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Complete mitochondrial genomes elucidate phylogenetic relationships of the deep-sea octocoral families Coralliidae and Paragorgiidae

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ABSTRACT

In the past decade, molecular phylogenetic analyses of octocorals have shown that the current morphological taxonomic classification of these organisms needs to be revised. The latest phylogenetic analyses show that most octocorals can be divided into three main clades. One of these clades contains the families Coralliidae and Paragorgiidae. These families share several taxonomically important characters and it has been suggested that they may not be monophyletic; with the possibility of the Coralliidae being a derived branch of the Paragorgiidae. Uncertainty exists not only in the relationship of these two families, but also in the classification of the two genera that make up the Coralliidae, *Corallium* and *Paracorallium*. Molecular analyses suggest that the genus *Corallium* is paraphyletic, and it can be divided into two main clades, with the *Paracorallium* as members of one of these clades. In this study we sequenced the whole mitochondrial genome of five species of *Paragorgia* and of five species of *Corallium* to use in a phylogenetic analysis to achieve two main objectives; the first to elucidate the phylogenetic relationship between the Paragorgiidae and Coralliidae and the second to determine whether the genera *Corallium* and *Paracorallium* are monophyletic. Our results show that other members of the Coralliidae share the two novel mitochondrial gene arrangements found in a previous study in *Corallium konojoi* and *Paracorallium japonicum*; and that the *Corallium konojoi* arrangement is also found in the Paragorgiidae. Our phylogenetic reconstruction based on all the protein coding genes and ribosomal RNAs of the mitochondrial genome suggest that the Coralliidae are not a derived branch of the Paragorgiidae, but rather a monophyletic sister branch to the Paragorgiidae.

While our manuscript was in review a study was published using morphological data and several fragments from mitochondrial genes to redefine the taxonomy of the Coralliidae. *Paracorallium* was subsumed into *Corallium* and the genus *Hemicorallium* was resurrected. This left two disjunct clades as *Corallium*, making that genus paraphyletic. One of the clades includes the type specimens of *Corallium*, indicating that clade should remain *Corallium*. For the other clade, we support the resurrection of the genus *Pleurocorallium* to fix the paraphyly of *Corallium*. Based on congruent phylogenies in both studies, the genus *Pleurocorallium* includes the species *C. secundum*, *C. kishinouyei*, *C. konojoi*, *C. elatius*, and *C. niveum*.

1. Introduction

Corals are among the dominant megafaunal taxa in areas of hard substrate in the deep sea, and have been noted in particular abundance on seamounts (e.g. Genin et al., 1986; Stocks, 2004), and in canyons (e.g. Hecker, 1990). Like their shallow-water counterparts, deep-sea corals act as ecosystem engineers (sensu Jones et al., 1994) with octocorals often forming dense thickets referred to as “beds” or “gardens”. These corals provide essential habitat for a variety of invertebrates and fishes (Messing et al., 1990; Genin et al., 1992; Jones et al., 1994; Rogers, 1994; Probert et al., 1997; Stocks, 2004; DeVogelaere et al., 2005; Leverette and Metaxas, 2005; Baco, 2007; Mortensen and Buhl-Mortensen, 2005; Buhl-Mortensen et al., 2010; Roberts et al., 2010; Baillon et al., 2012).

Despite their ecological importance, corals are heavily impacted by fisheries activities (e.g. Koslow et al., 2001; Clark and Rowden, 2009), potentially by deep-sea mining for cobalt-rich manganese crusts (e.g. Hein, 2002; Hein et al., 2009) and likely by climate change and ocean acidification (e.g. Guinotte et al., 2006). Corals are slow-growing, long-lived, and existing evidence suggests many are recruitment-limited (Grigg, 1988; Krieger, 2001; Roark et al., 2006, 2009; Sun et al., 2010). Thus they are very vulnerable to anthropogenic impacts and slow to recover from them (e.g. Williams et al., 2010).

Because of their fragility and the exploitation threats, international efforts have begun to focus on the conservation of deep-sea corals and seamount fauna (Watling and Norse, 1998; Morgan et al., 2006; Lumsden et al., 2007). Recent reviews of seamount fauna and

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deep-sea corals have concluded that the global deficiency of scientific expertise in morphological taxonomy is a significant impediment to the understanding of deep-sea coral diversity, coral biogeography, and seamount ecology (e.g. Morgan et al., 2006; Parrish and Baco, 2007; Rogers et al., 2007).

Molecular phylogenetic analyses of octocorals have shown that the current morphological taxonomic classification of these organisms needs to be revised (Berntson et al., 2001, Sanchez et al., 2003; McFadden et al., 2006, 2010). McFadden et al. (2006) presents the most comprehensive molecular phylogenetic analysis of octocorals to date. They demonstrate based on two mitochondrial protein coding genes (*nad2* and *mutS*) that most octocorals can be divided into three clades. One of these clades contains the scleraxonian *Corallium* and the alcyoniina *Anthomastus*, along with several other genera mostly belonging to the family Alcyoniidae (McFadden et al., 2006). The scleraxonian *Paragorgia* likely belongs to this clade too as suggested by the phylogenetic analysis of Berntson et al. (2001) based on the nuclear 18S gene. A close relationship between the Coralliidae and Paragorgiidae has been further demonstrated by the phylogenetic analysis of Herrera et al. (2010) and by that of Brockman and McFadden (2012), both based on several mitochondrial and nuclear markers. These two families share several taxonomically important characters and it has been suggested that they may not be monophyletic; with the possibility of the Coralliidae being a derived branch of the Paragorgiidae (Sanchez, 2005). Additionally, the study by Brockman and McFadden (2012) places the *Paragorgia* and *Corallium* in one clade with *Paracorallium* as a sister branch, adding uncertainty to the current taxonomic position of the Paragorgiidae and Coralliidae and questioning the validity of the genera *Paracorallium* and *Corallium* within the Coralliidae.

