Non-contact competition between soft and hard corals: a transcriptomic perspective

Thesis submitted by **Natalia Alexandra Andrade Rodríguez**

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College of Public Health, Medical and Veterinary Sciences

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Dedication

I dedicate this thesis to my family and friends.

“L’essentiel est invisible pour les yeux”

Antoine de Saint-Exupéry, 1943, Le Petit Prince

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- Dr. Aurélie Moya, ARC Center of Excellence for Coral Reef Studies, James Cook University
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I certify that the present thesis

Non-contact competition between soft and hard corals: a transcriptomic perspective

is, to the best of my knowledge and belief, original and my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

Natalia Alexandra Andrade Rodríguez

Abstract

Ecological interactions affect species evolution and, acting in combination with environmental factors, determine the composition of an ecosystem. In the case of coral reefs, the interactions of species with the corals (Anthozoa) is essential in shaping the ecosystem. Competition is particularly intense in coral reef communities because of the limited availability of space where conditions are appropriate (e.g. depth, substrate, currents) for settlement and growth. Space limitation makes the interaction between corals an essential element determining coral assemblages. Competitive interactions are difficult to analyses due to the number and diversity of factors (e.g. environment, life history, genotype) affecting outcomes. In the case of corals, research on competitive interactions has mostly focused on visible signs of aggression, such as measuring the damaged tissue next to a competitor or reporting visual competitive behaviours (e.g. mesenteric filaments). However, competition (particularly non-contact competition) does not always lead to visible symptoms, which has led in some cases to the underestimation of the extent of competitive interactions. For example, many soft corals (Octocorallia) produce secondary metabolites that may be used to compete for space; the production of secondary metabolites is unlikely to be visually obvious, and their impact on competitors may be subtle or cryptic. The outcomes of competitive interactions between individual corals will also be affected by the health and history of those individuals. For example, individuals that are already immune-compromised are unlikely to be able to compete as efficiently as healthier individuals. The immune system is assumed to be a critical component of competitive mechanisms. Research on coral immunity has focused, with few exceptions, on hard corals (Scleractinia), very little information being available on soft corals immune systems. The lack of basic research on soft corals extends to many aspects of their biology, despite the importance and abundance of these organisms in reef ecosystems. More research on soft corals immunity is important in order to better understand how these organisms respond to environmental factors or competition and to better predict the future composition of coral reefs. In this thesis, I have attempted to advance the knowledge of soft coral biology and non-contact competition between soft and hard corals. I analysed, at a transcriptomic level, the response of the soft coral *Lobophytum pauciflorum* to challenge with the defined immunogen MDP and the effects on both *L. pauciflorum* and the hard coral *Porites cylindrica* (hard corals) when these were in non-contact competition. The response of the soft coral to MDP was variable and unexpectedly dominated by genes likely to have functions in the nervous system. Non-contact competition

triggered general stress and immune responses in soft corals, as well as differential expression of genes likely to function in secondary metabolite production and others genes that may be involved in tissue remodelling. The transcriptomic response of the hard coral, *Porites*, on the other hand, suggested cellular stress combined with resistance and aggressive responses. This research also highlights the role of the coral nervous system and behaviour in the stress response, suggesting that neuro-related pathways are closely linked to the immune system. Similarities between the transcriptomic responses to non-contact competition identified here and previously reported responses to environmental stressors (e.g. ubiquitination, antioxidant production), is consistent with the recruitment of common gene repertoires; therefore climate change is likely to effects competitive interactions in complex ways. Finally, the research presented in this thesis demonstrates the extent of variation in the responses of individual corals to stress (immune challenge and competition) and the challenges that this poses particularly for the investigation of the molecular bases of competition. In the future, individual variation needs to be better accommodated for molecular investigations into coral research, which means increasing biological replication and stopping the practice of discarding outliers.

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Chapter 1 - General Introduction

1.1 Background

Coral reef ecosystems are highly important for human wellbeing and prosperity; activities related to the Great Barrier Reef (Australia), for example, contribute more than 5 billion dollars per year to the Australian economy (Day and Dobbs, 2013). Reefs all over the world are the main source of revenue and food for many communities (Hicks and Cinner, 2014). Therefore the collapse of these ecosystems would bring catastrophic human and economic consequences (Hughes et al., 2017a).

The recent series of bleaching events (2015 and 2016) and the associated loss of hundreds of square kilometres of coral reef highlight the importance to preserve the reefs that are left and that there is an urgent need to mitigate human impacts on them (Hughes et al., 2017b). Research on coral reef ecosystem functions is necessary to understand future scenarios. However, ecosystem-scale research needs to be combined with empirical and molecular investigations of coral biology to fully understand the potential of corals and reef fishes to adapt to future environmental conditions.

Cnidarians from the class Anthozoa, including Hexacorallia (hard corals and anemones) and Octocorallia (soft corals and gorgonians), are responsible for much of the complexity of reef ecosystems, but these are also amongst the simplest animals (Figure 1.1). Members of the hexacorallian order, Scleractinia (Bourne, 1900) are often referred to as hard corals or reef-building corals as the aragonite skeletons that they deposit create much of the structure of the reef (Graham and Nash, 2013). Shapes and sizes of the different hard coral species are the most obvious factor determining the structural complexity of a particular reef and much of the research effort on reef structure so far has focussed on this group (Alvarez-Filip Lorenzo et al., 2011; Coker et al., 2014; Graham and Nash, 2013; Jones et al., 1994). Octocorallia, on the other hand, have received much less attention in this respect despite the evidence that they not only contribute to reef structural complexity (Richardson et al., 2017a), but also to habit diversity – for example, by providing habitat and refuge to many species of reef fish (Ferrari, 2017; Jeng et al., 2011; Richardson et al., 2017b).

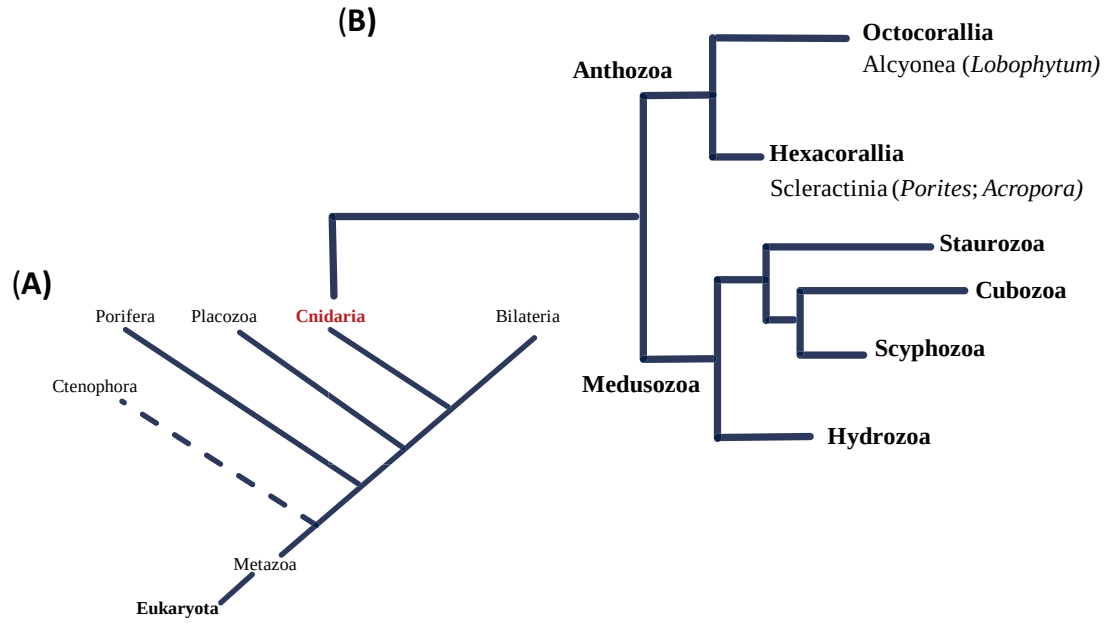

Figure 1.1: Schematic phylogenetic trees showing (A) The position of the phylum Cnidaria in the kingdom Metazoa and (B) the evolutionary relationship of hard (Scleractinia) and soft (Alcyonea) corals within Cnidaria (Zapata et al., 2015)**.**

Much of the focus of molecular studies on octocorals have principally focused on two areas of research: (1) the use of molecular phylogenetics to resolve taxonomic uncertainties (McFadden et al., 2010) and, (2) drug discovery – the search for pharmacologically relevant secondary metabolites (Chapter 3, Introduction). However, we are still very far from getting a full understanding of octocorals' ecology and biology. For example; little is known about the effects of stressors on soft corals and molecular mechanisms by which they respond (Fabricius, 1999). The few recent studies that are available provide some insights into the molecular defence mechanisms of octocorals. These include the transcriptomic response of the gorgonian *Gorgonia ventolina* to a natural parasite (Burge et al., 2013); the effects of environmental stressors on immune responses in the same organism (Mann, 2014) and lesion healing following artificial wounding in two gorgonians (Shirur et al., 2016). More information about immunology research in soft corals is provided in chapter 2.

Soft corals are often considered to be relatively resistant organisms due to their high growth rate and ability to colonise areas where hard corals have been decimated by *Acanthaster planci* (crown-of-thorns starfish) outbreaks or other catastrophic events, such as cyclone damage (Fabricius, 1997). For these reasons, soft corals have sometimes been described in the literature as better competitors for space than are hard corals (Alino et al., 1992). However, the ability

of soft corals to opportunistically occupy space provides only limited support for the idea that they will do far better than other cnidarians in the long term under increasingly severe environmental conditions (Fabricius, 1999, 1997). In fact, in the mass bleaching events of 2015 and 2016, high mortality was observed on soft coral dominated reefs (Hughes et al., 2017b; Richardson et al., 2018). Additionally, we have only a very limited understanding of how hard and soft corals interact, so it would be premature to speculate as to whether one group of corals has a significant advantage over the other.

1.1.1 Interactions between soft and hard corals

Competition for space is a major ecological pressure that shapes ecosystems like coral reefs. Significant factors in determining the outcome of a competitive interaction are biological characteristics that have been established over evolutionary time, effectively resulting in a natural hierarchy amongst species (Abelson and Loya, 1999; Chadwick and Morrow, 2011; Crowley et al., 2005). Nevertheless, it is the fitness of an individual organism that determines the outcome of a competitive scenario. For example, an individual whose fitness is already challenged because of a disease or another external stressor will be less likely to win a competitive encounter than would be a healthier individual of the same species. Additionally, the competitiveness of individuals within a species will vary with genotypic diversity (Elliott et al., 2016; Mitarai et al., 2014).

The variability of environmental or genotypic factors that could affect competitive outcomes makes it difficult to predict how anthropogenic stressors such as climate change are likely to compromise the capacity of an organism to compete (Evensen and Edmunds, 2016; Horwitz et al., 2017; Inoue et al., 2013). Using a reductionist approach to investigate the cellular processes that corals activate while competing for space is an essential first step in understanding how additional stressors might affect competitive outcomes (Horwitz et al., 2017).

Since corals are sessile organisms, they compete with each other for the limited space with appropriate light, substrate and current conditions that they need to grow and reproduce (Connell et al., 2004; Gambrel and Lasker, 2016). In the evolutionary history, corals have acquired a diverse range of efficient competitive strategies. At least four distinct competitive strategies have been identified (reviewed by Lang and Chornesky 1990 and Chardwick and Morrow, 2011): (1) overtopping of competing corals, essentially starving them of light, (2) deployment of mesenteric filaments to externally digest the competitor, (3) elongation of

polyps to enable tentacle contact with competing organisms followed by discharging of nematocysts and/or (4) development of sweeper tentacles to again enable nematocyst discharge (Chadwick and Morrow, 2011; Lang and Chornesky, 1990).

Soft corals can overtop other corals (Alino et al., 1992) in order to compete for space, and there have been reports of sweeper tentacles in gorgonians (Sebens and Miles, 1988). One particular characteristic of octocorals is the production of a diverse range of toxic chemicals or secondary metabolites that accumulate in their tissues, and when in contact with other colonies these compounds can cause tissue necrosis to their neighbours (Coll and Sammarco, 1983; Sammarco and Coll, 1992; Sammarco et al., 1983). Some soft corals can release those toxins into the water column to damage a distant enemy or to increase the tissue area affected by their chemicals (Sammarco et al., 1983). The strategy of using toxic chemicals to compete is known as allelopathy (Chadwick and Morrow, 2011; Coll et al., 1985).

Although competition clearly occurs between corals that are not in contact, research on competitive strategies in corals has overwhelmingly focussed on interactions that involve contact (Chornesky, 1983; Fleury et al., 2004; Sebens and Miles, 1988; Shearer et al., 2012; Tanner, 1995). Physical contact with a foreign tissue results in activation of the innate immune system of the coral, involving self- *vs* non-self-recognition (Frank et al., 1996; Hennessey and Sammarco, 2014; Hildemann et al., 1977). In a non-contact scenario, however, it is necessary to consider how the interacting organisms recognise the potential threat. Soft corals are an interesting group in which to study non-contact competition because they may react to the presence of another coral by releasing toxic chemicals to overcome the distance barrier. Note, however, that research on non-contact competition in soft corals has mainly focused on quantifying the effect of competition rather than understanding how and why the competition was triggered (Aceret et al., 1995; Coll and Sammarco, 1983; La Bare et al., 1986; Maida et al., 1995; Sammarco et al., 1983). Investigating the mechanisms used by corals to identify potential threats and competitors at a distance should, therefore, be a research priority. Hypothetical schemes for how such interactions might occur between soft and hard corals are explored in chapters 3 and 4 respectively.

In the work described in the following chapters, transcriptomics was used to investigate the cellular mechanisms involved in the responses of both soft and hard corals to non-contact competition. In addition, a similar approach was used to understand the response of soft corals

to immune challenge. *Lobophytum pauciflorum* (*Lobophytum*; Ehrenberg, 1834) was used as a representative of soft corals in these investigations (Figure 1.2). *Lobophytum* is widely distributed throughout the Indo-West Pacific in shallow waters and is particularly abundant at specific sites on the Great Barrier Reef (Benayahu, 2002; Tursch and Tursch, 1982). The genome of *Lobophytum pauciflorum* has been sequenced by collaborators and was available for this study, facilitating the process of transcriptome annotation (unpublish). Additionally, it has been reported that *Lobophytum pauciflorum* can affect potential competitors, including *Porites cylindrica*, at a distance (Sammarco et al., 1983); making this pair of species a particularly attractive system in which to study non-contact competition. The hard coral *Porites cylindrica* (*Porites*, Dana 1846) is relatively common on the Great Barrier Reef and other Pacific reefs (Dizon and Yap, 2005; Jompa and McCook, 2002; Palmer et al., 2011). Several competition studies using *Porites* provide a baseline of the behaviour and potential competitive outcomes (Aceret et al., 1995; Coll and Sammarco, 1983; Rinkevich and Sakamaki, 2001; Sammarco et al., 1985).

Transcriptomics analysis has been used in this thesis across all data chapters as a tool to understand the differences in gene expression between control and treatment samples. Next-generation sequencing technologies allow obtaining information about the behaviour of hundreds of thousands of genes due to a specific treatment on a particular time-point. This large-scale data serve to analyse the cellular response of non-model organisms like corals on a transcriptomic level. Transcriptomics has been used to understand the corals' response to stressors like an infection (Burge et al., 2013; Mohamed et al., 2018) and environmental stressors (Bellantuono et al., 2012; Oakley et al., 2017). These transcriptomic studies have demonstrated the power transcriptome-wide gene expression analysis in identifying the cellular pathways and specific genes that corals might be using to react to the stressor. Other methods like microarray (Shearer et al., 2012) have been used to characterise the response of the hard coral *Acropora millepora* to contact competition with algae; the limitations that this type of technic presented is that only targeted genes are analysed. Conversely, transcriptomics allows unbiased analysis of genes affected by a stressor; such broad analysis allow identification of specific genes of interest for more deep analysis (e.g. cloning).

1.2 Thesis structure and objectives

The primary goal of this thesis was to advance knowledge on the soft coral biology and improve our understanding of non-contact coral competition using transcriptomics analysis as a tool. The thesis comprises five chapters: a general introduction (this chapter); three data chapters (Chapter 2, 3 and 4) and a general discussion (Chapter 5). The three data chapters are intended for publication in peer-reviewed journals after format modification.

The objectives of Chapter 2 were to investigate differential gene expression in *Lobophytum* following challenge with a defined immunogen and to compare those results with the ones obtained in *Acropora millepora* challenged with the same immunogen (Weiss et al., 2013). This was achieved by challenging fragments of *Lobophytum* with highly purified muramyl dipeptide (MDP), a bacterial cell wall derivative (immunogen). David Miller, Aurélie Moya and I developed the experimental design. I performed the experiment and Aurélie contributed to laboratory analysis. Ira Cooke and I analysed the data. We all contributed to the data interpretation.

The objective of Chapter 3 was to determine the transcriptomic response of *Lobophytum* to non-contact competition with *Porites*. A non-contact competition experiment was set up to simulate a competitive scenario; tissue samples were taken for transcriptomics analysis.

David Miller, Aurélie Moya and I developed the experimental design. I performed the experiment and Aurélie contributed to the tissue sampling and laboratory analysis. Ira Cooke and I analysed the data. We all contributed to the data interpretation.

Chapter 4 is essentially an investigation of the other side of the *Lobophytum*/*Porites* interaction, focusing this time on the hard coral. The objective of chapter 4 was to improve our understanding of the molecular mechanisms by which *Porites* reacts to non-contact competition. The same experimental approach as we used in the previous chapter was applied here. Ira Cooke and I analysed the data, and we all contributed to the data interpretation.

Rhondda Johns and I analysed the data from the polyp activity.

Chapter 2 - Transcriptomic analysis of *Lobophytum pauciflorum* under immune challenge

2.1 Introduction

The immune system (IS) is crucially important to animal health. Vital animal traits such as growth, reproduction and survival, rely on the correct functioning of this system. An unhealthy animal will be more susceptible to predators, for example, or might not be strong enough to fight a competitor for space or mating (Vollmer and Kline, 2008; Wright et al., 2017). Immunity contributes to an organism's health by acting against pathogens; although many studies also suggest that the central role of this system is to control the "healthy" microbiome community associated with each species (Bosch, 2014).

In the face of climate change, understanding immunity in cnidarians is increasingly important to predict coral reef resilience and resistance in response to pathogens and anthropogenic stressors (Mydlarz et al., 2010; Pinzón et al., 2015; Reed et al., 2010). Rising ocean temperatures and ocean acidification put corals under physiological stress making them susceptible to infections that might be lethal (Bruno et al., 2007). As mentioned in the previous chapter, octocorals (soft corals) are an essential component of the reef community, providing food and habitat for many fishes. However, most of the studies on coral disease and bleaching have focused on scleractinian corals or anemones, with comparatively little attention given to soft corals despite their ecological importance (Shirur et al., 2016).

Transcriptomic analysis has been used in recent years to characterise the coral innate immune repertoire, and these studies have provided insights into the evolutionary origins and functions of the IS. Miller et al. (2007) and Mydlarz et al. (2016) reviewed immunity in cnidarians, summarising the various gene families shared and likely common cellular mechanisms with vertebrates and mammals, such as the nucleotide-binding oligomerisation (NOD)-like receptor (NLR) and Toll-like receptor (TLR) signalling pathway components. Some vertebrate immune gene families, absent in model organisms like *Drosophila* and *Caenorhabditis elegans*, are present in the hard coral *Acropora millepora* (Weiss et al., 2013). This fact enhances the importance of coral research to better understand immunity in higher animals.

Burge et al. (2013) used transcriptomic analysis to investigate the immune response of an octocoral (*Gorgonia ventalina*) when exposed to a parasite (*Aplanochytrium)*. They found that

G. ventalina shared many homologous genes and immune signalling pathways with scleractinian corals. For example, the immune challenge to the octocoral stimulated the expression of likely immune receptor pattern recognition molecules (e.g. tachylectin-5A) and immune effectors including candidate antimicrobial peptides (e.g. a homolog of arenicin) that have also been found in the immune responses of other cnidarians (Burge et al., 2013).

Conversely, some differences were observed between the octocoral and hexacoral responses. For example, *G. ventalina* under parasitic infection up-regulated metabolic processes such as cellular respiration, while Weiss et al. (2013) suggested that *A. millepora* was suppressing metabolism under immune challenge. This and other differences between soft and hard coral responses to immune challenge highlight the importance of further investigation of soft coral immunity.

While the work of Burge et al. (2013) on *G. ventalina* essentially sets a baseline for further investigation on soft coral immunity, there are limits or caveats to what can be learnt about immunity by characterising the host response to a parasite. One important consideration is that the pathogen might be reacting to the host defence mechanisms, altering the host's general immune response (Norris and Evans, 2000). Since this kind of alteration is specific to the host-pathogen relationship, it limits our conclusions about the soft coral immune response to bacterial pathogens or response to symbiotic bacteria.

The coral immune system presumably detects pathogens via receptors that will be activated by specific molecules associated with bacteria (Miller et al., 2007). Pathogen-associated molecular patterns (PAMPs) are molecules present in the cell walls and/or membranes of Gram-negative or/and Gram-positive bacteria that are detected by the host receptors, activating an immune response. The use of purified PAMPs to immune challenge corals is a technique to examine a specific aspect of the host response, whereas during challenge with whole bacteria or pathogens the response will be directed to a diverse and undefined range of molecules (Girardin et al., 2003).

Muramyl dipeptide (MDP) is a PAMP present in Gram-positive and Gram-negative bacteria that has been found to activate the NLR signalling pathway in mammalian cells (Girardin et al., 2003). Weiss et al. (2013) used MDP to immune challenge *Acropora millepora* nubbins. Their findings showed that MDP stimulation of *A. millepora* caused up-regulation of some immune-related genes one hour after injection and demonstrated the common involvement of GiMAP/IAN genes in the early immune responses of corals and mammals (Weiss et al., 2013).

This study aims to break the knowledge imbalance between soft and hard corals, as well as improve the understanding of the similarities and differences of the immune system across the phylum Cnidaria. To archive these aims the specific objectives included : (1) to characterise the early immune response of *Lobophytum pauciflorum* (soft coral) to PAMPs and (2) to replicate the Weiss et al. (2013) experiment on *L. pauciflorum* in an attempt to compare early immune responses of hard and soft corals.

2.2 Materials and Methods

2.2.1 Sample collection and experimental design

Six colonies of the soft coral *Lobophytum pauciflorum* (*Lobophytum*) were collected in the reefs around Orpheus Island (18'34' S;146'29'E) and transported to Orpheus Island Research Station (OIRS) for fragmentation (GBRMPA Permit No. G16/38499.1). Each colony was divided into 18 pieces of approximately five centimetres in length obtaining a total of 108 fragments of *Lobophytum*. These soft coral fragments (lobes) were then placed into 36 individual tanks for three weeks to recover from the collection. Each tank had a volume of 1.5L and held three fragments from the same colony.

After the three-week recovery period, the lobes were subjected to the immune challenge experiment. Lobes were either injected with 200ml of a solution of the immunogen muramyl dipeptide (MDP, InvivoGen; Cat# tlrl-mdp) in PBS (immune challenge fragments) or with PBS only (control). The immunogen was prepared at a concentration of 10µl/ml as described in Weiss et al. 2013. Fragments were injected on the top of the lobe, as shown in Figure 2.1. This technique was first tested by injecting cooking dye into spare coral fragments to visualise the spread of dye into the soft coral tissue. During the injection process water flow and air bubbling supply was stopped for all tanks to facilitate manipulation of the lobes and to maximise the time of MDP exposure in case the solution injected was expelled. After all fragments were injected, air bubbling was renewed, and the water temperature was controlled by keeping the tanks on a bain-marie with high water flow.

The samples were collected for RNA analysis at three time-points: 1hr post-injection, 6hr post-injection, 24hrs post injection, by cutting approximately three centimetres of tissue around the injected area and immediately snap-freezing it in liquid nitrogen. All samples were stored at -

80 °C until processed for further analysis. In total three technical replicates of six biological replicates were sampled per time point.

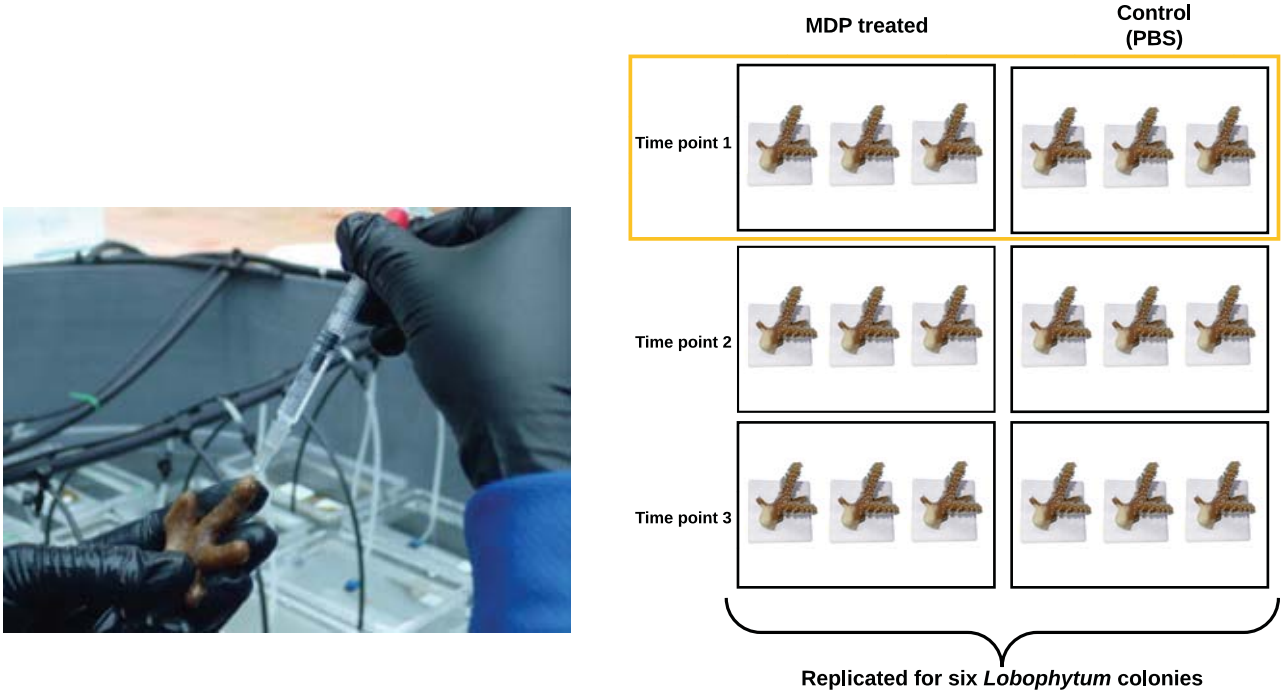

Figure 2.1: Photograph showing the technique for injection of soft coral fragments (right). Diagram explaining experimental design, yellow panel corresponds to the time point (one hour post-injection) analysed in this chapter (left).

2.2.2 RNA extraction, library preparation and sequencing

Previous observations of *A. millepora* immune challenge with MDP by Weiss *et al.* (2013) showed that the hard coral reacted at a gene expression level to the treatment one-hour post-injection. *Lobophytum* samples that were exposed for an hour to the immune challenge were therefore chosen for RNA extraction, sequencing, and analysis. A total of six immune challenged and six control samples were crushed using a hydraulic press in liquid nitrogen. The RNA extraction was performed from the tissue powder with TRIzol Reagent (Ambion, catalogue Number 15596-026) following the supplier protocol (Chomczynski and Sacchi, 2006).

The quality of the RNA extraction was assessed with the Agilent Tapestation with RNA ScreenTapes, and the concentrations of each extraction were normalised to 80ng in 12.5 µl of miliQ water. Library preparation was done using an Illumina NeoPrep machine with a TruSeq Stranded mRNA Library Prep for NeoPrep kit and yields were verified on the Tapestation using D5000 ScreenTapes. Final library concentrations were set to 15nM in 25 µl and sent to the Australian Genomic Research Foundation (AGRF) for paired-end sequencing on a HiSeq2500 Illumina machine with a target sequencing volume of approximately 20 million reads per sample.

2.2.3 Transcriptome analysis

Reads from each sample were corrected for random sequencing errors using the software Rcorrector (Song and Florea, 2015). Sequences were then mapped against the *Lobophytum pauciflorum* transcriptome assembled for Chapter 3. Details of the quality of the assembly and annotation methods are provided in Chapter 3. Bowtie2 version 2.2.4 (Langmead & Salzberg, 2012) was used to map the reads from immune challenged and control samples against the available transcriptome. The mapping used recommended settings (end to end alignments, report all alignments, minimum alignment score 0.3) to suit downstream quantification and clustering with Corset version1.05 (Davidson & Oshlack, 2014).

2.2.4 Gene expression analysis

Reads mapped with Bowtie2 (including multi-mapping reads) were analysed with the software Corset to cluster transcripts and aggregate read counts for each cluster. An annotation score based on the length and the information available for each transcript was used to choose one transcript per cluster to transfer annotations from transcripts to clusters.

The package DESeq2 (Love et al., 2014), run in the R software version 3.3.0 (R Core Team, 2016), was used to normalise read counts between samples and to perform differential expression analysis on the basis of cluster counts obtained with Corset.

A Principal component analysis (PCA) was performed using the transformed read counts obtained after running DESeq2 with a null model and using the variance stabilising transformation tool from the same package. This preliminary analysis revealed relationships between samples based on gene expression of the whole transcriptome and suggested that the six colonies of *Lobophytum* could be divided into two groups (Table 2.1). The groups were

obtained by examining differences between treated/untreated samples for the same colony, and observing the distribution pattern that the samples had in the PCA. These observations seemed to divide the colonies into two consistent groups. These groups were used to create a factor "Group" to model accounting for different gene expression responses to MDP between groups. Full details of this model are provided in Table 2.2.

Results were then interpreted by extracting differentially expressed genes for specific model terms as follows. Results from "Group1-MDP" factor corresponded to differentially expressed genes (DEG) found in the contrast analysis between samples of Group 1 treated with MDP (Group1-MDP) when compared to control sample from Group1 (Group1-control). Similarly, the model factor "Group2-MDP" represented the DEG when contrasting Group 2 samples treated with MDP (Group2-MDP) to control samples from Group 2 (Group2-control). The factor "Group1 vs Group2" corresponded to the DEG when comparing samples from Group1 to Group2 irrespectively from the treatment (Table 2.1).

Adjusted p-values (padj) for differential gene expression were obtained using the Benjamini Hochberg procedure for multiple testing correction. Power to detect differentially expressed genes was optimised using independent filtering based on the mean of normalised counts as a filter statistic. The padj threshold recommended by DESeq2 and use for this study was of 0.1 (Love et al., 2014). It is relevant to mention that this is a discovery study where interpretations are not based on individual genes, but instead, on patterns across multiple related genes. Under these circumstances, a small number of false positives is unlikely to distort the overall conclusions. The DEG found in the model factor "Group2-MDP" were used for the analysis to infer gene function because samples of Group 2 were behaving more consistently in the PCA analysis than samples from Group 1 (section 2.3.2).

Table 2.1: *Lobophytum* samples grouped based on PCA results. "ID" corresponds to field and sequencing labelling of each colony; "Colony" corresponds to the labelling of each colony used for DESeq analysis and plotting. In the column "Treatment": "T" represent samples immune challenged with MDP and "C" control samples that did not receive MDP. "Group" represents the classification of each colony depending on its behaviour observed in the PCA and "ind.n" accounts for the colony identity within each one of the groups.

ID	Colony	Treatment	Group	ind.n
LG_C	C1	C	G1	1
LG_T	C1	T	G1	1
LY_C	C5	C	G1	2
LY_T	C5	T	G1	2
LR_C	C6	C	G1	3
LR_T	C6	T	G1	3
LB_C	C2	C	G2	1
LB_T	C2	T	G2	1
LN_C	C3	C	G2	2
LN_T	C3	T	G2	2
LW_C	C4	C	G2	3
LW_T	C4	T	G2	3

Table 2.2: Model and variables used for gene expression analysis with DESEq2

Function		Variables	Description
Model	~ Group +Group:ind.n +Group:Treatment	Intercept	
		Group G2 vs G1	Differences between groups irrespectively of the treatment
		GroupG1 ind.n2	
		GroupG2 ind.n2	
		GroupG1 ind.n3	
		GroupG3 ind.n3	
		GroupG1 Treatment-MDP	Treatment effect on colonies from Group1
		GroupG2 Treatment-MDP	Treatment effect on colonies from Group2

2.2.5 Analysis to infer gene function

The R package "GOSeq" was used to perform an enrichment analysis to determine whether differentially expressed genes involved in specific cellular processes, biological components and molecular functions were overrepresented based on the Gene ontology terms (GO-terms) of the annotated clusters (Young et al., 2010).

Genes found to be differentially expressed between Group2-MDP and Group2-control samples were manually classified into four categories: 1. Immune-related genes, 2. Neuro-related genes, 3. Extracellular matrix(ECM)-related genes and 4. Transcription-related genes. The gene categorisation was based on literature review of the gene function, GO-terms, best BLAST hit, protein domains and KEGG (Kyoto Encyclopaedia of Genes and Genomes) Orthology corresponding to the gene annotation for each cluster (See Chapter 3 for details of the transcriptome annotation process).

The 52 DEG identified by Weiss et al. (2013) in *A. millepora* when treated with MDP were BLAST searched (E-value<10^{-5}) against the genes differentially expressed in *Lobophytum* (Group2-MDP) using the program Geneious v. 9.1.5 (Kearse et al., 2012). This analysis aimed to compare the gene expression profile of soft and hard corals in response to MDP challenge.

2.3 Results

2.3.1 RNA analysis, sequencing and transcriptome analysis

Five out of six colonies of *Lobophytum* recovered from the fragmentation stress. One-third of soft coral fragments from colony C6 died due to unknown reasons. Nevertheless, there were enough healthy lobes from all the colonies (including C6) to run the experiment and get tissue samples for the first two time points: 1hr post-injection and 6hr post-injection. The mortality of colony C6 did not affect the results discussed here because only samples for time point one were analysed in this chapter.

RNA extraction and library preparation from samples collected an hour post-injection was carried out successfully. Sequencing of the twelve samples yielded approximately 750 million paired-end reads (~50 million pairs per sample). The mapping rate of the corrected reads to the transcriptome assembly generated as described in Chapter 3 was ~ 55%. Approximately 56% of the clusters were annotated with 10,114 unique UniProt gene IDs, and 53 % of clusters had

associated gene ontology terms. Finally, use of the Corset software produced 107,087 clusters that were analysed to identify differentially expressed genes.

2.3.2 Gene expression analysis

The exploratory PCA analysis shows that the principal component (PC) 1 explains 34% of the variance between samples, while the PC2 explains 19% of the variance (Figure 2.2). The PCA analysis did not resolve samples into control and treatment groups; rather, for each colony, controls were directed on an angle to the corresponding treatments. Samples grouping by colony in transcriptomic analysis have frequently been observed in hard coral studies, illustrating the high colony variability within species regardless of the treatment (Aguilar et al., 2017; Bertucci et al., 2015). In this PCA plot, two groupings were apparent; colonies C1, C5 and C6 formed one group (Group1) and colonies C2, C3 and C4 the second (Group 2). These two groups differed in the direction of change between treatment and control in the PCA plot; in Group 1, *Lobophytum* samples treated with MDP were situated above the corresponding colony control sample, whereas the opposite direction of change was observed for colonies in Group 2 (Figure 2.2, Table 2.1). It is important to note that in Group 1 colony C5 is following the same directions on the PC2 axes than the other colonies in this group; but in PC1, it is situated to the right of the control and not to the left like the rest of the group members. Possibly meaning that this particular colony might be regulating some genes differently than the rest of the colony group.

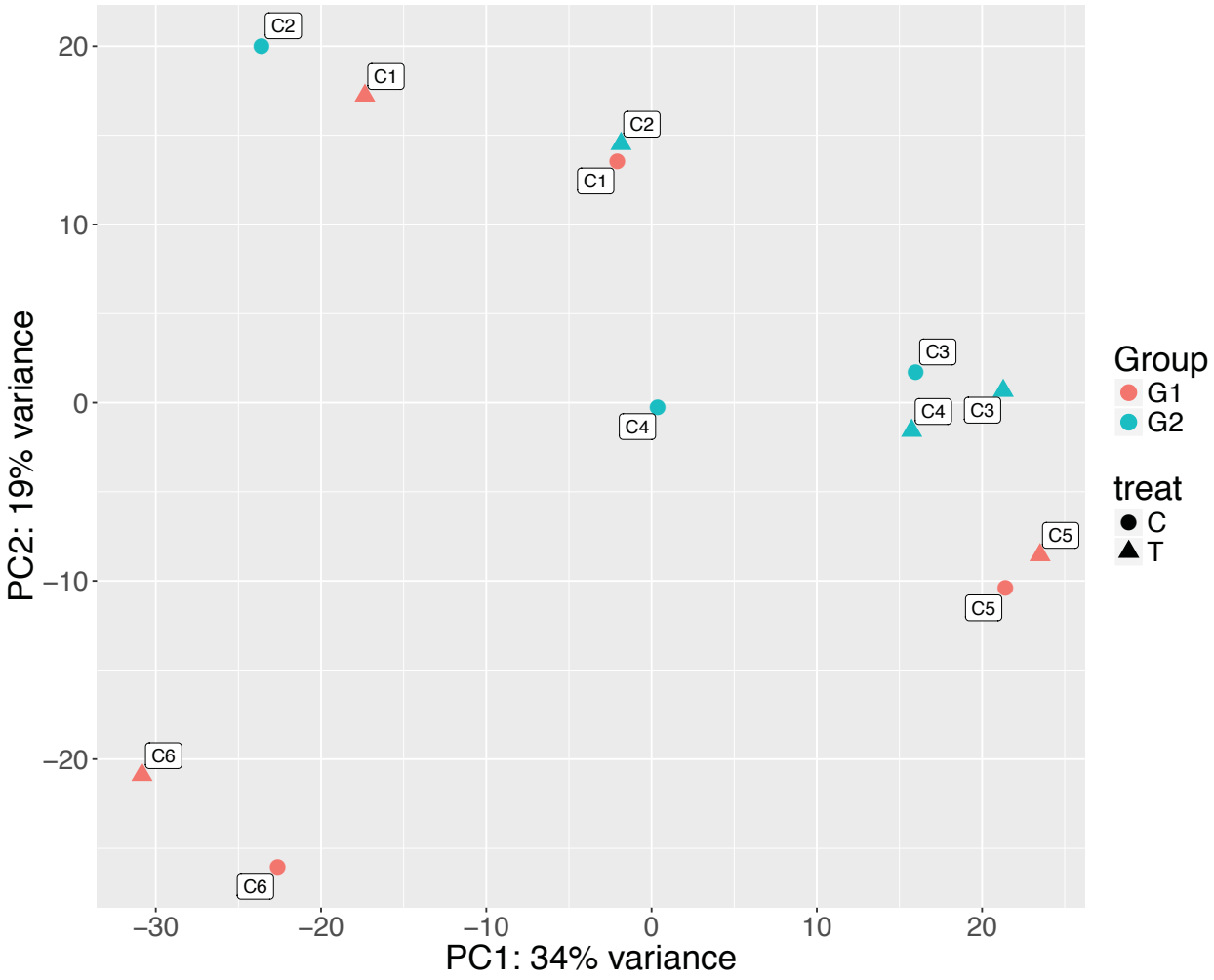

Figure 2.2: Principal component analysis based on normalized, variance stabilized counts for all *Lobophytum* samples. Red=colonies from Group1, blue= colonies from Group2, with labels showing the competing *Lobophytum* colonies. Circles = control samples, triangle = samples immune challenged with MDP.

Group 1 and Group 2 were used to define the "Group" variable for DESeq2 analysis (Table 2.1), specifying in the model the variation of responses between the sets of samples.

The DESeq2 analysis found that a total of 78 genes were responsible for the differences between Groups 1 and 2, irrespective of treatment. Only two genes were differentially expressed when comparing Group1-MDP to Group1-control. Conversely, 75 genes were differentially expressed between Group2-MDP and Group2-control (Figure 2.3).

A total of 41 genes explained the difference between groups irrespective of treatment and were also responsible for the variation between treatment and control for samples in Group 2 (Group2-MDP vs Group2-control; Figure 2.3). The overlap in Figure 2.3 was expected because the grouping was based on the differences observed in the PCA between the two set of samples in terms of how they responded to the treatment (Figure 2.2).

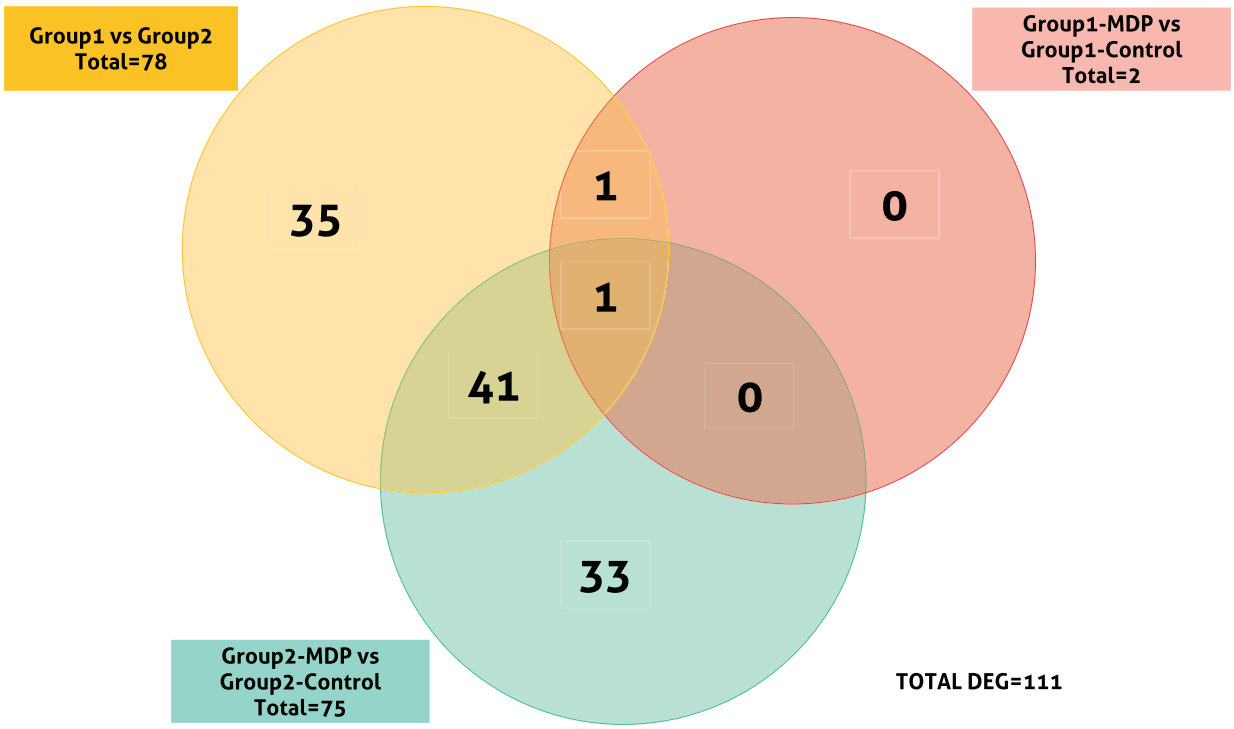

Figure 2.3: Venn diagram of differentially expressed genes (DEG) in *Lobophytum* under an immune challenge. 'Group1 vs Group2' corresponds to DEG when comparing Group1 and Group2 irrespective of treatment.

The colony grouping performed based on the PCA results helped to find the genes responsible for the variations between Group2-MDP and Group2-control. Close examination of the PCA plot shows that the direction of change between controls and treatments was far more consistent for Group 2 than for Group 1. This explains why very few genes (two) were found to be differentially expressed between treatments and controls for Group 1, whereas 75 DEG were found for Group 2. Nonetheless, 33 of the DEG down-regulated in Group2-MDP compared to Group2-control, were up-regulated in Group1-MDP compared to Group1-control (but with

limited statistical support, data not shown). This opposite response suggests that DEG in Group2-MDP might also contribute to the differences seen in the PCA analysis between Group1-MDP and Group1-control samples, but that in general colonies from Group1 had inconsistent gene expression profiles (different genes up or down-regulated).

2.3.3 Analysis to infer gene function

2.3.3.1 Ontology enrichment analysis

Enrichment analysis of the genes differentially expressed between Group2-MDP and Group2-control identified nine gene ontology terms (GO-terms) that were overrepresented with at least three UniProt IDs per term (Table 2.3).

Four clusters that were down-regulated in the Group2-MDP treatment relative to controls were annotated as homologs of nitric oxide synthase (NOS; Cluster-32814.5; Cluster-56627.2) and agrin (Cluster-61500.0, Cluster-60630.0), and these were responsible for the enrichment of the GO-terms "synapse [GO:0045202]", "ion binding [GO:0044325]" and "regulation of cardiac muscle contraction [GO:0055117]".

NOS is an oxidoreductase responsible for the production of nitric oxide (NO) from arginine. Nitric oxide is an important signalling molecule involved in various cellular processes such as immune defence and nervous transmission (Colasanti et al., 2010). In *Lobophytum pauciflorum,* NOS has been localised predominantly in the gastroderm (i.e. endoderm) (Safavi-Hemami et al., 2010) rather than the ectoderm (as might be expected in the case of a nervous system function). Safavi-Hemami et al. (2010) suggest that NO signalling is unlikely to be involved in a nervous reaction in this soft coral (Safavi-Hemami et al., 2010).

Conversely, studies in other cnidarians suggest functions in the nervous system; NO stimulation caused tentacle retraction in *Aiptasia pallida* (Salleo et al., 1996), feeding behaviour in *Hydra vulgaris* (Colasanti et al., 1997) and peristaltic activity in the sea pansy *Renilla koellikeri* (Anctil et al., 2005).

