EXPRESSED GENES OF THE DEEP-WATER CORAL, *LOPHELIA PERTUSA*

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Key Words: Deep-sea coral, gene expression, cDNA, Hox, ParaHox, DM, calcium L-channel, *Lophelia pertusa*, Gulf of Mexico

ABSTRACT

While some functional genomic studies have been conducted on shallow-water corals, these kinds of studies on deep-water corals are virtually absent and little or nothing is known about how deep-water organisms such as *Lophelia pertusa* perform the basic life functions of growth, differentiation, and reproduction. A suite of assays that could be used to measure and assess the physiological status of these life functions in key coral species would be a useful management tool. Utilizing polymerase chain reaction, the expression of several gene families was investigated to study the molecular mechanisms functioning in *Lophelia* that are known from studies of other metazoan species to be associated with vegetative growth, division, gamete development, and skeletal biomineralization. Four, previously unknown expressed genes were discovered from the deep-sea coral, *Lophelia pertusa*. Expression of these genes were compared in budding (presumably immature) and unitary (presumably mature) polyps. Two members of the *Hox/paraHox* gene family, thought to be associated with segmentation and neuronal development were found to have elevated expression in budding polyps. An L-type calcium channel gene associated with the importation of Ca^{2+} into calcioblastic cells was also more highly expressed in budding as opposed to unitary polyps. In contrast, a *DM*-containing gene, a member of a family of genes notably associated with sexual development and gamete differentiation, was strongly expressed in both budding and unitary polyps. Interestingly, the samples analyzed were taken at a time thought to be the approximate spawning period for *Lophelia pertusa* in the Gulf of Mexico.

INTRODUCTION

Whether they exist in shallow or deep water, coral reefs provide habitat for numerous species. Unfortunately, corals are in worldwide decline due to the impacts of numerous stressors, many of which are anthropogenic in origin. Incidences of coral bleaching and disease are widespread, and many scientific studies have been initiated to investigate the role of abiotic factors such as elevated water temperatures and reduced water quality in the generation and exacerbation of these problems. The slow growth rates of many coral species make recovery from insults slow. Therefore, successful management of coral reef resources is best done by intervention to identify and eliminate stress to the degree possible. Modern, molecular methods can aid in these efforts by providing strategies for the investigation of physiological processes and how these processes are impacted by environmental stressors. While some functional genomic studies have been conducted on shallow-water corals, these kinds of studies on deep-water corals are virtually absent. Little or nothing is known about how deep-water organisms such as *Lophelia pertusa* 'do business'. Many questions exist, including: 1) what biochemical pathways are active; 2) what genes are expressed under basal and stressed conditions; 3) what strategies do these organisms employ to perform the basic life functions of growth and reproduction; and 4) are these strategies the same as those of their shallow-water 'cousins'? Life functions are, of course, many and varied yet can be categorized broadly as growth, differentiation, and reproduction. In reef building corals such as *Lophelia,* growth can be that of soft tissue and/or the biomineralization of calcium to produce a skeleton. Reproduction can be vegetative or mediated sexually by the production of gametes. Differentiation produces specialized structures such as tentacles and stinging cells or can produce a new branching pattern in the skeleton. A suite of assays that could be used to measure and assess the physiological status of these life functions in key coral species would be a useful management tool. This study was initiated to begin to develop such a tool.

MATERIALS AND METHODS

Samples of *Lophelia pertusa* were collected using the Johnson Sea-Link submersible from the northern Gulf of Mexico location known as Viosca Knoll during the course of two cruises in 2004 and 2005 [USGS-GM-2004-03 (July 29 to August 5, 2004) and USGS-GM-

2005-04 (September 15-21, 2005)]. The sites, Viosca Knoll 862 and 826, were sampled both years and are described in detail elsewhere in this report.

SAMPLE COLLECTION.—Samples collected in 2004 were recovered using a variety of methods using the standard sampling containers such as the 'Daisy Wheel', 'BioBox', or simple baskets. Additional samples were taken using the 'Kellogg Sampler' (KS) as described in Chapter 6 of this report. Samples taken using the KS were either stabilized at depth using a DMSO-Salt based preservative (Dawson et al. 1998; Seutin et al. 1991), or recovered alive. Samples collected in 2004 were crushed, transferred to RNA*later*® (Ambion, Austin, TX), frozen, and transported to the USGS-Leetown Science Center in a refrigerated container that maintained the samples in a frozen state. Samples collected and analyzed in 2005 were recovered alive using the KS or the 'BioBox' and frozen immediately in liquid nitrogen vapors. Individual polyps were categorized (Fig. 5-1) as budding or unitary. Budding polyps were transferred whole to 2 ml screw-capped polypropylene vials and frozen. Due to their size, unitary polyps were 1) wrapped in aluminum foil and frozen whole, or 2) had their tissue removed with a cotton swab that was rapidly transferred to a 2 ml screw-capped vial and frozen. For comparison purposes, some samples were transferred to RNA*later*® for storage. All samples from 2005 were transported frozen to the USGS-Leetown Science Center in a liquid nitrogen dry shipper.

