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DNA Sequencing of 18s Ribosomal RNA Genes of the Northern Star Coral (*Astrangia poculata*) Collected from New Jersey Artificial Reefs.

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Abstract

The purpose of this work was to investigate the distribution and molecular evolution of the Northern Star coral *Astrangia poculata* on artificial reefs and shipwrecks in the local coastal ocean. This region is generally devoid of natural hard structure habitat, consisting primarily of gently sloping sandy plains. Most corals live in tropical to sub-tropical waters above 20ºC and cannot tolerate high turbidity. The Northern Star Coral is an exception in that it is found from the sub-tropics to the temperate zone on hard surfaces. Northern Star Coral colonies can be encrusting, massive or branching. Individual coral colonies were sampled from seven collection sites offshore of Atlantic City, New Jersey at depths from 20-30 m. Coral samples containing at least 5-10 corallites per sample were collected by scuba diver. Between 10-30 mg of tissue was extracted from individual polyps using a Qiagen DNA extraction kit (Qiagen Corp). DNA was amplified by the polymerase chain reaction (PCR) using eukaryotic universal primers for the small subunit (18s) ribosomal RNA gene. PCR products were cloned in pGemT (Promega) and plasmid preparations were cycle sequenced to determine the exact DNA base sequence of the individual being typed. This DNA sequence information was aligned and analyzed to determine the genetic relationship of the individuals using a reference *A. poculata* sequence from GenBank. In general, genetic variation between the local sites was found to be very low, but will serve as a baseline for collections over a wider geographic range.

Keywords: DNA sequencing, NJ artificial reef

Introduction

A prominent member of the invertebrate assemblage colonizing shipwrecks and artificial reefs in the coastal waters offshore of New Jersey is the Northern Star coral *Astrangia poculata*, synonyms *A. danae* and *A. astreiformis*, Phylum Cnidaria, Class Anthozoa (Peters et al., 1988). This region is generally devoid of natural hard structure habitat, consisting primarily of gently sloping sandy plains. Most scleractinian corals, subclass Hexacorallia, Order Scleractinia, live in tropical to sub-tropical waters above 20ºC and cannot tolerate high turbidity. However, an exception is the Northern Star Coral which is found from the sub-tropics to the temperate zone on the Northwest Atlantic coast. Northern Star Coral colonies can be encrusting, massive or branching but are not considered to be reef forming as they generally only cover existing structures. The individual coral skeletons or corallites are well defined, circular, compact, and up to 10 mm in diameter. The polyps or living coral tissue may be translucent to dark brown depending on the presence and number of zooxanthellae (Peters et al., 1988). The corals may reproduce both asexually by budding within established colonies and by sexual reproduction with planktonic dispersal followed by settling on hard surfaces such as rocks, pilings and shipwrecks. The purpose of this study was to look at genetic differentiation of star

coral in relation to their wide ecological amplitude and their habit of colonizing a substrate that is extremely patchy.

Methods

Coral Collection

Individual coral colonies were sampled from seven collection sites offshore of Atlantic City, New Jersey, USA (39°23'N: 74°26'W) at depths from 20-30 m on shipwrecks and ships sunk as artificial reefs (Figure 1). The sites, followed by their sinking dates are: Jet Trader- 2005, Lemuel Burrows-1942, Almirante-1918, John Marvin-1992, Sea Girt-1990, Car Float-unknown and Glory (possibly Lake Frampton)-1920. Coral samples containing at least 5-10 corallites per sample were collected by scuba diver (Figure 2). Corals were kept in aquaria with filtered seawater and aeration until sampling (Figure 3).

