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Saudi Journal of Biological Sciences

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Original article

The complete mitochondrial genome of *Plesiastrea versipora* (Scleractinia, Plesiastreidae) sheds light on its phylogeny and taxonomy of the family PlesiastreidaeWentao Niu^{1,*}, Jianguang Xiao¹, Peng Tian, Feng Guo

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ARTICLE INFO

Article history:

Received 5 March 2020

Revised 22 April 2020

Accepted 23 April 2020

Available online 30 April 2020

Keywords:

Complete mitogenome

Plesiastrea versipora

Plesiastreidae

Phylogenetics

ABSTRACT

The genus *Plesiastrea* used to be a member of the traditional family Faviidae, falling into the challenging 'Bigmessidae' clade, and was re-established until recent molecular phylogenies published. The entire mitogenome of the symbiotic coral *Plesiastrea versipora* (Lamarck, 1816), the type species of the family Plesiastreidae, was sequenced. The length of the mitochondrial genome is 15,320 bp and it includes thirteen protein-coding genes (PCGs), two rRNAs and two tRNAs. The nucleotide composition of GC is 32%. We perform phylogenetic reconstruction based on maximum likelihood (ML) and Bayesian analysis (BI) using all PCGs. Our result indicates that *P. versipora* clusters closely with species which belong to Mussidae, Merulinidae and Lobophylliidae. Our phylogenetic analyses provide solid evidence for phylogenetic placement of *P. versipora* and the evolutionary relationships among different families within the traditional robust clade of Scleractinia. In addition, the mitogenome data provide useful information for further molecular systematic investigations on Plesiastreidae as well as conservation biology research of *P. versipora*.

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1. Introduction

By integrating genetic data and new morphological features, modern coral taxonomy has been greatly developed (Kitahara et al., 2016; Richards et al., 2019; Oku et al., 2019). Conventional morphology-based phylogenetic classification does not show all the phylogenetic relationships in Scleractinia groups, which accordingly has resulted in ambiguity about the taxonomic position of these groups. Molecular phylogenetic studies have therefore become increasingly important to provide solid evidence to organize coral taxa among scleractinian (Benzoni et al., 2011; Huang et al., 2014; Kang et al., 2020). The advantages of mtDNA over nuclear DNA (nDNA) in phylogenetics are the asserted orthology of all genes and the small genome structure being relatively

conserved (Kayal et al., 2013). Due to their intrinsic properties, the mitochondrial genome played an important role in scleractinian corals phylogeny (In-Young et al., 2020; Niu et al. 2020).

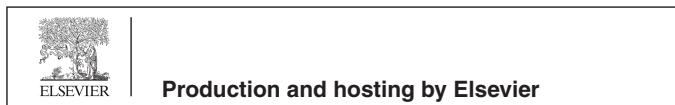
Milne Edwards and Haime (1848) originally depicted the genus *Plesiastrea* on the basis of a specimen from the Indian Ocean which was named *Astrea versipora*. Traditionally, the genus *Plesiastrea* was considered belonging to the family Faviidae and morphologically closely related to the genus *Montastraea* (Benzoni et al., 2011). However, genetic data have shown that there is no close phylogenetic relationship between them and *Montastraea* is polyphyletic (Huang et al., 2014). *Plesiastrea versipora* (Lamarck, 1816), one of the two extant species of this genus, is evolutionarily contrasting to other robust group in Scleractinia (Fukami et al., 2008). Based on the molecular phylogenetic studies, a new family Plesiastreidae (Dai and Horng, 2009) including the genera of *Plesiastrea*, *Physogyra*, *Plerogyra*, and *Blastomussa* was originally established (Dai and Horng, 2009). Benzoni et al. (2014) moved the three genera (*Physogyra*, *Plerogyra* and *Blastomussa*) to *Incertae sedis* based on the morphology and molecular data. There is only one genus *Plesiastrea* in the Plesiastreidae now.

The phylogenic history of the genus *Plesiastrea* was well summarized in Benzoni et al. (2011), together with a series of analyses on the species *P. versipora* based on morphological and genetic

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characteristics. Here, the whole mitogenome sequence of *P. versipora* has been sequenced and assembled for the first time using next-generation sequence (NGS). The phylogenetic status and taxonomy of the family Plesiastreidae was analyzed based on the protein-coding genes (PCGs) of the mitogenomes.

2. Materials and methods

2.1. Sampling collection and genomic DNA extraction

The specimen of *P. versipora* (Fig. S1) was collected by SCUBA diving from Daya Bay in Guangdong Province, China. Specimen was determined according to identification guides (Chan et al., 2005; Dai and Horng, 2009). DNA extraction was carried out according to the manual of the Qiagen's DNeasy Tissue Kit.