There also appears to be a positive correlation between NOS activity (and NO concentration) with bleaching (Trapido-Rosenthal et al., 2001) and, on this basis, it has been suggested that under stress (e.g. heat stress) the host might increase the activity of NOS, resulting in higher NO levels and thus triggering the disruption of symbiosis (bleaching) (Perez and Weis, 2006; Ross, 2014) . The NOS homologs in *Lobophytum* (Cluster-32814.5; Cluster-56627.2) and three

other genes related to redox activity and nervous responses were annotated under the enrichment terms “regulation of neurogenesis [GO:0050767]” and “heme binding [GO:0020037] “. Heme binding is important for cellular oxidant metabolism due to its relationship with iron cycling and is essential for detoxification (Table 2.3). Most of the clusters in this group were down-regulated in Group2-MDP when compared with Group2-control.

Agrin homologs and another seven genes down-regulated in response to treatment (Table 2.3), had the associated GO-terms: “calcium ion binding [GO:0005509]”; “extracellular region [GO:0005576]” and “extracellular matrix [GO:0005576]”. Amongst these seven genes were three extracellular matrix (ECM) constituents involved in cell-cell communication (agrin, collagen alpha-6(VI) and a cartilage matrix protein; Table 2.3). Several other genes implicated in immune defence and nervous responses, including myeloperoxidase (MPO), phospholipase A2 (PLA2) and the neural pentaxin-2 (NPTX2), were also down-regulated upon MDP-challenge (Table 2.3). Conversely, *Lobophytum* homologs of five other genes IDs annotated with these same GO-terms (“GO:0005509”; “GO:0005576” and “GO:0005576”) were up-regulated in Grp2-MDP relative to controls. The up-regulated clusters corresponded to genes potentially involved in mucus production like deleted in malignant brain tumour 1 (dmbt1) and the von Willebrand factor (vWF); or in cell adhesion and recognition (fibrillin-2 and protocadherin Fat 4, respectively). Finally, homologs of the cartilage matrix protein (Matrilin-1) and to a discoidin domain receptor 2 were down-regulated in *Lobophytum* from Group2-MDP . These genes were annotated with the GO-term “regulation of bone mineralisation [GO:0030500]”and have a role in the reorganization of the extracellular matrix.

Table 2.3: Nine gene ontology terms overrepresented in DEG found between Group2-MDP and Group2-control samples.

GO	Over represented pvalue	Description	Ontology	Number of clusters	Number of Gene IDs	Uniprot ID
GO:0045202	5.31E-05	synapse	CC	5	2	NOS1_RAT NOS1_HUMAN AGRIN_MOUSE
GO:0044325	3.47E-05	ion channel binding	MF	4	2	NOS1_RAT NOS1_HUMAN AGRIN_MOUSE
GO:0055117	1.20E-05	regulation of cardiac muscle contraction	BP	3	2	NOS1_HUMAN AGRIN_MOUSE
GO:0005509	6.03E-07	calcium ion binding	MF	12	8	AGRIN_MOUSE MATN1_HUMAN PA2GA_MOUSE EFCB1_LOTGI DLL1_RAT FBN2_MOUSE FAT4_MOUSE
GO:0005576	4.60E-10	extracellular region	CC	16	10	AGRIN_MOUSE MATN1_HUMAN PA2GA_MOUSE EFCB1_LOTGI FBN2_MOUSE PERM_HUMAN NPTX2_HUMAN DMBT1_HUMAN VWF_CANLF
GO:0031012	3.21E-05	extracellular matrix	CC	6	4	AGRIN_MOUSE FBN2_MOUSE VWF_CANLF CO6A6_MOUSE
GO:0050767	2.31E-05	regulation of neurogenesis	BP	3	2	NOS1_RAT NOS1_HUMAN DLL1_RAT
GO:0020037	1.38E-05	heme binding	MF	5	4	NOS1_RAT NOS1_HUMAN PERM_HUMAN PXDN_DROME NGB_CHAAC
GO:0030500	5.69E-05	regulation of bone mineralization	BP	3	2	MATN1_HUMAN DDR2_MOUSE

2.3.3.2 Manual gene categorization

The GO-term enrichment analysis (Table 2.3) provides an overview of the biological processes, cellular components and molecular functions that were overrepresented in the set of DEG. Nevertheless, manual classification and annotation of the DEG revealed more details about the reaction of *Lobophytum* samples from Group-2 to treatment (Table 2.4). As mentioned previously, four categories of genes can be identified in this dataset: 1. Immune-related genes, 2. Neuro-related genes, 3. ECM-related genes and 4. Transcription-related genes. These categories are explored in more detail below.

Five genes classified as immune-related were up-regulated in the MDP treatment of Group2 when compared to control samples of the same group (Table 2.4). Three of the five genes were signalling components related to recognition and cell survival (Cluster-24631.0; Cluster-33002.5337; Cluster-39559.2). The remaining two clusters corresponded to the deleted in malignant brain tumour protein (dmbt1) and laccase-4. Dmbt1 has been found up-regulated in corals under immune challenge, and it has also been suggested that it has a function in the recognition and maintenance of symbionts (Wright et al., 2017).

Laccase participates in melanin synthesis, and it has been reported that a laccase homolog was up-regulated in *Pocillopora damicornis* exposed to either non-virulent or virulent bacteria after 12 days of exposure (Vidal-Dupiol et al., 2014). Palmer et al. (2012) found less laccase activity in bleaching and disease-susceptible corals than in non-susceptible. Conversely, five other genes nominally associated with immune responses were down-regulated in Group2-MDP versus Group2-control samples (Table 2.4). Most of these genes have functions in removal of reactive oxygen species (ROS) typically generated during a cellular stress response; peroxidasin, for example, controls detoxification of ROS. Peroxidasin was up-regulated in several hard coral species exposed to heat stress (Libro et al., 2013; Louis et al., 2017; Voolstra et al., 2009) and a peroxidasin homolog was also up-regulated in *Gorgonia ventalina* following challenge with *Aplanochytrium* (a parasite) (Burge et al. 2013).

A total of fifteen clusters potentially involved in nervous system function or development based on their annotation were differentially expressed upon MDP-challenge. Of these, twelve were down-regulated and three (homologs of the protocadherin Fat 4 (Fat 4), delta-like protein 1 (delta1) and SCO-spondin) were up-regulated in Group2-MDP compared with Group2-control (Table 2.4). Protocadherins function in axogenesis in vertebrates (Liebeskind et al., 2017), and Fat 4 is a component of the ECM that functions in cell adhesion but may also be involved in Wnt signalling (Magie and Martindale, 2008). Note that Wnt signalling is involved in patterning the nervous system of *Nematostella* (Bosch et al., 2017). Delta is the receptor for the notch ligand, and this signalling system was discovered in the context of early neurogenesis in *Drosophila* (Artavanis-Tsakonas et al., 1995; Lehmann et al., 1981). Roles for delta/notch signalling in neurogenesis are common in metazoans, and in *Nematostella* the delta homolog *Nvdelta* is an inhibitor of embryonic neurogenesis (Layden et al., 2012; Layden and Martindale, 2014). If the similarity between the delta homologs of *Lobophytum* and *Nematostella* is consistent with conservation of function, the up-regulation of delta1 observed in *Lobophytum* upon MDP challenge likely reflects inhibition of neurogenesis. Manual annotation of Cluster-

27610.0 implies that it is a homolog of SCO-spondin, a protein involved in the modulation of neural aggregation (UniProt). A homolog of this gene has been shown (by *in situ* hybridization) to be expressed in the head region of *Hydra* (Hamaguchi-Hamada et al., 2016).

Amongst the twelve down-regulated clusters in the neuro-related genes category, two clusters were annotated as a homolog of the glycine receptor subunit alpha-2 (GLRA2; Table 2.4). GLRA2 has roles in the peristaltic contraction of the epitheliomuscular cells and chemosensory responses of *Hydra* (Pierobon, 2012; Watanabe, 2017). Thus the down-regulation of the GLRA2 homolog observed in *Lobophytum* upon MDP challenge suggests suppression of neural signalling. Two sox gene homologs were also down-regulated: Sox-8 (Cluster-31038.0) also annotated as *AmSoxE1* from *Acropora millepora*, and *Sox9A* (Cluster-52616.0) also annotated as *SoxE1* from *Hydractinia echinata* (Table 2.4). Cnidarian SoxE genes have been implicated in neuronal development (Shinzato et al., 2008) and the *Lobophytum* SoxE expression data are again consistent with the idea of suppression of neuronal signalling and development under immune challenge. Two genes previously discussed (Results section 2.3.3.1), agrin and NOS, were also categorized as neuro-related genes. As mentioned above, NOS being implicated in nervous signalling. Agrin is a ligand produced by motor neurons and has a role in the mammalian neuro-muscular connection (Zhang et al., 2011) . The *Hydra* serine protease inhibitor (kazal1) resembles agrin in terms of domain structure (Chera et al. 2006). Kazal2, a sequence similar but not identical to kazal1, from *Hydra magnipapillata* has been found to have antimicrobial properties (Augustin et al., 2009; Mydlarz et al., 2016). The antimicrobial properties of the *Hydra* kazal-type protein in the immune defence and the presence of kazal-type domain in the *Lobophytum* homolog of agrin suggests that this protein may have a role or roles in the immune and/or nervous systems.

Six clusters classified as ECM components or involved in ECM-based signalling pathways were down-regulated upon immune challenge (Table 2.4). Most of the genes in the ECM category were involved in calcium binding and/or mineralisation based on their associated GO-terms. The ECM component fibrillin-2 was the only protein in this category to be up-regulated upon challenge.

Nine clusters differentially expressed upon MDP challenge were classified as being related to transcription (Table 2.4). Two clusters (Cluster-1141.1; Cluster-56145.5) that lacked annotation but contained reverse transcriptase domains were up-regulated in this category, whereas a forkhead domain-containing protein and a homolog of the nucleolar protein 73 (NNP73) were both down-regulated (UniProt).

Table 2.4: Genes differentially expressed in Group2-MDP. Blue=down-regulated genes and red=up-regulated genes.

Biological characteristic	Category	Cluster ID	UniProt ID	BEST E-value	Protein names	log2 Fold Change Grp2	padj Grp2	Domain name	Domain e-value	Accession ccd
Signalling component / Stress / ROS/ Defence	IMMUNITY	Cluster-30490.3	THIO_PLAF7	1.00E-26	Thioredoxin	-1.0	8.15E-02	TRX family	2.67E-35	cd02947
Signalling component / Nervous / Cell fate /Defence	IMMUNITY	Cluster-55251.0	PXDN_DROME	3.00E-115	Peroxidasin (EC 1.11.1.7)	-5.4	2.73E-02	Anperoxidase	0.00E+00	pfam03098
Signalling component / Recognition / Adhesion / mucus Defence	IMMUNITY	Cluster-33002.5471	NGB_CHAAC	5.00E-18	Neuroglobin	-0.8	4.39E-02	Globin likesuperfamily	2.30E-42	cl21461
Signalling component / SARC / Defence	IMMUNITY	Cluster-38295.0	PERM_HUMAN	1.00E-38	Myeloperoxidase (EC 1.11.2.2)	-6.5	8.38E-04	An peroxidase	0.00E+00	pfam03098
Signalling component / Nervous / Cell fate / Defence	IMMUNITY	Cluster-32573.0	ALDO2_ARATH	1.49E-117	Indole-3-acetaldehyde oxidase (EC 1.2.3.7) (Aldehyde oxidase 2)	-1.4	3.17E-02	PLN00192	6.22E-171	PLN00192
Signalling component / mucus	IMMUNITY	Cluster-24631.0	VWF_CANLF	8.00E-81	von Willebrand factor	2.8	1.16E-03	VWA	2.83E-43	pfam00092
Signalling component / ECM	IMMUNITY	Cluster-39559.2	TNNC2_PELES	1.00E-05	Troponin C, skeletal muscle	3.5	5.20E-03	EFh	4.07E-10	cd00051
Signalling component / Coagulation	IMMUNITY	Cluster-33002.390	DMBT1_HUMAN	3.00E-14	Deleted in malignant brain tumors 1 protein	2.1	9.48E-02	SR	2.30E-27	smart00202
Signalling component / Coagulation	IMMUNITY	Cluster-33002.5337	.	.	.	2.7	1.84E-02	FReD superfamily	5.60E-06	cl00085
Signalling component / Coagulation	IMMUNITY	Cluster-61829.0	LAC4_THACU	1.25E-20	Laccase-4	1.4	7.90E-03	CuRO 3 tcLLC2 insect-like	2.01E-55	cd13905

Biological characteristic	Category	Cluster ID	UniProt ID	BEST E-value	Protein names	log2 Fold Change Grp2	padj Grp2	Domain name	Domain e-value	Accession ccd
Receptor / mineralization / Apoptosis / Cell proliferation / MAPK / mineralization	NERVOUS	Cluster-52616.0	SOX9A_XENLA	3.00E-31	Transcription factor Sox-9-A	-1.8	1.12E-02	High Mobility Group (HMG)-box	3.05E-25	
Receptor / Nervous /	NERVOUS	Cluster-31038.0	SOX8_XENLA	6.00E-35	Transcription factor Sox-8	-3.7	4.05E-04	SOX-TCF HMG-box	1.49E-30	
Signalling component / transcription	NERVOUS	Cluster-32814.5	NOS1_HUMAN	0	Nitric oxide synthase, brain (EC 1.14.13.39)	-1.1	2.61E-02	NOS oxygenase superfamily	0.00E+00	cl00254
Receptor / mineralization	NERVOUS	Cluster-56627.2	NOS1_RAT	0	Nitric oxide synthase, brain (EC 1.14.13.39)	-1.3	2.05E-02	NOS oxygenase superfamily	0.00E+00	cl00254
Signalling component / Transcription	NERVOUS	Cluster-61309.2	GLRA2_HUMAN	4.87E-53	Glycine receptor subunit alpha-2	-8.0	3.66E-04	.	.	
Signalling component / Transcription	NERVOUS	Cluster-61309.1	GLRA2_HUMAN	4.87E-53	Glycine receptor subunit alpha-2	-7.7	8.90E-04	.	.	
Signalling component / Transcription	NERVOUS	Cluster-61500.0	AGRIN_MOUSE	6.00E-05	Agrin	-1.0	3.72E-02	GON domain is found in the ADAMTS	6.39E-63	pfam08685
Recognition / Lectin-like / Immune	NERVOUS	Cluster-60630.0	AGRIN_MOUSE	6.00E-05	Agrin	-1.0	1.60E-02	GON	6.39E-63	pfam08685
Signalling component / Cell survival / Immune	NERVOUS	Cluster-6158.3	.	.	.	-1.5	2.72E-02	Neuromodulin N super family	9.25E-07	
Signalling component / Cell fate / Nervous / Secretion / Muscle	NERVOUS	Cluster-6158.4	.	.	.	-1.4	6.79E-02	Neuromodulin N super family	9.25E-07	
Signalling component	NERVOUS	Cluster-20146.0	.	.	.	-1.9	1.40E-02	Na Ca ex superfamily	1.22E-04	cl27511
Signalling component / Cell fate	NERVOUS	Cluster-20146.4	.	.	.	-1.2	8.84E-02	Na Ca ex superfamily	1.22E-04	cl27511

Biological characteristic	Category	Cluster ID	UniProt ID	BEST E-value	Protein names	log2 Fold Change Grp2	padj Grp2	Domain name	Domain e-value	Accession ccd
Signalling component / Transcription	NERVOUS	Cluster-27610.0	SSPO_RAT	2.30E-60	SCO-spondin	0.9	4.67E-02	FA58C	2.83E-49	cd00057
Signalling component /	NERVOUS	Cluster-41331.0	FAT4_MOUSE	1.00E-15	Protocadherin Fat 4	1.1	9.20E-04	Cadherin repeat	9.34E-16	cd11304
Receptor / Nervous /	NERVOUS	Cluster-15490.6	DLL1_RAT	1.00E-17	Delta-like protein 1	3.1	2.05E-02	TLD superfamily	4.47E-11	cl02144
Signalling component / transcription	ECM	Cluster-36837.0	PA2GA_MOUSE	1.00E-24	Phospholipase A2 (EC 3.1.1.4)	-3.5	5.95E-04	Phospholipase A2	5.69E-33	pfam00068
Signalling component / transcription	ECM	Cluster-49405.0	FGFR3_PLEWA	9.00E-68	Fibroblast growth factor receptor 3 (EC 2.7.10.1)	-1.6	9.05E-02	.	.	
Signalling component / transcription	ECM	Cluster-22544.2	DDR2_MOUSE	9.00E-28	Discoidin domain-containing receptor 2 (EC 2.7.10.1)	-0.9	9.44E-02	.	.	
Signalling component / ECM	ECM	Cluster-28607.0	CO6A6_MOUSE	4.00E-22	Collagen alpha-6(VI) chain	-0.7	4.14E-02	VWA	1.25E-32	pfam00092
Signalling component / Cell fate / Nervous / Secretion / Muscle /	ECM	Cluster-61948.29	MATN1_HUMAN	3.00E-13	Cartilage matrix protein (Matrilin-1)	-2.6	2.40E-03	VWA	1.14E-21	smart00327
Signalling component /	ECM	Cluster-61948.13	MATN1_HUMAN	3.00E-13	Cartilage matrix protein (Matrilin-1)	-1.4	9.44E-02	VWA	1.14E-21	smart00327
Signalling component / Ligand / NOTCH / Nervous / Immune	ECM	Cluster-47263.6	FBN2_MOUSE	3.00E-72	Fibrillin-2	1.7	4.31E-02	VWA	6.81E-37	pfam00092
Effector / Stress / Oxidative response / Immune	TRANSCRIPTION	Cluster-33002.6881	ZMYM1_HUMAN	3.00E-24	Zinc finger MYM-type protein 1	-3.5	5.09E-03	DUF4371 super family	1.06E-23	
Effector / Stress / Inhibit	TRANSCRIPTION	Cluster-28526.3	RTJK_DROME	7.00E-17	RNA-directed DNA polymerase from mobile element jockey (EC 2.7.7.49)	-1.4	1.17E-02	.	.	

Biological characteristic	Category	Cluster ID	UniProt ID	BEST E-value	Protein names	log2 Fold Change Grp2	padj Grp2	Domain name	Domain e-value	Accession ccd
Effector / Stress / Inhibit	TRANSCRIPTION	Cluster-33002.3719	POLX_TOBAC	9.00E-09	Retrovirus-related Pol polyprotein from transposon TNT 1-94 (EC 3.4.23.-); (EC 2.7.7.49); Endonuclease	-1.0	8.81E-02	.	.	
Effector / Stress / Oxidative response	TRANSCRIPTION	Cluster-42273.1	UTP20_HUMAN	3.00E-138	Novel nucleolar protein 73 (NNP73)	-3.0	4.81E-02	Down-regulated in metastasis;	2.38E-173	
Effector / Immune / Mucus	TRANSCRIPTION	Cluster-38201.0	FD4_DROME	1.00E-26	Fork head domain-containing protein FD4	-2.2	7.85E-03	Forkhead domain	1.80E-47	
Signalling component / Factor / Transcription / growth	TRANSCRIPTION	Cluster-42273.0	.	.	.	-5.5	2.36E-05	RecF/RecN/SMC N termi.l domain	5.24E-07	
Signalling component / Factor / Nervous / transcription	TRANSCRIPTION	Cluster-21272.0	.	.	.	-3.1	1.36E-03	RT_like superfamily	1.06E-10	cl02808
Effector / Transcription	TRANSCRIPTION	Cluster-56145.5	.	.	.	0.8	2.37E-02	RT_like superfamily	1.18E-10	cl02808
Effector / Transcription	TRANSCRIPTION	Cluster-1141.1	.	.	.	3.0	4.81E-02	RT_like superfamily	4.66E-07	cl02808

2.3.4 Comparison with the acute response of Acropora millepora to MDP treatment

Despite some difficulties in identifying orthologs between the hard and soft corals on the basis of annotations, six clusters appeared to be differentially expressed in both *Acropora millepora* and *Lobophytum* Group2 after MDP stimulation (Table 2.5). Two *A. millepora* clusters (Cluster008297 and Cluster001272) matched the *Lobophytum* agrin homolog and these were down-regulated in both species (Table 2.5). However, note that the annotations of these clusters were not congruent; in *A. millepora*, Cluster001272 was annotated as an homolog of SCO-spondin and Cluster008297 as a homolog pentraxin-like protein.

In the case of the four other clusters, the direction of change upon MDP-stimulation differed between the *A .millepora* and *Lobophytum* homologs. Two *A. millepora* clusters (Cluster023274 and Cluster013871) were up-regulated upon MPD challenge whereas their homologs were down-regulated in *Lobophytum* (Table 2.5). Conversely, the other two *A. millepora* clusters (Cluster015890 and Cluster000397) were down-regulated under MDP challenge but their *Lobophytum* homolog were up-regulated (Table 2.5).

Table 2.5 Differentially expressed genes in *Acropora millepora* under MDP treatment that had homologs amongst the DEG on *Lobophytum* Group2-MDP. "Protein name *Lobophytum*" shows annotation found for the *Lobophytum* sequence. Blue: in "Fold change *A. millepora*' corresponds to genes down-regulated in Weiss et al (2013) and in "Protein name *Lobophytum*" represents down-regulates genes in the present experiment. Red= same specification as blue but genes were up-regulated.

Cluster *A. millepora*	Protein name *A.millepora*	e-value *A. millepora*	Fold change *A. millepora*	Length *A.millepora* (bp)	Length *Lobophytum* (bp)	e-value BLAST *A.millepora vs Lobophytum*	Protein name *Lobophytum*
Cluster008297	Notch/pentraxin -like unknown	6.0E-51	2.46E-01	2461	60492	6.53E-08	Agrin
Cluster001272	SCO-spondin / hemicentrin	0.0E+00	2.24E-01	5393	60492	3.14E-35	Agrin
Cluster023274	Serine protease	1.0E-42	Inf	1169	4617	1.65E-13	CUB and sushi domain-containing protein 2
Cluster015890	Hypothetical polydom like	4.0E-66	2.13E-01	1617	8532	2.08E-84	Fibrillin-2
Cluster000397	EGF-containing unknown	4.0E-132	2.42E-01	7649	8532	4.00E-35	Fibrillin-2
Cluster013871	No significant hits	.	1.09E+01	1797	5631	2.11E-08	RNA-directed DNA polymerase from mobile element jockey (EC 2.7.7.49) (Reverse transcriptase)

2.4 Discussion

Lobophytum pauciflorum colonies showed great variation in response to MDP challenge. Similar results have been observed in experiments analysing the transcriptional responses of hard corals (e.g. *Acropora millepora*) to stressors (e.g. bacterial challenge; (Aguilar et al., 2017; Wright et al., 2017). The significance of genotypic variation has only recently started to be realised in coral biology after the discovery of common responses across resisting corals that could be attributed to the genotype (Granados-Cifuentes et al., 2013). The strong influence of genotypic variation is one of the over-riding themes of this thesis and is discussed further in chapter 5.

The response of Group2 *Lobophytum* individuals to MDP stimulation was mainly negative; 81% of DEGs were down-regulated. In theory, an immune stimulus triggers the up-regulation of a cascade of genes alerting the organism to defend itself from threat (Hato and Dagher, 2015). Experiments with *A. millepora* suggest that this is an oversimplification. An hour after being exposed to viral mimic poly I:C, *A. millepora* responded by down-regulating many genes (Weiss et al., 2013), however, the same study found that more genes were up-regulated than down-regulated an hour after MDP challenge. This example demonstrates that cnidarian immune responses vary depending on the nature of the stimulus as well as between individuals. These results also support the idea that the innate immune system of corals features subtle and complex mechanisms, allowing these organisms to respond differently to a variety of stressors (Hildemann et al., 1977; Vidal-Dupiol et al., 2014).

Homologs of key genes involved in the control of oxidative stress in other animals (e.g. peroxidasin, myeloperoxidase, aldehyde oxidase 2) were down-regulated in *Lobophytum* upon MDP challenge, suggesting a suppression of the stress response (Davies et al., 2016; Oakley et al., 2017; Vidal-Dupiol et al., 2014). By contrast, in a number of studies where hard corals have been exposed to immune challenges, the ROS-removal machinery was up-regulated (Oren et al., 2010; Vidal-Dupiol et al., 2011; Weiss et al., 2013). Burge et al. 2013 also reported up-regulation of antioxidants, including peroxidasin, in *Gorgonia ventalina* after exposure to a parasite. Why the *Lobophytum* response to MDP stimulation differs in terms of antioxidant expression is unclear at this stage but it is possible that timing of response differs between species. It should be noted that the *Lobophytum* data are from one hour post treatment and that those specific proteins (anti-oxidants) might be up-regulated at a later time point. A general

down-regulation of transcription has often been reported (Seibel and Walsh, 2003) as an acute response to stress and in order to enable reallocation of energy to more immediate needs.

Nevertheless, some genes likely to have immune functions were up-regulated in coral lobes challenged with MDP. A closer look at the annotation of these genes implied that some were involved in recognition or signalling (dmbt1, vWF, fibrinogen domain). The up-regulation of recognition molecules can be rationalised as a strategy allowing the assessment of the threat.

Interestingly, laccase, an oxidoreductase involved in melanin production, was up-regulated. It seems contradictory that, immediately after an immune challenge, the soft corals down-regulated several other immune responses but up-regulated laccase activity. As mentioned in the results section, laccase-type activity was enhanced in less susceptible corals (i.e. to bleaching or diseases) and up-regulated in *Pocillopora damicornis* after challenge with virulent or non-virulant bacteria (Palmer et al., 2012). It has been suggested that laccase-based melanin synthesis might primarily function as a defence/ resistance mechanism in corals, whereas tyrosinase activation might be an "attack" response aimed at eliminating pathogens (Palmer et al., 2012). Taking into account previous studies and the observed up-regulation of a laccase homolog in *Lobophytum* upon MDP challenge, it seems possible that *Lobophytum* nubbins were responding to external stimulus with the production of melanin through a laccase-type pathway to protect themselves of a potential threat but avoiding the possible hazardous effects of tyrosine-based system. Considering that the sampling for this experiment was an hour after injection, it will be interesting to assess whether the soft corals transition to a tyrosine-type melanin pathway later in the response, or turn off laccase expression, effectively down-regulating the ability to produce melanin.

Consideration of all the results together suggests that Group2 *Lobophytum* responded to the MDP challenge by limiting the immune response so as to avoid self-inflicted damage and resist the stressor (Bellantuono et al., 2012; Libro and Vollmer, 2016; Reed et al., 2010; Seneca and Palumbi, 2015). On the basis of apparent down-regulation of a number of genes related to neural development, differentiation and signalling, it is likely that MDP-challenged corals from Group2 also down-regulated neural functions (Salzet et al., 2000), suggesting a close relationship between the immune and nervous systems in *Lobophytum* that will be discussed further in Chapters three and five. Potential cross-talk between the immune and nervous systems is also supported by the down-regulation of agrin and nitric acid synthase after immune challenge, as both of these proteins are involved in the control of nervous system responses in other organisms.

It is important to note that Group1 *Lobophytum* colonies responded mostly in the opposite direction than did Group2 colonies after MPD challenge with respect to expression of some anti-oxidant and neural-related genes. These different responses could reflect prior exposure differences, or differences in the time required to initiate a response (Marshall and Baird, 2000). A different approach needs to be taken in the future to elucidate with statistical support which genes are responsible for treatment response in colonies from Group1. It will be necessary to determine the genotypes of the colonies to group them accordingly in the model. It will be interesting to see the extent to which genotypic variation correlates with the Group1 and Group2 molecular responses.

This study illustrates high variability within a species with respect to the innate immune response to a defined immunogen at a single time point. A much better understanding of the functions of cnidarian genes is needed to understand the flexibility and limits of the innate immune response in corals.

Chapter 3 - Transcriptomic analysis of *Lobophytum pauciflorum* under competition

3.1 Introduction

An important characteristic of octocorals is the presence of secondary metabolites (SMs). Indeed, the literature describing the chemical constituents of octocorals by far exceeds that on the biology of these organisms. As mentioned in Chapter 1, secondary metabolites are obvious candidates as mediators of soft coral competition, but the natural roles of many of these compounds are currently unknown.

Secondary metabolites (SMs) are produced by many organisms, and while originally as they may have arisen as by-products of essential metabolic processes, they often acquire some selective advantage over time (Lotina-Hennsen et al., 2006). For example, some plant secondary metabolites (SMs) have been shown to have insect repellent properties (Pichersky and Gang, 2000) and thus can be a significant factor in structuring plant communities (Reigosa et al., 2006; Sinkkonen, 2006; Weidenhamer, 2006). Much of the research on secondary metabolites to date has focused on characterising their chemical structures and testing their cytotoxic effects, often with the aim of identifying potential pharmaceuticals or herbicides (Kabera et al., 2014). However, there remains a knowledge gap around the biological and ecological roles of most SMs.

Secondary metabolites are also common in marine environments and are produced by organisms ranging from bacteria to molluscs (Blunt et al., 2015). Soft corals are a particularly rich source of SM, many of which have been investigated for their pharmacological use. For example, Lobocrassin B from the soft coral *Lobophytum crassum* (Lin et al., 2017; Mariottini, 2016) and a cembranoid type diterpene extracted from *Lobophytum sp* have been shown to have anticancer activity (Al-Footy et al., 2016) . However, the level of toxicity of soft coral SMs depends not only on the nature of the compound but also on the target. It has been found, for example, that the antibacterial activity of isoprenoids (a group of SMs) extracted from *Lobophytum sp.* against *Pseudomonas aeruginosa, Staphylococcus aureus, S. epidermis and S. pneumonia* differed substantially (Al-Footy et al., 2016).

The abundance of biologically active SMs in soft corals may result from the anatomical characteristics of octocorals, which typically lack a hard skeleton and have only weak nematocyst capabilities. These characteristics might make soft corals more vulnerable to predation, algal competition, and coral competition (Tarrant et al., 2009). However, these vulnerabilities have resulted in the evolution of a range of toxins with which soft corals protect themselves from those ecological pressures (Aceret et al., 1995; La Bare et al., 1986; Sammarco and Coll, 1992) .

A wide variety of SMs, particularly complex terpenoids and ceramides (sphingolipids) are present in soft corals (Figure 3.1) (Blunt et al., 2017; Farag et al., 2016). It has been shown that ceramides are synthesized in the endoplasmatic reticulum (ER) from non-sphingolipid precursors (Gault et al., 2010). The sphingolipid family also includes sphingomyelin and glycosphingolipids (Hannun, 1996; Hannun and Luberto, 2000). Sphingolipid have a range of general functions in signal transduction and cell regulation in animal cells, but small structural modifications can give rise to secondary metabolites with cytotoxic or other activities (Maceyka and Spiegel, 2014). For example, the position and number of double bonds, and hydroxylation at key positions can impart secondary metabolite function to sphingolipids (Figure 3.1) (Muralidhar et al., 2003).

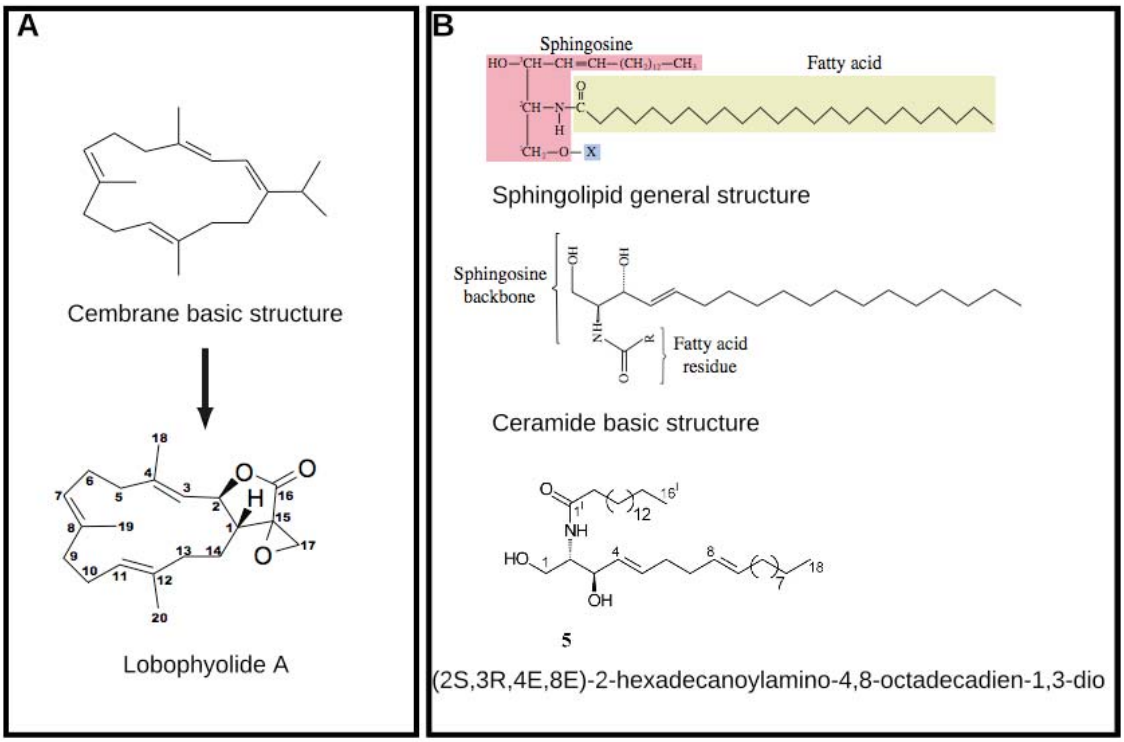

Figure 3.1: Example of cytotoxic secondary metabolites from *Lobophytum sp.* (A) Cembranoid (Lobophyolide A) extracted from *Lobophytum crassum* (Lai et al 2017); (B) Sphingolipid found in *Lobophytum sp.* (Muralidhar et al 2005).

Despite major efforts to characterise the SMs repertoires of octocorals, their ecological roles have been explored far less extensively (Chadwick and Morrow, 2011). Nevertheless, it is known that soft coral extracts (containing SMs) can reduce or truncate the settlement of larvae of other cnidarian species (Atrigenio and Aliño, 1996; Maida et al., 2001, 1995). Similarly, the toxicity of octocoral secondary metabolites on fish (Pawlik et al., 1987) and their role in competition has also been shown (Chapter 1). Available research to date implies that SMs may have significant impacts on reef community structure by modulating coral and algae species richness, as well as the species of fishes living and feeding around a SMs producing coral.

Although SMs are likely to be major mediators of soft coral ecology, many aspects of their biosynthesis and activity remains unknown. In the case of non-contact competition, an increase in secondary metabolite production is expected (Chapter 1), but there is neither the empirical evidence to support this nor an understanding of the

mechanisms behind it (Fleury et al., 2000). In fact, very little is known about the molecular pathways that corals use during an ecological interaction.

Based on a review of the current literature (mentioned in Chapter 1), I have attempted to synthesise current knowledge of non-contact competitive interactions from the soft coral perspective (Figure 3.2). This hypothetical scheme uses *Lobophytum pauciflorum* and *Porites cylindrica* as a pair of model species to describe the different aspects of soft coral biology that might be affected by non-contact competition with a hard coral. The interaction between these two species is likely to happen if they are in close proximity to each other. In fact, a range of secondary metabolites have been identified in *Lobophytum*, including ceramides and terpenoids likely to have cytotoxic properties. As mentioned in Chapter 1, *Lobophytum* can cause tissue damage to *Porites* even in non-contact situations, possibly as a consequence of SMs effects. Additionally, Coll and Sammarco (1983) found that pure terpenes extracted from *Lobophytum* tissue killed *Acropora formosa* and *Porites cylindrica (Porites andrewsi)* at low concentrations ranging from 5ppm to10ppm.

When soft and hard corals are near to each other, the first step in their interaction is the recognition of a potential threat (without recognition of an enemy there will be no interaction). As explained in Chapter 1, contact competition will trigger the innate immune system, however there is no evidence as to whether non-contact recognition can invoke an immune response (Hennessey and Sammarco, 2014). Nevertheless, it is assumed that if a stressor is recognised, the immune system will be activated. Secondary metabolites may then be deployed as part of a defence mechanism. If this occurs, it is likely that SMs would need to be transported in the soft coral to specific tissue areas under threat and released (Sinkkonen, 2006). Under this scenario, how the competitor (hard coral) responds is likely to dictate what happens next. For the soft coral, maintaining a constant immune response would be energetically costly, so it may choose to avoid conflict by appropriately regulating its growth and behaviour after establishing the position of its competitor (Hennessey and Sammarco, 2014; La Bare et al., 1986; Sammarco et al., 1985). Conversely, an aggressive response from the competing organism could lead to maintenance or enhancement of SMs biosynthesis and transport. As a result of these potential variables responses, non-contact competition between soft and hard corals is poorly understood, however it may play a significant role in structuring coral reef communities.

The aim of this chapter is to clarify molecular mechanisms (presented in the hypothetical scheme; Figure 3.2) that soft corals might use on a non-contact competition. Because these interactions are particularly challenging to study, of necessity a reductionist approach was adopted; therefore, the objective of this chapter was to determine the transcriptomic response of *Lobophytum* to non-contact competition with *Porites*. The results presented here should guide future studies that fill in the detail of the preliminary sketch below.

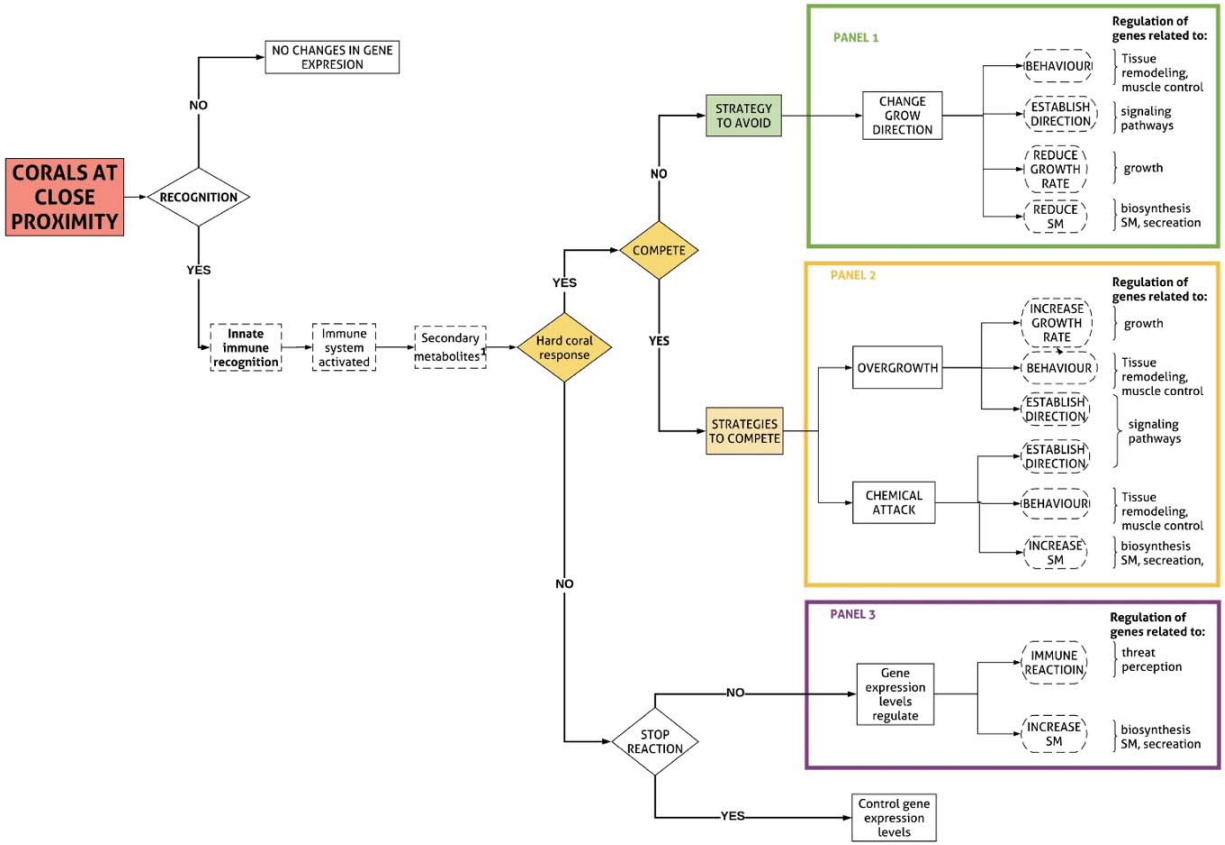

Figure 3.2: Hypothetical steps and cellular responses that a soft coral might experience under a non-contact competition scenario with a hard coral. Discontinuous lines correspond to elements that have not been experimentally tested ([1]Secondary metabolites are constantly produce but an increase of genes related with vesicle transport and release could be expected).

3.2 Materials and methods

3.2.1 Experimental design

The competition experiment was conducted at Orpheus Island Research Station (OIRS), in the central Great Barrier Reef (GBR), Australia (18'34' S;146'29'E) . Five colonies of the soft coral *Lobophytum* were collected from reefs around Orpheus Island, (GBRMPA Permit No. G16/38499.1) and transported to OIRS aquaria facilities. In addition, 54 nubbins (~3 cm) from three colonies (18 per colony) of the hard coral *Porites* were collected in the field. Species will be referred as genus names hereafter. After collection, the hard coral nubbins were fixed onto ceramic tiles with super glue. Each soft coral colony was cut into 12 pieces containing one or two lobes/fingers (~5 cm). Previous experience has shown that attaching *Lobophytum* can cause necrosis and compromise recovery post-fragmentation (personal communication, W. Wessels). Therefore, the segments of soft coral were placed on top of the tiles but not attached. The hard and soft coral fragments were then allowed to recover for 3 weeks prior to the start of the experiment.

After the acclimatization period, corals were placed in experimental tanks (1300ml) for a 60-day period. The setup was an open system where each tank received a free flow of 400 ml/min of filtered seawater (10μm). In each experimental tank, a soft coral piece and a hard-coral piece were placed ≤ 3 cm apart from each other (Figure 3.3), while isolated hard and soft corals were used as control. This pair-wise design was built with five biological replicates of the soft coral (colonies: La, Lb, Lc, Ld, Le) and three biological replicates of *Porites* (colonies: Pd, Pe, Pf). In total, the experiment was composed of 15 biological combinations of interacting corals (e.g. La-Pd, La-Pe), plus 8 non-interacting corals: 5 soft corals (e.g.: La-Control) and 3 hard corals (e.g. Pd-Control). For each combination and control there were 3 technical replicates/clones (Figure 3.3). The 15 combinations of interacting-corals, the control corals and their 3 technical replicates resulted in a total of 69 experimental tanks. Combinations will be referred as *Lobophytum*-Pd, Pe or Pf to refer to *Lobophytum* samples competing with a particular colony of *Porites.* Control samples will be referred as *Lobophytum*-control.

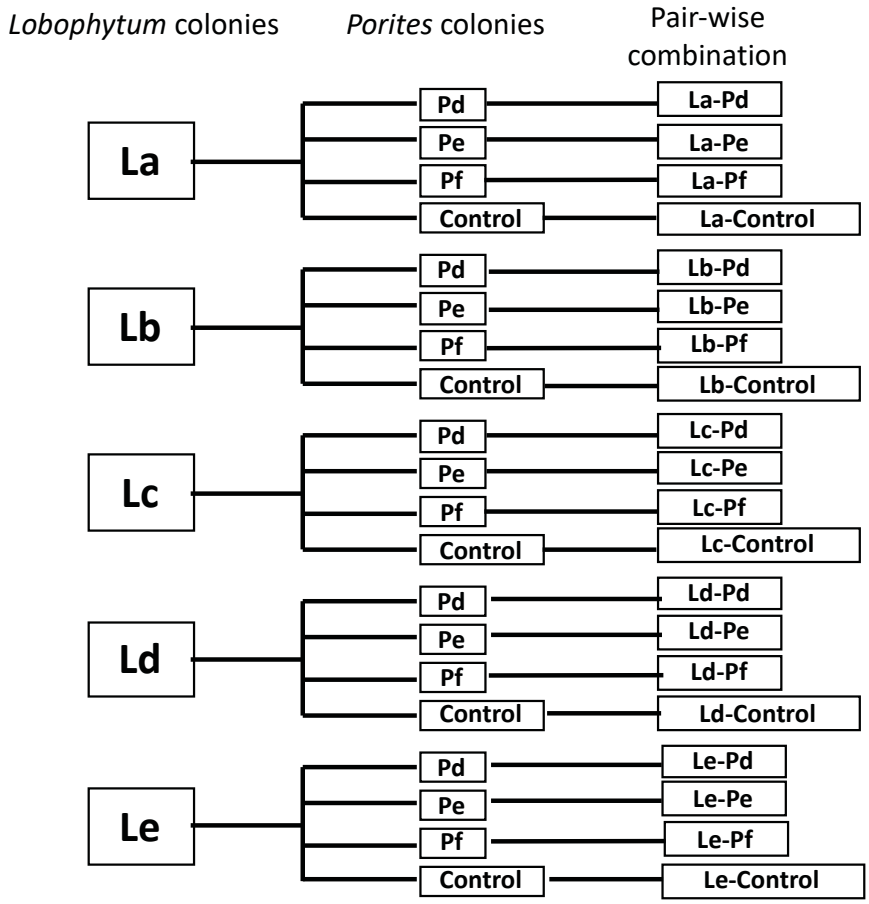

Figure 3.3: Coral competition experimental design showing the pair-wise interacting corals and controls, made with five colonies of *Lobophytum* and three colonies of *Porites*.

3.2.2 Collection and analysis of Porites behavioural data

Polyp activity and notes about both soft and hard corals behaviours were recorded eight days after starting the experiment and then daily until the end of the experiment (52 days). Polyp activity and aggressive behaviour was considered only for *Porites* because soft coral's competitive strategies are not physically visible. Previous competition experiments observed that *Porites* elongates its polyps to make physical contact with the competitor and cause local tissue damage (Rinkevich and Sakamaki, 2001; Sammarco et al., 1985). Therefore, elongated polyps and any other aggressive behaviour was recorded (Chadwick and Morrow, 2011). Detailed methods to analyse these data are described in Chapter 4.

3.2.3 Tissue sampling for gene expression analysis

To characterize the coral gene expression in a non-contact competition, tissues were sampled on day 4 (time point 1), day 30 (time point 2) and day 60 (time point 3) after the interaction started.

