MOLECULAR ASSESSMENT OF DEEP-SEA SCLERACTINIAN CORAL BIODIVERSITY AND POPULATION STRUCTURE OF *LOPHELIA PERTUSA* IN THE GULF OF MEXICO

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ABSTRACT

Geographic patterns of genetic diversity in *Lophelia pertusa* were examined by quantifying genetic diversity present in populations, and assessing levels of genetic differentiation within the Gulf of Mexico (GOM, 5 sampling locations, <1-290 km apart). Patterns of differentiation observed within GOM *Lophelia* were compared to *Lophelia* populations from the Atlantic Ocean continental slope off the Southeastern U.S. (SEUS, 6 sampling locations, 18-990 km apart) and with northeast Atlantic Ocean (NEAO) populations off the coast of Europe (5,400-7,900 km away from sampled U.S. populations). Multilocus genotypes for 190 *Lophelia* individuals were determined using a suite of nine microsatellite markers developed for GOM *Lophelia*. Eighteen individuals (9%) with identical multi-locus genotypes were identified as clones. Populations of *Lophelia* harbored substantial genetic diversity. The majority of populations had unique alleles indicative of little gene flow. Pairwise chord distances were high among all populations and regional groupings of populations resulted from a neighbor-joining clustering analysis. North versus south areas of Viosca Knoll (VK) 826, the most intensively sampled area, had fixation index estimates significantly greater than zero, suggesting little larval mixing. Comparisons of all GOM *Lophelia* populations with the shallowest site, VK862, produced significant fixation indices. Quantitative estimates of hierarchical gene diversity (AMOVA) indicated significant population structure at every level: between the three regions examined; between GOM and SEUS regions; and within the GOM and SEUS regions. Mantel tests identified significant correlations between geographic and genetic distance (an isolation-by-distance pattern) at larger spatial scales, but not within regions. Thus, dispersal of *Lophelia* larvae is generally localized, with occasional long distance dispersal occurring such that some genetic cohesion is retained regionally within the GOM and SEUS. Genetic differentiation observed between these regions indicates more restricted gene flow than

expected, and that the most effective management plan for *Lophelia* may be regional reserve networks.

GOM deep-sea scleractinian coral biodiversity was put into a phylogenetic framework by comparison of 16S mitochondrial DNA (mtDNA) sequences. Four basal lineages were revealed, including the 'complex' and 'robust' corals, the genus *Anthemiphyllia*, plus several species belonging to the family Caryophylliidae. The latter basal coral lineage appears diverse since three GOM species grouped within this clade. Members of the family Caryophylliidae were not monophyletic, but appeared in six clades; the majority of which were in the 'robust' coral group. The high estimate of mtDNA genetic distance reported previously between *Lophelia* in different oceanic regions was not supported.

INTRODUCTION

The spatial scale and pattern of demographic connection has profound implications for survival, regeneration, and evolution of marine communities (Palumbi 2003; Shanks et al. 2003; Cowen et al. 2006). The resilience to disturbance populations may exhibit depends upon the supply of new recruits. If populations are mostly self-seeded, with new recruits produced within the local area, impacts to this area can have serious long-term consequences due to reduced chances for re-colonization. If exchange of recruits is common among populations, chances for re-colonization following disturbance is greater. For corals, which are sessile as adults, our ability to forecast how reefs will respond to changes in the environment requires knowledge of larval dispersal, including the extent to which reefs are self-seeding versus accumulating recruits from elsewhere. However, tracking movements of tiny larvae is difficult, and as a result, there are few studies that have documented actual dispersal distances, and these studies have done so for species with a short larval life (hours to days) and for very short distances (Sammarco and Andrews 1989; Shanks et al. 2003).

Over the past several decades, genetic markers have been utilized for indirect estimates of dispersal ability in a variety of marine species (see reviews by Hellberg et al. 2002; Palumbi 2003). Migration or recruitment results in a genetic signal detectable through comparisons of geographically distributed populations, providing the means for an indirect estimate of population connectivity. Genetic connectivity is the extent to which populations of a species are linked by juvenile or adult exchange (Palumbi 2003). Interpretation of genetic connectivity relies on an understanding of the sensitivity of particular molecular markers and the population genetic models that provide a basis for analyses, including acknowledgement of the powers and limits of each (Hellberg et al. 2002).

The majority of studies of connectivity among corals have been based upon allozymes because more variable markers, such as microsatellites (Fig. 4.1), have proven difficult to develop due to the presence of zooxanthellae that harbor their own DNA (Baums et al. 2005a; Shearer et al. 2005). Generally, evidence of panmixia (high gene flow) has only been observed at small spatial scales (several tens of kilometers or less; see van Oppen and Gates 2006 for review). Surprisingly, brooding corals (with lower expected dispersal ability), as well as those that broadcast spawn and have longer-lived pelagic larvae, often show similar patterns dominated by localized recruitment with only sporadic long distance dispersal retaining some

connectivity over evolutionary timescales (Ayre and Hughes 2000). The localized gene flow observed in many corals is consistent with simulation studies of population-genetic data for marine organisms that demonstrate the spatial scale of connectivity to be smaller than assumed based upon larval dispersal potential (Warner and Cowen 2002; Palumbi 2003; Cowen et al. 2006).

The occurrence of the deep-sea scleractinian coral species *Lophelia pertusa* over vast distances and in different ocean basins may imply either high larval dispersal ability or suitable spacing of reef areas to allow for larval mixing. Although a number of species have previously been described in the genus *Lophelia,* they have either been found to be synonymous with *L. pertusa* (e.g. *L. prolifera*) or have been reclassified to other genera, such that *Lophelia* is considered a monotypic genus (the only species is *L. pertusa;* Zibrowius 1980). *Lophelia* has most commonly been collected in the NEAO, but has also been collected in the Mediterranean, off the coast of West Africa and South America, the Caribbean, the western Atlantic Ocean, the GOM, South of New Zealand, and the Pacific and Indian Oceans (Rogers 1999). Increased current speeds at crests of lithoherms have been documented at *Lophelia* sites (see Rogers 1999), and likely influence its distribution and dispersal. Limited information is available about sexual reproduction and larval type and duration in *Lophelia.* Sexes are separate (gonochoristic), gamete production appears to be seasonal, and oocyte size is indicative of longer-lived, self-feeding (lecithotrophic) larvae, but larval duration is unknown (Waller and Tyler 2005). Generally, coral larvae are poor swimmers and are thought to disperse passively (Harrison and Wallace 1990). Since we know little about larval duration and behavior, or the consistency and strength of local current flows, prediction of *Lophelia* dispersal remains challenging.

The only previous study of *Lophelia* population genetics found substantial structuring between offshore and fjord populations in the NEAO surveyed using microsatellite markers (LeGoff-Vitry et al. 2004a). Moderate, but significant, genetic differentiation was observed among continental margin populations, indicating that the majority of recruitment is highly localized.

Many other coral species were encountered in the GOM along with *Lophelia*, and evolutionary relationships among these coral species were estimated using DNA sequences. For scleractinian corals, high species diversity combined with a weak understanding of homologies among skeletal characters traditionally used in classification makes estimation of evolutionary

relationships difficult (discussed in Romano and Cairns 2000). Fortunately, this knowledge gap can be partially filled through studies of molecular phylogenetics, or the creation of coral "family trees" based upon similarities in gene sequences.

In recent years, a few molecular phylogenetic analyses have produced hypotheses regarding relationships among scleractinian corals. However, the molecular data do not support morphologically-based hypotheses of relationships above the family level, and many families are not supported. For example, coral species examined using 16S mtDNA fall into two major clades, the 'complex' (less calcified skeletons, long 16S gene) and 'robust' (heavily calcified skeletons, short 16S gene; Romano and Palumbi, 1996, 1997; Romano and Cairns 2000; LeGoff-Vitry 2004b). But these clades do not correspond to the seven morphologically-derived suborders (Veron 2000).

Other coral families investigated using molecular techniques have not matched hypotheses based on morphological characters either. Fukami et al. (2004a) found that Atlantic and Pacific corals in the families Faviidae and Mussidae grouped by ocean basin, not by family. The authors concluded that morphological convergence has obscured our understanding of basic taxonomy and such incorrect hypotheses may hamper conservation measures. Clearly, there is much more to learn about anthozoan relationships and molecular techniques provide additional tools to do so.

Deep-sea scleractinian corals are diverse, representing about 40% of known coral species, (615/1482 species; S. Cairns, pers. comm.). The majority of these azooxanthellate corals are solitary and live attached to the substrate between 200 and 1000 m in depth. In molecular phylogenies, deep-sea taxa are found intermingled among other shallow-water coral taxa (Romano and Cairns 2000, LeGoff-Vitry et al. 2004b). The coral family Caryophylliidae, to which *Lophelia* and many other deep-sea species belong, does not appear to be a natural grouping as members of the family are interspersed in both major clades of the coral "family tree" (LeGoff-Vitry et al. 2004b). Family Caryophyllidae is defined morphologically by a combination of skeletal characters that grade into each other and not by one unifying feature (Wells 1956).

STUDY OBJECTIVES

The objectives of this study were three-fold:

1. To document biodiversity of *Lophelia* and other scleractinian corals using informative nuclear and mtDNA markers and appropriate phylogenetic analyses;
2. To develop variable microsatellite DNA markers for Gulf of Mexico *L. pertusa*; and;
3. To use microsatellite markers to quantify local and regional patterns of genetic variation in *Lophelia,* including an assessment of genetic connectivity between reefs, relative contributions of clonal (asexual) and sexual reproduction, and inferred larval dispersal patterns.

MATERIAL AND METHODS

CORAL SAMPLES - Corals were collected at depth (400-800 m) during cruises on the R/V Seward Johnson using the submersible Johnson-Sea-Link (JSL) from Harbor Branch Oceanographic Institute. GOM coral samples were obtained through participation aboard two cruises led by Continental Shelf Associates, Inc. (CSA), and aboard two USGS cruises (USGS-GM-2004-03 and USGS-GM-2005-04; see Chapter 1 of this report, for additional details on the latter USGS cruises). The CSA-led cruises visited three alpha sites, Green Canyon (GC) 354, GC234, and VK826, and four beta sites, Mississippi Canyon (MC) 709, MC885, GC184/185 "Bush Hill" and VK862 (see Fig. 4.2 for locations). Funding provided through NOAA Ocean Exploration (OE) to W. Schroeder allowed the exploration of 3 additional sites in 2005: MC929, VK862-S, and VK826-NE. The USGS cruises immediately followed the CSA-led cruises in 2004 and 2005, and visited the most eastern of the sites on the Upper DeSoto Slope subprovince, VK826 and VK862. Details of GOM cruises and JSL sub dives can be found in final reports to MMS by Continental Shelf Associates, Inc. (2007) and USGS (Chapter 1 of this document and supplemental DVD).

Small pieces of *Lophelia* were collected using the JSL. Although sampling had to be opportunistic in order to meet all dive objectives, attempts were made to move at least 5m between *Lophelia* samples in order to avoid re-sampling of the same genetic individual (clone).

Collections were usually videotaped and location fixes were established at each collection. Once on board the vessel, specimens were assigned identification numbers and were photographed using a digital camera, capturing both branching structure and calicular views. A small piece of tissue was excised from each coral and preserved in duplicate, in DMSO buffer, and on an FTA® Technology Classic card (Whatman®). FTA® cards are impregnated with chemicals that allow capture of nucleic acids that remain stable at room temperature for years. A piece of the skeleton was placed in 95% ETOH as a voucher, and served as back-up of additional tissue for DNA extraction as necessary. We obtained 128 *Lophelia* tissue samples from the four GOM cruises in 2004-2005 described above. The number of *Lophelia* samples obtained from each site was as follows: VK826=90; VK862=28; MC885=1; MC929=2; GC185=1; GC234=4; and GC354=2. The VK826 samples were taken from three regions of the knoll: the southern area where the majority of sub dives were conducted (VK826-S); an extensive *Lophelia* thicket on the northwest region of the knoll, called "Big Blue Reef" by our USGS science team (VK826-NE); and an area on the northwestern region of the knoll near the topographic high (VK826-NW).

Tissue samples were also collected from nine other GOM coral species and identifications were verified by Dr. Stephen Cairns (National Museum of Natural History, Smithsonian Institution): *Caryophyllia berteriana* Duchassaing; one specimen of what is likely a new species of *Caryophyllia*; *C. polygona* Pourtalès; *Labyrinthocyathus langae* Cairns; *L. facetus* Cairns; *Tethocyathus cylindraceus* (Pourtalès); *Desmophyllum dianthus* (Esper); *Javania cailleti* (Duchassaing and Michelotti); and *Madrepora oculata* Linnaeus (see Appendix 4-I for specimen and location information and Appendix 4-II for photographs).

Lophelia can occur on humanmade structures such as oil rigs in the NEAO (Roberts 2002) and on World War II wrecks in the GOM. Coral tissue samples were preserved on FTA® cards by Will Schroeder on a cruise in 2004 exploring these deep Gulf wrecks in the GOM utilizing the ROV Triton XL-11 by Sonsub [cruise details at http://www.pastfoundation.org/ DeepWrecks]. Nine *Lophelia* samples were taken from the Tanker "Gulf Penn" in MC (MMS Lease Block 497) at a depth of approximately 533 m. Three samples of *Madracis myriaster* were collected from the Tanker "Halo" in MMS Lease Block Grand Isle 114 in approximately 146 m depth.

Collections of *Lophelia* were obtained from the SEUS aboard cruises funded through NOAA OE to Ross, Sulak, Nizinski and Baird in 2004 and 2005, and to Brooke, Reed and

Messing, 2005 (see http://www.oceanexplorer.noaa.gov/explorations and Life on the Edge 2005 and Florida Coast Deep Corals 2005 respectively for information on cruises). From these cruises, the following *Lophelia* samples were available for comparison with GOM populations: approximately 200 *Lophelia* samples from 8 sites from Southern Cape Canaveral, Florida to the North Carolina *Lophelia* Banks (cruises of Ross et al. mentioned above, Fig. 4.2) and 31 *Lophelia* samples from the continental slope off of southern Florida and Miami Terrace (Brooke et al. 2005 cruise mentioned above; see Reed et al. 2006 for area details).

Lophelia samples were obtained by an author (SWR) and by Andrew Davies from the Rockall Banks and Mingulay Reef, respectively, in the NEAO off of Scotland on cruises on the R/V Pelagia in 2006. Samples of *Lophelia* DNA from the North Sea have been provided by researchers Carl André and Tomas Lundälv from the Tjärnö Marine Biology Laboratory in Stömstad, Sweden, and have been used in phylogeographic comparisons and for testing mircosatellite markers.

DNA EXTRACTIONS - Total DNA was isolated from preserved coral tissue and/or FTA® card hole punches using the tissue protocol from the PureGene DNA extraction kit (Gentra Systems Inc., Minneapolis, Minnesota). DNA concentrations were determined by fluorescence assay (Labarca and Paigen 1980) and integrity of the DNA was visualized on 1% agarose gels (Sambrook et al. 1989). Comparisons were made between DNA extractions resulting from several tissue preservation methods (DMSO buffer, 95% ETOH, FTA® cards). All produced satisfactory DNA product for subsequent polymerase chain reactions (PCR).

DNA SEQUENCING - DNA sequences were obtained for the 16S mtDNA gene region which allowed for comparisons with large number of scleractinian coral sequences from work of Romano and Cairns (2000), and LeGoff-Vitry et al. (2004b), among others. PCR primers for the 16S mtDNA gene region included universal primers 16Sar and 16Sbr of Palumbi et al. (1991) and/or *Lophelia*-specific 16S primers LP16SF and LP16SR (Le Goff-Vitry et al. 2004b).

The internal transcribed spacer regions (ITS-1 and ITS-2), separating structural ribosomal DNA genes, were amplified from genomic DNA using universal primers 1S and A4 (Chen et al. 1996) in combination with *Lophelia*-specific primers ITS2FA and ITS2RA (LeGoff-Vitry et al. 2004a). In *Lophelia*, some intra-individual variation was observed as heterozygous bases in sequence chromatograms and these sites were coded as ambiguities in consensus sequences. We therefore considered it inappropriate to create a haplotype network for *Lophelia* using these data

because multiple haplotypes were observed for certain individuals. However, we were able to amplify and sequence the ITS regions in both 'complex' and 'robust' corals and have used these data to generate phylogenetic hypotheses to compare with those based on the mtDNA 16S gene. A subset of available sequences that included multiple individuals of *Lophelia* from the NEAO (LeGoff-Vitry et al. 2004a) was included in our analyses.

PCR reactions were carried out using 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 20 mM KCl), 2 mM $MgCl_2$, 0.2 mM of dNTPs, 0.375 µM of each primer, 0.01 mg/mL bovine serum albumin, 2.5 U of Taq polymerase, and ca. 100 ng of template DNA in a 20 µL reaction volume. Thermal cycling used an MJ-Research (Watertown, Massachusetts) PTC-200 thermocycler using the following cycle parameters: initial denaturing at 94 °C for 2 min; followed by 35 cycles of 94°C denaturing (1 min), 52–56°C annealing (1 min), 72°C extension (1 min), ending with a 5 min extension at 72°C. PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase (Promega Corp., Madison, WI) and then used as templates in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, California). Sequencing reactions were analyzed by capillary electrophoresis using the ABI PRISM 3100™ Genetic Analyzer and DNA Sequencing Analysis Software (Applied Biosystems, Foster City, CA).

SEQUENCE ANALYSES - Forward and reverse sequences were assembled for each individual and gene region using Sequencher v. 4.0 (Gene Codes Corporation, Ann Arbor, Michigan). Multiple sequence alignment was carried out using Clustal X v. 1.4b (Thompson et al. 1997), with default gap opening/extension penalties. The resulting alignment was checked by eye using MacClade v. 4.08 for OS X (Maddison and Maddison 2005).