2.2. Genome sequencing and analyses

The next generation sequencing was in accordance with the protocol (Niu et al. 2020). Purity and concentration of PCR products were tested with NanoDrop 2000 and Qubit 2.0 fluorometer. A total of 2 µg DNA after the quality control steps were fragmented to ~ 550 bp by M220 system. DNA fragment's size proportion was quantified with the Agilent Bioanalyzer 2100. According to instruction manual of manufacturer's, library for MiSeq was prepared by TruSeq DNA PCR-free Sample Preparation Kit. Concentration of the library was confirmed by real-time qPCR. A de novo assembly for raw reads was performed by using Geneious V9, to yield a single stranded, circular configuration of entire mitochondrial genome.

2.3. Mitochondrial genome annotation and analyses

The assembled mitogenome was preliminarily annotated with MITOS (Bernt et al., 2013) web servers and DOGMA (Wyman et al., 2004). Genes annotation of protein-coding and rRNA refer to homologous genes of other available scleractinian mitogenomes. Annotation of the tRNA genes were conducted with tRNA scan-SE. MEGA 7.0 (Kumar et al., 2016; Gao et al., 2017a, 2017b; Liang et al., 2018; Wang et al., 2017) was used for statistical analysis of nucleotide frequencies and codon usage. AT-skew and GC-skew analyses were used to indicate the nucleotide composition.

2.4. Phylogenetic analyses

To verify phylogenetic relationships, BI (Bayesian inference) and ML (maximum likelihood) were performed to reconstruct a tree based on the amino acid alignment of 13 PCGs using MEGA7.0. The entire mitogenome sequences of *P. versipora* and additional 15 representative robust clade species of scleractinian (Table S1) were used together for phylogenetic analysis. Three species of complex clade of Scleractinia including *Acropora aspera*, *Agaricia humilis* and *Galaxea fascicularis* were used as the outgroups (Table S1). 1000 bootstrap replicates were performed to estimate the node reliability for ML analysis. 4 chains (1 cold and 3 heated chains) were set to run at the same time for 10 million generations for the BI procedure. Each set was sampled every 100 generations after the initial 25% run were abandoned, then the consensus tree was obtained from the remaining samples.

3. Results and discussion

3.1. General characteristics of the mitochondrial genome

The length of the mitochondrial genome sequence obtained from *P. versipora* is 15,320 bp (GenBank accession number: MH025639) including unique 13 PCGs, 2 rRNA and 2 tRNA genes (tRNA^{Met}, tRNA^{Trp}) (Table 1, Fig. 1). Actually, *P. versipora* had the shortest mitogenome excepting *Astrangia poculata* (14,853 bp, NC008161) in all sequenced Scleractinia species. The variation in intergenic nucleotides led to the length differences. The base composition is A 25%, C 12.1%, G 19.9% and T 43%, The A + T content (68%) is distinctly higher than the GC content (32%) (Table 2). The lowest level of base in the mitochondrial genome is C (Fig. S2). The mitogenome of *P. versipora* doesn't provide special construction; its gene feature, number and arrangement are same as most of other available hard coral mitochondrial genomes (Niu et al., 2020). According to the results of GC- / AT-skews analysis (Fig. S3), the absolute value of GC-skew is smaller than AT-skew.

3.2. Protein-coding genes and codon usages

The overall length of all PCGs was 11,556 bp, with base composition of A 21.9%, C 12.2%, G 20% and T 45.9%. The intron insertion of ND5 was 9,396 bp. Most of the PCGs started with ATG. In addition to COI and ND2 starting with ATT, and Cyt *b* starting with TTA.

Table 1
Organization of the mitochondrial genome of *Plesiastrea versipora*.

Gene	Position		Length (bp)	Anticodon	Codon		Intergenic nucleotides	Strand
	From	To			Start	Stop		
tRNA ^{Met}	1	72	72	UAC			232	H
16S rRNA	256	1957	1702				183	H
ND5 5'	2041	2751	711		ATG		83	H
ND1	2860	3807	948		ATG	TAG	108	H
Cyt <i>b</i>	3816	4949	1134		TTA	TAA	8	H
ND2	5158	6261	1104		ATT	TAA	208	H
ND6	6263	6823	561		ATG	TAA	1	H
ATP6	6823	7500	678		ATG	TAA	-1	H
ND4	7500	8939	1440		ATG	TAG	-1	H
12S rRNA	9076	9985	910				136	H
COIII	9979	10,758	780		ATG	TAA	-7	H
COII	10,761	11,468	708		ATG	TAG	2	H
ND4L	11,450	11,749	300		ATG	TAA	-19	H
ND3	11,752	12,093	342		ATG	TAA	2	H
ND5 3'	12,150	13,253	1104			TAG	56	H
tRNA ^{Trp}	13,252	13,321	70	ACU			-2	H
ATP8	13,325	13,522	198		ATG	TAA	3	H
COI	13,540	15,087	1548		ATT	TAA	17	H

