged, exert, and widely spaced. Walls are solid. Columellae are absent. Tentacles are extended only at night. The septal vesicles of Physogyra are considerably smaller and more numerous when compared to the closely related Plerogyra. The colour of Physogyra lichtensteini is pale grey or sometimes dull green (Veron 2000), while in Plerogyra sinuosa, the colonies are flabello-meandroid with valleys somewhat connected by a light, blisterly coenosteum. Living parts of colonies are sometimes separated by dead basal parts. Vesicles are the size of grapes and usually have the shape of grapes but may be tubular, bifurcated, or irregular, depending primarily upon the state of inflation. The colour of Plerogyra sinuosa is cream or bluish grey. Plerogyra sinuosa is a prominent species and reasonably common in protected reefs, and it is easily recognised by its bubbly appearance (Veron 2000; Rowlett 2020).

In this research, the complete mitochondrial genomes of Physogyra lichtensteini and Plerogyra sinuosa are sequenced and their genome structures are analysed for the first time. The phylogenetic analyses of these two species, based on 13 protein coding genes
(PCGs) of the mitogenome, in combination with another 42 species of other genera of Scleractinia and two species of Corallimorphidae Hertwig 1882 (order Corallimorpharia) as outgroups, because they are closely related to Scleractinia in evolutionary terms. This helps determine their phylogenetic status and facilitate further study on stony coral evolutionary and phylogenetic relationships.

**Materials and methods**

**Sample collection and genomic DNA extraction**

Samples of *Physogyra lichtensteini* (Fig. 1A, C) and *Plerogyra sinuosa* (Fig. 1B, D) were obtained in 2019 from a coral mariculture company in China, which originally obtained mother stock from Negeri Sabah of Malaysia. Their specimens were maintained in our Coral Sample Repository with the codes 20191207-J2 (*Physogyra lichtensteini*) and 20191207-Y1 (*Plerogyra sinuosa*). Total genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Shanghai, China). Electrophoresis with 1% agarose gel was used to measure the integrity of their genomic DNA, and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA) was used to measure their genomic DNA concentration.

**Mitogenome sequencing, annotation, and analyses**

In this study, two methods were used to obtain the mitogenomes of *Physogyra lichtensteini* and *Plerogyra sinuosa*, respectively. The complete mitogenome of *Plerogyra sinuosa* was obtained through a PCR approach using the primer pairs designed by Lin et al. (2011). The complete mitogenome of *Physogyra lichtensteini* was obtained from high-throughput sequencing with a HiSeqX Ten platform (Illumina, San Diego, CA, USA) with a paired-end 150 bp approach according to Tian et al. (2021), and a total of 102,074 of 116,026,504 raw reads (approximately 0.09%) were de novo assembled to produce a single, circular form of the complete mitogenome with an average coverage of 892 X. The circularised contig of these two species were then submitted to the MITOS WebServer (Bernt et al. 2013; http://mitos.bioinf.uni-leipzig.de/index.py) for preliminary mitochondrial genome annotation. We identified and annotated their 13 PCGs and two tRNA genes by alignments of homologous mitogenomes from other scleractinians that had been recovered through BLAST searches in NCBI, and we also validated the tRNA genes using ARWEN (Laslett and Canbäck 2008). The genome structures were mapped using the online CGView Server (Stothard and Wishart 2005; https://proksee.ca/). Base composition, nucleotide frequencies, and codon usage were obtained through MEGA7 (Kumar et al. 2016). The skewing of the nucleotide composition was measured in terms of AT skews and GC skews according to the following formulae: AT skew = (A – T) / (A + T) and GC skew = (G – C) / (G + C) (Perna and Kocher 1995). The mitogenome sequences of *Physogyra lichtensteini* and *Plerogyra sinuosa* are available in GenBank under accession numbers MW970409 and MW936598.
Phylogenetic analyses