MOLECULAR ANALYSES -The expression of several gene families was investigated to study the molecular mechanisms functioning in *Lophelia* that are known to be associated with vegetative growth, division, gamete development, and skeletal formation via biomineralization of Ca^{2+}. This was accomplished by the application of degenerate oligonucleotide primers to direct the amplification and detection of messenger RNA sequences of targeted gene families utilizing polymerase chain reaction (Mullis et al. 1986; Saiki et al. 1985).

RNA EXTRACTION - In 2004, several combinations of commonly used extraction methods and differentially preserved tissue samples were tested for yield and quality of RNA. These combinations fell generally into two categories based on the extraction method used. The first method tested used no organic solvents (RNeasy; Qiagen, Valencia, CA) while the second method tested was the widely-used Tri Reagent® (Molecular Research Center, Inc., Cincinnati, OH) method (Chomczynski 1993; Chomczynski and Sacchi 1987) with modifications (Chomczynski and Mackey 1995a; Chomczynski and Mackey 1995b). Tissue samples preserved

in DMSO-Salt, RNA*later*®, as well as frozen, unfixed samples were extracted following the manufacturers' directions. Quality and yield were assessed by native electrophoresis on 1.5% agarose gels containing 0.5 µg/ml ethidium bromide. Native, as opposed to denaturing gel electrophoresis, was employed because most invertebrates' 28S rRNA contains a so-called 'hidden break' (Ishikawa 1977) that results in the two halves of the denatured 28S rRNA molecule migrating at the same position as the intact 18S rRNA molecule. Thus, the commonly employed criterion for 'good' RNA quality of a 28S band twice as intense as the 18S rRNA cannot be determined under denaturing conditions. Samples from the 2005 cruise were extracted using protocols optimized in 2004. Tissue samples from individual polyps (either whole, roughly broken polyps, or tissue adhered to cotton swabs) were quickly transferred to 1 ml Tri Reagent® in polypropylene scintillation vials and dissolved by vigorous vortexing at room temperature for two min followed by four additional cycles of vortexing for approximately 30 s every two min. Supernatants were recovered and transferred to pre-weighed polypropylene microcentrifuge tubes for determination of losses due to holdup of the reagent in skeletal material or cotton fibers. Fresh Tri Reagent® equal in volume to that not recovered during the first transfer was added to the extraction vials. The vials were vortexed, and the rinses were recovered and combined with the primary extracts to restore the extract volume to 1 ml. Removal of proteins and DNA using the bromo-chloropropane modification (Chomczynski and Mackey 1995b) followed the manufacturer's directions. Total RNA was then precipitated from the recovered aqueous phase using the high-salt modification (Chomczynski and Mackey 1995a), again following the manufacturer's directions. The RNA was dissolved in 50 µl RNase-free water and re-extracted with 1 ml Tri Reagent® as before. The RNA from this second-round extraction was further purified by dissolving the pellet in 100 µl RNase-free water and precipitating the RNA by the addition of an equal volume of 12 M lithium chloride and storage at -20 °C for 30 min. Precipitated RNA was collected by centrifugation at 12,000 x gravity (g) for 15 min at room temperature and the pellet was washed once with 0.5 ml 80% ethanol, dried, and dissolved in 60 µl RNase-free water. Residual DNA was removed by DNase treatment (TURBO DNA-*free*™; Ambion, Austin, TX) following the manufacturer's directions. An aliquot (10 µl) of each sample was reserved for use as a 'no-reverse transcriptase control' (NRTC) to verify that PCR-amplified gene fragments were from messenger RNA and not the result of spurious amplification of residual genomic sequences.

REVERSE TRANSCRIPTION - RNA (25 µl) was reverse-transcribed using Ready-To-Go You-Prime First-Strand Beads (GE Healthcare, Piscataway, NJ) and random hexanucleotides according to the manufacturer's directions. Reactions were performed in duplicate, combined, and residual RNA was removed by adding 10 µl 0.5 M EDTA and 10 µl 1.0 N NaOH. Incubation at 65°C for 15 min served to hydrolyze the RNA. Complementary DNA (cDNA) was recovered from this reaction by use of a Clean and Concentrator-5 Kit (Zymo Research, Orange CA). Binding buffer (1 ml) was combined with the crude cDNA and applied to a Zymo-Spin I centrifugal column in two additions and washed twice following the manufacturer's instructions. The purified cDNA was eluted with three successive additions of 20 µl TE buffer (10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA) to maximize recovery. Each aliquot of TE buffer was allowed to incubate on the column for 1 min before being collected with 30 s of centrifugation at maximum speed. The concentration of the recovered cDNA was determined by measuring absorbance at 260 nM and/or by fluorescence using a Quant-iT™ OliGreen® ssDNA Assay Kit (Invitrogen, Carlsbad, CA).