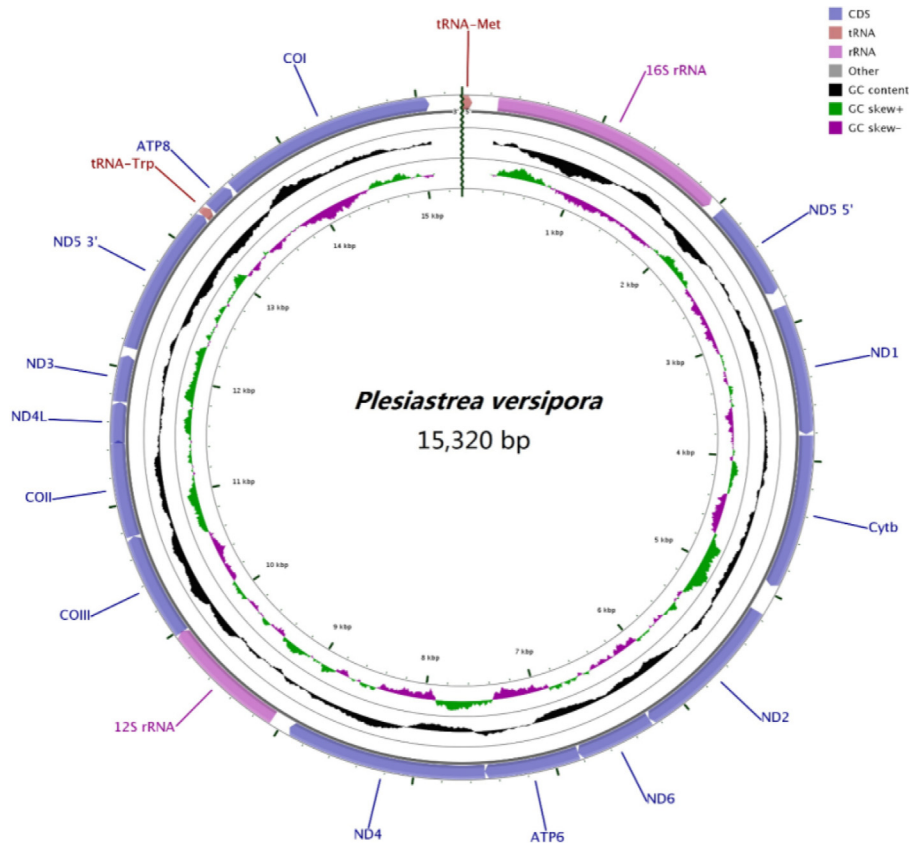


Fig. 1. The mitochondrial genome of *Plesiastrea versipora*. Gene order and positions were shown, all the genes were encoded on H-strand. COI, COII, COIII referred to the cytochrome oxidase subunits, Cyt *b* referred to cytochrome *b*, ND1-ND6 referred to NADH dehydrogenase components.

Table 2
Nucleotide composition in different regions of the mitochondrial genome of *Plesiastrea versipora*.

Gene/Region	T(%)	C(%)	A(%)	G(%)	A + T(%)	Size(bp)
ND5	47.2	11.2	22.5	19.2	69.6	1815
ND1	45.2	12.8	21.1	21.0	66.3	948
Cyt <i>b</i>	47.1	12.2	21.6	19.1	68.7	1134
ND2	48.9	11.4	20.9	18.8	69.8	1104
ND6	48.8	11.2	22.1	17.8	70.9	561
ATP6	47.5	12.7	21.4	18.4	68.9	678
ND4	47.1	12.2	20.2	20.5	67.3	1440
COIII	42.8	14.5	20.8	21.9	63.6	780
COII	39.7	12.6	24.7	23.0	64.4	708
ND4L	45.0	11.0	25.0	19.0	70.0	300
ND3	49.7	9.9	18.4	21.9	68.1	342
ATP8	45.5	9.1	34.9	10.6	80.3	198
COI	42.6	13.7	22.4	21.3	65.0	1548
PCGs	45.9	12.2	21.9	20.0	67.8	11,556
1st	36.1	13.2	22.5	28.3	58.6	3852
2st	47.9	18.4	18.1	15.6	66.0	3852
3st	53.7	5.0	25.2	16.0	78.9	3852
tRNA	24.7	20.4	32.4	22.5	57.0	142
rRNA	32.7	11.7	36.0	19.6	68.7	2612
Overall	43.0	12.1	25.0	19.9	68.0	15,320