The phylogenetic positions of *Physogyra lichtensteini* and *Plerogyra sinuosa* were inferred using 13 tandem mitogenome PCG sequences (ND5 + ND1 + Cytb + ND2 + ND6 + ATP6 + ND4 + COIII + COII + ND4L + ND3 + ATP8 + COI) (Tian et al. 2021)
Complete mitogenomes of *Physogyra lichtensteini* and *Plerogyra sinuosa* together with another 42 species of other genera of Scleractinia and two species belonging to two genera of Corallimorpharia that we obtained from GenBank (Table 1). We used MEGA 7 to select the best-fitting model based on the Akaike Information Criterion (AIC) and then constructed a maximum likelihood (ML) tree with 500 bootstrap replicates.

| Species                     | Family             | Mitogenome length (bp) | GenBank accession number |
|-----------------------------|--------------------|------------------------|--------------------------|
| *Physogyra lichtensteini*   | Plerogyridae       | 17,286                 | MW970409                 |
| *Plerogyra sinuosa*         | Plerogyridae       | 17,586                 | MW936598                 |
| *Acropora horrida*          | Acroporidae        | 18,480                 | NC_022825                |
| *Alveopora japonica*        | Acroporidae        | 18,144                 | MG851913                 |
| *Astreopora explanata*      | Acroporidae        | 18,106                 | KJ634269                 |
| *Isopora palifera*          | Acroporidae        | 18,725                 | KJ634270                 |
| *Montipora cactus*          | Acroporidae        | 17,887                 | NC_006902                |
| *Agaricia fragilis*         | Agariciidae        | 18,667                 | KM051016                 |
| *Agaricia humilis*          | Agariciidae        | 18,735                 | NC_008160                |
| *Pavona clavus*             | Agariciidae        | 18,315                 | NC_008165                |
| *Pavona decussata*          | Agariciidae        | 18,378                 | KP231535                 |
| *Desmophyllum pertusum*     | Caryophylliidae    | 16,150                 | FR821799                 |
| *Solenomilia variabilis*    | Caryophylliidae    | 15,968                 | KM609293                 |
| *Dendrophyllia arbuscula*   | Dendrophylliidae   | 19,069                 | KR824937                 |
| *Tubastrea coccinea*        | Dendrophylliidae   | 19,094                 |                         |
| *Duncanopsammia peltata*    | Dendrophylliidae   | 18,966                 | NC_024671                |
| *Fimbriaphyllia ancora*     | Euphylliidae       | 18,875                 | NC_015641                |
| *Galaxea fascicularis*      | Euphylliidae       | 18,751                 | NC_029696                |
| *Colpophyllia natans*       | Faviidae           | 16,906                 | NC_008162                |
| *Musia angulosa*            | Faviidae           | 17,245                 | DJ643834                 |
| *Fungiacyathus stephanus*   | Fungiacyathidae    | 19,381                 | JF825138                 |
| *Gardineria hawaiiensis*    | Gardineriidae      | 19,430                 | MT376619                 |
| *Echinophyllia aspera*      | Lobophylliidae     | 17,697                 | MG792550                 |
| *Dipastrea rotomana*        | Merulinidae        | 16,466                 | MH119077                 |
| *Hydnophora exxa*           | Merulinidae        | 17,790                 | MH086217                 |
| *Orbicella faveolata*       | Merulinidae        | 16,138                 | AP008978                 |
| *Platygyra cariosa*         | Merulinidae        | 16,463                 | JX911333                 |
| *Letepsammia formosissima*  | Micrabaciidae      | 19,048                 | MT705247                 |
| *Letepsammia superstit*     | Micrabaciidae      | 19,073                 | MT706035                 |
| *Rhombopamminia niphada*    | Micrabaciidae      | 19,542                 | MT706034                 |
| *Madrepora oculata*         | Oculinidae         | 15,841                 | JX236041                 |
| *Plesiastrea versipora*     | Plesiastreidae     | 15,320                 | MH025639                 |
| *Pocillopora eydoux*        | Pocilloporidae     | 17,422                 | EF526303                 |
| *Seriatopora hystrix*       | Pocilloporidae     | 17,059                 | EF63600.2                |
| *Madracis mirabilis*        | Pocilloporidae     | 16,951                 | NC_011160                |
| *Stylophora pistillata*     | Pocilloporidae     | 17,177                 | NC_011162                |
| *Goniopora columna*         | Poritidae          | 18,766                 | JF825141                 |
| *Porites fontanensis*       | Poritidae          | 18,658                 | NC_037434                |
| *Porites lobata*            | Poritidae          | 18,647                 | KU572435                 |
| *Porites rus*               | Poritidae          | 18,647                 | NC_027526                |
| *Psammocora profundacella*  | Psammocoridae       | 16,274                 | MT576637                 |
| *Astrangia poulata*         | Astrangidiidae     | 14,853                 | NC_008161                |
| *Pseudoiderastrea taenina*  | Siderastreidae     | 19,475                 | KP260633                 |
| *Siderastrea radians*       | Siderastreidae     | 19,387                 | NC_008167                |
| *Corallimorphus profundus*  | Corallimorphidae   | 20,488                 | KP398440                 |
| *Corynactis californica*    | Corallimorphidae   | 20,715                 | NC_027102                |
Results and discussion