Phylogenetic analyses of DNA sequences were carried out using PAUP* 4.0b10 (Swofford 2002) and MrBayes v. 3.04 (Bayesian analysis; Huelsenbeck and Ronquist 2001). Three tree reconstruction methods were utilized in PAUP, including neighbor-joining, maximum parsimony, and maximum likelihood analyses. For parsimony analyses, heuristic searches were run using unweighted, parsimony-informative characters with alignment gaps treated as missing data and the following settings: starting trees for branch swapping were obtained via stepwise addition, 50 random addition sequences per run, and tree bisection-reconnection (TBR) branch swapping on best trees. The most appropriate model of evolution was determined for each data set using the Akaike information criterion (AIC) implemented in the program Modeltest v. 3.7

(Posada and Crandall 1998), and this model was approximated for distance-based analyses and implemented for maximum likelihood and Bayesian analyses. Nodal support was assessed using 1,000 bootstrap replicates (Felsenstein 1985) for distance and maximum parsimony. Additionally, Bayesian inference was used to assess scleractinian relationships with model settings following the best AIC model for the data determined using Modeltest. The analysis was run for 10,000,000 generations with random starting trees, default priors, two Markov chains and sampling every 1,000 generations. Stationarity of the MCMC analyses was determined by plotting negative log likelihood values and parameter estimates against generation times. Trees from this burn-in (3,500 trees) were discarded before posterior probabilities were calculated.

LOPHELIA GENOTYPING - Attempts were made to use microsatellite markers developed for NEAO *Lophelia* (LeGoff and Rogers 2002). Little amplification resulted using the LeGoff microsatellite primers for GOM *Lophelia* samples. Therefore, we developed a new set of markers for GOM *Lophelia*.

Four microsatellite-enriched libraries were prepared for GoM *Lophelia* by Genetic Identification Services, Inc., Chatsworth, CA, USA (GIS; http://www.genetic-id-services.com/) using magnetic bead capture technology and the AAC (A library), AAAT (B library), TAGA (C library), and TGAC (D library) microsatellite motif capture molecules, following methods of Peacock et al. (2002). Isolation and sequencing of plasmid DNA from each library were performed as described in Eackles and King (2002). Approximately 300 clones were screened for usable microsatellite DNA. Of this group, 81 were deemed unique, of sufficient length (>10 repeats), and possessed adequate flanking regions for primer development. Eleven primer pairs (i.e., markers) have produced unambiguous PCR products interpreted as allelic variation (i.e., are polymorphic, Table 4.2) and forward primers were synthesized with a 5' end modification of one of three fluorescent dyes (FAM, HEX or NED ABI labels). Six additional primer pairs have been screened in a test panel of *Lophelia* but have not been used in this report. The PCR consisted of 100-200 ng of genomic DNA, 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2 mM $MgCl_2$, 0.2 mM of dNTPs, 0.5 μM of each primer, 0.01 mg/mL bovine serum albumin, 0.5 U of Taq polymerase, in a 10-20 uL reaction volume. Thermal cycling used an MJ-Research (Watertown, Massachusetts) PTC-200 thermocycler using the following cycle parameters: initial denaturing at 94°C for 2 min; followed by 35 cycles of 94°C denaturing (40 s), 58°C annealing (40 s), 72°C extension (1 min), ending with a 5 min extension at 72°C.

Amplified, labeled PCR products were subjected to capillary electrophoresis on an ABI Prism™ 3100 Genetic Analyzer (Applied Biosystems). Genescan v. 3.7 Analysis software and Genotyper v. 3.6 Fragment analysis software (Applied Biosystems) were used to score, bin, and output alleleic (and genotypic) data (see King et al. 2001 for details).

POPULATION GENETIC ANALYSES - Loci were tested for fit to statistical assumptions of population genetic analyses. Individuals with identical multilocus genotypes were identified using the program GENECAP (Wilberg and Dreher 2004). All individuals with identical multilocus genotypes collected from the same site were regarded as clones and were removed from the dataset for subsequent analyses so that each unique multilocus genotype was represented once. The probability of identity (PI), or the probability of two individuals sharing the same genotype, was calculated using GENECAP. For this calculation, a match probability of $P_{SIB}<0.05$ was used, as suggested by Woods et al. (1999). Allele frequencies, allelic patterns and expected heterozygosities under Hardy-Weinberg (H-W) equilibrium, and the number of private alleles were calculated using GenAlEx v6 (Peakall and Smouse 2006). Loci were tested for linkage disequilibrium using the randomization method of Raymond and Rousset (1995) for all pairs of loci in GENEPOP v. 3.2 (Raymond and Roussett 1995). Sequential Bonferroni adjustments for multiple tests (Rice 1989) were used on these and other multiple tests.

Several techniques were used to describe genetic relations between populations or individuals as estimated from microsatellite data. Levels of heterozygosity and polymorphism by locus and population were compared between populations in GenAlEx. Differences in allele frequencies between populations were tested for statistical significance using the genic differentiation randomization test in GENEPOP. Results were combined over loci using Fisher's method (Sokal and Rohlf 1994). Pairwise genetic distances between populations were calculated using the chord distance (Cavalli-Sforza and Edwards 1967) in BIOSYS (Swofford and Selander 1981). Chord distances were subjected to a neighbor-joining algorithm using the NEIGHBOR program in Phylip v. 3.6 (J. Felsenstein, University of Washington), resulting in an unrooted phylogram representing relationships among populations that was visualized using TREEVIEW (Page 1996). The strength of support for nodes in the phylogram was tested by bootstrapping over loci in NJBPOP (J.-M. Cornuet, Institut National de la Recherche Agronomique, Montpellier, France).

Genetic structure was quantified using *F*-statistics (Weir and Cockerham 1984). F_{ST} provides a measure of the genetic differentiation among populations by identifying the proportion of the total genetic diversity that separates populations, which is assumed to accumulate from migration and gene drift. F_{ST} ranges between 0 (undifferentiated, with similar allele frequencies) and 1 (alleles are not shared between populations). Another measure of genetic differentiation, R_{ST} (Slatkin 1995), which measures mutational differences between alleles (i.e. taking into account differences in allele size), was calculated using GenAlEx. Pairwise F_{ST} and R_{ST} estimates were tested for significance (difference from zero) by adjusted random permutations using GenAlEx.

Pairwise F_{ST} and R_{ST} estimates were used in analyses of molecular variance (AMOVA; Excoffier et al. 1992) as calculated using GenAlEx. AMOVA allows for hierarchical partitioning of genetic variation among populations and regions. Separate analyses were run for comparisons of all 13 *Lophelia* populations grouped into three regions (GOM, SEUS, and NEAO), on 11 GOM and SEUS populations, and on five GOM populations and six NWAO populations without a regional component. GenAlEx was used to test statistical significance of observed differentiation through 9,999 random permutations.

Isolation by distance (IBD) was examined by comparing matrices of genetic (chord distance, F_{ST} and R_{ST}) and geographic distances between each pair of collections. Separate analyses were run based on the groupings used for AMOVA above. A geographic distance matrix was estimated using ArcView GIS v. 3.2 (Environmental Systems Research Institute 2001) as the shortest ocean distance between collections sites, which included distance around the Florida peninsula for comparisons between GOM and Atlantic Ocean populations. Mantel randomization tests (Mantel 1967) were used to assess significance of the correlation in GenAlEx.

RESULTS

CORAL PHYLOGENY - Our multiple sequence alignment of mtDNA 16S sequences included 128 sequences representing all 7 scleractinian sub-orders in 20 of the 24 extant families (Appendix 4-I) and was 830 base pairs in length. Data generated for this study added 15 species in five families relative to previous analyses. Following a recent analysis (Medina et al. 2006),

two octocoral species (sub-class Octocoralia) were used as outgroups. The resulting topologies from Bayesian analysis (Fig. 4.3) and parsimony analysis of 384 parsimony informative sites (Fig. 4.4) were similar to previous analyses (discussed above) in that most coral species fall into two clades, referred to as the 'complex' (which have longer 16S gene sequences and are generally less calcified) and the 'robust' corals (short 16S gene, more heavily calcified). Bootstrap support was high for the 'robust' corals (100%), but was lower for the 'complex' corals (72%) unless representatives of the Order Corallimorpharia are included with the 'complex' group (bootstrap support 92%, Fig. 4.4). A strong relationship between the Corallimorpharia and the 'complex' scleractinians was also reported by Medina et al. (2006) in an analysis of entire mitochondrial genomes. Several caryophylliid species form a basal clade in the 'complex' corals, though this placement in the parsimony tree is not well supported (Fig. 4.4, bootstrap=64%). Included in this basal clade are the newly sequenced GOM species *Tethocyathus cylindraceus* and two species of *Labyrinthocyathus*, together with *Ceratotrochus*, *Odontocyathus* and *Vaughnella*, all deep-sea species from the Family Caryophylliidae, subfamily Caryophylliinae. In the phylogenetic analysis presented by Romano and Cairns (2000), the placement of included members of this group (*Ceratotrochus*, *Odontocyathus* and *Vaughnella*) was ambiguous, but in the analysis by LeGoff-Vitry et al. (2004b), these caryophylliids were allied with the 'complex' corals. Results from Bayesian analysis of our 16S data allies this caryophylliid group with the 'robust' corals instead of the 'complex' corals (Fig. 4.3, posterior probability=93). Taken together, it is most conservative to conclude that there are up to four basal lineages of scleractinian corals: 'complex' (including corallimorphs) and 'robust' corals, the monoytypic genus *Anthemiphyllia* (family Anthemiphylliidae), and the caryophylliid clade containing *Labyrinthocyathus* and *Tethocyathus*.

Similar to previous phylogenetic analyses, in both Bayesian (Fig. 4.3) and parsimony (Fig. 4.4) analyses, there was little support for monophyly of scleractinian sub-orders, with the exception of sub-order Dendrophylliina, represented by 10 species in the family Dendrophylliidae. When multiple individuals of a species were included, they grouped together in the phylogeny, including cases where the same species was collected from different ocean basins (e.g. *Lophelia* and *Madrepora oculata* from the GOM, SEUS and NEAO). Coral lineages within the 'robust' clade were more divergent relative to lineages within the 'complex' corals, as branch lengths between clades are longer and posterior probabilities are higher (Fig. 4.3).

Due to the difference in mtDNA 16S sequence length between the major scleractinian clades, 'complex' and 'robust' corals were aligned separately. Overall topologies of estimated relationships were similar with all alignments, but consistency indices were higher for separate alignments, indicating less homoplasy. Therefore, phylogenetic analyses performed on the 'complex' and 'robust' alignments are discussed to highlight details of relationships within the two major clades.

The alignment of 'robust' corals included 68 sequences and was 549 bases in length, of which 191 sites were parsimony-informative. Six well-supported clades (or groupings) were recovered from parsimony analysis (Fig. 4.5), yet relationships between the clades remains ambiguous since bootstrap support was lacking. Five individuals of *Madrepora oculata* collected from the GOM, SEUS and NEAO form a well supported clade, however *Oculina patagonica*, also belonging to the Oculinidae, did not group with *Madrepora*, as noted by LeGoff-Vitry et al. (2004b). *Madracis myriaster* individuals from the GOM and SEUS group together and are the sister group to *Pocillopora* from the same family, Pocilloporidae. Between well-supported clades of 'robust' corals (e.g. *Madrepora* and *Lophelia*), sequence divergence ranged from 8-16% (data not shown).

In contrast to the substantial genetic distance at mtDNA 16S reported between *Lophelia* samples from the NEAO and the southwest Atlantic Ocean (SWAO) off the coast of Brazil (approximately 7% sequence divergence, LeGoff-Vitry et al., 2004b), we found little differentiation between these *Lophelia* samples or any others from the GOM and SEUS included in our phylogenetic analysis. Upon reexamination of the sequence data for inclusion in this report, it was apparent that the substantial genetic differences reported previously by LeGoff-Vitry et al. (2004b) were due to gaps in their sequence alignment, not by nucleotide substitutions. Since gaps were not included as characters in our parsimony analysis, the *Lophelia* 16S sequences from LeGoff-Vitry et al. (2004b) were identical to those we report. The only substitutions among *Lophelia* samples occurred from those collected from the wreck "Gulf Penn" and one from the North Sea, yet sequence divergence remained less than 1% when compared with other *Lophelia* samples (data not shown, but note branch lengths in Fig. 4.5). It is possible that the high divergence estimates reported previously resulted from poor sequence alignment or interpretation.

The genus *Desmophyllum* was undifferentiated relative to *Lophelia* and both species belong to the same subfamily, Desmophyllinae (Vaughan and Wells 1943). *Caryophyllia* species, including individuals of *C. berteriana* from the GOM and SEUS, *C. inornata*, *C. ambrosia* and a new *Caryophyllia* species from the GOM formed a cluster that included the genera *Cyathoceras and Cristopatrochus,* and all of these genera belong to the same subfamily, Caryophylliinae. *Caryophyllia berteriana* and a new GOM *Caryophyllia* species were undifferentiated (bootstrap=84, Fig. 4.5). Despite their inclusion in different subfamilies within the Caryophylliidae (Desmophyllinae and Caryophylliinae, respectively), estimates of sequence divergence between *Lophelia* and *Caryophyllia* species were small (1.2-1.5%, data not shown).

Our alignment for 'complex' corals included 63 16S sequences and was 830 bases in length, of which 322 sites were informative for parsimony analysis. A phylogram of one of 4,763 most-parsimonious trees is shown in Figure 4.6. Again, basal relationships in the phylogeny, including *Anthemiphyllia* and the caryophylliid grouping including *Labyrinthocyathus* and *Tethocyathus,* remain ambiguous in placement, with the exception that the corallimorphs are now supported as the sister group of the 'complex' corals (bootstrap = 92%, Fig. 4.6). When multiple individuals from a taxon were included, they grouped together (e.g. *Javania cailleti*, family Flabellidae; *Enallopsammia* species, family Dendrophylliidae and *Labyrinthocyathus,* family Caryophylliidae). In many cases, species belonging to the same family clustered together supporting their evolutionary relatedness (e.g. Flabellidae, Dendrophylliidae, Fig. 4.6), yet bootstrap support was observed only for the following families: Fungiacyathidae, Turbinoliidae, Acroporidae, and Agariciidae. *Thecopsammia socialis* and *Bathypsammia fallosocialis*, both in the family Dendrophylliidae and common in the SEUS, are very similar morphologically and genetically at the 16S gene, yet they are distinguishable (bootstrap = 95%, Fig. 4.6). Two other dendrophylliid species, *Enallopsammia profunda* (collected at less than 1,000 m in the SEUS) and *E. rostrata* (collected at 1,460 m at GC862) grouped together and differed by approximately 1% sequence divergence. *Polymyces fragilis* and *Javania cailleti*, both in the family Flabellidae, fell into the same clade along with other genera from the Flabellidae.

We have attempted to amplify several non-coding regions of the mitochondrial genome that have been useful in previous studies of coral phylogeography (Table 4.1). Despite attempts using different PCR conditions and DNAs, little success has resulted, likely due to the distant

relationship between *Lophelia* and the acroporid coral species used to design most primers. We have amplified and sequenced clones of *Lophelia B*-tubulin, and high intra-individual variation was present. One of the single-copy nuclear gene markers designed by Severance et al. (2004), maSC12, has been amplified and sequenced in *Lophelia*, *Dasmophyllum*, and *Balanophyllia,* but variation within species was low.

For the nuclear ITS regions, sequences from the 'robust' and 'complex' coral clades were too divergent to reliably align and were analyzed separately. The sequence alignment for 'robust' corals included 106 sequences, was 1,628 bp in length, and 363 sites were informative for parsimony analysis. Analyses performed with different optimality criteria (parsimony, maximum likelihood and neighbor-joining) produced different topologies with regards to relationships among genera and this is reflected in the low bootstrap support (Fig. 4.7). Little differentiation within clades (or groupings comprised of genera) resulted in any analyses. For comparison with the phylogram presented in LeGoff-Vitry et al. (2004a), maximum likelihood results of the ITS analysis are shown in Fig. 4.7. Little differentiation or phylogeographic structure was observed at ITS in *Lophelia* samples from the GOM, SEUS, and NEAO, which is similar to results obtained by LeGoff-Vitry et al. (2004a). Two caryophylliid genera fell within the *Lophelia* clade, *Desmophyllum* (subfamily Desmophyllinae, like *Lophelia*) and *Solenosmilia* (subfamily Parasmiliinae). Two *Caryophyllia* species were the sister group to the *Lophelia* clade, as in the 16S analysis (Fig. 4.5). *Madracis myriaster* from the GOM wreck "Halo" formed a separate clade within the *Madracis* clade.

There were 25 sequences representing 'complex' corals in our alignment of nuclear ITS, which was 1,098 bp in length. The topology of the phylogenetic tree was stable when different analysis types were used, and a parsimony tree is shown in Figure 4.8. Coral families were well supported. *Javania cailleti* from GC was differentiated from SEUS *Javania* (1.2% sequence divergence, Fig. 4.8). The morphologically similar species *Thecopsammia socialis* and *Bathypsammia profunda* were easily distinguishable by their ITS sequences (14% divergence, note branch lengths in Fig. 4.8). Two other dendrophylliid species, *Enallopsammia profunda* (collected at less than 1,000 m in the SEUS) and *E. rostrata* (collected at 1,460 m at GC 862) grouped together as they did with 16S sequence data (Fig. 4.8), yet they were more divergent at ITS (approximately 4.3% sequence divergence).

LOPHELIA POPULATION GENETICS - Eleven polymorphic microsatellite loci were developed from the *Lophelia* microsatellite-enriched DNA libraries. Locus designation, GenBank accession numbers, repeat motifs, PCR product sizes, numbers of alleles observed, and primer sequences for these microsatellite markers are listed in Table 4.2. Markers were highly polymorphic and ranged from 11-53 alleles per locus, with an average of 27.4 alleles per locus (Table 4.2).

