Eguchipsammia fistula Microsatellite Development and Population Analysis

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ABSTRACT

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Deep water corals are an understudied yet biologically important and fragile ecosystem under threat from recent increasing temperatures and high carbon dioxide emissions. Using 454 sequencing, we develop 14 new microsatellite markers for the deep water coral *Eguchipsammia fistula*, collected from the Red Sea but found in deep water coral ecosystems globally. We tested these microsatellite primers on 26 samples of this coral collected from a single population. Results show that these corals are highly clonal within this population stemming from a high level of asexual reproduction. Mitochondrial studies back up microsatellite findings of high levels of genetic similarity. CO1, ND1 and ATP6 mitochondrial sequences of *E. fistula* and 11 other coral species were used to build phylogenetic trees which grouped *E. fistula* with shallow water coral *Porites* rather than deep sea *L. Petusa*.

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Chapter 1: Introduction

1.1 Deep Water Corals

Deep water corals, often referred to as cold water corals, are generally considered to include corals found deeper than 50m (Cairns 2007, Wilson 2009) although some have different definitions (Rogers 2004). Of the approximately 1482 species of described Scleractinian corals, 615 species occur deeper than 50m and are thus are considered deep water species (Cairns 2007). Deep water corals are azooxanthellate corals generally found in temperatures ranging from 4°-12°C; this would limit their depth to 1000m in higher latitudes and to 4000m around equatorial regions (Roberts et al. 2006). Concern for the health of coral reefs and the importance for marine protected areas is being realized for reefs and their associated organisms (Jones et al. 2009). Many economically important animals utilize seamount habitats. On seamounts, deep water corals create the primary structures of interest for these animals. These habitats are often used as sources of food and protection in an otherwise vast open ocean. For example, long lining studies have shown that fish are more populous above deep water coral reefs compared to surrounding areas (Husebø et al. 2002).

Deep water corals, however, are particularly vulnerable to climate change impacts. Anthropogenic carbon dioxide emissions have increased the acidity of the world's oceans and will continue to do so even under optimistic scenarios in the region. Ocean acidification is leading to a decrease in the amount of carbonate ions, which leads to a decrease in aragonite saturation in deeper water. Aragonite is a key mineral in the

formation of Scleractinian coral skeletons. As acidity increases the deep sea corals may even start dissolving which will threaten their survival (McCulloch et al. 2012).

1.2 The unique environment of the Red Sea

The physical characteristics of the Red Sea are different from other oceans in the world in several ways. Since the Red Sea is small and enclosed, more evaporation occurs relative to other oceans in the world (Privett 1959). The amount of runoff from surrounding land is low because much of the surroundings are desert. This, coupled with the region's high temperatures, leads to typically higher salinity than most seas. The Red Sea is relatively shallow, reaching a maximum depth of about 2000m. The temperature of the Red Sea deep water is unusually warm; even at great depths, the temperature is above 20°C (Edwards et al.1987). The temperature actually increases at greater depths because of brine pools of extremely high temperatures and salinity at the bottom of the Red Sea (Mantyla & Reid 1983). The Red Sea is also relatively oligotrophic. Deep water corals have rarely been studied in the Red Sea and most of what is known about deep water corals from the Red Sea comes from dredging studies. Marenzeller discovered *Eguchipsammia fistula* in the Red Sea on an expedition between 1895 and1897. Later expeditions led to findings of mostly "sub-fossilized" corals with few living specimens (Taviani et al. 2007). Most of the corals living in the Red Sea were believed to have gone extinct around the Last Glacial Maximum lowstand 19,500 years before present (BP) and all coral fauna found living in the Red Sea today must have re-colonized the Red Sea starting from approximately 10,000 yrs BP.

1.3 Coral Reproduction

Most species of Scleractinian corals have the capability to reproduce sexually. There are relatively few species of corals that are hermaphroditic or gonochronistic brooders, i.e. meaning that each colony will produce either the eggs or the sperm at one time (Harrison 2011). Hermaphroditic spawning species are the most common followed by gonochronistic spawning species. Sexual reproduction is both beneficial and detrimental for an organism (Crow 1994). Although it is energetically more costly to reproduce sexually, the benefits of the variation that is the result of sexual reproduction are sufficient for most organisms to practice it (Crow 1994). Some shallow water corals in the Red Sea reproduce only sexually although this is not the norm (Maier et al. 2005). There is very little research done on the reproduction of deep water corals (Waller et al. 2005). As of 2005 only 15 species of deep water Scleractinian corals have had their reproduction studied. In contrast to shallow water species, few (only three of these 15) species are hermaphroditic. However, hermaphroditism in deep water species is not the same as hermaphroditism in shallow water corals. These deep water species are cyclical hermaphrodites in which both spermacysts and oocytes are developed at the same time in the same mesentery but either the eggs or sperm are released at one time (Waller et al. 2005). Seventy-five percent of the fifteen deep water corals examined by Waller et al. (2005) are gonochronistic. Asexual reproduction is also very important and common in most coral species. Reproduction by fragmentation occurs when a fragment breaks off an adult colony and, instead of dying, forms a new viable individual (Highsmith 1982). Many corals, particularly the Acroporidae and Poritidae, are thought to reproduce mainly through fragmentation (Highsmith 1982). According to unpublished work by Jessica

Bouwmeester, *Eguchipsammisa fistula*, produces gametes and is gonochronistic. Histological samples did not have any brooded planulae, so it was inferred that this species is a broadcast spawner.

