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Homoplasious colony morphology and mito-nuclear phylogenetic discordance among Eastern Pacific octocorals ☆

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ABSTRACT

Octocorals are a diverse and ecologically important group of cnidarians. However, the phylogenetic relationships of many octocoral groups are not well understood and are based mostly on mitochondrial sequence data. In addition, the discovery and description of new gorgonian species displaying unusual or intermediate morphologies and uncertain phylogenetic affinities further complicates the study of octocoral systematics and raises questions about the role played by processes such as plasticity, crypsis, and convergence in the evolution of this group of organisms. Here, we use nuclear (i.e. 28S rDNA) and mitochondrial (*mtMutS*) markers and a sample of Eastern Pacific gorgonians thought to be remarkable from a morphological point of view to shed light on the morphological diversification among these organisms. Our study reveals the loss of the anastomosed colony morphology in two unrelated lineages of the seafan genus *Pacifigorgia* and offers strong evidence for the independent evolution of a whip-like morphology in two lineages of Eastern Pacific *Leptogorgia*. Additionally, our data revealed one instance of mito-nuclear discordance in the genera *Leptogorgia* and *Eugorgia*, which may be the results of incomplete lineage sorting or ancient hybridization–introgression events. Our study stresses the importance of comprehensive taxonomic sampling and the use of independent sources of evidence to address the phylogenetic relationships and clarifying the evolution of octocorals.

1. Introduction

Homoplasious evolution, the occurrence of the same character state in distinct lineages by means of independent events (Fitch, 2000), represents an important process shaping the phenotypic evolution of corals (Class Anthozoa, Phylum Cnidaria). In both hexacorals (Fukami et al., 2004; Arrigoni et al., 2012) and octocorals (Sánchez et al., 2003a; Kim et al., 2004; McFadden et al., 2006; France, 2007; Prada et al., 2008; Dueñas and Sánchez, 2009; McFadden and van Ofwegen, 2012; Prada and Hellberg, 2013; Bilewitch et al., 2014; Rowley et al., 2015; Yasuda et al., 2015), molecular phylogenetic analyses have revealed multiple instances of morphological homoplasy at different taxonomic levels. Whether such homoplasies can be attributed to convergent or to parallel evolution remains contentious, since the distinction between these two terms is not clear-cut or it changes depending on the author (Powell, 2007; Arendt and Reznick, 2007; Scotland, 2011; Martin and Orgogozo, 2013). Terminology aside, the seemingly generalized emergence of similar phenotypes among unrelated coral taxa suggests that traits often used as diagnostic for taxonomic classification might be evolutionary labile and homoplasious (Sánchez et al., 2003a; McFadden et al., 2006, 2010; Dueñas and Sánchez, 2009; McFadden and van Ofwegen, 2013; Bryce et al., 2015; Wirshing and Baker, 2015).

Among cnidarians, members of the subclass Octocorallia are of special interest for the study of morphological evolution due to their broad environmental tolerance and wide geographic and bathymetric distribution, occurring in all of the world's oceans from zero to more than 6600 m deep (Watling et al., 2011;

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Williams, 2011; Pante et al., 2012). Traditionally, the taxonomy of octocorals has been mainly based on a combination of traits derived from the analysis of the morphology of the colony (e.g. color, branching pattern) and of the study of the sclerome, that is, the inventory of calcium carbonate microskeletal elements called sclerites present in an octocoral taxon (Breedy and Guzman, 2002; Molodtsova, 2013; see also Carlo et al., 2011). The phylogenetic validity of these characters is usually taken at face value for taxonomic purposes, ignoring the evolutionary history of the traits and their potential homoplasy. For example, among Eastern Pacific gorgoniids, the genera *Leptogorgia* and *Pacifigorgia* possess similar scleromes but differ in their colony morphology. In *Pacifigorgia*, the branches anastomose to form fan-like colonies (Breedy and Guzman, 2002). *Leptogorgia* species, in contrast, generally form tree-like colonies. The genus *Eugorgia* is similar to *Leptogorgia* in its colony morphology but its sclerome, dominated by sclerites with fused warts (Breedy and Guzman, 2007; Breedy et al., 2009), is clearly different from that of *Leptogorgia* and *Pacifigorgia*. Molecular phylogenetic studies have shown a close phylogenetic relation and the monophyly of these three genera, potentially corroborating the synapomorphic status of the morphological characters used to support their taxonomy. However most phylogenetic analyses of the Eastern Pacific octocoral fauna published to date (e.g. Wirshing et al., 2005; Vargas et al., 2014) are exclusively based on mitochondrial markers and only include a very restricted taxonomic sampling of *Eugorgia* and *Leptogorgia*. Moreover, several species have been described only recently and more await identification and formal description (Breedy and Guzman, 2002, 2003, 2004, 2007, 2008, 2013; Breedy et al., 2009). Within *Leptogorgia* in particular, a number of species of unclear phylogenetic affinities, and remarkably variable morphologies—e.g. colonies resembling those of *Eugorgia* or other gorgoniid genera, forming loose anastomoses as in *Pacifigorgia*, or showing a whip-like morphology with bidirectional growth and no attachment points—occur along the Eastern Pacific, opening questions about the role played by homoplasy and lability in the morphologial evolution of these genera. *Leptogorgia* Milne-Edwards & Haime, 1857, *Pacifigorgia* Bayer, 1951 and *Eugorgia* Verrill, 1868 are important structural components of rocky habitats and high-energy environments where they can dominate the seascape. These genera account for the majority of species reported for the tropical eastern Pacific (e.g. Breedy and Guzman, 2002, 2003, 2004, 2007; Breedy et al., 2009). Thus, clarifying the phylogenetic relationships and the evolutionary processes that led to their diversification and shaped their morphologies is pivotal to our understanding of these important members of the eastern Pacific shallow water communities and will contribute to clarify octocoral phenotypic evolution in general.