This uncertainty in the classification of the two genera that make up the Coralliidae has been demonstrated in previous molecular analyses which show that the genus *Corallium* is paraphyletic, (that is, a group which is missing a subset of the descendants of a common ancestor) and it can be divided into two main clades, one of these clades having a mix of species of *Corallium* with *Paracorallium* (Herrera et al., 2010). To help resolve the classification of the members of the Coralliidae, Uda et al. (2011) sequenced the entire mitochondrial genome of *Corallium konojoi* and *Paracorallium japonicum*. Their study shows that *C. konojoi* and *Paracorallium japonicum* each have a unique mitochondrial gene arrangement, different from each other and from the two previously known arrangements in octocorals. Based on these two different mitochondrial gene arrangements and on their phylogenetic analysis of all mitochondrial protein-coding regions, Uda et al. (2011) conclude that the morphological classification that separates these two genera is valid. But it must be emphasized that they only sequenced the mitochondrial genome of one species each of *Corallium* and *Paracorallium*. In addition to their whole mitochondrial genome analysis, they performed a phylogenetic analysis based on two mitochondrial genes (*mutS* and *nad2*) that included more species of *Corallium*. This analysis showed the same results as Herrera et al. (2010) with two *Corallium* clades and the *Paracorallium* appearing as a member of one of these. Therefore the status of *Corallium* and *Paracorallium* as monophyletic (that is, containing all of the descendants of a common ancestor) genera remains in question.

In this study we sequenced the whole mitochondrial genome of five species of *Paragorgia* and of five species of *Corallium* to use in a phylogenetic analysis to achieve two main objectives; the first to elucidate the phylogenetic relationship between the Paragorgiidae and Coralliidae and the second to determine whether the genera *Corallium* and *Paracorallium* are monophyletic. Our two hypotheses are: (1) that the family Coralliidae is not just a derived branch of the Paragorgiidae, these two families are separate, monophyletic clades and (2) that the genus *Corallium* is paraphyletic, including *Paracorallium* within one of its clades.

2. Data and methods

2.1. Collections

Five species of the genus *Corallium* and five unique and distinct morphotypes of the genus *Paragorgia* (Table 1) were used for this study. Samples were collected from the seafloor using the submersibles Pisces V or Alvin from seamounts in the Northwestern Hawaiian Islands or Gulf of Alaska, respectively. Corals were placed in insulated bioboxes for return to the surface and subsamples were frozen at −80 °C. The remainder of each specimen was deposited at the Smithsonian.

2.2. DNA extraction, PCR, sequencing and assembly

Total genomic DNA was extracted from each specimen using Qiagen's DNeasy Blood and Tissue Kit. Two methods were used to sequence the entire mitochondrial genome of each specimen: Next generation sequencing technology was used for two of the specimens, *Corallium secundum* and *Corallium laauense*; while multiple overlapping PCRs were used for the remaining eight specimens.

The mitochondrial genome of *Corallium secundum* and *Corallium laauense* were obtained as a byproduct of a separate project that is using next generation sequencing technology to obtain partial

Table 1
Specimens of Octocorallia used for this study. Each of the five specimens of *Paragorgia* represent a unique morphotype that was easily distinguished based on colony morphology and/or color.

Species	Smithsonian USNM #	Collection date	Location	Genbank Acession #	Sequence from
Corallium imperiale[a]	1072449	Oct 2003	Smt E of Necker Island, NWHI	KC782355	Present Study
Corallium imperiale[a]	1072448	Oct 2003	Smt E of Necker Island, NWHI	KC782352	Present Study
Corallium laauense[a]		Oct 2003	Pioneer Bank, NWHI	KC782348	Present Study
Paracorallium japonicum[a]				AB595189	GenBank
Corallium kishinouyei[a]	1072441	Oct 2003	Smt Se of Laysan Island, NWHI	KC782353	Present Study
Corallium secundum[a]		Oct 2003	Pioneer Bank, NWHI	KC782347	Present Study
Corallium konojoi[a]				NC015406	GenBank
Paragorgia sp.	1075769	Aug 2004	Giacomini Smt, GOA	KC782349	Present Study
Paragorgia sp.	1072339	Oct 2003	Pioneer Bank, NWHI	KC782354	Present Study
Paragorgia sp.	1075761	Aug 2004	Pratt Smt, GOA	KC782350	Present Study
Paragorgia sp.	1072362	Oct 2003	Smt E of Necker Island, NWHI	KC782351	Present Study
Paragorgia sp.	1075741	Aug 2004	Dickins Smt, GOA	KC782356	Present Study
Keratoisinidae sp.				EF622534	GenBank
Acanella eburnea				EF672731	GenBank

[a] Ardila et al. (2012) subsumed *Paracorallium* into *Corallium* (changing the genus for *P. japonicum*) and resurrected *Hemicorallium* (changing the genus for *C. laauense* and *C. imperiale*). Adittionally we propose the resurrection of *Pleurocorallium* (changing the genus for *C. secundum*, *C. kishinouyei*, and *C. konojoi*).