University of Derby

Marine Fungi and their Diversity in Coral Reefs

Charlotte David

A submission in partial fulfilment of the requirements of the University of Derby for the award of the degree of Masters of Philosophy

School of Science and Engineering.

Masters of Philosophy January 2024

List of Contents

List of Figures:

Statement

The writing of the thesis is all my own work. The research and data in Chapter 2 is all my own, the data in Chapter 3 was provided by and allowed to be used by James Guest, Adriana Humanes, Elizabeth Beauchamp, Eveline van der Steeg and Helios Martinez. For both chapters the DNA extractions were sent to Barts and The London Genome Centre, Queen Mary University of London for amplification and sequencing. This thesis has been ethically approved (See Appendix 1.2).

Summary of Thesis

Reef-building corals support a wide range of species from microorganisms to reef sharks and manta rays. However, the impact of anthropogenic stress is beginning to show, with increases in the frequency of coral bleaching having knock-on effects for the wider reef community. In recent years, research has begun to show interest in the role microbes play within reefs. Some are suggesting they may hold the answer to the crisis that is unfolding (e.g. the use of microbes to mitigate heat stress or disease susceptibility in corals) (Peixoto et al., 2021). To date, most of this research has focused on prokaryotes and the coral algal symbionts, Symbiodiniaceae, while other microbial groups such as protists and fungi have received considerably less attention (Ainsworth et al., 2017; Bonacolta et al., 2023). The few studies that have explored the impacts of fungal communities on corals have tended to focused on the pathogenic nature (Le Campion-Alsumard et al., 1995), and only recently have people proposed they may be beneficial although a lot is still unknown (Roik et al., 2022). This thesis addresses several aims, including, aiming to further understanding of fungal diversity throughout the coral, as well as aiming to understand how fungal communities within the coral respond to external factors, all of which intend to increase our overall understanding of fungi in corals. Chapter 1 consists of an overview of our knowledge of fungi in the marine environment and fungi associated with corals. Chapter 2 explores how fungal diversity is distributed across coral compartments by separating different compartments (mucus, tissue and skeleton), and using molecular techniques to identify the fungal species found in each compartment. Here I found there were significant differences of the fungal diversity between the compartments (mucus and skeleton), but not between the mucus and tissue and the tissue and skeleton. There were also similarities between mucus and the surrounding environmental pools (sediment and water). In Chapter 3, the final chapter, spatial and temporal factors affecting coral fungal diversity were investigated, by collecting samples from multiple reefs, over multiple time points and across different depths, using molecular techniques to analyse the fungal communities. Surprisingly and in contrast to bacteria, the mycobiome was unchanged across space and time. However, similarities between the fungal diversity found in the coral mucus and the environmental pools of in situ corals were shown, supporting the findings of Chapter 2, which also saw similarities between the fungal communities in the mucus and environmental pools.

Therefore, this thesis adds to our understanding of coral associated fungal species in terms of compartmentalisation and response to temporal and spatial factors.

Acknowledgements:

Throughout my masters I have received an overwhelming amount of help and support from those around me. Specifically, Mike Sweet who has provided great insight into my project and guided me throughout the whole project. As well as Joe Taylor and Alessia Bani who were patient and integral to my understanding of statistical analysis. Hayley Marshall and the wonderful team of technicians at the University of Derby who made my projects possible, while supporting me with their invaluable knowledge and experience. I would like to acknowledge the team who collected and provided the data for the second project of my thesis; James Guest, Adriana Humanes, Elizabeth Beauchamp, Eveline van der Steeg and Helios Martinez. As well as thanking them for allowing me to analyse and use this data. I would also like to thank the team at Barts and The London Genome Centre, Queen Mary University of London for producing the sequences from DNA collected from both projects.

I would also like to acknowledge the professional and emotional support I received from my lab mates, friends, and family. I would be completely lost without their support and encouragement.

Chapter 1: A Background on Fungi and What is Known of Their Presences in the Marine Environment

Microorganisms contribute approximately 15% of the world's biomass, the majority of which is made up of the prokaryotes i.e. bacteria (Bar-On et al., 2018). Fungi, although fewer in number than bacteria are thought to contribute as much as animals, that is 12 Gigatons of the world's total biomass (Bar-On et al., 2018). Indeed, the number of fungal species is thought to be between 2.2 and 13.2 million, with some even suggesting this larger estimation remains an underestimation and the real value could exceed 1 trillion (Hawksworth and Lücking, 2017; Wu et al., 2019). However, only a small percentage of these species have actually been described (~150,000 species at the time of writing) (Niskanen et al., 2023; Phukhamsakda et al., 2022).

Most of the general public would associate fungi with their fruiting bodies (the mushroom), but this is a small proportion of the fungi itself, such as, an individual's mycelium (the underground network of hyphae) can spread unseen within the soil (Zabel and Morrell, 2020). This often means fungi can become contenders for the largest organisms on Earth. For example, *Amillaria ostoyae*, spans across 2,385 acres in Oregon, USA and is thought to be 1900 to 8650 years old (Ferguson et al., 2003). Fungi are not just restricted to soil and are found across a wide range of environments, such as marine oxygen minimum zones (Velez et al., 2022), deep sea thermal vents (Amend, 2014; Nagano and Nagahama, 2012; Velez et al., 2022; Xu et al., 2018), and the polar caps (Rosa et al., 2020; Tsuji et al., 2022). There are many reasons hypothesised to explain their success, including but not limited to, their nutritional acquisition capabilities and their numerous physiochemical traits (Bahram and Netherway, 2022).

Fungi have a wide range of methods for nutrition acquisition, fungi can utilise three main methods of assimilation: saprotrophy, parasitism, and mutualism. Saprotrophic fungi feed by breaking down dead organic material including leaf litter, making much needed resources such as carbon and other nutrients available to higher trophic levels (Averill et al., 2014). Parasitic fungi feed on their host, such as *Ophiocordyceps unilateralis*, also known as the zombie-ant fungus. This fungus dramatically controls the host's relocation outside of its nest, allowing *O. unilateralis* to develop by digesting the host and releasing spores onto other foraging ants (Hughes et al., 2011; Kobmoo et al., 2015). In contrast, mutualistic fungi create mutually beneficial partnerships with their hosts. This method of nutritional acquisition can be further divided into epiphytic (on the surface) and endophytic (within tissue). Many mycorrhizal fungi (such as those found associated with the roots of plants), often fit into these categories and can provide their hosts with essential nutrients such as phosphorus and nitrogen, and even control the composition of their immediate environment (Averill et al., 2019, 2014; Humphreys et al., 2010; Netherway et al., 2021). Within mutualistic fungi there are those which are described as 'symbiotic'. Probably the best example of this would be lichens. There are approximately 3600 species now described, and all of them consist of a fungi and an algae (sometimes with the addition of cyanobacteria). The fungi provide protection from predation and desiccation, whilst the algal partner or photobiont, provides photosynthetic derived carbohydrates (Pichler et al., 2023; Spribille et al., 2022).

Although fungi are widespread across environments, most studies have been terrestrial focused with the marine biome remaining largely under explored with many species likely undescribed. That said, it is known that fungi can be found in large numbers and with high diversity in marine sediment (Mouton et al., 2012; Orsi et al., 2013; Zhang et al., 2015) and the water column (Taylor and Cunliffe, 2016; Tisthammer et al., 2016a). They have also been shown to parasitise marine mammals (Huckabone et al., 2015; Huggins et al., 2020), crustaceans (Duc et al., 2009; Vicente et al., 2012), seagrass (Ettinger and Eisen, 2019; Mouton et al., 2012; Orsi et al., 2013; Zhang et al., 2015) and phytoplankton (Frenken et al., 2017; Sime - Ngando, 2012). The mutualistic roles of marine fungi remain less well understood, although they are thought to be key drivers in nutrient cycles such as the marine carbon cycle (MCC) (Amend et al., 2019; Sen et al., 2022; Taylor and Cunliffe, 2016). Culture-based studies have started to reveal the immense range of biochemical properties that they possess. For example, the ability to biodegrade microplastics (Paço et al., 2017), petroleum based products (Nogueira et al., 2024), whilst others are sources of novel anti-bacterial, anti-fungal and anti-inflammatory compounds (Hassan et al., 2020; Xu et al., 2015).

Although the presence of fungi in the marine environment has been known for the last 40 years (Ramos-Flores, 1983; Strake et al., 1988). A major contributor to the lack of understanding is due to the difficulties associated with isolating and culturing marine fungi. Which is integral to describing new species as well as understanding their functions (Alain and Querellou, 2009; Schultz et al., 2022). Combined with the possibilities of contamination due to the extent of fungal distribution, such as the presence of *Malassezia*. Previously, this genus was well known for being present on mammalian skin, linked to conditions such as eczema and dandruff. It is often found in next generation sequence studies in the marine realm - for example deep sea hydrothermal vents (Amend, 2014; Steinbach et al., 2023), and therefore it is not clear if the detection related to the deep sea is genuine or due to contamination. The expansion and development of next generation sequencing (NGS) has allowed researchers to better understand the sheer extent of fungi within the marine environment. Although NGS allows researchers to understand what is there it provides little insight into the roles of fungi in the marine environment, but other advancements such as the study of metabolomics can begin to understand potential functionalities.

Currently, it is estimated there could be 10,000 species of fungi which dwell in the marine environments, although there are only around 2125 species identified as of 12/01/2025 (Amend et al., 2019; Jones, 2011; Jones et al., 2019, 2015). Marine fungi have been found to span six phyla including; Aphelidiomycota, Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, and Mucoromycota (Jones et al., 2015). Most species are from the Ascomycota phylum, whereas Basidiomycota has the genus (*Aspergillus*) with the highest species richness. Chytridiomycota are also well-studied, due to their parasitism of phytoplankton, likely affecting food webs as well as nutrient cycles in the open ocean (Frenken et al., 2017; Sime - Ngando, 2012).

The coral itself is an oasis for several different microscopic organisms including fungi, often referred to as a holobiont which consists of a host and their associated microorganisms (Ainsworth et al., 2017; Blackall et al., 2015; Bonacolta et al., 2023; Galand et al., 2023; Simon et al., 2019). These microorganisms, including bacteria, algae, viruses, archaea, protists, and fungi, can be symbiotic, mutualistic, or parasitic depending on the conditions and the nature of the organism. The presence of these associated microorganisms in corals are well documented with previous research focusing on symbiotic algae (Symbiodiniaceae) and the better understood bacteria.

The associated microorganisms can be found throughout the coral but are also thought in some cases to be specific to compartments of the coral. For the purpose of this thesis, I will focus on the broader terms; the mucus, tissue and skeleton layers also known as compartments, each of these compartments provide different environments due to their different functions. The corals surface mucus layer (or SML) is nutrient rich and known to trap free floating nutrients as well as acting as a layer of protection against disease and pathogens (Glasl et al., 2016; Nelson et al., 2013). The tissue, consists of the tubular network of gastrovascular canals which connect to the gastrovascular cavity (Engelen et al., 2018). In the same compartment is the basal body wall containing calicoblastic cells which secretes an organic matrix resulting in the formation of the corals skeleton (Galloway et al., 2007; Tambutté et al., 2007). The skeleton is a porous material built with fibrous aragonite crystals. These form small spaces forming a highly suitable microhabitat for coral-associated microbiota (Góes-Neto et al., 2020; Golubic et al., 2005; Pernice et al., 2020).