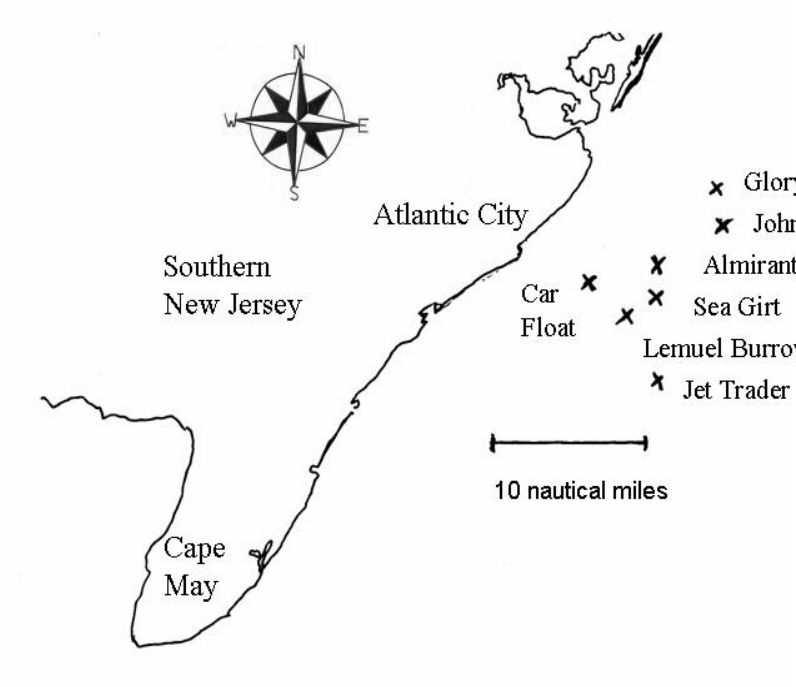

Figure 1. Dive sites for *Astrangia poculata* collection off of the southern New Jersey, USA coast

Figure 2. *Astrangia poculata* collection from deck plates on the wreck of the Almirante. Photo credit: David Roche (used with permission).

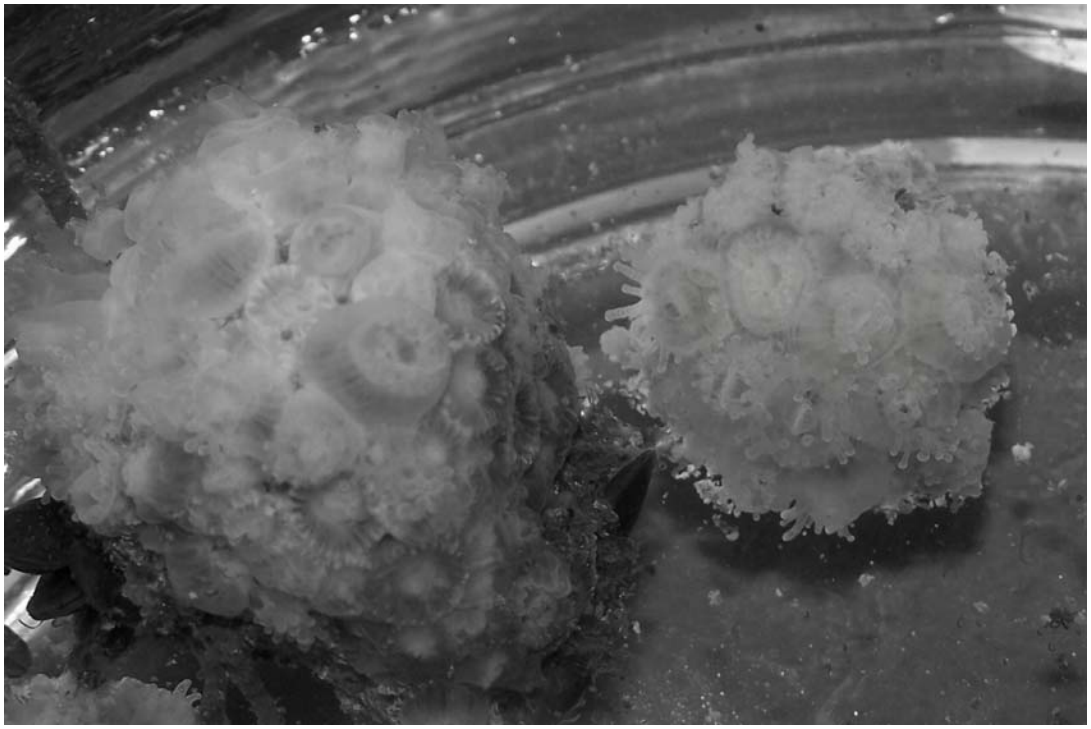

Figure 3. *Astrangia poculata* colonies with extended polyps. Photo credit: Peter Straub (used with permission).