A total of 190 *Lophelia* individuals representing 15 collections from the GOM, SEUS and NEAO were genotyped at the 11 *Lophelia* microsatellite loci developed for this project. Two loci, LpeC126 and LpeC149, had significant deviations from Hardy-Weinberg equilibrium expectations (heterozygote deficits) for most populations that are likely due to the presence of null alleles (alleles that do not amplify), and were dropped from further analyses (data not shown). In order to obtain a minimally adequate sample size for western GOM populations, three individuals from GC, two individuals from MC and five individuals from the wreck Gulf Penn from MC were combined as one 'population'. With these individuals combined, the data were re-tested for conformance to Hardy-Weinberg equilibrium expectations to insure that the number of significant deviations did not increase as would be expected with a mixture of different populations.

Of the 190 *Lophelia* genotyped, 10 pairs of individuals and two triplets had identical multi-locus genotypes. The probability that two unrelated individuals sampled from the same population could have identical multi-locus genotypes is very low since the estimate of P_{SIB} was very small (2.5×10^{-6}). Given that all identical genotypes were from the same collection site, these individuals were considered clonemates and only one of each unique multilocus genotype was included in further analyses.

For the remaining nine loci, numbers of alleles, observed and expected heterozygosity, and fixation indices by locus and population are shown in Table 4.3 and summarized graphically in Figure 4.8. Generally, expected levels of heterozygosity were high (between 0.65 and 0.8, Fig. 4.9) in all populations. Among the Atlantic Ocean populations, the Jacksonville Lithoherms population had the highest mean number of alleles (14, Table 4.3, Fig. 4.9) and VK826-S had the highest mean number of alleles from GOM populations (13.4, Table 4.3, Fig. 4.9). By comparing the mean number of common alleles with overall numbers, it can be seen that populations with more alleles have a larger proportion of rare alleles. For example, for the

Jacksonville Lithoherms, the Stetson Banks, and VK826-S *Lophelia* populations, the mean number of common alleles was less than half of the overall number of alleles, whereas other populations, such as Miami Terrace, the Western GOM and the two NEAO populations, were comprised of common alleles (Fig. 4.9).

Values of F_{IS} are expected to be close to zero with random mating. A positive F_{IS} value is indicative of a heterozygote deficit whereas a negative F_{IS} value indicates an excess of heterozygotes. Both negative and positive F_{IS} values resulted for each locus in different populations, though most were not significant after correction for multiple tests. Significant heterozygote deficits (positive F_{IS} values) were detected at five of the nine loci, but at a maximum of two out of 13 populations scored (Table 4.3). Since heterozygote deficits were not detected across all populations for any locus, results were not consistent with the presence of null alleles. The deviations from Hardy-Weinberg expected heterozygote proportions observed could be explained by either of two possible scenarios. The first is sampling error, which seems plausible given the small sample sizes at most sites relative to the large numbers of alleles observed for the markers. The second involves the presence of several random-mating populations sampled at a site in which any variance in allele frequency among them would produce a deficit of heterozygotes, termed a Wahlund effect (Wahlund 1928). For corals, heterozygote deficiencies have been observed in many studies (see Discussion).

Minimal linkage disequilibrium was detected as one out of 455 tests (0.2%) was significant after adjustment for multiple tests for the combination of LpeC151 and LpeC52 in the Jacksonville Lithoherms *Lophelia* population. The resulting disequilibrium may have resulted from sampling error, mixing of year classes in our sample, population mixing, or a combination of these, rather than physical linkage among these loci.

Genetic structure was detected over small and large spatial scales between sites. Private alleles (alleles unique to that population) were detected at all sites except Miami Terrace and the Western GOM (Table 4.3, Fig. 4.9), providing an indication of subdivision among sampling sites. Among SEUS populations, Jacksonville Lithoherms and Stetson Banks South had the most private alleles (11 and 9 respectively), and among GOM populations, VK826-S had the most private alleles (6, Table 4.3). For both Stetson Banks South and VK826-S, this high number of private alleles is especially interesting given the close proximity of other sampled sites (Stetson Banks North 40 km; VK826-NE and VK826-NW, less than 2 km, respectively).

Similarly, there were private alleles at each of the Cape Lookout *Lophelia* Banks sites that are located about 18 km apart. Private alleles occur at every locus and for some loci, private alleles occur regionally at one end of the allele size spectrum (data not shown).

To investigate evolutionary relationships in allele frequencies, pairwise genetic distances (chord distance, D_C, Cavalli-Sforza and Edwards 1967) were calculated between all sites (Table 4.4, above diagonal). Chord distance performs well at generating correct phylogenetic tree topology (Takezaki and Nei 1996) but also may have sample size related bias (Ruzzante 1998). Estimates of D_C were high among all populations. Among GOM populations, chord distances ranged between 0.42 – 0.51, whereas estimates among SEUS populations were slightly higher, ranging from 0.45-0.60. Trans-Atlantic comparisons produced the highest chord distances (0.602 – 0.687). The underlying genetic structure of the chord distance matrix is illustrated with an unrooted neighbor-joining tree (Fig. 4.10). *Lophelia* populations group by ocean region with bootstrap support for the clustering of GOM populations. Reflective of high chord distances among populations and unique genetic signatures, branch lengths leading to all populations were large. The longest branches were those between Mingulay Reef and Rockall Banks from the NEAO that had a chord distance of 0.62 (Fig. 4.10, Table 4.4).

Heterogeneous allele frequencies were observed throughout the study area, yet there were fewer significant differences within regions. When testing allele frequency heterogeneity between GOM populations, 30% of the comparisons were significant after correction for multiple tests ($\alpha = 0.05$, $P < 0.0015$). VK862 was significantly differentiated from the Western GOM population and from two of the three VK826 sites (south and northeast; data not shown). Comparing SEUS populations, 40% of comparisons exhibited departures from homogeneity in allele frequencies. Significant comparisons involved Stetson Banks North and South, plus the Cape Lookout *Lophelia* Banks South population and all others. All comparisons between the GOM and SEUS populations were significant, but 70% remained significant after Bonferroni correction for multiple tests. VK862 had significant heterogeneity in allele frequencies when compared with all SEUS sites and most comparisons between VK826 sites and SEUS sites were also significant, with the exception of Cape Lookout *Lophelia* Banks North. In comparisons of trans-Atlantic Ocean *Lophelia* populations, 75% were significant.

Fixation index (F_{ST}) estimates between populations were more variable than chord distances, ranging from 0 – 0.12 (Table 4.4, below diagonal). Among SEUS populations,

estimates of F_{ST} ranged from 0.001-0.06, with highest estimates from comparisons with Miami Terrace, which were all significantly greater than zero. Among GOM populations, estimates of F_{ST} ranged from 0 – 0.056 with all comparisons with VK826-NE and with VK862 (with the exception of VK826-NW) significantly greater than zero. Some populations that were in close proximity had relatively high pairwise estimates of F_{ST}. For example, the F_{ST} estimate between VK826-S and VK826-NE, separated by less than two kilometers, was 0.029, which was significantly greater than zero. Similarly, the F_{ST} estimate between the NE and NW VK826 sites was also high (0.049; Table 4.4) and was significantly greater than zero. In the SEUS, the Cape Lookout *Lophelia* Banks North and South populations were separated by 18 km and had F_{ST} = 0.024. Conversely, some populations appeared well connected at larger geographic distances. For example, in the GOM, both VK826-S and NW had low estimates of F_{ST} when compared with the Western GOM though separated by close to 300 km, and in the SEUS, the Stetson Banks *Lophelia* populations had low F_{ST} estimates when compared with the Jacksonville Lithoherms population, approximately 250 km apart. Estimates of F_{ST} between GOM and SEUS populations ranged from 0.019 – 0.077 and nearly all were significant, indicating genetic discontinuities between the GOM and SEUS. Trans-Atlantic Ocean F_{ST} estimates were all significant and ranged from 0.064 – 0.117 (Table 4.4).

Quantitative estimates of hierarchical gene diversity (AMOVA) indicated significant genetic population structure at all levels tested, with the greatest variance within collections (Table 4.5). When collections were grouped into the three ocean regions from which they were collected (13 populations: GOM, SEUS, NEAO), 2% of the genetic variation was distributed among regions, 3% between collections within regions, and 95% ($P < 0.0001$) within collections, with an overall F_{ST} estimate of 0.047 (Table 4.5). AMOVA results from GOM and SEUS populations (11 populations) also detected significant structuring, with more variance within populations (97%), and slightly less among regions (1%) and within regional populations (2%), with an F_{ST} estimate of 0.038. Significant structuring was also detected through AMOVA in both the GOM (5 populations) and the SEUS (6 populations), with 2% of the variance between populations and 98% within populations. Estimates of F_{ST} were considerably lower than the regional AMOVA estimates (GOM = 0.015; SEUS = 0.016). All of the AMOVAs were also significant when R_{ST} was used instead of F_{ST}. In three out of the four AMOVAs, the overall R_{ST} was about twice the F_{ST} estimate, the exception being within the GOM, where both estimates of

differentiation were similar (Table 4.5). This indicates that the differentiation among most of the populations examined is considerable as mutational processes have been sufficient to increase differentiation over that observed through random genetic drift.

Mantel tests identified a statistically significant correlation between pairwise matrices of geographic (ocean, km) distances and genetic (chord) distances among all sampled *Lophelia* populations (R^2=0.53, P=0.0002; Fig. 4.11A). Similarly, a significant correlation was detected when geographic and genetic distances were compared among GOM and SEUS populations, though the correlation was not as strong (R^2 =0.26, P=0.002; Fig. 4.11B). These correlations were also significant when F_{ST} was used as the genetic distance (not shown). These findings generally conform to the expectations of the stepping stone model of population structure (Slatkin 1987), in which gene flow occurs among nearest neighbor populations and those at greater distances become more differentiated. Such a pattern is called 'isolation-by-distance' (Wright 1943).

Mantel tests did not identify statistically significant correlations between pairwise matrices of geographic distances and genetic (chord) distances among *Lophelia* populations in either the SEUS or GOM (R^2=0.31, P=0.148 and R^2= 0.004 P=0.73; Figs. 4.12A and B, respectively). The Mantel results were significant among SEUS populations when F_{ST} was used as the genetic distance (R^2=0.58, P=0.021; not shown). At smaller spatial scales (less than about 600 km), population structure is more consistent with an island model (Wright 1969), where all populations are equally linked, yet hierarchical structuring detected through AMOVA does not support an island model.

DISCUSSION

CORAL PHYLOGENY - Evolutionary relationships of 10 GOM and nine SEUS scleractinian coral species were put into a phylogenetic framework including other deep and shallow water corals through analysis of mitochondrial 16S sequence data. The same four basal scleractinian lineages identified in our 16S phylogenetic analysis ('complex' and 'robust' corals, basal caryophylliids, plus *Anthemiphyllia*) were recognized by Romano and Cairns (2000). Romano and Palumbi (1996) concluded that the separation between the 'complex' and 'robust' clades was ancient, taking place at least 300 Ma, before the first appearance of the scleractinian

skeleton in the fossil record (240 Ma). The ancient nature of the basal scleractinian lineages may imply that the defining characteristic, the aragonitic calcium carbonate skeleton, has arisen independently up to four times (Romano and Cairns 2000).

The majority of GOM species included in the phylogeny were from the family Caryophylliidae, a speciose genus including many deep sea taxa that do not form a single evolutionary lineage (Romano and Cairns 2000; LeGoff-Vitry et al. 2004b). Our analyses support the polyphyletic origin of this family as six unique caryophylliid lineages were observed that weakly correspond to morphologically defined subfamilies. One of these caryophylliid lineages appears ancient in origin due to it's basal placement between 'complex' and 'robust' clades, and included three western GOM species whose relationships had not been estimated using molecular data previously (*Tethocyathus cylindraceus*, *Labyrinthocyathus langae* and *L. facetus*). These GOM caryophylliids grouped with other deep sea corals (*Ceratotrochus*, *Odontocyathus* and *Vaughanella*), and all belong to the subfamily Caryophylliinae. The inclusion of these GOM species in the basal group containing deep sea caryophylliids implies higher biodiversity within this ancient lineage than previously thought. Besides *Thalamophyllia* that grouped with species belonging to the Agariciidae in the 'complex' clade, the majority of caryophylliids fall within the 'robust' coral clade. It is apparent that the use of a combination of morphological characters to classify caryophylliid species does not adequately reflect their evolutionary histories. The use of molecular data may be especially useful in interpretation of the evolution of morphological features and historical relationships in deep sea scleractinian species.

Interpretation of skeletal homologies and evolutionary relationships for some deep sea corals from families other than the caryophylliids and oculinids (*Madrepora*) may be less complicated. For example, *Javania* and *Polymyces* fell within the monophyletic family Flabellidae. Similarly, *Thecopsammia*, *Bathypsammia*, and *Enallopsammia* formed a monophyletic grouping with members of the family Dendrophylliidae.

Samples of *Lophelia* from the Atlantic Ocean, GOM, and Brazil were minimally differentiated at 16S. These results stand in contrast to the large genetic distance previously reported between *Lophelia* from NEAO and SWAO (7%; LeGoff-Vitry et al. 2004b). The discrepancy between results could be explained by the quality of the sequence alignments or from difference in computation of genetic distance. When we used similar methods of distance calculation, none of the *Lophelia* samples were greater than 1% divergent. Our results are more

congruent with the slow rate of mutation observed in mtDNA previously (e.g. less than 1% divergence between three species of *Montastraea* in the entire mitochondrial genome; Fukami and Knowlton 2005), and in particular with results based upon the 16S gene region, which have not provided resolution below the family level (Romano and Cairns 2000).

Although scleractinian biodiversity generally decreases from East to West in the GOM (Cairns 1978; 1979; Cairns et al. 1993), the majority of the species obtained were from Green and Mississippi Canyons where *Lophelia* is less abundant. At the VK sites, *Lophelia* was the only scleractinian collected. The West Florida Slope is the most biodiverse region for GOM scleractinian coral species (Cairns et al. 1993).

The search for regions of DNA sequence that are variable within species has frustrated coral biologists (Baums et al. 2005a; Shearer et al. 2005). Unlike most other animals surveyed where protein-coding or mtDNA genes are commonly utilized to describe patterns of variation within species, anthozoan mtDNA evolves slowly and as a result, little variation within species exists (Romano and Palumbi 1997; van Oppen et al. 1999; Shearer et al. 2002; Fukami and Knowlton 2005). The search for DNA sequence variability within species may be especially difficult for 'robust' corals since they are more ancient and quite divergent from the complex corals most studied (Medina et al. 2006). Our limited success using primers designed for 'complex' corals on *Lophelia* demonstrates this difficulty. The ITS sequence data presented here were not highly variable within the species examined, but did highlight the differentiation among major clades.

LOPHELIA POPULATION GENETICS - This study, which represents the first population genetic comparison of *Lophelia* from different ocean basins, does not support the null hypothesis of a homogeneous gene pool among populations inhabiting the GOM, the SEUS, and the NEAO. Instead, preliminary results indicate discontinuities between ocean basins, or phylogeographic breaks, that may indicate regional adaptation or vicariant events. At this broad scale, a weak pattern of isolation by distance was detected indicating that gene flow is restricted among geographically distant populations. Thus, regional reserve networks would be the most effective management strategy for *Lophelia* in the GOM and SEUS.

Within regions, patterns of connectivity between *Lophelia* populations were more 'complex' than can be explained by restricted gene flow with increasing ocean distance (isolation by distance). Significant structuring was detected within each region, yet surprisingly,

some populations in close proximity to one another had relatively high pairwise estimates of F_{ST} (e.g. VK826-S and VK826-NE), while other populations seemed well connected (with lower estimates of F_{ST}) at larger geographic distances (e.g. VK826-NW and Western GOM, Stetson Banks and Jacksonville Lithoherms).

Incomplete genetic mixing, like that observed in GOM *Lophelia,* has been reported for broadcast spawning corals (Ayre and Hughes 2000, 2004; LeGoff-Vitry et al. 2004a; Whitaker 2004), many other sessile marine invertebrates (reviewed by Hellberg et al. 2002; Palumbi 2003), continental slope species (Rogers 2002), and fishes (Cowen et al. 2006; Selkoe et al. 2006; Froukh and Kochzius 2007). These findings were attributed to restricted larval dispersal (or limited mixing) despite the fact that these planktonic larvae are capable of long distance dispersal (see Swearer et al. 2002). In addition to limited larval mixing, life history characteristics of many marine species include high fecundity and a sweepstakes-like chance for matching favorable oceanographic conditions for spawning, fertilization, larval survival and dispersal, and recruitment success, such that only a few individuals actually contribute to an annual cohort (Hedgecock 1994a, b). Large variance in reproductive success has been shown to reduce the effective population size (Hedrick 2005). Limited larval mixing, patchy environmental selection in the plankton, and sweepstakes reproductive success, may act in tandem, creating a pattern termed “chaotic genetic patchiness” observed in many coastal marine species (Selkoe et al. 2006).

For corals, non-random mating may occur frequently due to restricted mixing of gametes. Gamete concentrations become more diffuse with increasing time spent in the water column, resulting in decreased fertilization success with time since spawning. Therefore, successful fertilization may be more likely between individuals in close proximity and mating between relatives may occur often. Such a scenario would lead to small effective population sizes, and ultimately, locally adapted gene complexes, maintaining genetic subdivision (Whitaker 2004). Such a scenario would also lead to heterozygote deficits, which have been observed in most population genetic studies of corals (see Ayre and Hughes 2000; Van Oppen and Gates 2006 for reviews). Non-random mating may be occurring in some GOM *Lophelia* populations as evidenced by several instances of heterozygote deficits. Spatial autocorrelation analysis would provide information about relatedness among individuals in relation to the distance separating samples, but would require even sampling along transects.

As discussed above, larval dispersal will be highly influenced by prevailing hydrodynamics during and following spawning. However, little is known about local hydrodynamics regimes around topographic features such as deep coral mounds. A recent study did report that bottom currents reverse at 6 h intervals at the base of the slope of Great Bahama Bank at depths of 600-800 m despite the north-flowing Gulf Stream at the surface (Grasmueck et al. 2006). If this type of localized circulation occurs at deep coral banks, water would be retained in the area longer and could effectively 'hold' larvae in the region.