Only a handful of studies have investigated fungi associated within the coral holobiont, previously focusing on the potentially pathogenic effects of fungi (Le Campion-Alsumard et al., 1995). But interests have shifted in recent years due to a growing understanding that fungi could provide potentially beneficial roles within the coral holobiont, such as novel metabolites (i.e. antimicrobials (Li et al., 2014; Wang et al., 2017)) with a wide range of uses in the terrestrial world as well as within the reef itself (Peixoto et al., 2021, 2017). However, little is known about the general diversity of fungi within coral holobionts, whether fungi are species specific to the coral or potentially specific to the reef, whether the fungi fluctuate over time or during stressor events such as bleaching, and even on a smaller scale the diversity, distribution, and functionality of fungi within the coral themselves. With this thesis being to our knowledge the first to specifically look at the diversity between the three compartments of the same coral. Previous studies have focused on one, potentially two compartments, none have investigated all three from the same coral. But all have shown the presence of fungi throughout the coral.

In contrast, we have a much better understanding of other well studied microorganisms and their presence within the coral holobiont. Such as bacteria and the coral symbiotic algae Symbiodiniaceae. For example, both communities are known to fluctuate within corals, shifting during and after thermal stress events (Bourne et al., 2008; Brener-Raffalli et al., 2018; Grottoli et al., 2018; Sun et al., 2022). Other studies show that bacterial community compositions are often host-specific, with some species of corals showing a more diverse or flexible microbiome, whilst others have a more stable microbiome even when experiencing stressor events (Góes-Neto et al., 2020; Pogoreutz et al., 2018; Tong et al., 2020; Ziegler et al., 2019). The coral symbiotic algae Symbiodiniaceae is by far the best documented for its response to thermal stress in corals, with research showing there are multiple clades which have a wide range of thermal tolerance, often swapping and shifting in relative abundance depending on sea surface temperatures (SST) (Díaz-Almeyda et al., 2017; Grégoire et al., 2017; Kemp et al., 2014). SST is just one of many factors impacting the microbiome of any marine organism (Maire et al., 2022; Pootakham et al., 2018; Wang et al., 2018), such as levels of sedimentation (Fifer et al., 2022), nutrient enrichment (Wang et al., 2018; Ziegler et al., 2019), pH and CO_2 concentration (Biagi et al., 2020).

This thesis endeavours to increase our understanding of marine fungi, specifically fungi associated within the coral holobiont. To do this, two studies have been carried out; the first to use molecular tools to characterise the fungal diversity throughout the coral, which has been done by separating the three main compartments (mucus, tissue, and skeleton). The second is to also use molecular tools to understand how spatial (reef and depth) and temporal (seasonal) factors can affect coral fungal diversity, in which samples were taken from three separate reefs in Palau across a 17-month period. The intention of these studies is to provide a starting point for further research, a first step in addressing the current lack of understanding of fungal diversity in the marine environment and more specifically in corals. Increasing our knowledge of what fungal species are present in the coral microbiome may be a useful stepping stone to aid future research in marine natural product discovery, the role fungi may play in the coral holobiont and how marine fungi respond to environmental change.

Chapter 2: Fungal Diversity Throughout Coral Compartments.

2.1) Abstract:

Fungi have been recorded in corals for decades but interest in them has been overshadowed by other microorganisms such as bacteria and the corals algae symbiont, Symbiodiniaceae. However, recent studies are beginning to show the potential benefits of coral associated fungi. Here I aimed to explore the distribution of fungi associated within different coral compartments (mucus, tissue, and skeleton), to help develop an understanding of the potential functional roles of the associated fungi. There were significant differences in fungal diversity between the mucus and skeleton of *Porites lobata* and *Porites lutea* ($p < 0.02$). However, this was not the case in *Galaxea fascicularis* ($p > 0.2$). Interestingly, there was a strong similarity between the fungal composition of the mucus of both *Porites* species and the water samples ($p > 0.9$). In terms of the different fungal compositions between compartments, the next step will be to determine its cause i.e. are the fungi actively selecting locations or is a similar environmental pool being differentiated due to coral influences such as their defensive systems?

2.2) Introduction:

Corals are complex organisms known commonly as a ‘holobiont’, consisting of the Cnidarian host and a vast array of bacteria, algae, viruses, protists, archaea and fungi (Ainsworth et al., 2017; Blackall et al., 2015; Bonacolta et al., 2023; Galand et al., 2023). These microorganisms have been shown to be able to carry out various functions for the coral. For example, Symbiodiniaceae which are symbiotic partners which supply the coral host with carbon rich photosynthetic products in exchange for nitrogenous waste (Morris et al., 2019; Ros et al., 2020). The second most well described associates of the coral holobiont are bacteria. Importantly bacteria have been shown to compartmentalise within the host i.e. separate into distinct community profiles in, for example, the mucus, tissue and skeleton (Glasl et al., 2016; Góes-Neto et al., 2020; Sweet et al., 2011).

A few studies have already hinted that fungal community structures may vary between compartments. For example, the prevalence of endolithic fungi in the skeleton (Góes-Neto et al., 2020; Golubic et al., 2005; Pernice et al., 2020), with other fungal species found more commonly within the tissue (Bonthond et al., 2018; Rabbani et al., 2021; Work et al., 2016) and/or showing a preference for the mucus (Kannan et al., 2017). However, to date there have been no studies which have systematically explored fungal diversity across all three compartments within the same coral fragment (Roik et al., 2022). This is not surprising, as until recently marine fungi were thought to primarily be pathogenic in nature. For example, fungi have been associated with dark spot syndrome (Sweet et al., 2013), black band disease (Yarden et al., 2007), and sea fan disease in gorgonian corals (Alker et al., 2001), although, the latter has been questioned by researchers (Sheridan et al., 2013). However, recent research is starting to uncover evidence that fungi can also provide beneficial services for corals, such as antibacterial properties (Li et al., 2014; Wang et al., 2017) and nitrogen cycling (Wegley et al., 2007). That said, it is generally recognised that there is a substantial lack of understanding of the functional traits of coral-associated fungi, as well as many novel species which have yet to be described. This means that our knowledge gap impedes upon how much can be learnt from future studies (Amend et al., 2012; Bonthond et al., 2018). In this thesis, I intend to broaden the understanding of the distribution of fungal species within the coral compartments, using DNA sequencing to visualise fungal communities with the goal of aiding future studies on fungal functionality within the coral microbiome.

2.3) Methods:

The corals used in this study were from the Aquatic Research Facility (ARF), University of Derby. Three species (*Porites lutea*, *Porites lobata* and *Galaxea fascicularis*) were chosen due to their high abundance in the wild, therefore being representative of large proportions of many coral communities, and recent research describing *G. fascicularis* as an ideal model for coral symbiotic research (Puntin et al., 2023). Further all the corals can be easily fragmented to provide nubbins of a similar size (~2cm) for experimentation. Each nubbin was housed in one of two purpose-built tanks, each with slightly different conditions to simulate different natural ranges the corals might be exposed to in situ. The first containing a Deltec 400i DC protein skimmer, two Fluval E Series 300 W thermostatic heaters and Jerod 4 channel doser DS4) for two weeks kept at 26.3 °C (SD ± 0.59) at a depth of 91cm (from light source to coral surface) with an average PAR reading during the day of 1339.86 lux (SD ± 751.8), at ~34 ppt under a 12:12 hour light dark regime supplied by two 32D Hydra reef lights. The other tank being; two D&D titanium 250 w heaters and a Teco 2000 chiller, mechanical X Filter 1.7, *Chaetomorpha sp*. algae refugium lit by 24" XHO, Ultra Reef UKS-200 protein skimmer, chemical filtration by rowaphos and activated carbon and Sicce 9.0 return pump, with Alkatonic and Dosetronic suppling Triton alkalinity, magnesium, calcium and trace element solutions) with an average temperature of 28.1 °C (SD ± 0.39), at ~34 ppt and at a shallower 42 cm depth (from light source to coral surface) with an average PAR reading during the day of 1582.63 lux (SD ± 811.9). Using a Neptune Systems Apex aquarium controller to maintain 12:12 hours light and dark regime using 60" XHO reef lights. Using the two different tanks to provide an insight into natural variation the corals may experience in situ. These were maintained for three weeks, throughout this period temperature and light intensity data were recorded using HOBO data loggers.

After the nubbins were separated by compartments (mucus, tissue, and skeleton). To collect the mucus, the method from Sweet et al., (2011) was followed, using the "snot sucker". Each sample had 360ml of sterile artificial sea water washed over it, the water was then run through a 0.22 µm polycarbonate filter, the filter was then stored in 100 % molecular grade EtOH until further analysis. The nubbins were flash frozen in liquid nitrogen immediately after mucus collection and stored in EtOH. To collect the tissue, the nubbins were placed into a sterile Ziplock bag, an airgun and Ethylenediaminetetraacetic acid (EDTA) at a concentration of 0.5 M and a pH of 8, were utilised to blast the tissue off the skeleton. Volumes over the range 1.5 – 3 ml of EDTA were used dependant on the size of the sample. The tissue slurry was then collected in a 2 ml Eppendorf tube, preserved in EtOH, and stored at -20 °C. The remaining skeleton was crushed up using a sterile pestle and mortar, the paste was transferred to a 2 ml Eppendorf also preserved in EtOH and stored at -20 °C. Water from each tank was collected (3 X 1 L from each, combined to a total of 3 L), and this water was filtered through 0.22 µm polycarbonate filter and stored in EtOH at -20 °C.

DNA extraction and ITS rRNA gene amplicon sequencing:

For all the samples DNA was extracted using the Qiagen DNeasy PowerSoil Pro Kit using the manufacturers protocol. ITSS rRNA genes from cDNA were amplified using the primers CS1-5.8S-Fun-2-for (5'-CAAGCAGAAGACGG CATACGAGAT-NNNNNNNNNNNN-AGTCAGTCAG-GG-AACTT TYRRCAAYGGATCWCT-3') and CS2-ITS4-FUN-2-rev ('5=-AATGATACGGCG ACCACCGAGATCTACAC-TATGGTAATT-AA-AGCCTCCGCTTA TTGATATGCTTAART-3'). The extracted DNA was then sent to the sequencing facility (Barts and The London Genome Centre, Queen Mary University of London) for quality control, PCR, library preparation, and pair-end sequencing with Illumina MiSeq platform v3 (2 × 300 bp). A contamination control was included at each step of the process. The sequences collected in this study have been deposited in the NCBI Sequence Read Archive under the accession number PRJNA1183395.

Histology:

Nubbins were fixed in Hartman's/Davidson's Fixative for 48 hours, then decalcified in either 5 % EDTA / 0.5 % fixative solution or in Calci-Clear (National Diagnostics). The decalcifying solutions were changed three times a week until the skeleton had fully dissolved which varied from 3 - 5 days. The samples were then dehydrated in sequential ethanol washing from 75 % - 100 %. The nubbins were embedded in paraffin and 5 µm sections were cut using a microtome (Thermo Scientific, Microm HM 325). The sections were stained with Lactophenol cotton blue, Hematoxylin and Eosin and DAPI (4',6-diamidino-2-phenylindole). Images were visualised by the Keyence VHXS750E (cotton blue and H&E) and Olympus IX83 inverted fluorescent (DAPI). The images were assessed in ImageJ2 (Rueden et al., 2017).

Statistical Analysis:

The data was analysed in R Studio (Version 4.3.1, RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/). The sequences were trimmed and quality checked using DADA2 (Callahan et al., 2016). All OTU's were retained for downstream analysis. The abundance graphs were produced using phyloseq (McMurdie and Holmes, 2013). For alpha diversity Chao1, evenness, Shannon and Simpson were all calculated and a graph was produced using ggplot 2 and vegan package (Oksanen et al., 2009; Wickham, 2016). For the beta diversity, PCoA plots were produced using the microeco package (Liu et al., 2021), Adonis and PERMANOVA were used to check for significant differences followed by Tukey post-hock tests.