Deep sea corals in the Red Sea are rarely studied and have never been studied with molecular techniques before. In this study I address a population *of Eguchipsammia fistula* to see how genetically similar the corals we collected are. The assumption is that the genetic relatedness, along with the histology work already done (by Jessica Bouwmeester), will tell us about the reproduction methodology of this species. Microsatellite loci will be the most useful in discerning the genetic differences between samples from a small population and to unveil the position of *Eguchipsammia fistula* in a phylogenetic tree of corals we applied a marker that evolves slower than microsatellites. A variety of mitochondrial markers are available, and for this study I collected markers from a GenBank database for several other coral species.

Chapter 2: Materials and Methods

Eguchipsammia fistula was described by Alcock in 1902 (Figure 1). It has been found from depths of 80 meters to 910 meters (Cairns 1984) and is distributed throughout the world's oceans including throughout the Indo-West Pacific, New Zealand and in the Red Sea (Evenhuis & Eldredge 2006; Gordon 2009; Vine 1986). This species has also been called *Eguchipsammia oaheansis* by Vaughan in 1907 according to the World Registry of Marine Species (Cairns 2009), although this name is not scientifically accepted anymore.

The samples for this study were collected from Glider Ridge (22°17.835'N, 38°53.815'E) in the Red Sea at 319 m depth using a remotely operated vehicle (ROV) from the AEGAEO research vessel on December 9, 2011. These samples were collected using a scoop on the manipulator of the ROV; unfortunately this method did not allow us to collect individual colonies, but only large groups with several colonies adjacent to each other. Two groups of samples were collected approximately 35 meters apart from each other. Thirty branches were preserved in 70 percent ethanol so we would be able to extract DNA from them. Samples were preserved by branches, because by the time we preserved them whether they were from the same colony or not was unknown; if one fragment had more than one branch we preserved these branches together in the same falcon tube. Coral tissue was blown off the skeleton using an airbrush. To increase DNA concentration coral tissue was collected using 1X Tris EDTA buffer and centrifuged for 3 minutes at 13,000rpm. Excess TE was removed and the concentrated pellet was used in the QIAGEN blood and tissue DNA extraction kit. The protocol was followed exactly but

the amount of reagent was always halved. This ensured us a higher concentration of DNA, which could later be diluted for purposes that do not require a high concentration of DNA such as PCR. Out of the 30 samples we preserved in ethanol we were able to successfully extract DNA from 26 of them. Of these 26 fragments, two samples had multiple branches preserved in the falcon tubes. We successfully extracted seven branches from one of the 26 fragments and 3 branches from another.

2.1 Microsatellite Development

Library construction and 454 FLX titanium shotgun sequencing was carried out in the Bioscience Core Lab at King Abdullah University of Science and Technology following a protocol described in the Rapid Library Preparation Method Manual of the GS FLX Titanium Series from October 2009. Our DNA was multiplexed with DNA from five other species. A total of 194,796 reads were obtained for *E. fistula*. The program MSATCOMMANDER (version 0.8.2) was used to identify reads containing at least eight repeats of di-, tri-, or tetranucleotide sequences (Faircloth 2008). This program then uses Primer 3 to design primers for suitable sequences where possible (Rosen & Skaletsky 2000). A total of 5,593 reads had repeats that met our criteria (at least 8 repeats of di, tri or tetranucleotides). For 1450 of these reads MSATCOMANDER was able to design primers pairs. Thirty-eight primer pairs were selected for initial screening of 26 individuals. These primer pairs were then tested to see whether they would amplify for all samples and see whether they were polymorphic or not. Initial PCR products were tested using the QIAXEL from QIAGEN. Instead of performing gel electrophoresis, high resolution cartridges were used. We found that 25 primer pairs amplified for all of our

coral samples. Eleven of these loci had multiple bands in the PCR product rendering then useless for our population studies. From the 14 remaining loci, 12 were polymorphic for the samples we had whereas two were monomorphic.

These loci were analyzed on 26 samples with dye labeled forward primers. PCRs were performed using the PCR-multiplex kit from QIAGEN following the manufacturer's protocol. PCRs were multiplexed into three different sets using a thermal profile consisting of an initial denaturing step of 15 minutes at 94°C followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 57-62°C (depending of the primer) and 30 seconds at 72°C. Samples were analyzed on a ABI PRISM 3730 sequencer using the GeneScan-500 LIZ internal size standard and genotyped using PEAKSCANNER(v 1.0). ARLEQUIN 3.1(Excoffier et al. 2005) was used to estimate the observed (Ho) and the expected (He) levels of heterozygosity and to evaluate deviations from Hardy-Weinberg Equilibrium (HWE). The number of alleles and linkage disequilibrium were estimated using GENEPOP 4.0.01(Raymond & Rousset 1995). The data was first put into an excel sheet and manipulated with the Microsoft excel Add-in GENALEX (Peakall & Smouse 2006). MICROCHECKER was used to detect null alleles could be the most probable cause of HWE departures.