DNA Extraction and Amplification

Between 10-30 mg of tissue was extracted and purified from individual polyps using a DNeasy Blood and Tissue extraction kit (Qiagen Corp., Valencia, CA). Genomic DNA was eluted in sterile water and quantified by UV spectrophotometer at 260 nm. DNA was amplified by the polymerase chain reaction (PCR) using eukaryotic universal primers for a portion of the small subunit (18s) ribosomal RNA gene with primers NS1 (White et al., 1990) and Euc 516r (Amann et al., 1990). The amplification by PCR was performed in 50 µl reactions with 100 ng template DNA, 5 UN Taq polymerase and 1X coraload buffer (Qiagen Corp., Valencia, CA), 200 µM each dNTPs, 0.2 µM each primer NS1 and Euc 516r. Conditions for PCR were as follows: denature 1 min at 94ºC; followed by 35 cycles of 94ºC for 1 min, 52ºC for 1:30 min and 72ºC for 2:00 min; ending with a final 10 min at 72ºC to ensure complete synthesis.

Purification and Cloning of PCR Products

PCR products were analyzed by agarose gel electrophoresis to ensure amplification of single products and then purified using a column cleanup with a Wizard SV Gel and PCR Clean-up kit (Promega, Madison, WI) to remove primers and template. Purified PCR products were then ligated into the plasmid pGemT Easy using a pGemT Easy cloning kit (Promega, Madison, WI) taking advantage of the non-template 'A' nucleotide addition commonly added to PCR products by Taq polymerase and single 'T' nucleotide overhangs on the prepared pGemT Easy vector. Ligation products were transformed into ultra-competent JM109 *E. coli* cells (Promega, Madison, WI) by heat shock for 50 s at 42ºC, grown for 60 min in LB broth with shaking. Transformants were then plated on LB agar medium supplemented with 100 µg/ml ampicillin and 80 µg/ml X-Gal and 0.5 mM IPTG for blue-white color selection and grown overnight at 37ºC. Individual white colonies were picked and grown overnight at 37ºC in 5 mL broth cultures of LB medium with 50 µg/ml ampicillin. Plasmid DNA was prepared using a column method from 1.5 mL of bacterial cultures with a Wizard Plus Miniprep DNA Purification System (Promega, Madison, WI). Plasmid DNA, eluted in water, was quantified on a UV spectrophotometer at 260 nm and diluted to100 ng/µl for DNA sequencing.

DNA Sequencing

A Genome Lab Dye Terminator Cycle Sequencing quick start kit (Beckman-Coulter, Fullerton, CA) was used with 200 ng of plasmid preparations for each reaction plus T7 or SP6 flanking sequencing primers (Promega, Madison, WI). Sequence reactions were subjected to 30 cycles of: 96ºC for 20 s, 50ºC for 20 s, and 60ºC for 4 min. After cycling, sequence reactions were purified by ethanol precipitation, dried and resuspended in sequence loading solution as per the Genome Lab instructions. Cycle sequence reaction products were separated and sequenced on a Beckman Coulter CEQ 8000 automated capillary genetic analyzer.

Sequence Analysis

Individual DNA sequence results were edited in Vector NTI (Invitrogen, Carlsbad, CA) to remove vector sequence and to resolve conflicts in forward and reverse sequences to produce consensus sequence for each template. Individual sequences were uploaded to the BLAST server at the National Center for Biotechnology Information and compared with known sequences deposited in GenBank for confirmation of identity. Individual confirmed consensus sequences were aligned using the Align-X application of Vector NTI (Invitrogen, Carlsbad, CA) and checked manually. The DNA sequence information was aligned and analyzed to determine the genetic relationship of the individuals using a reference *Astrangia danae* (*=A. poculata*) sequence from Genbank [AY039209] (Podar et al., 2001) and nine other Scleractinian coral sequences. Alignments were exported to PAUP 4.10 (Swofford,

2003) for phylogenetic analysis via MacClade (Maddison, 2005), and a Maximum Parsimony tree was inferred based on these 554 base pairs.