Four of the 13 PCGs have the conventional stop codons TAG, 9 PCGs have the stop codons TAA. Among thirteen PCGs, the longest and the shortest were ND5 gene (1,815 bp) and ATP8 gene (198 bp), respectively. Among 3852 codons for twenty amino acids, use frequency of codons for L, V, F, G and S accounting for 53.12% was higher. Nonpolar amino acid (A, G, I, L, M, F, V, Y, W) was the maximum, accounting for 67.86%, followed by polar amino acid (C, N, P, Q, S, T) and polarity charged amino acids (D, E, H, K, R), accounting for 20.56% and 11.24% (Fig. S4).

3.3. Ribosomal and transfer RNA genes

The genes which encode 16S rRNA and 12 rRNA were 1,702 bp and 910 bp in size, respectively. The length of total rRNA was 2,612 bp, and its base composition was A 36%, T 32.7%, G 19.6% and C 11.7%. The two transfer RNA length were 70 bp tRNA^{Trp} and 72 bp for tRNA^{Met} respectively. They included amino acid accept arm, anticodon arm, T Ψ C arm and DHU arm (Fig. S5), which is the classic cloverleaf structure.

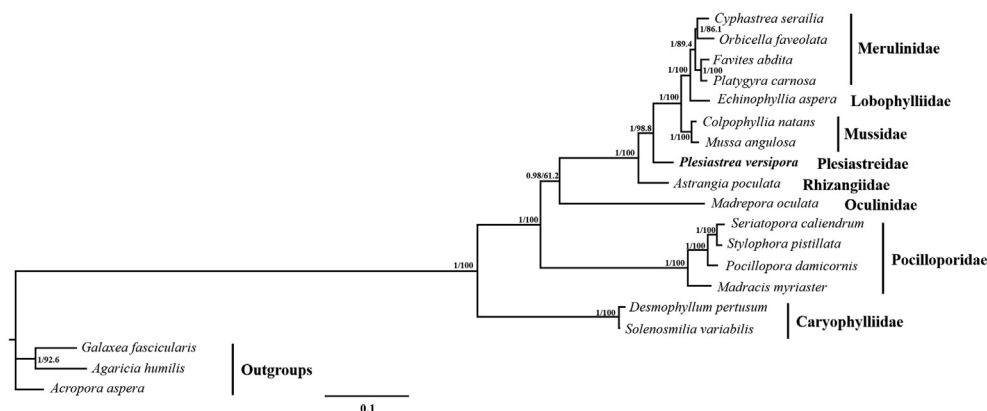


Fig. 2. Inferred phylogenetic relationships based on the concatenated nucleotide sequences of 13 mitochondrial protein-coding genes using Bayesian inference (BI) and maximum likelihood (ML). Numbers on branches are Bayesian posterior probabilities and bootstrap percentages.

3.4. Phylogenetic analyses

Both of BI and ML tree, which included the newly sequenced mitogenomes of *P. versipora* and other 15 robust species and three outgroup taxa retrieved from GenBank, were done based on nucleotide data. The results of the two analyses gave similar tree topologies (Fig. 2). The resultant topology supported that *P. versipora* clusters closely with species which belong to Mussidae, Merulinidae and Lobophylliidae. Then Oculinidae, Pocilloporidae and Caryophylliidae clustered into the main clade successively. We can easily get the relationship information among different families through phylogenetic tree. Indeed, the combination of molecular markers and classical morphological characteristics can help enhance modern taxonomic system within scleractinian corals (Kitahara et al., 2016).

4. Conclusions

We have sequenced the whole mitogenome of *P. versipora* for the first time, one of the two extant species of the family Plesiastreidae (Hoeksema and Cairns, 2020). The mitogenome of *P. versipora* is 15,320 bp in size. The composition in gene order, low GC content and size is similar to other mitochondrial genomes which have been sequenced in Scleractinia. The phylogenetic analysis based on the entire mitogenomes of *P. versipora* and related species shed light on its phylogeny and taxonomy of the family Plesiastreidae. Lastly, the whole mitochondrial genome of the *P. versipora* obtained in the study provides important and essential molecular data for scleractinian corals phylogenetic and evolutionary analysis in the further.

Acknowledgements

This work was supported by the Scientific Research Foundation of Third Institute of Oceanography, Ministry of Natural Resources (2019003), the People's Republic of China.

Author Contributions

The authors are responsible for the content and writing of the paper.

Declaration of Competing Interest

The authors declare that they have no competing of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2020.04.041>.

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