For the purposes of cross-validating these primers, we also collected Dendrophylliidae samples from shallow water reefs. Three samples we collected were from the genus *Tubastrea*, and the others were from various different genera. Twenty-five samples were from the genus *Balanophyllia* and the same species. We were unable

to identify the species for any of these corals. We chose corals that all belong to the family Dendrophylliidae because this is the same family that *E. fistula* is in.

2.2 Mitochondrial sequences

Four different regions of the mitochondria were sequenced for population genetic analysis. To amplify the ND1 region and part of the Cytochrome b region we used the forward primer Cs-F2 (CCATTGCTTATCACAGTAGCT) and reverse primer Cs-R2 (TAATGCATGGACAAAAAGCACC) (Lin et al. 2011). To amplify the ATP6 region of the mitochondria we used the forward primer Cs-F6 (TATGATCATCTTCATGGTGTCG) and the reverse primer Cs-R6 (GGGATCAATATGCCCTCAAA). The final region we amplified from the mitochondria is cytochrome oxidase 1. We used the forward primer Cs-F18 (GGACACAAGAGCATATTTTACTG) and the reverse primer Cs-R18 (CTACTTACGGAATCTCGTTTGA). PCR was performed on 6 individual samples with the following thermal profile 94°C for 15 minutes, followed by 36 cycles of 30 seconds at 95°, 1 minute and 30 seconds at 56°, and 30 seconds at 72°, and ending with 30 minutes at 60°. The PCR products were then run on a gel to make sure amplification occurred. The PCR products were then purified using QIAGEN gel purification kits. These were then Sanger sequenced by the Bioscience Core Lab at KAUST. Chromatograms were viewed and sequences edited using BIOEDIT 5.0. 6 (Hall 2001). Mitochondrial sequences corresponding to our sequences were collected for several species of Scleractinian corals from NCBI GenBank. We collected the ND1, CO1 and

Cytb from *Porites okinawensis, Colpophyllia natans, Siderastrea radians, Mussa angulosa, Porites porites, Montastraea franksi, Euphyllia ancora, Lophelia pertusa, Montipora cactus, Stylophora pistillata* and *Madrepora oculata*. The sequences were then aligned using ClustalW in MEGA. MEGA was then used to construct neighbor joining trees and UPGMA trees with 500 replicates of bootstrap to see phylogenetic relationships.

Chapter 3: Results

3.1 Microsatellite Development

Seven of our loci were observed to deviate from Hardy-Weinberg equilibrium. Observed number of alleles per locus ranged from 1 in loci DSC3 and DSC28 to 4 in locus DSC32. Ho and He varied from 0.000 to 1.000 and from 0.112 to 0.642 respectively (Table 1). Seven out of 14 loci deviated significantly from HWE after Bonferroni corrections. According to MICROCHECKER loci DSC6, DSC9, and DSC28 have an excess of homozygotes that may be caused by the presence of null alleles. These loci also appear to be highly linked according to ARLEQUIN. Loci DSC6, DSC9, DSC11 and DSC28 are significantly linked to 8 of the other loci.

Tubastrea coral samples were used to for cross validation testing of microsatellite primers because like *Eguchipsammia fistula* they belong to the family Dendrophylliidae. Most microsatellites amplified well in *Tubastrea.* The three samples of *Tubastrea* did not have any differences between them, but were distinct from the deep sea coral alleles. We included all of the loci that were polymorphic for the samples collected from Glider Ridge. We also included 1 locus that was monomorphic for these samples. We were able to check for clones using GENALEX in Microsoft Excel. Results showed that ten of our samples were clones of each other.

3.2 Mitochondrial Sequences

The mitochondrial sequences ATP6, CO1, and ND1 for our six samples showed no variability among each other. Phylogenetic trees (Figure 2A) according to ATP6 sequence showed that our coral sequence was most closely related to the two *Porites*

organisms. This is also true for the trees according to ND1 (Figure 2B) and CO1 (Figure 2C), with bootstrap number different slightly and showing that CO1 is the most variable part of the mitochondrial genome out of the 3 genes we sequenced. The one other deep sea coral in our dataset, *Lophelia pertusa,* was not the most closely related to *E. fistula*. Although there were slight differences among the phylogenetic trees according to the sequence sets, there was an overall similarity in the branching patterns.

Chapter 4: Discussion

We found that our dataset consisted largely of clones. This could be for reasons such as the reproductive behavior of our corals, or it could be related to our sampling methods. Asexual reproduction is likely why more than half of our samples have clones within our sample set. Cloning is found in turbulent environments more than any others (Heyward & Negri 2012). Newly formed embryos in turbulent surface waters are in many cases shredded into pieces. This however does not result in the death of the embryo but in the formation of clones. The fragmented pieces of embryo continue to divide and form smaller larvae that can still settle and grow into a normal adult colony (Miller et al. 2004). Since this population is found to be highly clonal with little genetic variation it will be difficult for recovery to occur in case of disaster such as decreasing the aragonite saturation in deeper water.

