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TECHNICAL NOTE

Variation in the whole mitogenome of reef-building *Porites* corals

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Abstract The study of complete mitochondrial genomes (mitogenomes) revealed different gene rearrangements, highly variable markers, and delineated clades that have aided the understanding of the evolutionary history in corals. In this study, we examined mitogenomic variation of reef-building *Porites* corals and designed 34 primer pairs to target high diversity regions and to amplify the complete mitogenome of a widely-distributed Indo-Pacific species of *Porites* (*P. lobata*) and two endemic species of the Eastern Pacific (*P. sverdrupi* and *P. panamensis*). All primer pairs amplified for each species and the mitogenomes assembled yielded the same gene order as obtained from next-generation sequencing. Mitogenomic variation in *Porites* corals was three to ten times higher than in *Acropora* or *Pocillopora*, two other major reef builders. In contrast to these corals, the nucleotide variation in *Porites* species was distributed evenly along the mitogenome. Primers designed here are useful to amplify regions with the highest variation possible as well as to assemble the whole mitogenomes of different *Porites* species. The resulting mitogenomes should improve our understanding of evolutionary relationships, delimitation of species, and conservation within the genus *Porites*.

Keywords Mitochondrial genome · *Porites* corals · Eastern Pacific · Scleractinian corals

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The animal mitochondrial genome (mitogenome) generally exhibits several characteristics that make it suitable for phylogenetic analysis: high substitution rate, maternal inheritance, and lack of recombination. In contrast, most of the mitochondrial genes studied in Class Anthozoa (i.e. anemones, corals and sea pens) show slow rates of nucleotide substitution, sometimes yielding few or no differences between closely related species (Shearer et al. 2002; Hellberg 2006; Prada et al. 2014). However, the recent study of the complete mitochondrial genome in this group have revealed different mitochondrial gene rearrangements (Lin et al. 2014; Figueroa and Baco 2015), the discovery of some variable mitochondrial markers (Flot and Tillier 2007; Luck et al. 2013), and congruence between mitochondrial and morphological groups of species (Luck et al. 2013; Schmidt-Roach et al. 2014) have helped in the understanding of their evolutionary history (Medina et al. 2006; Lin et al. 2011, 2014). Levels of mitogenomic variation appear to differ among coral genera due to gene rearrangements, high diversity regions, and the presence of indels (Flot and Tillier 2007; Luck et al. 2013, 2015)

Porites is one of the most taxonomically challenging and ecologically important genera of reef-building corals (Veron 2000; Forsman et al. 2009). Although these corals can grow under many environmental conditions, brooding

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Table 1 Primer information to amplify regions with the highest variation and to ensemble the complete mitogenome in *Porites* corals