2.4) Results:

Fungal communities within compartments:

There were no significant differences found between the corals from the two tanks for any of the statistical analysis and the data will be combined. *Galaxea fascicularis* showed no significance difference in the fungal communities across the compartments (p > 0.2) (Figure 2.1C). *Porites lobata* and *Porites lutea* in contrast did show differences in their fungal communities between compartments with a significant difference in fungal diversity between the mucus layer and the skeleton (p < 0.02). For both *P. lobata* (Figure 2.1A) and *P. lutea* (Figure 2.1B) the mucus fungal diversity was found to be very similar (p > 0.9) to the fungal diversity of the water samples.

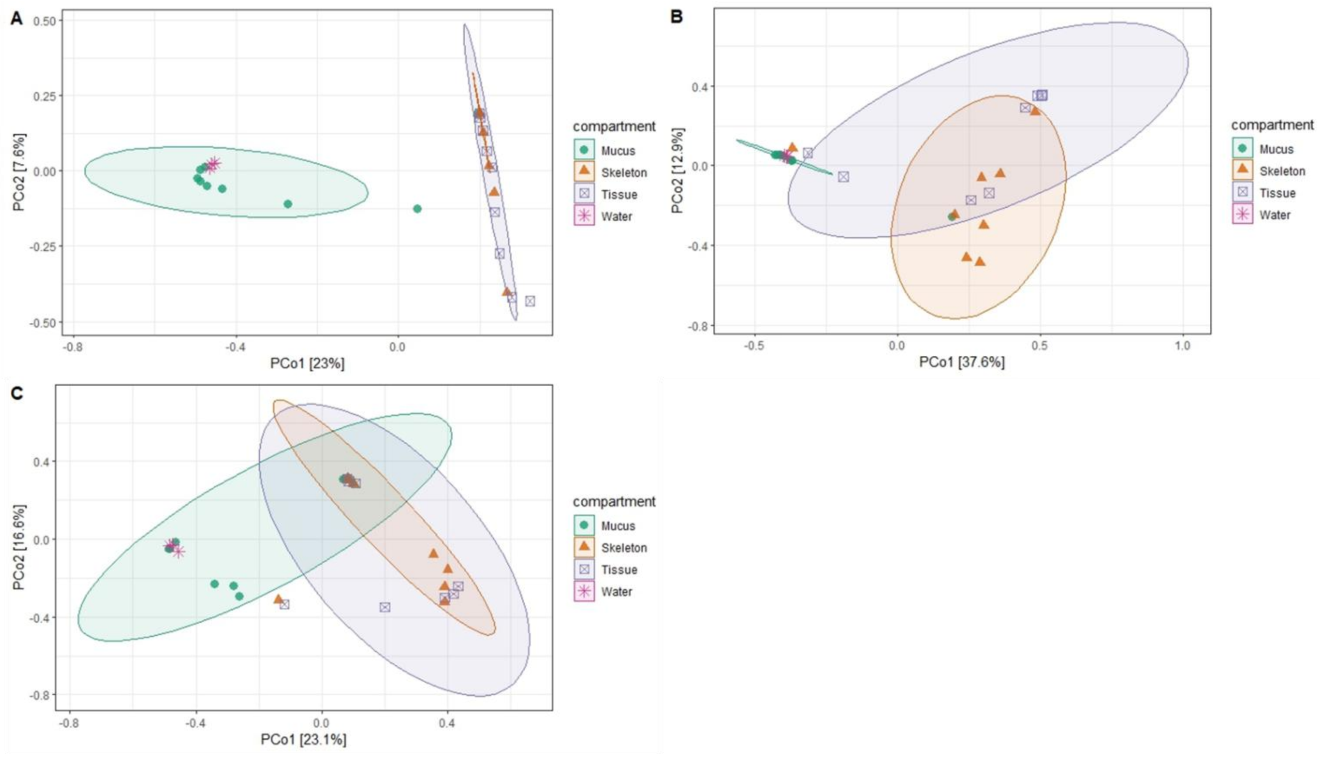


Figure 2.1: PCoA plots comparing the fungal community composition between the compartments (mucus, tissue, skeleton and water) of each of the coral species, A) *Porites lobata*, B) *Portites lutea,* C) *Galaxea fascicularis.*

Fungal communities in compartments across coral species:

There were no significant differences between fungal communities associated with the mucus of all three species (P > 0.5) (Figure 2.2A). There was however a significant difference from the tissue samples of *P. lutea* and *P. lobata* (p < 0.04), but no significant different between *P. lutea* and *P. lobata* to *G. fascicularis* ($p > 0.08$) (Figure 2.2B). The skeleton fungal communities for all three species were also significantly different ($p < 0.05$) from each other, with the largest significant difference between *P. lobata* and *P. lutea* ($p < 0.00006$) (Figure 2.2C).

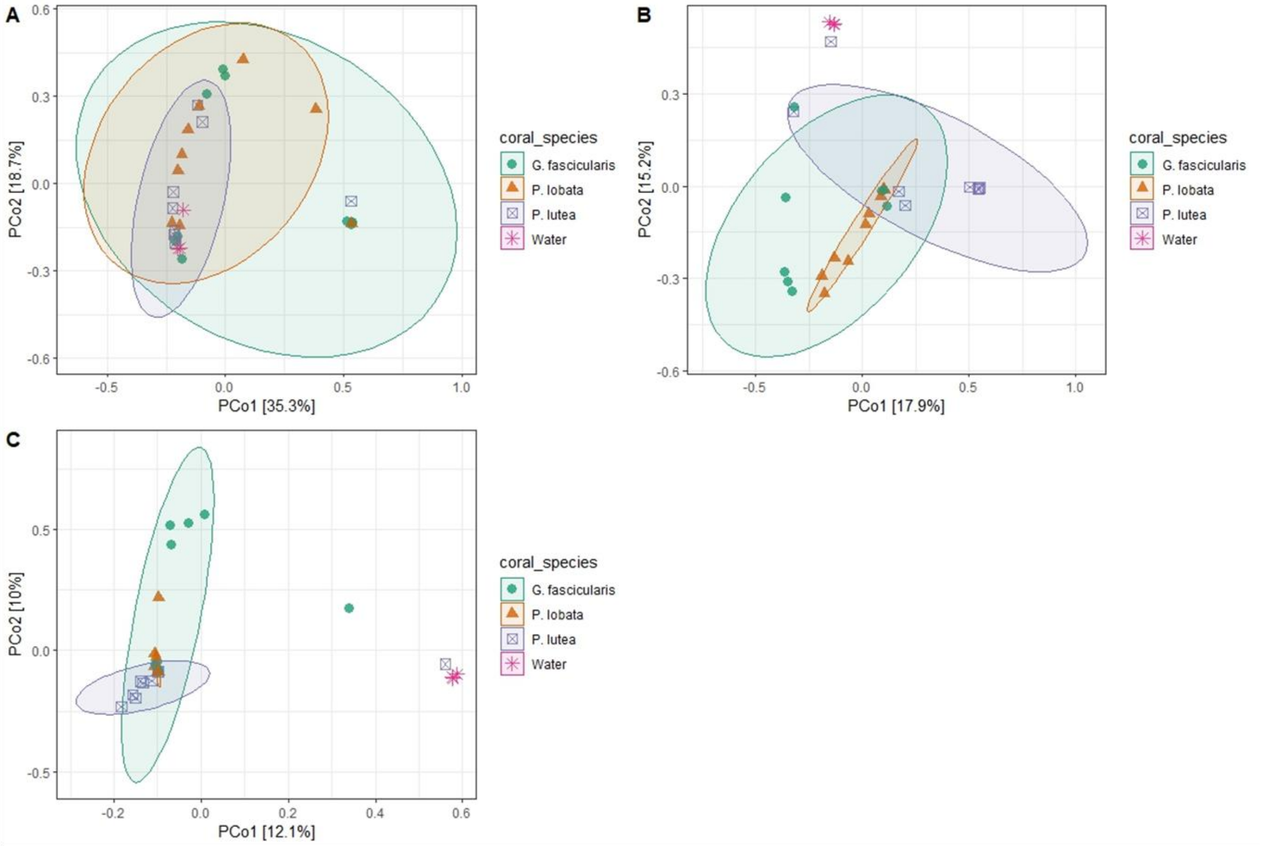


Figure 2.2: PCoA plots comparing the fungal community composition found in each compartment of the three coral species, A) Mucus, B) Tissue, C) Skeleton.

Diversity and richness of coral associated fungi:

The fungal community alpha diversity indices included Chao1 (richness), Simpsons and Shannon alongside evenness (Figure 2.3). The alpha diversity plots showed that the highest diversity and richness of fungal species was found in the water and *P. lutea* (mucus, tissue, and skeleton) samples, with *P. lobata* skeleton samples having the lowest richness and diversity. Most of the samples showed high evenness, suggesting that there is no dominant OTU within any given coral species, with the exception of skeleton samples from *P. lobata*.

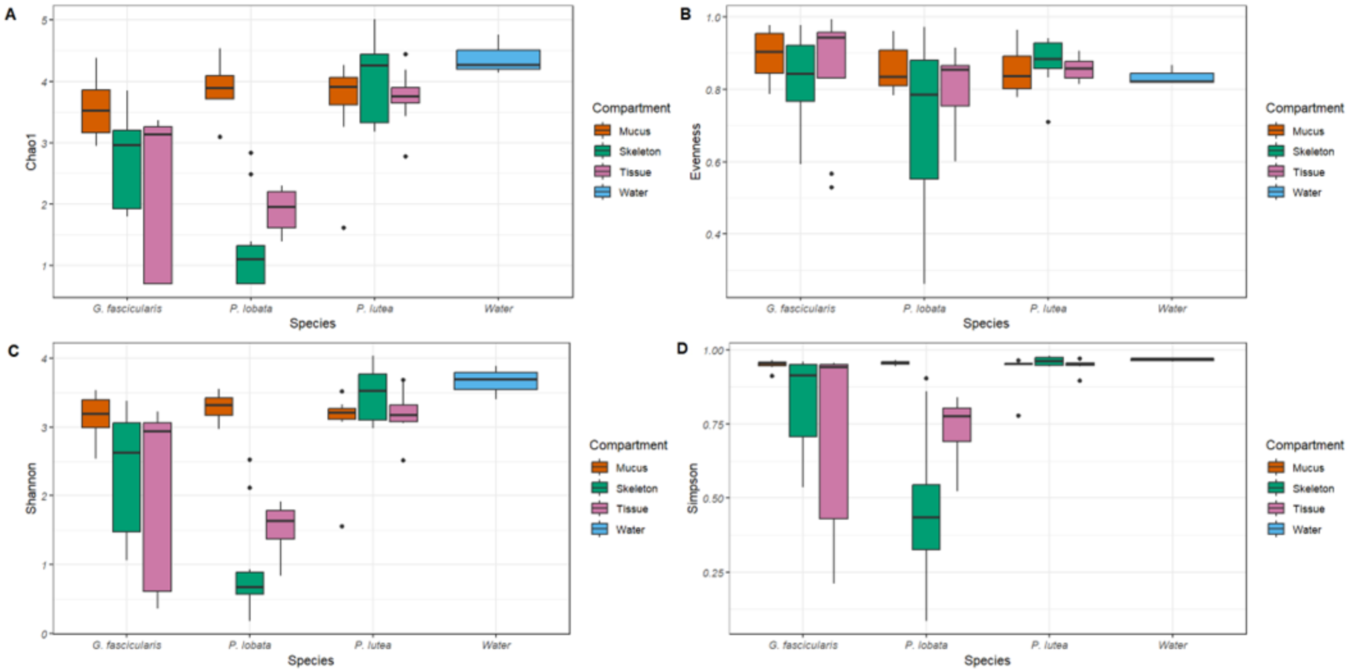


Figure 2.3: The diversity and composition of each coral species and their compartments at a fungal OTU level. A) Chao1 Index, B) Eveness distribution, C) Shannon Index, and D) Simpson Index.