Although we have a large amount of clones within our sample set, we also do have some genetic variation. Among our 26 samples we have 15 unique haplotypes which means sexual reproduction definitely has to be occurring. Our study species as a gonochronistic spawner in the deep sea faces some challenges. Deep water species also have a reduced number of eggs that are generally larger than their shallow water counterparts. This may be because populations of corals in the deep sea are more widely dispersed and the amount of food in the deep sea is usually less than in shallow water. Due to these conditions, species may be more likely to persist if they are brooding than if they are spawners (Waller et al. 2005). However, for any conclusions to be drawn about whether this species is predominantly sexually reproducing or asexually reproducing, or

whether the mode of reproduction is dependent on the environment we need to continue this work with a larger sample size.

We started out with a very large number of potential microsatellite loci, but many of these were subsequently eliminated. Although 25-30 samples collected from a population should be enough to know the extent of diversity in a population using microsatellites (Hale et al. 2012), our sampling method may have been problematic since we collected samples from only 35 meters apart. We do not know if we have captured all of the genetic variation in this population, but it is very likely that we did not. It is also possible that when collecting and sorting samples we separated branches from the same colony and considered them separate samples. This sampling method could be the reason why so many of our loci are not in HWE. However, other deep water coral populations have been found to deviate from HWE (Rodriguez et al. 2004).

Although we initially tested 38 primer pairs, we ended up with only 14 working ones. A large number of these were excluded outright because they did not amplify for all 26 samples. Out of the ones that did, however, we found that a large majority had multiple bands. Although for microsatellites two bands are acceptable (in the case of a heterozygous individual), multiple bands means that there is more than one region of the genome being amplified by one set of primers. There are many reasons as to why this intracolonial variation could happen. It is possible for coral colonies to grow into each other and fuse, this often results in one colony having more than one genotype or allogenic fusion (Maier et al. 2012). Although we do not have sufficient evidence to fully

address this hypothesis, future studies delving into this topic could be important. Another obvious possibility for this variation is somatic mutation (Maier et al. 2012).

We also learned that *E. fistula* is more closely related to shallow water corals than it is to other deep sea corals such as *L. pertusa*. The most widely studied deep sea coral is *Lophelia pertusa*. According to phylogenetic sequences this species is not very closely related to *Eguchipsammia fistula* (Goff-vitry et al. 2004). When looking at these species however, they are very morphologically similar. Yet they are not even in the same suborders. *Lophelia* is in the suborder Caryophiliidae while *E. fistula* is in the suborder Dendrophyllidae. These deep sea corals are more closely related to other shallow water corals than they are to each other. The fact that deep water corals are not all phylogenetically closer to each other than to shallow water corals has been noted in many cases (Kitahara et al. 2010; Lindner et al. 2008). Research suggests that shallow water *Stylasteridae* corals evolved from deep sea corals three different times (Lindner et al. 2008). The most basal lineages of Scleractinian corals are seen to be corals from the families Gardineriidae and Micrabaciidae, both of which are solitary azooxanthellate species which seem to be adapted for life in the deep sea (Kitahara et al. 2010). This shows us the importance of continuing to look for answers in the deep sea about the evolutionary history of corals and maybe other species.

To make these results conclusive it will be necessary to increase the sample size. Larval connectivity and population structure have been examined in a few species of deep water corals. For a true connectivity study we must collect samples of *E. fistula* from locations other than glider ridge. Deep water corals species sometimes do have large

amounts of genetic structure among populations (Baco et al. 2005; Goff-vitry et al. 2005). The connectivity and structure of our coral species would be worth studying, but the sampling method must be improved so that we can not only increase the sample size but get a true idea of the heterozygosity of the population.

Chapter 5: Conclusions

We found that our sample set of *E. fistula* was had high clonality. This could mean that our coral reproduces predominantly asexually. These clones could also be the result of incorrect sampling procedures. Although we have a many clones, we also found 15 different haplotypes within our sample set of 26, meaning sexual reproduction is occurring.

According to mitochondrial DNA data, *E. fistula* is more closely related to shallow water coral species than to other deep water coral such as *L. pertusa*.

