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Molecular evolution of calcification genes in morphologically similar but phylogenetically unrelated scleractinian corals

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ABSTRACT

Molecular phylogenies of scleractinian corals often fail to agree with traditional phylogenies derived from morphological characters. These discrepancies are generally attributed to non-homologous or morphologically plastic characters used in taxonomic descriptions. Consequently, morphological convergence of coral skeletons among phylogenetically unrelated groups is considered to be the major evolutionary process confounding molecular and morphological hypotheses. A strategy that may help identify cases of convergence and/or diversification in coral morphology is to compare phylogenies of existing "neutral" genetic markers used to estimate genealogic phylogenetic history with phylogenies generated from non-neutral genes involved in calcification (biomineralization). We tested the hypothesis that differences among calcification gene phylogenies with respect to the "neutral" trees may represent convergent or divergent functional strategies among calcification gene proteins that may correlate to aspects of coral skeletal morphology. Partial sequences of two nuclear genes previously determined to be involved in the calcification process in corals, "Cnidaria-III" membrane-bound/secreted α-carbonic anhydrase (CIII-MBSα-CA) and bone morphogenic protein (BMP) 2/4, were PCR-amplified, cloned and sequenced from 31 scleractinian coral species in 26 genera and 9 families. For comparison, "neutral" gene phylogenies were generated from sequences from two protein-coding "non-calcification" genes, one nuclear (β-tubulin) and one mitochondrial (cytochrome b), from the same individuals. Cloned CIII-MBSα-CA sequences were found to be non-neutral, and phylogenetic analyses revealed CIII-MBSα-CAs to exhibit a complex evolutionary history with clones distributed between at least 2 putative gene copies. However, for several coral taxa only one gene copy was recovered. With CIII-MBSα-CA, several recovered clades grouped taxa that differed from the "non-calcification" loci. In some cases, these taxa shared aspects of their skeletal morphology (i.e., convergence or diversification relative to the "non-calcification" loci), but in other cases they did not. For example, the "non-calcification" loci recovered Atlantic and Pacific mussids as separate evolutionary lineages, whereas with CIII-MBSα-CA, clones of two species of Atlantic mussids (*Isophyllia sinuosa* and *Mycetophyllia* sp.) and two species of Pacific mussids (*Acanthastrea echinata* and *Lobophyllia hemprichii*) were united in a distinct clade (except for one individual of *Mycetophyllia*). However, this clade also contained other taxa which were not unambiguously correlated with morphological features. BMP2/4 also contained clones that likely represent different gene copies. However, many of the sequences showed no significant deviation from neutrality, and reconstructed phylogenies were similar to the "non-calcification" tree topologies with a few exceptions. Although individual calcification genes are unlikely to precisely explain the diverse morphological features exhibited by scleractinian corals, this study demonstrates an approach for identifying cases where morphological taxonomy may have been misled by convergent and/or divergent molecular evolutionary processes in corals. Studies such as this may help illuminate our understanding of the likely complex evolution of genes involved in the calcification process, and enhance our knowledge of the natural history and biodiversity within this central ecological group.

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1. Introduction

1.1. Scleractinian phylogenetics

Scleractinian corals are critical to the health and stability of tropical reef ecosystems worldwide (Knowlton and Jackson, 2008; Sheppard et al., 2009). Their fossil record dates back

Abbreviations: CIII-MBSα-CA, membrane-bound/secreted alpha carbonic anhydrase specific to cluster "Cnidaria III"; BMP, bone morphogenic protein.

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~240 My and they have been the dominant builders of reefs since the Paleogene (~67 Mya) (Veron, 2000; Wood, 1999). As a result of their robust fossil record, and the fact that museum collections of corals are principally comprised of their skeletal coralla, conventional evolutionary hypotheses concerning scleractinian corals are primarily based on the morphology of their calcium carbonate skeletons (e.g., Veron, 2000; Wells, 1956). However, molecular systematic studies have dramatically changed evolutionary and taxonomic perspectives within the Scleractinia. These studies have shown that superficial similarity in gross skeletal morphology can often belie deeper evolutionary distinctiveness. For example, Romano and Palumbi (1996) discovered two distinct mitochondrial lineages among scleractinians whose members did not correspond to any of the five traditional morphological suborders (see Vaughan and Wells, 1943; Wells, 1956) or families within the Scleractinia (see Veron, 1995, 2000). Instead, each lineage, termed complex and robust (the robust lineage comprises corals that deposit a skeleton that is solid and heavily calcified, and the complex lineage consists of corals with more porous and less heavily calcified skeletons), correlated with the overall construction of their calcium carbonate skeletons (Romano and Palumbi, 1996). Subsequent studies using nuclear ribosomal loci (Cuif et al., 2003b; Fukami et al., 2008; Romano and Cairns, 2000) and other mitochondrial loci (Chen et al., 2002) have also corroborated the complex and robust coral lineages. Consequently, non-homologous and morphologically plastic characters (see Todd, 2008; Veron, 1995) have likely contributed to the pervasive polyphyly that has been documented using molecular systematic approaches. Efforts have been made to rectify many of the confounded coral families (e.g., Benzoni et al., 2007; Huang et al., 2009, 2011), and major taxonomic revisions of some of the most problematic taxa have been made (see Budd et al., 2012). Re-analyzes of taxonomically informative characters used among scleractinians have revealed micromorphological characters (subcorallite features mostly involving septal characteristics), as well as coral microstructure (the arrangement of calcification fibers within corallite septa), to be the most promising morphological characters with which homologies among coral taxa may be found (reviewed by Budd et al., 2010). This contrasts with conventional morphological approaches, which primarily utilize macromorphological characters (general corallite and colony architecture) that are likely to be homoplasious (i.e., a result of convergent evolutionary processes) (Veron, 1995).