A total of 1359 OTU's were identified, which consisted of three fungal phyla (Figure 2.4D), and 29 genera (Figure 2.4A-C). The dominant phylum was Ascomycota, with it accounting for a relative abundance of over 90 % of the OTU's in the majority of the samples, apart from the skeleton samples from both *Porites* species. Ascomycota was still the most prominent in *P. lobata* (49 %), followed by Basidiomycota (25 %), 'unidentified' fungi (21.7 %) and Chytridiomycota (3.8 %). The skeleton of *P. lutea* was the only compartment that was predominantly Chytridiomycota (53 %), followed by Ascomycota (40 %), with 7 % being classified as unidentifiable. The water samples were dominated by Ascomycota (75.5 %), along with Chytridiomycota (19.5 %) and unidentified (4 %). At a family level the greatest diversity was seen in the mucus, in which Clavicipitaceae (species: *Metarhizium sp*.) dominated across all three species (83 – 96 %). However, there were 12 other families present albeit all at low abundance. The tissue for all three species was also dominated by Clavicipitaceae, although with a lower abundance in the two *Porites* species (58.6 – 68.9 %), followed by Metschnikowiaceae (species: *Clavispora lusitaniae*, *Metschnikowia sp.*) (17 – 20 %). In the skeleton Clavicipitaceae also dominated in *G. fascicularis* (37.8 %), followed by Mycosphaerellaceae (species: *Ramularia unterseheri*) (34.6 %), Extremaceae (species:, *Vermiconia antarctica*) (21.4 %) along with several other families all in low abundance. In *P. lobata* the main fungal families were Bulleribasidiaceae (species: *Dioszegia crocea*) (25.3 %), Clavicipitaceae (25 %) and unidentified (23 %). *P. lutea* was dominated by an unknown Chytrid family/ies (53 %) and Clavicipitaceae (27.9 %). The water samples were also dominated by Clavicipitaceae (75.8 %) followed by unidentified (19.7 %) and unidentified Chytrid family/ies (3.8 %). It is worth noting that throughout all coral species and compartments, between 41 % - 99 % of the OTU's were unidentified to a genus level. With the highest relative abundance of unidentified OTU's seen in the tissue of *G. fascicularis*.

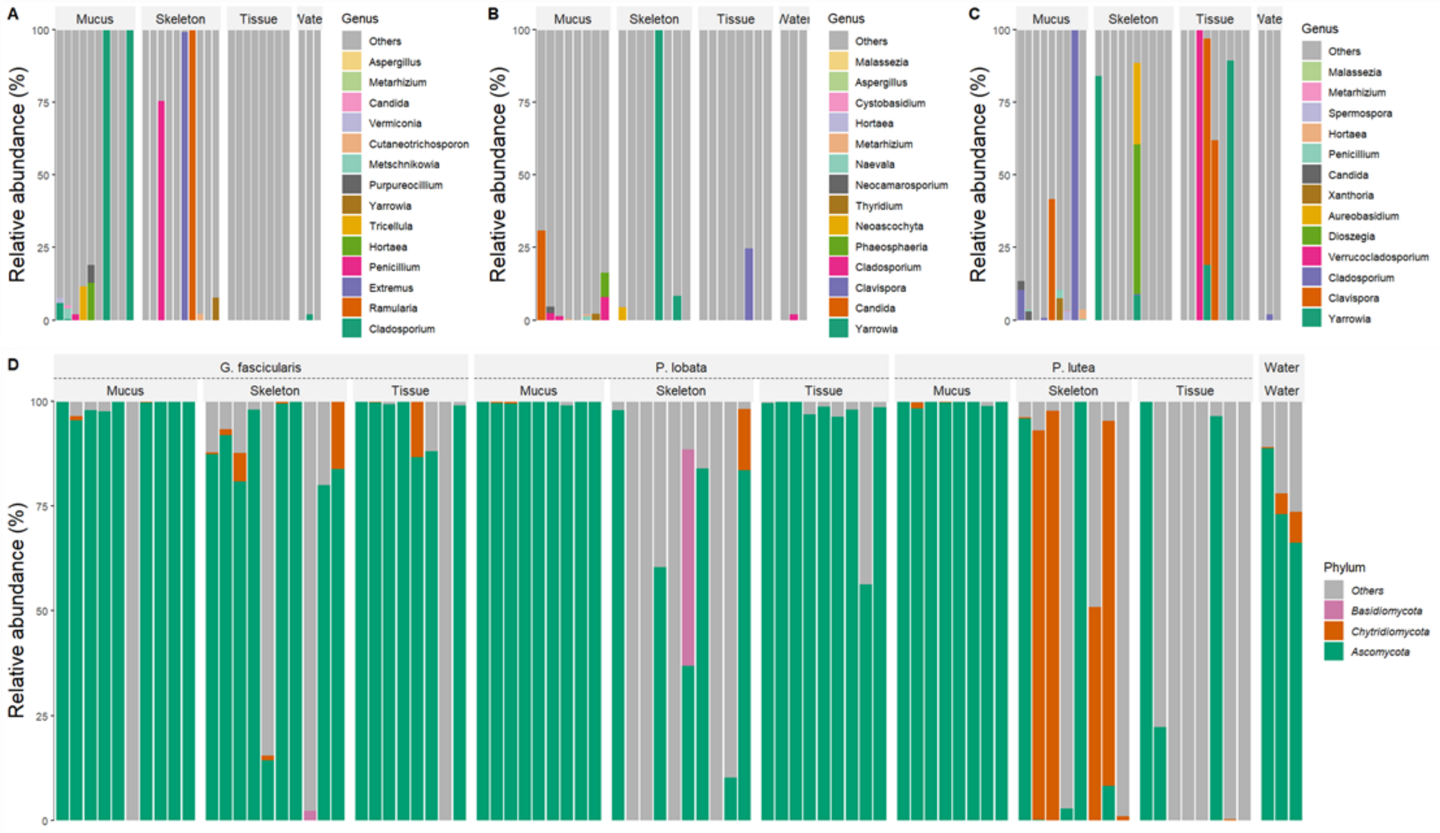


Figure 2.4: Relative abundance of the top 15 genus found in each coral species: A) *Galaxea fascicularis*, B) *Porites lobata*, C) *Porites lutea*, and D) relative abundance of fungal phyla for each of the three coral species and their compartments.

Histological Results:

Many of the sections showed at least some signs of fungal growth. Lactophenol cotton blue, a specifically fungal stain, stained slides showed the strongest evidence, with hyphae seen in much of the *P. lobata* samples/slides (Figure 2.5G-L). Evidence of fungal growth was much weaker in *G. fascicularis* samples/slides, other than a suspected fungal hyphae bundle found in just one section (Figure 2.5A-C).

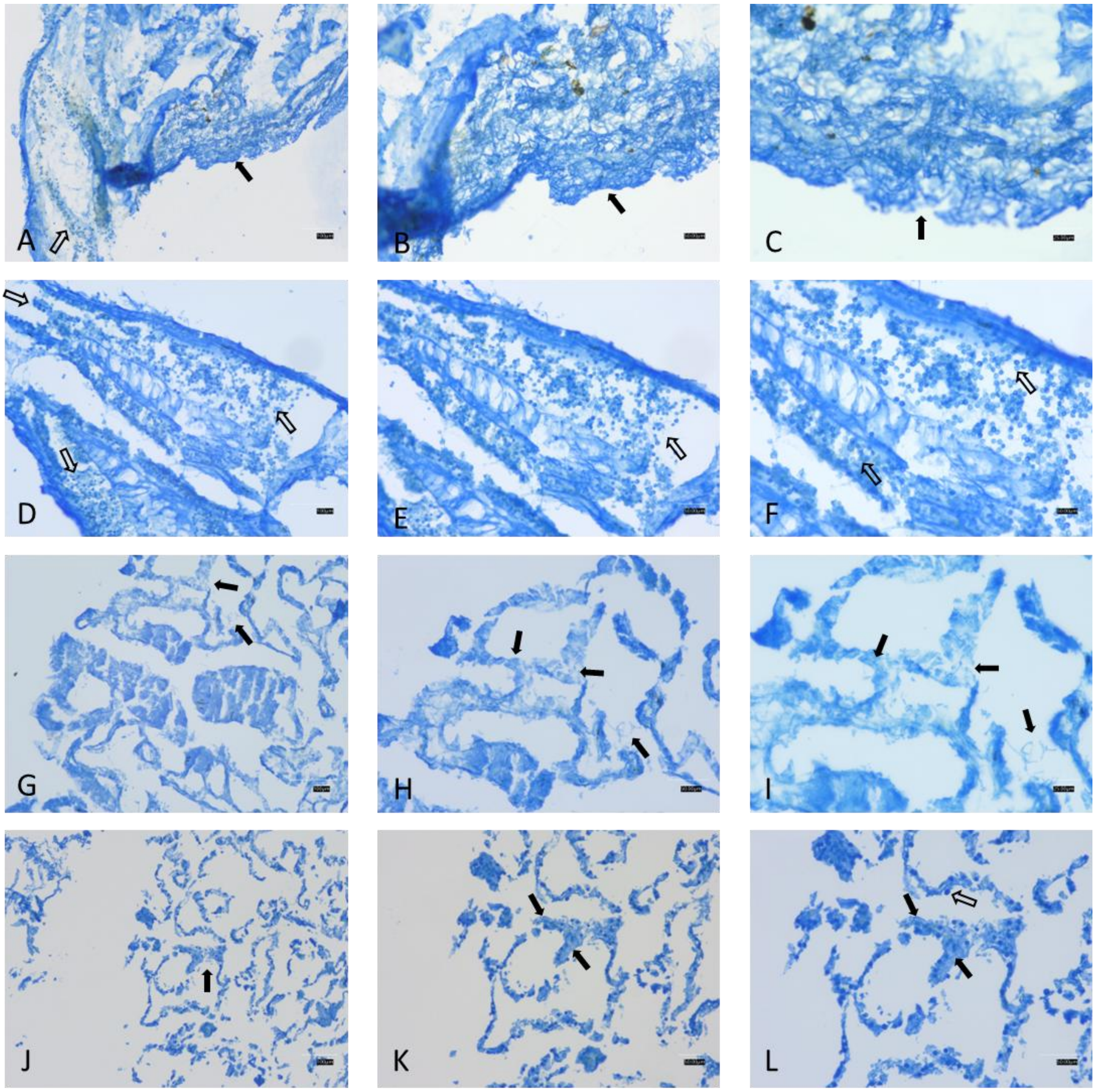


Figure 2.5: Microscope images of coral nubbins stained with lactophenol cotton blue, possible fungal hyphae shown by ⬆, Symbiodiniaceae shown by ⇧. A) x 30 *Galaxea fascicularis*, B) x 70 *Galaxea fascicularis*, C) x 100 *Galaxea fascicularis* D) x 40 *Galaxea fascicularis,* E) x 50 *Galaxea fascicularis*, F) x 70 *Galaxea fascicularis*, G) x 40 *Porites lobata*, H) x 70 *Porites lobata,* I) x 100 *Porites lobata*, J) x 30 *Porites lobata*, K) x 50 *Porites lobata*, L) x 70 *Porites lobata*.

The slides stained by Hematoxylin and Eosin did not show any signs of fungi (Figure 2.6), and neither did the slides stained using fluorescent DAPI (Figure 2.7).