POLYMERASE CHAIN REACTION - Expressed gene fragments were amplified using degenerate primers designed from known, conserved amino acid motifs of gene families. Several gene families were targeted that are important to growth and development, skeletal formation, and reproduction. Specifically, the targeted gene families were the *Hox* and related genes, genes associated with calcium transport and biomineralization, and members of the *DM* gene family known to be involved with gamete differentiation. The designation, *DM*, is derived from the *doublesex* gene discovered in the fruit fly and another gene with a similar structure, *Mab-3,* from the nematode *Caenorhabditis elegans.* *Hox* genes were amplified using methods previously employed to survey these genes in the sea anemones, *Nematostella vectensis* and *Metridium senile* (Finnerty and Martindale 1997). A calcium channel α1 subunit fragment was amplified following a procedure used successfully to clone this gene from a reef-building coral, *Stylophora pistillata* (Zoccola et al. 1999). *DM* gene family members were amplified using primers originally described by Raymond et al. (1999a) and subsequently used to identify members of this gene family associated with sex determination in *Acropora millepora* (Miller et al. 2003). Amplification of two additional genes was also attempted. The first was a *dpp/BMP2/4* ortholog identified in *Acropora millepora* apparently associated with body axis determination (Hayward et al. 2002) and the second was a PMCA P-type calcium ATPase cloned

from *Stylophora pistillata* (Zoccola et al. 2004) and involved with calcium transport and biomineralization. Amplification conditions were generally as described in the relevant citations except that PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare, Piscataway, NJ) were used for all reactions. Because of the paucity of information regarding coral gene expression, equal inputs of cDNA template were used in comparisons rather than the relative comparisons to the expression of a single reference gene as are commonly used. Generally, no-reverse transcriptase controls were performed using pools of the RNA from the group of samples being analyzed. Pools were tested for the presence of residual genomic DNA as opposed to individual sample RNAs because of the extremely limited amounts of available material. Pooled cDNA was used in preliminary experiments to determine the range of cycle numbers that produced linear amplification. Comparisons of gene expression between budding and unitary polyps were conducted using cycle numbers in the middle of the linear range and quantified from ethidium bromide-stained agarose gels using TotalLab vers. 2.01 (NonLinear USA, Inc., Durham, NC) image analysis software.

CLONING AND SEQUENCING - Amplified gene fragments were cloned into chemically competent *Escherichia coli* strain TOP10 using a TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA). Transformed colonies were screened by PCR and those bearing correct fragments were reserved for sequence analysis. Cloned *Hox* and related genes were sequenced from plasmid preparations purified from overnight broth cultures using a PerfectPrep Plasmid Isolation Kit (Eppendorf North America, Westbury, NY). Other sequences were obtained from PCR-amplified inserts from picked colonies using primers homologous to the M13 primer binding sites of the vector to direct the amplification. M13 primer sites were used for cycle sequencing in all cases using BigDye® (Applied Biosystems, Foster City, CA) methodology and analyzed using capillary electrophoresis instrumentation (Applied Biosystems, Foster City, CA). Sequences were submitted to BLAST (Altschul et al. 1990; Altschul et al. 1997) at http://www.ncbi.nlm.nih.gov/BLAST/ for identification of homologies with other known gene sequences.

RESULTS

SAMPLE COLLECTION, STORAGE AND RNA ISOLATION - Results of studies on the 2004 samples demonstrated that there was an absolute necessity to provide thermal stability during

collection. 'Daisy Wheel' and open basket samples taken in 2004 were degraded dramatically to the point of being nearly unusable. In contrast, samples collected using the Kellogg Sampler were of excellent quality as a result of the thermal isolation provided by the thick plastic construction of the device. No apparent differences were detected in quality between samples fixed at depth or recovered alive, although yields were typically better from samples recovered live. Aqueous-based extraction using the Qiagen RNeasy Kit was not suitable for RNA isolation from *Lophelia*. Skeletal material and mucus interfered with isolations by impeding tissue dissolution and clogging columns thereby causing reduced yields. The Tri Reagent®-based extraction produced good yield and quality of RNA when high-salt precipitation was employed, although two rounds of extraction and a final precipitation from lithium chloride were required to produce maximal product purity. Extraction of samples that included skeletal material stored in RNA*later*® was problematic. The solution degraded the skeletal components (as a result of the decalcification action of the EDTA component) and often caused phase inversions during Tri Reagent® extractions due to carryover of excessive amounts of salts trapped in the coral skeleton. In contrast, tissue material removed from the coral skeleton (by forceps or with cotton swabs) and stored in RNA*later*® yielded good quality RNA. The majority of samples taken in 2005 were collected without fixation at depth (live), frozen immediately in liquid nitrogen vapors upon return to the ship, and transported to the laboratory in a liquid nitrogen dry shipper. These produced RNA of excellent quality as did the tissues sampled with cotton swabs and stored in RNA*later*®.