The first time point was determined based on a pilot study, which showed that hard corals start reacting to the presence of the soft coral after ≥4 days of interaction. Time points 2 and 3 were chosen based on literature showing signs of bleaching and reduction of growth one and two months after exposure to the competitor respectively (Chadwick and Morrow, 2011; Connell et al., 2004; Sammarco et al., 1983). At each time point, one technical replicate from the 15 pair-wise interacting corals was sampled, plus one from the controls. Tissue samples were snap-frozen in liquid nitrogen and stored at -80 °C until processed for gene expression analysis. Only one time point was analysed for financial reasons and based on the results described below in section "Behavioural observations of *Porites*" .

3.2.4 RNA extraction and transcriptome assembly

Soft coral samples from time point 2 (30 days) were processed for the genomic analysis. This time point was chosen based on the changes in behaviour of *Porites* around this period in the experiment (see Chapter 4, Figure 4.5) implying that *Lobophytum* could have been changing its behaviour as well. In total, 20 tissue samples of *Lobophytum* were extracted; 4 fragments from each of the interacting soft coral colonies and one from the control. RNA extraction, library preparation and quality control were done as specified in Chapter 2. Final library concentrations were set to 15nM in 25 µl and sent for paired-end sequencing on a HiSeq2500 Illumina machine, targeting approximately 20 million reads per sample (Ramaciotti Center for Genomics, University of New South Wales). The sequencing of mRNA extracted from soft coral tissues, after 30 days of interaction, yielded 10 million paired-end reads per sample.

Sequencing data of the 20 soft coral samples were initially mapped to the *Lobophytum pauciflorum* genome (unpublished) but, due to the low mapping rate possibly caused by the heterogeneity of the samples (data not shown), a *de novo* transcriptome assembly was generated with Trinity V2.3.2 software (Grabherr et al., 2011). Symbiont

transcripts were removed from the transcriptome using the machine learning software, Psytrans V3 (Forêt and Ong, 2014) with reference to the *Symbiodinium clade B* transcriptome (Shoguchi et al., 2013). The completeness of the clean assembly was tested with the software BUSCO V2 (Simão et al., 2015) resulting in 87%, 9% and 3.9% complete, fragmented and missing core metazoan genes respectively. Mapping and alignment of the transcripts to the new transcriptome was done with the software Bowtie2 V2.2.4 (Conesa et al., 2016; Langmead and Salzberg, 2012) resulting in an average mapping rate of 63%. After mapping, a matrix of counts per cluster was produced with the program Corset V1.05 (Davidson and Oshlack, 2014). The assembly resulted in over 152,000 contigs after clustering.

3.2.5 Transcriptome annotation

The *Lobophytum pauciflorum* genome contained more complete sequences than the transcriptome assembly from this study, therefore the genome annotations were used for our transcriptome. *Lobophytum* transcriptome was blasted (BLAST-x $E < 10^{-5}$) against predicted transcripts obtained from the genome data (unpublished). The software Geneious v. 9.1.5 was used to perform this analysis (Kearse et al., 2012). Additionally, the software Trinotate V3.0 was also used to annotate the *Lobophytum* transcriptome (https://trinotate.github.io/). More details about Trinotate annotations are described in Chapter 4.

Genome and Trinotate annotations were used to find the best BLAST hit. If the transcript was blasted against a predicted transcript from the genome, the corresponding annotation was chosen as the best BLAST. If the transcript did not have a genome BLAST hit, then the Trinotate annotation was chosen. It is important to point out that in each scenario the BLAST hit with the lowest e-value amongst the BLAST-p or the BLAST-x was chosen. The best BLAST hit was used for downstream analysis. Approximately 52% of the *Lobophytum* contigs were annotated (BLAST-n $E<10^{-05}$) onto the *Lobophytum pauciflorum* genome, and 2% of the genes not annotated with the genome had a Trinotate hit (BLASTX and BLASTP $E <10^{-05}$).

Gene Ontology IDs and terms (GO terms) as well as KEGG (Kyoto Encyclopaedia of Genes and Genomes) Orthology terms (KO terms) of the best annotation were retrieved from the UniProt web site (The UniProt Consortium, 2017).

3.2.6 Gene expression analysis

The package DESeq2 V1.16.1 (Love et al., 2014), run in the R software V3.3.0 (R Core Team, 2016), was used to find differentially expressed genes (DEG) between the soft coral samples under competition and controls. The model (Model1) chosen to run this analysis included the five biological replicates of soft coral considering the three biological replicates of hard corals they were interacting with, plus controls (isolated soft corals) (Table 3.1, Table 3.2, Model 1: ~ "Soft coral" + " Hard coral control").

The DESeq2 function "contrast()" was used to explore the genes DE at three levels of the experimental design. Comparing first, gene expression of all interacting soft corals to controls, second all soft coral colonies interacting with a specific *Porites* colony to control and finally, differences in gene expression amongst soft coral. In this case, there were ten combinations that were analysed using a principal component analysis (PCA).

The results of the gene expression analysis performed with the above model (Model 1) identified DEGs only in the soft coral fragments that were interacting with *Porites* colony Pd. In order to identify these genes, a second gene expression model (Model 2: ~"Soft coral" + "Pd Others", Table 3.1) was run comparing the soft corals interacting with Pd to the soft corals interacting with all the other colonies (i.e. those interacting with Pe, Pf or the soft coral control colonies) (Table 3.1, Table 3.2).

The DEG were obtained using the Benjamini Hochberg procedure that finds the adjusted p-values (padj). Only genes with an padj minor to 0.1 were used for downstream analysis.

Table 3.1: Samples of *Lobophytum* used for gene expression analysis with DESeq2. "Soft coral" identifies the *Lobophytum* colony the sample came from, "Hard coral control" shows which colony of *Porites* the soft coral sample was interacting with or if it was an isolated fragment for control. "Pd Other" indicates if the sample was competing with *Porites* colony Pd (Pd) or if it was interacting with any other *Porites* colony or was a control (Other). The column highlighted in blue corresponded to the variables used to fit model 1 in DESeq2. The column highlighted in yellow detail the variables used to fit model 2 (see Table 3.2).

Sample ID	Soft coral	Hard coral control	Pd Other
La_Control	La	Control	Other
La_Pd	La	Pd	Pd
La_Pe	La	Pe	Other
La_Pf	La	Pf	Other
Lb_Control	Lb	Control	Other
Lb_Pd	Lb	Pd	Pd
Lb_Pe	Lb	Pe	Other
Lb_Pf	Lb	Pf	Other
Lc_Control	Lc	Control	Other
Lc_Pd	Lc	Pd	Pd
Lc_Pe	Lc	Pe	Other
Lc_Pf	Lc	Pf	Other
Ld_Control	Ld	Control	Other
Ld_Pd	Ld	Pd	Pd
Ld_Pe	Ld	Pe	Other
Ld_Pf	Ld	Pf	Other
Le_Control	Le	Control	Other
Le_Pd	Le	Pd	Pd
Le_Pe	Le	Pe	Other
Le_Pf	Le	Pf	Other

Table 3.2: Models and functions used to find genes differentially expressed in *Lobophytum* samples after 30 days of interaction with *Porites*.

Model	Function	Variable for contrast	Arguments contrasted
Model 1	~ "Soft coral" + "Hard coral control"	"Hard coral control"	Pd vs Control Pe vs Control Pf vs Control Pd vs Pe Pd vs Pf Pe vs Pf
Model 2	~ "Soft coral" + "Pd Other"	"Pd Other"	Pd vs Other

3.2.7 Co-expression network analysis

To investigate the relationship between the DEG in the soft coral fragments interacting with *Porites* colony Pd and the rest of the transcriptome, a network of co-expression analysis was performed with the R package petal (Petereit et al., 2016). To ensure that the neighbours of the DEG were relevant for the experiment, a network was built with a subset of the soft coral transcriptome that was affected by the interaction with colony Pd. This subset corresponded to the genes with a padj<0.5, found in the contrast analysis between Lobophytum- Pd and Lobophytum-control (Table 3.2).

The package petal optimized a threshold to build a scale-free and small world co-expression network with the data provided. The network analysis was run with the expression data for all 20 samples of *Lobophytum* after a variance stabilizing transformation was done with DESeq2 (Love et al., 2014).

Once the network was obtained, the DEG common to both models (Model 1 and 2; Table 3.2) were analysed running a vicinity network (VN) analysis with the petal package (Petereit et al., 2016). This analysis aims to find small groups of genes that are highly correlated by their gene expression patterns amongst samples (Petereit et al., 2016).

A sub-network of the DEG found in both models and their direct neighbours detected by the VN analysis was created with Cytospace V3.6.1 (Shannon et al., 2003). The component (fully connected group of genes/nodes) with the vast majority of genes/nodes from this sub-network was used for downstream gene function analysis.

3.2.8 Analysis to infer gene function

The genes obtained from the co-expression network analysis were analysed and classified with consideration to the hypothesis of the soft coral response to a non-contact competition explained previously (Figure 3.2). *Lobophytum* samples under competition were expected to be regulating genes involved with the perception and response to threat, as an innate immune response. Genes involved in the regulation of growth and behaviour, as well as genes related to secondary metabolite production and secretion were also expected to be affected after 30 days of interaction. The potential functions of the DEG were classified according to: 1. information from UniProt ID annotation (functions, ontology and orthology reference), 2. function of protein domains found with NCBI conserved domain finder (*e*-value<1E-3) and 3. literature relevant to the gene/protein function in Cnidaria or other metazoans (appendix B). Transcripts without annotation from the DEG list were manually searched using UniProt BLAST tool (*e*-value<1E-5).

Finally, Gene ontology terms (GO-terms) enrichment analysis was executed with the R package GOSeq to analyse if any functionality was over-represented within the set of genes differentially expressed between *Lobophytum*-Pd and *Lobophytum*-control (Young et al., 2010).

3.3 Results

After three weeks of acclimatization, all 60 *Lobophytum* lobes showed new tissue growth over the wounded area and some had attached to the tiles. Similarly, the *Porites* nubbins also had new tissue growing on the tiles. These observations indicated that the corals had recovered from the collection and fragmentations stress.

3.3.1 *Behavioural observations of Porites*

During the 60 days of interaction, competitive behaviours towards *Lobophytum* were recorded in six of the 54 *Porites* nubbins (Table 4.4, Chapter 4). More information about the aggressive behaviour (Figure 4.3) and detailed results of the *Porites* polyp activity are provided in Chapter 4. Briefly, there were statistically significant differences in the activity of *Porites* polyps, with controls control nubbins showing

greater polyp activity than those exposed to *Lobophytum*. Additionally, it was observed that when exposed to *Lobophytum*, nubbins from colony Pd were consistently less active than both the controls and the nubbins from other *Porites* colonies (Figure 4.5, Chapter 4).

3.3.2 *Genes differentially expressed in Lobophytum under competition*

Initial comparisons between interacting soft corals and control, failed to identify any *Lobophytum* genes differentially expressed between controls and those exposed to *Porites* nubbins (padj< 0.1). Similarly, contrast analysis found no significant differences in gene expression profiles between competing lobes *Lobophytum*-Pe or *Lobophytum*-Pf and *Lobophytum*-control lobes. Conversely, 265 genes were differentially expressed (padj<0.1) between samples of *Lobophytum*-Pd and *Lobophytum*-controls.

Subsequent gene expression contrast analyses of the soft coral in interactions with the three colonies of *Porites* showed: 1149 genes DE between *Lobophytum*-Pd compared to *Lobophytum*-Pe; 364 genes DE between *Lobophytum*-Pd compared with *Lobophytum*-Pf and; 2 genes DE between *Lobophytum*-Pe and *Lobophytum*-Pf (Figure 3.4). Interestingly, there were 131 genes consistently differentially expressed in *Lobophytum*-Pd when compared to *Lobophytum*-Pe, Pf or control (Figure 3.4).

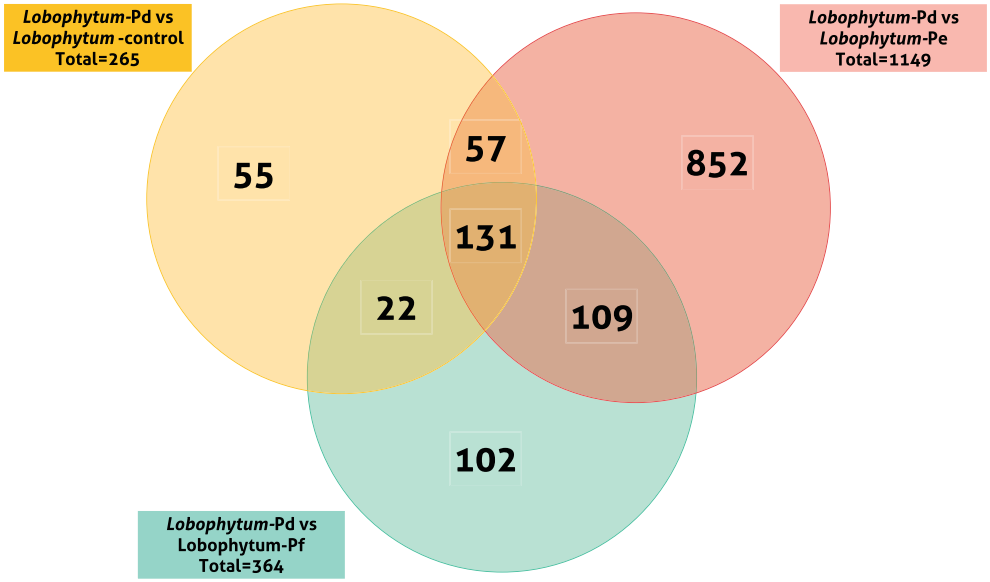

Figure 3.4: Common DEG in *Lobophytum* samples interacting with colony Pd contrasted with *Lobophytum* samples interacting with other colonies or in control.

During the PCA analysis, samples were grouped by soft coral colony rather than by treatment. Colonies Lb and Lc had similar gene expression profiles, as did colonies Ld and Le (Figure 3.5). However, colony La appeared to have a different gene expression profile from the other four colonies and is thus distinct on the PCA plot (Figure 3.5). The PCA analysis showed that 66% of the variance in gene expression is driven by the differences between soft coral colonies. In the case of *Lobophytum* samples interacting with *Porites* colonies Pe and Pf, gene expression was unaffected by the presence of the hard coral. However, samples of *Lobophytum*-Pd consistently differed from the other four soft coral samples, with the exception of colony Lb (Figure 3.5, triangles). Additionally, 509 genes were found to be differentially expressed (padj<0.1) when comparing *Lobophytum*-Pd to all the other soft coral samples. Importantly, of the 131 genes consistently DE in *Lobophytum*-Pd (Model 1, Table 3.2), 130 were also included in the 509 DE genes from the second model. This highlights the role of these 130 genes on the soft coral reaction when in presence with colony Pd.

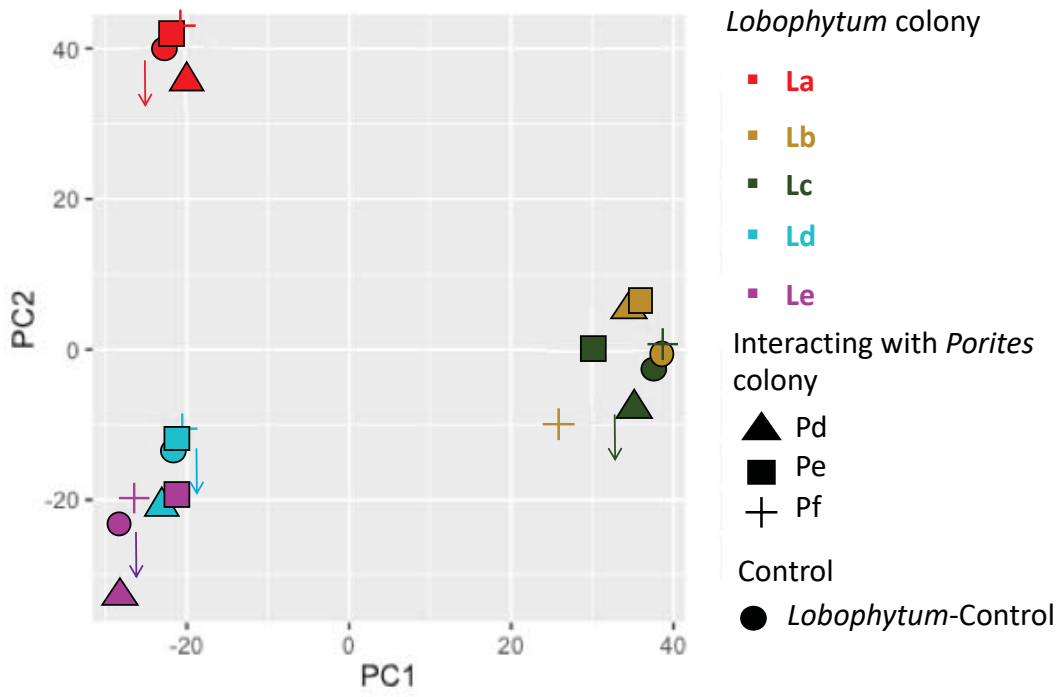

Figure 3.5: PCA analysis showing the distribution of soft coral colonies based on their gene expression profiles. The arrows indicate the predominant direction of change between *Lobophytum* controls and those exposed to nubbins of *Porites* colony Pd.

Co-expression network analysis results

Contrast analysis identified 1180 DEG between *Lobophytum*-Pd and *Lobophytum*-controls (padj< 0.5). Co-expression analysis (see Methods section 3.2.7) of this subset of genes identified a network of 1013 genes connected with a threshold of 0.803 (Appendix Figure B. 1). In this network, there were 122 DEG in common between Model 1 (*Lobophytum*-Pd compared to *Lobophytum*-control) and Model 2 (*Lobophytum*-Pd compared to *Lobophytum*-Other).

Vicinity network analysis showed that these 122 DEG had 239 direct neighbours, resulting in a total of 361 nodes (genes). The component with the vast majority of the genes had 339 nodes, of which 305 were up-regulated and 34 down-regulated in the *Lobophytum*-Pd interaction. Moreover, down and up regulated genes were respectively grouped together in the network despite the fact that the direction of expression of the genes used was not the same in all samples (Figure 3.6). The 339 genes comprising this network were subjected to further analysis as described below.

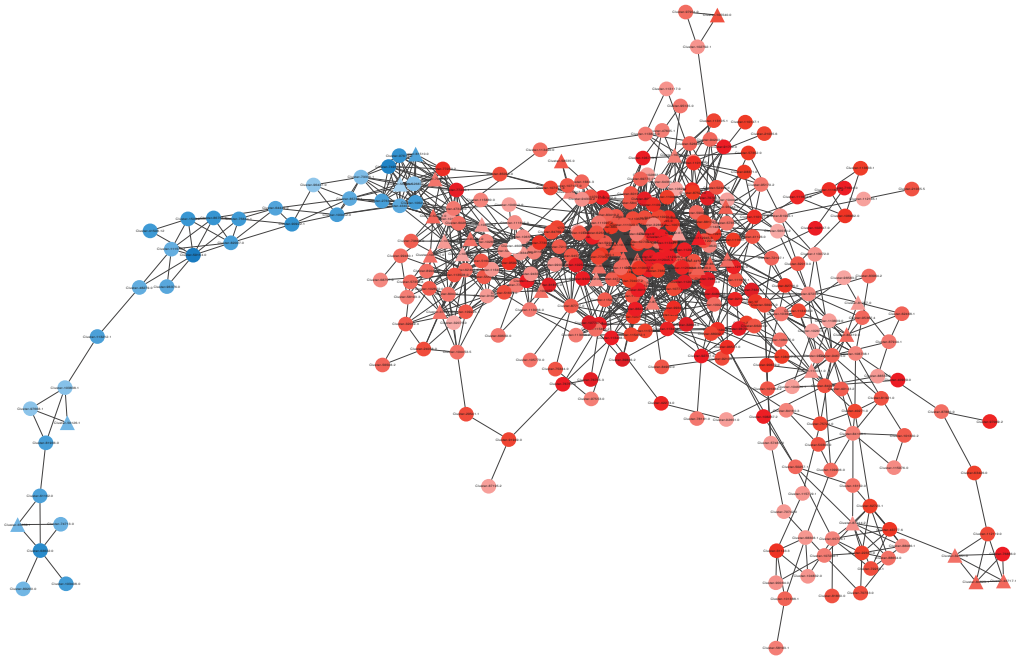

Figure 3.6: Co-expression network of 339 differentially expressed genes (triangle =padj<0.1; circles =0.1 <padj<0.5) in *Lobophytum*-Pd compared *Lobophytum*-control. Genes up-and down-regulated in *Lobophytum*-Pd samples are shown in red and blue respectively.

3.3.3 Analyses to infer gene function (Lobophytum-Pd)

3.3.3.1 Gene ontology enrichment analysis

Enrichment analysis of the gene ontology (GO) annotations was used to look for over-representation amongst the 339 genes comprising the network above (Figure 3.6). However, using the thresholds recommended by the software GOseq (Young et al., 2010), no significant GO term over-representation was detected in biological process (BP), cellular component (CC) and molecular function (MF) GO terms. Therefore, manual research based on UniProt information of the best BLAST hit, protein domain analyses and database/literature searches focused particularly (but not exclusively) on cnidarians was undertaken, in an attempt to better understand the effect of non-contact competition in the soft coral.

3.3.3.2 Gene classification analysis

The analysis of the 339 genes was undertaken based on three classes of response that are corollaries of the non-contact competition hypothesis previously presented (Figure 3.2). These are: (1) threat perception and generalised response to stress, (2) tissue remodelling - genes with implied functions in growth and tissue movement, and (3) secondary metabolite production and secretion. Of the 237 annotated genes in the network, a total of 207 could be classified into one of these functional groups. The 30 remaining annotated genes either had general functions, such as transport, transcription and translation, or had annotations which were insufficiently informative for functions to be assigned.

3.3.3.2.1 Threat response related genes

Of the 207 genes considered here, a total of 53 could be classified under the threat response category (Table 3.3), and the overall composition of this group suggests that the response of *Lobophytum* to the presence of *Porites* colony Pd is a rather generalised reaction to threat or stress. Several proteins involved in innate immune recognition were up-regulated (10 out of 11 genes), including pattern recognition receptors (PRR) that sense pathogens and molecules associated with cell damage in corals and other organisms (Hamada et al., 2013). In contrast to the other 10 receptors, NLRC5 (a NOD-like receptor) was down-regulated. Several NLRs were up-regulated in disease-resistant staghorn corals (Libro and Vollmer, 2016), and NLRC5 was up-regulated in *Acropora aculeus* after 12 hr under heat (32 ˚C) stress (Zhou et al., 2017). NLRC5 may therefore have various functions in different anthozoans.

In contrast to NLRC5, ten other putative innate immune genes were up-regulated (Table 3.3). This group included three clusters matching the *Acropora millepora* mannose receptor 1 (MMR) homolog (Kvennefors et al., 2008). Proteins of this type have been implicated in innate immune recognition in a wide range of invertebrates. In *A. millepora*, mannose-binding properties have been demonstrated and is probably essential for the innate immune response of coral to pathogens (Kvennefors et al., 2008). A homolog of the *Lobophytum* pentraxin receptor (Cluster-106713.0) has been found to be up-regulated in *Pseudodiplora strigosa* following an immune challenge (Ocampo et al., 2015) and in *Acropora cervicornis* found to be resistant to white band

disease (Libro and Vollmer, 2016). Cluster-16207.1 is predicted to encode an acetyl group-binding receptor with a fibrinogen domain, the latter of which has been considered a hallmark of recognition molecules (Doolittle et al., 2012; Hayes et al., 2010).

Three other receptors likely to be involved in stress response perception and transmission were up-regulated (Table 3.3). Two of these contained the 7tmB3 Methuselah-like domain, a component of GPCRs that play essential roles in stress signalling in *Drosophila* (Lin et al., 1998), and have been proposed to have a role in immune responses in the hard coral *Stylophora pistillata* (Voolstra et al., 2017). Additionally, Cluster-75720.0 was classified as a tyrosine-protein kinase (TPK) receptor, homologs of which were up-regulated in *A. millepora* preconditioned to moderate thermal stress (Bellantuono et al., 2012) (Table 3.4).

Lobophytum individuals under competition were up-regulating activators, factors and intermediate proteins related to pathogen defence and cellular stress (Table 3.3). It has been shown that corals increase melanin production to protect themselves against microorganisms during infection or during wound healing (Mydlarz et al., 2016; Weiss et al., 2013). Factors related to melanin biosynthesis were found amongst the DEG in the present experiment. Three clusters were annotated against the same genome predicted transcript (Cluster-78941.0, Cluster-112028.5, Cluster-112028.2) manually annotated as tyrosinase (e-value: 7E-72) and up-regulated in competing corals. This enzyme is essential for melanogenesis and it has been identified in several cnidarians (Dunlap et al., 2013; Esposito et al., 2012; Voolstra et al., 2017). However, to the best of my knowledge an up-regulation of tyrosinase in corals under cellular stress has not been reported previously.

In competing corals, ten transcriptome clusters encoding seven genes involved in secretion or intermediate production of defence molecules were up-regulated (Table 3.3). This category of genes included at least two homologs of known antimicrobial peptides. Von Willebrand factor (vWF) has previously been found to be up-regulated in *Stylophora pistillata* reacting to allogeneic challenge (Oren et al., 2010) and is thought to be involved in secretory processes. Lactoperoxidase (LPO), also up-regulated here, has the potential to generate the antimicrobial agent hypothiocyanous acid.

Microbial infection and environmental stressors can cause major disruptions of normal cellular functions – for example, misfolding of proteins, high levels of ROS, DNA damage etc. - that may result in cell death. Several genes with probable functions in controlling these imbalances were up-regulated in *Lobophytum* under competition. In fact, genes related to detoxification (Cluster-105770.0), ubiquitination (Cluster-106624.0) and antioxidants (Cluster-112610.0) were up-regulated. For example, a homolog of the dual oxidase maturation factor 1 (a membrane trafficking molecule involved in the inflammatory response) was up-regulated in competing corals, as was a homolog of dual oxidase 2 (DUOX2), which is involved in converting reactive oxygen species (ROS) molecules to hydrogen peroxide (H_2O_2) and water (Mone et al., 2014) (Table 3.3).

The group of genes considered to be effectors of a generalised threat response included antimicrobial peptides, radicals produced due to the cellular stress response (CSR), antioxidant molecules involved in limiting ROS damage and proteins involved in the production of secondary metabolites used for defence. Given the central importance of SMs in the stress response and competition in soft corals (Chadwick and Morrow, 2011; Coll, 1992; Coll et al., 1985; Coll and Sammarco, 1983), this topic is discussed at length below (section 3.3.3.3).

Soft coral lobes exposed to nubbins of *Porites* colony Pd up-regulated genes encoding at least two potential antimicrobial peptides (AMP). Not only are AMPs essential in the defence of corals against pathogens (Mydlarz et al., 2016; Wenger et al., 2014), but are also likely to modulate the associated microbial community to the benefit of the host species (Bosch, 2013). One of the up-regulated *Lobophytum* genes encodes a clear homolog of hydramacin (Cluster-82736.0), an antimicrobial peptide from the hydrozoan cnidarian Hydra (Jung et al., 2009). Hydramacin is the founding member of the macin family of AMPs, which is represented in molluscs and Folsomia (Arthropod) as well as in several other cnidarians. As with other members of this AMP family, the *Lobophytum* hydramacin has a leader sequence and contains ten cysteine residues whose spacing is conserved across Anthozoa. Hydramacins are highly active against both gram-positive and gram-negative bacteria (Bosch et al., 2009).

A homolog of the polychaete AMP arenicin-2 (Cluster-53819.0) was also identified in competing soft corals. The polychaete arenicin-2 has antimicrobial activity against fungi and bacteria (gram positive/negative). The *Lobophytum* arenicin-2 protein

contains a BRICHOS domain proteins, a type of domain generally associated with membrane-anchored proteins and thought to function in secretory pathways (Johansson et al., 2006; Sánchez-Pulido et al., 2002). A gene encoding Arenicin-2 homolog was also up-regulated in the sea fan *Gorgonia ventalina* in response to infection with the parasite *Aplanochytrium* (Burge et al., 2013).

Other up-regulated genes considered to be effectors of a generalised threat response in *Lobophytum* included two hydrolases acting on peptide bond and related with pro-apoptotic regulation. Fist, stromelysins that can degrade extracellular matrix proteins and are involved in strong inflammation reactions (Gentile and Liuzzi, 2017), and second, cysteine proteinase 3 that has a lysosomal function, but also has essential roles in apoptosis. A homolog of this enzyme was up-regulated in *A. pallida* under heat stress, and has been identified in the immune-transcriptome of *Pseudodiploria strigose* (Jouiaei et al., 2015b; Kitchen and Weis, 2017; Ocampo et al., 2015). These published data support the hypothesis that stromelysin and cysteine proteinase 3 might be produced as a consequence of immune activation in *Lobophytum* under competition.

Two other hydrolases were up-regulated in competing corals, but these enzymes act on ester bonds rather than peptide bonds. PP2C-like domain-containing proteins have been found to be regulated in *Hydra* as part of an injury-induced immune response (Wenger et al., 2014). PPC2 is a Mg^{2+}/Mn^{2+}-dependent serine/threonine phosphatase, which are class of proteins considered to have essential roles in stress response pathways and in regulation of the cell cycle (Stern et al., 2007).

Amongst the cellular stress response genes up-regulated in competing *Lobophytum*, two oxidoreductases which act on a peroxide acceptor were up-regulated: a peroxidasin proposed to be a heat stress biomarker (Louis et al., 2017) and a homolog of myeloperoxidase (MPO), an antioxidant with an important role in controlling free radicals (Mydlarz et al., 2016); Table 3.3).

Table 3.3: Genes encoding receptors potentially involved in recognition of a general threat response. Blue and red are used to indicate genes down and up-regulated respectively. "Biological characteristic" was assigned considering best BLAST hit annotation and the NCBI domain functions.

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	Best Blast e-value	Log2 Fold Change	padj
Receptor / Stress / GPCR	Cluster-111919.1	AGRG6_DANRE	Adhesion G-protein coupled receptor G6	7.00E-19	1.002	9.49E-02
Receptor / Innate / GPCR	Cluster-90084.0	CASR_RAT	Extracellular calcium-sensing receptor	2.00E-111	0.456	7.81E-03
Receptor / Innate	Cluster-86901.3	MRC1_MOUSE	Macrophage mannose receptor 1	1.00E-03	1.462	2.19E-04
Receptor / Innate	Cluster-86901.1	MRC1_MOUSE	Macrophage mannose receptor 1	1.00E-03	1.403	1.39E-03
Receptor / Innate / Secretion	Cluster-72255.1	MRC1_MOUSE	Macrophage mannose receptor 1	0.00E+00	1.035	3.34E-03
Receptor / Innate / Nervous system / Vesicles / Glutamate related	Cluster-106713.0	NPTXR_RAT	Neuronal pentraxin receptor	5.30E-06	1.652	3.12E-05
Receptor / Innate / Nervous system / Glutamate related	Cluster-69770.0	P2RX7_HUMAN	P2X purinoceptor 7	1.00E-09	0.843	1.29E-02
Receptor / Stress / GPCR	Cluster-92381.0	A0A2B4R645_STYPI	Putative G-protein coupled receptor Mth-like 3	1.60E-59	0.735	6.89E-01
Receptor / Innate	Cluster-85743.1	NLRC5_ICTPU	Protein NLRC5	6.00E-20	-0.879	5.52E-02
Signalling component / Ligand / Stress / Channel	Cluster-33940.2	ANO4_BOVIN	Anoctamin-4	6.00E-161	0.753	3.33E-01

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	Best Blast e-value	Log2 Fold Change	padj
Signalling component / Factor / Stress / Protein modification	Cluster-105770.0	CRERF_HUMAN	CREB3 regulatory factor	2.00E-33	0.702	2.35E-02
Signalling component / Innate / Secretion / Symbiosis	Cluster-104453.5	DMBT1_MOUSE	Deleted in malignant brain tumors 1 protein	2.00E-13	0.726	8.44E-02
Signalling component / Innate / Secretion / Symbiosis	Cluster-104453.3	DMBT1_MOUSE	Deleted in malignant brain tumors 1 protein	2.00E-13	0.741	1.23E-01
Signalling component / Ligand / Stress / Behaviour / Proline	Cluster-113809.0	DPP4_FELCA	Dipeptidyl peptidase 4 (EC 3.4.14.5)	7.00E-136	0.321	6.79E-03
Signalling component / Factor / Stress / Transport	Cluster-112610.0	DOXA1_HUMAN	Dual oxidase maturation factor 1	2.00E-47	1.376	1.91E-03
Signalling component / Factor / Stress / Protein modification	Cluster-106624.0	CBLBB_XENLA	E3 ubiquitin-protein ligase CBL-B-B (EC 2.3.2.27)	0	0.601	1.44E-01
Signalling component / Recognition / Innate / Protein modification	Cluster-16207.1	FBCD1_MACFA	Fibrinogen C domain-containing protein 1	7.00E-32	0.773	1.16E-01
Signalling component / Innate / AMP-agent	Cluster-84004.0	PERL_MESAU	Lactoperoxidase (EC 1.11.1.7)	3.00E-115	1.022	1.62E-04
Signalling component / Factor / Stress / Transcription co-regulators	Cluster-84900.0	LITAF_DANRE	Lipopolysaccharide-induced tumor necrosis factor-alpha factor homolog	7.00E-28	0.960	2.74E-03
Signalling component / Ligand / Innate	Cluster-96644.0	NAL12_HUMAN	NACHT, LRR and PYD domains-containing protein 12	2.00E-40	0.894	NA

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	Best Blast e-value	Log2 Fold Change	padj
Signalling component / Factor / Stress / Transport	Cluster-114298.2	SOS2_HUMAN	Son of sevenless homolog 2 (SOS-2)	0	0.552	1.61E-02
Signalling component / Factor / Stress / Melanin	Cluster-78941.0	A0A2B4RPF7_STYPI	Tyrosinase	7.00E-72	1.284	1.53E-03
Signalling component / Factor / Stress / Melanin	Cluster-112028.5	A0A2B4RPF7_STYPI	Tyrosinase	7.00E-72	1.565	5.91E-05
Signalling component / Factor / Stress / Melanin	Cluster-112028.2	A0A2B4RPF7_STYPI	Tyrosinase	7.00E-72	1.541	9.44E-05
Signalling component / Recognition / Stress / Secretion	Cluster-75720.0	TIE1_HUMAN	Tyrosine-protein kinase receptor Tie-1 (EC 2.7.10.1)	1.00E-50	1.094	2.33E-03
Signalling component / Innate	Cluster-76765.4	VWF_MOUSE	von Willebrand factor	2.00E-57	1.557	4.96E-05
Signalling component / Innate	Cluster-76765.3	VWF_MOUSE	von Willebrand factor	2.00E-57	1.959	1.42E-07
Signalling component / Innate	Cluster-76765.0	VWF_MOUSE	von Willebrand factor	2.00E-57	1.937	2.18E-07
Effector / Stress / Secretion	Cluster-63426.0	.	.	.	1.148	2.01E-03
Effector / Innate / AMP	Cluster-53819.0	ANN2_AREMA	Arenicin-2	1.00E-05	0.819	1.30E-01
Effector / Stress / Apoptosis / Secretion	Cluster-40143.2	CYSP3_SOLLC	Cysteine proteinase 3 (EC 3.4.22.-)	4.00E-40	0.908	2.29E-02
Effector / Stress / Sphingolipids	Cluster-103842.1	PA24A_RABIT	Cytosolic phospholipase A2 (cPLA2) (EC 3.1.1.5)	4.00E-33	0.688	1.83E-02

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	Best Blast e-value	Log2 Fold Change	padj
Effector / Stress / Protein modification	Cluster-107427.1	DNJB1_MOUSE	DnaJ homolog subfamily B member 1 (HSP40)	1.00E-65	0.641	3.64E-01
Effector / Stress / Protein modification	Cluster-105325.1	DJC25_XENLA	DnaJ homolog subfamily C member 25	2.00E-101	0.363	2.01E-01
Effector / Innate / AMP / mucus	Cluster-70407.2	DUOX2_PIG	Dual oxidase 2 (EC 1.11.1.-) (EC 1.6.3.1)	0	1.194	1.18E-02
Effector / Innate / AMP / mucus	Cluster-97212.0	DUOX2_PIG	Dual oxidase 2 (EC 1.11.1.-) (EC 1.6.3.1)	6.00E-34	1.467	1.40E-04
Effector / Stress	Cluster-95995.1	RN213_HUMAN	E3 ubiquitin-protein ligase RNF213 (EC 2.3.2.27)	4.00E-118	1.286	1.55E-03
Effector / Stress / Secretion	Cluster-77230.1	XYNA_STRLI	Endo-1,4-beta-xylanase A (EC 3.2.1.8)	4.00E-04	1.788	2.53E-06
Effector / Stress / Secretion	Cluster-77230.2	XYNA_STRLI	Endo-1,4-beta-xylanase A (EC 3.2.1.8)	4.00E-04	1.302	1.46E-03
Effector / Stress / Protein modification	Cluster-65736.1	EDEM1_MOUSE	ER degradation-enhancing alpha-mannosidase-like protein 1	0	0.557	2.10E-03
Effector / Stress / Secretion	Cluster-80426.1	GSXL1_ARATH	Flavin-containing monooxygenase FMO GS-OX-like 1 (EC 1.8.-.-)	7.00E-64	0.701	2.52E-03
Effector / Innate / AMP / Secretion	Cluster-82736.0	HYDMA_HYDVU	Hydramacin-1	1.00E-06	1.268	8.29E-03
Effector / Stress	Cluster-84769.0	WNK1_MOUSE	Isoform 4 of Serine/threonine-protein kinase WNK1	7.5E-161	1.069	6.93E-02

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	Best Blast e-value	Log2 Fold Change	padj
Effector / Stress	Cluster-87195.2	KLHDB_ANOGA	Kelch-like protein diablo	4.00E-77	0.319	1.78E-02
Effector / Stress / Secretion	Cluster-96501.0	PERM_HUMAN	Myeloperoxidase (EC 1.11.2.2)	1.00E-38	1.306	3.32E-04
Effector / Stress	Cluster-105783.0	PXDN_XENTR	Peroxidasin (EC 1.11.1.7)	3.00E-133	1.229	2.88E-03
Effector / Stress	Cluster-47728.0	Y9801_DROME	PP2C-like domain-containing protein CG9801	0.003	1.117	2.01E-03
Effector / Stress	Cluster-100338.3	Y9801_DROME	PP2C-like domain-containing protein CG9801	0.003	1.253	1.38E-05
Effector / Stress / Apoptosis	Cluster-89200.0	R13L2_ARATH	Putative disease resistance RPP13-like protein 2	0.004	-0.733	6.48E-02
Effector / Stress / Apoptosis / Secretion	Cluster-32013.0	MMP3_HUMAN	Stromelysin-1 (EC 3.4.24.17)	2.00E-50	0.958	3.48E-02
Effector / Protein modification / Secretion	Cluster-99075.0	QSOX1_MOUSE	Sulfhydryl oxidase 1 (EC 1.8.3.2)	2.00E-104	0.445	7.32E-02
Effector / Stress / Protein modification	Cluster-110992.2	A0A015KNY6_9GLOM	Ubiquitin-ribosomal 60S subunit protein L40B fusion protein	4.70E-48	1.307	1.91E-03
Effector / Stress / Protein modification	Cluster-110992.0	A0A015KNY6_9GLOM	Ubiquitin-ribosomal 60S subunit protein L40B fusion protein	4.70E-48	1.337	1.94E-03
Effector / Stress	Cluster-75641.1	WRN_HUMAN	Werner syndrome ATP-dependent helicase (EC 3.6.4.12)	1.00E-15	-1.107	4.06E-02

3.3.3.2.2 Genes related to tissue remodelling and growth

Of the 207 *Lobophytum* genes classified into the three functional groups considered here, 98 could be related to tissue remodelling and growth (Table 3.4). Two major groups were considered in this category; first, genes with functions in the nervous system and second, genes involved in wound healing and growth.

Genes involved in nervous system function

Amongst the genes with possible nervous system-related functions were homologs of receptors which in bilaterians are capable of activating a signalling cascade related to muscle movement or vasocontraction/vasodilatation. These included four clusters encoding rhodopsin-like GPCRs. For both Cluster-111570.1 and Cluster-110221.0, the best BLAST hit was a beta-2 adrenergic receptor (e-value: 2.00E-21 and 2.00E-27, respectively), however, Cluster-111570.1 was down-regulated whilst 110221.0 was up-regulated (Table 3.4). The beta-2 adrenergic receptor plays a role in calcium signalling pathways that are involved in a cascade of reactions leading to tissue remodelling.

Another rhodopsin-like receptor found up-regulated in competition was alpha-1B adrenergic receptor (Cluster-52996.0; e-value: 4.00E-17). This is a receptor from the Ca2+ sensing pathway, and is involved in signalling muscle contraction and vasoconstriction (The UniProt Consortium, 2017). Adrenergic receptors have been found to be regulated on the sea anemone *Calliactis polyps* after an injury induced immune response (Stewart et al., 2017).

Two members of the neuroactive ligand-receptor interaction pathway were also differentially expressed, a homolog to melanocortin receptor 5 and a homolog to nicotinic acetylcholine receptor subunit alpha-9 (NACHR). Melanocortin receptor 5 homolog (MC5-R; e-value: 6.00E-07) was up-regulated in competing corals. Specific binding sites have been found for melanocortin GPCRs in *Acropora millepora*, suggesting that the functionality described in other organisms could be the same in corals (Anctil et al., 2007). In mice and humans, this type of receptor is related to pheromone signalling (Morgan and Cone, 2006). MC5-R is present in human peripheral tissues and is mainly involved in exocrine function, related to sebaceous gland secretion (Yang, 2011). Although cnidarians do not have an endocrine system, it is possible that

the melanocortin receptor-like gene might have a role in sensing specific cues related to coral behaviour (Tarrant, 2005).

The homolog of NACHR was also up-regulated. In vertebrates this neuroactive receptor is related to muscle contraction. A NACHR homolog has been found to be expressed in the apical organ of *Nematostella* (Sinigaglia et al., 2015). There are also studies showing that acetylcholine (Ach) affects muscle-epithelial contraction in cnidarians (Lentz and Barrnett, 1961; Scappaticci and Kass-Simon, 2008; Watanabe, 2017).

A homolog of the ephrin-A receptor 2, a gene involved in axon guidance as well as inflammation signalling, was down-regulated in corals under competition. Activation of ephrin ligand and ephrin receptor (bidirectional signalling) enhanced neuron differentiation and possibly also cell adhesion in higher animals (Kullander and Klein, 2002; Ryan et al., 2013). A homolog of the ephrin receptor is present in *Nematostella* (Ryan et al., 2013), but the function of these genes in cnidarians is not known. In higher animals, all of the receptors mentioned above have been related to muscle control or behavioural changes, which would have required tissue modification to happen.

Under competition, *Lobophytum*-Pd also differentially expressed genes which encode proteins involved in nervous system responses as well as possible effectors. These included several intermediates of nervous signalling pathways such as synaptic vesicle components or activators of neuron differentiation (Table 3.4).

Some Sox genes were up-regulated in soft corals under competition. Sox genes are present in cnidarian genomes, but their functions are not well understood. Cluster-113421.0 was annotated as Sox9, but homologs of this have been identified in other cnidarians, including *Acropora millepora* (AmSoxE1), where the expression pattern suggests a role in the development of the nervous system (Shinzato et al., 2008).

Another group of genes involved in pathways related to movement, behaviour and general muscle control were also differentially expressed in corals under competition (Table 3.4). Fukutin, for example, is a putative transmembrane protein that is ubiquitously expressed, although at higher levels in skeletal muscle, heart and brain. It is localised to the cis-Golgi compartment where it is involved in the biosynthesis of phosphorylated O-mannosyl trisaccharides, a structure present in alpha-dystroglycan (DAG1) which is required for binding laminin G-like domain-containing extracellular proteins. The dystroglycan complex is essential for anchoring muscle fibres to the

extracellular matrix in bilaterians. This protein has been found in cnidarian genomes, but its function is unknown (Leclère and Röttinger, 2017).

While some of the genes listed above might have functions in innervating cnidarian "muscles", other differentially expressed genes may have roles in muscle specification or function. Cluster-97901.0 encoded a protein containing a LIM domain-binding (e-value:1.86E-23) and was up-regulated in soft corals under competition. This protein is essential for muscle functionality in higher animals (Leclère and Röttinger, 2017; Martindale et al., 2004), and Nv-muscle-LIM domain genes are expressed in the endodermal lining of the developing tentacles in *Nematostella*. Conversely, myosin-2 light chain, another gene related to muscle contraction was down-regulated in soft coral under competition (Crowder et al., 2017); Cluster-97668.1). A homolog of myosin-2 light chain has also been found to be down-regulated in *Acropora palmata* under heat stress (DeSalvo et al., 2010; Louis et al., 2017).

Finally, in the tissue remodelling group related to nerve net development or behavioural changes, four clusters were classified as effectors. It is difficult to distinguish these genes from modulators of the nervous system due to the interconnectivity found in this system. Nevertheless, the up-regulation of cholinesterase and cyclin-dependent kinase 17 in competing corals might be activated by the genes previously mentioned to be involved in the nervous system. Cholinesterase is involved in neurotransmitter recycling and will inhibit signalling via acetylcholine. Even if there is significant evidence of the participation of acetylcholine related proteins in chemical transmission in cnidarians, acetylcholine itself has never been isolated from corals (Kass-Simon and Pierobon, 2007a; Oren et al., 2014; Watanabe, 2017). Cholinesterase has other roles, including regulation of apoptosis, cell adhesion and cell migration (Falugi et al., 2012).

Genes involved in growth and cell fate

Soft corals under non-contact competition regulated the expression of several genes whose bilaterian homologs function in growth and cell fate. However, these homologues were not necessarily related to the nervous system (Table 3.4). These genes include receptors of the mitogen-activated protein kinase (MAPK), Ras (GTPases) and the transforming growth factor-beta (TGF-ß) signalling pathways, all of which were up-regulated.

Cluster-32075.0 encodes a TGF-ß receptor. The TGF-ß signalling pathway has a variety of roles in proliferation, apoptosis, differentiation and migration in insects, worms and mammals, and also has a role in immunity in vertebrates (Detournay et al., 2012; Technau et al., 2005). TGF-ß signalling also appears to have a diverse range of functions in cnidarians (Technau et al., 2005). Also relevant to TGF-ß signalling, a homolog of the Ski oncogene, known to facilitate SMAD binding (Petersen et al., 2015), was up-regulated in competing *Lobophytum*. SMADs are transcription factors that act downstream of TGF-ß signalling, and play roles in development and symbiont tolerance and maintenance in cnidarians (Detournay et al., 2012; Samuel et al., 2001).