	Primer order	Primer name	Mitogenome region	Position genome (NC_008166)	Primer sequences (5′–3′)	Product size (bp)
•01	1-1	01_S	ND1	847–1554	F: CAGGTAACAAAATTGGAGGT	707
					R: AGAGCCAACACACAATAGAA	
02	1-2	02_O	ND1-CYTB	1377–2304	F: AGGGGTTTTGTATATTTTGG	927
					R: AAGCACCCTGTTATGATCTG	
03	2-2	03_F	ND1-CYTB	1997–2953	F: CTAGTGTTCTTTTGGGGTTT	956
					R: CACTGGAGTTACCAACGAG	
•04	3-3	04_I	CYTB	2657–3586	F: TTTAGTGTCTCTGGGGCTAC	929
					R: CCATTATACCCAACGTCAAA	
05	4-4	05_A	CYTB-ND2	3202–4102	F: AATTGGGCAGTTAGTCTCTC	900
					R: CCGTAAATCCACACAATAAA	
06	4-5	06_A	ND2	3803–4787	F: AAAAGCTAATTTTCCCAAAG	984
					R: TAATAGACACCAGCCACAAC	
07	5-5	07_N	ND2-ND6	4304–5451	F: TTGGTTATTTTTCTATTTTGGTT	1147
					R: CGCCTCCATATTTTGATACT	
08	5-6	08_A	ND2-ND6	4918–5900	F: GGATTTACAATGCTCTCTCC	982
					R: AATACTTTAACCCCTCAGACC	
•09	6-6	09_I	ND6-ATP6	5597–6597	F: TTCAAACAAGTCGGTAAAAA	1000
					R: ATTAAGAGCCCATTTAGCAG	
10	6-7	10_D	ATP6-ND4	6070–7359	F: TAGTGTTTTTATGCCGAGTG	1289
					R: GAAGCCAAATATGAAATGGT	
11	7-7	11_P	ND4	7187–8010	F: ATCGGTGTTTATGCTTTTG	823
					R: CCCGCCTATTTAAGTCTCT	
•12	7-8	12_A	ND4-12S rRNA	7318–8356	F: TAGCGGTTAAAATTCCTCAA	1038
					R: AGTGAAAATGTGGCTCCTAA	
13	8-8	13_Z	ND4-12S rRNA	8061–8966	F: TTTCCTTTTGTACTCATAGACC	905
					R: ACCTGACTTCATCCAATAGAC	
14	8-9	14_S	12S rRNA-COX3	8412–9521	F: TTTGTGCAATATACGAAAGTAA	1109
					R: CAAAAGAGGTCAAGGAGAAG	
15	9-9	15_A	12S rRNA-COX3	9066–9900	F: GGAATATAACGGAAAGTTGG	834
					R: AAGTAACAGTACCCCCAGAA	
•16	9-10	16_N	COX3-COX2	9707–10859	F: TGGAATGCTTTTATTTATACTCTC	1152
					R: CATCCATTTTTACACCCAAG	
17	10-10	17_C	COX2-ND4L	10429–11164	F: TGTTATTGTAGTAGTAGTGTTGTGG	735
					R: TAAAATGCCCTCTATTAAGAAC	
18	10-11	18_H	COX2-NAD4L-ND3-ND5	10908–11958	F: GGACGTTTTATGGTCAATGT	1050
					R: TAAAGCAAATGGAGCTTGTT	
19	11-11	19_E	ND3-ND5	11415–12217	F: GGTCTTTTCGTTTTATTGGT	802
					R: CCCCCATCTTTCTTATATCTT	
•20	12-12	20_Z	ND5-tRNA-Trp-ATP8-COX1	12160–13510	F: AGTGCGGGGTCTGTTATT	1350
					R: ACGTACCAATGTCTTTATGGT	
21	13-13	21_M	ND5-tRNA-Trp	12035–13044	F: AAGTTATTGCTTATTCGACTTG	1009
					R: TTGAAGGCTAACGGTCTACT	
22	13-14	22_I	ND5-tRNA-Trp-ATP8-COX1	12637–13552	F: GCGGTGTTAGTTATTGTTCTT	915
					R: CTGTACCGAGCATACCTG	
23	14-14	23_H	ND5-tRNA-Trp-ATP8-COX1	12955–13612	F: TGGGGAGTTGTTTAGTTTTT	657
					R: GATCGTCTCCTAACATAGCC	
•24	14-15	24_E	COX1	13476–14389	F: GGCGTTTTCTACTAACCATAA	913
					R: AGCAGGACAAAGCTCAAA	

Table 1 continued

	Primer order	Primer name	Mitogenome region	Position genome (NC_008166)	Primer sequences (5′–3′)	Product size (bp)
25	15-15	25_R	COX1	13837–14689	F: ACGGTTTATCCTCCTCTATCT	852
					R: AAAGCCTCTGACACCATAA	
•26	15-16	26_M	COX1	14484–15500	F: ATCAGTGGGAAACTAAGACC	1016
					R: TACAACTCCAGTCAAACCAC	
27	16-16	27_O	COX1	14701–15614	F: TTCAATCTGGCGTAGTGTAG	913
					R: AAAATAAAACCCACCAAAAA	
28	16-17	28_S	COX1-tRNA-Met-16S rRNA	15192–16269	F: AGTGATGAAAAGTAAATGAAAAA	1077
					R: GTCTCCGCATTGAAACAC	
29	17-17	29_A	Cox1-tRNA-met-16S rRNA	15792–16751	F: TCTTTGGGCTCTACTATTTCA	959
					R: GAAAACCAGCTATCTCCAAG	
•30	17-18	30_H	16S rRNA	16452–17345	F: TGAAGGAAAGTTGAAAGAGAC	893
					R: GCGTTTATTATTATCACCCATC	
31	18-18	31_I	16S rRNA	16901–17796	F: TTTAAGGGGGATAGACTTTG	895
					R: AAGCCACATAAGTTTCCAGT	
32	19-19	32_J	16S rRNA	17678–18268	F: ACGAGGGTCTCACTGTCTT	590
					R: TGTTACCACGCTTTTAACTC	
•33	20-20	33_A	16S rRNA-ND5	18214–173	F: TTGGTCTGTTCGTCCATT	609
					R: TGTGTCGTAGAAAAACTTAAAAC	
34	20-1	34_=)	ND5	66–991	F: ATTAGGAGAAAAAGGTGCTG	925
					R: ATAAAGTAGAATCACAAAAAGTCTC	

