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Complete mitochondrial genome of *Muricea crassa* and *Muricea purpurea* (Anthozoa: Octocorallia) from the eastern tropical Pacific

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Abstract

We sequenced the complete mitogenomes of two eastern tropical Pacific gorgonians, *Muricea crassa* and *Muricea purpurea*, using NGS technologies. The assembled mitogenomes of *M. crassa* and *M. purpurea* were 19,586 bp and 19,358 bp in length, with a GC-content ranging from 36.0% to 36.1%, respectively. The two mitogenomes had the same gene arrangement consisting of 14 protein-coding genes, two rRNAs and one tRNA. Mitogenome identity was 98.5%. The intergenic regions between *COB* and *NAD6* and between *NAD5* and *NAD4* were polymorphic in length with a high level of nucleotide diversity. Based on a concatenated dataset of 14 mitochondrial protein-coding genes we inferred the phylogeny of 26 octocoral species.

Key words: mitogenome, Muricea crassa, Muricea purpurea, NGS

Muricea crassa (Verrill, 1869) and *Muricea purpurea* (Verrill, 1864) are two shallow water gorgonians of the family Plexauridae. Their distribution is limited to the eastern tropical Pacific where they are abundant members of coral communities and littoral zones (Guzman et al., 2004). Samples were collected as part of an ecological and biodiversity survey undertaken in the Coiba National Park (Panama). Genomic DNA was extracted from ethanol-preserved samples and was used to construct genomic libraries using the Accel-NGS 1S DNA Library kit (Swift Biosciences, Ann Arbor, MI, USA) following the manufacturers instructions. These libraries were sequenced (100bp PE) on an Illumina HiSeq

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(Illumina Inc., San Diego, CA). The quality of the reads obtained was assessed with FastQC (Andrews, 2010), low quality reads and Illumina adaptors were trimmed using Trimmomatic 0.3.2 called from Trinity RNA-Seq 2.0.6 (Grabherr et al., 2011). Despite its original purpose, the Trinity RNA-Seq assembler was used after normalization to 50X coverage for *de-novo* mitogenome assembly. The assembly resulted in a single mitochondrial contig in both species. Initial annotation was performed with the ORF finder function implemented in Geneious 8.1.7 (Kearse et al., 2012) and was corroborated by comparison with published octocoral mitogenomes. The presence of DNA repeats was assessed with the tandem repeats finder server 4.08 available at https://tandem.bu.edu/trf/trf.html (Benson, 1999). The complete mitogenomes of M. crassa (LT174652) and M. purpurea (LT174653) were 19,586 bp and 19,358 bp long, with a GC-content of 36.0% (M. purpurea) and 36.1% (M. crassa), respectively. Both mitogenomes had gene arrangement of type "A" (see Brockman and McFadden, 2012). In total, the Coding DNA Sequences (CDSs) spanned about 76% of the mitogenome in both species. Among protein-coding genes, the highest level of nucleotide diversity (0.4%) was found in NAD1, NAD6 and COX2, whereas no nucleotide substitutions were found in NAD3, ATP6 and ATP8. Except for NAD2 and NAD5 (13bp overlap), the other protein-coding genes were separated by intergenic regions (IGRs) of different lengths. In both species, the shortest IGRs were those located between 12S rRNA and NAD1 and between 16S rRNA and NAD2, while the longest was found between NAD5 and NAD4. The latter IGR was also the most diverse region with a nucleotide diversity of 6.2%. Length polymorphism was found in the COB-NAD6 IGR, which was 184bp shorter in M. purpurea than in M. crassa. Sequencing of indel-rich IGRs such as that between COB and NAD6 may result useful for molecular species-identification in the genus Muricea. Finally, we found a 37 bp tandem repeat in the IGR between NAD4 and tRNA of M. crassa.

The two newly sequenced complete mitogenomes were used to assess the phylogenetic relationships among 26 different octocoral species. A concatenated nucleotide alignment of 14 protein-coding genes (15,249 bp in total) for 41 taxa was generated with MUSCLE (Edgar, 2004) using the default options provided in Seaview (Gouy et al., 2010). The maximum likelihood tree was inferred in RAxML 7.2.8 (Stamatakis, 2006) under the GTR+GAMMA substitution model. Node support was estimated using 1000 bootstrap pseudoreplicates. The phylogenetic tree (Figure 1) was re-rooted using three calcaxonians and two pennatulaceans as outgroup (not shown in Figure 1). Tree topology is consistent with recently published studies (Figueroa and Baco, 2015). The phylogenetic placement of *Muricea* spp. sister to Nephtheidae (*Dendronephthya* spp. and *Scleronephthya* spp.) is likely an artifact caused by poor taxon sampling in the family Plexauridae.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The project was partially supported by the LMU München German Excellence Initiative Junior Research Funds to SV, the Smithsonian Tropical Research Institute and the Secretaría Nacional de Ciencias y Tecnología (SENACYT) de Panamá to HMG. Collection and exporting permits of eastern Pacific material were issued by the Autoridad de Recursos Marinos de Panamá and by the Ministerio de Ambiente de Panamá.

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> Figure 1: Phylogenetic tree of 41 octocorals based on a concatenated alignment of 14 mitochondrial protein-coding genes. The calcaxonians (Keratoisidinae sp., Acanella eburnea, Narella hawaiinensis and Junceella fragilis) and pennatulaceans (Renilla muelleri and Stylatula elongata) Dendronephthya castane IQ694727 Echinogorgia complexa JX023274 Sinularia peculiaris — HQ694728 Euplexaura crassa — DQ640646 Pseudopterogorgia bipinnata ndronephth ea purpurea 100C LT174653 Muricea purpur LT174652 Muricea crassa IQ290079 GI 10478 90H 100 5 GU04 were used to re-root the tree but are not shown here. Numbers at the nodes indicate bootstrap values. laauer Corallium imperiale KC782355 Corallium imperiale sp. gorgia sp. Paragorgia Corallium rallium konojo. KC782353 Corallium kishinouvei allium elatiu: 8 Corallium elatii KC782354 Paragorgia sp KM015354 Sibogagorgia cauliflora secundum 93 KF785800 Paragorgia coralloides HF785801 Paragorgia coralloides aponicum JX508792 Paraminabea aldersladei Anthomastus sp. 15353 Anthomastus sp. 100 AB595190 Con AB700134 Cor 100 AB700135 Con Daradordia Corallium <C782 100 KC78 crallium AB700136 Corallium DQ640649 Briareum asbestinum KC782349 KC782347 - KM015352 / ----- KM01 ⁹⁸ <u>7</u>2 100 0.02 100 100 100 100 100 100 8 0

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