At least ten clusters had annotations related to the MAKP and Ras signalling pathways. Five clusters up-regulated in *Lobophytum* under competition were annotated as fibroblast growth factor receptor 1 (FGFR-1) and one as fibroblast growth factor receptor 3 (FGFR3). FGFR activation might lead to signalling via the MAKP and Ras pathways. These two pathways (MAKP and Ras) play many roles in cell differentiation and migration in cnidarians. FGFRs are expressed during gastrulation and in the development of the apical tuft (a chemosensory structure present in planula stages) in *N. vectensis*, suggesting roles in neural induction (Matus et al., 2007). In addition to the FGFRs, there were three clusters up-regulated and annotated as RalA-binding protein 1 (RalBP1). RalBP1 is activated by the Ras pathway and enhances metastasis in mammals (Wu et al., 2010), but its cnidarian homologs have not been characterized.

In addition to the signalling pathway components discussed above, a Wnt ligand (Wnt-4, Cluster-115880.0) was up-regulated in competing corals (Table 3.4). Wnts are known to be involved in developmental regulation and tentacle formation, and may also participate in skeleton formation in corals (Hemond et al., 2014).

A homolog of cytosolic 10-formyltetrahydrofolate dehydrogenase (FDH, Cluster-111305.0) was up-regulated in soft corals under competition. This oxidoreductase functions in one-carbon metabolism and is important for purine and thymidine synthesis, and for the conversion of homocysteine to methionine (Fox and Stover, 2008; Lewin et al., 2017). FDH was up-regulated during regeneration following injury in *Montastrea cavernosa* (Horricks, 2017), and may have similar functions (wound repair and cell fate determination) in *Lobophytum.*

The only growth effector-related gene down-regulated in competing soft corals was a homolog of p52tIPK (Cluster-27684.2), a repressor of cell growth in mammals. This protein has been shown to be responsible for the inhibition of PKR (interferon-inducible, double-stranded RNA-dependent protein kinase) which can lead to protein translation shutdown in mammalian cells (Peel, 2004). PKR is an early cellular responder to viral infection and responsible for a strong immune response which can induce cell death (Peel, 2004). Assuming that the cnidarian protein has a similar function, the down-regulation of a growth repressor may probably help to maintain or enhance growth in *Lobophytum* under competition.

Table 3.4: Genes with potential functions in tissue remodelling. Blue and red indicate genes down and up-regulated respectively. "Biological characteristic" was assigned considering best BLAST hit annotation and the NCBI domain functions.

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Receptor / Other / GPCR / Tissue	Cluster-29541.1	AGRD1_BOVIN	Adhesion G-protein coupled receptor D1	4.00E-28	1.059	6.62E-02
Receptor / Other / GPCR / Tissue	Cluster-65069.0	AGRD1_BOVIN	Adhesion G-protein coupled receptor D1	1.00E-73	0.869	1.30E-01
Receptor / Other / GPCR / Tissue	Cluster-111496.0	AGRG4_HUMAN	Adhesion G-protein coupled receptor G4	6.00E-64	1.170	4.29E-03
Receptor / Nervous system / Vasoconstriction / GPCR	Cluster-52996.0	ADA1B_HUMAN	Alpha-1B adrenergic receptor	4.00E-17	0.994	3.60E-02
Receptor / GPCR / Vasocontraction	Cluster-110221.0	ADRB2_BOVIN	Beta-2 adrenergic receptor	2.00E-27	1.103	1.12E-02
Receptor / GPCR / Vasocontraction	Cluster-111570.1	ADRB2_MACMU	Beta-2 adrenergic receptor	2.00E-21	-0.827	8.04E-02
Receptor / Nervous system / RTK	Cluster-61510.0	EPHA2_MOUSE	Ephrin type-A receptor 2 (EC 2.7.10.1)	1.00E-116	-0.983	1.37E-01
Receptor / Cell fate / Growth / RAS / Secretion	Cluster-50735.0	FGFR1_CHICK	Fibroblast growth factor receptor 1 (EC 2.7.10.1)	2.00E-58	1.007	2.09E-02
Receptor / Cell fate / Growth / RAS / Secretion	Cluster-60199.0	FGFR1_CHICK	Fibroblast growth factor receptor 1 (EC 2.7.10.1)	2.00E-58	0.996	9.22E-02

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Receptor / Cell fate / Growth / RAS / Secretion	Cluster-77355.0	FGFR1_CHICK	Fibroblast growth factor receptor 1 (EC 2.7.10.1)	2.00E-58	1.116	8.30E-03
Receptor / Cell fate / Growth / RAS / Secretion	Cluster-77355.1	FGFR1_CHICK	Fibroblast growth factor receptor 1 (EC 2.7.10.1)	2.00E-58	1.273	2.05E-04
Receptor / Cell fate / Growth / RAS / Secretion	Cluster-97243.0	FGFR1_MOUSE	Fibroblast growth factor receptor 1 (EC 2.7.10.1)	4.00E-24	1.341	4.79E-04
Receptor / Cell fate / Growth / RAS / Secretion	Cluster-67745.2	FGFR3_PLEWA	Fibroblast growth factor receptor 1 (EC 2.7.10.1)	9.00E-68	1.039	7.51E-02
Receptor / Cell fate / Growth	Cluster-47635.1	ITB1_SHEEP	Integrin beta-1	4.00E-163	0.612	5.93E-03
Receptor / Behaviour / Nervous system / GPCR / Cell fate	Cluster-40343.0	MC5R_MOUSE	Melanocortin receptor 5	6.00E-07	1.181	1.55E-03
Receptor / Nervous system / Cell fate	Cluster-22331.0	NOTC1_DANRE	Neurogenic locus notch homolog protein 1 (Notch 1)	2.00E-21	1.140	1.50E-02
Receptor / Behaviour / Nervous system / Cell fate	Cluster-113490.0	ACHA9_CHICK	Neuronal acetylcholine receptor subunit alpha-9	6.00E-57	0.758	3.84E-04

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Receptor / Sense / Behaviour / Nervous system / Cell fate	Cluster-95367.4	PTPRD_MOUSE	Receptor-type tyrosine-protein phosphatase delta (EC 3.1.3.48)	8.00E-146	0.808	3.98E-03
Receptor / Cell fate / Growth / TGF-b	Cluster-32075.0	TGFR1_RAT	TGF-beta receptor type-1 (EC 2.7.11.30)	6.00E-165	0.232	7.01E-02
Receptor / Cell fate / Growth	Cluster-65058.1	CAD96_DROME	Tyrosine kinase receptor (EC 2.7.10.1)	2.00E-43	-0.737	1.26E-01
Signalling component / Cell fate / Nervous system / Immunity / Secretion	Cluster-106982.0	AGRIN_MOUSE	Agrin	6.00E-05	1.265	3.84E-04
Signalling component / Cell fate / Nervous system / Immunity / Secretion	Cluster-111607.0	AGRIN_MOUSE	Agrin	6.00E-05	1.379	2.46E-06
Signalling component / Cell fate / Nervous system / Immunity / Secretion	Cluster-111607.1	AGRIN_MOUSE	Agrin	6.00E-05	1.205	4.39E-05
Signalling component / Cell fate / Nervous system / Immunity / Secretion	Cluster-111607.2	AGRIN_MOUSE	Agrin	6.00E-05	1.327	2.51E-05

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Cell fate / Nervous system / Immunity / Secretion	Cluster-113633.1	AGRIN_MOUSE	Agrin	6.00E-05	1.172	7.85E-03
Signalling component / Cell fate / Nervous system / Immunity / Secretion	Cluster-91949.0	AGRIN_MOUSE	Agrin	6.00E-05	1.105	9.87E-03
Signalling component / Ligand / Sense / Behaviour	Cluster-87717.0	ARRD1_HUMAN	Arrestin domain-containing protein 1	2.00E-10	0.925	2.98E-03
Signalling component / ECM	Cluster-56741.5	A0A2B4R7B4_STYPI	Basement membrane-specific heparan sulfate proteoglycan core protein	3.60E-45	-0.327	1.00E+00
Signalling component / Cell fate / Exosome / Secretion	Cluster-59857.0	CO6A6_MOUSE	Collagen alpha-6(VI) chain	4.00E-22	1.048	1.26E-04
Signalling component / Ligand / Movement / Behaviour / Nervous system	Cluster-83447.0	CNTN6_MOUSE	Contactin-6	8.00E-68	0.396	3.60E-03
Signalling component / Nervous system / Cell fate / Proline	Cluster-102822.0	CSMD3_MOUSE	CUB and sushi domain-containing protein 3	9.00E-41	0.921	2.14E-04

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Nervous system / Cell fate / Proline	Cluster-57350.3	CSMD3_MOUSE	CUB and sushi domain-containing protein 3	9.00E-41	0.793	2.18E-03
Signalling component / Nervous system / Cell fate / Proline	Cluster-83964.2	CSMD3_MOUSE	CUB and sushi domain-containing protein 3	3.00E-66	0.786	9.80E-02
Signalling component / Nervous system / Cell fate / Proline	Cluster-57350.5	CSMD3_MOUSE	CUB and sushi domain-containing protein 3	9.00E-41	0.707	1.94E-02
Signalling component / Factor / Cell fate / Nervous system / Secretion	Cluster-94243.0	CRIM1_MOUSE	Cysteine-rich motor neuron 1 protein	2.00E-07	0.803	4.83E-03
Signalling component / Binding / Transport / Endocytosis / Secretion	Cluster-102615.1	EHBP1_HUMAN	EH domain-binding protein 1	7.00E-42	0.285	1.12E-02
Signalling component / Nervous system / Secretion / Immunity	Cluster-87880.0	EAA2_MOUSE	Excitatory amino acid transporter 2	4.00E-132	0.748	1.01E-04
Signalling component / Factor / Transcription / Growth	Cluster-40909.0	FD3_DROME	Fork head domain-containing protein FD3	5.00E-25	1.676	1.04E-05

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Factor / Transcription / Growth	Cluster-97039.0	FD4_DROME	Fork head domain-containing protein FD4	1.00E-26	1.908	7.27E-09
Signalling component / Muscle / Protein modification	Cluster-44719.1	A0A2B4SP55_STYPI	Fukutin-related protein	1.10E-23	0.904	7.86E-02
Signalling component / Muscle / Protein modification	Cluster-52660.1	FKRP_MOUSE	Fukutin-related protein	0.001	0.845	2.41E-02
Signalling component / Calcification	Cluster-112945.1	A8C9K2_MONCP	Galaxin	2.10E-06	0.941	1.59E-02
Signalling component / Calcification	Cluster-112945.3	A8C9K2_MONCP	Galaxin	2.10E-06	1.471	NA
Signalling component / Calcification	Cluster-112945.4	A8C9K2_MONCP	Galaxin	2.10E-06	1.432	1.06E-04
Signalling component / Calcification	Cluster-40271.0	A8C9K2_MONCP	Galaxin	2.10E-06	1.113	2.14E-02
Signalling component / Binding protein / Nervous system / Cell fate	Cluster-89045.0	RIT1_HUMAN	GTP-binding protein Rit1	2.00E-37	0.756	2.21E-02
Signalling component / Ligand / Sense / Behaviour / Melanosome /	Cluster-51695.0	GNAO_BOVIN	Guanine nucleotide-binding protein G(o) subunit alpha	6.00E-101	1.047	1.88E-02

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Ligand / Sense / Behaviour / Melanosome	Cluster-51695.4	GNAO_BOVIN	Guanine nucleotide-binding protein G(o) subunit alpha	6.00E-101	0.952	2.91E-03
Signalling component / Tissue / Secretion / Mucus	Cluster-87944.1	HPSE_HUMAN	Heparanase (EC 3.2.1.166)	5.00E-107	0.669	2.44E-02
Signalling component / Tissue / Secretion / Mucus	Cluster-87944.2	HPSE_HUMAN	Heparanase (EC 3.2.1.166)	5.00E-107	0.467	1.66E-01
Signalling component / Factor / Remodelling / Cell fate	Cluster-113105.1	INF2_HUMAN	Inverted formin-2	1.00E-74	1.107	2.88E-03
Signalling component / Factor / Remodelling / Cell fate	Cluster-115783.0	INF2_HUMAN	Inverted formin-2	1.00E-74	1.133	1.10E-03
Signalling component / Factor / Nervous system / Protein modification	Cluster-111605.0	KLH12_BOVIN	Kelch-like protein 12	4.00E-58	0.474	1.75E-03
Signalling component / Cell fate / Vesicles	Cluster-94675.0	KIF23_MOUSE	Kinesin-like protein KIF23	6.00E-138	0.869	1.92E-06

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Ligand / Muscle / Behaviour	Cluster-97901.0	LDB3_MOUSE	LIM domain-binding protein 3	2.00E-05	0.769	5.91E-05
Signalling component / Binding / Muscle / Exosome	Cluster-97668.1	MLC2_DROME	Myosin-2 light chain	3.00E-37	-0.523	5.49E-04
Signalling component / Ligand / Cell fate / Nervous system	Cluster-106212.1	NRX4_DROME	Neurexin-4	4.00E-29	-1.081	6.09E-02
Signalling component / Ligand / Cell fate / Nervous system	Cluster-74955.6	NRX4_DROME	Neurexin-4	5.00E-22	-1.400	5.91E-05
Signalling component / Ligand / Cell fate / Nervous system	Cluster-97612.0	NRX4_DROME	Neurexin-4	5.00E-22	-1.216	8.96E-03
Signalling component / Factor / Transcription / Nervous system	Cluster-76290.1	PAX3B_XENLA	Paired box protein Pax-3-B	4.00E-74	1.227	8.39E-03
Signalling component / ECM / Muscle	Cluster-79563.3	PPN_DROME	Papilin	0.003	0.686	1.35E-01

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling protein / Nervous system / Protein modification / Immunity	Cluster-81996.1	NECB_HYDVU	PC3-like endoprotease variant B (EC 3.4.21.-)	0	0.475	3.47E-02
Signalling component / Sense / Behaviour / Glutamate related	Cluster-68308.1	KCNK1_RABIT	Potassium channel subfamily K member 1	1.00E-50	0.395	5.52E-02
Signalling component / Transport	Cluster-99480.1	DISP_DROME	Protein dispatched	3.00E-37	0.757	2.98E-03
Signalling component / Cell fate / Remodelling	Cluster-105540.0	ECT2_MOUSE	Protein ECT2	2.00E-19	1.026	1.00E-01
Signalling component / Muscle / Nervous system	Cluster-94577.0	PP12C_MOUSE	Protein phosphatase 1 regulatory subunit 12C	7.00E-17	0.972	8.30E-03
Signalling component / Ligand / Growth	Cluster-115880.0	WNT4_CHICK	Protein Wnt-4	2.00E-95	0.563	1.11E-02
Signalling component / Adhesion / Exosome	Cluster-108106.2	FAT1_HUMAN	Protocadherin Fat 1	8.50E-40	0.374	2.24E-01
Signalling component / Nervous system / Cell fate	Cluster-21035.5	FAT4_HUMAN	Protocadherin Fat 4	0	1.004	3.24E-07

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Nervous system / Cell fate	Cluster-21035.6	FAT4_HUMAN	Protocadherin Fat 4	5.00E-09	1.121	7.22E-08
Signalling component / Nervous system / Cell fate	Cluster-42274.0	FAT4_HUMAN	Protocadherin Fat 4	9.00E-22	1.380	1.45E-06
Signalling protein / Cell fate	Cluster-113066.0	RBP1_HUMAN	RalA-binding protein 1	7.00E-75	0.653	1.83E-02
Signalling protein / Cell fate	Cluster-113066.2	RBP1_HUMAN	RalA-binding protein 1	7.00E-75	0.644	7.18E-02
Signalling protein / Cell fate	Cluster-113066.5	RBP1_HUMAN	RalA-binding protein 1	7.00E-75	1.029	4.61E-03
Signalling component / Ligand / Growth / Transcription	Cluster-107202.1	RFC2_RAT	Replication factor C subunit 2	0	0.632	2.67E-05
Signalling component / Binding protein / Cell fate / Secretion	Cluster-59123.2	SCUB2_MOUSE	Signal peptide, CUB and EGF-like domain-containing protein 2	5.00E-13	0.572	7.37E-03
Signalling protein / Growth / TGF-b	Cluster-59833.0	SKI_XENLA	Ski oncogene	2.00E-56	0.518	1.79E-02

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Factor / Growth	Cluster-64366.0	SOCS4_BOVIN	Suppressor of cytokine Signalling 4	7.00E-54	0.494	1.49E-02
Signalling component / Factor / Cell fate	Cluster-68824.3	SVEP1_HUMAN	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	8.00E-05	0.943	7.45E-02
Signalling component / Nervous system / Vesicles	Cluster-97553.2	SYT1_PONAB	Synaptotagmin-1	1.00E-50	0.634	6.18E-02
Signalling component / Nervous system / Vesicles	Cluster-97553.0	SYT1_PONAB	Synaptotagmin-1	1.00E-50	0.608	7.19E-02
Signalling component / Ligand / Nervous system / Vesicles	Cluster-113824.0	SY63_DIPOM	Synaptotagmin-C	7.00E-53	0.642	5.41E-02
Signalling component / Ligand / Exosome / Nervous	Cluster-91603.0	STXB1_RAT	Syntaxin-binding protein 1	0	0.362	4.80E-03
Signalling protein / TGF-b / Growth	Cluster-62501.3	K1PV46_CRAGI	Thrombospondin-1	1.2E-21	0.654	1.21E-02

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Stress / Behaviour / Nervous system / Secretion /	Cluster-62021.2	TSP4_HUMAN	Thrombospondin-4	1.00E-151	0.722	9.94E-02
Signalling component / Stress / Behaviour / Nervous system / Secretion /	Cluster-62021.3	TSP4_HUMAN	Thrombospondin-4	1.00E-151	0.682	1.72E-01
Signalling component / Factor / Transcription / Growth	Cluster-102547.0	RFX4_HUMAN	Transcription factor RFX4	5.00E-143	1.657	3.31E-05
Signalling component / Factor / Nervous system / Transcription	Cluster-110067.0	SOX10_CHICK	Transcription factor SOX-10	1.00E-34	1.259	3.29E-03
Signalling component / Factor / Nervous system / Transcription	Cluster-60151.0	SOX8_XENLA	Transcription factor Sox-8	6.00E-35	1.602	7.24E-06
Signalling component / Factor / Transcription / Nervous system	Cluster-113421.0	SOX9_MOUSE	Transcription factor SOX-9	1.00E-34	1.540	9.82E-05
Signalling component / Factor / Nervous system / Transcription	Cluster-96004.0	SOX9A_XENLA	Transcription factor Sox-9-A	3.00E-31	1.500	3.88E-06

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Signalling component / Cell fate / Secretion	Cluster-113528.0	TF29_SCHPO	Transposon Tf2-9 polyprotein	1.00E-73	1.004	1.11E-02
Signalling component / Protein modification / Nervous system	Cluster-96376.0	TRIM2_RAT	Tripartite motif-containing protein 2 (EC 2.3.2.27)	9.00E-26	-0.915	3.47E-02
Signalling component / Nervous system / Vesicles	Cluster-61548.0	VTI1A_HUMAN	Vesicle transport through interaction with t-SNAREs homolog 1A	8.00E-71	0.354	1.22E-01
Effector / Growth / Cell fate / Protein modification	Cluster-27684.2	P52K_HUMAN	52 kDa repressor of the inhibitor of the protein kinase (p52rIPK)	5.00E-38	-1.033	2.14E-02
Effector / Nervous system / Secretion	Cluster-86531.1	CHLE_PANTT	Cholinesterase (EC 3.1.1.8)	1.00E-49	0.907	2.63E-01
Effector / Nervous system / Secretion	Cluster-96324.0	CHLE_PANTT	Cholinesterase (EC 3.1.1.8)	1.00E-49	0.953	8.04E-05
Effector / ECM / Tissue / proline	Cluster-102524.0	CTHR1_HUMAN	Collagen triple helix repeat-containing protein 1	6.00E-34	1.855	3.11E-08
Effector / Nervous system / Cell fate	Cluster-72197.1	CDK17_HUMAN	Cyclin-dependent kinase 17 (EC 2.7.11.22)	7.00E-175	0.841	4.41E-02
Effector / Nervous system / Cell fate	Cluster-72197.0	CDK17_HUMAN	Cyclin-dependent kinase 17 (EC 2.7.11.22)	7.00E-175	0.937	1.31E-02

Biological characteristic	Cluster ID	UniProt ID	Protein names	Best BLAST e-value	LOG2 Fold Change	padj
Effector / Cell fate / Protein modification	Cluster-111305.0	AL1L1_XENLA	Cytosolic 10-formyltetrahydrofolate dehydrogenase (EC 1.5.1.6)	0	0.808	1.43E-02
Effector / Cell fate / Exosome /	Cluster-91183.0	AMPE_BOVIN	Glutamyl aminopeptidase (EC 3.4.11.7)	0	0.600	1.86E-02
Effector / Cell fate / Secretion / Proline	Cluster-104495.2	PCP_BOVIN	Lysosomal Pro-X carboxypeptidase (EC 3.4.16.2)	1.00E-138	0.292	7.70E-02
Effector / Cell fate / Sphingolipids / SMs	Cluster-56550.2	RADI_MOUSE	Radixin	0	0.245	1.36E-03

3.3.3.3 Genes related to secondary metabolite production and transport

As previously described, sphingolipids are involved in various cellular processes, and they have been associated with stress responses, immune reactions and wound healing (Adada et al., 2014; Gault et al., 2010) (Table 3.5). It is important to note that many of the genes discussed in this group could also be classified under any of the categories above. Nevertheless, the experiment described in this chapter aimed to stimulate allelopathic responses in *Lobophytum* through competition. It is therefore appropriate to have a specific focus on these genes due to their relationship with the sphingolipid signalling pathway and metabolism or secondary metabolite biosynthesis in general.

Considering the potential functions of sphingolipids as components of a defence mechanism, two transcripts annotated as opioid-like receptors (Cluster-113574.0, Cluster-115976.0) may be linked to sphingolipid pathways (Table 3.5). Opioid receptors are present throughout the Bilateria, but the effects of opioid stimulation have not been tested in Cnidaria (Sneddon, 2017). Manual BLAST analysis of Cluster-113574.0 via the UniProt website identified somatostatin receptor type 4 from *Stylophora pistillata* as best match (e-value: 6e-41). Somatostatin receptors have similarly been identified in other cnidarian genomes (Alzugaray et al., 2016a; Voolstra et al., 2017). It has been suggested that the activation of somatostatin receptors triggers a signalling system that is involved with the coordination of movement during feeding (Alzugaray et al., 2016b). These results illustrate the difficulties of extrapolating database search results to understand functions in non-model organisms.

When in competition with *Porites*, *Lobophytum*-Pd also up-regulated the transcription of proteins that could potentially enhance secondary metabolite production (Table 3.5). Consistent with the hypothesis that sphingolipid metabolism plays a role in allelopathic responses, sphingosine kinase 1 (SHK1; Cluster-113072.0) was up-regulated in *Lobophytum*-Pd. SHK1 is responsible for the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P), which is an activator of NF-kB, a transcription factor that controls the expression of many immune related genes (Mydlarz et al., 2016; Spiegel and Milstien, 2000). SHK1 is important for cell survival under heat stress but it is not related specifically to bleaching, demonstrating the importance of sphingolipids in primary metabolism (Kitchen and Weis, 2017).

At least ten clusters related to lipid transporters or lipid transport vesicles were up-regulated in *Lobophytum* Pd (Table 3.5). Ceramides, sphingolipid bases with amide-linked fatty acids, and cembranoids, complex diterpene derivatives, with a diverse range of biological activities, are the major secondary metabolites of *Lobophytum* spp. As these compounds generally have low water solubility, so their secretion is likely to be via lipid vesicles. It is tempting to hypothesise that at least part of the observed up-regulation of the vesicle machinery was due to the transport (exosomes) and biosynthesis (lysosomes) of secondary metabolites. However, more evidence is needed to confirm this hypothesis.

A suite of genes related to the urea cycle, ammonia transport and proline biosynthesis were also up-regulated in competing corals. These included carbamoyl-phosphate synthase (CPSase1), a urea cycle component with a role in arginine and proline biosynthesis. CPSase1 has been found to be up-regulated during the day on a day-night study with *Acropora cervicornis*, suggesting a role in nitrogen transfer between the coral and *Symbiodinium* (Hemond and Vollmer, 2015). Nevertheless, the production of aspartate, which is essential for arginine biosynthesis and the urea cycle were down-regulated which suggests that the observed up-regulation of CPSase1 was to facilitate only proline biosynthesis (asparginase down-regulation is explained latter on in the text).

The up-regulation of a clear homolog of Delta-1-pyrroline-5-carboxylate synthase (P5CS) indicates that proline biosynthesis was up-regulated in completing corals. P5CS was also up-regulated in *Acropora palmata* larvae following heat stress (Polato et al., 2013). Note that proline can be transformed into ornithine, which is a precursor of many secondary metabolites, including tropane, piperidine and pyridine alkaloids. Three highly down-regulated clusters which were annotated as asparaginase-like protein 1, could also theoretically participate in secondary metabolite biosynthesis (Table 3.5) because its product (L-aspartate) is a stepping point for the biosynthesis of a series of secondary metabolites in plants. It has been suggested that a homolog of this protein is involved in the transfer of ammonia to *Symbiodinium* in symbiotic *Aiptasia* (Oakley et al 2016). The down-regulation of asparaginase-like protein 1 is consistent with the idea that the production of aspartate is subject to complex control.

In the group of gene related with secondary metabolites production and release, three clusters annotated as endothelin were also up-regulated in competition (Table 3.5). Endothelin has been identified in both the tentacle transcriptome and the venom proteome of *Chrysaora fuscescens* (jellyfish, Ponce et al 2016), and is involved in the maturation of wasp venom peptide toxins (Brinkman et al 2012). Furthermore, endothelin in *Hydra* has been reported to be related to muscle contraction and development (Zhang et al., 2001).

Competing soft corals also regulated possible effector genes involved in lipid modification, including a homolog of serine/threonine-protein phosphatase 2A regulatory subunit B (PP2A), which may function in sphingolipid metabolism. This phosphatase (PP2A) can be triggered by ceramide to regulate apoptosis (Maceyka and Spiegel, 2014). A homolog of phospholipase DDHD1, which functions in lipid catabolism, was also up-regulated in competing soft corals. A homolog of DDHD1 has been found to be up-regulated in corals infected with WBD (Libro et al 2013), which is consistent with involvement in stress or immune responses in cnidarians.

Some complex glycosphingolipids with SMs activity that have been identified in Cryptococci (fungi) (Li et al., 2018), *Leptomonas samueli* (a protozoan) (Previato et al., 1994) and molluscs (Kojima et al., 2013) have been found to incorporate xylose. The xylose donor (UDP-xylose) for such lipid derivatives is generated by UDP-glucuronic acid decarboxylase 1 (UGD; Bar-Peled et al., 2001; Harper, 2002), a homolog of which was up-regulated in competing *Lobophytum* (Table 3.5). I hypothesise that in *Lobophytum* the up-regulation of UGD may play an indirect role in the transformation of sphingolipids into bioactive secondary metabolites by increasing UDP-xylose synthesis. The domain hit for this enzyme was SDR, which is involved in the activities of steroids, cofactors and lipids.

Table 3.5: Genes related to secondary metabolite production and transport. Blue and red are used to indicate genes down and up-regulated respectively. "Biological characteristic" was assigned considering best BLAST hit annotation and the NCBI domain functions

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	BLAST e-value	LOG2 Fold Change	padj
Receptor / GPCR / Opioids binding	Cluster-115976.0	NPFF2_MOUSE	Neuropeptide FF receptor 2	2.00E-40	0.511	6.85E-02
Receptor / Nervous system / GPCR / Sphingolipid	Cluster-113574.0	A0A2B4T088_STYPI	Somatostatin repector type 4	6.00E-41	1.074	6.62E-02
Signalling component / Transport	Cluster-88172.0	A0A2B4RV31_STYPI	Uncharacterized protein	8.90E-53	1.138	1.13E-02
Signalling component / Transport	Cluster-32864.0	ANXA4_MOUSE	Annexin A4	5.00E-23	0.599	4.92E-02
Signalling component / Transport	Cluster-56508.2	APOH_RAT	Beta-2-glycoprotein 1	1.00E-10	0.841	4.40E-02
Signalling component / Transport	Cluster-24038.0	APOH_RAT	Beta-2-glycoprotein 1	1.00E-10	1.051	1.81E-04
Signalling component / Protein modification / Toxin / Secretion	Cluster-88240.1	CALUA_DANRE	Calumenin-A	4.00E-55	1.482	7.24E-06
Signalling component / Protein modification / Toxin / Secretion	Cluster-88240.0	CALUA_DANRE	Calumenin-A	4.00E-55	1.491	1.33E-06
Signalling component / Protein modification	Cluster-56937.0	CALUA_DANRE	Calumenin-A	4.00E-55	1.071	5.49E-02
Signalling component / Steroid	Cluster-62723.1	CP17A_CHICK	Steroid 17-alpha-hydroxylase/17,20 lyase (EC 1.14.14.19) (EC 1.14.14.32)	2.00E-79	1.152	5.45E-04

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	BLAST e-value	LOG2 Fold Change	padj
Signalling component / Urea / Proline	Cluster-58193.3	CPSM_HUMAN	Carbamoyl-phosphate synthase (EC 6.3.4.16)	0	0.605	1.09E-02
Signalling component / Urea / Proline	Cluster-58193.1	CPSM_HUMAN	Carbamoyl-phosphate synthase (EC 6.3.4.16)	0	0.603	9.96E-02
Signalling component / Lipid / Vesicles	Cluster-70742.0	CYH1_HUMAN	Cytohesin-1	3.00E-162	0.378	2.01E-02
Signalling component / Lysosomes	Cluster-108295.0	GGA1_MOUSE	ADP-ribosylation factor-binding protein GGA1	3.00E-128	0.269	1.25E-02
Signalling component / Lysosome	Cluster-98583.0	HPS6_HUMAN	Hermansky-Pudlak syndrome 6 protein	6.00E-08	0.264	3.29E-02
Signalling component / Protein modification	Cluster-73336.1	M17L2_DANRE	Mpv17-like protein 2	8.00E-29	-0.646	7.03E-02
Signalling component / Factor / Transport	Cluster-108007.3	MOT10_DANRE	Monocarboxylate transporter 10	1.00E-37	1.183	1.76E-06
Signalling component / Factor / Transport	Cluster-108007.0	MOT10_DANRE	Monocarboxylate transporter 10	1.00E-37	0.721	6.73E-02
Signalling component / Urea / Proline	Cluster-77879.0	P5CS_PONAB	Delta-1-pyrroline-5-carboxylate synthase (EC 2.7.2.11)	0	0.616	6.90E-03

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	BLAST e-value	LOG2 Fold Change	padj
Signalling component / Protein modification / Sphingolipid	Cluster-113041.0	PLCB4_HUMAN	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-4 (EC 3.1.4.11)	1.00E-143	0.429	1.12E-01
Signalling component / Transport / Immunity	Cluster-84180.3	PLS2_BOVIN	Phospholipid scramblase 2	4.00E-117	0.564	2.17E-02
Signalling component / Transport / Immunity	Cluster-84180.1	PLS2_BOVIN	Phospholipid scramblase 2	8.00E-103	0.597	2.24E-03
Signalling component / Protein modification / Proline	Cluster-104664.1	PPIG_HUMAN	Peptidyl-prolyl cis-trans isomerase G (EC 5.2.1.8)	2.00E-82	0.336	1.12E-02
Signalling component / Urea / Transport / Secretion	Cluster-99345.0	RHCG_PIG	Ammonium transporter Rh type C	1.00E-68	1.323	4.25E-03
Signalling component / Stress / Sphingolipid	Cluster-100052.0	RHOA_RAT	Transforming protein RhoA	5.00E-39	0.506	3.61E-01
Signalling component / Binding protein / Sphingolipid	Cluster-101820.1	RHOAB_DANRE	Rho-related GTP-binding protein RhoA-B	2.00E-83	0.350	5.67E-02
Signalling component / Transport	Cluster-109906.0	S35B1_MOUSE	UDP-galactose transporter-related protein 1	4.00E-39	0.770	5.16E-03
Signalling component / Lysosomes / Proline	Cluster-32651.0	S36A1_HUMAN	Proton-coupled amino acid transporter 1	1.00E-17	0.731	5.74E-01
Signalling component / Transport / Vesicle	Cluster-86293.0	SGK3_PONAB	Serine/threonine-protein kinase Sgk3 (EC 2.7.11.1)	0	0.549	5.90E-02

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	BLAST e-value	LOG2 Fold Change	padj
Signalling component / Protein modification / Sphingolipid	Cluster-113072.0	SPHK1_ARATH	Sphingosine kinase 1 (EC 2.7.1.91)	2.00E-04	0.451	1.04E-02
Effector / Secretion	Cluster-112945.5	A0A0B2VMD4_TOXCA	Snake venom metalloprotease inhibitor 02A10	1.60E-13	1.483	5.91E-05
Effector / Secretion	Cluster-112945.0	A0A0B2VMD4_TOXCA	Snake venom metalloprotease inhibitor 02A10	1.60E-13	1.309	3.26E-04
Effector / Secretion	Cluster-86765.6	ASGL1_DANRE	L-asparaginase (EC 3.4.19.5)	1.00E-52	-1.167	7.52E-03
Effector / Secretion	Cluster-41508.12	ASGL1_MOUSE	L-asparaginase (EC 3.4.19.5)	1.00E-60	-1.109	4.44E-02
Effector / Secretion	Cluster-1509.2	ASGL1_MOUSE	L-asparaginase (EC 3.4.19.5)	1.00E-60	-1.148	2.48E-02
Effector / Catabolism	Cluster-50257.1	DDHD1_BOVIN	Phospholipase DDHD1 (EC 3.1.1.-)	1.00E-107	0.813	1.10E-03
Effector / Exosome / Secretion	Cluster-59025.1	ECE1_BOVIN	Endothelin-converting enzyme 1 (EC 3.4.24.71)	5.00E-150	0.823	1.91E-05
Effector / Exosome / Secretion	Cluster-1664.3	ECE1_BOVIN	Endothelin-converting enzyme 1 (EC 3.4.24.71)	5.00E-150	0.818	3.52E-02
Effector / Exosome / Secretion	Cluster-37437.1	ECE1_MOUSE	Endothelin-converting enzyme 1 (EC 3.4.24.71)	1.00E-65	0.700	5.64E-01
Effector / Immunity / Toxin	Cluster-76886.0	FGL2_MOUSE	Fibroleukin (Cytotoxic T-lymphocyte-specific protein)	9.00E-34	1.471	4.62E-04
Effector / Immunity / Carotenoid	Cluster-95179.2	GRDP1_ARATH	Glycine-rich domain-containing protein 1 (AtGRDP1)	5.00E-36	0.677	9.49E-03
Effector / Immunity / Toxin	Cluster-115462.3	MCCB_CAEEL	Probable methylcrotonoyl-CoA carboxylase beta chain (EC 6.4.1.4)	2.00E-161	0.929	5.16E-03

Biological characteristic	Cluster ID	UniProt ID/ NCBI ID	Protein names	BLAST e-value	LOG2 Fold Change	padj
Effector / Immunity / Toxin	Cluster-115462.0	MCCB_CAEEL	Probable methylcrotonoyl-CoA carboxylase beta chain (EC 6.4.1.4)	2.00E-161	0.829	4.11E-01
Effector / Immunity / Toxin	Cluster-115462.2	MCCB_CAEEL	Probable methylcrotonoyl-CoA carboxylase beta chain (EC 6.4.1.4)	2.00E-161	0.753	4.43E-02
Effector / Secretion / Toxin	Cluster-62468.1	NAS4_CAEEL	Zinc metalloproteinase nas-4 (EC 3.4.24.-) (Nematode astacin 4)	6.00E-39	0.855	1.85E-03
Effector / Secretion / Toxin	Cluster-62468.0	NAS4_CAEEL	Zinc metalloproteinase nas-4 (EC 3.4.24.-) (Nematode astacin 4)		0.752	1.50E-04
Effector / Protein modification / Sphingolipids	Cluster-75344.0	P2R3B_HUMAN	Serine/threonine-protein phosphatase 2A regulatory subunit B" subunit beta	1.00E-160	0.988	9.00E-02
Effector / Exosome	Cluster-107382.0	PGDH_MOUSE	15-hydroxyprostaglandin dehydrogenase (EC 1.1.1.141)	3.00E-62	1.451	4.03E-04
Effector / SMs / Carotenoid	Cluster-113117.0	PYRD2_HUMAN	Pyridine nucleotide-disulfide oxidoreductase domain-containing protein 2	4.00E-120	0.429	9.97E-02
Effector / Stress / Secretion / SMs	Cluster-92651.0	UXS1_MOUSE	UDP-glucuronic acid decarboxylase 1 (EC 4.1.1.35)	0	0.331	4.07E-03

3.4 Discussion

The results presented here show that transcriptomic analyses can be a powerful tool for the investigation of ecological interactions such as non-contact competition, and can provide insights into the cellular mechanisms that might be affected by this stressor.

This competition study clearly demonstrates the significance of individual (genotypic) variation in the outcome of coral interactions. Even though strong genotype effects are clearly evident in every transcriptomic experiment that has been carried out on adult corals (Aguilar et al., 2017; Bertucci et al., 2015; Granados-Cifuentes et al., 2013; Wright et al., 2017), it has been standard practice to pool data for biological replicates prior to analysis, seeking to elucidate general responses by minimising individual variation effects. In the present case, only one of three *Porites* genotypes (genotype Pd) induced a detectable response in *Lobophytum* explants, but this effect was seen in four out of five *Lobophytum* genotypes. The experimental design allowed this genotype-specific response to be detected, whereas pooling of all of the data led to swamping of this effect (results not shown). More insights about the importance of genotypic variation in coral reef studies are discussed in chapter 5.

Gene expression analysis showed the complexity of the *Lobophytum* molecular response to the presence of *Porites* colony Pd. The following discussion aims to provide biological and ecological context to the results presented above.

3.4.1 Non-contact competition triggers immune responses in Lobophytum

3.4.1.1 Cellular stress responses - signs and control

The hypothesis proposed here is that non-contact competition triggered a cellular stress response (CSR) in *Lobophytum* (Figure 3.2). In fact, during the experiment molecular signatures of cell damage were detected even in the absence of visual evidence of it (data not shown). CSR is commonly reported in corals facing environmental challenges (e.g. heat stress or pH stress) where their normal physiology is compromised (Kaniewska et al., 2012; Oakley et al., 2017). Injured or pathogen-infected corals also typically display a CSR, tissue disruption or physical damage presumably being responsible for initiating the response (Stewart et al., 2017; Wenger et al., 2014).

In a contact competition scenario, each organism will physically generate stress in their competitor for example, by allelopathy or external digestion. Non-contact competition effects,

however, are more subtle and the triggers of stress responses less obvious. Hard corals are unable to inflict physical damage on *Lobophytum* as there is no evidence of the of use allelopathy as a defence mechanism by the former. Nevertheless, CSR resulting from non-contact stressors has been demonstrated in other animals (Gunderson et al., 2017). In fact, predation pressure with no physical contact can cause this type of response in insects, *Daphnia* and toads (Gunderson et al., 2017). In these cases the CSR was triggered by molecular cues (kairomones) or visual stimuli (Gunderson et al., 2017). Kairomones or other molecular cues from *Porites* are presumably responsible for activating the *Lobophytum* CRS.

3.4.1.2 Immune response activation upon non-contact competition

One response to stress appears to be an activation of the immune system in competing corals. In this context, it is important to note that the analyses of gene expression were carried out after 30 days of interaction, essentially capturing immune responses at a relatively advanced stage. The number, diversity and nature of the immune genes expressed at this time suggest a very general response – essentially, these corals appear to be in a general state of alert and ready to confront any of a range of threats. A number of antimicrobial peptides and other effectors of a bacterial immunity (Destoumieux-Garzón et al., 2016; Mariottini and Grice, 2016; Mydlarz et al., 2016) were induced, as were proteins that have been seen to be up-regulated in heat stressed or injured corals. Based on these results it appears that *Lobophytum* mounted a general stress response when in competition with *Porites*. Nevertheless, evidence suggests that *Lobophytum* was also reacting to the stresses of competition in more specific ways that will be described below.

3.4.2 Competition effects on soft coral body movement controlled by nervous system

The hypothesis of non-contact competition described in the introduction (Fig 3.2), suggests that behaviour and growth may be modified in response to the competitive interaction. Consistent with this idea, the gene expression data suggest that *Lobophytum* under competition may be actively modifying its body shape and growth.

Interestingly, most of the genes classified in the tissue remodelling category were homologs of bilaterian genes involved in regulating movement/muscle contraction and nervous system signalling. Despite having very different origins, cnidarian muscles have shown to respond and function in very similar ways to bilaterian muscles (Leclère and Röttinger, 2017). Thus the up-

regulation of genes involved in muscle contraction in bilaterians suggests that these might likewise function in the polyp activity or to body movement in cnidarians (Leclère and Röttinger, 2017).

Body shape in soft corals is controlled not only by muscle contraction but also mostly by the release or uptake of water from/to the hydrostatic skeleton (Davis et al., 2015). Homologs of genes that function in vasocontraction and in cell homeostasis in bilaterians might possibly also be involved in regulating body movement in cnidarians (Davis et al., 2015; Fabricius and Alderslade, 2001). Body movement in *Lobophytum* normally has a diurnal cycle (shrinkage and expansion; personal observation), and the results from this study suggests that corals under competitive challenge might modify their normal behaviour in response to the stress. The up-regulation of genes related to nervous signals, synaptic vesicles and neuron differentiation in *Lobophytum* under competition, may serve to coordinate the coral movement. An active control of movement and body shape in competitive scenarios has been seen in other cnidarians (Hennessey and Sammarco, 2014; La Barre and Coll, 1982; Sammarco and Coll, 1992). *Lobophytum* might be modifying the shape of its soft body to regulate the distance to the competitor. This behavioural variation was not a reflex reaction to external stimulus because such reflex responses do not modify gene expression. Therefore it is suggested that there was an active control of movement and muscle contraction in *Lobophytum* through complex sequence of events involving regulation of a number of cellular pathways.

The ability of soft corals to extend or reduce size by pumping water in or out brings great difficulties when measuring growth. In this study, I did not attempt to measure the growth of the soft corals but I hypothesise that observed up-regulation of likely growth receptors and developmental genes (such as Wnt4; Table 3.4) in *Lobophytum* when in competition reflects tissue growth. A number of studies have reported decreases in growth rates of corals due to competitive interactions (Horwitz et al., 2017; Tanner, 1997), but one competitive strategy is to overgrow the opponent, which presumably requires increased growth rates (Álvarez-Noriega et al., 2018; Chadwick and Morrow, 2011). Consideration of these facts suggests that *Lobophytum* may have adopted an overgrowth strategy in this experiment, but some of the genes discussed in this section could also potentially function in production and release of secondary metabolites.

3.4.3 Evidence of secondary metabolite production

Although the bioactive properties of sphingolipids and cembranoids from several soft corals have been investigated, no information is available about their roles in competitive interactions (Al-Footy et al., 2016; Al-Lihaibi et al., 2010). This study proposed that the up-regulation of sphingolipid signalling and metabolism could have provided the substrate for SMs production in competing corals. Bioactive sphingolipids have been identified in *Lobophytum pauciflorum* tissue, and regulation of sphingolipid biosynthetic pathways is a plausible mechanism of (SMs-mediated) competition in this species (Muralidhar et al., 2005, 2003).

If sphingolipid derivatives function as competitive agents in soft corals, their production may require modification or transformation into the bioactive molecule.

Most of the bioactive sphingolipids found in soft corals are glycosides with one or more sugar residues (Muralidhar et al., 2005) . Several genes likely to function in amino sugar and nucleotide sugar metabolism, such as UGD, were up-regulated in *Lobophytum* Pd, suggesting potential involvement in SMs formation (Table 3.5).

At least ten DEG were candidates for roles in lipid transport and vesicle formation which leads us to suggest that, whatever their chemical nature, secreted secondary metabolites are deployed by competing *Lobophytum* colonies. Additionally, homologs of both a chaperone found in *N. vectensis* nematocysts (Moran et al., 2013) and snake venom inhibitors of metalloproteases (The UniProt Consortium, 2017) could serve to protect *Lobophytum* from its own toxins.

The lack of understanding of gene function in corals limits the scope of interpretation of the DEG found in this experiment. More transcriptomic studies related with ecological interactions are needed to get a more comprehensive understanding of the cellular machinery that soft corals use to compete.

3.4.4 How might a CSR trigger defence mechanisms?

A generalised cellular stress response might be the starting point to activate more specific responses, such as secondary metabolite production or the avoidance of the interaction. Under this scenario, it is reasonable to hypothesise that the nervous system might mediate the development of more specific responses following the general CSR. Evidence is emerging of extensive cross-talk between the immune and nervous systems in a diverse array of animals (Salzet et al., 2000). In oysters, for example, neurotransmitters such as acetylcholine can

modulate apoptosis and phagocytosis (Liu et al., 2016). The up-regulation of a possible homolog of beta-adrenergic receptor and other neurotransmitters was observed in an experiment investigating injury-induced immunity in the sea anemone *Calliactis polypus* (Stewart et al., 2017), suggesting cross-talk between the immune and nervous system in cnidarians. Both phagocytosis and beta-adrenergic receptor expression were activated in *Lobophytum* under competition, which is consistent with the idea of immuno-neuro crosstalk. A number of transcriptome clusters matching the mammalian protein agrin – which functions on the neuromuscular junction but also has a role in immune signalling (Trautmann and Vivier, 2001; Khan et al., 2001) – were also up-regulated in *Lobophytum* Pd, is also suggestive of immuno-neuro crosstalk. It is also tempting to speculate that this cross-talk could also underlie the enhanced movement and tissue remodelling observed in *Lobophytum* under competition.