Annealing temperature was 54 °C for all primers. Primer numbers of first column correspond to primers from the Fig. 1c

Porites species are vulnerable to local impacts due to genetic isolation and limited capacity of dispersal (Paz-García et al. 2012; Saavedra-Sotelo et al. 2013; Paz-García and Balart 2015). Genetic delineation of species in this group has been performed using a few nuclear markers and mitochondrial regions (e.g. control region and COI). Some such studies have sometimes found relationships congruent to morphology (Forsman et al. 2009), while others have revealed cryptic species (Boulay et al. 2014) or did not find genetic differences between named morphological species (Prada et al. 2014). Although next-generation sequencing has allowed the rapid generation of genomic resources for non-model organisms, genomic contamination may be an issue in corals due to their association with a wide variety of taxa (i.e. virus, bacterium, dinoflagellate). Thus, additional bioinformatics steps and genomic resources are necessary to screen an assembly for sequences that come only from that particular taxon (Willette et al. 2014).

The design of primers and direct sequencing of coral mitogenome could be a fast, low cost, and low risk approach if the low mitochondrial nucleotide variation in corals could be avoided. In this study, we aimed to examine the whole mitogenome variation in *Porites* corals, to design primers that amplify high diversity regions, and to allow future phylogenetic and phylogeographic studies to improve the evolutionary understanding of species in the eastern Pacific.

In a previous study, seventy-four primers pairs were developed to amplify the complete mitogenome in scleractinian corals (Lin et al. 2011). Here, ten primers pairs were designed to access regions with the highest variation in *Porites* corals and an additional twenty-four designed to assemble the whole mitogenome (see below). In total, thirty-four overlapping primers were designed using Primer3Web (http://primer3.ut.ee) (Untergasser et al. 2012) and the recently published complete mitochondrial genomes of *P. panamensis* (NC_024182) and *P. porites* (NC_008166) available on the NCBI database GenBank (Medina et al. 2006; Del Río-Portilla et al. 2014). Phylogenetic breadth of the utility of these primers was tested on three *Porites* species collected in the Eastern Pacific: *P. sverdrupi* (Bahía Concepción, central Gulf of California 26°38′27.16″N, 111°49′45.89″W), *P. panamensis* (Isla Despensa, northern Costa Rica 11° 0′10.10″N, 85°44′49.50″W), and *P. lobata* (Palmitas, Gulf of Papagayo, Costa Rica 10°38′41.60″N, 85°41′15.60″W). One small fragment (~3 cm^2) from the center of each colony was collected in July 2011 in Mexico (sampling permit DGOPA.05356.140710.3457) and in May 2013 in Costa Rica (Resolución no. 064-2013-SINAC) and preserved in salt-saturated DMSO buffer (Seutin et al. 1991).

Genomic DNA was isolated from each species according to protocols of the DNeasy Tissue Kit (QIAGEN, Valencia, CA, USA). Genomic DNA was amplified using each primer pair listed in Table 1. Amplifications were performed in 15 µl reaction volume containing 50 ng of template DNA, 0.4 mM of each primer, 2 mM $MgCl_2$, 0.3 mM dNTPs, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, and 50 mM KCl), and 1.5 units Taq DNA polymerase (Invitrogen Life Technologies). PCR consisted in an initial step of denaturation at 94 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 54 °C for 30 s, 72 °C for 75 s, and a further extension step of 72 °C for 10 min.

All three *Porites* species amplified successfully using all 34 pairs of primers. PCR products ranged in size from 590 to 1350 bp (Table 1). PCR products of *P. panamensis* were purified and Sanger sequenced (Genewiz, South Plainfield, NJ, USA). Sequences were verified and aligned in CodonCode Aligner (CodonCode Corp., Dedham, MA, USA) and compared to other coral species mitogenomes from NCBI GenBank (*P. panamensis* NC_024182, *P. porites* NC_008166, *P. okinawanensis* NC_015644, *P. rus* NC_027526). Mitogenomic variation among *Porites* species was examined by estimating the nucleotide diversity and number of polymorphic sites for 300 bp windows along the mitogenome using DnaSP v.5.10 (Librado and Rozas 2009).