On larger spatial scales, hydrodynamic patterns may lead to unexpected phylogeographic breaks in genetic continuity. For example, strong currents near Puerto Rico may serve as a barrier to gene flow between two regionally isolated populations of *Acropora palmata* in the eastern and western Caribbean (Baums et al. 2005b). Although direct comparisons are difficult, *A. palmata* F_{ST} estimates between these regions (F_{ST} = 0.036, Baums et al. 2005b) were comparable to those observed between GOM and SEUS *Lophelia* populations (F_{ST} = 0.038, Table 4.5). The *Acropora* results (Baums et al. 2005b) correspond to distinct regions of population isolation revealed using a high-resolution biophysical model for dispersal of larval fishes in the Caribbean region (Cowen et al. 2006).

A pattern of genetic structuring consistent with isolation by distance has only been observed in brooding coral species (*Balanophyllia*, Hellberg 1994, 1996; *Seriatopora*, Maier et al. 2005, but see Underwood et al. 2007 for contrasting results from the same species at smaller spatial scale in Western Australia). The weak isolation by distance pattern observed for *Lophelia* in this study is likely driven by large genetic distances between distant populations. A stepping stone model of gene flow was not consistent with patterns of genetic differentiation within the regions examined (e.g. between GOM or SEUS *Lophelia* populations). It follows that isolation by distance was not observed regionally in NEAO *Lophelia* (LeGoff-Vitry et al. 2004a).

CONSERVATION IMPLICATIONS. - GOM *Lophelia* sites VK862 and VK826 are especially deserving of protection. VK826 harbors substantial and highly complex genetic variation, and may act as a *Lophelia* recruitment source to other areas of the GOM. VK862 is the most genetically unique and isolated of the GOM populations surveyed. All *Lophelia* populations harbor substantial genetic diversity, thus providing the potential for adaptive evolution should environmental conditions change. But, within regions, genetic structuring among *Lophelia* populations was complex with incomplete genetic mixing. Gene flow between ocean regions

appears restricted. The majority of recruitment is likely to be localized, with long distance dispersal between sites sporadic but significant over long time frames. New recruits from other locations are unlikely to replenish destroyed reefs in the short term (ca. several years). Loss of any reef habitat should be regarded as a substantial loss to regional genetic diversity of *Lophelia*. These results indicate that the most effective management scheme for *Lophelia* is regional reserve networks in the GOM, SEUS and NEAO regions.

RECOMMENDATIONS

Obtaining additional genetic samples is a priority. In particular, samples from the *Lophelia* population on the West Florida Shelf (West Florida Lithoherms, see Reed et al. 2006) are needed to complete our sampling of known populations from the GOM and the SEUS. Additionally, samples from the Straits of Florida, Great Bahama Banks, and elsewhere in the Caribbean are necessary to increase our understanding of the extent of gene flow between the GOM and neighboring regions. More samples of *Lophelia* from MC and GC are needed before conclusions regarding the degree of gene flow between the western Gulf and the VK sites can be made. Targeting discrete samples from different areas of the shipwreck "Gulf Penn" in MC would help satisfy this goal and these samples have an added advantage of known maximum age.

Fine scale limits of connectivity can be addressed with additional *Lophelia* samples taken at evenly spaced intervals along transects and subsequent spatial autocorrelation analysis (Bertorelle and Barbujani 1995). This type of sampling can be accomplished using either manned submersibles or remotely operated vehicles and will require high quality, fine-scale maps of *Lophelia* reefs.

Weak genetic structuring has proven difficult to estimate accurately using population genetic statistics (Waples 1998). By increasing sample sizes for each population (N~50, Ruzzante 1998), sampling more populations, and increasing the number of microsatellite markers assessed, precision of our estimates is likely to increase. Also, placing the population genetic results into context with other components of the *Lophelia* studies may help to develop biologically meaningful estimates of the limits of gene flow between reef areas. For example, genetic differentiation reported for *Lophelia* in this study should be compared to that reported for *Lophelia*-associated microbial communities, and between *Lophelia* morphotypes ('brachycephala' vs. 'gracilis' forms). Long-term data of currents at scale of 10s of meters,

especially during the *Lophelia* reproductive season, would be informative. Such information could be obtained through the deployment of landers or other measurement devices.

Finally, comparative population genetic studies of other scleractinian coral or octocoral species that differ in larval type (i.e. dispersal potential) would broaden the ecological scope of this work. A good candidate for further population genetic investigation is the cosmopolitan scleractinian coral species *Madrepora oculata*. *Madrepora* differs from *Lophelia* in that it is a periodic rather than a seasonal spawner and likely differs in fecundity since *Lophelia* produces relatively large numbers of small oocytes and *M. oculata* produces fewer, larger oocytes (Waller and Tyler 2005). Similarly, examining patterns of genetic differentiation in *Lophelia*-associated invertebrates would add to our knowledge of connectivity in these deep reef habitats.

DISCLAIMER

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Table 4.1. Mitochondrial and nuclear DNA regions explored for utility in phylogeographic analyses of *Lophelia pertusa*.

Gene region	**Study**	**Primers**	**Target coral species**	**Success in *Lophelia***
mtDNA Control Region	van Oppen et al. 2001	rns, cox3	*Acropora* spp.	Little, cloned product
Control Region	Vollmer & Palumbi 2002	CRf, CO3r	*Acropora* spp.	
Control Region	Van Oppen et al. 2004	Ms_FP2, MON_RP2	*Montipora* spp.	Little
16S/COI intron	Fukami et al. 2004a	MNCf, r	*Montastarea* spp.	Little, cloned product
nad5/nad1 intron 1	Concepcion et al. 2006	3 sets	*Acropora, Pavona, Montipora, Mussa, Pocillopora*, others	Some, little variation
nad3/nad5 intron 2	Concepcion et al. 2006	2 sets	*Acropora, Pavona, Montipora, Mussa, Pocillopora*, others	Some, little variation
ITS	van Oppen et al. 2000	Acf, Acr	*Acropora*	None
ITS-1	Chen et al. 1996	1S, A4	Corallimorphs	Good
ITS-2	LeGoff et al. 2004	ITS2FA, ITS2RA	*Lophelia*	Good, no geogr. pattern
Calmodulin	Vollmer & Palumbi 2002	CalMf, CalMr2	*Acropora*	None
Mini-collagen	MacKenzie et al 2004	Mini-C FP1, RP1	*Acropora nasuta*	Little
Cnox2	MacKenzie et al 2004	Conx2 FP1, RP1	*Acropora nasuta*	Little
PaxC	van Oppen et al. 2000	PaxC intron FP1, RP1	*Acropora cervicornis* group	None
PaxC	van Oppen et al. 2004	Mont PAX-FP1	*Montipora* spp.	None
B-tubulin intron	Fukami et al. 2004b	Tubulin F, R	*Montastraea*	Cloned, high intra-indiv. variation
Single-copy nuclear	Severance et al. 2004	6 sets	*Montastraea*	Some for maSC12, little variation

Table 4.2. Characteristics of 11 *Lophelia pertusa* microsatellite DNA loci: Locus designation, repeat motif, size range of PCR products, number of alleles observed, and primers sequences. Annealing temperature for all loci was 58°C.

Locus	Repeat Motif	Size range (bp)	No. of alleles	Primer Sequence (5'→3')
*Lpe*A5	$(AAC)_{17}$†	277-311	11	F: NED-GGCATGGAAACCTATGTTGTTA R: ACAAGGAATGGAAACGAGAGAG
*Lpe*C44	$(TCAA)_{11}(ATCT)_8$	183-339	39	F: FAM-CAGTTTATCACGCCATGTTTG R: GGCGCATGATAGTTCTGGTAG
*Lpe*C52	$(TAGA)_{20}$	85-259	23	F: NED-AGATTGAATGTTTTGCAAGGTC R: CTTTTCGCTATAAGGCATTGAC
*Lpe*C61	$(TAGA)_{33}$	140-322	41	F: FAM-ATTTGATTCGTGACCTTCCTTC R: AATCGTGGCATTACATACCTG
*Lpe*C120	$(GATA)_5(GACA)_5(GATA)_9$	211-359	30	F: HEX-TCTTGATCGATCTTCGTCTTG R: GTTTTCCCACATGATAACGAAC
*Lpe*C126	$(GATA)_{12}$	211-259	12	F: HEX-CTGGCTTTCTCGAGGTATAATG R: TTTTAAATCGTGGCATTACCAG
*Lpe*C131	$(GATA)_7$	112-150	12	F: FAM-TTTTAAATCGTGGCATTACCTG R: ATCCTATTTATTGGCGCGTTG
*Lpe*C142	$(GATA)_{16}(GATAA)_5(GATA)_4$	160-244	25	F: NED-TAAACATATTGGAAGGCCTGTG R: ATCGACGTTATCTTCGTCATTG
*Lpe*C149	$(GATA)_{10}$	161-405	38	F: FAM-CTTAGCTCTCAAGTGTAAATGTGC R: CCACGTATAGTCCATTGTAGGG
*Lpe*C151	$(GATA)_9$	109-181	13	F: HEX-CGCTAAAGGTAAGTTATCAATCG R: GAGGCATACATGTAAGATAATCAGG
*Lpe*D3	$(TGAC)_{32}(TGAT)_2$	120-398	53	F: FAM-AACGCATGGACGCAATTATAT R: CACCCTCACAGGTTTTATGGA

†$(AAC)_2GCCACCATCACC(AAC)_{10}$

Table 4.3. Levels of polymorphism and heterozygosity by locus and *Lophelia* sampling site. Given are the number of *Lophelia* genotyped (*N*), the number of unique multi-locus genotypes (MLGs), the number of inferred clones genotypes (Clones), the number of observed alleles (*A*), the proportion of observed (H_O) and expected (H_E) heterozygotes per locus and site, and the inbreeding coefficient (F_{IS}) for each site and for each locus (All); numbers in bold represent significant deviations from Hardy-Weinberg equilibrium because of heterozygote deficits at the 0.05 level after sequential Bonferroni corrections. Also shown are average number of alleles per locus (N_A) and the number of private alleles (P_{VA}) per population. See text for site abbreviations.

Locus		Sampling Site													
		CLO-N	**CLO-S**	**STS-N**	**STS-S**	**JAX**	**MTR**	**VK826-S**	**VK826-NE**	**VK826-NW**	**VK862**	**WEST GULF**	**RB**	**MNG**	**ALL**
	N	15	15	15	16	24	8	25	16	8	23	13	6	6	190
	MLGs	11	14	15	16	22	8	25	12	8	19	10	6	6	172
	Clones	4	1	0	0	2	0	0	4	0	4	3	0	0	18
LpeA5	*A*	6	7	7	8	8	5	8	5	6	8	5	4	5	
	H_O	0.818	0.857	0.800	0.933	0.905	0.429	0.913	0.750	1.000	0.750	0.625	1.000	0.750	
	H_E	0.719	0.740	0.740	0.820	0.808	0.673	0.771	0.691	0.755	0.758	0.500	0.681	0.750	
	F_{IS}	-0.138	-0.159	-0.081	-0.138	-0.119	0.364	-0.184	-0.085	-0.324	0.010	-0.250	-0.469	0.000	-0.119
LpeC61	*A*	16	14	18	19	23	9	23	16	11	15	14	7	6	
	H_O	0.909	0.714	0.917	0.867	0.895	1.000	0.875	1.000	0.875	0.867	0.900	1.000	1.000	
	H_E	0.926	0.893	0.938	0.927	0.938	0.880	0.919	0.921	0.891	0.909	0.905	0.820	0.833	
	F_{IS}	0.018	0.200	0.022	0.065	0.046	-0.136	0.048	-0.085	0.018	0.046	0.006	-0.220	-0.200	-0.010
LpeC131	*A*	2	2	1	4	6	3	5	4	5	4	4	4	4	
	H_O	0.111	0.071	0.000	0.188	0.227	0.250	0.167	0.167	0.167	0.400	0.125	0.800	0.167	
	H_E	0.105	0.069	0.000	0.279	0.212	0.227	0.355	0.413	0.736	0.344	0.414	0.580	0.625	
	F_{IS}	-0.059	-0.037	NA	0.329	-0.073	-0.103	**0.531**	0.597	0.774	-0.161	**0.698**	-0.379	0.733	0.349
LpeC44	*A*	13	8	14	15	16	8	19	10	12	16	9	9	7	
	H_O	0.700	0.700	0.500	0.750	0.579	0.750	0.818	0.900	1.000	0.611	0.500	0.833	0.600	
	H_E	0.900	0.810	0.913	0.917	0.891	0.852	0.886	0.855	0.908	0.914	0.875	0.861	0.840	
	F_{IS}	0.222	0.136	0.452	0.182	0.350	0.119	0.077	-0.053	-0.101	0.331	0.429	0.032	0.286	0.191

Table 4.3 (continued)

Locus		Sampling Site													
		CLO-N	**CLO-S**	**STS-N**	**STS-S**	**JAX**	**MTR**	**VK826-S**	**VK826-NE**	**VK826-NW**	**VK862**	**WEST GULF**	**RB**	**MNG**	**ALL**
LpeC151	*A*	6	6	6	6	6	5	7	7	2	6	4	4	5	
	H_O	0.300	0.462	0.250	0.667	0.368	0.500	0.500	0.667	0.500	0.333	0.222	0.667	0.600	
	H_E	0.705	0.728	0.653	0.642	0.679	0.681	0.713	0.785	0.486	0.664	0.623	0.722	0.780	
	F_{IS}	0.574	0.366	0.617	-0.038	**0.457**	0.265	0.299	0.150	-0.029	0.498	0.644	0.077	0.231	0.319
LpeD3	*A*	14	15	21	15	22	9	22	11	10	11	12	9	10	
	H_O	0.889	0.714	0.800	0.533	0.773	0.500	0.960	1.000	0.875	0.684	1.000	1.000	0.667	
	H_E	0.914	0.855	0.936	0.918	0.931	0.867	0.886	0.864	0.836	0.717	0.889	0.833	0.889	
	F_{IS}	0.027	0.164	0.145	0.419	0.170	0.423	-0.084	-0.157	-0.047	**0.046**	-0.125	-0.200	0.250	0.083
LpeC52	*A*	7	13	5	9	13	5	14	9	6	7	6	2	1	
	H_O	0.875	0.917	0.857	0.750	0.579	0.857	0.826	1.000	0.571	0.667	0.500	0.167	0.000	
	H_E	0.781	0.861	0.622	0.774	0.668	0.622	0.787	0.861	0.633	0.785	0.695	0.153	0.000	
	F_{IS}	-0.120	-0.065	-0.377	0.031	**0.133**	-0.377	-0.049	-0.161	0.097	0.150	0.281	-0.091	NA	-0.004
LpeC120	*A*	11	10	15	15	17	7	8	5	5	7	7	5	3	
	H_O	0.889	0.769	0.867	0.733	0.850	1.000	0.500	0.273	0.714	0.909	0.667	0.600	0.667	
	H_E	0.889	0.861	0.911	0.907	0.891	0.840	0.659	0.446	0.622	0.818	0.599	0.600	0.500	
	F_{IS}	0.000	0.107	0.049	0.191	0.046	-0.190	**0.241**	0.389	-0.148	-0.111	-0.113	0.000	-0.333	0.011
LpeC142	*A*	9	13	15	13	15	3	15	11	9	11	7	6	10	
	H_O	1.000	0.818	1.000	1.000	0.947	0.667	0.958	0.875	0.875	0.786	0.667	0.800	0.833	
	H_E	0.867	0.884	0.909	0.910	0.902	0.500	0.901	0.867	0.844	0.819	0.809	0.760	0.889	
	F_{IS}	-0.153	0.075	-0.100	-0.099	-0.051	-0.333	-0.064	-0.009	-0.037	0.040	0.176	-0.053	0.062	-0.034
N_A		9.333	9.778	11.778	11.333	14.000	6.000	13.444	8.667	7.333	9.444	7.556	5.556	5.667	
P_{VA}		6	3	6	9	11	0	6	1	2	3	0	3	4	

Table 4.4. Genetic distance (chord distance, Cavalli-Sforza and Edwards 1967; above diagonal) and F_{ST} estimates (below diagonal) estimated from microsatellite genotypes among thirteen collections of *Lophelia pertusa*. See text for site abbreviations.

	Sampling Site												
	CLO-N	**CLO-S**	**JAX**	**MTR**	**STS-N**	**STS-S**	**VK826-S**	**VK826-NE**	**VK826-NW**	**VK862**	**WGULF**	**MNG**	**RB**
CLO-N		0.522	0.448	0.594	0.530	0.504	0.504	0.583	0.574	0.538	0.556	0.664	0.618
CLO-S	0.024		0.467	0.596	0.519	0.543	0.529	0.567	0.595	0.556	0.571	0.667	0.624
JAX	0.009	0.018*		0.545	0.479	0.457	0.458	0.529	0.542	0.522	0.519	0.624	0.615
MTR	0.043*	0.060*	0.032*		0.560	0.585	0.583	0.620	0.629	0.625	0.597	0.685	0.641
STS-N	0.017	0.020	0.007	0.053*		0.530	0.520	0.588	0.587	0.579	0.569	0.687	0.602
STS-S	0.010	0.020	0.001	0.031*	0.010		0.504	0.563	0.538	0.559	0.550	0.639	0.639
VK826-S	0.021*	0.026*	0.019*	0.053*	0.028*	0.022*		0.439	0.482	0.420	0.450	0.545	0.619
VK826-NE	0.033*	0.041*	0.051*	0.075*	0.053*	0.043*	0.029*		0.512	0.506	0.486	0.608	0.635
VK826-NW	0.031	0.054*	0.037*	0.077*	0.052*	0.029	0.011	0.046*		0.507	0.487	0.616	0.670
VK862	0.032*	0.044*	0.043*	0.052*	0.055*	0.044*	0.036*	0.048*	0.033		0.511	0.603	0.672
WGULF	0.037*	0.035*	0.037*	0.072*	0.045*	0.029*	0.009	0.033*	0.000	0.056*		0.603	0.671
MNG	0.076*	0.115*	0.087*	0.100*	0.094*	0.086*	0.085*	0.077*	0.088*	0.064*	0.114*		0.618
RB	0.076*	0.108*	0.070*	0.085*	0.084*	0.064*	0.070*	0.117*	0.057*	0.106*	0.086*	0.106*	

* = F_{ST} estimate significantly different from zero at = 0.05 ($P < 0.0006$)

Table 4.5. Analysis of molecular variance (AMOVA) among: (1) 13 *Lophelia pertusa* collections from the North Atlantic and Gulf of Mexico; (2) 10 *Lophelia pertusa* collections from the Gulf of Mexico (Gulf) and Southeastern U.S. (SEUS); (3) 5 *Lophelia pertusa* collections from the Gulf of Mexico; (4) 6 *Lophelia pertusa* collections from the SEUS. Each variance component and fixation index (F_{ST}) was significantly greater than zero at $\alpha = 0.05$. AMOVAs were also significantly greater than zero when R_{ST} was used in calculations. Overall R_{ST} values are shown for comparison.