The results presented here show the complexity of competitive interactions involving cnidarians even when visible signs are not evident, and that such complex phenomena are likely to be tractable using present day methods. We are a long way from understanding the molecular mechanisms underlying competitive interactions involving cnidarians and more research is needed to elucidate the mechanisms use by corals to recognise the threat, but the tools to carry out this kind of work are either available now or will be very soon.

Chapter 4 - Transcriptomic analysis of *Porites cylindrica* under competition

4.1 Introduction

The past 15 years of research have demonstrated that anthropogenic stressors are rapidly modifying coral reef ecosystems (Hughes et al., 2018). Therefore, changes in species composition due to these stressors are almost inevitable. Some Caribbean coral reefs, for example, have suffered significant changes to their species composition and shifts from coral to algae due to events of high ocean temperature have been observed (Hughes, 1994). It is urgent to understand how coral biology is altered by climate change stressors to try to predict the future outcomes for reef ecosystems.

Many studies indicate that not all coral species will be impacted in the same way (Fitt et al., 2009; Hughes et al., 2017b; Loya et al., 2001; Marshall and Baird, 2000; Obura, 2001). In fact, genera such as Acropora or Pocillopora are consistently found to be more susceptible to stressors than *Porites* or massive corals, which are often classified as bleaching-resistant (Hughes et al., 2017b; Loya et al., 2001). Despite the notorious differences between genera there are few specific traits that can predict if a coral is resistant or not.

Recent studies point out that intraspecific variation also plays a vital role on the severity of the effects of climate change on a particular coral species (e.g. disease, ocean acidification) (Sekizawa et al., 2017). Wright et al. (2017) found winners and losers amongst colonies of the same species (Acropora millepora) under bacterial challenge. Gene expression analysis revealed that the survivors were less responsive to the immune challenge, increasing their capacity to control the negative effects of the infection (Wright et al., 2017). Thus intraspecific variation adds another level of complexity when attempting to predict the effects of climate change on coral reef communities.

To predict the future composition of coral reef ecosystems it is essential to understand species interactions and how they may be affected by environmental changes (Chadwick and Morrow, 2011; Harris, 2016). As mentioned in previous chapters, competition is an ecological interaction that drives species and ecosystem evolution. Some studies have focused on understanding the interaction between coral and algae in view of the species shift (from coral to algae) that can occur when temperature increases (Jompa and McCook, 2002; Lirman, 2001;

Shearer et al., 2012; Tanner, 1995). Fewer studies have been done on intraspecific competition between corals and the effects of environmental stressors on these interactions (Evensen et al., 2015; Horwitz et al., 2017). The outcome of coral competition remains difficult to predict due to the specificity of the interaction.

As mentioned in Chapter 1, visual evidence of non-contact competition has been described, but more research is required into how corals engage into these interactions (Chadwick and Morrow, 2011; Sammarco et al., 1983). Consideration of previous literature provides a basis for hypotheses about the future effects of climate change on coral interactions. However, the cellular mechanisms that are behind the interactions are poorly understood.

These cellular mechanisms, controlled by gene expression, are ultimately responsible for coral reactions to stressors and interactions. In fact, when hard corals are in a non-contact competitive scenario, several steps or events can occur that will change the cellular mechanisms used by the animal to respond to the interaction and potentially determine the outcome (Figure 4.1). First, corals need to recognise the existence of the potential threat. If the competitor uses allelopathy, cytotoxins may cause cellular damage which will activate pathways to contain or repair the harm (Alino et al., 1992; Blunt et al., 2017; Shearer et al., 2012). Alternatively, the recognition process may be triggered by kairomones, which are more likely to activate cellular stress responses and subsequently alert other mechanisms of defence (Aceret et al., 1995; Agrawal et al., 1999; Sammarco et al., 1985, 1983). If the hard coral overcomes the cellular stress caused by the attack, it could either fight back or avoid the interaction (Figure 4.1). Unfortunately, in the absence of an understanding of mechanisms, it is difficult to predict responses to future competition scenarios, as different species (and colonies of the same species) may react differently.

A better understanding of the cellular pathways that are activated during non-contact competition may provide insights into biological limits or advantages that corals experience during the interaction. For this purpose, the response of *Porites cylindrica* to non-contact competition with the soft coral *Lobophytum* pauciflorum was assessed at a transcriptomic level.

The hard coral data described in this chapter is consistent with a stress response, showing impacts on polyp behaviour and genes related to it. I interpret other aspects of the transcriptome data as potentially indicative of both protective responses of *Porites* and aggressive reactions towards *Lobophytum*.

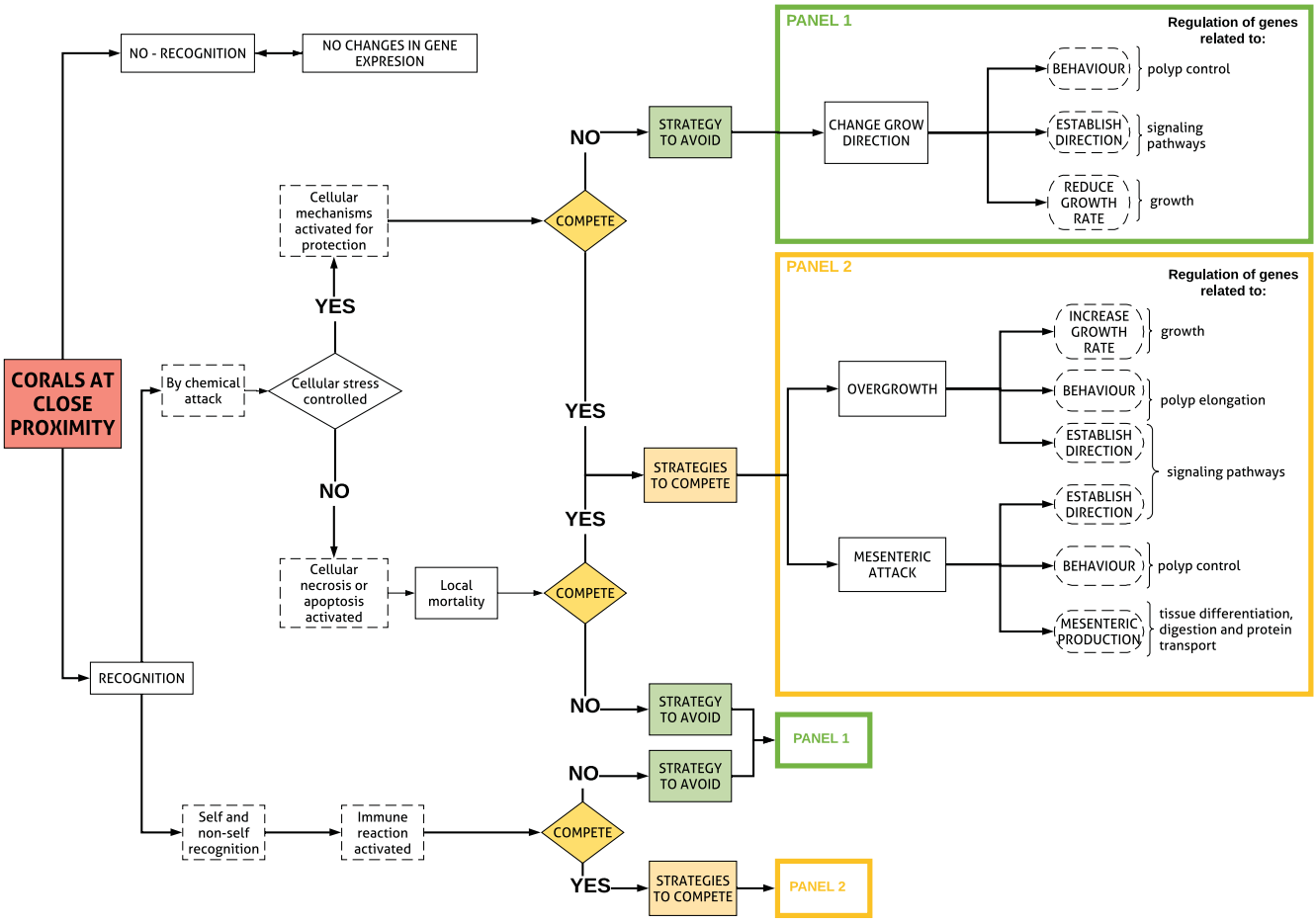

Figure 4.1: Hypothetical steps and cellular responses that a hard coral might experience under a non-contact competition scenario. Discontinuous line corresponds to elements that are not yet supported by experimental data.

4.2 Material and Methods

4.2.1 Experimental design

This experiment was run at Orpheus Island Research Station (OIRS) with colonies of both *Porites cylindrica* and *Lobophytum pauciflorum* collected from the surrounding reefs (GBRMPA Permit No. G16/38499.1). The full details of the experimental design are described in Chapter 2. In brief, each tank had a nubbin of *Porites* in close proximity (~3 cm) to a fragment of *Lobophytum*. These pairings were replicated for every combination of the three *Porites* colonies with the five *L. pauciflorum* colonies, with three technical replicates of each

of these combinations (Figure 4.2). Solitary fragments of both species were used as the no-competition controls. The corals were left in these pairings for 60 days.

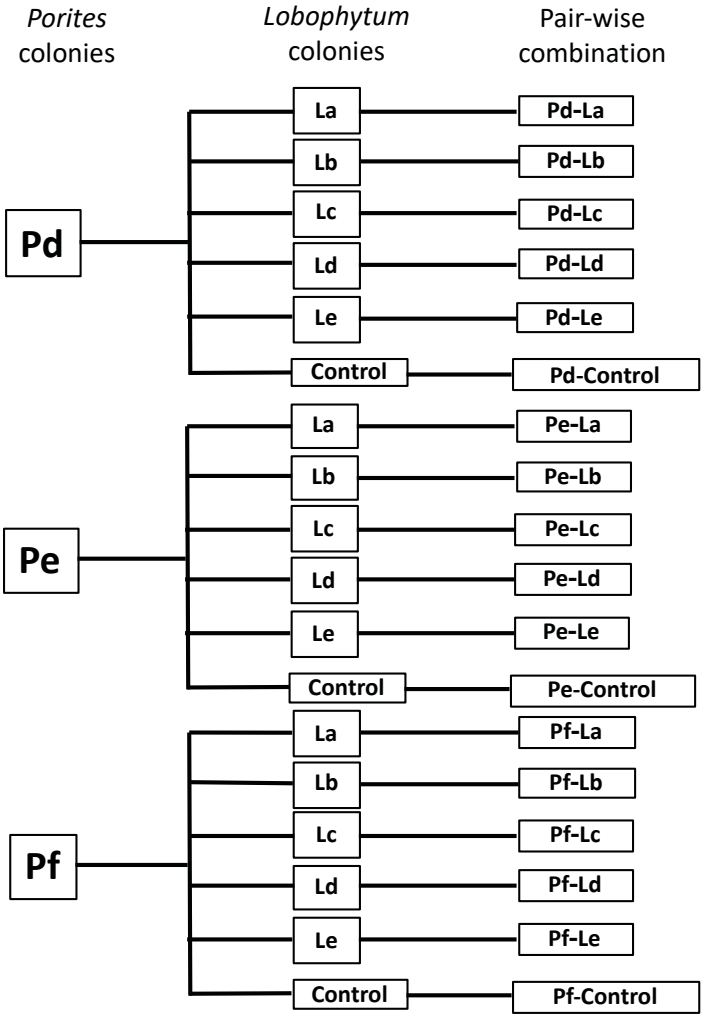

Figure 4.2: Diagram of the pairwise interacting corals and controls made with three colonies of *Porites* (Pd, Pe and Pf) and five colonies of *Lobophytum* (La, Lb, Lc, Ld, Le).

4.2.2 Collection and analysis of Porites behavioural data

Behavioural observations were recorded to determine if *Porites* interacting with *Lobophytum* were showing signs of competitive behaviour or were affected by the interaction. As mentioned in chapter 3, *Porites* polyp activity was observed 3 times per day (between: 8am to 11am, 12pm to 4pm and 6 pm to 9pm), starting from day 8 of the experiment and continuing until day 60. Polyp activity was categorized as open, partially open, or closed. The three daily polyp activity

measurements were summarized to a majority consensus value using the key shown in Table 4.1.

This data was analysed using cumulative link mixed effect models (clmm2) with the package 'ordinal' in the statistics program R, to determine if competition affected *Porites* polyp activity (Christensen, 2015). This analysis modelled polyp activity as an ordered factor (Closed < Partially open < Open) as a function of the following fixed effects; time categorized in eight groups of ~5 days each, the *Porites* colony the nubbin came from and the nubbin's treatment (competition or control). In addition, the tank the sample was in was modelled as a random effect.

Finally, competitive behaviour of *Porites* towards *Lobophytum* was recorded, as mentioned in chapter 3, to determine if these hard corals were showing signs of aggressive behaviour.

Table 4.1: Key used to summarize the three daily observations of polyp activity into a single activity per day. Variation of polyp activity corresponded to the possible combinations of activities on a 24h period: open (O), partially open (P), closed (C).

Variation of polyp activity	Summary of daily activity
O-O-O	Open
O-O-P	Open
O-O-C	Open
O-P-P	Partially open
O-P-C	Partially open
O-C-C	Closed
P-P-P	Partially open
P-P-C	Partially open
P-C-C	Closed
C-C-C	Closed

4.2.3 RNA extraction and transcriptome assembly

In chapter 3, I found that after 30 days of interaction, *Lobophytum* colonies competing with the *Porites* colony Pd showed over-expression of genes involved in signalling, sensory pathways, and innate immune response (see Chapter 3 section 3.3.3). To determine the effects of competition on *Porites* gene expression at the same time point, tissue samples were snap-frozen after 30 days of interaction and stored at -80˚C.

RNA extractions of the *Porites* fragments were performed with TRIzol Reagent (Ambion, catalogue Number 15596-026) according to the manufacturer protocol (Chomczynski and Sacchi, 2006). RNA quality check and library preparation were performed as described in Chapter 2. High-quality RNA extractions were obtained for nubbins from two out of the three colonies of *Porites* used in the experiment (Pd and Pf). It was not possible to extract RNA from nubbins of colony Pe, therefore 12 samples (10 nubbins in competition and 2 nubbins in control) from colonies Pd and Pf were sequenced.

The samples were sequenced by AGRF (Melbourne, Australia) using 2 lanes of an Illumina HiSeq2500 to produce 700 million, 100bp paired-end reads, which equates to approximately 14.5 million reads per sample.

A *de novo* transcriptome for *Porites* was assembled following the Oyster River protocol (MacManes, 2016). Random sequencing errors were corrected using the software Rcorrector before running the assembly analysis (Song and Florea, 2015). Independent assemblies were performed for each *Porites* colony using the software Trinity (Grabherr et al., 2011) and then merged together using the software TransFuse (https://github.com/cboursnell/transfuse).

The merged transcriptome was analysed with the software TransRate which optimized and scored the assembly based on contigs and mapping metrics (Smith-Unna et al., 2016). Symbiont transcripts were removed from the optimized assembly using software Psytrans (Forêt and Ong, 2014) and the completeness of the clean assembly was tested with the software BUSCO (Simão et al., 2015).

The corrected transcripts were mapped to the merged transcriptome using Bowtie2 with recommended settings (end to end alignments, report all alignments, min alignment score 0.3) to suit downstream quantification with the software (Corset 1.05) used to obtain counts and clusters. Details of the quality of the transcriptome assembly and mapping rate are described in the Results section.

4.2.4 Transcriptome annotation

The software Trinotate V3.0 was used to annotate the *Porites* transcriptome. Full details of the Trinotate protocol are described on the Trinotate website (https://trinotate.github.io/). Briefly, protein prediction was done with TransDecoder and homologs to proteins in the SwissProt database were identified using both BLAST-P on predicted proteins and BLAST-X on raw transcripts ($E < 10^{-5}$), signal peptides were identified using SignalP version 4.1.

Since the transcriptome assembly is likely to contain many incomplete sequences, Trinotate annotations were supplemented with annotations from predicted genes from the whole genome sequence of *Porites lutea*. To do this, *Porites* transcriptome sequences were blasted (BLAST-X E < 10^{-5}) against predicted transcripts obtained from the genome data (unpublished) using Geneious v. 9.1.5 (http://www.geneious.com, (Kearse et al., 2012).

The BLAST hit with the lowest e-value amongst Trinotate annotations and genome annotations was considered the best BLAST hit and used for downstream analysis. Gene Ontology IDs and terms (GO terms) as well as Kegg Orthology terms (KO terms) of the best annotation were retrieved from UniProt (The UniProt Consortium 2013).

4.2.5 Gene expression analysis

The software Corset was used to cluster transcripts based on multi-mapping reads reported by Bowtie2, and to obtain read counts for each cluster suitable for the analysis of differentially expressed genes (Davidson and Oshlack, 2014) . An annotation score based on the length and the information available for each contig was used to choose one contig per cluster for the purpose of transferring annotations from contigs (see above) to clusters.

The analysis to obtain the genes differentially expressed between control and treatment was done with the R package DESeq2 (Love et al., 2014). To take into account the high intraspecific variation observed in the Principal Component Analysis (PCA) from this experiment (see results, Figure 4.6), the variable 'Hard coral' was merged with the condition 'Treatment' creating a new variable 'Hard coral treatment' (Table 4.2). The former was used to fit the model in DESeq2 (Table 4. 3).

The genes differentially expressed were found with the 'contrast' function from DESeq2 comparing gene expression of competing nubbins from colony Pd and colony Pf against their respective controls (colonies Pd and Pf). Then, the genes consistently up or down-regulated between replicates were used for the downstream analysis and with an adjusted p-value (padj) <0.1. It is important to point out that the differences between *Porites* colony Pd and Pf were not analysed in this experiment because the origin of colony variation is unknown in this case and do not necessarily reflect variations due to the treatment.

Table 4.2: Samples of *Porites* to be used for gene expression analysis with DESeq2. "Hard coral" denotes the *Porites* colony the sample came from, "Soft coral control" shows which colony of *Lobophytum* the sample was interacting with, "Treatment" indicates if the sample was competing (T) or was a control (C) and the highlighted column "Hard coral treatment" corresponds to the variable used to fit the model in DESeq2.

Hard coral	Soft coral control	Treatment	Hard coral treatment
Pd	La	T	Pd_T
Pd	Lb	T	Pd_T
Pd	Lc	T	Pd_T
Pd	Ld	T	Pd_T
Pd	Le	T	Pd_T
Pd	Control	C	Pd_C
Pf	La	T	Pf_T
Pf	Lb	T	Pf_T
Pf	Lc	T	Pf_T
Pf	Ld	T	Pf_T
Pf	Le	T	Pf_T
Pf	Control	C	Pf_C

Table 4.3: Functions to analyse gene expression of *Porites* under competition using DESeq2.

	Function	Variables
Model	~ Hard coral treatment	Intercept Pd-T Pd-C Pf-T Pf-C
Contrast	contrast(0,1,-1,1,-1)	Intercept (0) Pd-T (1) Pd-C (-1) Pf-T (1) Pf-C (-1)

4.2.6 Analysis to infer gene function

The hypothesis of the steps and outcomes of distant competition were used to understand how *Porites* was reacting to the presence of *Lobophytum* (Figure 4.1). In summary, if *Porites* reacted to the presence of *Lobophytum* because of a chemical attack, this would presumably lead to differential expression of genes involved in cellular stress responses and detoxification (Shearer et al., 2012). Allelopathy can cause bleaching and tissue necrosis, therefore these cellular processes were investigated amongst the DEG. Finally, as mentioned in Chapter 3, *Porites* colonies showed some aggressive behaviour, therefore the presence of genes related to this behaviour were analysed.

The putative functions of the DEG in *Porites* under competition were analysed considering the same information as in Chapter 3: UniProt ID annotation (functions, ontology, KEGG reference); function of protein domains found with NCBI conserved domain finder (*e*-value<1E-3) and, literature relevant to the gene/protein function in Cnidarian or other metazoans (appendix Table A). Gene ontology term enrichment analysis was executed with the R package GOSeq to analyse if any functionality was over-represented within the genes differentially expressed between the control and competing corals (Young et al., 2010).

4.3 Results

4.3.1 *Aggressive behavioural observations of Porites*

As mentioned in the previous chapter, *Porites* showed aggressive behaviour towards *Lobophytum* (Table 4.4). Two thirds of the reacting nubbins used mesenteric filaments to attack the soft coral while a third (two out of six) showed elongated polyps at the base of the coral nubbin (Figure 4. 3).

Table 4.4 *Porites* nubbins interacting with *Lobophytum* that showed a visual aggressive behaviour. Day of observation shows how long the corals had been interacting before the behaviour was observed. Day of tissue sampling indicates the day that the nubbins were collected for genetic analysis.

		Competitive behaviour			
Porites colony	*Lobophytum colony*	Mesenteric filaments	Elongated polyps	Day of observation	Day of tissue sampling
Pd	Lb	✓	.	23, 25	30
Pd	La	✓	.	26	30
Pd	Lc	✓	.	41	60
Pe	Lc	.	✓	6	30
Pe	Ld	.	✓	6, 24	30
Pf	Ld	✓	.	50	60

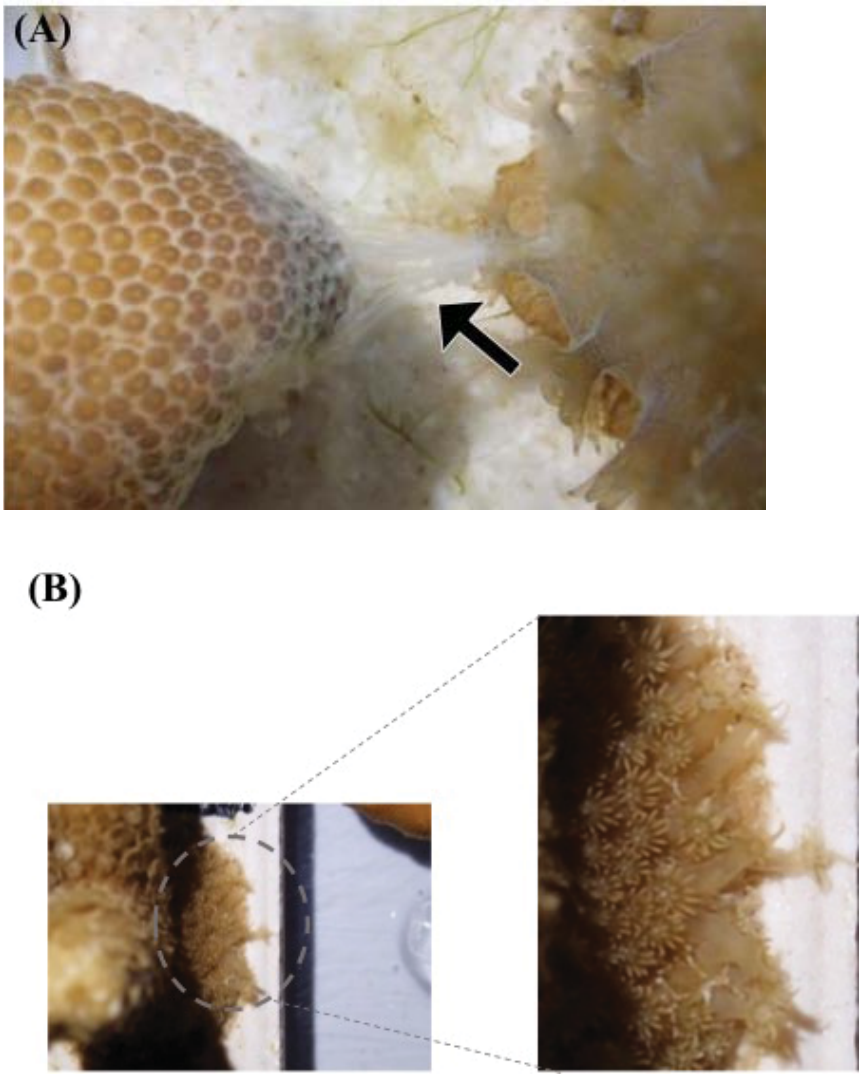

Figure 4.3: Aggressive behaviour of *Porites* towards *Lobophytum*. (A) *Lobophytum* (left) being attacked by mesenteric filaments of *Porites* (right). (B) Base elongated polyps from the hard coral interacting with *Lobophytum*.

4.3.2 Analysis of Porites polyp activity data

Competition impacted polyp activity, with nubbins under competition spending a higher proportion of their time with partially open or closed polyps compared to controls (Figure 4.4). This result was supported by the results of cumulative link mixed model analysis (Table 4.5). This model predicts the probability for a polyp to be in a particular category (open, closed or partially open) depending on experimental covariates such as the treatment, time category and genotype.

The treatment term in this model is significantly different from 0 (P = 8.2E-3) and negative, indicating that polyps from nubbins under competition are more likely to be in the category "closed". This pattern can also be seen visually in Figure 4.4, where both closed and partially open categories are more frequent under competition (Figure 4.4-B) than in controls (Figure 4.4-A).

Both competing and non-competing nubbins showed increased polyp activity over time, with a reduction in closed and partially open polyps during the second half of the experiment (Table 4.5, Estimate increase from 0.21 to 2.98; Figure 4.4). Nevertheless, polyps of nubbins in competition remained less active than the control nubbins until the end of the experiment (Figure 4.4).

Finally, significant differences were also seen in polyp activity between the three colonies of *Porites* (Table 4.5, Colony Pe, P=5.3E-04; Colony Pf, P=9.5E-04). At every time point, colonies Pe and Pf were significantly more likely to have their polyps open than nubbins from colony Pd (Table 4.5, Figure 4.5).

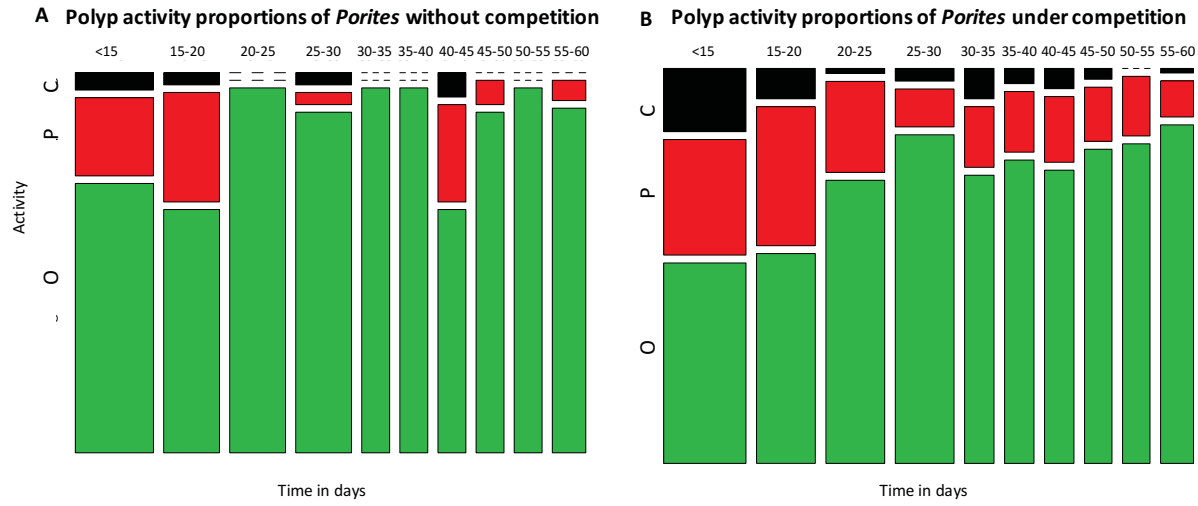

Figure 4.4: Mosaic plot showing the proportion of open (O, green), partially open (P, red) and closed (C, black) nubbins of *Porites* in control condition –no competition (A) and in competition with *L. pauciflorum* (B) over duration of the experiment. Polyp activity is shown as a proportion of observations within a given time period (x axis). Changes in bar width at day 30 represents a reduction in the number of samples (n) due to sampling at day 30, n=36 (days 0-30), n=15 (days 31-60).

Table 4.5: Coefficients for the cumulative link mixed effect model fitted for *Porites* polyp activity data. The intercept of the model was: days 0-15, no competition control for *Porites* and colony Pd.

	Estimate	**Std error**	**Z**	**P-value**
15-20 days	0.21	0.20	1.01	3.0E-01
20-25 days	1.43	0.24	6.03	1.6E-09
25-30 days	2.08	0.28	7.43	1.1E-13
30-35 days	1.29	0.31	4.12	3.7E-05
35-40 days	1.56	0.33	4.75	2.1E-06
40-45 days	1.03	0.30	3.49	4.9E-04
45-50 days	1.73	0.35	5.01	5.6E-07
50-55 days	1.97	0.36	5.39	7.1E-08
55-60 days	2.28	0.37	6.10	1.0E-09
Competition	-1.01	0.38	-2.64	**8.2E-03**
Colony Pe	1.15	0.33	3.47	**5.3E-04**
Colony Pf	1.09	0.33	3.30	**9.5E-04**

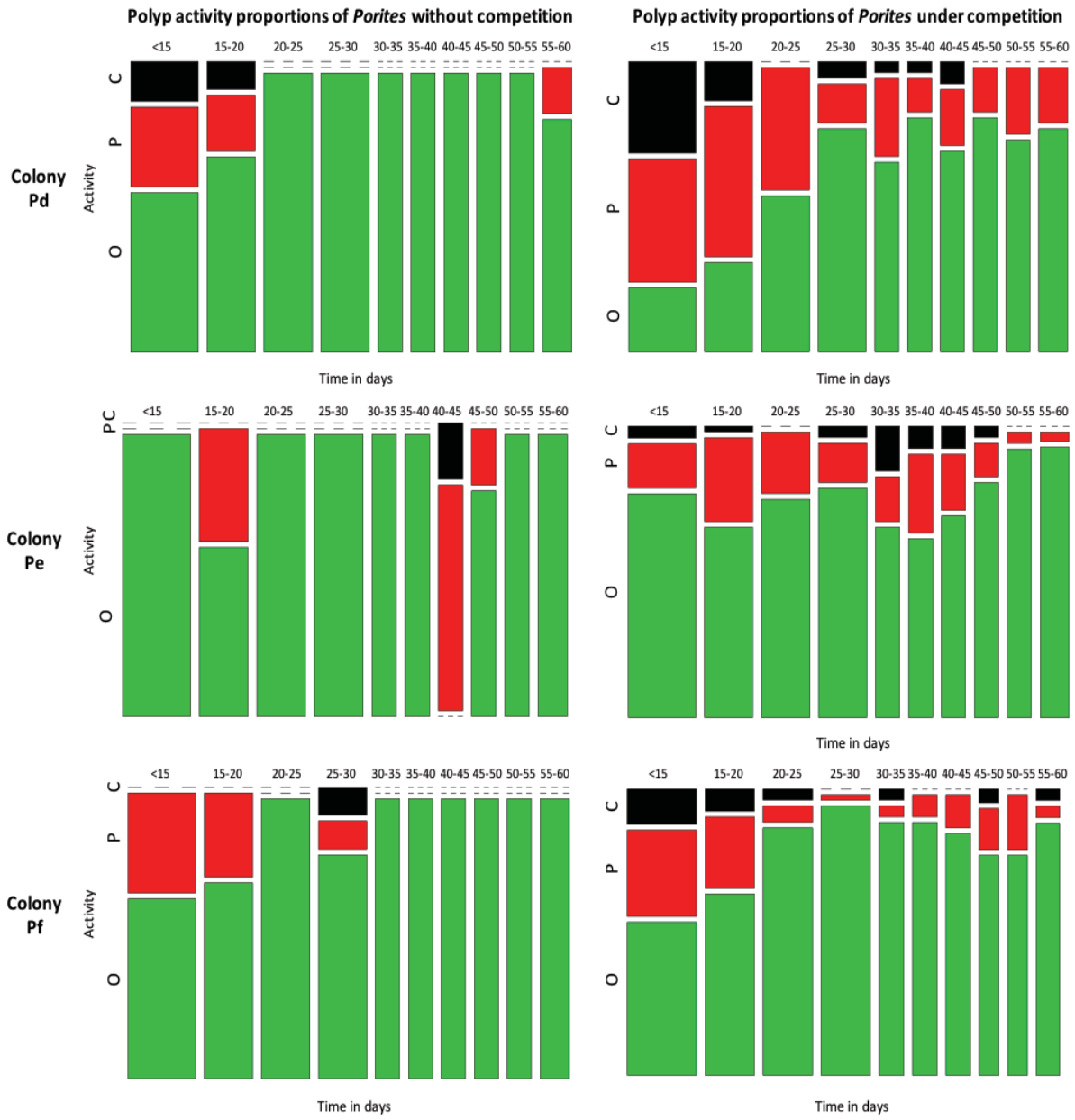

Figure 4.5: Mosaic plot showing the proportion of open (O, green), partially open (P, red) and closed (C, black) nubbins of *Porites* colonies in control condition –no competition (left panels) and in competition with *Lobophytum* (right panels) over the duration of the experiment. Polyp activity is shown as a proportion of observations within a given time period (x axis). Changes in bar width at day 30 represents a reduction in the number of samples (n) due to sampling at day 30. n=36 (days 0-30), n=15 (days 31-60).

4.3.3 Transcriptome assembly and annotation

Assembly of the *Porites* transcriptome (see methods) resulted in 406,531 contigs with an average length of 1069 bp and a GC content of 44%. Assessment of the assembly with

Transrate yielded an overall score of 0.1119 with 234,238 good contigs used for downstream analysis. Transrate estimated that 57% of transcripts contained open reading frames. The mapping rate of the raw corrected reads to the new transcriptome was ~50%. Assembly completeness was 95% according to BUSCO based on conserved metazoan gene content. This percentage of completeness is similar to those for other *de novo* coral assemblies, such as *Acropora millepora* (95%) or *Fungia concinna* (97%). Corset analysis resulted in 144,087 clusters. Sixty-two percent of these clusters were successfully annotated with an UniProt ID.

4.3.4 Gene expression analysis

Initial data exploration showed that the sample from colony Pd interacting with colony Ld was an outlier, showing variation that could not be interpreted (appendix C, Figure C.1). Therefore, this sample was excluded when fitting the model for the gene expression analysis with DESeq2 and subsequent Principal Component Analysis (PCA). The other eleven samples showed congruent variation as explained below.

Principal components analysis showed that the largest source of variation between samples was the genotype of the colony. This can be clearly seen in Figure 4.6 where samples from colonies Pd and Pf are separated at opposite ends of PC1 which explains 89 % of the variance. Differences amongst samples within each colony appeared to be responsible for variation captured by PC2 which accounted for 5% of the total variance (Figure 4.6). The control nubbins are similarly located along PC2 component, suggesting gene expression similarity between controls from colony Pd and Pf. Then treatment samples were divided into two groups in the PC2 scale, those located towards the positive area and those in the negative zone (Figure 4.6).

A total of 193 genes were found to be differentially expressed (DE) between corals in competition and control corals (based on the contrast detailed in Table 4.3 (Methods)). When focusing on the differentially expressed genes (DEG) that had a similar expression pattern for both colonies Pd and Pf (described in methods), 52 genes were always down regulated in samples experiencing competition compared to samples in control (appendix C Table C.1). Similarly, 90 genes were consistently up-regulated in nubbins under competition versus control (appendix C Table C.1).

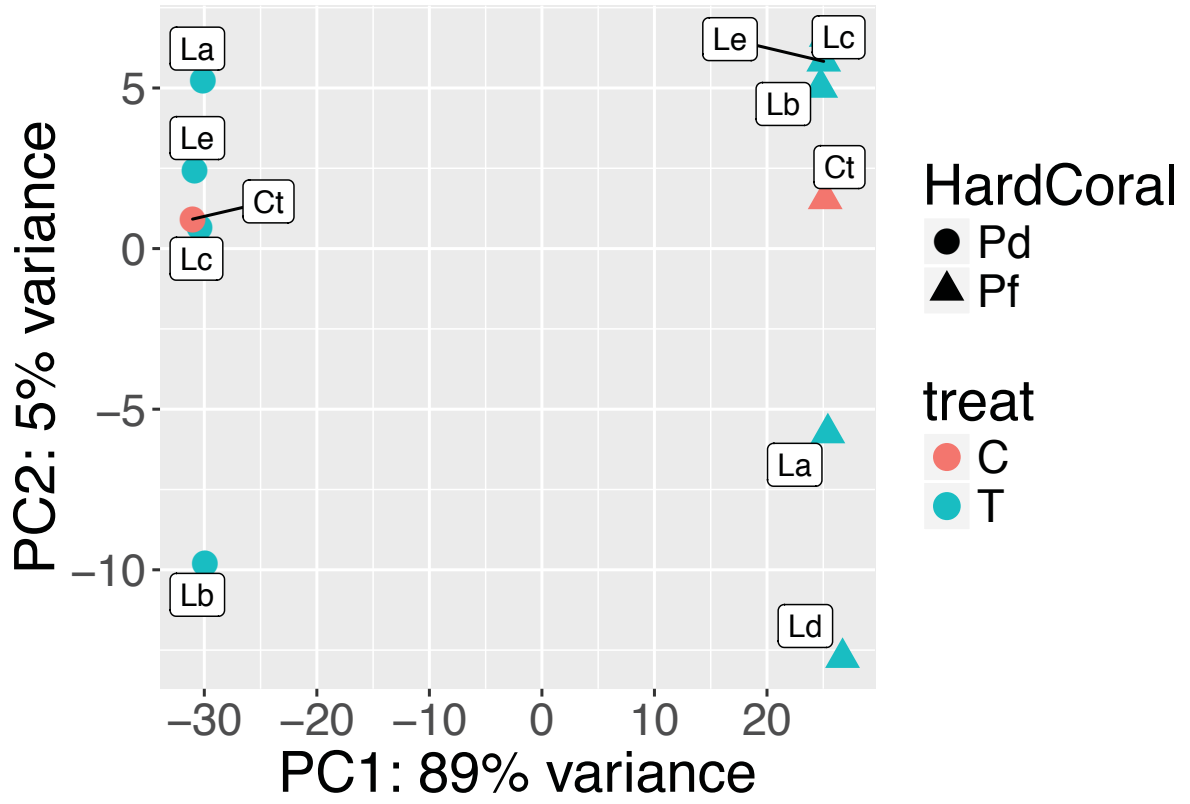

Figure 4.6: Principal component analysis based on normalized, variance stabilized counts for all samples. C=no-competition control, T=competition treatment of *Porites* interacting with *Lobophytum*, with labels showing the competing *Lobophytum* colony. Circles = *Porites* colony Pd, triangle = *Porites* colony Pf.

4.3.5 Analysis to infer gene function

The gene ontology enrichment analysis did not find significant over-represented GO terms between competing and non-competing *Porites* nubbins. Therefore a targeted approach was used to give sense to the data, focusing on four manually curated gene categories (see Methods section 4.2.6): (1) cellular stress genes, (2) genes involved in behavioural changes, (3) genes related to resisting cellular damage and (4) genes associated with an aggressive response to the interaction. In discussing the results, genes referred to as up- or down-regulated correspond to those genes expressed at higher or lower levels in competing *Porites* compared to control nubbins.

4.3.5.1 Cellular stress response

Twenty-two of the differentially expressed genes were potential antioxidants or putatively involved in ubiquitination, mucus production or apoptosis (Table 4.6). Regulation of redox proteins is characteristic of corals under cellular stress (Oakley et al., 2017; Shearer et al.,

2012). Peroxidasin, an oxidoreductase also involved in apoptosis and immunity, was up-regulated in competing corals (Libro et al., 2013; Louis et al., 2017; Voolstra et al., 2009). Eight genes involved in ubiquitination and mucus production were also up-regulated, as well as seven apoptotic genes (Table 4.6).

The only cluster down-regulated was annotated as FANK1_HUMAN (Table 4.6). Although to the best of my knowledge a protein containing both domains has not been reported in any cnidarian, FN3 and ankyrin domains have both been reported as likely to function in cnidarian immunity (Burge et al., 2013; Ocampo et al., 2015). In humans, FANK1 has been reported to act as an anti-apoptotic factor (Wang et al., 2011).

Three transcripts up-regulated in competing corals were classified as corresponding to two pro-apoptotic genes IDs: clathrin interactor 1 (Cluster-65721.45376) and tetratricopeptide repeat protein 28 (TPR repeat protein 28; Cluster-65721.16878 and Cluster-65721.36430) (Table 4.6). Pro-apoptotic functions of these genes have been established only in mammals, therefore extrapolating their function to cnidarians may appear questionable. However, much of the apoptotic machinery is well conserved between corals and humans (Moya et al., 2016), suggesting that these genes may have similar functions in corals. Additionally, proteins related to clathrin-coated vesicles have been recorded to be up-regulated within the first two hours after *Hydra* was injured, suggesting that the vesicle pathway contributed to cleaning up apoptotic cells and other cellular debris (Wenger et al., 2014). Clathrin interactor 1 regulation in competition might be related to apoptosis as well.

Table 4.6: Genes differentially expressed in *Porites* under competition and related with signs of cellular stress. Blue and red correspond to genes down and up-regulated respectively. "Biological characteristic" was assigned considering the Best blast hit annotation and the NCBI domain functions.

Biological characteristics	Cluster ID	UniProt ID	Protein names	E.value	log2 fold change	padj
Anti-Apoptotic	Cluster-65721.46053	FGFR3_HUMAN	Fibroblast growth factor receptor 3	3.6E-84	2.96	6.8E-02
Anti-Apoptotic	Cluster-67822.0	TIM50_DANRE	Mitochondrial import inner membrane translocase subunit TIM50	1.8E-87	1.90	9.9E-02
Anti-Apoptotic	Cluster-65721.6587	TIM50_DANRE	Mitochondrial import inner membrane translocase subunit TIM50	1.8E-87	4.41	8.5E-02
Anti-Apoptotic/ immunity activation	Cluster-35329.24	FANK1_HUMAN	Fibronectin type 3 and ankyrin repeat domains protein 1	3.9E-89	-1.56	4.1E-03
Pro-Apoptotic	Cluster-65721.45376	EPN4_BOVIN	Clathrin interactor 1	1.8E-101	4.79	6.4E-02
Pro-Apoptotic	Cluster-65721.36430	TTC28_HUMAN	Tetratricopeptide repeat protein 28	1.5E-25	2.38	9.9E-02
Pro-Apoptotic	Cluster-65721.16878	TTC28_HUMAN	Tetratricopeptide repeat protein 28	9.2E-105	3.63	2.1E-02

Biological characteristics	Cluster ID	UniProt ID	Protein names	E.value	log2 fold change	padj
Apoptosis / Immunity	Cluster-65721.19733	PXDN_XENTR	Peroxidasin	1.4E-14	3.95	7.7E-02
Immunity activation	Cluster-54747.2	AKNA_MOUSE	AT-hook-containing transcription factor	3.7E-14	-1.67	1.2E-04
Ubiquitination / Immunity activation	Cluster-65721.38798	ERAP2_BOVIN	Endoplasmic reticulum aminopeptidase 2	2.2E-31	2.10	5.1E-02
Ubiquitination	Cluster-65721.8683	BTBD6_MOUSE	BTB/POZ domain-containing protein 6	1.8E-50	2.41	2.0E-02
Ubiquitination	Cluster-65721.26350	DZIP3_MOUSE	E3 ubiquitin-protein ligase	1.5E-12	3.98	9.0E-02
Ubiquitination	Cluster-65721.28005	KCMF1_XENLA	E3 ubiquitin-protein ligase	4.1E-16	3.99	5.6E-02
Mucus	Cluster-65721.27776	FBP3_STRPU	Fibropellin-3	1.9E-35	1.72	6.5E-02
Mucus	Cluster-65721.37056	FUK_HUMAN	L-fucose kinase	2.5E-167	1.40	6.9E-02
Mucus	Cluster-65721.34748	MUC5A_HUMAN	Mucin-5AC	5.1E-10	2.17	7.8E-02

Biological characteristics	Cluster ID	UniProt ID	Protein names	E.value	log2 fold change	padj
Mucus	Cluster-65721.34213	MUC5A_HUMAN	Mucin-5AC	5.1E-10	3.54	3.9E-02
Antioxidant	Cluster-65721.20113	CAHZ_DANRE	Carbonic anhydrase	4.2E-46	1.83	2.1E-02
Antioxidant	Cluster-65721.24988	DHRS7_MOUSE	Dehydrogenase/reductase SDR family member 7	4.4E-81	2.90	9.9E-02
Antioxidant	Cluster-60667.0	MTRR_MOUSE	Methionine synthase reductase	2.8E-144	1.68	7.6E-02
Antioxidant	Cluster-65721.12981	Y8969_DICDI	FAD-linked oxidoreductase	6.2E-41	-1.63	7.7E-02
SOS response	Cluster-46927.1	RECQ_HAEIN	ATP-dependent DNA helicase RecQ	4.7E-11	2.52	5.6E-02

4.3.5.2 Genes involved with behavioural changes

Under competition, seven *Porites* GPCRs and four other sensory genes potentially involved in behaviour regulation were differentially expressed (Table 4.7). Amongst these genes, a serotonin receptor was found to be down-regulated (log2 fold change: -2.45). Serotonin plays a role in muscle contraction in *Cladonema* (hydroid) and is classified as a excitatory neurotransmitter having in cnidarians (Mayorova and Kosevich, 2013; Watanabe, 2017). An homolog of syntaxin, a protein involved in synaptic vesicle transport, was also down-regulated in competing *Porites* (Table 4.7). Investigations on cnidarian nervous systems imply that vesicular transport of neurotransmitters is critical for cellular communication and polyp behaviour (Smith et al., 2014; Watanabe, 2017).

Cluster-65721.37966 and Cluster-51347.0 were up-regulated in competing *Porites*, and were annotated as orexin (Table 4.7). Orexin has been found to be up-regulated in *Acropora digitifera* at the setting phase during spawning, suggesting that it might have a role in the coral temporal information processing according to changes in light intensity (Rosenberg et al., 2017). Orexin is a member of the CCKR-like group that corresponds to the annotation found using the NCBI conserved domain finder as cholecystokin receptor (Table 4.7).

Cholescystokin receptor stimulation has been associated with inhibition of food consumption in insects (Schoofs et al., 2017). This receptor has been found in *Hydra attenuata* sensory nerve cells and could be mediating feeding responses in hydra as well (Grimmelikhuijzen et al., 1980). Although the roles of cholescystokin in Cnidaria remain somewhat unclear, functions in the regulation of behaviour seem likely. Additionally, acid-sensing channel 4 which was also up-regulated in *Porites* colony Pd under competition, is another possible behaviour regulator, as its hydra homolog is involved in feeding behaviour (Assmann et al., 2014).