EXPRESSED GENE FRAGMENTS IDENTIFIED - Positive amplifications were obtained using PCR to detect *Hox/paraHox* and *DM* family members as well as to detect a Ca^{2+} L-type voltage-gated channel. Control RNA samples that were not reverse transcribed (NRTCs) produced no detectable amplification products thereby verifying the absence of genomic DNA and that the amplification products produced from reverse-transcribed samples were indeed from messenger RNAs. Twenty-two clones bearing *Hox/paraHox* -like genes were analyzed. Twenty of the 22 were identical in nucleotide sequence. The amino acid translation of this sequence was identical to *pox3*, a member of the anterior *Gsx/Cnox2* gene family, identified from *Parazoanthus parasiticus* (Hill et al. 2003). An alignment with similar genes is shown in Fig. 5-2. The other two sequences revealed the presence of a novel *Hox* gene whose amino acid translation most closely resembles *engrailed* homologs, *Hox* genes often found to be associated with

segmentation. An alignment of this sequence with similar genes is shown in Fig. 5-3. The Ca^{2+} L-type voltage-gated channel from *Lophelia pertusa* was found to most closely resemble (Fig. 5-4) that from the reef-building coral, *Stylophora pistillata* (Zoccola et al. 1999) with significant homologies to other L-type calcium channel molecules. Finally, a protein-coding sequence containing a *DM* domain was recovered that possessed significant homologies (Fig. 5-5) to many *doublesex-mab3* proteins including another *DM*-containing protein from *Acropora millepora.* No *dpp/BMP2/4* orthologs or PMCA P-type calcium ATPase sequences were recovered despite numerous attempts. Redesigned primers using the annealing control primer (Hwang et al. 2003) and CODEHOP (Rose 2005; Rose et al. 2003) approaches also failed to direct the amplification of a product in the case of PMCA P-type calcium ATPase. Amplified fragments sequences are available under the GenBank accession numbers DQ975345, DQ975346, EF199992, and EF222276.

DIFFERENTIAL GENE REGULATION - When possible, the differential regulation of the described genes was examined by comparing the level of expression of unitary (presumably mature) polyps and budding (presumably immature) polyps. Although opportunities for comparisons were limited, both *Hox/paraHox* genes and the Ca^{2+} L-type voltage-gated channel gene described here appeared to be more highly expressed in budding as opposed to unitary polyps.

When compared to unitary polyps, expression of the *Hox/paraHox* genes was 6-fold higher in budding polyps (Fig. 5-6) and 3.2-fold higher for the calcium channel gene (data not shown). Surprisingly, the *DM* family gene was highly expressed uniformly in both unitary and budding polyps (Fig. 5-7).

DISCUSSION

Little is known about the factors that regulate the development of corals; however, genes that are known to be present in corals and that do affect development are members of the so-called *Hox/paraHox* gene family. *Hox/paraHox* genes belong to a phylogenetically widespread family of regulatory genes that have been shown to play important roles in pattern formation and cell-fate specification in several model systems including *Drosophilia, Caenorhabditis, Hydrozoa, Nematostella,* and *Metridium*. These genes have been found in all metazoan phyla that

have been surveyed, as well as in plants and fungi. They encode proteins which serve as transcription factors and consequently regulate other genes and gene products. Interruption of these gene functions by mutation or modulation results in dramatic changes in body plan and development. To begin to investigate the molecular aspects of *Lophelia pertusa* development, we have analyzed the structure of members of the *Hox/paraHox* family in this cnidarian. Although appropriate samples for comparison purposes were limited, early data seems to indicate that the expression of this gene is elevated in obviously budding as opposed to unitary polyps. Two different *Hox/paraHox* genes were found to be expressed in budding *Lophelia* polyps. The most prominently expressed of these was one that was identical in amino acid sequence, but different in nucleic acid sequence (Fig. 5-2), to a member of the anterior *Gsx/Cnox2* gene family that had been previously identified in *Parazoanthus parasiticus* (Hill et al. 2003). *Cnox-2Am* has been found to be expressed in cells thought to be neurons in *Acropora millipora* planula (Hayward et al. 2001). In *Hydra*, *cnox-2* was found to be upregulated in head but not foot regeneration (Gauchat et al. 2000). The discovery of *pox3* in *Lophelia pertusa* adds support to the existence of a novel, anthozoan-specific, anterior *paraHox* cluster the significance of which is not currently known. The second *Hox/paraHox* gene identified from *Lophelia pertusa* budding polyps in this study was a novel gene most closely related to known *engrailed* genes. These are genes associated with segmentation and skeletogenesis across a wide range of bilaterian phyla (Jacobs et al. 2000).