Characteristics and composition of mitogenome

The mitochondrial genome sizes of *Physogyra lichtensteini* and *Plerogyra sinuosa* are 17,286 bp and 17,586 bp, respectively, both including 13 PCGs, two tRNA (tRNA\textsubscript{Met}, tRNA\textsubscript{Trp}), and two rRNA genes (Tables 2, 3; Fig. 2). Their mitogenomes offer no distinct structure and their gene orders are same as other typical scleractinians (Lin et al. 2012). All genes are encoded on the H-strand. The base composition of the complete mitogenome is 24.75% A, 13.32% C, 21.75% G, and 40.17% T for *Physogyra lichtensteini*, and 24.87% A, 13.16% C, 22.01% G, and 39.96% T for *Plerogyra sinuosa*. Both species show a higher AT content than GC content (Fig. 3; Table 4).

Protein-coding genes

The lengths of all 13 protein-coding genes (PCGs) are 11,598 bp and 11,574 bp for *Physogyra lichtensteini* and *Plerogyra sinuosa*, respectively. In both species, the ND5 gene and COI gene have intron insertions, and the start and stop codon of all 13 PCGs are exactly the same except for the COI gene. Their shortest gene is in both ATP8, and their longest gene is ND5 (Tables 2, 3). According to the AT-skew and GC-skew analyses

**Table 2.** Organisation of the mitochondrial genome of *Physogyra lichtensteini*.

| Gene       | Position | Length (bp) | Anticodon | Codon | Intergenic nucleotides* | Strand† |
|------------|----------|-------------|-----------|-------|------------------------|---------|
| tRNA\textsubscript{Met} | 1        | 72          | 72        | CAU   | 1228                   | H       |
| 16S rRNA   | 270      | 1967        | 1698      |       | 197                    | H       |
| ND5 5'     | 1998     | 2708        | 711       | ATG   | 30                     | H       |
| ND1        | 2817     | 3764        | 948       | ATG   | 108                    | H       |
| Cytb       | 3767     | 4906        | 1140      | ATG   | 2                      | H       |
| ND2        | 5124     | 6218        | 1095      | TTA   | 217                    | H       |
| ND6        | 6219     | 6779        | 561       | ATG   | 0                      | H       |
| ATP6       | 6779     | 7453        | 675       | ATG   | -1                     | H       |
| ND4        | 7453     | 8892        | 1440      | ATG   | -1                     | H       |
| 12S rRNA   | 8890     | 9800        | 911       |       | -3                     | H       |
| COIII      | 9794     | 10573       | 780       | ATG   | -7                     | H       |
| COI        | 10576    | 11283       | 708       | ATG   | 2                      | H       |
| ND4L       | 11265    | 11564       | 300       | ATG   | -19                    | H       |
| ND3        | 11567    | 11908       | 342       | GTG   | 2                      | H       |
| ND5 3'     | 11996    | 13099       | 1104      | TAG   | 87                     | H       |
| tRNA\textsubscript{Trp} | 13098    | 13166       | 69        | UCA   | -2                     | H       |
| ATP8       | 13170    | 13367       | 198       | ATG   | 3                      | H       |
| COI 5'     | 13385    | 14095       | 711       | ATT   | 17                     | H       |
| COI 3'     | 15173    | 16057       | 885       | TAG   | 1077                   | H       |