A gene annotated as a histamine receptor, which was down-regulated in *Porites* under competition, has been implicated in the discharge of nematocysts that is directly related to polyp behaviour (Kass-Simon and Pierobon, 2007b). The up-regulation of neuropeptide FF observed in corals under competition (Table 4.7) was considered to also be related with polyp behaviour. Rfamides have various roles in cnidarian nervous systems, including the perception of photo-stimuli able to modify larval behaviour (Katsukura et al., 2004; Plickert and Schneider, 2004; Watanabe et al., 2009) and neuropeptide FF is involved in the regulation of Rfamides neuropeptides in man (Bray et al., 2014).

Another GPCR up-regulated in competition was latrophilin-3 (Table 4.7), previously identified in *Nematostella vectensis* (Krishnan and Schioth, 2015). Although its role in Cnidaria is unknown, the human protein has been shown to regulate the number of synapses in neuron cultures (O'Sullivan et al., 2012), and could have a similar function in cnidarian nervous systems.

Homologs of two genes involved in controlling rhythmic behaviour were differentially expressed in competing corals; the human glycoprotein hormone receptor (LGR4) and casein kinase-1 were down and up-regulated respectively (Table 4.7). An LGR4-like glycoprotein hormone receptor has previously been identified in *Anthopleura elegantissima* (sea anemone), but its function is unknown (Vibede et al., 1998). Homologs of casein kinase-1 are implicated in circadian gene regulation in a wide variety of animals, including corals (Bhattacharya et al., 2016), and appear to also be involved in circatidal regulation in *Aiptasia* (Sorek et al., in press).

Finally, three clusters from the DEG list were annotated as hemicentin, and were up-regulated in competition (Table 4.7). Cluster-65721.42025 best BLAST was against hemicentin-1,thise *Porites* sequence had fascin (e-value=2.7e-9) and thrombospondin type 1 (e-value=1.14e-8) domains. Previous work on corals has implicated hemicentin-1 as an EMC protein involved in cell adhesion and skeleton attachment (Bertucci et al., 2015; Drake, 2015; Goldberg, 2001; Ramos-Silva et al., 2013), but it has also been associated with immune recognition in symbiosis establishment (Schwarz et al., 2008) and is expressed at lower levels in disease susceptible colonies of *A. millepora* (Wright et al., 2017).

Like Cluster-65721.42025, an uncharacterized protein containing fascin and thrombospodin domains is expressed in the hypostome of *Hydra vulgaris,* a region which has organizer-like properties (Hamaguchi-Hamada et al., 2016), suggesting the possibility of other roles than immunity or cell adhesion for the *Porites* protein.

Table 4.7: Genes differentially expressed in *Porites* under competition and related with coral behaviour Blue and red correspond to genes down and up-regulated respectively. "Biological characteristic" was assigned considering the Best blast hit annotation and the NCBI domain functions.

Biological characteristic	Cluster ID	Best BLAST hit	Protein name	E-value	log2 fold change	padj
GPCR	Cluster-65721.18038	5HT2A_CRIGR	Serotonin receptor 2A	2.4E-17	-2.45	6.2E-02
GPCR	Cluster-65721.3350	LGR4_HUMAN	Leucine-rich repeat-containing G-protein coupled receptor 4 (glicoprotein hormone receptor)	1.2E-132	-1.42	7.8E-02
GPCR	Cluster-65721.23813	HRH2_PONPY	Histamine H2 receptor (Gastric receptor I)	1.6E-19	-1.83	1.2E-02
GPCR	Cluster-57352.0	NPFF2_HUMAN	Neuropeptide FF receptor 2	5.1E-51	3.58	5.3E-02
GPCR	Cluster-58555.1	AGRL3_BOVIN	Latrophilin-3	1.2E-31	2.30	1.6E-02
GPCR	Cluster-65721.37966	OX2R_RAT	Orexin receptor type 2	1.4E-42	3.85	3.6E-02
GPCR	Cluster-51347.0	OX2R_RAT	Orexin receptor type 2	1.4E-42	3.48	4.2E-02

Biological characteristic	Cluster ID	Best BLAST hit	Protein name	E-value	log2 fold change	padj
Sensory / Nervous	Cluster-69557.2	ASI4A_DANRE	Acid-sensing ion channel 4	2.3E-27	4.37	3.6E-02
Sensory / Nervous	Cluster-65721.42025	HMCN1_HUMAN	Hemicentin-1	1.2E-07	2.55	6.1E-02
Sensory / Nervous	Cluster-65721.27743	STX1B_SHEEP	Syntaxin-1B	1.5E-106	3.12	9.6E-02
Sensory / Nervous	Cluster-55143.1	KC1D_RAT	Casein kinase I isoform delta	0.0E+00	3.83	9.6E-02

4.3.5.3 Genes involved in resistance to cellular damage

Autophagy related genes

Five transcripts associated with autophagy and/or lysosomal vesicles were up-regulated in corals under competition (Table 4.8). Autophagy is inhibited/prevented by TOR; conversely therefore, when TOR is inactivated the autophagy pathway will be activated. Two possible inhibitors of TOR, KICSTOR and phosphatidylinositol phosphatase (PP), were up-regulated suggesting that autophagy was activated in competing corals.

KICSTOR has been found to inhibit TORC1 in mammalian cells (Yao et al., 2017), and its cnidarian homolog may enhance autophagy in competing *Porites* nubbins. A second candidate activator of autophagy is phosphatidylinositol phosphatase (PP), whose metabolic role is cleavage of a phosphate group from phosphatidylinositol (PI). Up-regulation of PP will decrease levels of PI in the cells, decreasing the availability of the substrate for phosphatidylinositol kinase (PK). Inactivation of PK induced autophagy in yeast and *Hydra* (Chera et al., 2009; Noda and Ohsumi, 1998), and starving it of substrate (PI) presumably has the same effect. The observed up-regulation of a PP homolog in *Lobophytum* might therefore indirectly permit activation of autophagy.

Lysosomes are essential for protein degradation in the final steps of autophagy. The SID1 transmembrane protein has been associated with the reduction of lysosomal organelles in mammals. The down-regulation of SID1 on corals under competition (Table 4.8) could have enhanced lysosomal presence in competing corals and reinforced autophagy (Beck et al., 2017; Jialin et al., 2010; Nguyen et al., 2017). The formation and detection of lysosomes are essential to maintain organelle integrity and autophagy process (Yao et al., 2017). The positive regulation of genes related with lysosome membrane integrity provides additional evidence of a possible activation of autophagy in competing samples (Table 4.8).

4.3.5.4 Immune genes related to stress-resisting genotypes

The gene expression analyses showed eight genes classified as immune activators to be down-regulated and protein NLRC3 (considered an immune suppressor) to be up-regulated in competition (Table 4.8). Counterintuitively, there are papers that imply that low-mortality or disease-tolerant coral colonies are relatively unresponsive at the immune level (Wright et al., 2017), whereas highly responsive individuals may be particularly susceptible. These reports

suggest that the apparent suppression of immunity observed here may actually be a survival strategy on the part of competing *Porites*.

In this context, it is interesting to note that allene oxide synthase-lipoxygenase (AOSL), which was strongly up-regulated in *Acropora cervicornis* colonies during a white-band disease outbreak that caused extensive mortality (Libro et al., 2013), was down-regulated in *Porites* in competition (Table 4.8).

A hemicentin-1 was down-regulated in disease-resistant corals (Wright et al., 2017), while a hemicentin-2 up-regulated in thermally tolerant colonies (Barshis et al, 2003). The respective down- and up-regulation of homologs of hemicentin-1 (Cluster-65721.41772) and hemicentin-2 (Cluster-65721.13816) in corals under competition might similarly indicate resistance or tolerance to soft coral competition (Table 4.8). The two hemicentin-1 homologs in *Porites* had differences in domain composition and different regulation; which could probably mean that functions for this annotations should not be considered the same without analysis protein domains.

Catalase was down-regulated in *Porites* under competition (Table 4.8). Catalase has been found to be up-regulated to avoid harmful concentrations of reactive oxygen species (ROS) as a consequence of melanin production to fight pathogens or protect injured tissue to be infected (Wright et al., 2017).The up-regulation of catalase is positively correlated to the immune response of corals to stress (Mydlarz and Palmer 2011; Mydlarz et al 2016; Moya et al 2012). Nevertheless, low catalase production has been related to low-mortality in *Acropora millepora* and to colonies with an overall low sensibility to infection (Wright et al., 2017). Therefore, a down-regulation of catalase in competition could imply that the corals were controlling their immune reaction.

At least three pro-apoptotic genes were down-regulated, and a homolog of the adenosine receptor A2 (a candidate anti-apoptotic protein) was up-regulated in competing corals, suggesting suppression of the apoptotic process (Table 4.8). Whilst a tumour necrosis factor receptor (TNR)-related gene was up-regulated in competition, and some mammalian TNFRs are triggers of apoptosis, many TNFRs are present in hard corals and no roles have been assigned to them. In fact a TNFR has been found to be up-regulated in stress resisting corals (Mydlarz et al., 2016).

Finally, a coral homolog of the dmbt1 gene (deleted in metastasis brain tumor 1) was up-regulated in nubbins in competition (Table 4.8). Dmbt1-related genes have been associated

with the establishment and regulation of symbiosis in marine invertebrates (Neubauer et al., 2016; Wright et al., 2017). Dmbt1 has been found up-regulated in resistant colonies of *A. millepora* challenged with a bacterial infection; or in control corals when compared to susceptible colonies (Wright et al., 2017). This gene has an important function in coral immunity, and it has been suggested that it might play a role in symbiotic relationship with *Symbiodinium* (Wright et al., 2017). Additionally, dmbt1 has been found to be down-regulated in *A. digitifera* larvae when infected with *Chromera* (Mohamed et al., 2018). The coral response to *Chromera* is essentially hostile (Mohamed et al., 2018), thus the down-regulation of dmbt1 is consistent with roles in symbiotic recognition and coral immunity (Mohamed et al., 2018). Interestingly, dmbt1 was also up-regulated in *Lobophytum* competing with colony Pd and in *Lobophytum* immune challenged with MDP (Chapters 2 and 3).

Table 4.8: Genes differentially expressed in *Porites* under competition that have been shown to be differentially expressed in bleached and disease-resistant corals in the literature or that might have a role in controlling the negative effects of competition. Blue and red text indicate genes down and up-regulated respectively. "Biological characteristic" was assigned considering the best BLAST hit and NCBI domain functional annotation.

Biological characteristic	Cluster ID	UniProt ID	Protein name	E-value	log2 fold change	padj
Autophagy	Cluster-65721.7813	ANAG_HUMAN	Alpha-N-acetylglucosaminidase	0.0E+00	0.81	3.7E-02
Autophagy	Cluster-65721.16456	CL066_HUMAN	KICSTOR complex protein	6.5E-117	2.26	2.9E-02
Autophagy	Cluster-52076.0	HPS1_HUMAN	Hermansky-Pudlak syndrome 1 protein	2.3E-74	3.54	3.3E-03
Autophagy	Cluster-65721.11203	PTPRQ_MOUSE	Phosphatidylinositol phosphatase PTPRQ	2.2E-99	3.62	6.4E-02
Autophagy	Cluster-65721.43695	DIRC2_XENLA	Disrupted in renal carcinoma protein 2 homolog	1.1E-78	4.35	3.7E-02
Autophagy/ immunity activation	Cluster-59959.0	SIDT2_HUMAN	SID1 transmembrane family member 2	1.7E-12	-1.57	3.9E-02
Immunity activation/antioxidant	Cluster-69901.1	AOSL_PLEHO	Allene oxide synthase-lipoxygenase protein	0.0E+00	-2.80	7.3E-08
Immunity activation	Cluster-33162.0	AVR7_CHICK	Avidin-related protein 7	1.7E-17	-2.55	1.0E-03

Biological characteristic	Cluster ID	UniProt ID	Protein name	E-value	log2 fold change	padj
Immunity activation	Cluster-49904.0	MBLC2_HUMAN	Metallo-beta-lactamase domain-containing protein 2	6.9E-70	-1.85	2.7E-02
Immunity activation	Cluster-65721.23340	RPC4_BOVIN	DNA-directed RNA polymerase III subunit RPC4	7.3E-29	-1.66	9.6E-02
Immunity activation	Cluster-61415.1	GATM_XENTR	Glycine amidinotransferase, mitochondrial	0.0E+00	-1.03	9.6E-02
Immunity activation	Cluster-65721.41772	HMCN1_HUMAN	Hemicentin-1	1.8E-08	-0.79	2.9E-02
Immune Suppression	Cluster-65721.27799	NLRC3_MOUSE	Protein NLRC3	6.0E-40	2.42	1.6E-02
Immunity/antioxidant	Cluster-31830.0	CATA_DROME	Catalase	0.0E+00	-3.57	7.9E-03
Apoptosis	Cluster-65721.13816	HMCN2_HUMAN	Hemicentin-2	1.3E-12	2.11	2.6E-02
Pro-apoptotic	Cluster-65721.31170	UQCC1_XENLA	Ubiquinol-cytochrome-c reductase complex assembly factor 1	4.5E-50	-3.00	5.3E-02
Pro-apoptotic	Cluster-58194.2	DNAS1_OREMO	Deoxyribonuclease-1	2.5E-60	-1.90	6.0E-03

Biological characteristic	Cluster ID	UniProt ID	Protein name	E-value	log2 fold change	padj
Pro-apoptotic	Cluster-63940.0	CLSPN_HUMAN	Claspin	2.3E-39	-1.84	1.1E-02
Pro-apoptotic/ immunity	Cluster-65721.30303	TNR6_HUMAN	Tumor necrosis factor receptor superfamily member	1.9E-06	2.71	3.3E-02
Symbiosis/immunity	Cluster-65721.5619	DMBT1_HUMAN	Deleted in malignant brain tumours 1 protein	2.8E-71	1.13	7.4E-02
Symbiosis/immunity/ anti-apoptotic/GPCR	Cluster-59651.0	AA2AR_CANLF	Adenosine receptor A2a	6.5E-16	3.66	5.5E-02
Signalling	Cluster-57694.1	I5P1_HUMAN	Type I inositol 1,4,5-trisphosphate 5-phosphatase	2.9E-89	-3.32	1.9E-08
Vesicle	Cluster-65721.31362	SAR1B_BOVIN	GTP-binding protein SAR1b	2.3E-105	-1.49	1.0E-03
Vesicle	Cluster-65721.30552	SBP1_RAT	Selenium-binding protein 1	2.0E-133	-0.91	5.9E-02

4.3.5.5 Genes potentially involved in the aggressive response to the interaction

As mentioned above, *Porites* colonies showed signs of aggressive behaviour. The enhancement of production of potential toxins could be associated with an aggressive response. Alpha-*N*-acetylglucosaminidase (N-acetyl-alpha-glucosaminidase) and small cysteine-rich protein 2 (Amil-SCRiP2) were up-regulated in *Porites* nubbins when competing with *Lobophytum.* Amil-SCRiP2 is a well-studied toxin that has been found in hard corals tissue (Jouiaei et al., 2015a).

4.4 Discussion

This study investigated, for the first time, the molecular responses of a hard coral to distant competition at a transcriptomic level. Non-contact competition between *Porites* and *Lobophytum* altered the behaviour of the hard coral and the expression of genes related to it. Comparative analysis gave insights into how hard corals modified the expression of genes involved in cellular stress responses and immunity to resist the challenges of non-contact interaction.

Competition with the soft coral *Lobophytum* induces in *Porites* a transcriptomic response similar to what has been shown in hard corals exposed to environmental stressors (Bellantuono et al., 2012; Davies et al., 2016; Oakley et al., 2017). Markers of cellular stress including genes involved in ubiquitination, oxidative stress responses, apoptosis, and production of mucus were enhanced in competing *Porites*, suggesting that non-contact competition was causing a cellular stress response (CSR).

Ubiquitination is an evolutionarily conserved pathway that cells use to flag damaged or toxic proteins that need to be destroyed to avoid more extensive cellular damage. Genes involved in ubiquitination in this study have also been found to be up-regulated in corals under chemotoxic attack (contact competition with algae), heat stress, or where corals were immune challenged (Hahn et al., 2004; Shearer et al., 2012; Wright et al., 2017). This suggests that non-contact competition might be as stressful for coral cells as any of the other stressors mention above.

Porites under competition seemed to be increasing the production of antioxidants, such as peroxidasin. Peroxidasin has been found to be up-regulated under heat stress in both larval and adult corals (Libro et al., 2013; Louis et al., 2017; Voolstra et al., 2009). Peroxidasin also functions in containing post-apoptotic damage, suggesting that its up-regulation in competition serves to contain damage to *Porites* cells caused by harmful soft coral chemicals (Nelson et al., 1994).

Apoptosis is a conserved immune defence mechanism that serves to limit the extent of damage resulting from injury or cellular insult (Clarke et al., 2005; Kaniewska et al., 2012; Moya et al., 2016). I hypothesise that apoptosis was induced during the interaction with *Lobophytum* to remove *Porites* cells that were damaged by the soft coral toxins in order to avoid necrosis and more extensive tissue loss.

The observed up-regulation of genes involved in mucus production suggests that competing hard corals were probably using mucus to protect themselves against soft coral attack. Increases in mucus production were not visually apparent in the daily observations of competing corals, but several studies have reported increased mucus production in corals under stress (Bythell and Wild, 2011), leading us to hypothesise that molecular signatures of increased mucus production are an additional sign of cellular stress.

Competing *Porites* nubbins were under cellular stress, which might have triggered changes in their behaviour. *Porites* nubbins interacting with *Lobophytum* displayed decreased polyp activity, possibly in an attempt to protect tissue from chemical attack. To withdraw the polyps and "pack" them inside the skeleton is a behaviour frequently used by corals to protect tissue from potential predators or physical damage. In the context of non-contact competition, closing the polyps or having them partially extended from the corallites effectively reduces the surface area exposed to the soft coral toxins. The reduction of *Porites* polyp activity may therefore be a behavioural strategy, supported by changes in expression of several genes potentially involved in polyp activity, including casein kinase-1 and a glycoprotein hormone receptor; both implicated in the rhythmic control of behaviour in a wide variety of organisms. Circadian rhythm genes influence many cellular processes in corals, including feeding and therefore polyp activity (Bertucci et al., 2015). Changes in the expression of genes involved in rhythmic behaviour might also explain why polyps were less active under competition.

The observed differential expression of GPCRs (see above) may also underlie changes in *Porites* polyp behaviour. Differential expression of orexin might be particularly significant in this context, possibly participating in keeping *Porites* polyps closed or less active than controls by inhibiting feeding responses (Grimmelikhuijzen et al., 1980; Hamaguchi-Hamada et al., 2016; Rosenberg et al., 2017; Watanabe, 2017; Watanabe et al., 2009). Experimental studies in *Hydra* indicating that amiloride delays the feeding reaction, implicated acid-sensing ion channels (ASICs) in feeding behaviour. The observed up-regulation of an homolog of ASIC in *Porites* under competition is consistent with a role for ASICs in control of polyp activity in corals (Assmann et al., 2014; Osmakov et al., 2013; Rahman and Smith, 2014).

Differential expression of many genes implicated in behavioural changes in other organisms implies that non-contact competition triggers behavioural changes in *Porites* alongside the cellular stress response.

The negative effects of low polyp activity and of cellular stress in competing *Porites* were accompanied by activation of autophagy and suppression of immune responses. The use of autophagy is a common survival response activated in starvation scenarios (Bellantuono et al., 2012; Chera et al., 2009). While activation of this pathway has been shown in other cnidarians (Beck et al., 2017; Jialin et al., 2010; Nguyen et al., 2017), it has never previously been observed as a symptom of competition. It is important to point out that ubiquitination has also been related with directed autophagy in mammals (Dupont et al., 2010; Shaid et al., 2012). Therefore, the up-regulation of genes implicated in ubiquitination might also be a response to the low polyp activity and possible starvation of *Porites* under competition.

In the non-contact competition scenario, *Porites* did not show signs of tissue loss or bleaching, suggesting that it might have mechanisms to resist soft coral attack. The observed differential expression of "resistance related genes" (Table 4.8) supports the idea that immune reactions and inflammation were supressed in competing corals (Libro et al., 2013).

Suppression of immune and inflammatory response genes has been implicated in the resistance of corals to environmental stressors (Barshis et al., 2013; Wright et al., 2017). Under the present scenario, damage to *Porites* tissue inflicted by *Lobophytum* might be expected to activate immune reactions, and limiting the strength of these responses might be a strategy of the hard coral to tolerate the stressor (competition).

As mentioned earlier, several genes nominally associated with apoptosis were differentially expressed in *Porites* under competition; while this could be interpreted as a simple damage response, it could also be regarded as symptomatic of resistance to stress, as has been suggested by (Mydlarz et al., 2016)(Table 3.6). Other studies have found that bleaching resistant coral colonies may effectively block apoptosis (Ainsworth et al., 2007; Libro et al., 2013; Pinzón et al., 2015), which cannot be ruled out in the present case.

In summary, the complexity of the responses to competition in terms of apoptosis-related genes is difficult to interpret, but may enable *Porites* to contain potentially damaging effects of competition.

Control of the apoptotic process could also explain the absence of visual signs of tissue damage in corals which exhibited symptoms of stress at the molecular level.

Other studies on soft and hard coral competition have recorded that bleaching is generally one of the first indications that zooxanthellate corals (both scleractinians and octocorals) are under stress (Alino et al., 1992, 1992; Sammarco et al., 1983), but was not observed in the present

case. Dmbt1 and adenosine receptors have previously been implicated in the establishment and maintenance of symbiosis in corals (Mohamed et al., 2018; Neubauer et al., 2016; Wright et al., 2017), and the up-regulation of these genes observed under competition might underlie the absence of bleaching in the present study.

All of the above imply that *Porites* nubbins responded to competition using defensive mechanisms, essentially avoiding or limiting tissue damage. Additionally, behavioural data showed that *Porites* competing increased their polyp activity over time and after day 30 (when the samples analysed here were taken), indicating that recovery was underway. By contrast, observational and behavioural data indicate aggression from *Porites* towards *Lobophytum*. The most obviously aggressive behaviour was observed in the case of *Porites* colony d, where mesenteric attacks were being detected at 23, 25, 26 and 41 days in the competitive scenario. Despite the obvious aggression, in colony d, polyp activity was affected more strongly by competition than were the other two colonies. These results suggest that by day 30, *Porites* d may have passed a defence / offense threshold – essentially transitioning from a phase of damage control to a more aggressive mode. Consistent with it being the most aggressive genotype, *Porites* d was the only colony to stimulate a strong reaction in the majority (four of five) of *Lobophytum* colonies it was exposed to (Chapter 3). The interaction between *Porites* d and *Lobophytum* colonies is explored at greater length in the General Discussion (Chapter 5).

During the course of the experiment, *Porites* colony f also deployed mesenteries to attack *Lobophytum*, but this was observed only after day 30 of interaction; this colony responded more slowly (possibly passing the hypothetical defence / offense threshold later), but in essentially the same way as colony d (Table 4.4). These results illustrate the complexity of competitive interactions and the difficulty of predicting or interpreting outcomes. Whilst *Porites* f had no detectable effect on *Lobophytum* colonies, gene expression analysis demonstrates that *Porites* d and *Porites* f had the same gene expression profile when exposed to *Lobophytum*. These results suggest that, whilst both *Porites* colonies were attacked by the soft coral, at the time point chosen, only *Porites* d affected the soft coral.

One clear molecular signature of aggression on the part of competing hard corals was up-regulation of small cysteine-rich protein 2 (Amil-SCRiP2). Whilst the SCRiPs were originally thought to function in calcification, recent work (reviewed in Jouiaei et al., 2015b) shows that they are potent neurotoxins. The expectation is that up-regulation of SCRiPs reflects increased production of nematocysts or gland cells (Jouiaei et al., 2015b) , these presumably being required for an aggressive attack that has not previously been described in hard corals.

However, if the GPCRs down-regulated in the present study really are associated with nematocyst discharge, up-regulation of Amil-SCRiP2 might reflect a non-nematocyst function such as an allelopathic agent secreted by gland cells (Columbus-Shenkar et al., 2018). So far there are no reports of hard corals using allelopathy in competitive interactions but toxic chemicals have been found in hard coral tissues (Chadwick and Morrow, 2011). It is difficult to know whether there are no reports of allelopathy in scleractinians because they do not use this strategy or because the symptoms (tissue damage) have always been assumed to be associated with mesenteric or cnidocyte attack. In this case, the strong reaction from the *Lobophytum* colonies to *Porites* d might be associated with up-regulation of this toxin.

The presence of *Lobophytum* initially suppressed polyp activity in *Porites* nubbins, and the gene expression profiles of *Porites* when competing with *Lobophytum* resembled those of other hard corals following exposure to environmental stressors. Competition appeared to trigger a cellular stress response in the hard coral nubbins, but immunity and bleaching were suppressed, and polyp activity increased later in the experiment. The gene expression activated by *Porites* under competition was comparable to the one of bleaching or disease resistant corals. Some *Porites* colonies deployed mesenteries to attack *Lobophytum*, but the timing of this behaviour differed amongst colonies, Porites d being the first to initiate such a response. The results illustrate the complexity and heterogeneity of competitive interactions involving cnidarians, and the challenges posed to unravelling their molecular bases

Chapter 5 - General Discussion

Colony response diversity

Data presented throughout the three data chapters (chapters 2, 3 and 4) illustrate the diversity of responses shown by both the soft coral *Lobophytum* and the hard coral *Porites*. Two types of colony variation were detected overall: a quantitative and a qualitative variation.

The quantitative variation type between colonies caused differences in gene expression levels (number of counts of a particular transcript) but did not affect the direction of gene expression response (gene up-or down-regulated because of the treatment) of the colonies. For example, *Lobophytum* colonies affected by *Porites* colony Pd responded to competition by up- or down-regulating the same genes across colonies, but the levels of expression of these genes were variable between colonies (Chapter 3, Figure 3.5).

Similar effects to those shown here (i.e. high variability in responses; effects of variation between colonies being much greater than effects of treatment vs control) have been observed in a number of RNAseq experiments conducted on adult corals. For example, Bertucci et al. (2015) encountered more variability between colonies than between treatments and the corresponding controls when studying gene expression differences between day and night in *Acropora millepora*. The authors overcame the difficulties of modelling such variation by using two programs to find DEG: sSeq (Yu et al., 2013), which enables analyses of small (n=3, in this case) data sets (but sometimes results in false positives), and EdgeR (Robinson et al., 2010), which uses a more conservative approach to find DEG (Bertucci et al., 2015). The combined analyses allowed identification of 497 genes differentially expressed between day and night, but the overlap between the EdgeR and sSeq datasets was relatively low (13% of the 497 DEG). The Bertucci et al. (2015) study illustrates not only the extent of variability between colonies of the same coral species, but also that the problem is not necessarily intractable - statistical programs might have the capacity to overcome the variation. Whilst qualitative variation between the *A. millepora* colonies cannot be completely discounted, it is unlikely that colonies differ qualitatively in terms of the genes expressed in response to the diurnal day/night cycles. One factor that may have significantly contributed to the limited overlap between the DEG datasets is the difficulty of including colony variation in models when only three biological replicates are available.

Whilst other influences (including history of exposure to biological and/or physical challenges) undoubtedly contribute to the observed variation in responses, the high levels of polymorphism known to characterise a number of coral species (Torda et al., 2017) is presumably a major factor driving this variation. Despite the heterogeneity in responses being well-documented, individual variability has typically been overlooked and/or ignored in coral genomics and ecological studies (Ainsworth et al., 2007; Horwitz et al., 2017; Weiss et al., 2013).

Observed variations in gene expression levels sometimes translate into differences in phenotypic responses (which was not the case for Bertucci et al (2015)). Barshis et al. (2013) analysed differences in gene expression patterns between *Acropora hyacinthus* colonies from thermally resilient populations (HV) and from more sensitive populations (MV) when exposed to heat stress. One key finding of that study was that the HV corals had higher baseline levels of expression of key genes than did the more susceptible MV colonies, suggesting that this "front loading" effect might explain why HV corals manage to survive natural bleaching events (Barshis et al., 2013). This study demonstrates the importance of considering colony quantitative variation in "treatment vs control" experiments to better understand coral biology.

Chapter 4 illustrates the importance of taking into account this type of variation in coral interaction studies. In fact, intraspecific variation was also observed between the three *Porites* colonies used to challenge *Lobophytum* explants in the competition experiment. Only one of the two *Porites* colonies (Pd) for which sequence data are available induced a strong and consistent response in *Lobophytum*, affecting four of the five *Lobophytum* individuals. These two colonies of *Porites* (Pd and Pf) had qualitatively similar but quantitatively different responses in competition, up-and down-regulating the same genes but with different levels of expression. The observed quantitative differences were presumably the reason why *Porites* colony Pd effected responses from four of the *Lobophytum* colonies whereas *Porites* colony Pf did not. The higher responsiveness on the part of *Lobophytum* to Pd than to other *Porites* colonies was reflected in lower polyp activity data, colony Pd displaying lower polyp activity compared to the other two colonies (Chapter 4; Fig. 4.5). Note that the competitive impact of *Porites* Pd in this scenario was statistically detectable only due to the level of biological replication employed - each of the (three) *Porites* colonies was exposed to five different *Lobophytum* colonies - and the fact that colony effects were taken into account in the model (Chapter 4, table 4.2).

Molecular work on corals typically features only limited biological replication (Bertucci et al., 2015; Sammarco et al., 1985; Vidal-Dupiol et al., 2009). The results presented here highlight

the need for appropriate levels of biological replication, and the importance of accommodating genotypic variation. Generalisations about how coral species might respond to a stressor cannot be based on simple "treatment vs control" experiments on a single genotype (Vidal-Dupiol et al., 2013).

The second type of difference between colonies was qualitative variation (i.e. different genes responding in different colonies) rather than quantitative. In chapter 2 – Immune challenge, genes up-and down-regulated by one group of colonies (Group1) differed from those differentially expressed in the second set of colonies (Group2). Qualitative variability in responses between *Lobophytum* colonies was a major difficulty when attempting to identify a generic (or typical) response of the organism to MDP treatment. In fact, classical "treatment vs. control" models, in which data for each of the colonies were pooled, were unable to identify consistent differences in gene expression in MDP treated *Lobophytum* samples compared to the controls, between individual variation swamping treatment effects. A more complex model, using groups based on hypothetical genotypes, permitted identification of some genes that were consistently differentially expressed between treatments and the corresponding controls in Group2 *Lobophytum* individuals (Chapter 2, table 2.4). No consistent differential expression could be detected in Group1 individuals, presumably because of high heterogeneity within the group (Chapter 2, figure 2.3). Results presented in Chapter 2 illustrate once again the importance of biological replication, and of looking for consistent patterns within subsets of biological replicates. An example of the variability within the same coral species is that of Wright et al (2017), who found different responses to the same stressor (bacterial challenge) in *Acropora millepora* colonies. In this study, a single group of *A.millepora* colonies showed changes in gene expression when comparing treatment vs control (unchallenged colonies); whilst another set of colonies were unresponsive to the treatment (Wright et al., 2017).

Consideration of the results presented in this thesis and some of the recent literature implies that a real understanding of the molecular responses of corals will require a change of approach that involves increasing biological replication and an end to the practices of pooling samples and eliminating outliers without adequate consideration about how informative they can be.

The genotype might define "winners" and "losers"

In the coral ecology literature, the terms “winner” and “loser” have been extensively used in the context of relative sensitivity of species to climate change (Fabricius et al., 2011; Loya et al., 2001). Several studies imply that there are also “winner” and “loser” genotypes within a species (Barshis et al., 2013; Wright et al., 2017). “Winners” are species or genotypes that resist and survive the stressor (e.g. bleaching, heat-stress, disease), while “losers” are those that do not. The definition of winners might also take into account resistance to subsequent stress events (Hughes et al., 2017b), as in the case of some survivors of the 2015 mass bleaching event not surviving the 2016 bleaching event (Hughes et al., 2018).

Some of the genes differentially expressed in *Lobophytum* after MDP treatment appear to have homologs that show similar expression characteristics in bleaching and/or disease-resistant corals (Bellantuono et al., 2012; Libro and Vollmer, 2016; Palumbi et al., 2014; Reed et al., 2010; Vollmer and Kline, 2008); this similarity was also observed for *Porites* nubbins under non-contact competition (chapters 2 and 4 respectively). These results suggest the possibility of similar strategies of stress tolerance in octocorals and scleractinians. However, given the limited number of species that have been studied to date, such generalisations may be premature.

5.1.1 The nervous and immune systems work together to maintain coral health

Not only were genes differentially expressed in corals subjected to either MDP-immune challenge or competition, but clear behavioural responses were also observed. Under competition, *Porites* displayed less polyp activity than controls, and the differential expression of several nervous system-related genes suggests some involvement in this change of behaviour (Chapter 4, Figure 4.4 and 4.5).

It is necessary to take a step back and understand how this complex behaviour could have been triggered. The first step of the competition hypotheses presented in Chapters 3 and 4 was recognition of a potential threat (Figure 3.2 and Figure 4.1). This first step is likely to be a cellular stress response and, under the schemes presented as Figs 3.2 and 4.1, this alerts the immune system to activate a series of cellular processes that will lead to a change in behaviour. Connection and coordination between the immune and nervous systems appear vital for coral

behaviour and a critical element of non-contact competition as these might determine the outcome of the interaction. In fact, studies on coral competition regularly mention that soft corals reshape their bodies to move away or toward the enemy (Hennessey and Sammarco, 2014; La Barre and Coll, 1982)

Connection between the nervous and the immune systems in *Lobophytum* was implied by the results of the immune challenge experiment (Chapter 2). Differential expression of genes known to be involved in cross-talk between the immune and nervous systems in other animals (e.g. NOS, pentraxin and agrin; see Chapter 2, table 2.4) supports the idea that this cross-talk also occurs in *Lobophytum* (Anctil et al., 2005; Bosch et al., 2017; Ross, 2014; Trautmann and Vivier, 2001).

Lobophytum samples from the competition experiment differentially expressed both genes associated with tissue remodelling in other organisms, and genes likely to be associated with a non-specific immune response (Chapter 3, table 3.3 and 3.4). Again, based on the soft coral competition hypothesis presented in Chapter 3 (Figure 3.2), the most likely succession of events might be that the immune activation was the starting point of a complex nervous response that leads to behaviour.

Changes in coral behaviour during competition have previously been observed (Hennessey and Sammarco, 2014; Sammarco and Coll, 1992, 1990), but the underlying cellular mechanisms are unknown/have not been investigated. Recent work suggests that cross-talk between the immune and nervous systems primarily functions in maintenance of an appropriate microbiome, and that all three components together are responsible for maintaining the animal in good health (Bosch, 2013; Cryan and Dinan, 2014, 2014).

With few exceptions, coral behavioural biology has been in limbo for too long, and research to better understand how stressors affect behaviour is urgently required.

Non-contact competition between Lobophytum and Porites: a hypothesis

In chapter 3 and 4, the transcriptomic responses of *Lobophytum* and *Porites* reveal the cellular mechanisms that both species might use to react to non-contact competition after 30 days of interaction. Based on these results, a hypothetical model was developed (Fig 5.1) to account for what might have been occurring during the interaction.

Under the scenario presented, the interaction is initiated when secondary metabolites (SMs) that are normally released by *Lobophytum* reach *Porites* tissue (Figure 5.1(1)). Cytotoxic effects of the SMs on *Porites* then might trigger a cellular stress response (CSR), as described in Chapter 4, which subsequently might lead to other responses in *Porites*, including up-regulation of toxin expression (e.g. SCRiPs; Figure 5.1 (1)). The presence of *Porites* toxins could then alert the soft coral to the proximity of a potential threat (Figure 5.1 (2)).

The response of *Lobophytum* to cues or toxins from *Porites* results in up-regulation of general threat response genes (Chapter 3; figure 5.1 (2)). The activation of the *Lobophytum* immune system might subsequently activate a series of cellular pathways including the sphingolipid signalling pathways and the vesicle secretory machinery, potentially leading to increases in the production and release of SMs respectively (Chapter 3; figure 5.1(2)).

After 30 days of interaction, attack by *Lobophytum* caused *Porites* to decrease its polyp activity; activate autophagy to resist the chemical attack and showed a gene expression profile comparable to resisting corals (e.g. controlling immune reaction; Chapter 4; figure 5.1 (3)). The aggressive behaviour observed on the part of *Porites* (e.g. mesenteric filament attack or toxin expression) might be an indicator that the hard coral was not only resting but fighting back (figure 5.1 (3)). On the other hand, at the same time point, *Lobophytum* showed a high general immune response combined with the genes related to the regulation of the nervous system and many GPCR. These results imply that *Lobophytum* activated its immune defence mechanisms and that it was modulating *movement* and directionality (figure 5.1 (3)).

Porites polyp activity increased gradually in the duration of the experiment almost reaching control nubbins activities at the end of the 60 days of interaction (Chapter 4, figure 4.4 and 4.5). Two possible explanations for this are either that the hard corals managed to resist and overcome any toxic effects of *Lobophytum* chemicals, or that the attack by *Lobophytum* was weakened.

To be unresponsive to the interaction might not be convenient for corals in the case that the competitor represents an actual threat. However, to stop the effects of the interaction after assessing the danger might be a strategy use to save energy and may represent an initial step towards coexistence between two corals (Álvarez-Noriega et al., 2018; Chadwick and Morrow, 2011; González-Rivero et al., 2016).

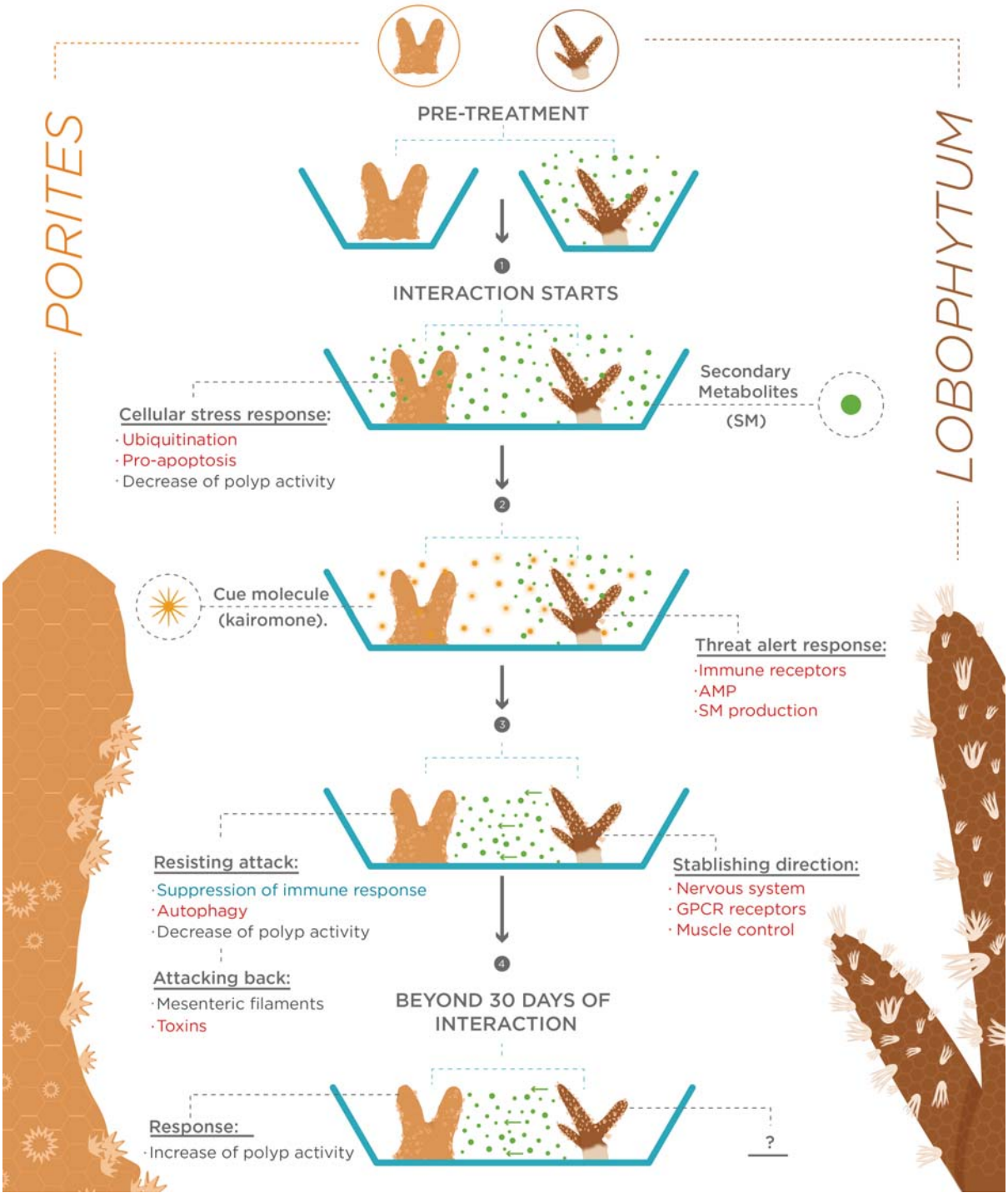

Figure 5.1: Hypothetical succession of cellular events that occurred during the 30 days of interaction between *Lobophytum* and *Porites* based on gene expression analyses described in chapters 3 and 4 and. Text in red and blue represent elements that were up-or down-regulated (respectively) in *Porites* or *Lobophytum* under non-contact competition compared to control.

Parallels between the impacts of environmental stressors and competition.

Results presented in Chapters 3 and 4 show that components of the response to non-contact competition resemble a cellular stress response. As mentioned in Chapter 1, contact competition between corals directly results in an immune reaction based on self- *vs* non-self-recognition (Buss et al., 2012; Rinkevich and Sakamaki, 2001) and it has been assumed that non-contact competition also triggers an immune reaction (Chadwick and Morrow, 2011). The present study provides some support for this - some evidence of an immune reaction was observed (eg. Immune related genes, Chapter 3, table 3.3). It seems that non-contact competition might provoke an imbalance in cellular function, triggering a cellular stress response (CSR) (Chapter 4, table 4.6) and it is plausible that this CSR also launched the immune response. This would mean that non-contact competition affects corals in similar ways to an environmental factor. In both *Lobophytum* and *Porites,* homologs of proteins involved in ubiquitination and apoptosis and with antioxidant properties were differentially expressed in the competition scenario (Chapters 3 and 4). These pathways have also consistently been shown to be differentially expressed in corals exposed to heat (Davies et al., 2016; Fitt et al., 2009; Louis et al., 2017; Oakley et al., 2017) and other environmental stressors (Aguilar et al., 2017; Evensen et al., 2015; Moya et al., 2012). The involvement of the same pathways in the coral responses to physical stressors and to competition suggests that climate change might have significant – possibly synergistic - effects on competition outcomes (Horwitz et al., 2017). A coral that is already under cellular stress and/or immuno-compromised by environmental factors might not survive competition. Conversely, cellular stress triggered by competition could effectively precondition a coral to resist environmental stressors (Carilli et al., 2012; Nyström et al., 2001) .

It is important to mention that, although the response to non-contact interaction is in some respects non-specific (e.g. general stress response), other aspects of the response are not. For example, the differential expression of genes likely to have nervous system-related functions is likely to be a specific reaction to competition. It will be interesting to investigate the mechanisms that link a general cellular stress reaction to more specific responses – possibly even behavioural changes.

Recommendations and future research

The most obvious requirement for future work is to provide a time dimension to the types of experiments described here. A major limitation in the interpretation of the data presented in this thesis is that they represent single time points in what are undoubtedly dynamic as well as complex responses. Sampling along time series would allow much greater insights into the molecular responses to competition and immune challenge. In fact, having information about the gene expression of an earlier time point in competition might help to support the idea that the first step of the interaction is triggered by cellular stress. Conversely, data of a later time point for the immune challenged experiment (Chapter2) could allow to define if the two groups of *Lobophytum* (Group1 and Group2) were responding with a different set of genes or if the two groups required different time to respond to the stimulus.

As mentioned above, it is important that genotypic diversity be given more consideration in experimental coral research. Although the transcriptomic analyses presented here imply substantial genotypic diversity amongst the experimental corals, the true extent of genotypic diversity is unknown. Future work of this type should include some measures of genotypic variation in the experimental material, and would ideally be based on defined genotypes and the genetic distance between them. The discovery of cryptic species within many classically defined coral "species" means that reliance on morphology in the identification of individual corals is inadequate (McFadden et al., 2010). Soft coral genomics is in its infancy, but robust genome assemblies would greatly facilitate the kinds of work described in this thesis.

Another important factor that was not taken into account here was potential for variation in the microbiome to contribute to the observed differences in gene expression of the corals. The contribution of the microbiome to animal health and homeostasis has been established in recent years (Bosch, 2013; McFall-Ngai et al., 2013), but to date the potential impact of microbiome symbiosis on experiments involving marine invertebrates has rarely been considered. Wessels et al. (2017) characterized the microbiome of *Lobophytum* using 16S sequencing and found this to be dominated by spirochaetes. However, more recent work (PhD study in progress at JCU) suggests substantial differences between soft coral individuals, and that spirochaetes may be essentially absent from many individuals. As spirochaetes are typically intracellular parasites, their presence/absence could have a substantial impact on the molecular

responsiveness of individuals. Hence it would be advisable to conduct microbiome analysis on individual corals selected for experimental work, particularly in the case of *Lobophytum*.

A better understanding of the secondary metabolite profile of *Lobophytum,* their biological activities and pathways involved in their production and secretion is needed.

Whereas work to date on coral competition has largely been based on observation, transcriptomic analysis is clearly a powerful tool for the investigation of the mechanisms underlying competitive interactions. Nevertheless, it is important that, as soon as possible, gene expression analyses be combined with classical physiological measurements. The results presented here illustrate the complementarity of behavioural (e.g polyp activity, Chapter 4) and molecular (e.g. differential expression of genes involved in autophagy and muscle contraction) data. It is not necessarily the case that different tools answer different questions; rather, the combination of tools can provide deeper insights than can either in isolation.