Results

DNA of sufficient purity was recovered from most samples to readily amplify and obtain a ca. 550 bp PCR product. After cloning of the PCR product and DNA sequencing, both BLAST search and nucleotide alignment indicated that all of the samples collected belong to a Scleractinian coral, most likely *Astrangia poculata.* All sequences match the Northern Star Coral with no more than one or two base pair differences per sequence in this region, with very little variability either between or among populations (Figure 4). The inferred Maximum Parsimony tree constructed in PAUP includes both our collected coral sequences and available sequences from a number of related coral species within the Subclass: Hexacorallia; Order: Scleractinia (Figure 5).

Discussion

The DNA extracted, amplified and sequenced was identified as coral tissue. Despite using 'universal' eukaryotic primers, we did not clone or sequence any zooxanthellae specific DNA. This may be due to the fact that *A. poculata* has been reported as a facultative symbiotic partner or that our primers were not specific enough to detect the zooxanthellae symbiont due to an overabundance of host DNA. This suggests that symbiont-specific primers may be useful to amplify zooxanthellae DNA in this species. The coral DNA sequences from this study were aligned with one another and with nine other species of Scleractinian corals. In published studies, the use of nuclear 18S rDNA (Bernston et al., 1999) and 16 S mitochondrial rDNA (Romano and Palumbi, 1996) have been shown to effectively discriminate between Orders of the Class Anthozoa. The closest DNA sequence matches for the collected specimens, based on the maximum parsimony analysis, were with the three species *Astrangia poculata, Phyllangia mouchezii*, and *Fungia scutaria*. Both *A. poculata* and *P. mouchezii* are in the same family, Family Rhizangiidae, Suborder Faviina. *F. scutaria* is classified in Suborder: Fungiina, Family: Fungiidae. Given the morphological identification of our collected specimens as *A. poculata*, we are confident that the DNA identification as *A. poculata* is concordant. Neither *P. mouchezii* (east North Atlantic-Mediterranean) or *F. scutaria* (Indo-Pacific) are found in the Northwest Atlantic. Given these results, it was not possible to discriminate the corals below the level of Order Scleractinia. This would suggest that a more variable region of the small subunit rRNA, possibly including the internally transcribed spacer region may be a useful approach to defining an area with enough variability to discriminate between the populations. In addition, collection of samples of *A. poculata* from a wider geographical area could identify areas of greater variability in DNA sequence to the analysis.