*Data are number of nucleotides between the given gene and its previous gene; negative numbers indicate overlapping nucleotides.
†H indicates that the genes transcribed on the heavy strand.
Table 3. Organisation of the mitochondrial genome of *Plerogyra sinuosa*.

| Gene      | Position | Length (bp) | Anticodon | Codon | Intergenic nucleotides* | Strand† |
|-----------|----------|-------------|-----------|-------|-------------------------|---------|
| tRNA^5s  | 1        | 72          |           | CAU   | 1581                    | H       |
| 16S rRNA  | 272      | 1969        | 1698      |       | 199                     | H       |
| ND5 5'   | 2000     | 2710        | 711       |       | 30                      | H       |
| ND1      | 2819     | 3766        | 948       |       | 108                     | H       |
| Cytb     | 3769     | 4908        | 1140      |       | 2                       | H       |
| ND2      | 5125     | 6219        | 1095      | TTA   | 216                     | H       |
| ND6      | 6220     | 6780        | 561       |       | 0                       | H       |
| ATP6     | 6780     | 7454        | 675       |       | -1                      | H       |
| ND4      | 7451     | 8893        | 1443      |       | -4                      | H       |
| 12S rRNA  | 8891    | 9797        | 907       |       | -3                      | H       |
| COIII    | 9795     | 10574       | 780       |       | -3                      | H       |
| COII     | 10577    | 11284       | 708       |       | 2                       | H       |
| ND4L     | 11266    | 11565       | 300       |       | -19                     | H       |
| ND3      | 11568    | 11909       | 342       |       | 2                       | H       |
| ND5 3'   | 11997    | 13100       | 1104      |       | 87                      | H       |
| tRNA^3p  | 13099    | 13167       | 69        |       | UCA                     | H       |
| ATP8     | 13171    | 13368       | 198       |       | 3                       | H       |
| COI 5'   | 13368    | 14270       | 903       |       | -1                      | H       |
| COI 3'   | 15336    | 16004       | 669       |       | 1065                    | H       |

*Data are number of nucleotides between the given gene and its previous gene; negative numbers indicate overlapping nucleotides.
†H indicates that the genes transcribed on the heavy strand.

Table 4. Nucleotide composition in different regions of mitogenomes of *Physogyra lichensteini* (*P. l.*) and *Plerogyra sinuosa* (*P. s.*).