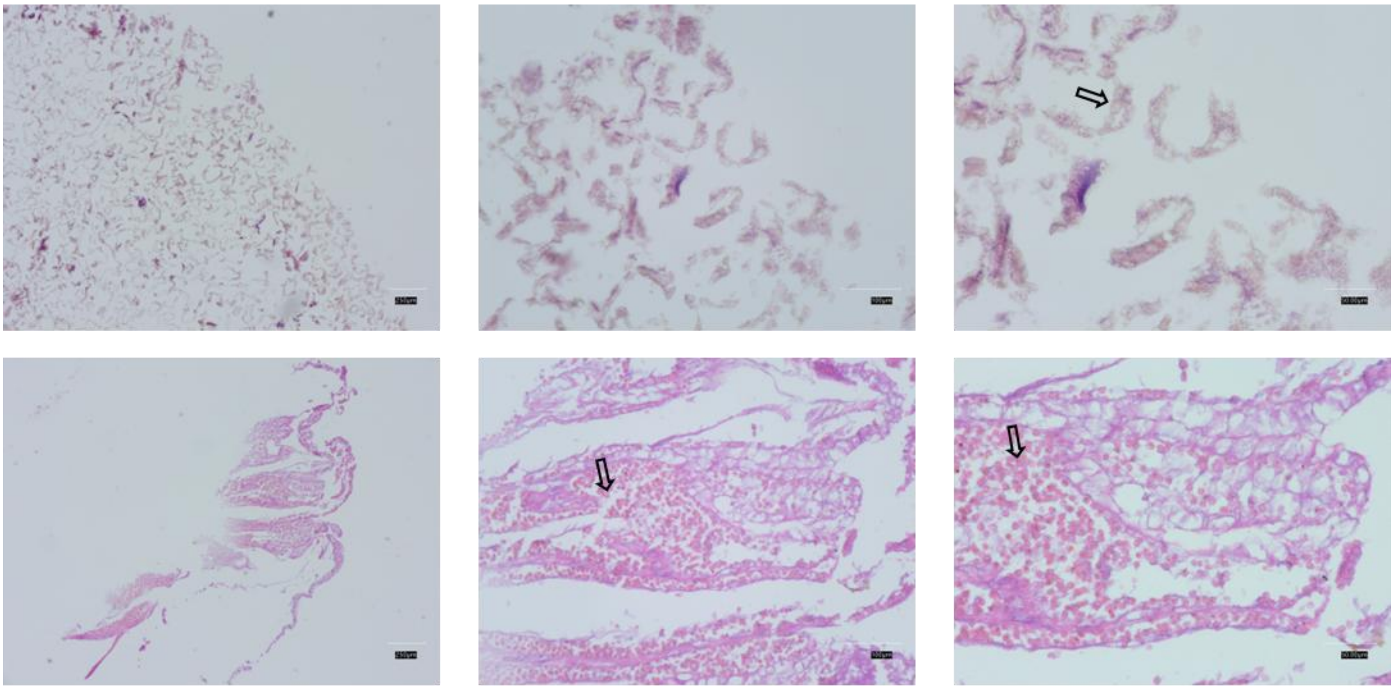


Figure 2.6: Microscopic images of coral nubbins stained with Hematoxylin and Eosin, Symbiodiniaceae shown by ⇑. A) x 10 *Porites lobata*, B) x 40 *Porites lobata*, C) x 70 *Porites lobata*, D) x 10 *Galaxea fascicularis*, E) x 40 *Galaxea fascicularis*, F) x 70 *Galaxea fascicularis*.

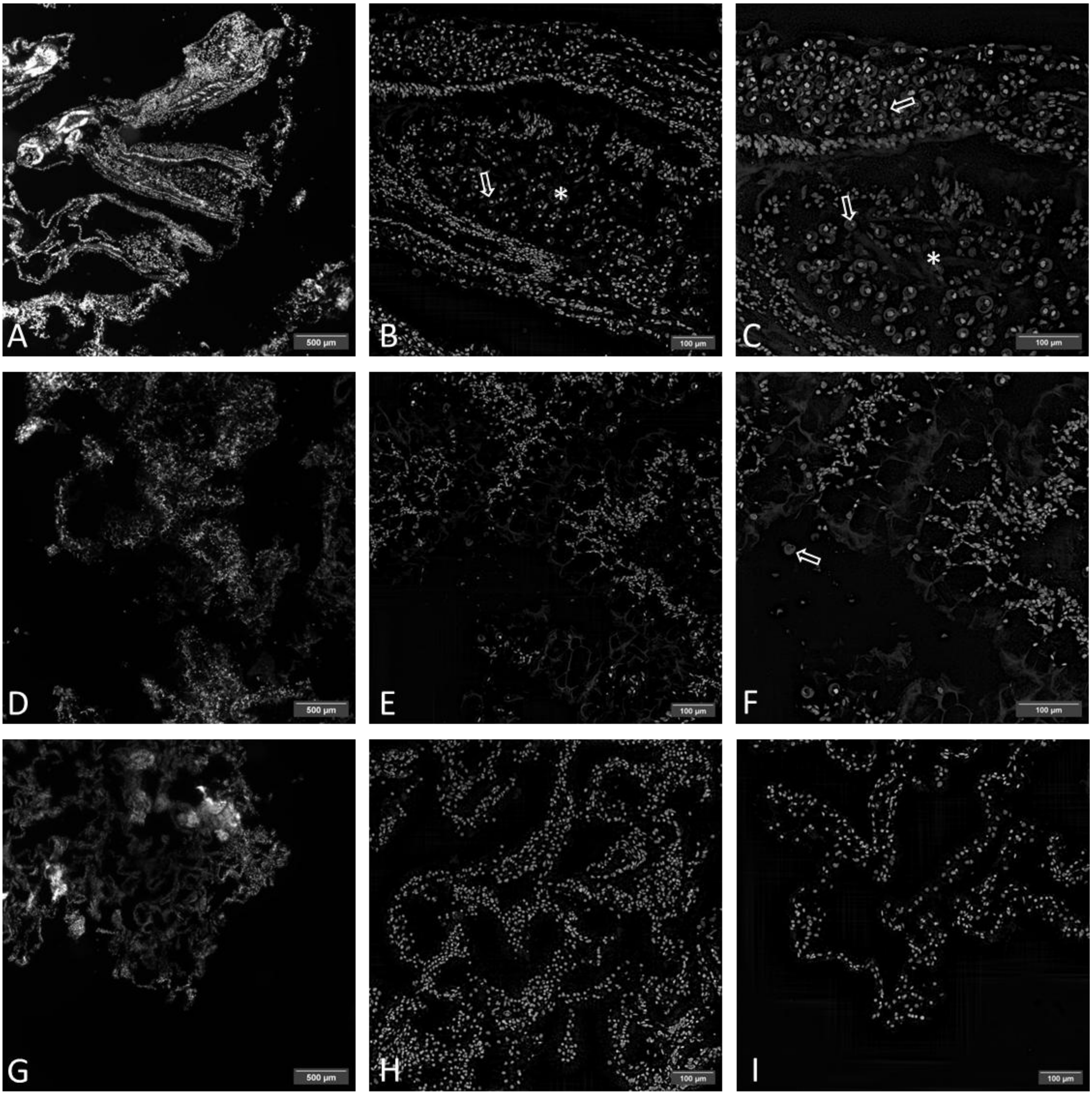


Figure 2.7: Microscope images of coral nubbins stained with fluorescent DAPI stain. Gastrovascular cavity shown with an asterisk (*), and Symbiodiniaceae shown with an ⇧. A) x10 *Galaxea fascicularis*, B) x40 *Galaxea fascicularis*, C) x60 *Galaxea fascicularis*, D) x10 *Galaxea fascicularis*, E) x40 *Galaxea fascicularis*, F) x60 *Galaxea fascicularis*, G) x10 *Porites lobata*, H) x40 *Porites lobata*, I) x40 *Porites lobata*.

2.5) Discussion:

This study aimed to gain further understanding into the communities of fungi associated with corals, and more specifically whether the fungal communities differed between compartments (mucus, tissue, and skeleton).

The fungal diversity throughout compartments:

The data showed that there were significant differences between the compartments for two of the three coral species assessed. Significant differences were seen between the mucus and the skeleton of both *Porites lobata* and *P. lutea*. Relating this to previous studies is difficult as this study is the first to separate all three compartments of the same coral fragment (Roik et al., 2022). That said, some studies have explored elements of fungal compartmentalization. For example, Bonthond et al. (2018) and Rabbani et al. (2021) examined fungal communities in the tissue. And the skeleton was explored by Góes-Neto et al. (2020). Apprill et al. (2016) also showed significant differences between the fungal communities of the tissue and mucus in five species of Caribbean corals, contrasting the findings in this study. One would expect differences to occur between compartments as each has its own microhabitat (for example, pH levels, oxygen, nutrient, and light levels). The mucus receives the most amount of light, has a large amount of carbohydrates, up to 50% of the corals photosynthetically fixed carbon (Wright et al., 2019), and is in contact with the surrounding water facilitating the transfer of fresh oxygen. In contrast, the coral tissues harbours the Symbiodiniaceae which provide oxygen and sugars during the day and CO2 during the night (Shashar et al., 1993). The skeleton likely experiences increased levels of nitrogen, phosphate, and ammonium (Pernice et al., 2020). Such difference in 'environmental' parameters will almost certainly govern what fungal species are able to survive and thrive in each compartment (Jones et al., 2022; Tedersoo et al., 2020). This may, at least in part, explain some of the observed differences seen in this study. Although compartmentalisation has been shown for coral bacterial communities (Glasl et al., 2016; Góes-Neto et al., 2020; Sweet et al., 2011), similar fungal diversity in these compartments might be due to the close proximity of the compartments.

Rather unsurprisingly, the fungal communities found in the mucus from both *Porites* species was similar to the water samples, and there was no significant difference between fungal communities in the mucus collected from all three species. Again, this is to be expected as mucus is known to harbour transient members of the corals holobiont, captured from the environmental pools like the water and/or the sediment (Glasl et al., 2016). The same has also been noted in other studies (Engelen et al., 2018).

Key fungal abundance across samples:

The diversity of fungal OTU's within the coral compartments was not as great as initially expected. Although this may be due to 77% of the OTU's (1043 out of 1359) being unidentifiable to the genus level. This certainly restricts what can be interpreted from the data. Although limiting, this is a common issue and one that is encountered across multiple marine fungal studies (Ainsworth et al., 2017; Amend et al., 2012; Bonthond et al., 2018; Roik et al., 2022). Of the OTU's that were identified, the most abundant family was Clavicipitaceae which dominated communities across most of the compartments, except from the skeleton of *P. lutea*. Within this family unidentified species from the genus *Meterhizium* were found. Interestingly, *Meterhizium* have been dubbed the "specialist-generalist" for their versatility. Indeed, it is thought to be a beneficial endophyte, commonly found in soil and the mycorrhizal communities of plants being entomopathogenic, infecting, disabling or killing insects and nematodes (St. Leger and Wang, 2020). This genus has already been identified within the marine environment and previously even been isolated from sponges, from which researchers found the presence of antibacterial compounds (Kong et al., 2013; Yao et al., 2022). The ability of this genus to be beneficial to a host, whilst providing protection from a large range of insects and nematodes, could be indicative when looking for biological agents to treat coral parasites (Kassam et al., 2022; Sullivan et al., 2022). Another potentially beneficial endophyte which was also found across many samples was *D. crocea* (family: Bulleribasidiaceae), which is also commonly discussed within the mycorrhizal communities of plants (Takashima et al., 2019). Finding endophytic fungal OTUs and suggests that fungi may have an important role to play in future research into beneficial functions of fungi within the coral holobiont (Peixoto et al., 2021, 2017).

Alongside the potentially beneficial species were known pathogens. For example, *Clavispora lusitaniae* (family: Metschnikowiaceae), which infects humans (Krcmery et al., 1999), and *Ramularia unterseheri* (family: Mycosphaerellaceae) which causes Ramularia leaf spot disease in a range of important crops such as barley (Videira et al., 2015). Other interesting finds come from the family Extremaceae, from which we identified two species which were both found in *G. fascicularis*, *Extremus adstrictus* and *Vermiconia antarctica*. *V. antarctica,* to the best of our knowledge was thought to be endemic to Antarctica (Coleine et al., 2021; Quaedvlieg et al., 2014). It is notable that *E. adstrictus* was found only in the skeleton of *G. fascicularis* as it has previously been found in calcium carbonate rich limestone rocks in Mallorca and Poland. However, not much is known about its function within limestone (Owczarek-Kościelniak, 2020; Quaedvlieg et al., 2014). The presence of this rock dwelling fungal species in the coral skeleton does however lend further evidence to some degree of compartmentalization due to selection by fungi.