Variation	df	Sum of Squares	Variance Component	% Variation	F_{ST}	R_{ST}
			Three regional groupings			
Among regions	2	26.39	0.074	2%	0.020	
Among populations within regions	10	63.51	0.104	3%	0.028	
Within populations	331	1187.75	3.588	95%	0.047	0.080
Total	343	1277.65	3.767			
			Gulf vs. SEUS			
Among Regions	1	14.82	0.052	1%	0.014	
Among populations within regions	9	55.10	0.090	2%	0.024	
Within populations	309	1111.50	3.597	96%	0.038	0.067
Total	319	1181.42	3.738			
			Gulf of Mexico			
Among populations	4	20.04	0.053	2%		
Within populations	143	500.42	3.499	98%	0.015	0.013
Total	147	520.46	3.553			
			Southeastern U.S.			
Among populations	5	25.59	0.056	2%		
Within populations	166	587.69	3.54	98%	0.016	0.034
Total	171	613.28	3.596			

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Figure 4.11. Mantel test results illustrating insignificant correlations between geographic and genetic distance for A) southeastern U.S. *Lophelia* populations B) Gulf of Mexico. *Lophelia* populations.

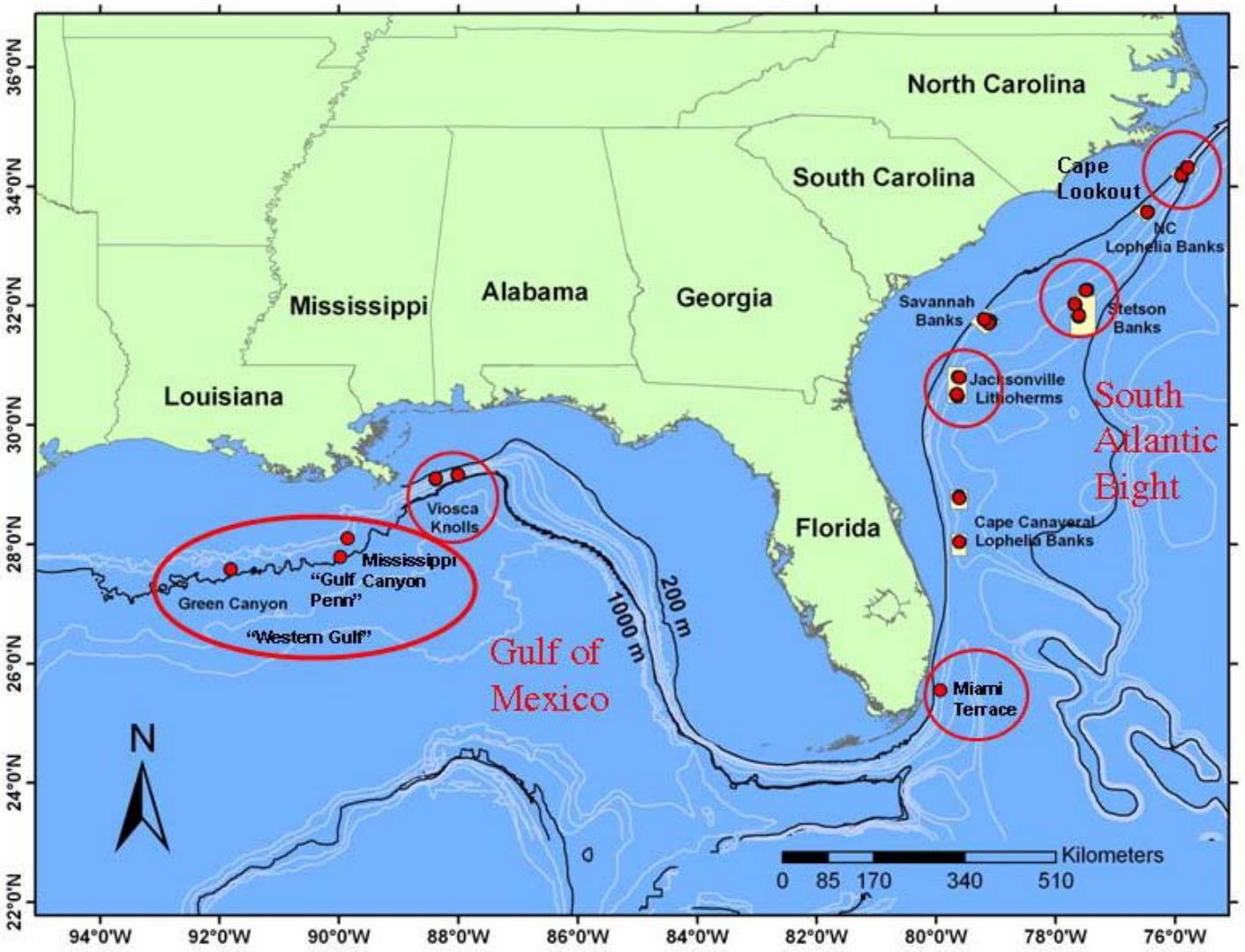

Figure 4.1. Map of *Lophelia* collection sites from the Gulf of Mexico and Southeastern U.S. continental slope. Red dots represent collection sites and circled sites are included in population genetic analyses.

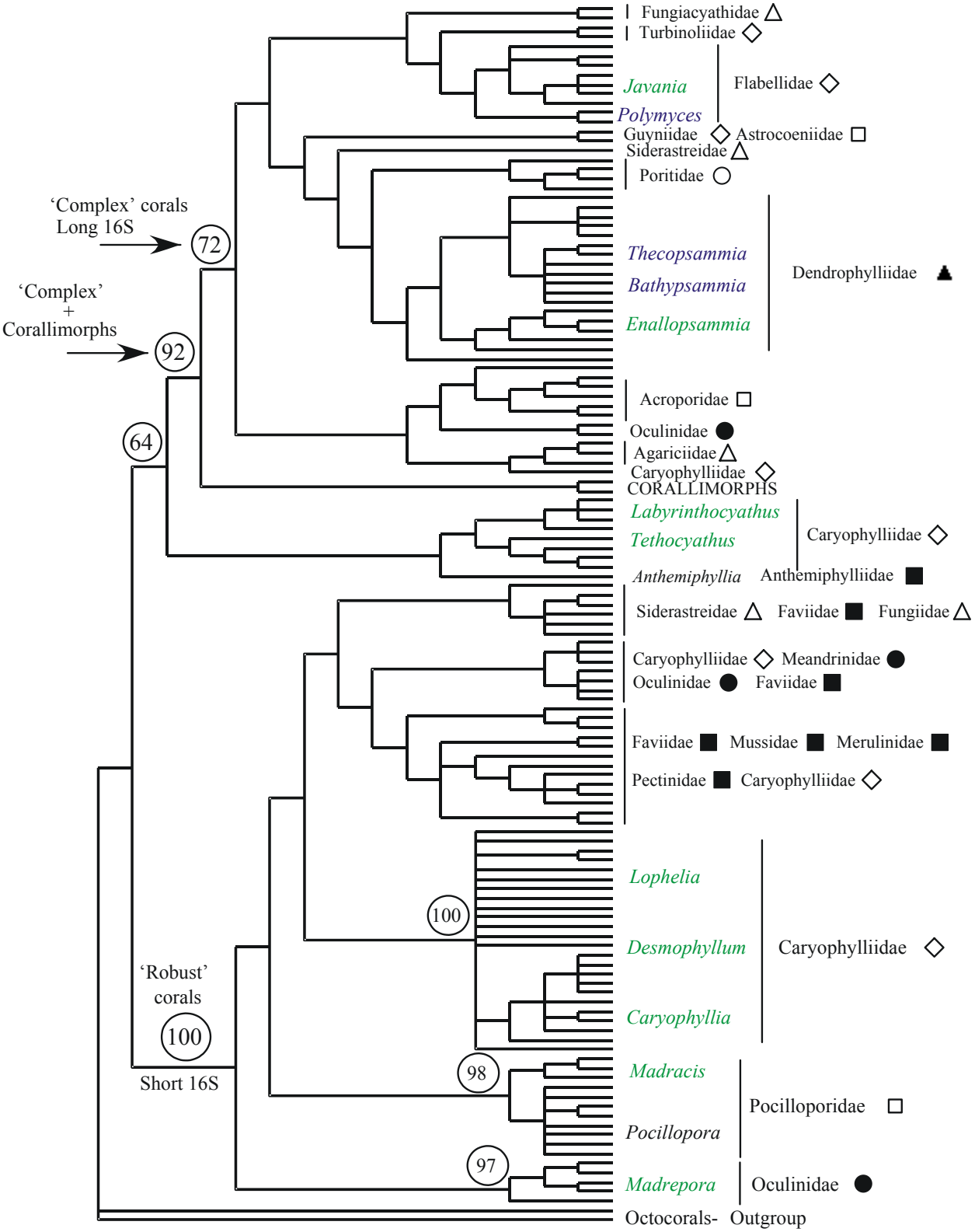

Figure 4.2. Phylogenetic hypothesis for scleractinian corals based upon maximum parsimony analysis of mitochondrial 16S sequence data. Numbers in circles represent bootstrap support. Colored genus names represent sequences unique to this study and colors refer to region of origin: green = Gulf of Mexico, blue = southeastern U.S. Please refer to Figure 4.3 for key to suborders.

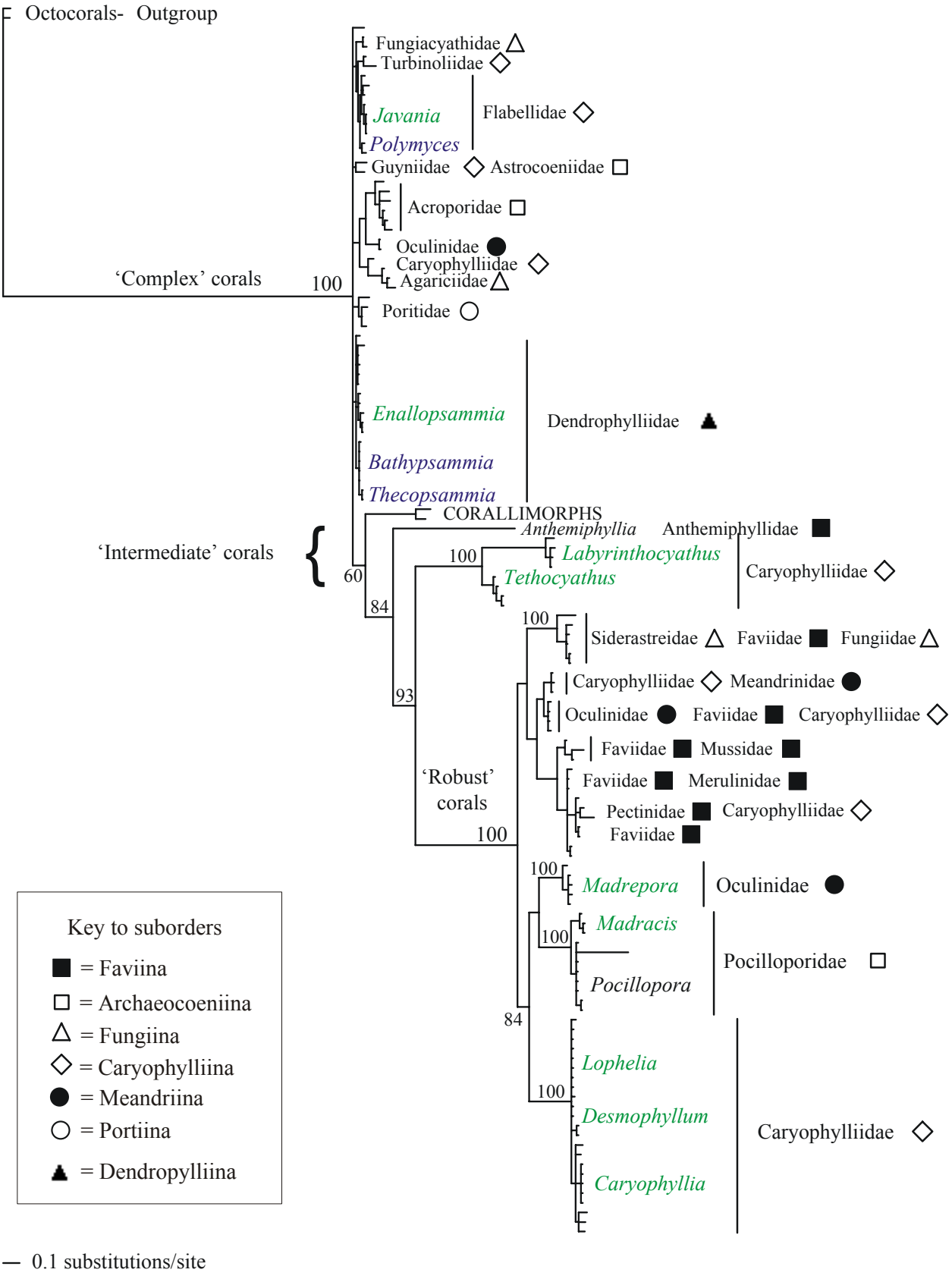

Figure 4.3. Phylogram resulting from Bayesian analysis of 16S sequence data for scleractinian corals. Colored genus names represent sequences unique to this study and colors refer to region of origin: green = Gulf of Mexico, blue = southeastern U.S.

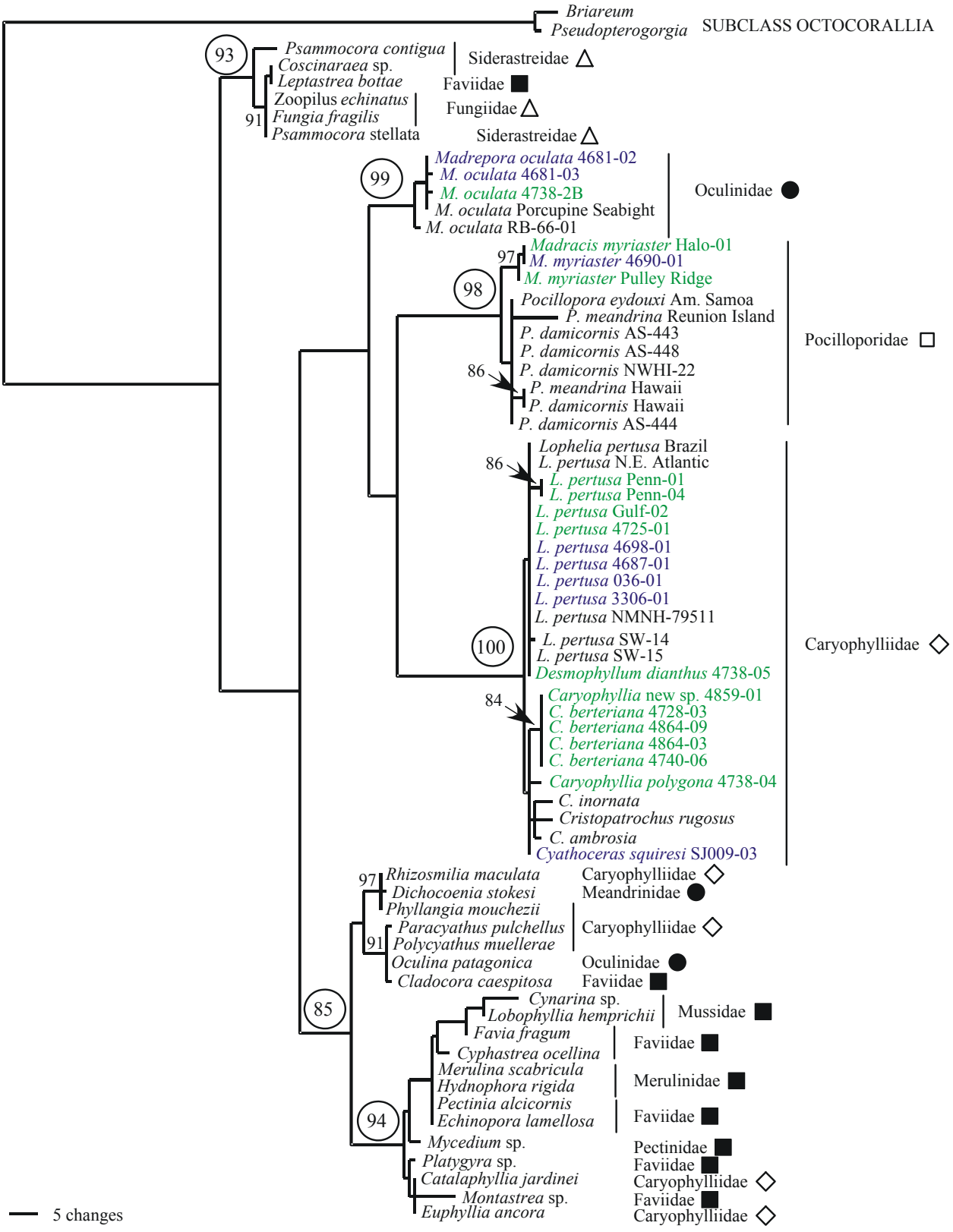

Figure 4.4. Phylogenetic hypothesis for 'robust' scleractinian corals based upon maximum parsimony analysis of mitochondrial 16S sequence data. Numbers represent bootstrap support for clade. Colored genus names represent sequences unique to this study and colors refer to region of origin: green = Gulf of Mexico, blue = southeastern U.S. Please refer to Figure 4.3 for key to suborders.

