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Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record

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ABSTRACT

The phylum Cnidaria is comprised of remarkably diverse and ecologically significant taxa, such as the reef-forming corals, and occupies a basal position in metazoan evolution. The origin of this phylum and the most recent common ancestors (MRCAs) of its modern classes remain mostly unknown, although scattered fossil evidence provides some insights on this topic. Here, we investigate the molecular divergence times of the major taxonomic groups of Cnidaria (27 Hexacorallia, 16 Octocorallia, and 5 Medusozoa) on the basis of mitochondrial DNA sequences of 13 protein-coding genes. For this analysis, the complete mitochondrial genomes of seven octocoral and two scyphozoan species were newly sequenced and combined with all available mitogenomic data from GenBank. Five reliable fossil dates were used to calibrate the Bayesian estimates of divergence times. The molecular evidence suggests that cnidarians originated 741 million years ago (Ma) (95% credible region of 686–819), and the major taxa diversified prior to the Cambrian (543 Ma). The Octocorallia and Scleractinia may have originated from radiations of survivors of the Permian–Triassic mass extinction, which matches their fossil record well.

1. Introduction

The phylum Cnidaria comprises over 9000 animal species, such as corals, sea anemones, sea fans, sea pens, jellyfish, and hydroids, that have simple body forms and produce cnidae, the diagnostic feature of the phylum (Daly et al., 2007; Fautin, 2009; Rogers, 2009). Cnidarians are taxonomically subdivided into approximately seven classes and 25 orders that exhibit diverse adaptations with respect to their morphology, reproduction, and ecology (Daly et al., 2007; Fautin, 2009; Rogers, 2009). Although they are distributed worldwide in shallow to deep ocean environments, some lineages also occur in fresh water (Fabricius and Alderslade, 2001). Cnidarians originated early in the history of metazoan evolution, as indicated by fossil evidence (Ausich and Babcock, 1998; Cartwright et al., 2007; Chen et al., 2002; Hagadorn and Waggoner, 2000; Han et al., 2010) and molecular phylogenies (Dunn et al., 2008; Peterson et al., 2004, 2008).

Considering their early phylogenetic position, the following questions arise: when did the Cnidaria first appear, and when did the major classes and subclasses diverge to produce the modern extant taxa? Although they are fascinating, these questions remain mostly unsolved. The hard calcareous skeletons of the Scleractinia, belonging to the Anthozoa, have left well-preserved fossils since their first appearance in the Triassic (Stanley, 2003; Veron et al., 1996; Veron, 1995); however, the origins of the soft-bodied taxa are poorly understood. Ambiguity exists in assigning the limited known fossils to relatives of the modern taxa, as exemplified by the soft corals of the Octocorallia (Ausich and Babcock, 2000; Bengtson, 1981).

During the past decade, a few molecular studies have attempted to estimate divergence times for the deeper cnidarian nodes (Cartwright and Collins, 2007; Medina et al., 2006; Peterson et al., 2004; Waggoner and Collins, 2004). Recent methodological advances in the estimation of divergence times have made it possible to estimate the ages of phylogenetic nodes beyond calibration points based on a limited number of fossil dates. Though the results must be carefully interpreted and guided by the fossil data, these advances provide opportunities to assess the origins of major cnidarian lineages using the limited fossil records (Cartwright and Collins, 2007; Peterson et al., 2007).

Classical molecular clocks (Zuckerkandl and Pauling, 1962), assuming that the evolutionary rates are constant, have been used to estimate divergence times for many taxa; for example, they have been employed to calculate deeper divergence times of metazoan phyla (Doolittle et al., 1996; Wray et al., 1996) and hard skel-

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etal corals of the Scleractinia (Romano and Palumbi, 1996). However, variability in mutation rates, generation lengths, effective population sizes, and functional constraints on gene products can produce different evolutionary rates for various taxonomic groups. This variability has led to more sophisticated estimation procedures that relax the assumptions about rate homogeneity. The "local clock" approach (Yoder and Yang, 2000) assumes the existence of multiple molecular clocks that are assigned to various taxonomic groups, within which evolutionary rates are assumed to be constant. Nonparametric and "penalized likelihood" methods (Sanderson, 1997, 2002) assume different evolutionary rates among lineages to estimate lineage-specific rates and divergence times. Rate heterogeneity among lineages was further investigated and modeled using a log-normal distribution under a Bayesian framework (Thorne et al., 1998). Nucleotide substitution rates in protein-coding sequences can be partitioned for nonsynonymous and synonymous changes that can be modeled with bivariate log-normal distributions (Seo et al., 2004). An advantage of the method described by Seo et al. over that of Thorne et al. is that changes in selective pressure can be properly estimated. Both methods adopt fixed tree topologies and simple strategies for the incorporation of fossil data, but other Bayesian methods can jointly estimate tree topologies and divergence times (Drummond and Rambaut, 2007) and incorporate statistical models that deal with the uncertainty of fossil dates (Yang and Rannala, 2006). To analyze cnidarian mitochondrial genomes, we adopted the methods of Seo et al. and Thorne et al. because they assume similar rate change models and particularly the former method provides an additional function for estimating synonymous and nonsynonymous rates.