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Appendix A: Chapter 2

Table A. 1: Protein domain from differentially expressed genes in *Lobophytum* colonies from Group2-MDP

Cluster ID	Domain name	Domain e-value	Accession ccd
Cluster-30490.3	TRX family	2.67E-35	cd02947
Cluster-38295.0	An peroxidase	0.00E+00	pfam03098
Cluster-33002.5471	Globin like superfamily	2.30E-42	cl21461
Cluster-55251.0	An peroxidase	0.00E+00	pfam03098
Cluster-32573.0	PLN00192	6.22E-171	PLN00192
Cluster-24631.0	VWA	2.83E-43	pfam00092
Cluster-33002.5337	FReD superfamily	5.60E-06	cl00085
Cluster-39559.2	EFh	4.07E-10	cd00051
Cluster-33002.390	SR	2.30E-27	smart00202
Cluster-61829.0	CuRO 3 tcLLC2 insect like	2.01E-55	cd13905
Cluster-6158.3	Neuromodulin N super family	9.25E-07	.
Cluster-6158.4	Neuromodulin N super family	9.25E-07	.
Cluster-20146.0	Na Ca ex superfamily	1.22E-04	cl27511
Cluster-20146.4	Na Ca ex superfamily	1.22E-04	cl27511
Cluster-41331.0	Cadherin repeat	9.34E-16	cd11304
Cluster-27610.0	FA58C	2.83E-49	cd00057
Cluster-61500.0	GON domain is found in the ADAMTS	6.39E-63	.
Cluster-60630.0	GON	6.39E-63	pfam08685
Cluster-15490.6	TLD superfamily	4.47E-11	cl02144
Cluster-31038.0	SOX-TCF_HMG-box	1.49E-30	.
Cluster-52616.0	High Mobility Group (HMG)-box	3.05E-25	.
Cluster-56627.2	NOS oxygenase superfamily	0.00E+00	cl00254
Cluster-32814.5	NOS oxygenase superfamily	0.00E+00	cl00254
Cluster-36837.0	Phospholipase A2	5.69E-33	pfam00068
Cluster-28607.0	VWA	1.25E-32	pfam00092
Cluster-61948.29	VWA	1.14E-21	smart00327
Cluster-61948.13	VWA	1.14E-21	smart00327
Cluster-47263.6	VWA	6.81E-37	pfam00092
Cluster-42273.0	RecF/RecN/SMC N termi.l domain	5.24E-07	.
Cluster-21272.0	RT_like superfamily	1.06E-10	cl02808
Cluster-56145.5	RT_like superfamily	1.18E-10	cl02808
Cluster-1141.1	RT_like superfamily	4.66E-07	cl02808
Cluster-33002.6881	DUF4371 super family	1.06E-23	.
Cluster-42273.1	Down-regulated in metastasis;	2.38E-173	.
Cluster-38201.0	Forkhead domain	1.80E-47	.

Cluster ID	Domain name	Domain e-value	Accession ccd
Cluster-41317.1	EF-hand_7	8.77E-08	pfam13499
Cluster-41317.3	EFh	2.76E-10	cd00051
Cluster-41317.15	EFh	2.76E-10	cd00051
Cluster-1200.1	LamG superfamily	1.30E-39	cl22861

Table A. 2: Literature related with DEG in *Lobophytum* colonies from Group2-MDP

Cluster ID	UniProt ID	Literature Cnidaria	Literature other organisms
Cluster-30490.3	THIO_PLAF7	Polato et al 2013	.
Cluster-38295.0	PERM_HUMAN	Mydlraz et al 2016	.
Cluster-33002.5471	NGB_CHAAC	.	Burmester and Hankeln 2002
Cluster-55251.0	PXDN_DROME	1.Voolstra et al., 2009; 2. Louis et al., 2017; 3. Libro et al 2013; 4. Burge et al 2013	Nelson et al 1994
Cluster-32573.0	ALDO2_ARATH	.	.
Cluster-24631.0	VWF_CANLF	Bythell and Wild 2011	.
Cluster-33002.5337	.	.	.
Cluster-39559.2	TNNC2_PELES	Leclere and Rottinger 2017	He et al 2017
Cluster-33002.390	DMBT1_HUMAN	1.Neubauer et al 2016; 2.Mohamed et al. 2018;3.	.
Cluster-61829.0	LAC4_THACU	1. Vidal-Dupiol et al 2014; 2. Palmer et al 2012	.
Cluster-41331.0	FAT4_MOUSE	1. Hemond et al 2014	.
Cluster-27610.0	SSPO_RAT	Schwarz et al 2008	.
Cluster-61500.0	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-61309.2	GLRA2_HUMAN	1. Watanabe 2017	.
Cluster-61309.1	GLRA2_HUMAN	.	.
Cluster-60630.0	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-15490.6	DLL1_RAT	1. Gahan et al 2017; 2. Layden and Martindale 2014	Riella et al 2011
Cluster-31038.0	SOX8_XENLA	Shinzato et al 2008	.
Cluster-52616.0	SOX9A_XENLA	Shinzato et al 2008	.
Cluster-56627.2	NOS1_RAT	1. Perez and Weis 2006; 2. Trapido-Rosenthal; 3. Kitchen and Weis 2017	.

Cluster ID	UniProt ID	Literature Cnidaria	Literature other organisms
Cluster-32814.5	NOS1_HUMAN	1. Perez and Weis 2006; 2. Trapido-Rosenthal; 3. Kitchen and Weis 2017	.
Cluster-36837.0	PA2GA_MOUSE	1. Talvinen and Nevalainen 2002; 2. Quinn et al 2017	.
Cluster-28607.0	CO6A6_MOUSE	Mandelberg et al 2016	.
Cluster-61948.29	MATN1_HUMAN	Bertucci et al 2015	.
Cluster-61948.13	MATN1_HUMAN	Bertucci et al 2015	.
Cluster-47263.6	FBN2_MOUSE	Reber-Müller et al 1995	Roberston et al 2011
Cluster-38201.0	FD4_DROME	Hayward et al 2015	Shimeld et al 2010
Cluster-41317.1	EFCB1_LOTGI	Hauck et al 2007	.
Cluster-1200.1	NPTX2_HUMAN	1. Ocampo et al 2015; 2. Bosch et al 2017	Davidson and Swalla 2002

Table A. 3: KEGG term retrieved from the UniProt ID found in as best BLAST in *Lobophytum* colonies from Group2-MDP

Cluster ID	Genome id	UniProt ID	BEST E-value	log2 fold change Grp2	padj Grp2	KO
Cluster-30490.3	s201_g51.t1	THIO_PLAF7	1.00E-26	-1.0	8.15E-02	K03671
Cluster-38295.0	s1081_g1.t1	PERM_HUMAN	1.00E-38	-6.5	8.38E-04	K10789
Cluster-33002.5471	s54_g32.t1	NGB_CHAAC	5.00E-18	-0.8	4.39E-02	K21893
Cluster-55251.0	s297_g19.t1	PXDN_DROME	3.00E-115	-5.4	2.73E-02	K19511
Cluster-32573.0	s603_g8.t1	ALDO2_ARATH	1.49E-117	-1.4	3.17E-02	K11817
Cluster-61309.2	s116_g41.t1	GLRA2_HUMAN	4.87E-53	-8.0	3.66E-04	K05194
Cluster-61309.1	s116_g41.t1	GLRA2_HUMAN	4.87E-53	-7.7	8.90E-04	K05194
Cluster-31038.0	s154_g28.t1	SOX8_XENLA	6.00E-35	-3.7	4.05E-04	K09270
Cluster-52616.0	s154_g27.t1	SOX9A_XENLA	3.00E-31	-1.8	1.12E-02	K18435
Cluster-56627.2	s127_g10.t1	NOS1_RAT	0	-1.3	2.05E-02	K13240
Cluster-32814.5	s127_g13.t1	NOS1_HUMAN	0	-1.1	2.61E-02	K13240
Cluster-36837.0	.	PA2GA_MOUSE	1.00E-24	-3.5	5.95E-04	K01047
Cluster-22544.2	s222_g31.t1	DDR2_MOUSE	9.00E-28	-0.9	9.44E-02	K05125
Cluster-28607.0	s503_g7.t1	CO6A6_MOUSE	4.00E-22	-0.7	4.14E-02	K06238
Cluster-42273.1	s7_g115.t1	UTP20_HUMAN	3.00E-138	-3.0	4.81E-02	K14772
Cluster-33002.3719	s104_g7.t1	POLX_TOBAC	9.00E-09	-1.0	8.81E-02	K16669
Cluster-38201.0	s34_g52.t1	FD4_DROME	1.00E-26	-2.2	7.85E-03	K09411
Cluster-24703.1	s178_g12.t1	CBPA4_MOUSE	4.00E-63	-1.3	9.44E-02	K08637
Cluster-30811.2	s369_g19.t1	CSMD2_HUMAN	1.00E-06	-1.0	8.37E-02	K17495
Cluster-24631.0	s367_g10.t1	VWF_CANLF	8.00E-81	2.8	1.16E-03	K03900
Cluster-33002.390	s345_g12.t1	DMBT1_HUMAN	3.00E-14	2.1	9.48E-02	K13912
Cluster-41331.0	s21_g17.t1	FAT4_MOUSE	1.00E-15	1.1	9.20E-04	K16669

Appendix B: Chapter 3

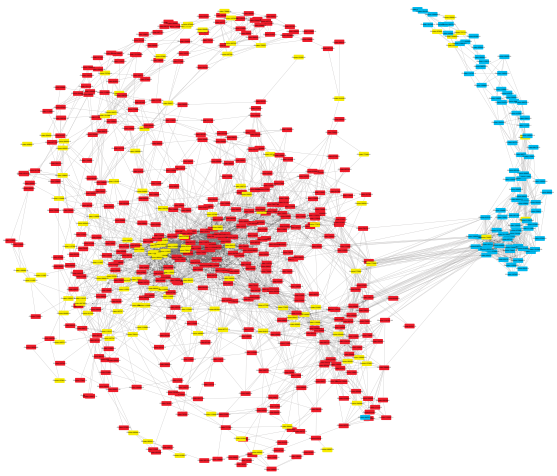

Figure B. 1: Network of co-expression of build with 1180 DEG between *Lobophytum*-Pd and *Lobophytum*-control. Red= DEG up-regulated and Blue= DEG down-regulated . Yellow= DEG with padj<0.1.

Table B. 1: Protein domain from differentially expressed genes in *Lobophytum* colonies interacting with *Porites* colony Pd

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-77355.1	PTKc	1.98E-109		cd00192
Cluster-77355.0	PTKc	1.98E-109		cd00192
Cluster-50735.0	PTKc	1.98E-109		cd00192
Cluster-60199.0	PTKc	1.98E-109		cd00192
Cluster-97243.0	PTKc	6.67E-76		cd00192
Cluster-67745.2	PTKc	5.09E-123		cd00192
Cluster-113066.5	RhoGAP super family	2.16E-59		cl02570
Cluster-113066.0	RhoGAP super family	2.16E-59		cl02570
Cluster-113066.2	RhoGAP super family	2.16E-59		cl02570
Cluster-32075.0	PKc_like super family	3.51E-178		cl21453
Cluster-59833.0	c-SKI_SMAD_bind	8.36E-42		pfam08782
Cluster-65058.1	PTKc	3.90E-79		cd00192
Cluster-81996.1	Peptidases S8	3.18E-155		cd04059
Cluster-62501.3	TSP1	2.89E-09		smart00209
Cluster-47635.1	vWFA super family	1.20E-127		cl00057
Cluster-65069.0	7tm_GPCRs super family	4.53E-92		cl28897
Cluster-29541.1	7tmB2_Adhesion	4.00E-59		cd15040

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-111496.0	7tmB2_Adhesion	8.45E-88		cd15040
Cluster-59857.0	VWA	1.25E-32		pfam00092
Cluster-115880.0	wnt	1.33E-121		pfam00110
Cluster-105540.0	CCCAP super family	2.00E-44		cl25735
Cluster-94675.0	Motor_domain super family	1.02E-133		cl22853
Cluster-107202.1	DUF229 super family	2.28E-30		cl27313
Cluster-97039.0	Forkhead	1.80E-47		pfam00250
Cluster-40909.0	Forkhead	3.34E-44		pfam00250
Cluster-102547.0	RFX_DNA_binding	7.05E-40		pfam02257
Cluster-87944.2	Glyco_hydro_79n super family	4.50E-28		cl04201
Cluster-87944.1	Glyco_hydro_79n super family	4.50E-28		cl04201
Cluster-64366.0	SH2 super family	5.57E-38		cl15255
Cluster-113528.0	ZnMc_astacin_like	1.94E-70		cd04280
Cluster-115783.0	FH2 super family	4.06E-73		cl19758
Cluster-113105.1	FH2 super family	4.06E-73		cl19758
Cluster-99480.1	Patched super family	7.29E-13		cl25655
Cluster-61510.0	PKc_like super family	5.91E-142		cl21453
Cluster-59123.2	CUB super family	2.59E-14		cl00049
Cluster-102615.1	DUF3585	7.42E-36		pfam12130
Cluster-56550.2	FERM_C_ERM	1.44E-71		cd13194
Cluster-94577.0	ANK	3.22E-13		
Cluster-22331.0	Neuromodulin_N super family	3.25E-09		cl26511
Cluster-40343.0	7tm_classA_rhodopsin-like	2.63E-24		cd00637
Cluster-95367.4	PTPc	9.52E-104		cd00047
Cluster-113490.0	FH2	6.00E-79		pfam02181
Cluster-52996.0	7tm_classA_rhodopsin-like	2.83E-35		cd00637
Cluster-102524.0	Collagen	2.45E-07		pfam01391
Cluster-91183.0	M1_APN_2	0.00E+00		cd09601
Cluster-111570.1	7tm_classA_rhodopsin-like	1.01E-48		cd00637
Cluster-110221.0	7tm_GPCRs super family	2.64E-49		cl28897
Cluster-52660.1	NO	.		
Cluster-44719.1	LicD super family	1.49E-06		cl01378
Cluster-97553.0	C2B_Synaptotagmin	5.15E-44		cd00276
Cluster-97553.2	C2B_Synaptotagmin	5.15E-44		cd00276
Cluster-79563.3	WAP	2.90E-10		pfam00095
Cluster-68308.1	Ion_trans_2	5.92E-15		pfam07885
Cluster-61548.0	SNARE_Vti1a	5.93E-26		cd15891
Cluster-87880.0	SDF	4.49E-114		pfam00375
Cluster-111607.0	GON	6.39E-63		pfam08685
Cluster-111607.2	GON	6.39E-63		pfam08685
Cluster-106982.0	GON	6.39E-63		pfam08685
Cluster-111607.1	GON	6.39E-63		pfam08685
Cluster-113633.1	GON	6.39E-63		pfam08685

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-91949.0	GON	6.39E-63		pfam08685
Cluster-96376.0	RING_Ubox super family	3.75E-15		cl17238
Cluster-113824.0	C2B_Synaptotagmin	2.50E-44		cd00276
Cluster-91603.0	Sec1	1.06E-95		pfam00995
Cluster-87717.0	Arrestin_N super family	4.22E-17		cl22903
Cluster-51695.0	G-alpha	8.15E-112		cd00066
Cluster-51695.4	G-alpha	8.15E-112		cd00066
Cluster-97901.0	LIM1_Enigma_like	1.86E-23		cd09361
Cluster-83447.0	Ig super family	1.44E-22		cl11960
Cluster-74955.6	SMC_N super family	7.01E-12		cl25732
Cluster-97612.0	SMC_N super family	7.01E-12		cl25732
Cluster-106212.1	SMC_N super family	1.04E-25		cl25732
Cluster-62021.3	TSP_C super family	1.06E-35		cl05347
Cluster-62021.2	TSP_C super family	1.06E-35		cl05347
Cluster-57350.5	CCP super family	2.82E-17		cl27761
Cluster-57350.3	CCP super family	2.82E-17		cl27761
Cluster-102822.0	CCP super family	2.82E-17		cl27761
Cluster-76290.1	HTH super family	4.54E-47		cl21459
Cluster-104495.2	Abhydrolase super family	3.47E-75		cl21494
Cluster-113421.0	SOX-TCF_HMG-box	9.72E-26		cd01388
Cluster-96004.0	HMG-box super family	3.05E-25		cl00082
Cluster-60151.0	SOX-TCF_HMG-box	1.49E-30		cd01388
Cluster-111605.0	BTB super family	1.47E-43		cl28614
Cluster-111305.0	ALDH-SF super family	0.00E+00		cl11961
Cluster-94243.0	KU	6.86E-15		smart00131
Cluster-83964.2	ZnMc super family	1.41E-36		cl00064
Cluster-110067.0	SOX-TCF_HMG-box	5.14E-28		cd01388
Cluster-97668.1	EFh_PEF super family	3.00E-30		cl25352
Cluster-89045.0	P-loop_NTPase super family	5.79E-14		cl21455
Cluster-42274.0	VWA	3.06E-55		pfam00092
Cluster-21035.6	VWA	3.06E-55		pfam00092
Cluster-21035.5	VWA	3.06E-55		pfam00092
Cluster-86531.1	COesterase	6.21E-132		pfam00135
Cluster-96324.0	COesterase	6.21E-132		pfam00135
Cluster-72197.1	PKc_like super family	0.00E+00		cl21453
Cluster-72197.0	PKc_like super family	0.00E+00		cl21453
Cluster-56741.5	TLD	8.41E-16		pfam07534
Cluster-74713.0	PAT1 super family	1.90E-03		cl25764
Cluster-76765.0	FA58C	1.95E-48		cd00057
Cluster-76765.4	FA58C	1.95E-48		cd00057
Cluster-76765.3	FA58C	1.95E-48		cd00057
Cluster-114298.2	RasGEF	1.31E-70		smart00147
Cluster-100052.0	Rho	5.51E-89		cd00157

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-101820.1	RHO	8.94E-100		smart00174
Cluster-86293.0	PKc_like super family	0.00E+00		cl21453
Cluster-75344.0	EF-hand_7	3.51E-08		pfam13499
Cluster-103842.1	Patatin_and_cPLA2 super family	1.35E-155		cl11396
Cluster-91702.0	NO	.		
Cluster-113072.0	LCB5 super family	4.85E-58		cl27661
Cluster-113117.0	COG1233	1.97E-35		COG1233
Cluster-77879.0	ProB super family	0.00E+00		cl25378
Cluster-104664.1	cyclophilin_ABH_like	1.39E-101		cd01926
Cluster-113041.0	PI-PLCc_beta	3.52E-107		cd08591
Cluster-95995.1	AAA	3.14E-07		cd00009
Cluster-88240.0	EFh_CREC super family	9.18E-77		cl25354
Cluster-88240.1	EFh_CREC super family	9.18E-77		cl25354
Cluster-56937.0	EFh_CREC super family	9.18E-77		cl25354
Cluster-95179.2	GRDP-like	1.65E-34		pfam07173
Cluster-105770.0	bZIP_AUREO-like	5.80E-10		cd14809
Cluster-96644.0	P-loop_NTPase super family	7.16E-19		cl21455
Cluster-88555.0	GOLGA2L5 super family	7.49E-29		cl25923
Cluster-98583.0	HPS6 super family	1.75E-10		cl24317
Cluster-105783.0	An_peroxidase	0.00E+00		pfam03098
Cluster-33940.2	Anoctamin	1.16E-131		pfam04547
Cluster-105325.1	DnaJ	2.56E-28		COG0484
Cluster-84900.0	zf-LITAF-like	7.19E-23		pfam10601
Cluster-70742.0	Sec7	3.53E-95		pfam01369
Cluster-87328.0	MDM1 super family	1.29E-07		cl28796
Cluster-85093.0	Diphthamide_syn	6.83E-71		pfam01866
Cluster-63426.0	FReD super family	3.42E-05		cl00085
Cluster-112610.0	DuoxA	1.69E-120		pfam10204
Cluster-32864.0	Rap_GAP	9.68E-60		pfam02145
Cluster-109906.0	UAA super family	4.46E-27		cl26745
Cluster-107747.1	DRIM	2.38E-173		pfam07539
Cluster-32651.0	SLC5-6-like_sbd super family	3.49E-25		cl00456
Cluster-116468.1	SMC_N super family	3.43E-20		cl25732
Cluster-106595.4	MC_N super family	3.43E-20		cl25732
Cluster-106595.7	MC_N super family	3.43E-20		cl25732
Cluster-104453.3	CUB	6.52E-15		cd00041
Cluster-104453.5	CUB	6.52E-15		cd00041
Cluster-50257.1	DDHD	3.72E-42		pfam02862
Cluster-97212.0	An_peroxidase_like super family	.1.58e-56		cl14561
Cluster-70407.2	dual_peroxidase_like	0.00E+00		cd09820
Cluster-73336.1	Mpv17_PMP22	5.64E-20		pfam04117
Cluster-41508.12	SRGL1_like	9.74E-110		cd04702

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-1509.2	Ntn_Asparaginase_2_like super family	8.45E-98		cl00635
Cluster-86765.6	Ntn_Asparaginase_2_like super family	1.22E-83		cl00635
Cluster-70733.0	P-loop_NTPase super family	2.70E-04		cl21455
Cluster-75720.0	PTKc	1.73E-120		cd00192
Cluster-84004.0	An_peroxidase	0.00E+00		pfam03098
Cluster-108295.0	VHS_ENTH_ANTH super family	6.21E-33		cl02544
Cluster-108738.1	MFS_1	5.02E-40		pfam07690
Cluster-107104.0	Chromo	2.14E-13		pfam00385
Cluster-75641.1	RT_like super family	2.72E-20		cl02808
Cluster-96501.0	An_peroxidase	0.00E+00		pfam03098
Cluster-99075.0	Thioredoxin_like super family	2.66E-39		cl00388
Cluster-87195.2	BTB super family	1.22E-50		cl28614
Cluster-65736.1	Glyco_hydro_47	8.56E-117		pfam01532
Cluster-84769.0	STKc_WNK	2.46E-165		cd13983
Cluster-107427.1	DnaJ	4.92E-48		COG0484
Cluster-62468.1	ZnMc_astacin_like	1.94E-70		cd04280
Cluster-92651.0	UGD_SDR_e	0.00E+00		cd05230
Cluster-111919.1	7tmB3_Methuselah-like	6.36E-65		cd15039
Cluster-108007.3	MFS	3.40E-41		cd06174
Cluster-108007.0	2A0111 super family	5.08E-29		cl26868
Cluster-104912.0	SMC_N super family	7.32E-06		cl25732
Cluster-97239.1	AA_permease_2 super family	7.53E-178		cl26159
Cluster-99345.0	Ammonium_transp super family	2.58E-29		cl03012
Cluster-72255.1	CLECT	3.18E-10		smart00034
Cluster-69770.0	NO	.		
Cluster-106624.0	Cbl_N	7.77E-65		pfam02262
Cluster-90084.0	Periplasmic_Binding_Protein_Type_1 super family	1.06E-112		cl10011
Cluster-92381.0	7tmB3_Methuselah-like	2.22E-64		cd15039
Cluster-113574.0	7tm_classA_rhodopsin-like	1.27E-33		cd00637
Cluster-115976.0	7tm_GPCRs super family	1.64E-66		cl28897
Cluster-102863.0	eIF-3c_N super family	0.00E+00		cl20295
Cluster-115462.3	Carboxyl_trans super family	0.00E+00		cl27613
Cluster-58193.3	Biotin_carb_N super family	0.00E+00		cl27719
Cluster-58193.1	Biotin_carb_N super family	0.00E+00		cl27719
Cluster-94339.3	PKc_like super family	2.33E-91		cl21453
Cluster-94339.2	PKc_like super family	2.33E-91		cl21453
Cluster-37437.1	M13	1.28E-110		cd08662
Cluster-59025.1	M13	0.00E+00		cd08662
Cluster-1664.3	M13	0.00E+00		cd08662
Cluster-32013.0	ZnMc_MMP	7.28E-78		cd04278

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-40143.2	Peptidase_C1A	6.11E-53		cd02248
Cluster-76886.0	FReD	5.09E-63		cd00087
Cluster-113809.0	DPPIV_N super family	6.19E-80		cl27623
Cluster-77230.2	RICIN	1.03E-18		cd00161
Cluster-77230.1	RICIN	1.03E-18		cd00161
Cluster-62723.1	p450	3.85E-99		pfam00067
Cluster-112028.5	Tyrosinase super family	4.07E-23		cl02830
Cluster-112028.2	Tyrosinase super family	4.07E-23		cl02830
Cluster-78941.0	Tyrosinase super family	4.07E-23		cl02830
Cluster-80426.1	K_oxygenase super family	9.46E-74		cl26174
Cluster-107382.0	SDR super family	6.22E-63		cl25409
Cluster-88172.0	SRPBCC super family	7.47E-13		cl14643
Cluster-24038.0	ZnMc super family	2.19E-40		cl00064
Cluster-56508.2	ZnMc super family	2.19E-40		cl00064
Cluster-84180.1	Scramblase	2.13E-107		pfam03803
Cluster-84180.3	Scramblase	2.13E-107		pfam03803
Cluster-98371.0	DUF4371 super familyl	5.14E-28		cl16778
Cluster-76316.1	DUF4371 super family	1.06E-23		cl16778
Cluster-102792.1	WD40	7.77E-70		cd00200
Cluster-96705.0	SMC_N super family	2.15E-10		cl25732
Cluster-47728.0	PP2Cc	9.67E-15		smart00332
Cluster-100338.3	PP2Cc	9.67E-15		smart00332
Cluster-43777.6	FAM181 super family	7.97E-09		cl24280
Cluster-112169.0	THAP	2.55E-15		pfam05485
Cluster-32039.4	SET	2.09E-08		pfam00856
Cluster-107147.0	SET	5.75E-04		pfam00856
Cluster-88654.0	RT_nLTR_like	1.48E-43		cd01650
Cluster-112165.1	P-loop_NTPase super family	1.28E-04		cl21455
Cluster-89200.0	NB-ARC super family	1.78E-05		cl26397
Cluster-85745.0	TonB_N	8.88E-05		pfam16031
Cluster-75643.1	RT_LTR	4.43E-67		cd01647
Cluster-62531.0	RT_LTR	2.41E-34		cd01647
Cluster-57484.0	WD40 super family	3.02E-11		cl25539
Cluster-106713.0	LamG super family	4.79E-15		cl22861
Cluster-85743.1	P-loop_NTPase super family	7.88E-21		cl21455
Cluster-62468.0	ZnMc_astacin_like	1.94E-70		cd04280
Cluster-115462.2	Carboxyl_trans super family	0.00E+00		
Cluster-115462.0	Carboxyl_trans super family	0.00E+00		
Cluster-107747.0	SMC_N super family	5.24E-07		cl25732
Cluster-81257.0	PLN02193 super family	1.15E-17		cl26061
Cluster-102854.0	ANK	4.40E-31		(REPEATS)cd00204
Cluster-82736.0	Macin super family	3.58E-05		cl20762
Cluster-47323.0	Reeler	6.76E-23		pfam02014

Cluster ID	Domain name	Domain	e-value	Accession ccd
Cluster-16207.1	FReD	9.85E-96		cd00087
Cluster-56126.1	WSC super family	2.27E-11		cl02568
Cluster-16150.0	FA58C	6.44E-34		cd00057
Cluster-53819.0	BRICHOS super family	5.61E-06		cl04394
Cluster-108267.2	CD20 super family	1.69E-04		cl04401
Cluster-111097.2	NTR_like super family	8.79E-09		cl02512
Cluster-110485.0	RPA_2b-aaRSs_OBF_like super family	6.44E-06		cl09930
Cluster-82641.1	NTR_like super family	7.27E-10		cl02512
Cluster-83419.0	DUF885	3.42E-71		pfam05960
Cluster-83419.2	DUF885	3.42E-71		pfam05960
Cluster-109225.1	DUF1759 super family	1.02E-08		cl04160
Cluster-103817.0	TMEM154 super family	7.16E-05		cl20971
Cluster-73595.0	FN3	3.31E-04		cd00063
Cluster-110992.2	ubiquitin	1.09E-10		pfam00240
Cluster-110992.0	ubiquitin	1.09E-10		pfam00240
Cluster-113488.3	Neuromodulin_N super family	9.25E-07		cl26511
Cluster-113488.1	Neuromodulin_N super family	9.25E-07		cl26511
Cluster-46080.1	NO	.		
Cluster-113488.0	Neuromodulin_N super family	9.25E-07		cl26511
Cluster-113212.1	COG2085 super family	1.25E-39		cl28110
Cluster-103452.0	DUF1759 super family	1.48E-10		cl04160
Cluster-43401.7	conj_TIGR03752 super family	3.25E-04		l26990
Cluster-104832.0	EBV-NA3 super family	1.06E-04		cl27975
Cluster-88086.1	SMC_N super family	1.49E-09		cl25732
Cluster-48279.0	GIY-YIG_PLEs	4.38E-20		cd10442
Cluster-41126.0	TauE	3.10E-13		pfam01925
Cluster-112221.2	C2 super family	1.83E-05		cl14603
Cluster-92134.1	RT_like super family	4.01E-03		cl02808
Cluster-57352.0	CD20 super family	6.12E-03		cl04401
Cluster-87526.0	RT_like super family	5.42E-50		cl02808
Cluster-31143.3	FA58C super family	4.04E-14		cl25480
Cluster-90679.0	DDE_Tnp_4	7.38E-47		pfam13359

Table B. 2: Literature related with DEG in *Lobophytum* colonies interacting with *Porites* colony Pd

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-77355.1	FGFR1_CHICK	Matus et al 2007	.
Cluster-77355.0	FGFR1_CHICK	Matus et al 2007	.
Cluster-50735.0	FGFR1_CHICK	Matus et al 2007	.
Cluster-60199.0	FGFR1_CHICK	Matus et al 2007	.
Cluster-97243.0	FGFR1_MOUSE	Matus et al 2007	.
Cluster-67745.2	FGFR3_PLEWA	Matus et al 2007	.
Cluster-113066.5	RBP1_HUMAN	.	Rojas & Valencia 2014
Cluster-113066.0	RBP1_HUMAN	.	Rojas & Valencia 2014
Cluster-113066.2	RBP1_HUMAN	Bosch 2007;	Rojas & Valencia 2014
Cluster-32075.0	TGFR1_RAT	1. Technau et al 2005; 2. Detournay et al 2012	.
Cluster-59833.0	SKI_XENLA	1. Samuel et al 2001; 2. Detournay et al 2012; 3. Matus etal 2006; 4. Peterson et al 2015	.
Cluster-65058.1	CAD96_DROME	1. Ocampo et al 2015	.
Cluster-81996.1	NECB_HYDVU	.	Salzet et al 2000
Cluster-62501.3	K1PV46_CRAGI	1. Hamaguchi-Hamada et al 2015	.
Cluster-47635.1	ITB1_SHEEP	.	1. Babonis and Martindale 2017
Cluster-115880.0	WNT4_CHICK	1. Lee et al 2006; 2. Hemond et al 2014	.
Cluster-105540.0	ECT2_MOUSE	.	Tatsumoto et al 1999
Cluster-68824.3	SVEP1_HUMAN	.	.
Cluster-94675.0	KIF23_MOUSE	.	Hirokawa N. et al 2009
Cluster-108106.2	FAT1_HUMAN	Frazão et al 2017	Nishikawa et al 2011
Cluster-107202.1	RFC2_RAT	.	.
Cluster-97039.0	FD4_DROME	Hayward et al 2015	Shimeld et al 2010
Cluster-40909.0	FD3_DROME	Hayward et al 2015	Shimeld et al 2010
Cluster-64366.0	SOCS4_BOVIN	Putnam et al 2007	.
Cluster-99480.1	DISP_DROME	Matus et al 2008	.
Cluster-61510.0	EPHA2_MOUSE	.	1. Ryan et al 2013; 2. Kullander and Klein 2002

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-56550.2	RADI_MOUSE	.	1. Adada et al 2014; 2. Neisch and Fehon 2011
Cluster-22331.0	NOTC1_DANRE	Käsbauer et al 2007	Murata and Hayashi 2016
Cluster-40343.0	MC5R_MOUSE	Anctil, et al 2007	1. Yang 2011; 2. Morgan and Cone 2006
Cluster-95367.4	PTPRD_MOUSE	.	.
Cluster-113490.0	ACHA9_CHICK	1. Grüder and Assmann; 2. Sinigaglia et al 2015; 2. Watanabe 2017	.
Cluster-52996.0	ADA1B_HUMAN	Stewart et al 2017	.
Cluster-27684.2	P52K_HUMAN	.	Peel 2004
Cluster-111570.1	ADRB2_MACMU	Stewart et al 2017	.
Cluster-110221.0	ADRB2_BOVIN	Elofsson and Carlberg 1089	.
Cluster-52660.1	FKRP_MOUSE	Leclère and Röttinger, 2017	.
Cluster-44719.1	A0A2B4SP55_STYPI	Leclère and Röttinger, 2017	.
Cluster-79563.3	PPN_DROME	.	1. Kramerova et al 2000; Campbell et al 1987
Cluster-68308.1	KCNK1_RABIT	Satterlie 2017	.
Cluster-61548.0	VTI1A_HUMAN	Bosch et al 2017	Liebeskind et al 2017
Cluster-87880.0	EAA2_MOUSE	.	.
Cluster-111607.0	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-111607.2	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-106982.0	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-111607.1	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-113633.1	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-91949.0	AGRIN_MOUSE	1. Libro et al 2013; 2. Crowder et al 2017	Trautmann and Vivier 2001
Cluster-96376.0	TRIM2_RAT	.	Khazaei et al 2010
Cluster-87717.0	ARRD1_HUMAN	1. Plachetzki et al 2012;	2. Gomez et al 2011
Cluster-51695.0	GNAO_BOVIN	.	Zang et al 2014
Cluster-97901.0	LDB3_MOUSE	1. Martindale et al 2004; 2. Lecière and Röttinger 2017	.
Cluster-83447.0	CNTN6_MOUSE	1. Bertucci et al 2015; 2. Pierobon et at 2012	Huang et al 2016

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-74955.6	NRX4_DROME	.	1. Reissner et al 2013; 2. Leys and Riesgo 2011
Cluster-97612.0	NRX4_DROME	.	1. Reissner et al 2013; 2. Leys and Riesgo 2011
Cluster-106212.1	NRX4_DROME	.	1. Reissner et al 2013; 2. Leys and Riesgo 2011
Cluster-57350.5	CSMD3_MOUSE	.	Stern et al, 2009
Cluster-57350.3	CSMD3_MOUSE	.	Stern et al, 2009
Cluster-102822.0	CSMD3_MOUSE	.	Stern et al, 2009
Cluster-113421.0	SOX9_MOUSE	Shinzato et al 2008	.
Cluster-96004.0	SOX9A_XENLA	Shinzato et al 2008	.
Cluster-60151.0	SOX8_XENLA	Shinzato et al 2008	.
Cluster-111305.0	AL1L1_XENLA	Horricks, R. A Thesis, 2017	Lewin et al. 2017
Cluster-97668.1	MLC2_DROME	1. Crowder et al 2017; 2. Louis et al 2017; 3.	.
Cluster-89045.0	RIT1_HUMAN	.	Rojas & Valencia 2014
Cluster-42274.0	FAT4_HUMAN	1. Bertucci et al 2015; 2. Hemond et al 2014	Hulpiau and van Roy 2011
Cluster-21035.6	FAT4_HUMAN	1. Bertucci et al 2015; 2. Hemond et al 2014	Hulpiau and van Roy 2011
Cluster-21035.5	FAT4_HUMAN	1. Bertucci et al 2015; 2. Hemond et al 2014	Hulpiau and van Roy 2011
Cluster-86531.1	CHLE_PANTT	1. Talesa et al. 1992	Falugi and Aluigi 2012
Cluster-96324.0	CHLE_PANTT	1. Talesa et al. 1992	Falugi and Aluigi 2012
Cluster-76765.0	VWF_MOUSE	Oren et al 2010	.
Cluster-76765.4	VWF_MOUSE	Oren et al 2010	.
Cluster-76765.3	VWF_MOUSE	Oren et al 2010	.
Cluster-100052.0	RHOA_RAT	.	Rojas and Valencia 2014
Cluster-86293.0	SGK3_PONAB	Bosch 2013	.
Cluster-75344.0	P2R3B_HUMAN	.	Maceyka and Spiegel 2014
Cluster-91702.0	RERGL_DANRE	Mohamed et al 2016	Rojas & Valencia 2014
Cluster-113072.0	SPHK1_ARATH	1. Rodriguez-Lanetty et al 2006; 2. Kitchen and Weis 2017	.
Cluster-113117.0	PYRD2_HUMAN	Dunlap et al 2013	.
Cluster-77879.0	P5CS_PONAB	Polato et al 2013	.

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-104664.1	PPIG_HUMAN	1. Shearer et al 2012; 2. Moran et al 2013	.
Cluster-113041.0	PLCB4_HUMAN	.	1. Chen et al 2016
Cluster-95995.1	RN213_HUMAN	1. Granados-Cifuentes et al 2013	.
Cluster-88240.0	CALUA_DANRE	1. Bellantuono et al 2012; 2. Libro et al 2013; 3. Oakley et al 2017	.
Cluster-88240.1	CALUA_DANRE	1. Bellantuono et al 2012; 2. Libro et al 2013; 3. Oakley et al 2017	.
Cluster-56937.0	CALUA_DANRE	1. Bellantuono et al 2012; 2. Libro et al 2013; 3. Oakley et al 2017	.
Cluster-105770.0	CRERF_HUMAN	.	Audas et al 2008
Cluster-105783.0	PXDN_XENTR	1.Voolstra et al., 2009; 2. Louis et al., 2017; 3. Libro et al 2013; 4. Burge et al 2013	Nelson et al 1994
Cluster-33940.2	ANO4_BOVIN	Elran et al 2014	Han et al 2017
Cluster-70742.0	CYH1_HUMAN	.	Wittinghofer 2014
Cluster-85093.0	DPH2_NEMVE	.	SU et al 2014
Cluster-63426.0	.	.	Pemberton et al 2004 ; 2. Yan et al 2013
Cluster-109906.0	S35B1_MOUSE	.	Oikari et al 2016
Cluster-107747.1	UTP20_HUMAN	.	.
Cluster-32651.0	S36A1_HUMAN	.	Yao et al 2017
Cluster-50257.1	DDHD1_BOVIN	Libro et al 2013	.
Cluster-97212.0	DUOX2_PIG	.	Bae et al 2010
Cluster-70407.2	DUOX2_PIG	.	Bae et al 2010
Cluster-73336.1	M17L2_DANRE	.	Löllgen and Weiher 2014
Cluster-41508.12	ASGL1_MOUSE	Oakley et al 2016	.
Cluster-1509.2	ASGL1_MOUSE	Oakley et al 2016	.
Cluster-86765.6	ASGL1_DANRE	Oakley et al 2016	.
Cluster-70733.0	NLRP3_BOVIN	Ocampo et al 2015; Mydlarz et al 2016	.
Cluster-75720.0	TIE1_HUMAN	1. Bellantuono et al 2012; 2. Pizon et al 2017	.
Cluster-84004.0	PERL_MESAU	Mohamed et al 2018	.
Cluster-96501.0	PERM_HUMAN	Mydlraz et al 2016	.
Cluster-99075.0	QSOX1_MOUSE	.	Limor et al 2013
Cluster-65736.1	EDEM1_MOUSE	Shearer et al 2012	.

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-111919.1	AGRG6_DANRE	1. De Mendoza et al 2016; 2. Dunlap et al 2013; 3. Voolstra et al 2017	Lin et al 1998
Cluster-108007.3	MOT10_DANRE	Sproles et al 2018	.
Cluster-86901.3	MRC1_MOUSE	Kvennefors et al 2008	Yang et al 2015
Cluster-86901.1	MRC1_MOUSE	Kvennefors et al 2008	Yang et al 2015
Cluster-72255.1	MRC1_MOUSE	Kvennefors et al 2008	Yang et al 2015
Cluster-92381.0	A0A2B4R645_STYPI	1.De Mendoza et al 2016; 2.Dunlap et al 2013; 3.Voolstra et al 2017	Lin et al 1998
Cluster-113574.0	OPRK_MOUSE	Alzugaray et al 2016	Sneddon 2018
Cluster-115976.0	NPFF2_MOUSE	Rosenberg et al 2017	.
Cluster-115462.3	MCCB_CAEEL	.	Feller and Feist 1962/ Ingenuity website
Cluster-58193.3	CPSM_HUMAN	Hemond and Vollmer et al 2015	.
Cluster-58193.1	CPSM_HUMAN	Hemond and Vollmer et al 2015	.
Cluster-37437.1	ECE1_MOUSE	1.Ponce et al 2016; 2.Zhang et al 2001	.
Cluster-59025.1	ECE1_BOVIN	1.Ponce et al 2016; 2.Zhang et al 2001	.
Cluster-1664.3	ECE1_BOVIN	1.Ponce et al 2016; 2.Zhang et al 2001	.
Cluster-40143.2	CYSP3_SOLLC	1.Kitchen and Weis 2017; 2.Jouiaei et al 2015; 3. Ocampo et al 2015	.
Cluster-76886.0	FGL2_MOUSE	Ocampo et al 2015	Doolittle et al 2012
Cluster-113809.0	DPP4_FELCA	Wenger et al 2014	.
Cluster-77230.2	XYNA_STRLI	1. Bellantuono et al 2012; 2. Schwarz wt al 2008	Pauchet & Heckel 2013
Cluster-77230.1	XYNA_STRLI	1. Bellantuono et al 2012; 2. Schwarz wt al 2008	Pauchet & Heckel 2013
Cluster-62723.1	CP17A_CHICK	Oakley et al 2016	.
Cluster-112028.5	PFX18679	Mydlraz et al 2016	.
Cluster-112028.2	PFX18679	Mydlraz et al 2016	.
Cluster-78941.0	PFX18679	Mydlraz et al 2016	.
Cluster-107382.0	PGDH_MOUSE	1.Koljak et al 2001; 2.Turk and Kem 2009	.
Cluster-24038.0	APOH_RAT	Bertucci at al 2015	Mather et al 2016
Cluster-56508.2	APOH_RAT	Bertucci at al 2015	Mather et al 2016

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-84180.1	PLS2_BOVIN	Wenger et al 2014	Han et al 2017; 2. Bevers and Williamson
Cluster-84180.3	PLS2_BOVIN	Wenger et al 2014	Han et al 2017; 2. Bevers and Williamson 2010
Cluster-47728.0	Y9801_DROME	Wenger et al 2014	Stern et al, 2009
Cluster-100338.3	Y9801_DROME	Wenger et al 2014	Stern et al, 2009
Cluster-107147.0	SETD9_HUMAN	Mohamed et al 2016	Dillon et al 2005
Cluster-106713.0	NPTXR_RAT	1.Ocampo et al 2015 ; 2.Hamaguchi-Hamada et al 2016; 3.Libro and Vollmer 2015	.
Cluster-85743.1	NLRC5_ICTPU	1.Zhou et al 2017; 2.Libro and Vollmer 2016	.
Cluster-62468.0	NAS4_CAEEL	1.Ponce et al 2016; 2.Pan et al 1998	.
Cluster-115462.2	MCCB_CAEEL	.	Feller and Feist 1962/ Ingenuity website
Cluster-115462.0	MCCB_CAEEL	.	Feller and Feist 1962/ Ingenuity website
Cluster-82736.0	HYDMA_HYDVU	Jung et al 2008	.
Cluster-16207.1	FBCD1_MACFA	Doolittle et at 2012	.

Table B. 3: KEGG term related to the UniProt ID found in as best BLAST *Lobophytum* - colonies interacting with *Porites* colony Pd.