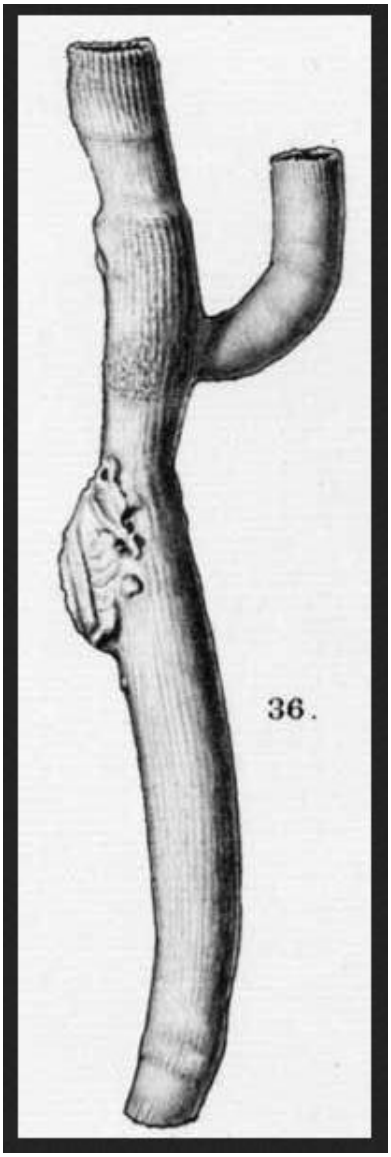

Figure 1: Eguchipsammia fistula by Alcock 1906

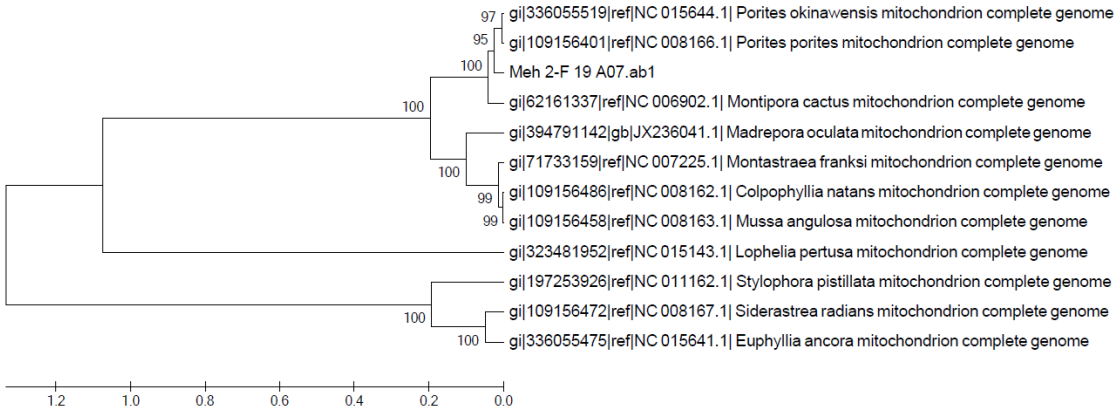

Figure 2A: Phyolgenetic tree according to ND1

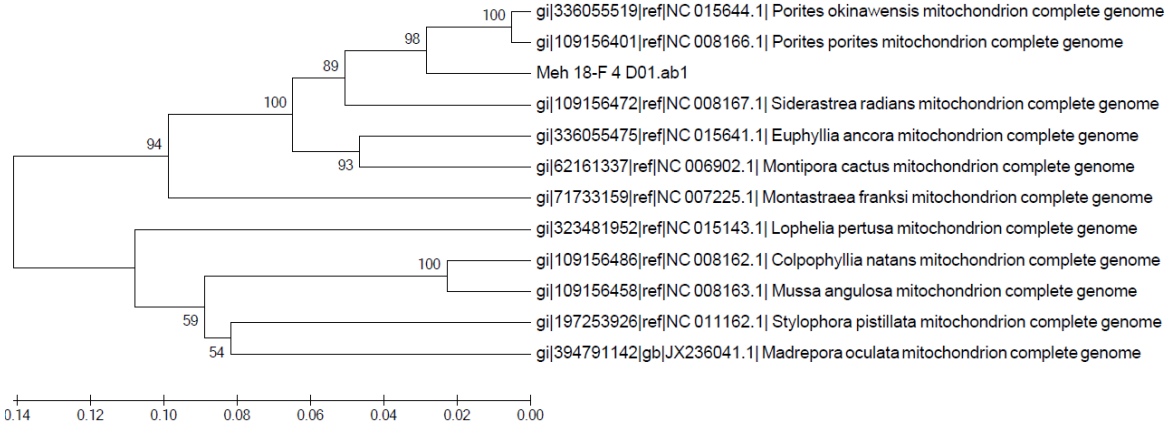

Figure 2B: Phylogenetic tree according to CO1

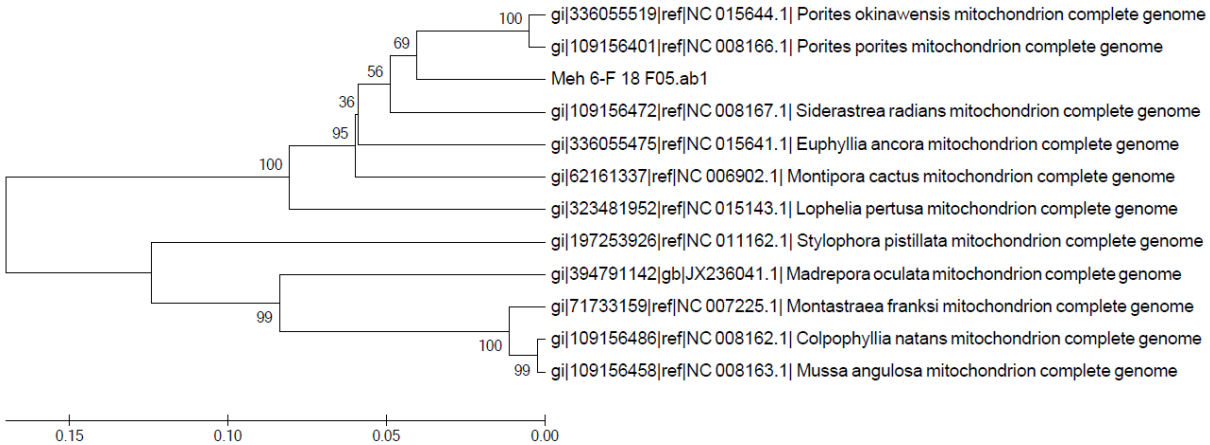

Figure 2C: Phylogenetic tree according to ATP6

Locus	Primer sequence	Repeat motif	N_A	Size range (bp)	HWE	H_O/H_E	Genbank accession no.
DSC3	F: TTCACGTGCTTTCTTGTGC[VIC]	$(AC)_6(AG)_1(AC)_8$	1	215			KC130924
	R: CTGGCAAGCTCTAAGTGGC						
DSC6	F: GTGACATGGCGGCTGAAC[PET]	$(AC)_9$	3	175-183	0.023	0.043/0.127	KC130925
	R: TCTGGCATACCACTGACCG						
DSC9	F: GCAACTCCTGAACTTGGGC[NED]	$(AT)_{12}$	2	143-157	0	0.000/0.487	KC130926
	R: GGTCGTTGTAAATCGAGGTAGC						
DSC11	F: ATTCGGTGACTGGCGAAGG[6FAM]	$(AT)_9$	2	169-171	1	0.280/0.246	KC130927
	R: GGATCCGATTTGTTTATCACGC						
DSC15	F: TTCGATTCCCACTCACGGC[PET]	$(TA)_9$	3	192-198	1	0.115/0.112	KC130928
	R: AGTCATTTAAGCTGGTTTGCTC						
DSC22	F: GGCTATCATCACAGTGCTCC[VIC]	$(AT)_8$	3	173-175	0	1.000/0.630	KC130929
	R: TGACTGTTGGCTTGGTAAACG						
DSC23	F: GAGTGGACCTAAGCAGAAAGG[NED]	$(AT)_9$	2	221-225	0.55	0.423/0.340	KC130930
	R: ACTTGAAATCTTAGTGACAGCCC						
DSC25	F: CGAAGGCCCGAGCGTAG[PET]	$(TA)_9$	2	320-332			KC130931
	R: CCATTTGGCGGAGTTTCGG						
DSC28	F: AGCATCTGCACGCTCCTAGT[VIC]	$(AAT)_{10}$	1	169			KC130932
	R: CAAACGAGCATGGTGTACCT						
DSC29	F: GAAGTTCTCGGGTCATTGGA[NED]	$(AGT)_{11}$	2	269-272	1	0.346/0.292	KC130933
	R: TCGCGATCTCCTTAACTCTGA						
DSC30	F: ATCATTAATCTGCAAACATTACGC[6FAM]	$(CAT)_{16}$	2	201-204	0.08	0.615/0.446	KC130934
	R: TGTGCTTATAATGGAAAAAGGTTG						
DSC31	F: CCAAAGAAAGTGTGCAATATCCC[PET]	$(AAT)_{12}$	2	167-173	0.558	0.360/0.301	KC130935
	R: GGTTAGGTCAGTCTCGCGG						
DSC32	F: ATCACGAATGTGGGAAGCC[NED]	$(AAT)_9$	4	244-259	0	0.423/0.642	KC130936