Histological findings:

Assessing community profiles using next generation sequences offers up a wealth of information. However, such studies that have been undertaken with prokaryotes fall short in showing specifically where these microbes are within the coral, with very few using methods other than molecular techniques (Work and Meteyer, 2014). This study has examined three compartments providing more specific location information from the sequence data. However, insights can also be gained from histology visually identifying the location of cells or fungal hyphae and how they connect between the tissue, skeleton, and/or mucus. Indeed, by using a relatively cheap, easy to use, and safe stain (Lactophenol cotton blue), fungal hyphae were successfully visualised within the coral tissue.

Limitations and future research:

The differences within compartments between *G. fascicularis* and the two *Porites* species suggests the importance of studying multiple coral species in the future. As well as provoking questions for future studies about the importance of the coral host in determining the diversity and location of their fungal community. Additionally, this study has demonstrated differences in fungal community composition between coral compartments, and highlights that within coral heterogeneities should be considered in future coral microbiome research. Undoubtably, the greatest limitations of this study and others focused on coral associated fungi is the scale of what we do not currently know, to the point where many OTUs are unable to be identified. Thus, much of the information from these studies cannot yet be utilised, severely limiting the extend of the conclusions that we can draw from the results (Ainsworth et al., 2017; Amend et al., 2012; Bonthond et al., 2018; Roik et al., 2022).

Conclusion:

This chapter provides a stepping stone to improving our knowledge of the importance and roles of fungi in Scleractinian corals, in particular, that communities may differ between compartments within a coral. The patterns observed appear to be host specific, as were differences between *G. fascicularis* and the two species of *Porites* in the structure of their associated fungi. Highlighting the importance for future studies to determine the extent to which the coral host plays in the role of fungal community dynamics. This study provides evidence of differences in fungal community structures between different coral compartments (mucus, tissue, and skeleton), as well as evidence of specialist fungi found in specific compartments and potentially beneficial fungi which could change the perspective of fungi only being pathogenic in corals.

Chapter 3: Spatial and temporal factors affecting fungal diversity in the Indo Pacific reef building coral *Echinopora lamellosa*.

3.1) Abstract:

Microorganisms associated with corals are diverse and yet the focus has primarily been on bacteria and the symbiotic algae, Symbiodiniaceae. Research has found that a number of factors can affect the diversity of coral associated microorganisms (specifically bacteria and Symbiodiniaceae), such as temperature, time of year and position of the coral (depth, geographical location, etc). This study builds on this knowledge by exploring the effects of spatial and temporal factors on the diversity of coral associated fungi. Samples of *Echinopora lamellosa* were taken from three different reefs in the Rock Islands, Palau at three time periods spanning 17 months. Surprisingly, there were no significant differences of fungal diversity between sites, depth, or time point ($p > 0.05$). Consistent with the findings of Chapter 2, there were significant differences in fungal communities between the coral samples and those taken from the surrounding environment ($p < 0.0009$). However, there were no significant differences between the environmental samples and opportunistically collected coral mucus ($p > 0.7$), suggesting substantial cross-exchange of microorganisms between the coral surface layer mucus and the surrounding environmental pools. Previous studies also support these findings, suggesting that the structure of coral associated fungal communities may be more strongly determined by the host coral species than by other external factors, including when these factors vary spatially and temporally.

3.2) Introduction:

In aquatic environments fungi have been largely overlooked, with a large proportion being undetected for many years. As a result, progress in understanding the diversity and community composition, let alone function, of marine fungi is much further behind than that of other marine microorganisms. Research efforts have so far focused on terrestrial species such as the symbiotic mycorrhizal fungi which are associated with nutrient transfer in plant roots (Rosendahl, 2008; Tedersoo et al., 2020). It is know that fungi are found across marine habitats, such as in open water for example (Taylor and Cunliffe, 2016; Tisthammer et al., 2016b). Fungi are also commonly encountered in sediments (from the deep seas to the shore line), and as with their terrestrial counterparts there are examples of mycorrhizal fungi in seagrass roots (Ettinger and Eisen, 2019; Mouton et al., 2012; Orsi et al., 2013; Zhang et al., 2015).

Indeed the presence of fungi in coral has been documented (Ramos-Flores, 1983; Strake et al., 1988), most early studies focused on fungi as potentially pathogenic agents (Le Campion-Alsumard et al., 1995). With the emergence of the holobiont theory (Simon et al., 2019), it is now widely accepted that some fungi could have potentially beneficial roles within the coral microbiome. But again, a lack of research focused on fungi in corals has resulted in a very poor understanding of fungal diversity and function within coral microbiome. A major contributor to the lack of understanding is due to the difficulties associated with isolating and culturing marine fungi. This is integral to describing new species as well as understanding their functions (Alain and Querellou, 2009; Schultz et al., 2022). The increase in next generation sequencing (NGS) has allowed for progress in deciphering the diversity of fungi found in corals, but it fails to provide much insight into the functional role of the fungi within the coral, or the extent and effects of interactions with the hundreds of other microorganisms that make up the coral microbiome. Metabolomics studies can provide insight into possible functions, with some finding that coral associated fungal species produce antibacterial metabolites (Li et al., 2014; Wang et al., 2017). Fungal species have also been found to be present throughout the whole coral, with the presence of fungi living in the coral skeleton (endolithic fungi) being well documented (Góes-Neto et al., 2020; Golubic et al., 2005; Pernice et al., 2020). The functions of the fungi within the coral skeleton are uncertain. It has been proposed that endolithic fungi may actually be pathogenic causing a skeletal disease (Le Campion-Alsumard et al., 1995), rather than providing a positive functional role for the host coral. Endolithic fungi could be beneficial, with studies suggesting that some are saprotrophic, potentially being able to break down wastes and facilitating cycling of nutrients back to the coral (Góes-Neto et al., 2020; Pernice et al., 2020). However, it is still unclear exactly what function many fungi offer to their coral hosts.

Even basic information about the relationships between fungi and coral is limited. For example, still little is known about the mycobiome composition, how it responds to stressor events such as coral bleaching, seasonal shifts, as well as whether the communities change between reefs, or even between coral species. Therefore, when attempting to assess a corals microbiome, it is important to assess across multiple time points (seasonal) and geographical locations to be able to assess how such factors can affect the microbiome structure (McLachlan et al., 2020). There have been a few studies that have touched on some of these aspects in relation to fungi. For example, impacts of temperature (Amend et al., 2012), bleaching events (Kusdianto et al., 2021), depth (Bonthond et al., 2018), as well as how diversity changes over an extended time period (Kim and Drew Harvell, 2004). However, few, if any have delved deeper in attempting to document and understand shifts and changes across spatial and temporal scales as well as taking in to account the communities in the neighbouring environments such as the water column and sediment. This study explores these aspects, focusing on the coral *Echinopora lamellosa,* which were sampled at three different locations in Palau, across three time points. The adjacent sediment and water column were also sampled to see if changes in the fungi associated with corals were matched by changes in their surrounding environment.

3.3) Methods:

58 colonies of *Echinopora lamellosa* were tagged across three sites in Palau in April 2018 at depths ranging from between 0.7 and 9 m. The three sites were Risong Bay (07°17’28.90’’N, 134°26'28.20''E), Mekeald (07°17’28.9’’N, 134°26'28.2''E), and Monkey Ball (07°13’51.30’’N, 134°22'88.60''E) (Figure 3.1). Samples, approximately 2 cm^2 were collected from each colony using sterile pliers and placed into individually labelled whirl pack bags. These were then transported in a cooler to the Palau International Coral Reef Centre, where they were placed in 100% molecular grade ethanol and preserved at -80°C until extraction and analysis (see below). At the same time sediment and water samples were also collected. For sediment, n = three replicates of 500µl were scooped into individual microcentrifuge tubes, filled with 100% molecular grade ethanol on the boat and stored at -80°C. For water, n = three sterile niskin bottles were utilised to sample one litre of water adjacent to the depth of most of the corals per site. This water was transported back to the lab in the same manner as the corals, then manually filtered through 0.22µm sterivex filters using individually packed 50 ml syringes.

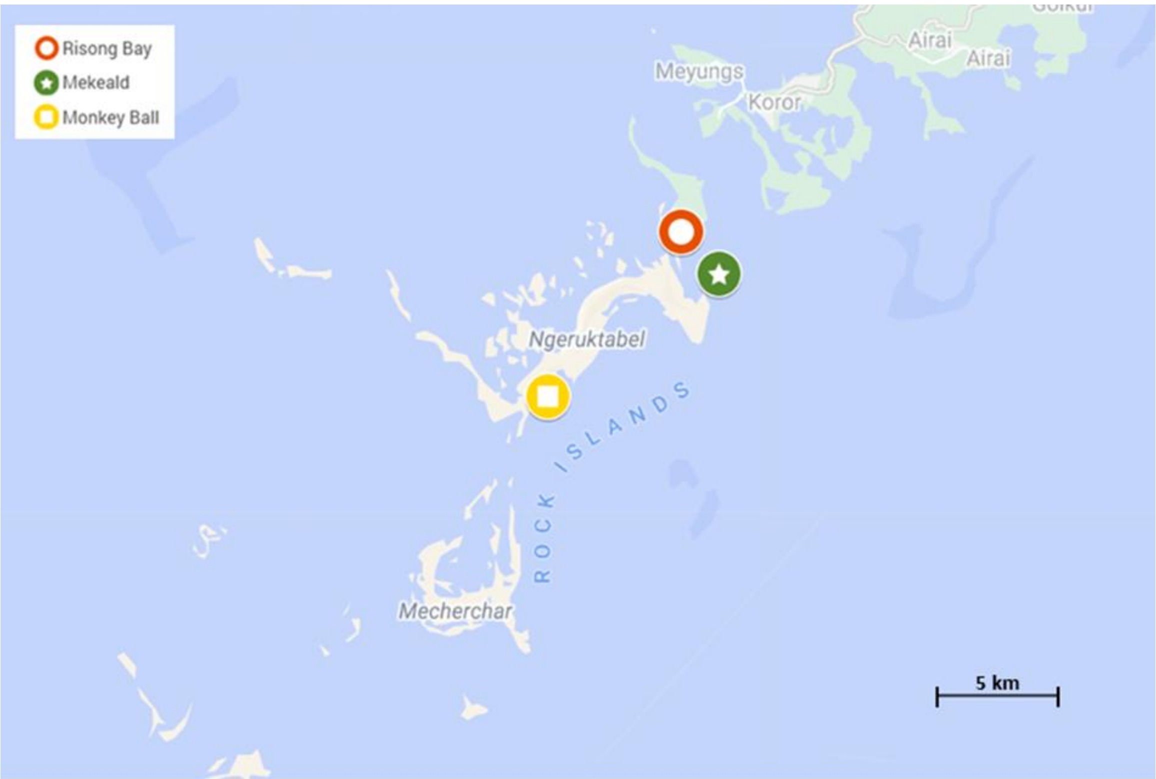


Figure 3.1: The three collection sites, Risong Bay (circle), Mekeald (star), Monkey Ball (square). Map produced from Google Maps (2023) *Collection sites*. Available from: https://www.google.com/maps/d/edit?mid=1Zoo03b5bFWB-OyT1dAdTjzKgN_tJ-f4&usp=sharing [Accessed 6th November 2023]

The process was repeated in April 2019 and September 2019. Opportunistically, mucus was also collected from four colonies (105, 113, 136 and 154). For this, a larger fragment, approximately 5 cm^2 was taken from the parental colony, inverted in air and the dripping mucus collected in the microcentrifuge tubes. This was undertaken as the corals were found to produce a significant amount of mucus during the transport back to the lab and it was viewed as an opportunity to examine similarity and differences between fungi associated with the mucus and fungi found in the whole tissue samples. HOBO data loggers were deployed at each of the three sites for continual temperature monitoring across three years (2018 – 2020).