Molecular analyses have uncovered the prevalence of morphological convergence within the Scleractinia. Fukami et al. (2004, 2008) found certain Atlantic mussids grouped with the Atlantic faviid genera *Favia*, *Diploria*, *Colpophyllia* and *Manicina* (subsequently submerged into the family Mussidae; Budd et al., 2012) in a single clade, and not with their Pacific congeners. This suggests the Atlantic and Pacific lineages of these groups each experienced their own intra-basin radiations accompanied by morphological convergence of their skeletons. Studies of the micromorphology and microstructure of the septal teeth of these groups show that, although they appear similar at the macromorphological level, each contains unique characteristics at finer morphological scales (Budd and Stolarski, 2009).

Similarly, using molecular data, Benzoni et al. (2007) found species within two genera of the family Siderastreidae, *Coscinaraea wellsi* and *Psammocora explanulata*, to group more closely with members of the family Fungiidae than with members of their own family. Moreover, micromorphological characters, including interstomatous septa and fulturae (buttress-like structures that join septal faces, similar to synapticulae), were more similar to those of fungiids than to other siderastreids, corroborating their molecular data (Benzoni et al., 2012). This indicates that the macromorphological characters currently used to classify *C. wellsi* and *P. explanulata* (*sensu* Veron, 2000) are not homologous, and the diversification and/or plasticity of their morphological characters may have led to their placement in the Siderastreidae.

1.2. Biomineralization in scleractinian corals

Early models of coral biomineralization (calcification) emphasized a purely mineralogical approach to the formation of calcium carbonate crystals. Bryan and Hill (1941) noticed the similarity of coral aragonitic fibers to those produced by inorganic crystalline systems. The role of the coral in structuring the morphology of the calcium carbonate crystals was restricted to functioning as an inhibitor of crystal expansion by preventing the unwanted growth of crystals from piercing the overlying cells of the coral polyp (Clode and Marshall, 2002). However, an alternate view asserts that organic compounds generated by the calicoblastic cell layer (commonly referred to as the "organic matrix"), as well as the transport of ions such as calcium and bicarbonates to the mineralizing site, both initiate calcium carbonate crystal formation and regulate their structural architecture in a "matrix mediated" model of crystal growth (Allemand et al., 2004; Cuif and Dauphin, 2005a, 2005b; Cuif et al., 2003a; Meibom et al., 2006, 2008). Consequently, the differences in coral skeletal morphology reflect differences in organic matrix properties among different taxa (Dauphin et al., 2008).

1.3. "Calcification" genes

Carbonic anhydrases (CA), enzymes that catalyze the interconversion of carbon dioxide (CO_2) to bicarbonate (HCO_3^-), and bone morphogenic proteins (BMP), proteins involved in signaling and which act as growth factors, are two groups of proteins whose members have been isolated from the organic matrix of corals (e.g., Ramos-Silva et al., 2013), and have been found to be involved in the calcification process (Moya et al., 2008; Zoccola et al., 2009). Although the physiological function of many CAs does not involve calcification (Henry, 1996), the association of some CAs to the process of calcification in corals has been recognized for >50 years (e.g., Goreau, 1959). However, their specific role is only beginning to be understood. For example, Jackson et al. (2007) determined that α-carbonic anhydrase enzymes were present in the first metazoans and were part of a core "skeletogenetic toolkit" upon which later metazoan lineages have elaborated for their own taxonomically unique biomineralization strategies. BMP homologs have also been isolated from invertebrates, including sea urchins (Hwang et al., 1999), other echinoderms, mollusks, and cnidarians (Lelong et al., 2001). Orthologs of BMP were found to affect dorso-ventral axis formation in a scleractinian coral (Hayward et al., 2002), and shell formation in mollusks (Nederbragt et al., 2002).

CAs have been shown to play important roles in the calcification of many invertebrates, e.g., mollusk shells (Gaume et al., 2011), as well as vertebrates, e.g., fish otoliths (Tohse et al., 2004). In corals, gene expression analyses found CAs to be expressed in post settlement polyps at the aboral disk (an area consistent with the onset of calcification), whereas in older polyps CAs were expressed in the septa (where calcification occurs through adulthood) (Grasso et al., 2008). Bioinformatic analyses of two CAs from the coral *Fungia scutaria* revealed these CAs to be membrane-bound/secreted (MBS) α-CA homologs whose function is associated with calcification (DeBoer et al., 2006). In addition, Moya et al. (2008) sequenced a MBS α-CA (STPCA), found to be localized to cells in the calicoblastic layer, from the coral *Stylophora pistillata* that likely plays an important role during the precipitation of calcium carbonate. Similarly, CAs were isolated within the organic matrices of the azooxanthellate coral *Tubastrea aurea* and the octocoral *Lobophytum crassum* (Rahman and Isa, 2005; Rahman et al., 2008; Tambutte