	R: GTCCGTACGCCATGACCTC						
DSC34	F: ACTCGTTTACTTGGAGACATCG[NED]	$(TTA)_{16}$	2	324-366	0	0.000/0.479	KC130937
	R: CTCAACTGGCCGATCAACTG						

Table1: Microsatellite primers and details. HWE P-values after Bonferroni correction

REFERENCES

Abdelkrim, J. et al., 2009. Fast, cost-effective development of species-specific microsatellite markers by genomic sequencing. *BioTechniques*, 46(3), pp.185–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19317661 [Accessed November 4, 2012].

Baco, A.R. & Shank, T.M., 2005. Population genetic structure of the Hawaiian precious coral Corallium lauuense (Octocorallia : Coralliidae) using microsatellites. *Cold-water Corals and Ecosystems*, pp.663–678.

Brown, W.M., George, M. & Wilson, a C., 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 76(4), pp.1967–71. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=383514&tool=pmcentrez&rendertype=abstract.

Chistiakov, D. a., Hellemans, B. & Volckaert, F. a. M., 2006. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. *Aquaculture*, 255(1-4), pp.1–29. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0044848605007313 [Accessed October 30, 2012].

Conrad, D.F. et al., 2006. A worldwide survey of haplotype variation and linkage disequilibrium in the human genome. *Nature genetics*, 38(11), pp.1251–60. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17057719 [Accessed November 1, 2012].

Costantini, F., Fauvelot, C. & Abbiati, M., 2007. Genetic structuring of the temperate gorgonian coral (Corallium rubrum) across the western Mediterranean Sea revealed by microsatellites and nuclear sequences. *Molecular Ecology*, 16, pp.5168–5182.

Crow, J.F., 1994. Advantages of sexual reproduction. *Developmental genetics*, 15(3), pp.205–13. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8062455.