| Gene/Region | T (%) | C (%) | A (%) | G (%) | A+T (%) | Size (bp) |
|-------------|-------|-------|-------|-------|---------|-----------|
|             | *P. l.* | *P. s.* | *P. l.* | *P. s.* | *P. l.* | *P. s.* | *P. l.* | *P. s.* |
| ND5         | 46.56  | 46.61 | 12.07 | 12.01 | 21.71  | 21.76  | 19.67  | 19.61  | 68.27  | 68.37  | 1815  | 1815  |
| ND1         | 43.35  | 43.46 | 14.14 | 14.03 | 19.20  | 19.09  | 23.31  | 23.42  | 62.55  | 62.55  | 948   | 948   |
| Cytb        | 44.91  | 44.82 | 13.68 | 13.68 | 20.88  | 20.88  | 20.53  | 20.61  | 65.79  | 65.70  | 1140  | 1140  |
| ND2         | 47.31  | 47.31 | 12.79 | 12.60 | 20.00  | 20.09  | 19.91  | 20.00  | 67.31  | 67.40  | 1095  | 1095  |
| ND6         | 44.56  | 44.56 | 13.37 | 13.35 | 22.28  | 22.28  | 19.79  | 19.61  | 66.84  | 66.84  | 561   | 561   |
| ATP6        | 46.81  | 46.37 | 13.19 | 13.33 | 22.22  | 22.22  | 17.78  | 18.07  | 69.03  | 68.59  | 675   | 675   |
| ND4         | 45.35  | 45.56 | 13.54 | 13.47 | 19.86  | 19.86  | 21.25  | 21.11  | 65.21  | 65.42  | 1440  | 1443  |
| COIII       | 42.69  | 42.69 | 15.38 | 15.51 | 19.74  | 19.62  | 22.18  | 22.18  | 62.43  | 62.31  | 780   | 780   |
| COII        | 39.69  | 39.55 | 13.28 | 13.28 | 24.01  | 23.87  | 23.02  | 23.31  | 63.70  | 63.42  | 708   | 708   |
| ND4L        | 44.33  | 44.33 | 12.00 | 12.00 | 24.67  | 24.67  | 19.00  | 19.00  | 69.00  | 69.00  | 300   | 300   |
| ND3         | 47.08  | 46.78 | 9.94  | 9.94  | 17.84  | 17.84  | 25.15  | 25.44  | 64.92  | 64.62  | 342   | 342   |
| ATP8        | 43.43  | 43.43 | 12.12 | 12.12 | 29.29  | 29.29  | 15.15  | 15.15  | 72.72  | 72.72  | 198   | 198   |
| COI         | 40.41  | 39.31 | 15.10 | 15.78 | 22.12  | 22.20  | 22.37  | 22.71  | 62.53  | 61.51  | 1596  | 1572  |
| PCGs        | 44.35  | 44.20 | 13.42 | 13.50 | 21.25  | 21.25  | 20.89  | 20.96  | 65.60  | 65.45  | 11574 | 11574 |
| 1st         | 35.59  | 35.56 | 13.63 | 13.69 | 21.99  | 21.90  | 28.79  | 28.85  | 57.58  | 57.47  | 3866  | 3858  |
| 2nd         | 47.31  | 47.15 | 18.65 | 18.61 | 17.93  | 18.01  | 16.11  | 16.23  | 65.24  | 65.16  | 3866  | 3858  |
| 3rd         | 50.16  | 49.90 | 7.99  | 8.19  | 23.82  | 23.82  | 18.03  | 18.09  | 73.98  | 73.72  | 3866  | 3858  |
| tRNA        | 24.82  | 24.82 | 23.40 | 23.40 | 27.66  | 27.66  | 24.11  | 24.11  | 52.48  | 52.48  | 141   | 141   |
| rRNA        | 31.70  | 31.67 | 12.50 | 12.48 | 35.19  | 35.12  | 20.62  | 20.73  | 66.89  | 66.79  | 2609  | 2605  |
| Overall     | 40.17  | 39.96 | 13.32 | 13.16 | 24.75  | 24.87  | 21.75  | 22.01  | 64.92  | 64.83  | 17286 | 17586 |
Figure 2. The mitogenome order and positions of *Physogyra lichtensteini* and *Plerogyra sinuosa*. COI, COII, and COIII refer to the cytochrome oxidase subunits, Cyt b refers to cytochrome b, and ND1–ND6 refers to NADH dehydrogenase components. All the genes are encoded on the H-strand.
Complete mitogenomes of *Physogyra lichtensteini* and *Plerogyra sinuosa*

(Fig. 4), both PCGs of *Physogyra lichtensteini* and *Plerogyra sinuosa* show a stronger nucleotide asymmetry, with AT skew higher than GC skew. Amongst L, F, V, G, and S in *Physogyra lichtensteini* and *Plerogyra sinuosa*, codon use frequency was higher, accounting for 53.5% and 53.4%, respectively. In their 20 amino acids, the majority are non-polar amino acids, and a minority are polarity-charged amino acids (Fig. 5).

**rRNA and tRNA genes**

The encoding genes 12S and 16S rRNA in *Physogyra lichtensteini* are 911 bp and 1,698 bp in size, and in *Plerogyra sinuosa* they are 907 bp and 1,698 bp in size. The base composition of rRNA in *Physogyra lichtensteini* was 35.19% A, 12.5% C, 20.62% G, and 31.7% T, and in *Plerogyra sinuosa* it was 35.12% A, 12.48% C, 20.73% G, and 31.67% T. The two tRNA encoding genes, tRNA$_{^{\text{Met}}}$ (72 bp) and tRNA$_{^{\text{Trp}}}$ (69 bp), are exactly the same in *Physogyra lichtensteini* and *Plerogyra sinuosa* (Tables 2, 3). They are folded into the classic cloverleaf structure which includes an amino acid accept arm, DHU loop, anticodon loop, and TΨC loop (Fig. 6).
Phylogenetic analyses