DNA extraction and Fungal ITS rRNA gene amplicon sequencing

DNA was extracted from both coral tissue, sediments and the water samples using the Qiagen DNeasy Blood and Tissue kit using the manufacturers protocol. ITSS rRNA genes from cDNA were amplified using the primers CS1-5.8S-Fun-2-for (5'-CAAGCAGAAGACGG CATACGAGAT-NNNNNNNNNNNN-AGTCAGTCAG-GG-AACTT TYRRCAAYGGATCWCT-3') and CS2-ITS4-FUN-2-rev ('5=-AATGATACGGCG ACCACCGAGATCTACAC-TATGGTAATT-AA-AGCCTCCGCTTA TTGATATGCTTAART-3'). PCRs were carried out in 25µl reaction mixtures with 5µl of template DNA using illustra PuReTaq ready-to-go PCR beads (GE Healthcare Life Sciences, Pittsburgh, PA, USA), with 15 replicates per sample, and using a different Golay index for each sample. The thermocycling conditions were initial denaturing at 96°C for 2 min, followed by 27 cycles of denaturation at 94°C for 30 s, 58°C for 40 s, 72°C for 2 min, and a final extension at 72°C for extracted DNA was sent to the sequencing facility for quality control, PCR, library preparation, and pair-end sequencing with Illumina MiSeq platform v3 (2 × 300 bp). A contamination control consisting of pure RNAlater buffer was included in all steps. The sequences collected in this study have been deposited in the NCBI Sequence Read Archive under the accession number PRJNA1184330.

Statistically analysis

The raw amplicon sequencing reads were processed using DADA2 (Callahan et al., 2016), then phyloseq (McMurdie and Holmes, 2013) was used for further analysis. All fungal OTU's were retained for downstream analysis. The relative abundance was visualised using phyloseq, microeco (Liu et al., 2021) and ggplot2 (Wickham, 2016). For alpha diversity

Chao1, evenness, Shannon and Simpson were all calculated and graphs were produced using ggplot 2 and vegan package (Oksanen et al., 2009; Wickham, 2016). The PCoA (principal coordinates analysis) plots were produced to show the difference in fungal community composition between samples, the results from this were then further analysed using Adonis from the vegan package (Oksanen et al., 2009) and PERMANOVA to look for statistically significant differences.

3.4) Results:

Variation in fungal community composition between sites, time periods and the surrounding environment:

Each collection site was exposed to the natural seasonal cycles, with the minimum temperature recorded at Mekeald of 28.11 °C, and a maximum temperature recorded at Monkey Ball of 32.73 °C (Figure 3.2). In 2018 small scale bleaching was reported in the Rock Islands of Palau (Colin and Johnston, 2020), and all of the three study sites showed a peak in temperature just after April 2018, when the first samples were collected (Figure 3.2).

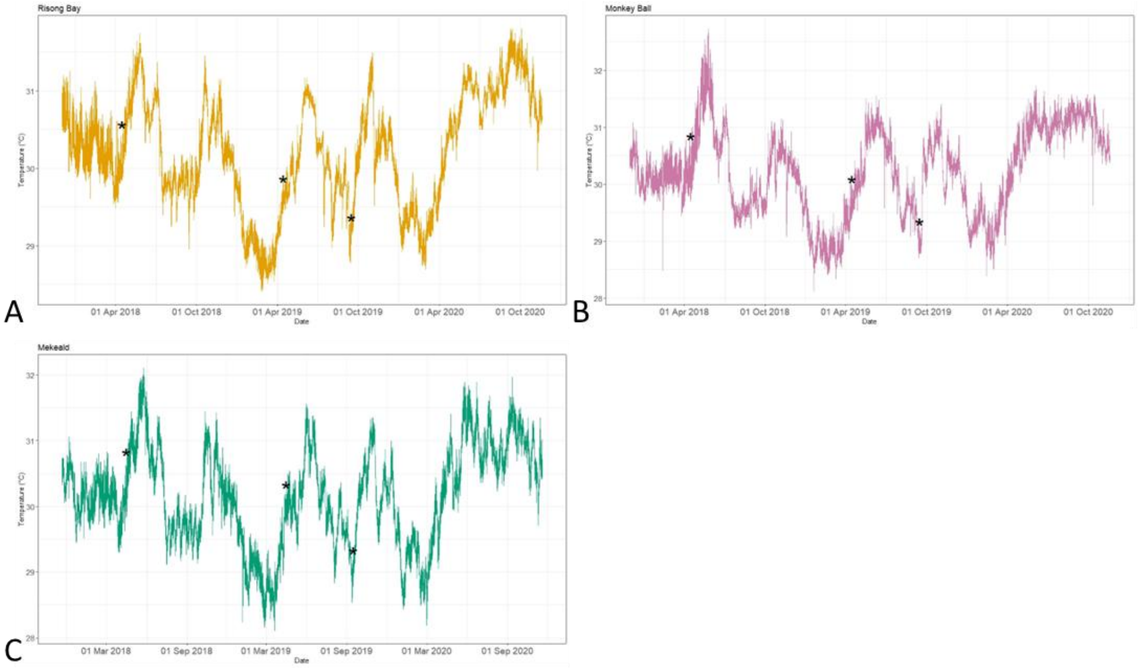


Figure 3.2: Sea surface temperatures (SSTs) recorded from each of the three collection sites from November 2017 to January 2021. The collection dates are indicated with an asterisk (*). A) Risong Bay, B) Monkey Ball, C) Mekeald.

There were no significant differences in the fungal community structures between coral samples from any of the collection dates ($p > 0.06$) (Figure 3.3A). There was also no significant difference between the communities across sites ($p > 0.2$), with Mekeald and Risong Bay having very similar fungal communities ($p > 0.99$) (Figure 3.3A). Additionally, there were also no significant differences between the samples from different depths ($p > 0.5$).

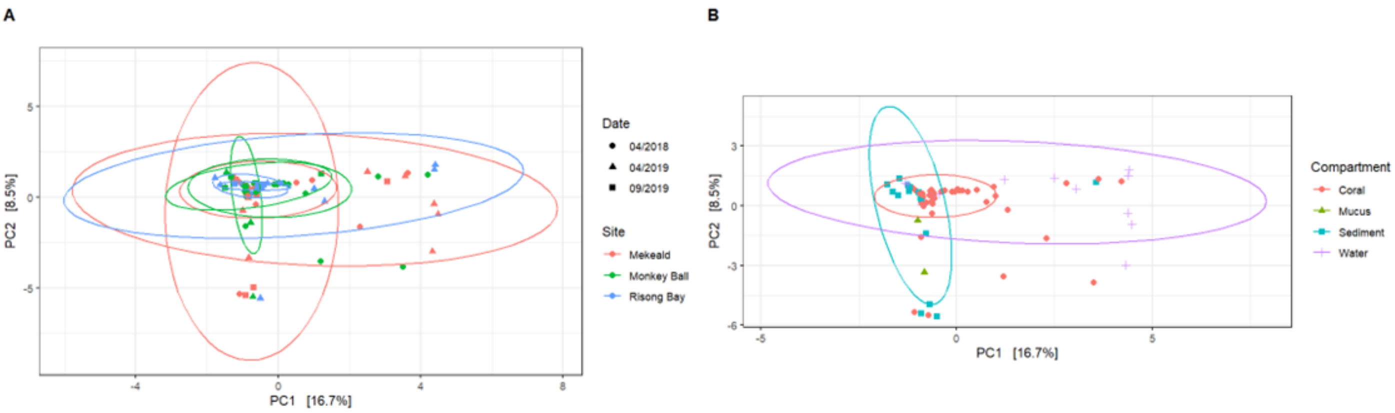


Figure 3.3: PCA graphs showing the fungal community diversity of, A) the three different sites and the three collection points, B) the different compartments that samples were collected from (coral, coral mucus, sediment and water)

There was a significant difference between the fungal communities in the coral samples and the water ($p < 0.0002$), and between those in the coral and those in sediment ($p < 0.0009$). Interestingly, there was no significant difference between sediment and water communities ($p > 0.7$), or between communities in the coral mucus and the water, sediment, and the coral ($p > 0.7$). The fungal communities in the coral mucus were most similar to that found in the sediment samples ($p > 0.9$) (Figure 3.3B).

Fungal diversity:

Across all samples, 1141 OTU's were retrieved. Of these, 841 could be assigned as fungal. The fungal OTU's were from five phyla (Ascomycota, Basidiobolomycota, Basidiomycota, Chytridiomycota, and Rozellomycota), and 68 genera (Figure 3.4). Taxa consistently found across samples were families, Cladosporiaceae (species: *Cladosporium austrohemisphaericum, C. dominicanum, C. endophyticum, C. halotolerans, C. herbarum, C. sphaerospermum*), Saccharomycetaceae (species: *Candida haemulonis, C. metapsilosis, C. parapsilosis, C. tropicalis*), Teratospaeriaceae (species: *Hortaea werneckii*), and Bulleribasidiaceae (species: *Dioszegia crocea, D. hungarica, Hannaella surugaensis, H. taiwanensis, Vishniacozyma carnescens, V. globispora, V. tephrensis, V. victoriae*) (Figure 3.4). The fungal OTU's in the water samples from all three sites were dominated by two key species; *Hortaea werneckii* (relative abundance of 42 – 52%) and *Candida spp*. (20%). The remainder of the community included a wide range of species, which were always low in relative abundance. In contrast, for the sediment samples (for both Risong Bay and Monkey

Ball) 50% of fungal OTUs were unidentified at genera level. The most abundant genera in the Mekeald sediment samples were *Hannaella spp*. (30%). The second most abundant genus for all three sites was *Cladosporium spp*. (19 – 45 %), also followed by several other genera with small percentages although the range of these rarer genera was not as diverse as that seen in the water samples (Figure 3.4).