Divalent calcium ($Ca^{2+)}$ is a physiologically important cation widely involved in the regulation of physiological processes. Small and transient uptake of Ca^{2+} into cells is sufficient for many cellular functions, but the continuous transport of large amounts of calcium is required for biomineralization and the building of skeleton. Whether intracellular or extracellular, the first step of biomineralization is the entry of Ca^{2+} ions into the cell. On the basis of pharmacological evidence, Zoccola et al. (1999) hypothesized the existence of a voltage-gated Ca^{2+} channel and successfully cloned the α1 subunit of this protein from *Stylophora pistillata.* These authors then localized this molecule using immunohistochemical analysis to the calcioblastic ectoderm, the site involved in calcium carbonate precipitation. Following their approach, a fragment of the analogous calcium L-channel was cloned in this study from *Lophelia pertusa* and was found to be 93% homologous in amino acid sequence to that from *Stylophora pistillata.* This gene appears to be upregulated in budding as opposed to unitary polyps. Another

molecule involved in Ca^{2+} transport, a PMCA P-type calcium ATPase, has also been cloned from *Stylophora pistillata* (Zoccola et al. 2004). The analogous gene was not recovered in this study despite numerous attempts and the use of several sets of primers redesigned using annealing control (Hwang et al. 2003; Kim et al. 2004) and CODEHOP (Rose 2005; Rose et al. 2003) approaches. Further investigations will be required to study the mechanism of Ca^{2+} export from the cell.

DM-containing proteins are zinc-finger proteins known to be involved in gender determination and sexual differentiation in many metazoans including worms, flies, fish, mammals, birds, and reptiles (Guo et al. 2005; Huang et al. 2002; Kettlewell et al. 2000; Raymond et al. 1999a; Raymond et al. 2000; Raymond et al. 1999b; Raymond et al. 1998; Ryan et al. 2006; Zhu et al. 2000). Recently, a *DM* domain protein was also recovered from a coral, *Acropora millipora* (Miller et al. 2003) and demonstrated to have elevated expression temporally (at spawning) and spatially (in branch tips as opposed to bases). As shown here, *Lophelia pertusa* also possesses a *DM* domain protein. Expression levels were robust in the samples analyzed from the mid-September collection of 2005, a time frame that corresponds to a hypothesized spawning period determined from histological studies (Booke et al. 2005). Additional analyses of samples taken in other seasons would be required to confirm the temporal, spawning-associated expression of this gene. In contrast to the findings from *Acropora millipora*, expression of the *Lophelia pertusa DM* gene was similar in budding and unitry polyps.

This study has resulted in the discovery of four, previously unknown expressed genes from the deep-sea coral, *Lophelia pertusa*. These data will add to the number of anthozoan sequences, provide further details of the evolution of *Hox/paraHox* genes, allow for studies of sexual differentiation and fecundity, and facilitate studies to unravel the complexities of biomineralization in this important reef-building coral. This suite of genes could provide the basis for analyses aimed at comparing the health status of *Lophelia* in different reef tracts or assessing coral status over time.

RECOMMENDATIONS

Future samples for studies of gene expression must be obtained using sampling equipment that, at a minimum, provides for thermal stability. As it is likely that samples may also be used for other purposes including population genetics and microbiology studies, it is imperative that samples be collected into individual, sterile collection chambers. Sampling equipment as exemplified by the prototype of the 'Kellogg Sampler' fulfill this need. Variations of this device could potentially increase capacity. Further studies into the issues of fixation at depth versus live retrieval should be undertaken to answer remaining questions of how best to recover samples representative of the in situ condition.

A limitation encountered in this study was the small amount of mRNA obtained from individual polyps, restricting the comparisons that could be performed and the opportunities for additional gene discovery. While polyps might be pooled to partially address this limitation, pooling would reduce the number of comparisons possible when samples are limited. A better approach for future studies might be the adoption of recently-developed methods for amplifying RNA and/or creating reusable template libraries attached to magnetic beads.