There are three distinct clades of Scleractinia in our ML tree, including “Complex”, “Robust”, and “Basal” clade. The ML topology tree of all the 47 species shows that *Physogyra lichtensteini* and *Plerogyra sinuosa* are clustered in family Plerogyridae which belong to the “Robust” clade with high bootstrap support (Fig. 7). Our finding is consistent with the results of Fukami et al. (2008) who placed *Plerogyra* and *Physogyra* in the “Robust” clade. From the ML tree we also find that *Physogyra lichtensteini* and *Plerogyra sinuosa* are a sister group with *Astrangia poculata*, which belongs to the family Astrangiidae Milne Edwards & Haime, 1857. Our MT tree of the Plerogyridae shows the same classification as used by Rowlett (2020). Single- or multi-gene analyses of mitochondrial genes have already been used to infer phylogenetic relationships amongst scleractinians (Kitahara et al. 2016; Arrigoni et al. 2020). The 13 tandem mitogenome PCG sequences we used in this research can provide important molecular information to understand

![Figure 4. The PCG AT skew and GC skew of the mitochondrial genomes of Physogyra lichtensteini and Plerogyra sinuosa.](image)
Figure 5. The PCG codon use frequency of the mitochondrial genomes of *Physogyra lichtensteini* and *Plerogyra sinuosa*.

Figure 6. Putative secondary structures of two tRNAs in *Physogyra lichtensteini* and *Plerogyra sinuosa*. 
Figure 7. Inferred phylogenetic relationships based on a maximum-likelihood analysis of concatenated nucleotide sequences of 13 mitochondrial PCGs. Numbers on branches are bootstrap percentages.

The evolutionary relationships amongst stony corals, especially at the family level. As fewer than a tenth of stony coral species have been sequenced at this time, more mitogenomes of other scleractinians are necessary before accurate family-level evolutionary relationships can be reconstructed. In the future, more advanced markers and more species should be used to confirm the evolutionary relationships among all scleractinians.

Conclusions

The complete mitochondrial genomes of Physogyra lichensteini and Plerogyra sinuosa were sequenced for the first time. Their mitogenomes show a similar gene order and composition with other typical Scleractinia. Our phylogenetic analysis of Physogyra lichensteini
and *Plerogyra sinuosa*, based on their 13 tandem mitochondrial protein-coding genes and including another 42 species of Scleractinia and two species of Corallimorpharia, help us to understand the evolutionary relationships amongst stony corals and facilitate further studies on stony coral evolutionary and phylogenetic relationships.

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**References**

Arrigoni R, Berumen ML, Berumen ML, Beck PS, Hulver AM, Montano S, Pichon M, Strona G, Terraneo TI, Benzoni F (2020) Towards a rigorous species delimitation framework for scleractinian corals based on RAD sequencing: The case study of *Leptastrea* from the Indo-Pacific. Coral Reefs 39(4): 1001–1025. [https://doi.org/10.1007/s00338-020-01924-8](https://doi.org/10.1007/s00338-020-01924-8)

Benzoni F, Arrigoni R, Waheed Z, Stefani F, Hoeksema BW (2014) Phylogenetic relationships and revision of the genus *Blastomussa* (Cnidaria: Anthozoa: Scleractinia) with description of a new species. The Raffles Bulletin of Zoology 62: 358–378.

Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsch G, Putz J, Middendorf M, Stadler PF (2013) MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69(2): 313–319. [https://doi.org/10.1016/j.ympev.2012.08.023](https://doi.org/10.1016/j.ympev.2012.08.023)

Budd AF, Fukami H, Smith ND, Knowlton N (2012) Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). Zoological Journal of the Linnean Society 166(3): 465–529. [https://doi.org/10.1111/j.1096-3642.2012.00855.x](https://doi.org/10.1111/j.1096-3642.2012.00855.x)

Dai CF, Horng S (2009) Scleractinia Fauna of Taiwan: II. The Robust Group. National Taiwan University, Taipei, 162 pp.