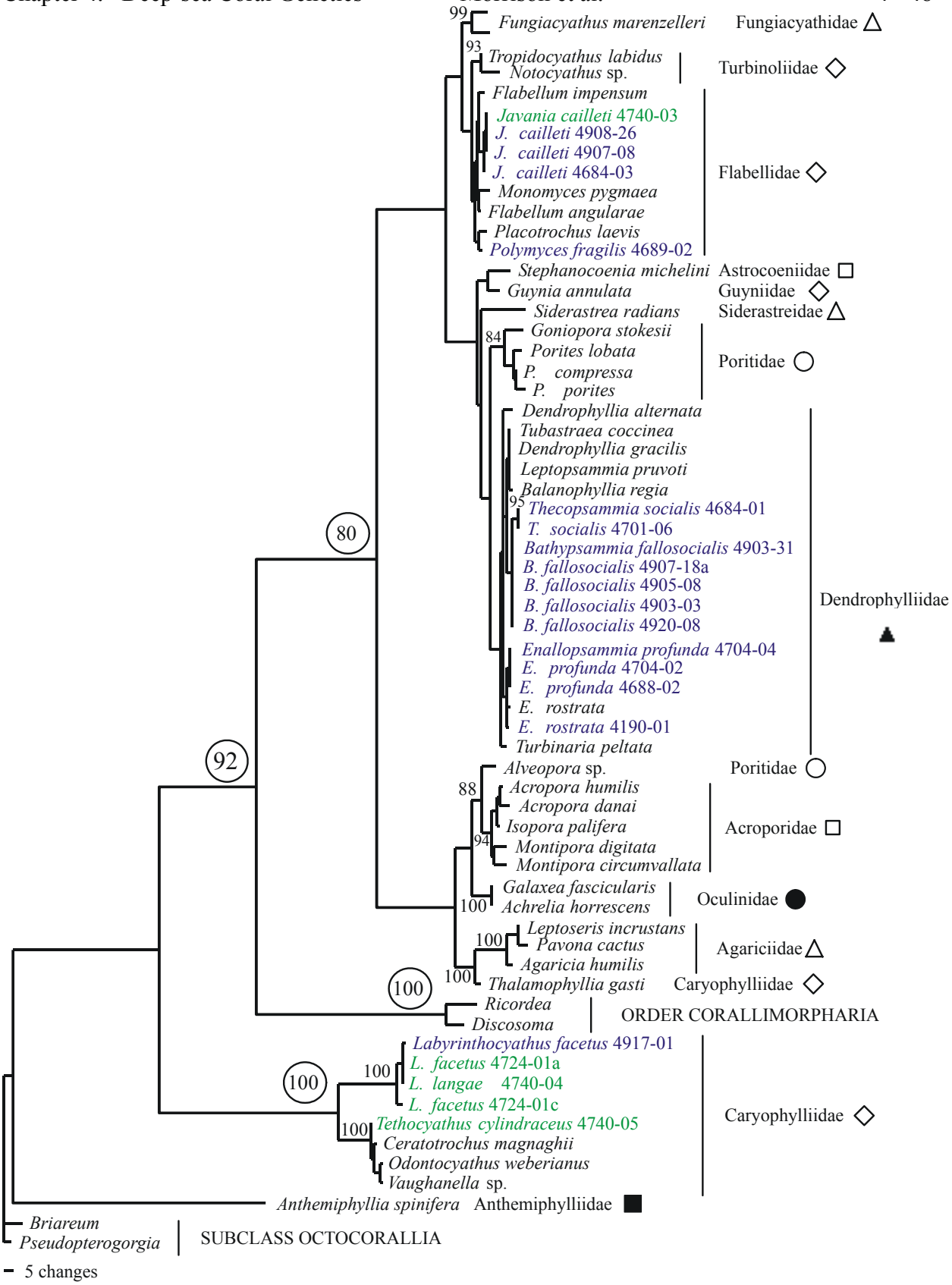

Figure 4.5. Phylogenetic hypothesis for 'complex' scleractinian corals based upon mitochondrial 16S sequence data and maximum parsimony analysis. Numbers represent bootstrap support for clade. Colored genus names represent sequences unique to this study and colors refer to region of origin: green = Gulf of Mexico, blue = southeastern U.S. Please refer to Figure 4.3 for key to suborders.

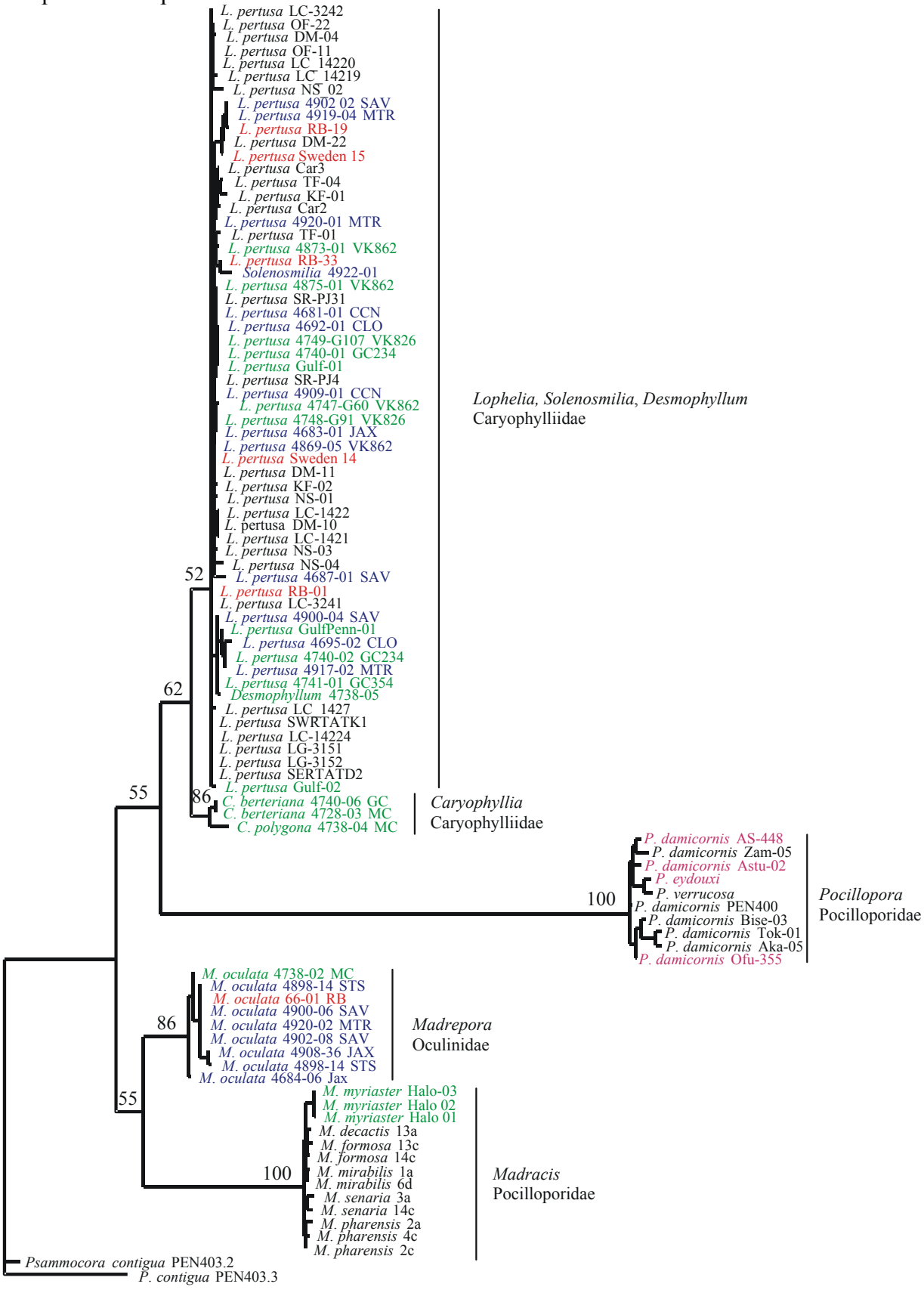

Figure 4. 6. Phylogenetic hypothesis of relationships among 'robust' corals based upon maximum likelihood analysis of nuclear internal transcribed spacer sequence data. Numbers represent bootstrap support. Colored genus names represent sequences unique to this study and colors refer to region of origin: green = Gulf of Mexico, blue = southeastern U.S, red = N.E. Atlantic, pink = Pacific.

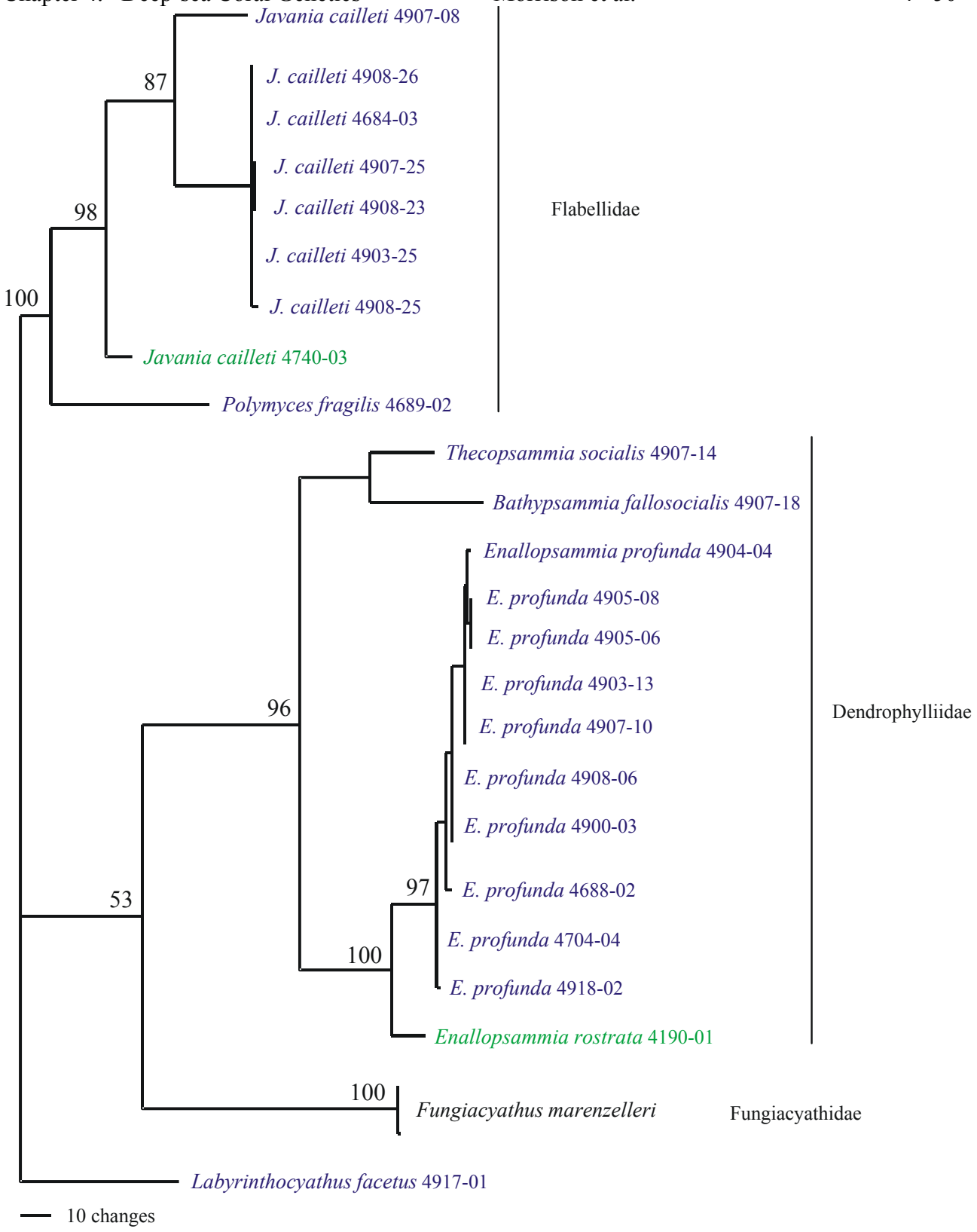

Figure 4.7. Phylogenetic hypothesis of relationships among 'complex' corals based upon maximum parsimony analysis of nuclear internal transcribed spacer sequence data. Numbers refer to bootstrap support. Colored genus names represent sequences unique to this study and colors refer to region of origin: green = Gulf of Mexico, blue = southeastern U.S.

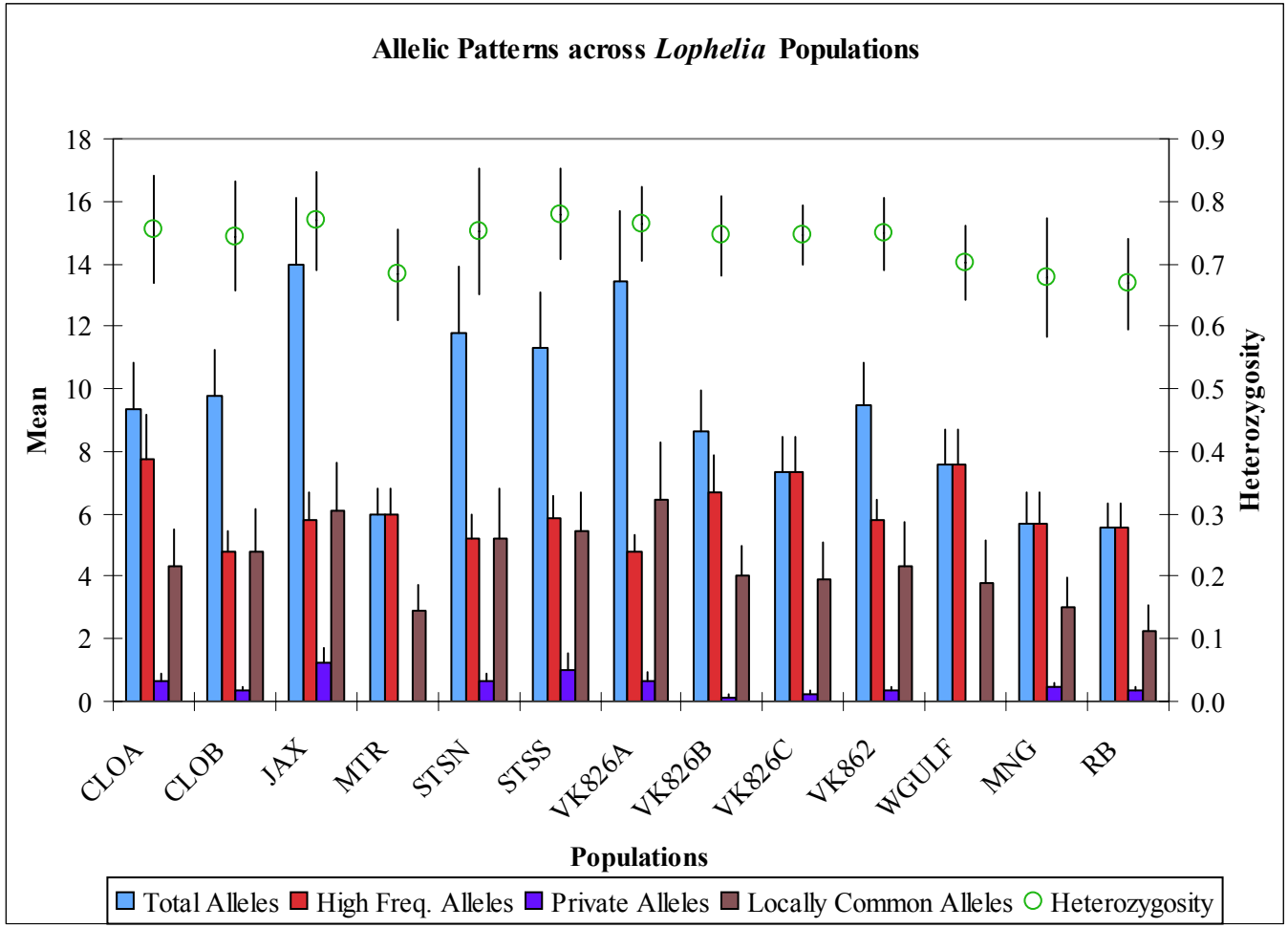

Figure 4.8. Allelic patterns for 13 *Lophelia* populations surveyed at nine microsatellite loci. Please see text for population abbreviations.