Routine comparisons of gene expression levels require one or more internal standards. These usually are provided by so-called 'housekeeping' genes known to be invariant in expression levels across tissue samples and physiological states. Because of the paucity of information regarding coral gene expression, equal inputs of cDNA template were used in comparisons in this study. This increased the complexity of analysis. Future studies should identify suitable reference genes for use in gene expression studies.

Four expressed genes were identified in this study that reflect *Lophelia pertusa* physiological status. This list could and should be expanded for *Lophelia* and other deep-sea corals. Additionally, more insights remain to be gleaned by further studies on the genes identified in this study. Cloning of the full-length genes and the corresponding genomic sequences might identify other forms, splice variants, and control mechanisms important to deep-sea physiology.

ACKNOWLEDGMENTS

I thank first the pilots and technicians of the submersible Johnson-Sea-Link, whose talents routinely produced successful results, returned samples and crews intact to the surface, and without whom this research could not have been conducted. The Harbor Branch Oceanographic Institution and the captains and crews of the R/V Seward Johnson I and II provided the comforts of home in a challenging environment. Thanks are extended to the other members of the USGS research crew for their logistical expertise, patience, and help. Special thanks go to Chris Kellogg for her efforts in developing and supplying a sampling device that was essential to the successful completion of this study, Jim Murray for his willingness to substitute for me on the 2005 cruise, and Deborah Iwonowicz for sequencing the calcium L-channel and an especially problematic DM gene. This investigation was supported by the USGS Outer Continental Shelf Ecosystem Program, and was sponsored and facilitated by the Minerals Management Service.

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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LIST OF FIGURES

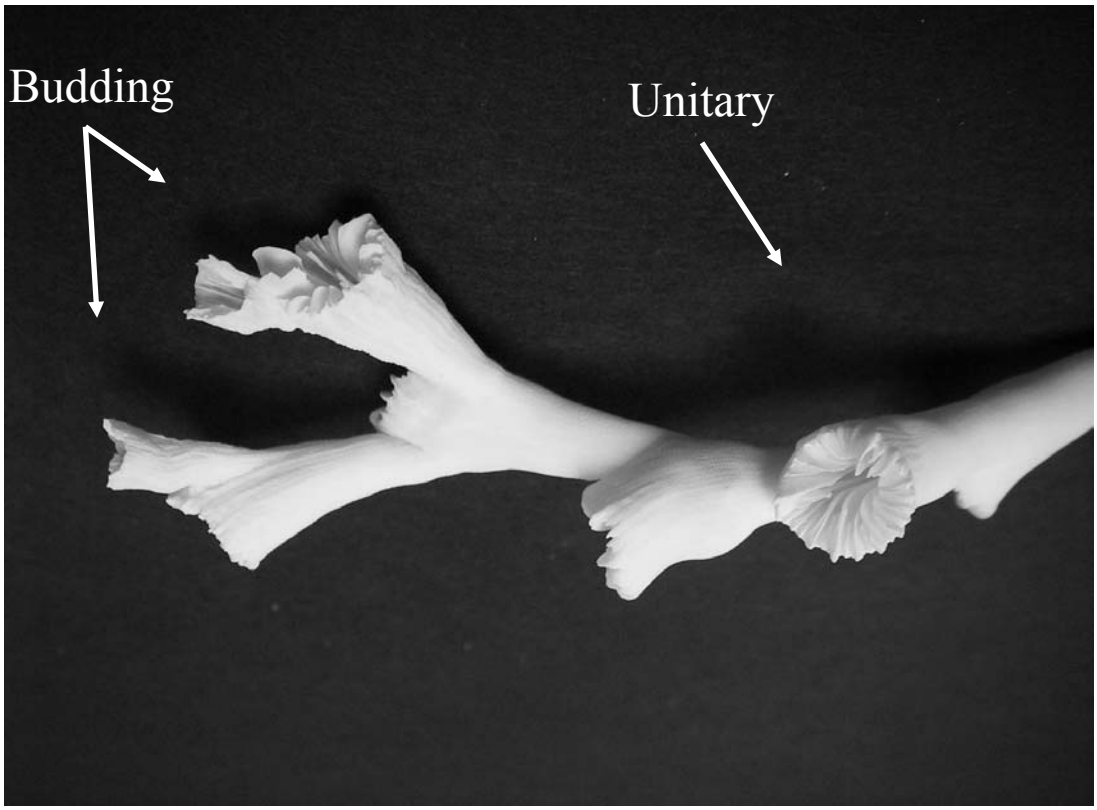

Figure 5.1. *Lophelia pertusa* branching. Budding (presumably immature) polyps are shown on the left and a unitary (presumably mature) polyp is shown on the right.

```
Lophelia pertusa              1   HYNRYLCRPRRIEIAQSLGLTEKQVKI  27
Parazoanthus parasiticus      1   ............................  27
Nematostella vectensis       21   ..................E.........  47
Drosophila heteroneura        3   .F.K...........A..D...R...   28
Metridium senile              1   ..................D......X.  27
Pecten maximus                1   .F.K...........A..D...R....  27
```