De Palmas S, Denis V, Soto D, Lin YV, Ho MJ, Chen CA (2021) Scleractinian diversity in the upper mesophotic zone of Ludao (Taiwan): A museum collection with new records from Taiwanese waters. Marine Biodiversity 51(5): 80. [https://doi.org/10.1007/s12526-021-01210-y](https://doi.org/10.1007/s12526-021-01210-y)

Fukami H, Chen CA, Budd AF, Collins A, Wallace C, Chuang YY, Chen C, Dai CF, Iwao K, Shepard C, Knowlton N (2008) Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). PLoS ONE 3(9): e3222. [https://doi.org/10.1371/journal.pone.0003222](https://doi.org/10.1371/journal.pone.0003222)

Hoeksema BW, Cairns S (2022) World List of Scleractinia. [https://www.marinespecies.org/scleractinia/aphia.php](https://www.marinespecies.org/scleractinia/aphia.php) [Accessed on: 2022-7-11]
Kayal E, Roure B, Philippe H, Collins AG, Lavrov DV (2013) Cnidarian phylogenetic relationships as revealed by mitogenomics. BMC Evolutionary Biology 13(1): e5. https://doi.org/10.1186/1471-2148-13-5

Kitahara MV, Cairns SD, Stolarski J, Blair D, Miller DJ (2010) A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data. PLoS ONE 5(7): e11490. https://doi.org/10.1371/journal.pone.0011490

Kitahara MV, Fukami H, Benzioni F, Huang D (2016) The new systematics of Scleractinia: integrating molecular and morphological evidence. In: Goffredo S, Dubinsky Z (Eds) The Cnidaria, Past, Present and Future. Springer, Cham, 41–59. https://doi.org/10.1007/978-3-319-31305-4_4

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Laslett D, Canbäck B (2008) ARWEN: A program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics 24(2): 172–175. https://doi.org/10.1093/bioinformatics/btm573

Lin MF, Luzon KS, Licuanan WY, Ablan-Lagman MC, Chen CA (2011) Seventy-four universal primers for characterizing the complete mitochondrial genomes of scleractinian corals (Cnidaria; Anthozoa). Zoological Studies 50: 513–524.

Lin MF, Kitahara MV, Tachikawa H, Fukami H, Miller DJ, Chen CA (2012) Novel organization of the mitochondrial genome in the deep-sea coral, Madrepora oculata (Hexacorallia, Scleractinia, Oculinidae) and its taxonomic implications. Molecular Phylogenetics and Evolution 65(1): 323–328. https://doi.org/10.1016/j.ympev.2012.06.011

Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution 41(3): 353–358. https://doi.org/10.1007/BF01215182

Romano SL, Palumbi SR (1996) Evolution of scleractinian corals inferred from molecular systematics. Science 271(5249): 640–642. https://doi.org/10.1126/science.271.5249.640

Rowlett J (2020) Indo-Pacific Corals. Rowlett (self-published), 809 pp.

Stolarski J, Kitahara MV, Miller DJ, Cairns SD, Mazur M, Meibom A (2011) The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. BMC Evolutionary Biology 11(1): e316. https://doi.org/10.1186/1471-2148-11-316

Stothard P, Wishart DS (2005) Circular genome visualization and exploration using CGView. Bioinformatics 21(4): 537–539. https://doi.org/10.1093/bioinformatics/bti054

Tian P, Xiao J, Jia Z, Guo F, Wang X, Wang W, Wang J, Huang D, Niu W (2021) Complete mitochondrial DNA sequence of the Psammocora profundacella (Scleractinia, Psammocoridae): Mitogenome characterisation and phylogenetic implications. Biodiversity Data Journal 9: e62395. https://doi.org/10.3897/BDJ.9.e62395

Veron JEN (2000) Corals of the World. Australian Institute of Marine Science, Townsville. http://www.coralsoftheworld.org/species_factsheets/ [Accessed on: 2022-7-11]

Waheed Z, Benzoni F, van der Meij SET, Terraneo TI, Hoeksema BW (2015) Scleractinian corals (Fungiidae, Agaricidae and Euphylliidae) of Pulau Layang-Layang, Spratly Islands, with a note on Pavona maldivensis (Gardiner, 1905). ZooKeys 517: 1–37. https://doi.org/10.3897/zookeys.517.9308