The *P. panamensis* mitogenome assembled from our 34 primer pairs and Sanger sequencing (Genbank accession number KU761953) yielded the same gene order as that obtained from next-generation sequencing (Illumina MiSeq, Del Río-Portilla et al. 2014) and similar to those of other scleractinian species (Medina et al., 2006; Lin et al., 2011). In total, 335 polymorphic sites and 221 parsimony informative sites were found along the seven *Porites* mitogenomes. This variation was three times higher than the variation found in the mitogenoma of *Acropora* species (Liu et al. 2015) and ten times higher than *Pocillopora* species mitogenomes (Flot and Tillier 2007). In contrast to coral mitogenomes from the family Acroporidae, Agariciidae, and Pocilloporidae (Flot and Tillier 2007; Luck et al. 2013; Liu et al. 2015), nucleotide diversity and polymorphic sites in *Porites* species were distributed evenly along the mitogenome (Fig. 1a).

The partial mitochondrial regions used to delineate *Porites* species in previous studies (Control Region and COI, Forsman et al. 2009, Prada et al. 2014) represented only the 7 % of the variation across the whole mitogenomes of *Porites* corals. This low level of variation may be why these studies failed to distinguish some *Porites* species from each other. The mitochondrial regions with higher variation were 16S rRNA, ND5, ND4, COX1, ND1, and CYTB. These correspond to 43.3 % of the total variation in *Porites* mitogenome (Fig. 1b).

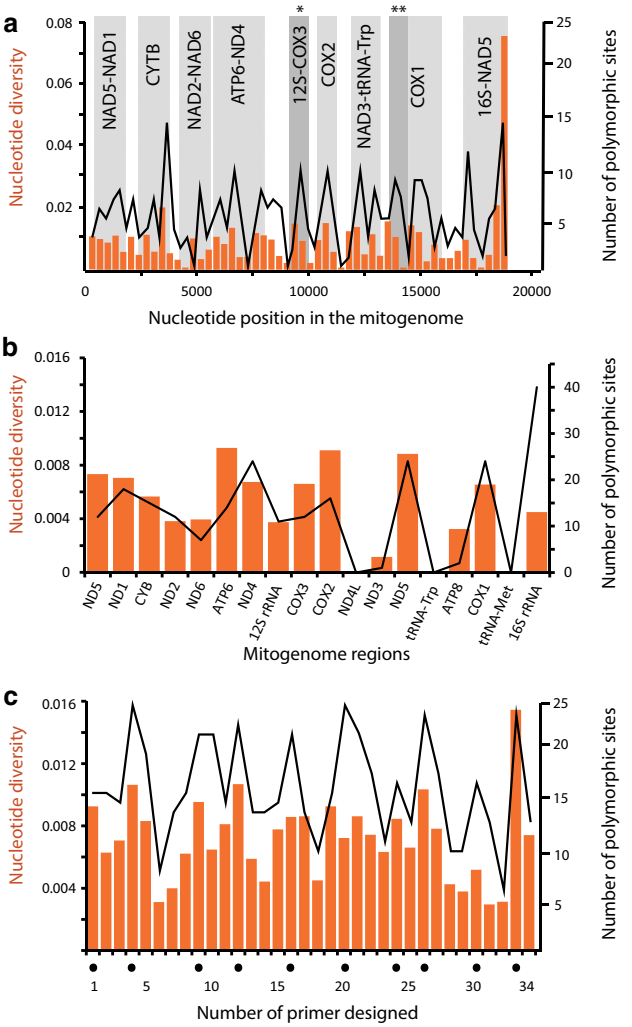

Fig. 1 Nucleotide diversity (*bars*) and polymorphic sites (*lines*) in the mitogenome of reef-building *Porites* corals. **a** Distribution of the variation in the whole mitogenome (each 300 bp). **b** Genes variation in the mitogenome (69.25 % of the total variation in the mitogenome). **c** Mitogenome variation accessed by primers designed in this study. Asterisks in **a** indicate partial mitochondrial regions used by Forsman et al. 2009 (*putative control region, **COI). Points in **c** indicate the ten primers that can be used to access the 64.18 % of the variation in the *Porites* mitogenome

The primers reported here were designed to amplify regions with the highest variation as well as to aid assembly of whole mitogenomes by providing access missing regions commonly uncovered by next-generation sequencing (i.e. Liu et al. 2015). For example, just ten primers pairs (Fig. 1c; Table 1) will reveal 64.2 % of the variation in the *Porites* mitogenome. The use of these markers will hopefully improve our understanding of evolutionary relationships, the delimitation of species, and conservation within the genus *Porites*.

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