As single genes or combinations of genes, mitogenomes contain useful markers for phylogenetic analyses. Considerable efforts were previously directed towards producing highly resolved trees for Anthozoa based on the mitochondrial *cox1*, *msh1* and *nad2* genes in the Octocorallia (McFadden et al., 2006, 2009, 2010) and *cox1* in the Scleractinia, which belongs to the Hexacorallia (Kitahara et al., 2010). Though low substitution rates for these genes may provide insufficient information to distinguish some species and genera of Octocorallia (McFadden et al., 2011), mitochondrial genes have long been used for phylogenetic studies to capture global phylogenetic relationships among representative anthozoan taxa from families to suborders and orders (France and Hoover, 2002; France et al., 1996; Hellberg, 2006; Shearer et al., 2002).

In general, mitochondrial genes of nonbilaterian animals, such as the Medusozoa and Anthozoa, have been useful for clarifying higher phylogenetic relationships (Gissi et al., 2008; McFadden et al., 2010). The combined information from several mitochondrial genes increases confidence in estimates of higher evolutionary relationships and deep divergence times in anthozoan lineages that could not be achieved using fewer individual genes (Brugler and France, 2007; Medina et al., 2006). While the small and large subunit ribosomal RNA genes are often chosen for phylogenetic studies of Medusozoa (Collins, 2002; Collins et al., 2006), a lack of mitogenomic data exists for this taxon. Before the present study, only one mitogenomic sequence for Scyphozoa and two for Hydrozoa were available in GenBank.

Here, we explore published mitogenomic data that have accumulated for 39 species to date (27 Hexacorallia, 9 Octocorallia and 3 Medusozoa) to estimate divergence times. We also sequenced and characterized the complete mitogenomes from seven octocoral species and two jellyfish species, resulting in a total of 48 cnidarians included in the present study. We investigated evolutionary rates and divergence times by applying sophisticated Bayesian methods to 13 protein-coding genes from all available mitogenomic data. By assuming a log-normal rate change model and separating nucleotide substitutions into synonymous and nonsynonymous partitions, we evaluated changes in selective pressures. The obtained divergence time estimates are compared with those estimated from previous studies.

2. Materials and methods

2.1. Sample collection and DNA extraction

2.1.1. Octocorals

We sequenced the complete mitogenomes of seven octocorals (*Dendronephthya castanea*, *Dendronephthya mollis*, *Dendronephthya putteri*, *Dendronephthya suensoni*, *Scleronephthya gracillimum*, *Euplexaura crassa*, and *Echinogorgia complexa*) from order Alcyonacea. Among these species, three (*D. castanea*, *D. suensoni*, and *S. gracillimum*) were collected at depths between 10 and 20 m below the sea surface on the submerged rock cliff of Munseom Island, Korea in 2005. The seven octocorals represent two families (Nephtheidae and Plexauridae) from the Alcyoniina–Holaxonia clade (McFadden et al., 2006) (Table 1). Several 2-cm long branches were dissected for each specimen and stored in 95% ethanol until use. Genomic DNA was isolated using a DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, USA) following the manufacturer's protocol. We obtained DNA samples of the remaining four species (*D. mollis*, *D. putteri*, *E. crassa*, and *E. complexa*) from the Korean Coral Resource Bank (Ewha Womans University, Korea). Voucher specimens of the seven octocorals were deposited at the Natural History Museum in Ewha Womans University, Korea (Table 1 and Supplementary Table S1).

2.1.2. Scyphozoans

The two jellyfish species examined here, *Aurelia aurita* and *Chrysaora quinquecirrha*, are distributed worldwide and have frequently been found in southern Korean coastal waters. Specimens of these two scyphozoans were collected from Korean coastal waters near Incheon and Geoje-do, respectively. Individuals were immediately preserved in absolute ethanol for dehydration and stored at room temperature until use. Genomic DNA was isolated from the stored tissues using Proteinase K treatment followed by chloroform extraction and isopropanol precipitation. The isolated DNA was further purified using the DNeasy tissue kit (Qiagen, Valencia, CA). The voucher sample of *A. aurita* was deposited at the Natural History Museum in Ewha Womans University, Korea (Table 1 and Supplementary Table S1).

2.2. PCR amplification and sequencing

2.2.1. Octocorals

To amplify and read the complete mitochondrial genome sequences (Fig. 1), we used 31 pairs of primers that were previously designed and successfully applied to for whole mitogenome sequencing of the soft coral species *Dendronephthya gigantea* (Park et al., 2010). PCR amplification reactions using the 31 primer pairs were performed with the following conditions: an initial denaturation step at 94 °C for 1 min followed by 35 cycles at 92 °C for 40 s, 50 °C for 1 min, and 72 °C for 1 min, with a final extension step at 72 °C for 7 min. The PCR products were purified with the LaboPass PCR purification kit (Cosmo Genetech Inc., Seoul, Korea) and sequenced using an ABI3730XL instrument (Applied Biosystems Inc., Carlsbad, USA).

2.2.2. Jellyfishes

We designed seven primer sets to amplify sequences from the two scyphozoan jellyfishes (*A. aurita* and *C. quinquecirrha*) using the published mitochondrial genome of *A. aurita* (GenBank accession no: NC_008446, Shao et al., 2006). PCR amplification reactions were performed with the following conditions: 40 cycles at 98 °C