Cluster ID	log2 fold change M2	padj_M2	Best BLAST	Best E-value	KO	Genome ID
Cluster-105770.0	0.702	2.35E-02	CRERF_HUMAN	2.00E-33	K21554	s29_g52.t1
Cluster-96644.0	0.894	NA	NAL12_HUMAN	2.00E-40	K20865	s151_g26.t1
Cluster-105540.0	1.026	1.00E-01	ECT2_MOUSE	2.00E-19	K20704	s173_g31.t1
Cluster-88555.0	0.372	2.14E-02	GOGA2_RAT	5.00E-21	K20358	s221_g20.t1
Cluster-98583.0	0.264	3.29E-02	HPS6_HUMAN	6.00E-08	K20192	s28_g60.t1
Cluster-52660.1	0.845	2.41E-02	FKRP_MOUSE	0.001	K19873	s943_g12.t1
Cluster-44719.1	0.904	7.86E-02	A0A2B4SP55_STYPI	1.10E-23	K19873	s102_g66.t1
Cluster-97901.0	0.769	5.91E-05	LDB3_MOUSE	2.00E-05	K19867	s376_g13.t1
Cluster-105783.0	1.229	2.88E-03	PXDN_XENTR	3.00E-133	K19511	s142_g18.t1

Cluster ID	log2 fold change M2	padj_M2	Best BLAST	Best E-value	KO	Genome ID
Cluster-33940.2	0.753	3.33E-01	ANO4_BOVIN	6.00E-161	K19499	s43_g1.t1
Cluster-105325.1	0.363	2.01E-01	DJC25_XENLA	2.00E-101	K19371	s899_g13.t1
Cluster-84900.0	0.960	2.74E-03	LITAF_DANRE	7.00E-28	K19363	.
Cluster-70742.0	0.378	2.01E-02	CYH1_HUMAN	3.00E-162	K18441	s154_g17.t1
Cluster-113421.0	1.540	9.82E-05	SOX9_MOUSE	1.00E-34	K18435	s154_g30.t1
Cluster-96004.0	1.500	3.88E-06	SOX9A_XENLA	3.00E-31	K18435	s154_g27.t1
Cluster-87328.0	0.632	1.05E-01	MDM1_CHICK	8.00E-06	K17886	.
Cluster-85093.0	1.052	6.62E-02	DPH2_NEMVE	9.00E-22	K17866	s825_g2.t1
Cluster-68824.3	0.943	7.45E-02	SVEP1_HUMAN	8.00E-05	K17495	s321_g21.t1
Cluster-83964.2	0.786	9.80E-02	CSMD3_MOUSE	3.00E-66	K17495	s292_g5.t1
Cluster-57350.5	0.707	1.94E-02	CSMD3_MOUSE	9.00E-41	K17495	s292_g18.t1
Cluster-57350.3	0.793	2.18E-03	CSMD3_MOUSE	9.00E-41	K17495	s292_g18.t1
Cluster-102822.0	0.921	2.14E-04	CSMD3_MOUSE	9.00E-41	K17495	s292_g18.t1
Cluster-94577.0	0.972	8.30E-03	PP12C_MOUSE	7.00E-17	K17457	s19_g136.t1
Cluster-94675.0	0.869	1.92E-06	KIF23_MOUSE	6.00E-138	K17387	s2573_g1.t1
Cluster-112610.0	1.376	1.91E-03	DOXA1_HUMAN	2.00E-47	K17233	s165_g13.t1
Cluster-91702.0	1.671	7.22E-08	RERGL_DANRE	4.00E-36	K17198	s340_g17.t1
Cluster-32864.0	0.599	4.92E-02	ANXA4_MOUSE	5.00E-23	K17093	s123_g40.t1
Cluster-62501.3	0.654	1.21E-02	K1PV46_CRAGI	1.2E-21	K16857	s20_g64.t1
Cluster-42274.0	1.380	1.45E-06	FAT4_HUMAN	9.00E-22	K16669	s32_g91.t1
Cluster-21035.6	1.121	7.22E-08	FAT4_HUMAN	5.00E-09	K16669	s32_g91.t1
Cluster-21035.5	1.004	3.24E-07	FAT4_HUMAN	0	K16669	s32_g91.t1
Cluster-108106.2	0.374	2.24E-01	FAT1_HUMAN	8.50E-40	K16506	s77_g28.t1
Cluster-103842.1	0.688	1.83E-02	PA24A_RABIT	4.00E-33	K16342	s42_g45.t1
Cluster-72197.1	0.841	4.41E-02	CDK17_HUMAN	7.00E-175	K15595	s32_g56.t1
Cluster-72197.0	0.937	1.31E-02	CDK17_HUMAN	7.00E-175	K15595	s32_g56.t1
Cluster-91603.0	0.362	4.80E-03	STXB1_RAT	0	K15292	s194_g43.t1
Cluster-113824.0	0.642	5.41E-02	SY63_DIPOM	7.00E-53	K15290	s3_g101.t1
Cluster-97553.0	0.608	7.19E-02	SYT1_PONAB	1.00E-50	K15290	s1_g98.t1
Cluster-97553.2	0.634	6.18E-02	SYT1_PONAB	1.00E-50	K15290	s1_g98.t1
Cluster-109906.0	0.770	5.16E-03	S35B1_MOUSE	4.00E-39	K15275	s602_g6.t1
Cluster-107747.1	2.153	1.62E-10	UTP20_HUMAN	3.00E-138	K14772	s7_g115.t1
Cluster-32651.0	0.731	5.74E-01	S36A1_HUMAN	1.00E-17	K14209	s43_g49.t1
Cluster-116468.1	0.405	8.52E-04	RRBP1_MOUSE	2.00E-21	K14000	s152_g49.t1
Cluster-106595.4	0.469	1.11E-02	RRBP1_MOUSE	2.00E-21	K14000	s152_g49.t1
Cluster-106595.7	0.293	1.57E-01	RRBP1_MOUSE	2.00E-21	K14000	s152_g49.t1
Cluster-104453.3	0.741	1.23E-01	DMBT1_MOUSE	2.00E-13	K13912	s84_g3.t1
Cluster-104453.5	0.726	8.44E-02	DMBT1_MOUSE	2.00E-13	K13912	s84_g3.t1
Cluster-50257.1	0.813	1.10E-03	DDHD1_BOVIN	1.00E-107	K13619	s14_g38.t1
Cluster-70407.2	1.194	1.18E-02	DUOX2_PIG	0	K13411	s165_g18.t1
Cluster-97212.0	1.467	1.40E-04	DUOX2_PIG	6.00E-34	K13411	s165_g15.t1
Cluster-86293.0	0.549	5.90E-02	SGK3_PONAB	0	K13304	s25_g36.t1

Cluster ID	log2 fold change M2	padj_M2	Best BLAST	Best E-value	KO	Genome ID
Cluster-70733.0	1.039	6.98E-02	NLRP3_BOVIN	8.00E-05	K12800	s3726_g1.t1
Cluster-77879.0	0.616	6.90E-03	P5CS_PONAB	0	K12657	s47_g54.t1
Cluster-84004.0	1.022	1.62E-04	PERL_MESAU	3.00E-115	K12550	s400_g12.t1
Cluster-108295.0	0.269	1.25E-02	GGA1_MOUSE	3.00E-128	K12404	s154_g32.t1
Cluster-108738.1	0.565	1.31E-02	S17A9_MOUSE	2.00E-94	K12303	s421_g18.t1
Cluster-75344.0	0.988	9.00E-02	P2R3B_HUMAN	1.00E-160	K11583	s174_g19.t1
Cluster-107104.0	0.969	1.16E-02	CBX2_MOUSE	4.00E-08	K11451	s886_g3.t1
Cluster-91183.0	0.600	1.86E-02	AMPE_BOVIN	0	K11141	s103_g7.t1
Cluster-96501.0	1.306	3.32E-04	PERM_HUMAN	1.00E-38	K10789	s1081_g1.t1
Cluster-99075.0	0.445	7.32E-02	QSOX1_MOUSE	2.00E-104	K10758	s84_g34.t1
Cluster-107202.1	0.632	2.67E-05	RFC2_RAT	0	K10755	s444_g8.t1
Cluster-87195.2	0.319	1.78E-02	KLHDB_ANOGA	4.00E-77	K10457	s106_g100.t1
Cluster-65736.1	0.557	2.10E-03	EDEM1_MOUSE	0	K10084	s647_g3.t1
Cluster-113117.0	0.429	9.97E-02	PYRD2_HUMAN	4.00E-120	K10027	s2_g53.t1
Cluster-104664.1	0.336	1.12E-02	PPIG_HUMAN	2.00E-82	K09566	s5_g52.t1
Cluster-107427.1	0.641	3.64E-01	DNJB1_MOUSE	1.00E-65	K09507	s24_g46.t1
Cluster-97039.0	1.908	7.27E-09	FD4_DROME	1.00E-26	K09411	s34_g52.t1
Cluster-40909.0	1.676	1.04E-05	FD3_DROME	5.00E-25	K09397	s34_g51.t1
Cluster-76290.1	1.227	8.39E-03	PAX3B_XENLA	4.00E-74	K09381	s17_g85.t1
Cluster-60151.0	1.602	7.24E-06	SOX8_XENLA	6.00E-35	K09270	s154_g28.t1
Cluster-110067.0	1.259	3.29E-03	SOX10_CHICK	1.00E-34	K09270	s154_g26.t1
Cluster-102547.0	1.657	3.31E-05	RFX4_HUMAN	5.00E-143	K09174	s180_g12.t1
Cluster-84769.0	1.069	6.93E-02	WNK1_MOUSE	7.5E-161	K08867	s178_g49.t1
Cluster-62468.1	0.855	1.85E-03	NAS4_CAEEL	6.00E-39	K08778	s306_g20.t1
Cluster-113066.5	1.029	4.61E-03	RBP1_HUMAN	7.00E-75	K08773	s510_g9.t1
Cluster-113066.0	0.653	1.83E-02	RBP1_HUMAN	7.00E-75	K08773	s510_g9.t1
Cluster-113066.2	0.644	7.18E-02	RBP1_HUMAN	7.00E-75	K08773	s510_g9.t1
Cluster-92651.0	0.331	4.07E-03	UXS1_MOUSE	0	K08678	s78_g11.t1
Cluster-61548.0	0.354	1.22E-01	VTI1A_HUMAN	8.00E-71	K08493	s156_g12.t1
Cluster-65069.0	0.869	1.30E-01	AGRD1_BOVIN	1.00E-73	K08465	s54_g35.t1
Cluster-29541.1	1.059	6.62E-02	AGRD1_BOVIN	4.00E-28	K08465	s36_g14.t1
Cluster-111919.1	1.002	9.49E-02	AGRG6_DANRE	7.00E-19	K08463	s1524_g5.t1
Cluster-111496.0	1.170	4.29E-03	AGRG4_HUMAN	6.00E-64	K08455	s250_g10.t1
Cluster-115976.0	0.511	6.85E-02	NPFF2_MOUSE	2.00E-40	K08375	.
Cluster-108007.3	1.183	1.76E-06	MOT10_DANRE	1.00E-37	K08187	s216_g1.t1
Cluster-108007.0	0.721	6.73E-02	MOT10_DANRE	1.00E-37	K08187	s216_g1.t1
Cluster-104912.0	0.327	4.59E-02	VNN1_BOVIN	1.00E-45	K08069	s19_g113.t1
Cluster-87944.2	0.467	1.66E-01	HPSE_HUMAN	5.00E-107	K07964	s513_g14.t1
Cluster-87944.1	0.669	2.44E-02	HPSE_HUMAN	5.00E-107	K07964	s513_g14.t1
Cluster-89045.0	0.756	2.21E-02	RIT1_HUMAN	2.00E-37	K07832	s761_g1.t1
Cluster-95367.4	0.808	3.98E-03	PTPRD_MOUSE	8.00E-146	K06777	s334_g6.t1
Cluster-83447.0	0.396	3.60E-03	CNTN6_MOUSE	8.00E-68	K06764	s207_g2.t1

Cluster ID	log2 fold change M2	padj_M2	Best BLAST	Best E-value	KO	Genome ID
Cluster-97239.1	0.528	6.88E-07	CND1_XENLA	0	K06677	s253_g23.t1
Cluster-99345.0	1.323	4.25E-03	RHCG_PIG	1.00E-68	K06580	s20_g41.t1
Cluster-72255.1	1.035	3.34E-03	MRC1_MOUSE	0.00E+00	K06560	s654_g2.t1
Cluster-86901.3	1.462	2.19E-04	MRC1_MOUSE	1.00E-03	K06560	s193_g7.t1
Cluster-86901.1	1.403	1.39E-03	MRC1_MOUSE	1.00E-03	K06560	s193_g7.t1
Cluster-59857.0	1.048	1.26E-04	CO6A6_MOUSE	4.00E-22	K06238	s503_g7.t1
Cluster-113041.0	0.429	1.12E-01	PLCB4_HUMAN	1.00E-143	K05858	s447_g9.t1
Cluster-56550.2	0.245	1.36E-03	RADI_MOUSE	0	K05762	s1480_g2.t1
Cluster-47635.1	0.612	5.93E-03	ITB1_SHEEP	4.00E-163	K05719	s76_g22.t1
Cluster-87880.0	0.748	1.01E-04	EAA2_MOUSE	4.00E-132	K05613	s261_g26.t1
Cluster-69770.0	0.843	1.29E-02	P2RX7_HUMAN	1.00E-09	K05220	s728_g16.t1
Cluster-75720.0	1.094	2.33E-03	TIE1_HUMAN	1.00E-50	K05120	s126_g28.t1
Cluster-67745.2	1.039	7.51E-02	FGFR3_PLEWA	9.00E-68	K05094	s95_g25.t1
Cluster-68308.1	0.395	5.52E-02	KCNK1_RABIT	1.00E-50	K04912	s123_g15.t1
Cluster-113490.0	0.758	3.84E-04	ACHA9_CHICK	6.00E-57	K04810	s196_g34.t1
Cluster-113072.0	0.451	1.04E-02	SPHK1_ARATH	2.00E-04	K04718	s67_g23.t1
Cluster-106624.0	0.601	1.44E-01	CBLBB_XENLA	0	K04707	s80_g9.t1
Cluster-64366.0	0.494	1.49E-02	SOCS4_BOVIN	7.00E-54	K04697	s49_g22.t1
Cluster-32075.0	0.232	7.01E-02	TGFR1_RAT	6.00E-165	K04674	s14_g70.t1
Cluster-62021.3	0.682	1.72E-01	TSP4_HUMAN	1.00E-151	K04659	s149_g8.t1
Cluster-62021.2	0.722	9.94E-02	TSP4_HUMAN	1.00E-151	K04659	s149_g8.t1
Cluster-90084.0	0.456	7.81E-03	CASR_RAT	2.00E-111	K04612	s2_g48.t1
Cluster-92381.0	0.735	6.89E-01	A0A2B4R645_STYPI	1.60E-59	K04599	s77_g82.t1
Cluster-51695.0	1.047	1.88E-02	GNAO_BOVIN	6.00E-101	K04534	s70_g39.t1
Cluster-51695.4	0.952	2.91E-03	GNAO_BOVIN	6.00E-101	K04534	s70_g39.t1
Cluster-101820.1	0.350	5.67E-02	RHOAB_DANRE	2.00E-83	K04513	s255_g28.t1
Cluster-100052.0	0.506	3.61E-01	RHOA_RAT	5.00E-39	K04513	s255_g13.t1
Cluster-77355.1	1.273	2.05E-04	FGFR1_CHICK	2.00E-58	K04362	s459_g9.t1
Cluster-77355.0	1.116	8.30E-03	FGFR1_CHICK	2.00E-58	K04362	s459_g9.t1
Cluster-50735.0	1.007	2.09E-02	FGFR1_CHICK	2.00E-58	K04362	s459_g9.t1
Cluster-60199.0	0.996	9.22E-02	FGFR1_CHICK	2.00E-58	K04362	s459_g9.t1
Cluster-97243.0	1.341	4.79E-04	FGFR1_MOUSE	4.00E-24	K04362	s459_g8.t1
Cluster-113574.0	1.074	6.62E-02	OPRK_MOUSE	2.00E-19	K04214/K04220	.
Cluster-40343.0	1.181	1.55E-03	MC5R_MOUSE	6.00E-07	K04203	s704_g7.t1
Cluster-110221.0	1.103	1.12E-02	ADRB2_BOVIN	2.00E-27	K04142	s325_g3.t1
Cluster-52996.0	0.994	3.60E-02	ADA1B_HUMAN	4.00E-17	K04136	s412_g3.t1
Cluster-76765.0	1.937	2.18E-07	VWF_MOUSE	2.00E-57	K03900	s197_g21.t1
Cluster-76765.4	1.557	4.96E-05	VWF_MOUSE	2.00E-57	K03900	s197_g21.t1
Cluster-76765.3	1.959	1.42E-07	VWF_MOUSE	2.00E-57	K03900	s197_g21.t1
Cluster-102863.0	0.341	3.05E-02	EIF3C_DANRE	0	K03252	s480_g4.t1
Cluster-114298.2	0.552	1.61E-02	SOS2_HUMAN	0	K03099	s60_g8.t1
Cluster-22331.0	1.140	1.50E-02	NOTC1_DANRE	2.00E-21	K02599	s515_g1.t1

Cluster ID	log2 fold change M2	padj_M2	Best BLAST	Best E-value	KO	Genome ID
Cluster-115462.3	0.929	5.16E-03	MCCB_CAEEL	2.00E-161	K01969	s58_g105.t1
Cluster-115462.2	0.753	4.43E-02	MCCB_CAEEL	2.00E-161	K01969	s58_g105.t1
Cluster-115462.0	0.829	4.11E-01	MCCB_CAEEL	2.00E-161	K01969	s58_g105.t1
Cluster-58193.3	0.605	1.09E-02	CPSM_HUMAN	0	K01948	s190_g20.t1
Cluster-58193.1	0.603	9.96E-02	CPSM_HUMAN	0	K01948	s190_g20.t1
Cluster-94339.3	0.470	2.55E-02	GCY3E_DROME	5.00E-174	K01769	s43_g63.t1
Cluster-94339.2	0.451	4.43E-02	GCY3E_DROME	5.00E-174	K01769	s43_g63.t1
Cluster-37437.1	0.700	5.64E-01	ECE1_MOUSE	1.00E-65	K01415	s79_g18.t1
Cluster-59025.1	0.823	1.91E-05	ECE1_BOVIN	5.00E-150	K01415	s151_g7.t1
Cluster-1664.3	0.818	3.52E-02	ECE1_BOVIN	5.00E-150	K01415	s151_g7.t1
Cluster-32013.0	0.958	3.48E-02	MMP3_HUMAN	2.00E-50	K01394	s227_g9.t1
Cluster-40143.2	0.908	2.29E-02	CYSP3_SOLLC	4.00E-40	K01366	s206_g10.t1
Cluster-76886.0	1.471	4.62E-04	FGL2_MOUSE	9.00E-34	K01314	s1400_g3.t1
Cluster-104495.2	0.292	7.70E-02	PCP_BOVIN	1.00E-138	K01285	s20_g47.t1
Cluster-113809.0	0.321	6.79E-03	DPP4_FELCA	7.00E-136	K01278	s447_g12.t1
Cluster-77230.2	1.302	1.46E-03	XYNA_STRLI	4.00E-04	K01181	s1265_g13.t1
Cluster-77230.1	1.788	2.53E-06	XYNA_STRLI	4.00E-04	K01181	s1265_g13.t1
Cluster-62723.1	1.152	5.45E-04	CP17A_CHICK	2.00E-79	K00512	s25_g11.t1
Cluster-112028.5	1.565	5.91E-05	PFX18679	7.00E-72	K00505	s156_g32.t1
Cluster-112028.2	1.541	9.44E-05	PFX18679	7.00E-72	K00505	s156_g32.t1
Cluster-78941.0	1.284	1.53E-03	PFX18679	7.00E-72	K00505	s156_g32.t1
Cluster-80426.1	0.701	2.52E-03	GSXL1_ARATH	7.00E-64	K00485	s326_g12.t1
Cluster-115880.0	0.563	1.11E-02	WNT4_CHICK	2.00E-95	K00408	s4_g87.t1
Cluster-111305.0	0.808	1.43E-02	AL1L1_XENLA	0	K00289	s38_g29.t1
Cluster-107382.0	1.451	4.03E-04	PGDH_MOUSE	3.00E-62	K00069	s46_g67.t1
Cluster-73336.1	-0.646	7.03E-02	M17L2_DANRE	8.00E-29	K13348	s96_g24.t1
Cluster-97668.1	-0.523	5.49E-04	MLC2_DROME	3.00E-37	K12751	s364_g10.t1
Cluster-96376.0	-0.915	3.47E-02	TRIM2_RAT	9.00E-26	K11997	s281_g13.t1
Cluster-75641.1	-1.107	4.06E-02	WRN_HUMAN	1.00E-15	K10900	s1199_g1.t1
Cluster-65058.1	-0.737	1.26E-01	CAD96_DROME	2.00E-43	K08252	s459_g10.t1
Cluster-61510.0	-0.983	1.37E-01	EPHA2_MOUSE	1.00E-116	K05103	s227_g56.t1
Cluster-111570.1	-0.827	8.04E-02	ADRB2_MACMU	2.00E-21	K04142	.
Cluster-41508.12	-1.109	4.44E-02	ASGL1_MOUSE	1.00E-60	K01424	s7_g52.t1
Cluster-1509.2	-1.148	2.48E-02	ASGL1_MOUSE	1.00E-60	K01424	s7_g52.t1
Cluster-86765.6	-1.167	7.52E-03	ASGL1_DANRE	1.00E-52	K01424	s7_g40.t1

Appendix C: Chapter 4

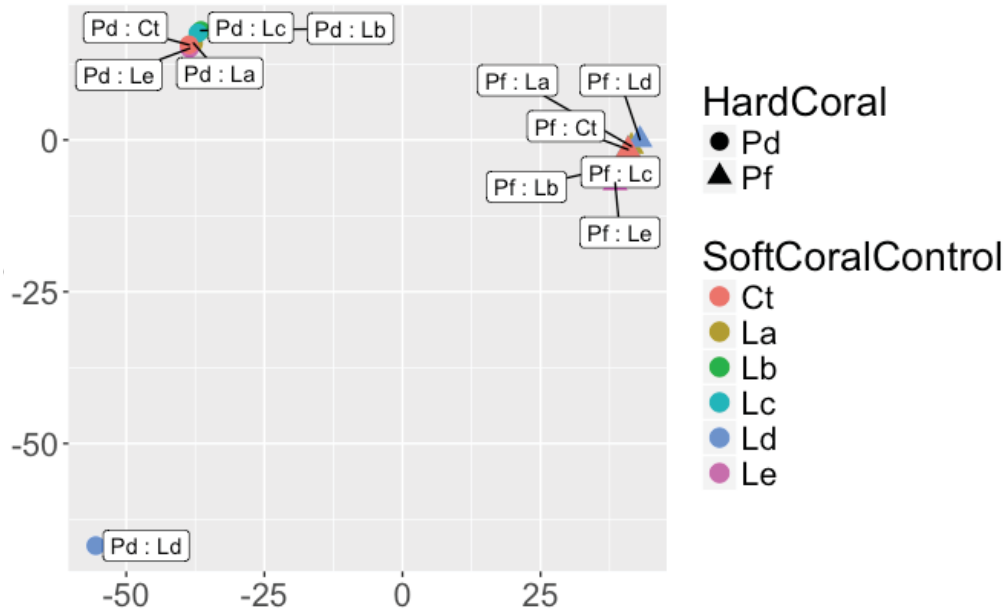

Figure C. 1: PCA of samples showing *Porites* samples from colony Pd and Pf interacting with the five colonies of *Lobophytum* or in control.

Table C. 1: Protein domain from differentially expressed genes in *Porites* colonies interacting with *Lobophytum*

Cluster ID	KO	Best BLAST	Best E-value	log2 fold change	padj	*Porites lutea* ID
Cluster-65721.7813	K01205	ANAG_HUMAN	0.0E+00	0.81	3.7E-02	plut2.m8.29081.m1
Cluster-65721.16456	NA	CL066_HUMAN	6.5E-117	2.26	2.9E-02	plut2.m8.20575.m1
Cluster-65721.43695	K15381	DIRC2_XENLA	1.1E-78	4.35	3.7E-02	plut2.m8.23281.m1
Cluster-52076.0	K20193	HPS1_HUMAN	2.3E-74	3.54	3.3E-03	plut2.m8.4300.m1
Cluster-65721.11203	K16910	PTPRQ_MOUSE	2.2E-99	3.62	6.4E-02	plut2.m8.25273.m1
Cluster-59651.0	K04266	AA2AR_CANLF	6.5E-16	3.66	5.5E-02	jamg1.model.xfSc0000340.5
Cluster-65721.5619	K13912	DMBT1_HUMAN	2.8E-71	1.13	7.4E-02	plut2.m8.12388.m1
Cluster-65721.30303	K04390	TNR6_HUMAN	1.9E-06	2.71	3.3E-02	plut2.m8.12488.m1
Cluster-65721.34213	K21125	MUC5A_HUMAN	5.1E-10	3.54	3.9E-02	plut2.m8.5961.m1
Cluster-65721.34748	K21125	MUC5A_HUMAN	5.1E-10	2.17	7.8E-02	plut2.m8.5961.m1
Cluster-65721.37056	K05305	FUK_HUMAN	2.5E-167	1.40	6.9E-02	plut2.m8.17968.m1

Cluster ID	KO	Best BLAST	Best E-value	log2 fold change	padj	*Porites lutea* ID
Cluster-65721.19733	K19511	PXDN_XENTR	1.4E-14	3.95	7.7E-02	plut2.m8.8042.m1
Cluster-65721.6587	K17496	TIM50_DANRE	1.8E-87	4.41	8.5E-02	plut2.m8.32379.m1
Cluster-67822.0	K17496	TIM50_DANRE	1.8E-87	1.90	9.9E-02	plut2.m8.32379.m1
Cluster-65721.38798	K13723	ERAP2_BOVIN	2.2E-31	2.10	5.1E-02	plut2.m8.32091.m1
Cluster-65721.26350	K10642	DZIP3_MOUSE	1.5E-12	3.98	9.0E-02	plut2.m8.26064.m1
Cluster-65721.8683	K10478	BTBD6_MOUSE	1.8E-50	2.41	2.0E-02	plut2.m8.16281.m1
Cluster-65721.46053	K05094	FGFR3_HUMAN	3.6E-84	2.96	6.8E-02	plut2.m8.17369.m1
Cluster-46927.1	K03654	RECQ_HAEIN	4.7E-11	2.52	5.6E-02	plut2.m8.2031.m1
Cluster-65721.20113	K18245	CAHZ_DANRE	4.2E-46	1.83	2.1E-02	plut2.m8.2750.m1
Cluster-65721.24988	K11165	DHRS7_MOUSE	4.4E-81	2.90	9.9E-02	plut2.m8.19207.m1
Cluster-60667.0	K00597	MTRR_MOUSE	2.8E-144	1.68	7.6E-02	plut2.m8.5327.m1
Cluster-66332.0	K14210	SLC31_MOUSE	3.2E-114	3.68	9.9E-02	plut2.m8.12639.m1
Cluster-65721.37966	K04239	OX2R_RAT	1.4E-42	3.85	3.6E-02	plut2.m8.21287.m1
Cluster-51347.0	K04239	OX2R_RAT	1.4E-42	3.48	4.2E-02	plut2.m8.21287.m1
Cluster-57352.0	K08375	NPFF2_HUMAN	5.1E-51	3.58	5.3E-02	plut2.m8.12434.m1
Cluster-58555.1	K04594	AGRL3_BOVIN	1.2E-31	2.30	1.6E-02	plut2.m8.3175.m1
Cluster-69557.2	K04831	ASI4A_DANRE	2.3E-27	4.37	3.6E-02	plut2.m8.7505.m1
Cluster-65721.721	K01719	HEM4_HUMAN	9.8E-52	2.28	6.4E-02	plut2.m8.523.m1
Cluster-65721.42025	K17341	HMCN1_HUMAN	1.2E-07	2.55	6.1E-02	plut2.m8.7088.m1
Cluster-55143.1	K08959	KC1D_RAT	0.0E+00	3.83	9.6E-02	plut2.m8.3451.m1
Cluster-65721.27743	K08486	STX1B_SHEEP	1.5E-106	3.12	9.6E-02	plut2.m8.12178.m1
Cluster-65721.35632	K06173	TRUA_MOUSE	3.2E-82	4.01	3.3E-03	plut2.m8.2928.m1
Cluster-65721.39009	K13288	ORN_RAT	6.0E-81	1.15	4.1E-02	plut2.m8.16230.m1
Cluster-34283.0	K07874	RIC1_ORYSJ	1.2E-25	3.50	9.9E-02	plut2.m8.8797.m1
Cluster-39072.7	K08745	S27A4_MACFA	0.0E+00	3.22	2.8E-02	plut2.m8.7981.m1
Cluster-65721.16213	K05020	OPUD_BACSU	4.4E-107	1.34	2.0E-02	plut2.m8.10342.m1
Cluster-65721.5330	K00452	3HAO_NEMVE	1.9E-101	4.40	9.4E-02	plut2.m8.20256.m1
Cluster-58182.13	K09428	ELF2_MOUSE	1.2E-28	1.63	7.5E-02	plut2.m8.6132.m1
Cluster-25635.0	K10695	RING2_PONAB	3.1E-137	3.80	6.0E-02	plut2.m8.8355.m1
Cluster-65721.15304	K05208	NMDZ1_MOUSE	1.7E-44	2.26	9.6E-02	plut2.m8.12962.m1
Cluster-61120.4	K07424	CP3AO_SHEEP	3.8E-113	1.80	8.7E-02	plut2.m8.11213.m1

Cluster ID	KO	Best BLAST	Best E-value	log2 fold change	padj	*Porites lutea* ID
Cluster-45841.0	K00167	ODBB_RAT	1.0E-166	2.29	1.6E-02	plut2.m8.6297.m1
Cluster-31851.2	K18189	TACO1_HUMAN	2.9E-41	2.12	8.8E-02	plut2.m8.3874.m1
Cluster-65721.38334	K16342	PA24A_DANRE	4.2E-176	1.87	5.7E-02	plut2.m8.106.m1
Cluster-54380.0	K04210	GPR83_MOUSE	5.3E-32	2.55	2.1E-02	plut2.m8.24999.m1
Cluster-65721.5139	K18437	PDE8B_HUMAN	0.0E+00	0.84	8.7E-02	plut2.m8.12353.m1
Cluster-65721.20654	K14948	PTBP2_RAT	2.4E-157	2.07	6.0E-02	plut2.m8.20808.m1
Cluster-65721.22170	K17922	SNX8_HUMAN	3.1E-70	3.73	1.2E-02	plut2.m8.16098.m1
Cluster-65721.26339	K14613	PCFT_DANRE	5.3E-60	3.12	8.5E-02	plut2.m8.24694.m1
Cluster-65721.17490	K15185	AFF4_HUMAN	1.1E-51	3.48	8.6E-02	plut2.m8.6867.m1
Cluster-34751.0	K15185	AFF4_HUMAN	1.1E-51	1.03	6.4E-02	plut2.m8.6867.m1
Cluster-48653.4	K14720	S39AE_MOUSE	3.7E-42	2.11	2.1E-02	plut2.m8.3622.m1
Cluster-65721.15833	K11292	SPT6H_HUMAN	0.0E+00	2.63	4.3E-02	plut2.m8.6508.m1
Cluster-31830.0	K03781	CATA_DROME	0.0E+00	-3.57	7.9E-03	plut2.m8.12455.m1
Cluster-61415.1	K00613	GATM_XENTR	0.0E+00	-1.03	9.6E-02	plut2.m8.7193.m1
Cluster-65721.41772	K17341	HMCN1_HUMAN	1.8E-08	-0.79	2.9E-02	plut2.m8.17656.m1
Cluster-57694.1	K01106	I5P1_HUMAN	2.9E-89	-3.32	1.9E-08	plut2.m8.3481.m1
Cluster-65721.31362	K07953	SAR1B_BOVIN	2.3E-105	-1.49	1.0E-03	plut2.m8.26983.m1
Cluster-65721.30552	K17285	SBP1_RAT	2.0E-133	-0.91	5.9E-02	plut2.m8.1304.m1
Cluster-54747.2	K21404	AKNA_MOUSE	3.7E-14	-1.67	1.2E-04	plut2.m8.278.m1
Cluster-72455.8	K11217	JAK1_HUMAN	5.7E-15	-3.22	7.8E-02	plut2.m8.3337.m1
Cluster-5692.0	K10048	CEBPB_HUMAN	2.8E-16	-6.38	1.4E-03	.
Cluster-65721.3350	K04309	LGR4_HUMAN	1.2E-132	-1.42	7.8E-02	plut2.m8.2161.m1
Cluster-65721.18038	K04157	5HT2A_CRIGR	2.4E-17	-2.45	6.2E-02	plut2.m8.4611.m1
Cluster-70152.1	K12275	SEC62_PONAB	3.0E-41	-0.96	7.8E-02	plut2.m8.7766.m1
Cluster-46579.6	K16733	RGAP1_HUMAN	1.6E-151	-1.09	7.8E-02	jamg1.model.Sc0000350.12
Cluster-72424.3	K00544	BHMT1_DANRE	0.0E+00	-1.14	9.6E-02	plut2.m8.13433.m1
Cluster-46719.0	K02183	CALM4_ARATH	2.6E-41	-2.54	6.4E-02	plut2.m8.30011.m1
Cluster-65721.40080	K12833	SF3B6_MOUSE	4.9E-70	-2.78	1.2E-02	plut2.m8.23447.m1
Cluster-34455.0	K01206	FUCO_BRAFL	0.0E+00	-1.16	9.6E-02	plut2.m8.25393.m1
Cluster-25123.0	K13126	PABP4_HUMAN	0.0E+00	-2.76	9.1E-02	plut2.m8.5023.m1
Cluster-5789.0	K03231	EF1A_DANRE	0.0E+00	-7.74	8.3E-06	plut2.m8.23228.m1

Cluster ID	KO	Best BLAST	Best E-value	log2 fold change	padj	*Porites lutea* ID
Cluster-65721.39850	K19372	DJC27_DANRE	5.6E-41	-1.23	9.3E-02	plut2.m8.7595.m1
Cluster-65721.31302	K13129	SMN_BOVIN	6.1E-27	-1.16	4.5E-02	plut2.m8.12426.m1
Cluster-65721.34954	K19909	SYT9_HUMAN	2.3E-23	-1.82	5.6E-03	plut2.m8.18540.m1

Table C. 2: Literature related with DEG in *Porites* colonies interacting with *Lobophytum*

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-31830.0	CATA_DROME	Wright et al., 2017	.
Cluster-49904.0	MBLC2_HUMAN	Wenger et al 2014	Pettinati et al., 2015
Cluster-65721.45376	EPN4_BOVIN	Wenger et 2014	Jha et al 2012
Cluster-57352.0	NPFF2_HUMAN	1.Watanabe et al., 2009; 2.Plicket & Schneider 2004	Bray et al 2014
Cluster-65721.3350	LGR4_HUMAN	Vibede et al ., 1998	Roch & Sherwood, 2014
Cluster-65721.25059	NA	Vibede et al ., 1998	.
Cluster-65721.24988	DHRS7_MOUSE	Tarrant et al 2009	.
Cluster-46719.0	CALM4_ARATH	Stewart el al 2017	.
Cluster-65721.42194	CPP1_ACRMI	SMART, Davidson and Swalla, 2002; Weiss et al.2013; Vidal-Dupiol et al 2011; Mydlarz et al 2016	.
Cluster-57694.1	I5P1_HUMAN	Shearer et al 2012	.
Cluster-65721.8923	IPO11_HUMAN	Shearer et al 2012	.
Cluster-5692.0	CEBPB_HUMAN	Sabourault, C et al 2009	.
Cluster-49093.0	SDK2_CHICK	Ramos-Silva et al 2013,2014	.
Cluster-65721.1210	C2CD5_HUMAN	Podobnik & Anderluh 2017	.
Cluster-60313.2	HTD2_HUMAN	Ontology	.
Cluster-45162.3	HTD2_HUMAN	Ontology	.
Cluster-65721.31170	UQCC1_XENLA	Moya et al 2016	Ernester & Forsmark-Andree 1993
Cluster-65721.20113	CAHZ_DANRE	Moya et al 2008	.
Cluster-65721.18038	5HT2A_CRIGR	Mayorova & Kosevich, 2013	Watanabe, 2017
Cluster-59651.0	AA2AR_CANLF	Mason et al 2012; 2. Mohamed et al., 2016	Ohta & Sitkovsky, 2001

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-65721.31362	SAR1B_BOVIN	Maor-Landaw et al 2014	NA
Cluster-65721.38798	ERAP2_BOVIN	Libro et al 2013	Lee, 2017
Cluster-59959.0	SIDT2_HUMAN	Li et al., 2012	1. Jialin et al 2010; 1. Beck et al 2017; Nguyen et al 2017
Cluster-54658.0	CSL1_ONCKE	Kvennefors et al, 2008	.
Cluster-58555.1	AGRL3_BOVIN	Kishnan & Schioth 2015	O'Sullivan et al 2012
Cluster-65721.23813	HRH2_PONPY	Kass-Simon & Pierobon 2007	1.Jouiaei et al, 2015
Cluster-65721.7803	CAHD1_HUMAN	Hemond et al 2014	NA
Cluster-65721.42025	HMCN1_HUMAN	Hammaguchi-Hamada et at 2016	.
Cluster-65721.11203	PTPRQ_MOUSE	Chera et al 2009	Noda and Ohsumi, 1998
Cluster-65721.13148	WDR91_DANRE	Chera et al 2009	.
Cluster-72424.3	BHMT1_DANRE	Brekhman et al 2015; Aguilar et al 2017	NA
Cluster-55143.1	KC1D_RAT	Bhattacharya et al, 2016	.
Cluster-65721.13816	HMCN2_HUMAN	Barshis et al 2013	.
Cluster-65721.37056	FUK_HUMAN	1.Wild et al 2010; 2.Meikle et al 1988	.
Cluster-65721.19733	PXDN_XENTR	1.Voolstra et al., 2009; 2. Louis et al., 2017; 3. Libro et al 2013	Nelson et al 1994
Cluster-65721.30552	SBP1_RAT	1.Shearer et al 2012; 2.Huibin 2011,thesis;	.
Cluster-65721.37966	OX2R_RAT	1.Rosenberg et al., 2017; 2. Grimmelikhuijzen et al 1980	1. Schoofs et al., 2017; 2. Sakurai et al 1998
Cluster-51347.0	OX2R_RAT	1.Rosenberg et al., 2017; 2. Grimmelikhuijzen et al 1980	1. Schoofs et al., 2017; 2. Sakurai et al 1998
Cluster-65721.30303	TNR6_HUMAN	1.Pinzon et al, 2016; 2. Mydlarz et al., 2016; 3. Libro et al 2013	.
Cluster-35329.24	FANK1_HUMAN	1.Ocampo et al 2015; 2. Burge el al., 2013	Wang et al., 2011
Cluster-65721.5619	DMBT1_HUMAN	1.Neubauer et al 2016; 2.Mohamed et al. 2018;3.	.
Cluster-69901.1	AOSL_PLEHO	1.Libro et al 2013; 2.	1.Neau et al 2009 ; 3. Mortimer etal 2006
Cluster-65721.27743	STX1B_SHEEP	1. Watanabe 2017	Smith et al 2014
Cluster-65721.5942	SCR2_ACRMI	1. Jouiaei et al 2015, 2. Jouiaei et al 2015	.
Cluster-65721.5945	SCR2_ACRMI	1. Jouiaei et al 2015, 2. Jouiaei et al 2015	.

Cluster ID	Best BLAST	Literature Cnidaria	Literature other organisms
Cluster-65721.41772	HMCN1_HUMAN	1. Bertucci et al., 2015; 2. Ramos-Silva et al., 2013; 3. Drake, J. L., 2015; 4. Wright et al., 2017 ; 5. Schwarz et al., 2008	.
Cluster-69557.2	ASI4A_DANRE	1. Assmann et al . 2014 ; 2. Rahman et al 2014; 3.Osmakov et al 2013	.
Cluster-60667.0	MTRR_MOUSE	1. Aguilar et at 2017, 2. Wang and Douglas 1999	.
Cluster-45780.0	HEM0_OPSTA	.	Tzou et al, 2014
Cluster-65721.27799	NLRC3_MOUSE	.	Schneider et al., 2012
Cluster-65721.43695	DIRC2_XENLA	.	Savalas et al 2011
Cluster-65721.7813	ANAG_HUMAN	.	Platt et al., 2015
Cluster-46579.6	RGAP1_HUMAN	.	Matsuura et al , 2013
Cluster-52076.0	HPS1_HUMAN	.	Martina et al 2003
Cluster-54747.2	AKNA_MOUSE	.	Ma et al., 2011
Cluster-65721.35632	TRUA_MOUSE	.	Hamma & Ferré-D'Amaré 2006
Cluster-65721.6587	TIM50_DANRE	.	Guo et al., 2004
Cluster-67822.0	TIM50_DANRE	.	Guo et al., 2004
Cluster-61415.1	GATM_XENTR	.	Grohmann et al. 2017
Cluster-70152.1	SEC62_PONAB	.	Fumagalli et al, 2016
Cluster-65721.16456	CL066_HUMAN	.	1. Yao et al 2017; 2.Pan et al 2012
Cluster-33162.0	AVR7_CHICK	.	1. Ladner et al., 2012; 2. Ahlroth et al., 2000
Cluster-63940.0	CLSPN_HUMAN	.	1. Clarke et al., 2005; 2. Azemha et al 2017

Table C. 3: KEGG term related to the UniProt ID found in as best BLAST in *Porites* colonies interacting with *Lobophytum*

Cluster ID	Domains names	Domain e-value	Accession ncbi
Cluster-65721.7803	WA	5.37E-16	smart00327
Cluster-16238.5	ZP	7.90E-35	smart00241
Cluster-65721.34213	VWD	3.01E-22	smart00216
Cluster-65721.34748	VWD	3.01E-22	smart00216
Cluster-65721.5619	SR	4.79E-40	smart00202
Cluster-45780.0	PRK09064	0.00E+00	PRK09064
Cluster-53487.0	LLC1	3.62E-36	pfam14945
Cluster-65721.19733	Ig 3	1.55E-17	pfam13927
Cluster-65721.16512	Methyltransf 25	4.74E-15	pfam13649
Cluster-65721.36430	CHAT	6.17E-48	pfam12770
Cluster-65721.16878	CHAT	6.17E-48	pfam12770
Cluster-62372.15	DUF2615	5.68E-38	pfam11027
Cluster-65721.43695	MFS 1	9.91E-10	pfam07690
Cluster-65721.7813	NAGLU	0.00E+00	pfam05089
Cluster-53936.0	Mpv17 PMP22	4.47E-17	pfam04117
Cluster-65721.31170	Ubiq cyt C chap	9.45E-43	pfam03981
Cluster-70152.1	Sec62	2.62E-55	pfam03839
Cluster-65721.6587	NIF	7.66E-40	pfam03031
Cluster-67822.0	NIF	7.66E-40	pfam03031
Cluster-72424.3	S-methyl trans	8.99E-46	pfam02574
Cluster-54658.0	Gal Lectin	9.06E-22	pfam02140
Cluster-65721.16213	BCCT	0.00E+00	pfam02028
Cluster-65721.31320	AMMECR1	2.11E-53	pfam01871
Cluster-33162.0	Avidin	3.28E-34	pfam01382
Cluster-69557.2	ASC	3.18E-46	pfam00858
Cluster-65721.27743	Syntaxin	4.63E-64	pfam00804
Cluster-65721.8683	BTB	1.18E-18	pfam00651
Cluster-65721.20113	Carb anhydrase	5.77E-93	pfam00194
Cluster-61120.4	p450	2.70E-122	pfam00067
Cluster-65721.37481	COG5048	8.91E-08	COG5048
Cluster-65721.12981	GlcD	2.91E-22	COG0277
Cluster-65721.23813	7tm GPCRs super family	1.08E-30	cl28897
Cluster-65721.18038	7tm GPCRs super family	2.43E-36	cl28897
Cluster-57352.0	7tm GPCRs super family	1.27E-74	cl28897
Cluster-65721.13816	I-set super family	2.93E-12	cl28434
Cluster-69901.1	Lipoxygenase super family	5.40E-72	cl27717
Cluster-65721.5942	igma70 r3 super family	5.63E-03	cl27146
Cluster-65721.5945	igma70 r3 super family	5.63E-03	cl27146
Cluster-46927.1	DEXDc super family	8.61E-18	cl26939
Cluster-65721.37056	Fucokinase super family	8.58E-54	cl26826

Cluster ID	Domains names	Domain e-value	Accession ncbi
Cluster-65721.8039	DNA pol3 gamma3 super family	2.25E-03	cl26386
Cluster-65721.27799	LRR RI super family	1.56E-40	cl26161
Cluster-54708.0	Acetyltransf 10 super family	5.88E-08	cl26092
Cluster-54747.2	SMC N super family	5.13E-04	cl25732
Cluster-65721.13148	WD40 super family	7.49E-19	cl25539
Cluster-46719.0	EFh PEF super family	2.42E-39	cl25352
Cluster-65721.42025	Fascin super family	2.67E-09	cl23781
Cluster-49904.0	metallo-hydrolase-like MBL-fold super family	6.70E-63	cl23716
Cluster-62828.13	LamG super family	6.55E-41	cl22861
Cluster-65721.30303	TNFRSF super family	2.77E-06	cl22855
Cluster-65721.30552	SBP56 super family	9.62E-171	cl22313
Cluster-65721.5330	cupin like super family	5.06E-66	cl21464
Cluster-65721.39850	P-loop NTPase super family,	8.18E-59	cl21455
Cluster-61415.1	Amidinotransf super family	1.24E-12	cl19186
Cluster-39072.7	AFD class I super family	0.00E+00	cl17068
Cluster-59959.0	SID-1 RNA chan super family	8.35E-24	cl16505
Cluster-58182.13	SAM superfamily super family	1.95E-08	cl15755
Cluster-65721.38798	GluZincin super family	1.73E-53	cl14813
Cluster-65721.29997	BCAS3 super family	1.09E-18	cl13871
Cluster-49093.0	Ig super family	4.40E-18	cl11960
Cluster-65721.16456	DUF2003 super family	1.09E-171	cl09652
Cluster-66332.0	AmyAc family super family	4.82E-170	cl07893
Cluster-65721.28005	zf-Di19 super family	1.87E-04	cl05267
Cluster-65721.24991	BRICHOS super family	1.97E-05	cl04394
Cluster-49150.0	Crystall super family	3.38E-07	cl02528
Cluster-73121.3	VWD super family	1.19E-17	cl02516
Cluster-52309.0	MM CoA mutase super family	5.69E-03	cl00817
Cluster-57694.1	EEP super family	6.95E-124	cl00490
Cluster-65721.26350	UBQ super family	1.52E-05	cl00155
Cluster-65721.29629	SCP super family	2.18E-26	cl00133
Cluster-65721.3350	7tmA Glyco hormone R	1.60E-142	cd15136
Cluster-65721.37966	7tmA CCKR-like	3.75E-76	cd14993
Cluster-51347.0	7tmA CCKR-like	3.75E-76	cd14993
Cluster-5692.0	bZIP CEBP	6.23E-26	cd14693
Cluster-55143.1	STKc CK1 delta epsilon	0.00E+00	cd14125
Cluster-65721.40080	RRM SF3B14	1.71E-47	cd12241
Cluster-58194.2	DNase1	4.59E-116	cd10282
Cluster-55964.0	GDPD GDE4	2.50E-143	cd08612
Cluster-31830.0	catalase clade 3	0.00E+00	cd08156
Cluster-65721.721	HemD	4.11E-43	cd06578
Cluster-60667.0	methionine synthase red	1.13E-168	cd06203
Cluster-65721.39009	Orn	8.68E-98	cd06135
Cluster-65721.24988	11beta-HSD1 like SDR c	1.36E-102	cd05332

Cluster ID	Domains names	Domain e-value	Accession ncbi
Cluster-46579.6	RhoGAP MgcRacGAP	7.96E-97	cd04382
Cluster-65721.45376	ENTH epsin	1.99E-61	cd03571
Cluster-60313.2	R hydratase	1.71E-41	cd03449
Cluster-45162.3	R hydratase	1.71E-41	cd03449
Cluster-65721.35632	PseudoU synth PUS1 PUS2	1.04E-82	cd02568
Cluster-65721.31362	Sar1	2.48E-118	cd00879
Cluster-59651.0	7tm classA rhodopsin-like	4.93E-31	cd00637
Cluster-35329.24	ANK	1.54E-33	cd00204
Cluster-65721.46053	PTKc	3.32E-119	cd00192
Cluster-65721.42194	Tryp SPc	8.20E-88	cd00190
Cluster-34283.0	Rab	1.26E-37	cd00154
Cluster-65721.41772	Ig	1.36E-09	cd00096
Cluster-65721.11203	FN3	6.17E-13	cd00063
Cluster-65721.37035	FN3	4.85E-16	cd00063
Cluster-65721.27776	EGF CA	5.28E-12	cd00054
Cluster-58555.1	CUB	2.65E-30	cd00041