In this study, we present an expanded molecular phylogeny of the Eastern Pacific gorgoniid octocorals based on nuclear (28S rDNA) and mitochondrial (*mtMutS*) markers, and including an increased taxonomic sampling of the genera *Leptogorgia* and *Eugorgia* as well as three un-described, whip-like specimens of as yet unconfirmed phylogenetic affinity. The nuclear gene 28S rDNA have been successfully used to resolve intrafamily relationships among stoloniferous octocorals (McFadden and van Ofwegen, 2012), suggesting that this marker could be of general interest for resolving the molecular phylogeny of other octocoral taxa. We re-evaluate the phylogenetic relationships and the monophyly of the three main genera of Eastern Pacific gorgoniids recently revised, namely *Leptogorgia*, *Eugorgia* and *Pacifigorgia*, and show that character lability and homoplasious colony evolution played a role on the morphological evolution and diversification of these gorgoniid genera and likely shape octocoral evolution in general at all taxonomic levels.

2. Materials and methods

2.1. Molecular procedures

All specimens used in this study (Table 1) were collected between 2008 and 2010 along the Eastern Pacific of Costa Rica (mainland and the Isla del Coco National Park) and Panama (Coiba National Park, Gulf of Chiriquí). After genomic DNA extraction (following Vargas et al., 2014), a standard three-steps PCR was used to amplify the 28S rDNA gene using the primers C2′ (forward; Chombard et al., 1998) and 28S-1260fw (reverse; Voigt et al., 2012). In case of failure, different combinations with primers 28S-NL2F (forward; Scott Nichols pers. comm., 5′-TACCGTGAGG GAAAGGTGAAA-3′), RD3a (forward; McCormack et al., 2002) and D2 (reverse; Chombard et al., 1998) were used. The PCR temperature regime was as follows: 95 °C for 3 min, 35 cycles at 95 °C for 30 s; 52–54 °C for 30 s; 72 °C for 1 min, and 72 °C for 5 min. PCR amplifications contained 5.9 μL ddH2O, 2.5 μL 5x GoTaq Flexi Buffer (Promega, Madison), 1.5 μL MgCl2 (25 mM), 0.5 μL dNTP (10 mM each), 0.5 μL of each primer (5 mM), 0.1 μL GoTaq Polymerase (5 units/μL, Promega, Madison), and 1 μL of sample DNA for a total volume of 12.5 μL. The amount of DNA used for PCR was variable, generally ranging between 20 and 150 ng.

PCR products were visualized on 1.5% agarose gels, and cleaned-up using a polyethylene–glycol precipitation. Briefly, 10 μL PCR reaction were thoroughly mixed with an equal amount of PEG solution (20% PEG 8000, 2.5 M NaCl), incubated at room temperature for 20 min, centrifuged for 15 min at maximum speed (12,000 rpm), and washed twice with 80% ethanol. The air-dried pellets were resuspended in 10 μL ddH2O. The purified products were sequenced in both directions using the BigDye Terminator 3.1 chemistry (Applied Biosystems) and the same primers used for PCR. Sequencing products were precipitated using Sodium acetate–Ethanol and analyzed in an ABI 3700 Genetic Analyser at the Department of Genetics of the Ludwig-Maximilians-Universität München, Germany. Traces were visualized and assembled using Geneious 6.1.5 (Biomatters, available from http://www.geneious.com/), and the taxonomic affiliation of each "contig" was checked using NCBI's BLAST (Johnson et al., 2008). In addition to the 28S rDNA marker, sequences of the *mtMutS* gene of families Gorgoniidae and Plexauridae were downloaded from Genbank or sequenced using protocols previously described (see Vargas et al., 2014). All sequences were deposited in the European Nucleotide Archive (see Table 1 for details).

2.2. Sequence alignment and model selection

Sequences were aligned using the MAFFT version 7 online server (Katoh et al., 2002; Katoh and Toh, 2008; http://mafft.cbrc.jp/alignment/server/) with default settings and the resulting alignments were visually inspected in Seaview version 4.5.4 (Galtier et al., 1996; Gouy et al., 2010). For the *mtMutS* alignment, the amino acid translation was used to detect and correct frameshifts. The 28S rDNA alignment contained ambiguous regions that were identified and discarded from the final matrix using the Gblocks (Castresana, 2000; Talavera and Castresana, 2007) implementation in Seaview, with the options for a less stringent filtering set. JModelTest version 2.1.3 (Darriba et al., 2012, and references therein) was used to estimate the likelihood of different substitution models, including 7 schemes, with base frequencies (+F), proportion of invariable sites (+I), and the gamma distribution with four categories of rate heterogeneity across sites (+G). The best model was chosen using both the corrected Akaike Information Criterion (AICc; Akaike, 1973; Sugiura, 1978; Hurvich and Tsai,