Edwards, Y.J. et al., 1998. The identification and characterization of microsatellites in the compact genome of the Japanese pufferfish, Fugu rubripes: perspectives in functional and comparative genomic analyses. *Journal of molecular biology*, 278(4), pp.843–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9614946.

Ellegren, H., 2000. Microsatellite mutations in the germline : implications for evolutionary inference. *TIG*, 16(12).

Ellegren, H., 2004. Microsatellites: simple sequences with complex evolution. *Nature reviews. Genetics*, 5(6), pp.435–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15153996 [Accessed November 1, 2012].

Estoup, A., Jarne, P. & Cornuet, J.-M., 2002. Homoplasy and mutation model at microsatellite loci and. *Molecular ecology*, 11, pp.1591–1604.

Evenhuis, N.L. & Eldredge, L.G., 2006. Records of the Hawaii Biological Survey for 2004-2005. *Bishop Museum Occasional Papers*, 87(l), p.66.

Eytan, R.I. et al., 2009. Nuclear sequences reveal mid-range isolation of an imperilled deep-water coral population. *Molecular Ecology*, 18, pp.2375–2389.

Flot, J. & Tillier, S., 2006. Molecular phylogeny and systematics of the scleractinian coral genus Pocillopora in Hawaii. *Proceedings of the 10th International Coral Reef Symposium*, pp.24–29.

Goff-vitry, M C Le, Rogers, A D & Baglow, D., 2004. A deep-sea slant on the molecular phylogeny of the Scleractinia. *Molecular Phylogenetics and Evolution*, 30, pp.167–177.

Goff-vitry, Marie C Le & Rogers, Alex D, 2005. Molecular ecology of Lophelia pertusa in the NE Atlantic. *Cold-water Corals and Ecosystems*, pp.653–662.

Goldstein, D.B. et al., 1995. Evaluation of Genetic Distances. *Genetics*, pp.463–471.

Gordon, D., 2009. *New Zealand Inventory of Biodiversity. Volume One: Kingdom Animalia.*,

Hale, M.L., Burg, T.M. & Steeves, T.E., 2012. Sampling for Microsatellite-Based Population Genetic Studies : 25 to 30 Individuals per Population Is Enough to Accurately Estimate Allele Frequencies. *PLOS one*, 7(9).

Hall, T., 2001. BioEdit Help Contents.

Harr, B. & Schlötterer, C., 2000. Long microsatellite alleles in Drosophila melanogaster have a downward mutation bias and short persistence times, which cause their genome-wide underrepresentation. *Genetics*, 155(3), pp.1213–20. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1461152&tool=pmcentrez&rendertype=abstract.

Harrison, P.L., 2011. Sexual Reproduction of Scleractinian Corals.

Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species.

Proceedings. Biological sciences / The Royal Society, 270 Suppl , pp.S96–9. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1698023&tool=pmcentrez&rendertype=abstract [Accessed October 29, 2012].

Heyward, A.J. & Negri, A.P., 2012. Turbulence, Cleavage, and the Naked Embryo: A Case for Coral Clones. *Science*, 335(March), p.2012.

Highsmith, R., 1982. Reproduction by Fragmentation in Corals. *Marine Ecology Progress Series*, 7(4), pp.207–226. Available at: http://www.int-res.com/articles/meps/7/m007p207.pdf.

Jeffreys, A.J. et al., 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. *Nature*, 332(17).

Jones, G.P. et al., 2009. Larval retention and connectivity among populations of corals and reef fishes : history , advances and challenges. *Coral Reefs*, 28, pp.307–325.

Kimura, M. & Ota, T., 1975. Distribution of allelic frequencies in a finite population under stepwise production of neutral alleles. *Proceedings of the National Academy of Sciences of the United States of America*, 72(7), pp.2761–4. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=432851&tool=pmcentrez&rendertype=abstract.

Kitahara, M.V. et al., 2010. A Comprehensive Phylogenetic Analysis of the Scleractinia (Cnidaria , Anthozoa) Based on Mitochondrial CO1 Sequence Data. *PLOS one*, 5(7).

Lin, M. et al., 2011. Seventy-four Universal Primers for Characterizing the complete Mitochondrial Genomes of Scleractinian Corals (Cnidaria ; Anthozoa). *Zoological Studies*, 50(4), pp.513–524.

Lindner, A., Cairns, S.D. & Cunningham, C.W., 2008. From Offshore to Onshore : Multiple Origins of Shallow- Water Corals from Deep-Sea Ancestors. *PLOS one*, 3(6).

Maier, E et al., 2012. Intracolonial genetic variation in the scleractinian coral Seriatopora hystrix. *Coral Reefs*, 31(2), pp.505–517.

Maier, Elke, Tollrian, Æ.R. & Rinkevich, Æ.B., 2005. Isolation by distance in the scleractinian coral Seriatopora hystrix from the Red Sea. *Marine Biology*, pp.1109–1120.

Mantyla, A.W. & Reid, J.L., 1983. Abyssal characteristics of the World Ocean waters. *Deep Sea Research*, 30(8).