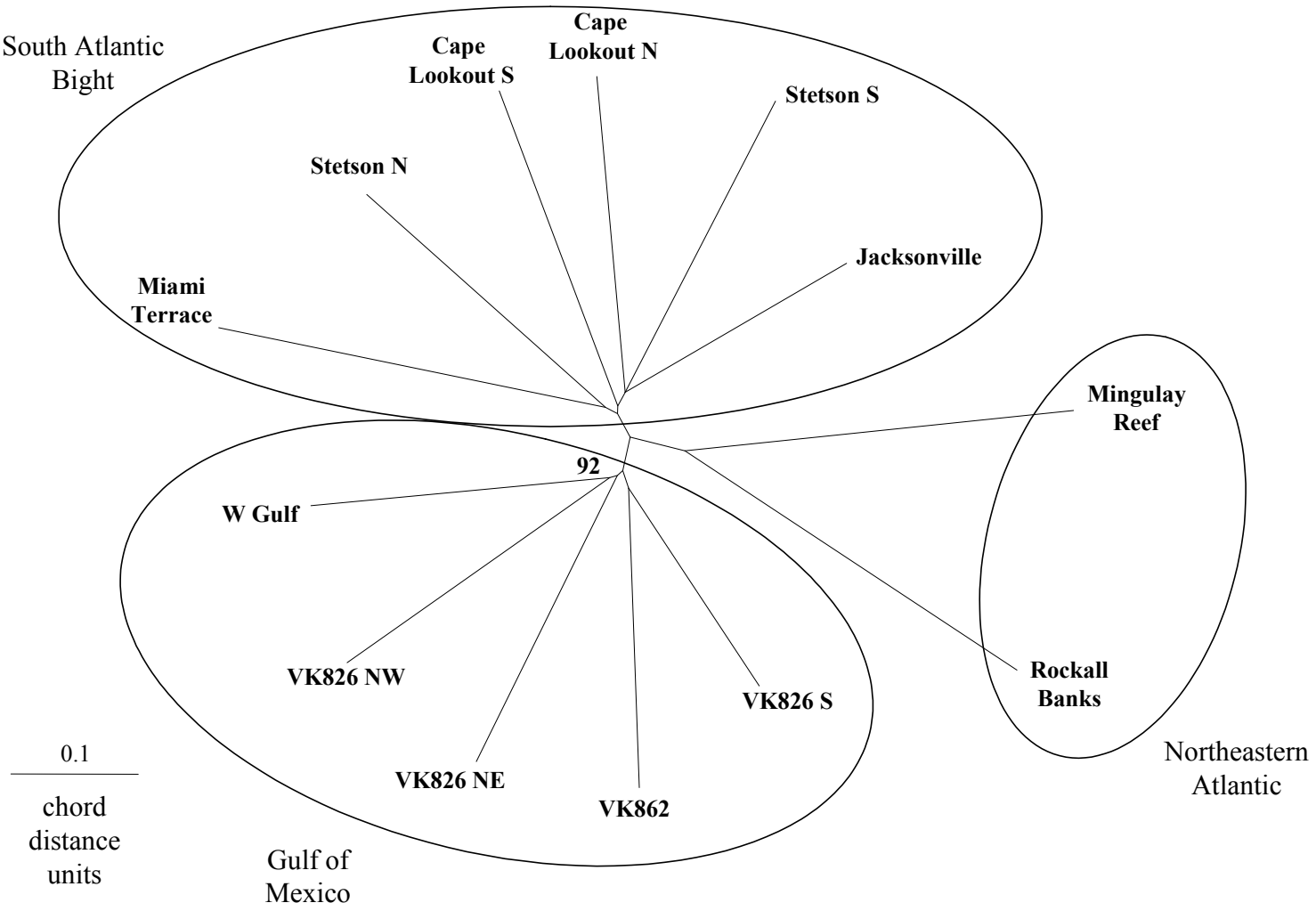

Figure 4.9. Unrooted neighbor-joining tree generated from pairwise genetic distances (chord distance, Cavalli-Sforza & Edwards 1967) of multilocus microsatellite genotypes between all *Lophelia* populations. The number at the Gulf of Mexico node represents bootstrap support.

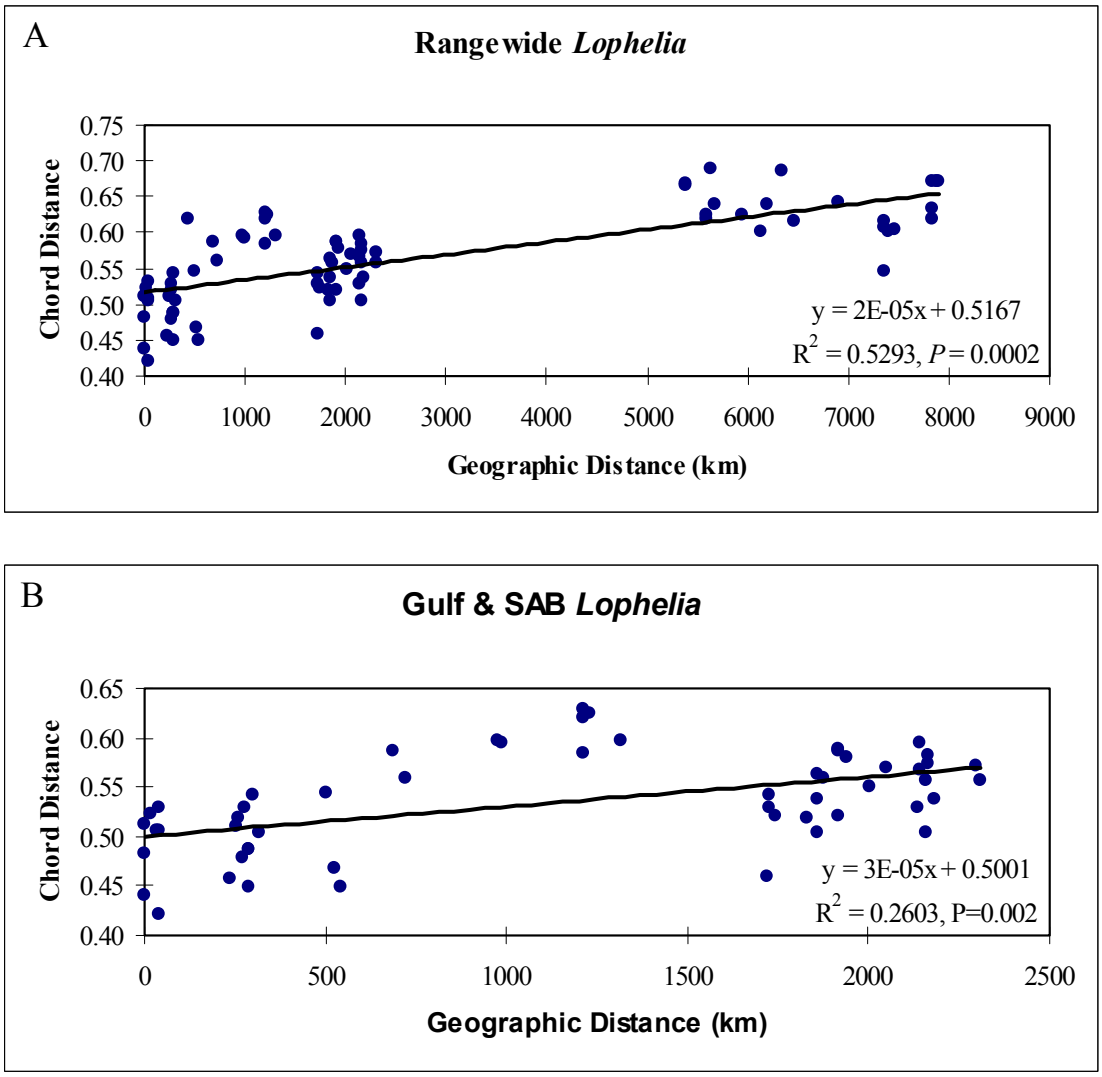

Figure 4.10. Mantel test results illustrating significant correlations between geographic distance (km) and genetic distance (chord distance; Cavalli-Sforza and Edwards 1967) for A) rangewide *Lophelia* populations surveyed, B) Gulf of Mexico and southeastern U.S. *Lophelia* populations.

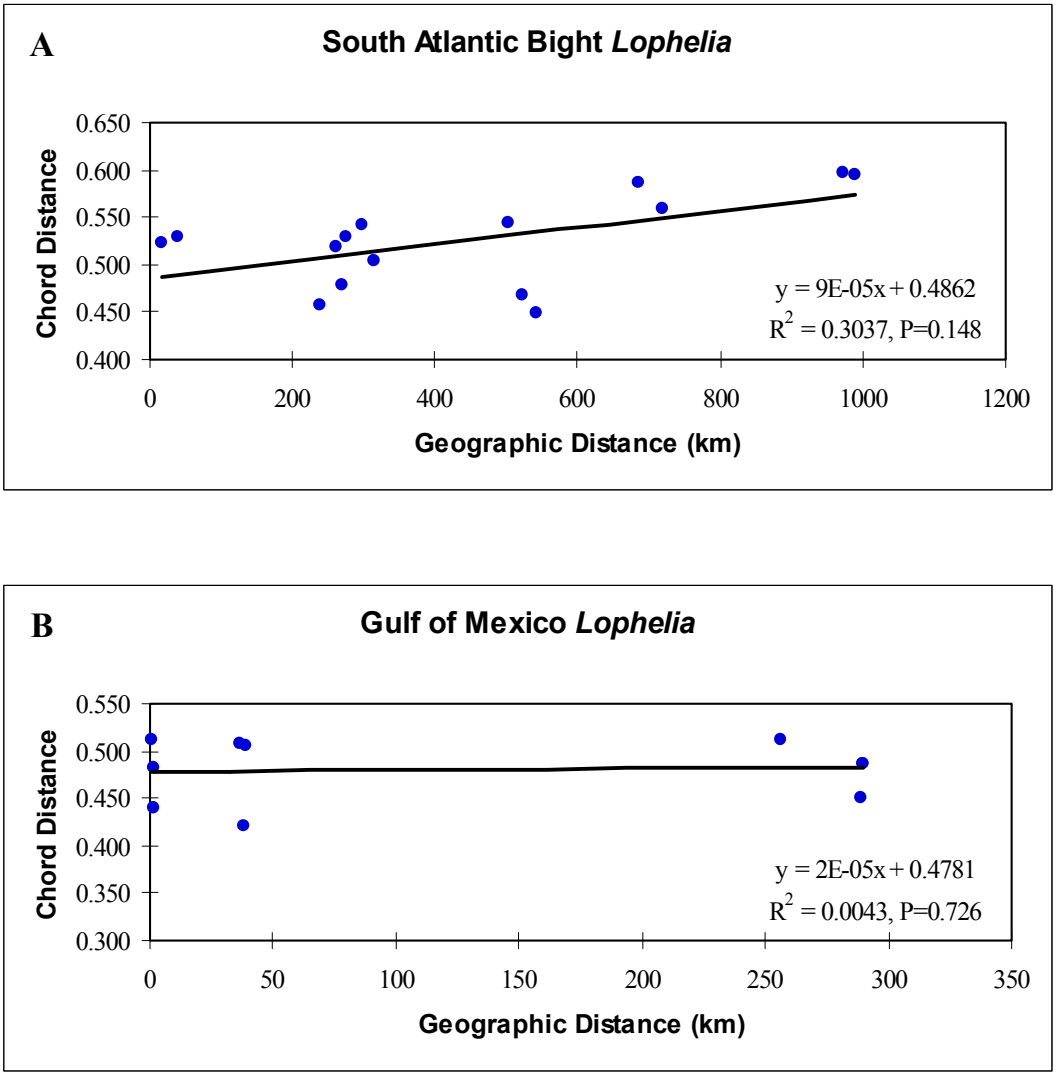

Figure 4.11. Mantel test results illustrating insignificant correlations between geographic distance (km) and genetic distance (chord distance; Cavalli-Sforza and Edwards 1967) for A) southeastern U.S. *Lophelia* populations B) Gulf of Mexico. *Lophelia* populations.

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Appendix 4-II. On-deck photographs of coral species used in genetic analyses.

Appendix 4-I. Species included in phylogenetic analyses, including taxonomic classification, sample source (when available), sequence authors, and Genbank accession numbers. (NEW= from present study, Genbank accession numbers to be obtained when publications submitted).

SUBORDER **Family** **Species**	**SOURCE**	**AUTHORS**	**SAMPLE ID#**	**Genbank Acc. No. 16S**	**Genbank Acc. No. ITS**
ARCHAEOCOENIINA					
Astrocoeniidae					
Stephanocoenia michelinii	Bahamas/HB	Romano and Cairns 2000	2-VII-87-2-022	AF265581	
Pocilloporidae					
Pocillopora damicornis	Hawaii	Romano and Palumbi 1996		L76019	
Pocillopora damicornis	Pac. -American Samoa, C. Kellogg	Morrison et al.	AS-448	**NEW**	**NEW**
Pocillopora damicornis	Pac.- American Samoa, C. Kellogg	Morrison et al.	AS-443	**NEW**	
Pocillopora damicornis	Pac.- American Samoa, C. Kellogg	Morrison et al.	AS-444	**NEW**	
Pocillopora damicornis	Pac.- NW Hawaiian Isl., C. Kellogg	Morrison et al.	NWHI-22	**NEW**	
Pocillopora damicornis	Pac.- Am. Samoa, Tutuila, C. Kellogg	Morrison et al.	Astu-02		**NEW**
Pocillopora damicornis	Pac.- Am. Samoa Ofu, C. Kellogg	Morrison et al.	Ofu-355		**NEW**
Pocillopora damicornis	Taiwan: Penghu Island	Chen et al. unpubl.	PEN400		AY722785
Pocillopora damicornis	Japan: Okinawa, Zamami	Hirose et al. unpubl.	Zam-05		AB214391

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Pocillopora damicornis	Japan: Okinawa, Bise	Hirose et al. unpubl.	Bise-03		AB214386
Pocillopora damicornis	Japan: Okinawa, Tokashiki	Hirose et al. unpubl.	Tok-01		AB214395
Pocillopora damicornis	Japan: Okinawa, Aka	Hirose et al. unpubl.	Aka-05		AB214393
Pocillopora meandrina	Hawaii	Romano and Palumbi 1996		L76018	
Pocillopora meandrina	Réunion Island	Le Goff-Vitry et al. 2004a		AF550373	
Pocillopora eydouxi	Pac.- American Samoa, C. Kellogg	Morrison et al.		**NEW**	**NEW**
Pocillopora verrucosa		Ridgway, unpubl.			AY139815
Madracis myriaster	GoM- Tanker Halo, W. Schroeder	Morrison et al.	MmyrHalo-01	**NEW**	**NEW**
Madracis myriaster	GoM- Tanker Halo, W. Schroeder	Morrison et al.	MmyrHalo-02		**NEW**
Madracis myriaster	GoM- Tanker Halo, W. Schroeder	Morrison et al.	MmyrHalo-03		**NEW**
Madracis myriaster	W. Atlantic, NC Ross et al. 2004	Morrison et al.		**NEW**	
Madracis myriaster	GoM- Pulley Ridge B. Halley	Morrison et al.		**NEW**	
Madracis mirabilis	Caribbean	Diekman et al. 2001	mir6d		AF251858

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Madracis mirabilis	Caribbean	Diekman et al. 2001	mir1a		AF251847
Madracis senaria	Caribbean	Diekman et al. 2001	sen3c		AF251909
Madracis senaria	Caribbean	Diekman et al. 2001	sen14		AF251906
Madracis decactis	Caribbean	Diekman et al. 2001	dec2c		AF251884
Madracis decactis	Caribbean	Diekman et al. 2001	dec13a		AF251879
Madracis formosa	Caribbean	Diekman et al. 2001	for13c		AF251891
Madracis formosa	Caribbean	Diekman et al. 2001	for14c		AF251894
Madracis pharensis	Caribbean	Diekman et al. 2001	pha2a		AF251927
Madracis pharensis	Caribbean	Diekman et al. 2001	pha4c		AF251928
Acroporidae					
Acropora humilis	Guam	Romano and Palumbi 1996		L75996	
Isopora palifera	Madang, PNG	Romano and Cairns 2000		AF265593	
Montipora circumvallata	Réunion Island	Le Goff-Vitry et al. 2004		AF550368	

Appendix 4-I (continued)

SUBORDER **Family** **Species**	**SOURCE**	**AUTHORS**	**SAMPLE ID#**	**Genbank Acc. No. 16S**	**Genbank Acc. No. ITS**
FUNGIINA					
Siderastreidae					
Psammocora stellata	Hawaii	Romano and Palumbi 1996		L76021	
Psammocora contigua	Réunion Island	Le Goff-Vitry et al. 2004a		AF550371	
Psammocora contigua	Taiwan: Penghu Island	Chen et al. unpubl.	PEN403.2		AY722783
Psammocora contigua	Taiwan: Penghu Island	Chen et al. unpubl.	PEN403.3		AY722784
Coscinaraea sp.	Solomon Islands			L76001	
Agariciidae					
Pavona cactus	Réunion Island	Le Goff-Vitry et al. 2004a		AF550370	
Leptoseris incrustans	Hawaii	Romano and Palumbi 1996		L76012	
Agaricia humilis	Florida	Medina et al. 2006		DQ643831	
Fungiidae					
Fungia fragilis	Hawaii	Romano and Palumbi 1996		L75998	
Zoopilus echinatus	Fiji	Romano and Palumbi 1996		L76024	

Appendix 4-I (continued)

SUBORDER **Family** **Species**	**SOURCE**	**AUTHORS**	**SAMPLE ID#**	**Genbank Acc. No. 16S**	**Genbank Acc. No. ITS**
Fungiacyathidae					
Fungiacyathus marenzelleri	N. Pacific	Romano and Palumbi 1996	USNM 93941	L76004	
FAVIINA					
Pectinidae					
Pectinia alcicornis	Palau	Romano and Palumbi 1996		L76017	
Mussidae					
Lobophyllia hemprichii	Palau	Romano and Palumbi 1996		L76013	
Cynarina sp.	Madang, PNG	Romano and Cairns 2000		AF265613	
Merulinidae					
Hydnophora rigida	Palau	Romano and Palumbi 1996		L76009	
Merulina scabricula	Fiji	Romano and Palumbi 1996		L76014	
Anthemiphyllidae					
Anthemiphyllia spinifera	Wallis & Futuna	Romano and Cairns 2000	USNM 98573	AF265652	
Faviidae					

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Cyphastrea ocellina	Hawaii	Romano and Palumbi 1996		L76132	
Echinopora lamellosa	Fiji	Romano and Palumbi 1996		L76003	
Leptastrea bottae	Hawaii	Romano and Palumbi 1996		L76010	
Cladocora caespitosa	Mediterranean	Romano and Cairns 2000		AF265612	
Platygyra sp.	Madang, PNG	Romano and Cairns 2000		AF265611	
Favia fragum		France et al. 1996		U40295	
CARYOPHYLLIINA Caryophliidae					
Catalaphyllia jardinei	Indo-Pacific	Romano and Palumbi 1996		L76000	
Euphyllia ancora	Palau	Romano and Palumbi 1996		L76002	
Rhizosmilia maculata	Bimini	Romano and Cairns 2000		AF265602	
Thalamophyllia gasti	Mediterranean	Romano and Cairns 2000		AF265590	