Figure 5.2. An alignment of *Lophelia pertusa pox 3* with similar Gsx/cnox2 genes from other species.

```
Lophelia pertusa              1    TECRYVTESRRAELASDLNLTETQVKT  27
Periplaneta americana       252   ..N..L..R..T...RE.G.N.A.I.   277
Haliotis asinine            167    D...L..Q..KD..LS.S...A.I.   191
Schistocerca gregaria       191   ..N..L..R..Q...RE.G.N.A.I.   216
Danio rerio                 192      ..I..Q..QS..QE...N.S.I.   214
Placopecten magellanicus     21     ....L..Q..LD..QE.....A.I.   45
Chaetopterus sp.            187      ..L..A..Q...AE...N.S.I.   209
```

Figure 5.3. An alignment of an *engrailed*-like gene from *Lophelia pertusa* with similar genes from other species.

```
Lophelia pertussa       1     IGMQVFGRIALDSDTSMNRNNNFQTFPQSLMVLFRSATGENWQQIMLACTHRDDVKCDPN  60
                              IGMQ+FGRIA++SDT++NRNNNFQTFPQSLMVLFRSATGENWQQIMLACTHRDDVKCD N
Stylophora pistillata   1313  IGMQMFGRIAINSDTAINRNNNFQTFPQSLMVLFRSATGENWQQIMLACTHRDDVKCDQN  1372

Lophelia pertussa       61    ADPQEDSGLCGSDFAYFYFVSFYSICSFLIINLFVAVIMDNFD  103
                              ADPQE SGLCGSDFAYFYFVSFYSICSFLIINLFVAVIMDNFD
Stylophora pistillata   1373  ADPQEPSGLCGSDFAYFYFVSFYSICSFLIINLFVAVIMDNFD  1415
```

Figure 5.4. Alignment of *Lophelia pertusa* calcium L-type channel amino acid sequence with that from *Stylophora pistillata*.

```
Lophelia pertusa              1    GMVSWLKGHKRYCRWRDCNCAQCTLIAERQRV  32
Tribolium castaneum           48   .V..A..............K.........  79
Anopheles gambiae             48   .V..A............V..K.........  79
Drosophila melanogaster       48   .V..A............V..K.........  79
Drosophila pseudoobscura      48   .V..A............V..K.........  79
Strongylocentrotus purpuratus 82   .V..A............I..K.........  113
Apis mellifera                55   .V..A............V..K.........  86
Brachionus calyciflorus       8    .V..A..............K.........  39
```

Figure 5.5. An alignment of a *DM*-containing gene from *Lophelia pertusa* with similar genes in other species.