McCulloch, M. et al., 2012. Resilience of cold-water scleractinian corals to ocean acidification : Boron isotopic systematics of pH and saturation state. *Geochimica Et Cosmochimica Acta*, 87, pp.21–34.

Mcfadden, C.S. et al., 2006. A molecular phylogenetic analysis of the Octocorallia (Cnidaria : Anthozoa) based on mitochondrial protein-coding sequences. *Molecular Phylogenetics and Evolution*, 41, pp.513–527.

Metzgar, D., Bytof, J. & Wills, C., 2000. Selection Against Frameshift Mutations Limits Microsatellite Expansion in Coding DNA Selection Against Frameshift Mutations Limits Microsatellite Expansion in Coding DNA. , pp.72–80.

Meyer, E. et al., 2009. Sequencing and de novo analysis of a coral larval transcriptome using 454 GSFlx. *BMC genomics*, 10, p.219. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2689275&tool=pmcentrez&rendertype=abstract [Accessed October 31, 2012].

Miller, K J & Ayre, D J, 2004. The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral Pocillopora damicornis. *Heredity*, 92(6), pp.557–68. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15100708 [Accessed October 18, 2012].

Miller, Karen J & Ayre, David J, 2008. Population structure is not a simple function of reproductive mode and larval type : insights from tropical corals. *Journal of Animal Ecology*, pp.713–724.

Nachman, M.W. & Crowell, S.L., 2000. Estimate of the mutation rate per nucleotide in humans. *Genetics*, 156(1), pp.297–304. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1461236&tool=pmcentrez&rendertype=abstract.

Neves, E. et al., 2008. Genetic variation and population structuring in two brooding coral species (Siderastrea stellata and Siderastrea radians) from Brazil. *Genetica*, 132(3), pp.243–254.

Peakall, R. & Smouse, P.E., 2006. genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1), pp.288–295. Available at: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1471-8286.2005.01155.x [Accessed October 4, 2012].

Pearson, C.E., Nichol Edamura, K. & Cleary, J.D., 2005. Repeat instability: mechanisms of dynamic mutations. *Nature reviews. Genetics*, 6(10), pp.729–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16205713 [Accessed November 3, 2012].

Pompanon, F. et al., 2005. Genotyping errors: causes, consequences and solutions. *Nature reviews. Genetics*, 6(11), pp.847–59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16304600 [Accessed October 29, 2012].

Privett, D.W., 1959. Monthly charts of evaporation from the N . Indian Ocean (including the Red Sea and the Persian Gulf). , 582, pp.424–428.

Richards, R.! & Sutherland, G.R., 1994. Simple repeat DNA is not replicated simply. *Nature Genetics*, 6.

Rodriguez, C.G. & Lasker, H.R., 2004. Microsatellite variation reveals high levels of genetic variability and population structure in the gorgonian coral Pseudopterogorgia elisabethae across the Bahamas. *Molecular Ecology*, 13, pp.2211–2221.

Schlotterer, C. & Tautz, D., 1992. Slippage synthesis of simple sequence DNA. *Nucleic Acids Research*, 20(2), pp.211–215.

Shearer, T.L. et al., 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, 11, pp.2475–2487.

Shearer, T.L. & Coffroth, M.A., 2008. Barcoding corals : limited by interspecific divergence , not intraspecific variation. *Molecular ecology resources*, 8, pp.247–255.

Sun, J.X. et al., 2009. Microsatellites are molecular clocks that support accurate inferences about history. *Molecular biology and evolution*, 26(5), pp.1017–27. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2734136&tool=pmcentrez&rendertype=abstract [Accessed November 13, 2012].

Takabayashi, M. et al., 1998. A coral-specific primer for PCR amplification of the internal transcribed spacer region in ribosomal DNA. *Primer Notes*, 7, pp.8–9.

Taviani, M. et al., 2007. Last Glacial Deep-Water Corals From The Red Sea. *Bulletin of Marine Science*, 81(3), pp.361–370.

Valdes, a M., Slatkin, M. & Freimer, N.B., 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. *Genetics*, 133(3), pp.737–49. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1205356&tool=pmcentrez&rendertype=abstract.

Vine, P., 1986. *Red Sea Invertebrates.*, London: Immel Publishing.

Vollmer, S.V. & Palumbi, S.R., 2004. Testing the utility of internally transcribed spacer sequences in coral phylogenetics. *Molecular Ecology*, 13, pp.2763–2772.

Waller, R.G., Tyler, P. a. & Gage, J.D., 2005. Sexual reproduction in three hermaphroditic deep-sea Caryophyllia species (Anthozoa: Scleractinia) from the NE Atlantic Ocean. *Coral Reefs*, 24(4), pp.594–602. Available at: http://www.springerlink.com/index/10.1007/s00338-005-0031-3 [Accessed October 4, 2012].

Weber, J. & Broman, K.W., 2001. Genotyping for Human Whole-Genome Scans: Past, Present, and Future. *Advances in Genetics*, 42.

Weersing, K. & Toonen, R.J., 2009. Population genetics , larval dispersal , and connectivity in marine systems. *Marine Ecology Progress Series*, 393, pp.1–12.