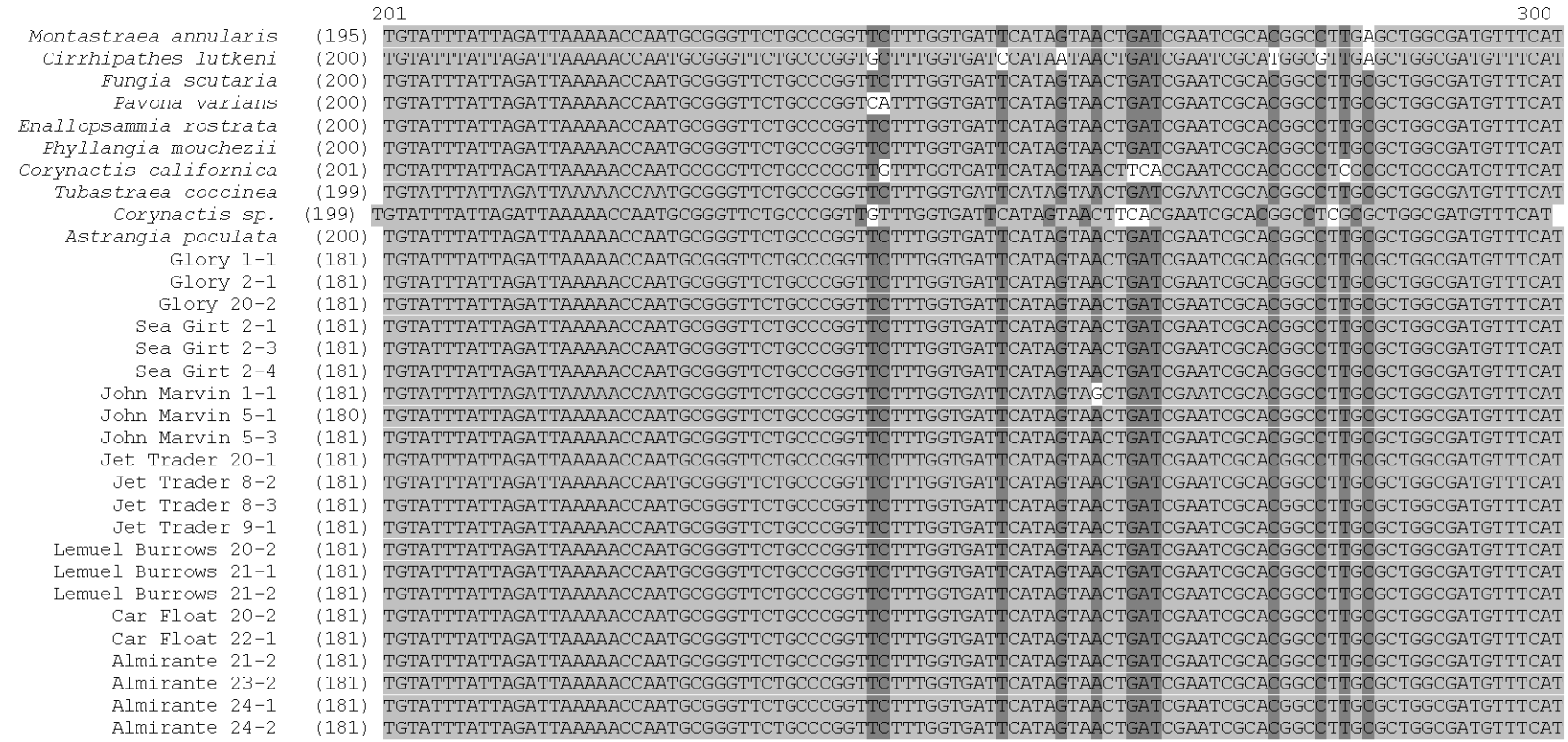

Figure 4. Alignment of 22 NJ artificial Reef samples with 1 full length *Astrangia poculata* (MA) and 9 other Scleractinian species (only part of alignment shown).