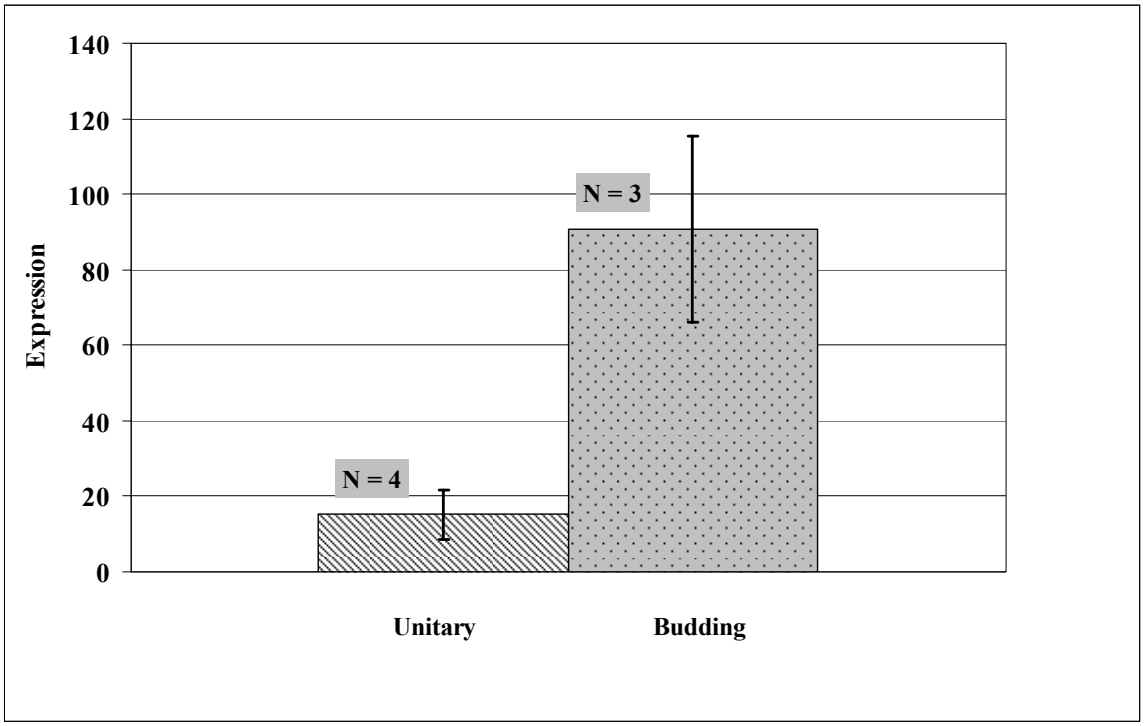

Figure 5.6. Comparison of *Hox/paraHox* expression levels in budding and unitary polyps. Expression is measured as quantity of PCR amplification product produced with equal inputs of cDNA. Error bars indicate one standard error of the mean.

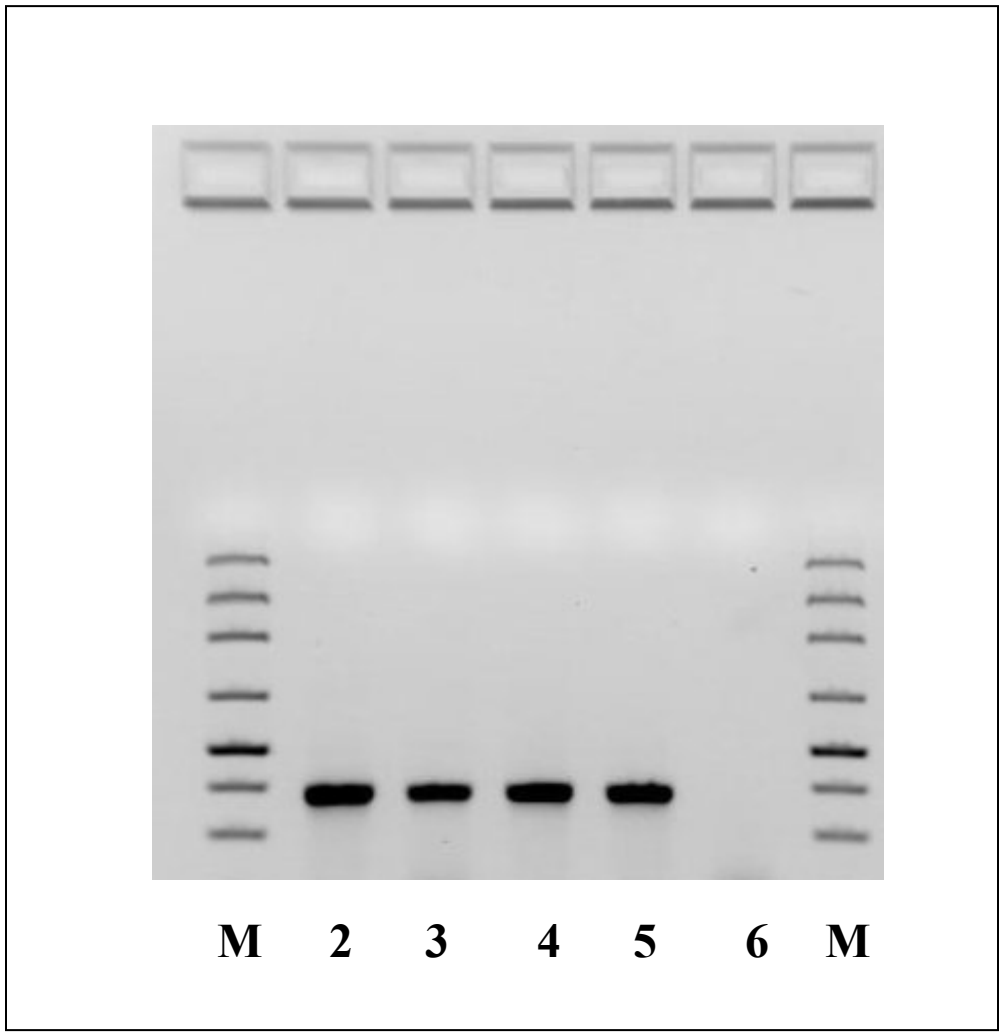

Figure 5.7. Expression of a *Lophelia pertusa DM*-containing gene. Lanes 2 and 3 are PCR-amplified products from budding polyps, while lanes 4 and 5 are PCR-amplified products from unitary polyps. Lane 6 is a NRTC and lanes marked M are molecular mass markers.