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Caryophyllia inornata	Mediterranean	Romano and Cairns 2000		AF265599	
Caryophyllia berteriana	GoM- MC929 NSF GoM 2004	Morrison et al.	Cbert-4728-03	NEW	NEW
Caryophyllia berteriana	GoM- GC234 CSA GoM 2004	Morrison et al.	Cbert-4740-06	NEW	NEW
Caryophyllia berteriana	GoM- MC929 CSA GoM 2005	Morrison et al.	Cbert-4864-03	NEW	
Caryophyllia berteriana	GoM- MC929 CSA GoM 2005	Morrison et al.	Cbert-4864-09	NEW	
Caryophyllia new sp.	GoM- GC234 CSA GoM 2005	Morrison et al.	CaryN-4859-01	NEW	
Caryophyllia polygona	GoM- MC885 CSA GoM 2004	Morrison et al.	Cpoly4738-04	NEW	NEW
Caryophyllia ambrosia	Porcupine Seabight	Le Goff-Vitry et al. 2004a		AF550362	
Phyllangia mouchezii	Mediterranean	Romano and Cairns 2000		AF265605	
Polycyathus muellerae	Mediterranean	Romano and Cairns 2000		AF265606	
Paracyathus pulchellus	Mediterranean	Romano and Cairns 2000		AF265603	
Cristopatrochus rugosus	Vanuatu	Romano and Cairns 2000		AF265600	
Odontocyathus weberianus	New Caldonia	Romano and Cairns 2000	Bathus 4-915	AF265594	

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Vaughanella sp.	Vanuatu	Romano and Cairns 2000		AF265595	
Ceratotrochus magnaghii	Mediterranean	Romano and Cairns 2000		AF265597	
Labyrinthocyathus langae	GoM- GC234 CSA GoM 2004	Morrison et al.	Llang4740-04	NEW	
Labyrinthocyathus facetus	GoM- GC234	Morrison et al.	Lpe4724-1a	NEW	
Labyrinthocyathus facetus	GoM- GC234	Morrison et al.	Lpe4724-1c	NEW	
Labyrinthocyathus facetus	W. Atlantic- Miami Brooke et al. 2005	Morrison et al.	Lpe4917-01	NEW	NEW
Tethocyathus cylindraceus	GoM- GC234 CSA GoM 2004	Morrison et al.	Tcyl4740-05	NEW	
Cyathoceras squiresi	W. Atlantic Ross et al. 2004	Morrison et al.	CsquSJ009-03	NEW	
Desmophyllum dianthus	GoM- MC885 CSA GoM 2004	Morrison et al.	Ddi4738-05	NEW	NEW
Solenosmilia variabilis	W. Atlantic- Miami Brooke et al. 2005	Morrison et al.	Svar4922-01		NEW
Lophelia pertusa	N.E. Atlantic	Le Goff-Vitry et al. 2004a		AF550367	
Lophelia pertusa	Brazil	Le Goff-Vitry et al. 2004a		AF550365	
Lophelia pertusa	N.E. Atlantic	Le Goff-Vitry et al. 2004b			AY257253- AY257337

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Lophelia pertusa	GoM- Tanker Gulf Penn, W. Schroeder	Morrison et al.	LpePenn-01	NEW	
Lophelia pertusa	GoM- Tanker Gulf Penn, W. Schroeder	Morrison et al.	LpePenn-04	NEW	
Lophelia pertusa	GoM- Sandra Brooke	Morrison et al.	LpeGulf-02	NEW	
Lophelia pertusa	W. Atlantic, Savannah Ross et al. 2004	Morrison et al.	Lpe4687-01	NEW	
Lophelia pertusa	W. Atlantic, NC Ross et al. 2003	Morrison et al.	Lpe3306-01	NEW	
Lophelia pertusa	W. Atlantic, Stetson Ross et al. 2004	Morrison et al.	Lpe4698-01	NEW	
Lophelia pertusa	W. Atlantic, NC Ross et al. 2003	Morrison et al.	Lpe036-01	NEW	
Lophelia pertusa	E. Atlantic, Sweden, T. Lundälv	Morrison et al.	LpeSW-14	NEW	
Lophelia pertusa	E. Atlantic, Sweden, T. Lundälv	Morrison et al.	LpeSW-15	NEW	
Lophelia pertusa	Gulf of Guinea	Morrison et al.	NMNH 79511	NEW	
Flabellidae					
Flabellum impensum	Antarctica	Romano and Cairns 2000	USNM 89307	AF265582	
Flabellum angularae	Porcupine Seabight	Le Goff-Vitry et al. 2004a		AF550363	

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Monomyces pygmaea	Mediterranean	Romano and Cairns 2000	USNM 98471	AF265583	
Placotrochus laevis	Beagle Gulf	Romano and Cairns 2000		AF265589	
Javania cailleti	GoM- GC234 CSA GoM 2004	Morrison et al.	Jca4740-03	NEW	NEW
Javania cailleti	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Jca4684-03	NEW	NEW
Javania cailleti	W. Atlantic, Ross et al. 2005	Morrison et al.	Jca4903-25		NEW
Javania cailleti	W. Atlantic, FL Ross et al. 2005	Morrison et al.	Jca4907-08	NEW	NEW
Javania cailleti	W. Atlantic, FL Ross et al. 2005	Morrison et al.	Jca4907-25		NEW
Javania cailleti	W. Atlantic, FL Ross et al. 2005	Morrison et al.	Jca4908-23		NEW
Javania cailleti	W. Atlantic, FL Ross et al. 2005	Morrison et al.	Jca4908-25	NEW	NEW
Javania cailleti	W. Atlantic, FL Ross et al. 2005	Morrison et al.	Jca4908-26	NEW	NEW
Polymyces fragilis	W. Atlantic, Stetson Ross et al. 2004	Morrison et al.	Pfrag4689-02	NEW	NEW
Turbinoliidae					
Tropidocyathus labidus	Wallis & Futuna	Romano and Cairns 2000	USNM 98759	AF265585	

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Notocyathus sp.	New Caldonia	Romano and Cairns 2000	Bathus 4-915	AF265584	
Guyniidae					
Guynia annulata	Mediterranean	Romano and Cairns 2000		AF265580	
MEANDRIINA Oculinidae					
Galaxea fascicularis	Guam	Romano and Palumbi 1996		L76006	
Acrhelia horrescens	Fiji	Romano and Palumbi 1996		L75994	
Oculina patagonica	Mediterranean	Romano and Cairns 2000		AF265601	
Madrepora oculata	Porcupine Seabight	Le Goff-Vitry et al. 2004a		AF550369	
Madrepora oculata	NE Atlantic, S. Ross Rockall Banks	Morrison et al.	MocuRB66-01	NEW	NEW
Madrepora oculata	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Mocu4681-02	NEW	
Madrepora oculata	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Mocu4681-03	NEW	
Madrepora oculata		Morrison et al.	Mocu4684-06		NEW
Madrepora oculata	GoM- MC885 CSA GoM 2004	Morrison et al.	Mocu4738-02B	NEW	NEW

Appendix 4-I (continued)

SUBORDER **Family** **Species**	**SOURCE**	**AUTHORS**	**SAMPLE ID#**	**Genbank Acc. No. 16S**	**Genbank Acc. No. ITS**
Madrepora oculata		Morrison et al.	Mocu4898-14		NEW
Madrepora oculata		Morrison et al.	Mocu4900-06		NEW
Madrepora oculata		Morrison et al.	Mocu4902-08		NEW
Madrepora oculata		Morrison et al.	Mocu4908-36		NEW
Madrepora oculata		Morrison et al.	Mocu4920-02		NEW
Meandrinidae					
Dichocoenia stokesi	Bahamas	Romano and Cairns 2000		AF265607	
PORITIINA Poritidae					
Porites compressa	Hawaii	Romano and Palumbi 1996		L76020	
Porites lobata	Reunion Island	Le Goff-Vitry et al. 2004a		AF550372	
Porites porites	Florida	Medina et al. 2006		DQ643837	
Goniopora stokesii	Palau	Romano and Palumbi 1996		L76008	
Alveopora sp.	Madang, PNG	Romano and Cairns 2000		AF265592	

Appendix 4-I (continued)

SUBORDER **Family** **Species**	**SOURCE**	**AUTHORS**	**SAMPLE ID#**	**Genbank Acc. No. 16S**	**Genbank Acc. No. ITS**
DENDROPHYLLIINA Dendrophylliidae					
Turbinaria peltata	Indo-Pac.	Romano and Palumbi 1996		L76023	
Tubastraea coccinea	Hawaii	Romano and Palumbi 1996		L76022	
Dendrophyllia gracilis	Bahamas	Romano and Cairns 2000	HB 20-XI-86-2-010	AF265588	
Dendrophyllia alternata	Brazil	Le Goff-Vitry et al. 2004a		AF550366	
Balanophyllia regia	Mediterranean	Romano and Cairns 2000		AF265587	
Leptopsammia pruvoti	Mediterranean	Romano and Cairns 2000		AF265579	
Enallopsammia rostrata	Bishop Seamount	France et al. 1996		U40294	
Enallopsammia rostrata	GC852	Morrison et al.	Eros4190-01	NEW	
Enallopsammia profunda	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Epro4704-02	NEW	
Enallopsammia profunda	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Epro4704-04	NEW	NEW
Enallopsammia profunda	W. Atlantic, Savannah Ross et al. 2004	Morrison et al.	Epro4688-02	NEW	NEW
Enallopsammia profunda	W. Atlantic, Stetson Ross et al. 2005	Morrison et al.	Epro4903-13		NEW

Appendix 4-I (continued)

SUBORDER Family Species	SOURCE	AUTHORS	SAMPLE ID#	Genbank Acc. No. 16S	Genbank Acc. No. ITS
Enallopsammia profunda	W. Atlantic, Stetson Ross et al. 2005	Morrison et al.	Epro4904-04		NEW
Enallopsammia profunda	W. Atlantic, Savannah Ross et al. 2005	Morrison et al.	Epro4905-06		NEW
Enallopsammia profunda	W. Atlantic, Savannah Ross et al. 2005	Morrison et al.	Epro4905-08		NEW
Thecopsammia socialis	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Tsoc4684-01	NEW	
Thecopsammia socialis	W. Atlantic, FL Ross et al. 2004	Morrison et al.	Tsoc4701-06	NEW	
Bathypsammia fallosocialis	W. Atlantic, Stetson Ross et al. 2005	Morrison et al.	Bfal4903-03	NEW	
Bathypsammia fallosocialis	W. Atlantic, Stetson Ross et al. 2005	Morrison et al.	Bfal4903-31	NEW	
Bathypsammia fallosocialis	W. Atlantic, Savannah Ross et al. 2005	Morrison et al.	Bfal4905-08	NEW	
Bathypsammia fallosocialis	W. Atlantic, FL Ross et al. 2005	Morrison et al.	Bfal4907-18a	NEW	
Bathypsammia fallosocialis	W. Atlantic, Miami Brooke et al. 2005	Morrison et al.	Bfal4920-08	NEW	

Appendix 4-II. On-deck photographs of coral species used in genetic analyses.

Desmophyllum dianthus (Caryophylliidae)
Site/Dive: Mississippi Canyon 885/ JSL-4738

Desmophyllum dianthus (Caryophylliidae)
Site/Dive: Mississippi Canyon 885/ JSL-4738

Caryophyllia polygona (Caryophylliidae)
Site/Dive: Mississippi Canyon 885/ JSL-4738

Caryophyllia polygona (Caryophylliidae)
Site/Dive: Mississippi Canyon 885/ JSL-4738

Caryophyllia berteriana (Caryophylliidae)
Site/Dive: Mississippi Canyon 929/ JSL-4864

Caryophyllia berteriana (Caryophylliidae)
Site/Dive: Mississippi Canyon 929/ JSL-4864

Caryophyllia new species (Caryophylliidae)
Site/Dive: Green Canyon 234/ JSL-4859

Caryophyllia new species (Caryophylliidae)
Site/Dive: Green Canyon 234/ JSL-4859

Lophelia pertusa (Caryophylliidae)
Site/Dive: Viosca Knoll 826/ JSL-4748

Lophelia pertusa (Caryophylliidae)
Site/Dive: Viosca Knoll 826 W/ JSL-4879

Solenosmilia variabilis (Caryophylliidae)
Site/Dive: Miami Terrace/ JSL-4922

Solenosmilia variabilis (Caryophylliidae)
Site/Dive: Miami Terrace/ JSL-4922

Madrepora oculata (Oculinidae)
Site/Dive: Cape Lookout NC North/ JSL-4891

Madrepora oculata (Oculinidae)
Site/Dive: Miami Terrace/ JSL-4920

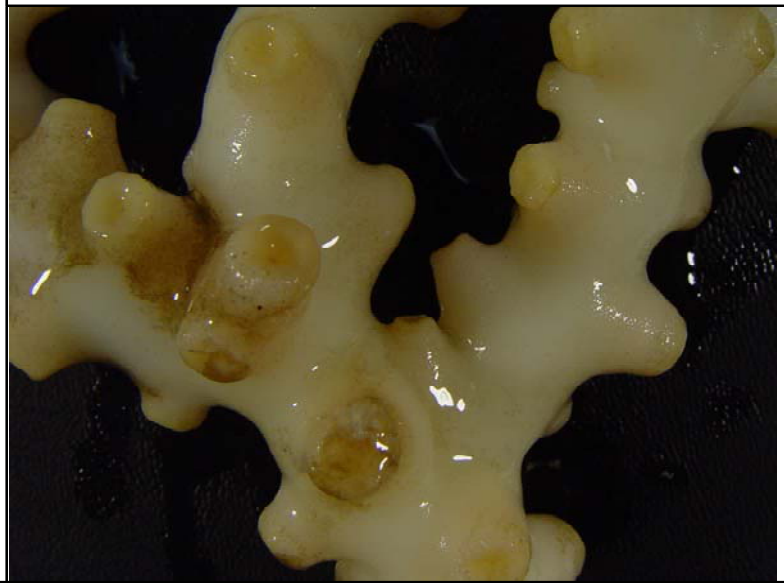
Madracis myriaster (Pocilloporidae)
Site/Dive: NC MPA/ JSL-4690

Labyrinthocyathus facetus (Caryophylliidae)
Site/Dive: Miami Sink Hole/ JSL-4917

Labyrinthocyathus facetus (Caryophylliidae)
Site/Dive: Miami Sink Hole/ JSL-4917

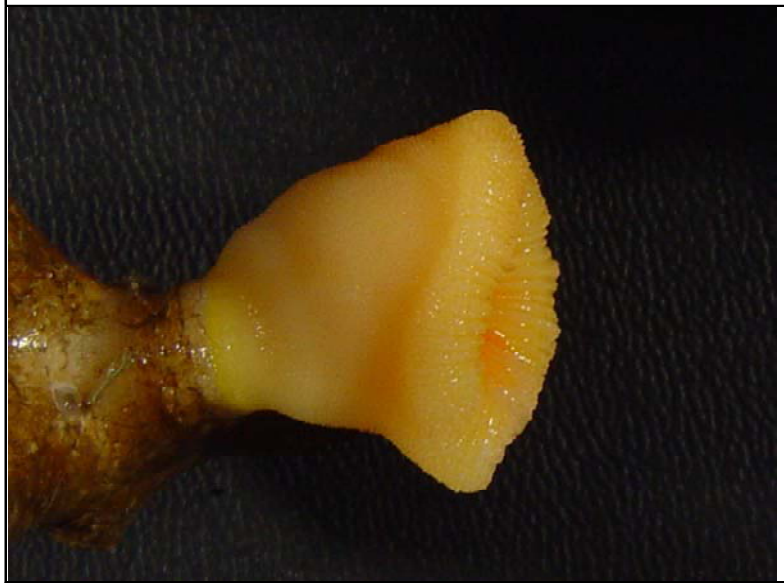
Enallopsammia profunda (Dendrophylliidae)
Site/Dive: Stetson Banks/ JSL-4903

Enallopsammia profunda (Dendrophylliidae)
Site/Dive: Stetson Banks/ JSL-4903



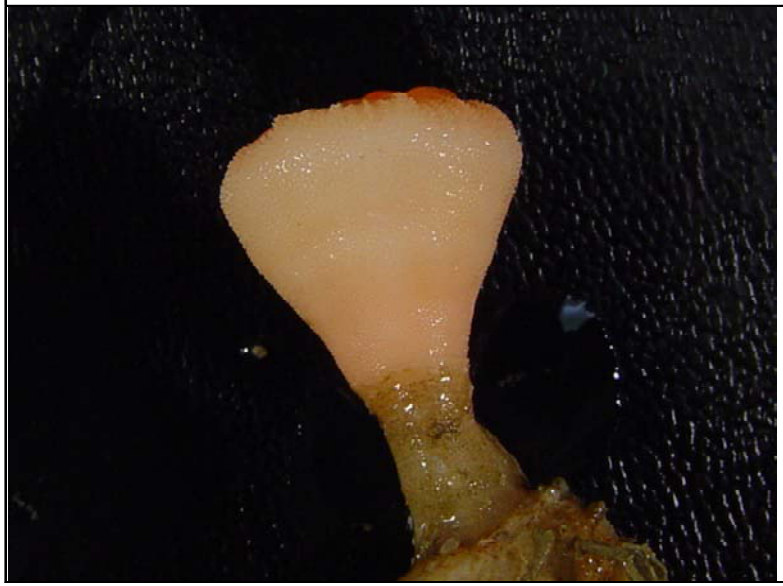
Thecopsammia socialis (Dendrophylliidae)
Site/Dive: Cape Fear, NC/ JSL-4896

Thecopsammia socialis (Dendrophylliidae)
Site/Dive: Cape Fear, NC/ JSL-4896



Bathypsammia fallosocialis (Dendrophylliidae)
Site/Dive: Stetson Banks/ JSL-4903

Bathypsammia fallosocialis
Site/Dive: Stetson Banks/ JSL-4903



Javania cailleti (Flabellidae)
Site/Dive: Jacksonville Lithoherms/ JSL-4685

Javania cailleti (Flabellidae)
Site/Dive: Jacksonville Lithoherms/ JSL-4907

Polymyces fragilis (Flabellidae)
Site/Dive: Stetson Banks/ JSL-4689