

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

Angelo Poliseno¹, Odalisca Breedy^{2,3,4}, Michael Eitel¹, Gert Wörheide^{1,5,6}, Hector M. Guzman⁴, Stefan Krebs⁷, Helmuth Blum⁷, and Sergio Vargas^{§1}

¹Department of Earth and Environmental Sciences, Palaeontology & Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner-Straße 10, 80333 München, Germany

²Centro de Investigación en Estructuras Microscópicas, Universidad de Costa Rica. P.O. Box 11501-2060, Universidad de Costa Rica, San José, Costa Rica

³Centro de Investigación en Ciencias del Mar y Limnología, Escuela de Biología, Universidad de Costa Rica. P.O. Box 11501-2060, Universidad de Costa Rica, San José, Costa Rica

⁴Smithsonian Tropical Research Institute, P.O. Box 0843-03092 Panama, Panama

⁵GeoBio-Center, Ludwig-Maximilians-Universität München, Richard-Wagner-Straße 10, 80333 München, Germany

⁶Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Straße 10, 80333 München, Germany

⁷Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-Universität München, Feodor-Lynen-Strasse 25, 81377 München, Germany

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

§Corresponding author: sergio.vargas@lmu.de



(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 penatulaceans as outgroup (not shown in Figure 1). Tree topology is consistent with recently published studies (Figuroa 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

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References

- Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>
- Benson G. (1999). Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res* 27:573-580.
- Brockman SA, McFadden CS. (2012). The Mitochondrial Genome of *Paraminabea aldersladei* (Cnidaria: Anthozoa: Octocorallia) supports intramolecular recombination as the primary mechanism of gene rearrangement in octocoral mitochondrial genomes. *Genome Biol Evol* 4:994-1006.
- Edgar RC. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792-1797.
- Figueroa FF, Baco AR. (2015). Octocoral mitochondrial genomes provide insights into the phylogenetic history of gene order rearrangements, order reversals, and cnidarian phylogenetics. *Genome Biol Evol* 7:391-409.

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Gouy M, Guindon S, Gascuel O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221-224.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, et al. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnol* 29:644-652.

Guzman HM, Guevara CA, Breedy O. (2004). Distribution, diversity, and conservation of coral reefs and coral communities in the largest marine protected area of Pacific Panama (Coiba Island). *Environ Conserv* 31:111-121.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.

Stamatakis A. (2006). RAxML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688-2690.

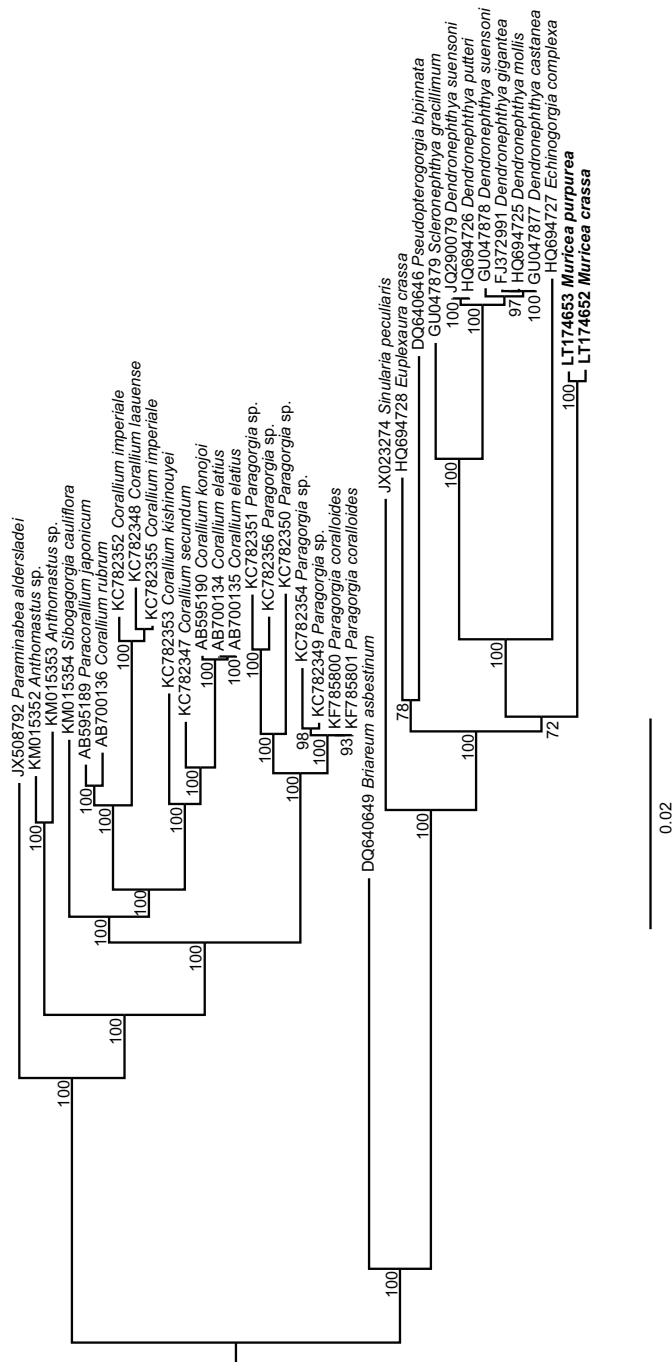


Figure 1: Phylogenetic tree of 41 octocorals based on a concatenated alignment of 14 mitochondrial protein-coding genes. The calcaxonians (*Keratoisidinae* sp., *Acanella eburnea*, *Narella hawaiiensis* and *Junceella fragilis*) and pennatulaceans (*Renilla muelleri* and *Stylatula elongata*) were used to re-root the tree but are not shown here. Numbers at the nodes indicate bootstrap values.