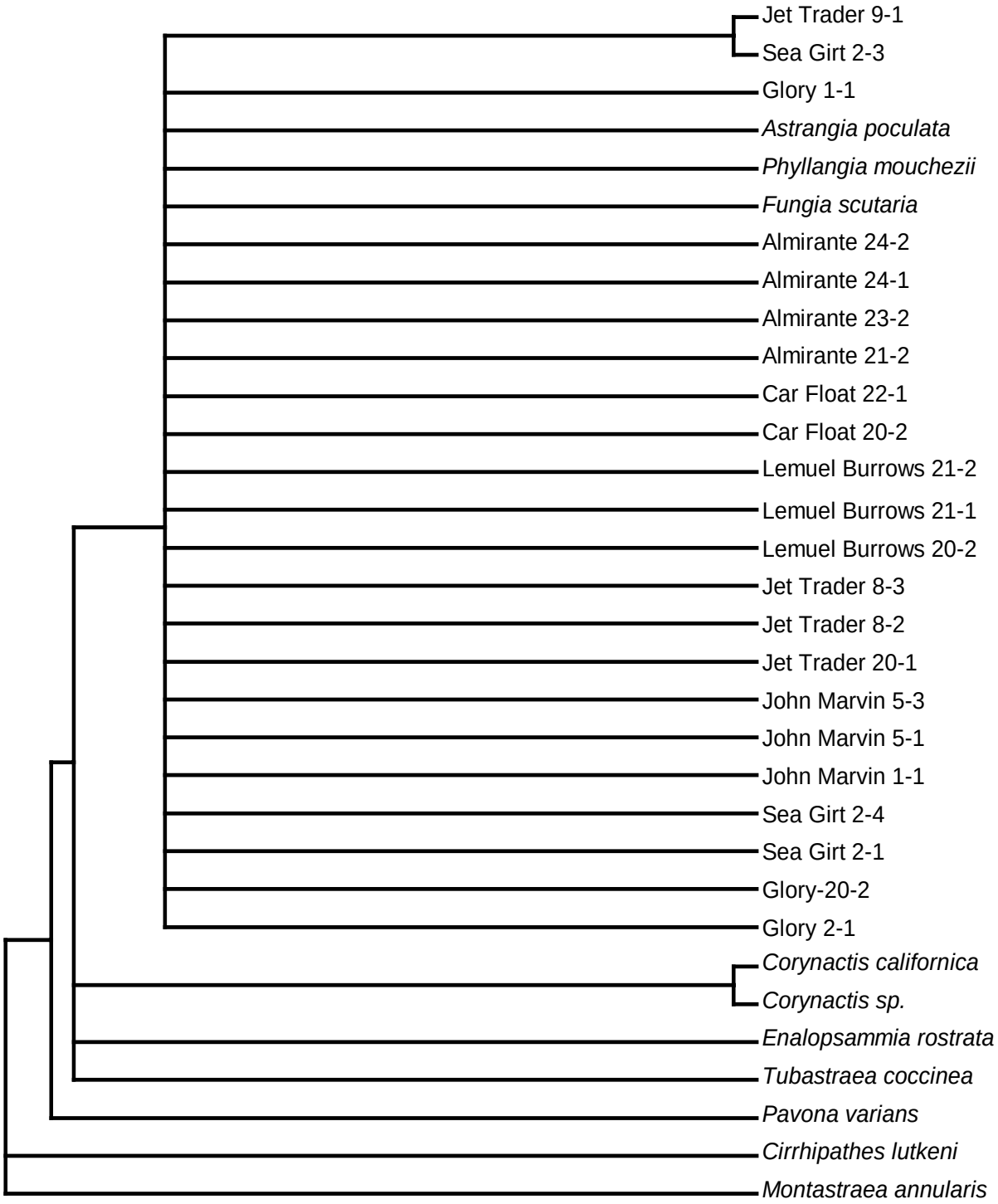

Figure 5. Maximum Parsimony analysis of 554 base pairs of the 18S rRNA gene from collected samples, as well as several related coral sequences

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References

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol. 1990; 56(6): 1919-1925.

Berntson EA, France SC, Mullineaux LS. Phylogenetic relationships within the Class Anthozoa (Phylum Cnidaria) based on nuclear 18S rDNA sequences. Mol Phylogenet Evol. 1999; 13(2): 417-433.

Maddison DR, Maddison WP. MacClade 4. Sunderland, Massachusetts: Sinauer Associates, 2005.

Peters EC, Cairns SD, Pilson MEQ, Wells JW, Jaap WC, Lang JC, Vasleski CE (Cummings), St. Pierre Gollahon L. Nomenclature and biology of *Astrangia poculata* (=*A. danae*, =*A. astreiformis*) (Cnidaria: Anthozoa). Proc Biol Soc Wash. 1988; 101(2): 234-250.

Podar M, Haddock SH, Sogin ML, Harbison GR. A molecular phylogenetic framework for the phylum Ctenophora using 18S rRNA genes. Mol Phylogenet Evol 2001; 21(2): 218-230.

Romano SL, Palumbi SR. Evolution of Scleractinian corals inferred from molecular systematics. Science. 1996; 271(5429):640-642.

Swofford DL. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sunderland, Massachusetts: Sinauer Associates. Version 4, 2003; CD-ROM.

White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJU, White TJ, eds. PCR Protocols. San Diego, CA: Academic Press, 1990: 315–322.