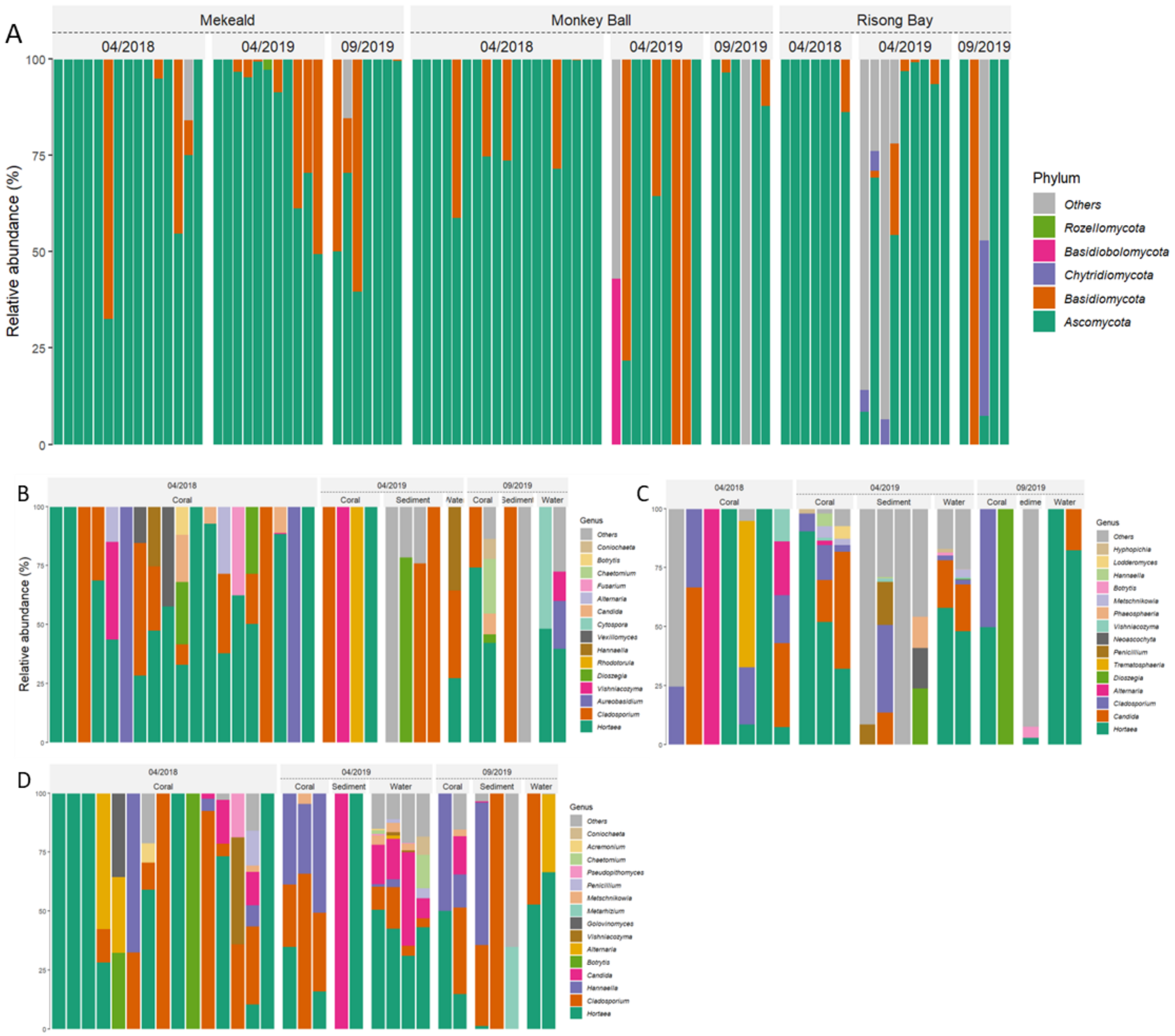


Figure 3.4: A) The relative abundance of phyla within the OTU's found in samples from the three sites (Mekeald, Monkey Ball and Risong Bay), B) the top 15 genera found in samples collected from Monkey Ball, C) the top 15 genera found in samples collected from Risong bay, D) the top 15 genera found in samples collected from Mekeald.

Alpha diversity indices were used to assess the richness, evenness, and distribution of the fungal communities, using Chao1 index (richness), evenness, Shannon index, and Simpsons index (Figure 3.5). These plots showed that over all richness of fungal OTUs was low. However, within the samples there was an even spread and a high diversity, suggesting many rare OTU's rather than a few dominant species.

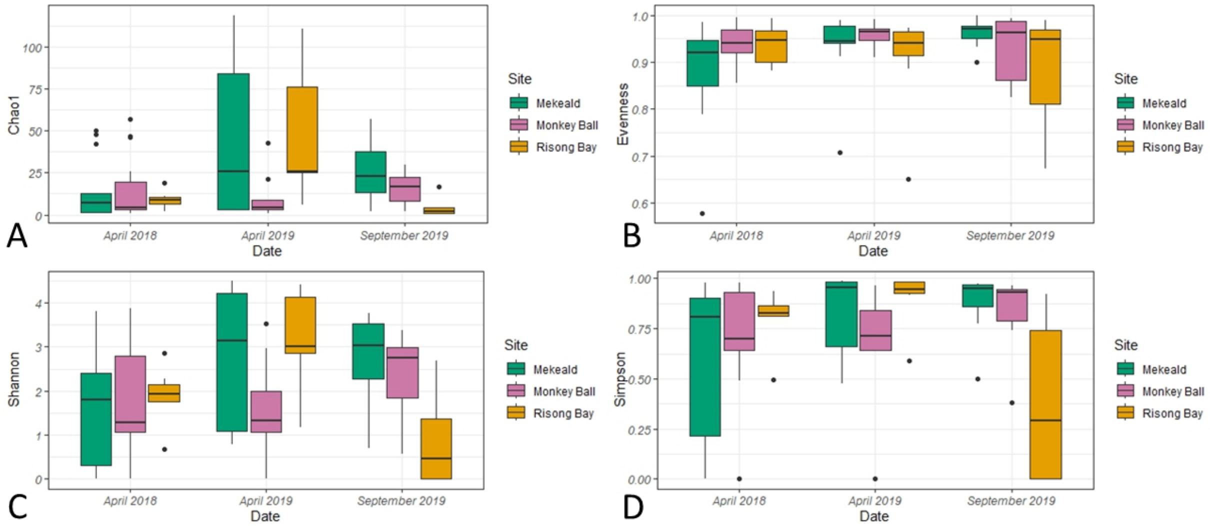


Figure 3.5: The diversity and composition of samples taken from each site at each time point, A) Chao1 index (richness), B) Evenness distribution, C) Shannon index, and D) Simpson index.

3.5) Discussion:

The intention of this study was to gain a greater understanding of how the fungal diversity within the coral microbiome may change over the course of time and whether there is a spatial influence. Samples were collected across multiple time points and from multiple sites in Palau. Potential relationships between the fungi associated with the coral and the pool of fungi in the surrounding environments (water and sediment).

The effect of spatial and temporal changes on coral fungal communities:

Surprisingly, there was no significant difference in the coral fungal community structures across the time points, as well as no significant difference between the fungal communities in coral samples from different sites. Similar findings have been shown in other studies (Amend et al., 2012; Rabbani et al., 2021). Interestingly, Amend et al. (2012) also highlighted that some key fungal taxa were consistently present in all the coral colonies sampled, which again reflects the findings from this study. However, the 'key' taxa in this study were different to those found by Amend et al. (2012), suggesting that corals may have a stable yet site- or species-specific grouping of fungi. Such a result mirrors what we know about coral-associated bacterial communities in that some species harbour stable communities whilst others can exhibit greater flexibility, especially when under instances of stress (Engelen et al., 2018; Voolstra and Ziegler, 2020). Indeed, one study has shown that stress (bleaching in this case) can cause shifts in the coral fungal communities (Kusdianto et al., 2021) and also shown for bacterial fractions (Gardner et al., 2019). When comparing and contrasting studies, it is important to note that few papers study the same coral species at the same location and this will likely be a factor in the contrasting findings. For example, again for the bacteriome, Dunphy et al. (2019) and Chu and Vollmer (2016) suggest the species of coral is more important in determining the microbiome composition then spatial and temporal differences.

The effects of the surrounding environments on the coral fungal diversity:

In contrast to the above, there were significant differences found between the coral samples and the associated 'environmental pools' (water and sediment) samples. Again, this is similar to other studies that have explored similar questions (for example, Kusdianto et al., 2021). However, interestingly, the mucus samples that were taken opportunistically did not show significant difference compared with the coral, water, and sediment samples. This suggest that the coral mucus is acting as a barrier, preventing the penetration or acquisition of fungi to the host tissue (Glasl et al., 2016). Others have highlighted a sharing of microorganisms across the corals mucus and the surrounding water, for example (Engelen et al., 2018), leading to multiple species of corals sharing very similar mucus fungal communities, but then having differing communities in other compartments (tissue and skeleton) (see Chapter 2).

Key fungal abundances across samples:

There was a high diversity of fungal taxa throughout the samples, some of which are already known to be found in various marine environments. For example *H. werneckii*, *D. hungarica, Cl. halotolerans*, (identified as dominant in our samples), have previously been cultured from corals at other locations (Bensch et al., 2012; Kaewkrajay et al., 2020; Marchetta et al., 2018; Takashima et al., 2001). With regards to what functions these dominant fungi may perform, we can only speculate at this point, but some of the species are known to be saprotrophic (such as *Chaetomium globosum* and *Cl. spp*.) (Abdel-Azeem, 2019; Bensch et al., 2012). Others are known to be pathogenic in nature. For example, *Alternaria metachromatica* is a known pathogen in tomato plants (Bashir et al., 2014), and *H. werneckii* which is known to infect human palms and soles (Bonifaz et al., 2008). From the perspective of coral health, of importance are the species which appear to be endophytes and are thought to live alongside hosts offering benefits. For example, *V. victoriae*, has been shown to enhance resistance to diseases in various fruits (Nian et al., 2023). Such species will be more likely candidates for use in coral probiotics, a field which is currently rapidly gathering momentum in coral biology (Peixoto et al., 2021). Similarly, *Ch. globosum* has been known to produce antibiotics and reduce infection in its plant host (Abdel-Azeem, 2019). Regardless of specific individual roles of certain fungal species or even genera, the sheer diversity found to be associated with corals suggests that there is extensive potential for coral host to access a range of beneficial functions. Thus, the fungal mycobiome could be an important component of the coral holobiont and likely to play a significant role in coral health, much in the same way as has been shown for coral associated bacteria (Peixoto et al., 2017; Santoro et al., 2021). For example, the saprotrophs fungi will be very interesting to explore in greater detail as they are known to be able to break down waste products and may even be involved in decomposing dead host cells and microorganisms within the coral skeleton and tissue (Góes-Neto et al., 2020). However, we are still very much in the infancy of understanding the roles of fungi within the coral microbiome, especially as many taxa being detected returned as ‘unknown’ in a BLAST search.

Limitations and future research:

Although this study sampled three time points, two were within the same year and two were the same month (different year). All sampling times missed the SST peaks (Figure 3.2) as well as the minor bleaching event that was witnessed at the location. If this had been captured during the sampling periods, it would have been possible to establish if more dramatic marked changes and shifts in the fungal communities occurred across time and space during stress events. Future research will certainly benefit from collection of samples throughout the year, to cover a wide range of environmental conditions including thermal stress events, as this will give a more complete record of fluctuations in fungal communities. This does not rule out the possibility the fungal communities within the coral microbiome may be more stable than their bacterial counterparts, which may suggest the (Bahram and Netherway, 2022). Long term studies with multiple collection points across different seasons will allow researchers to understand this further. Another interesting avenue to explore would be to compare the same species of corals from different reefs, with much greater distances between them. This, would give insight into whether species do prefer certain fungal taxa and are able to select for or exclude particular fungi.

Conclusion:

In conclusion, this study goes a long way to improving our knowledge of the diversity and composition of coral fungi across space and time. However, the preliminary conclusions suggest that at least for the coral species, *Echinopora lamellosa*, temporal and spatial changes may not be the main drivers of fungal diversity, rather it may other factors such as coral host, shown by the consistency of fungal diversity even in the face of multiple external factors, or strong niche separation within the fungi driving fungal community structure. However, the surrounding environments (sediment and water) do appear to play a small role in structuring fungal communities found in one compartment of the coral holobiont - the surface mucus layer.

General Discussion:

Thesis Findings:

The overarching aim for both studies in this thesis was to expand the knowledge of fungal community structure within the coral holobiont. More specifically, Chapter 2 intended to understand fungal diversity and distribution within the compartments of the coral. This study is the first of its kind to compare the fungal communities throughout all three compartments, with previous studies either only looking at the coral as a whole, or at a singular compartment – for example mucus or tissue (Apprill et al., 2016; Góes-Neto et al., 2020; Rabbani et al., 2021; Roik et al., 2022). The work of Chapter 3 provides an understanding of spatial and temporal effects on coral fungal composition, specifically comparing time points over a period of 17 months, different reef sites as well as different depths. This area has received more attention than that of fungal diversity in coral compartments but is still wildly under studied with very little known. Expanding our knowledge of how fungi are distributed throughout the coral and how external factors may affect their diversity will allow future researchers to gain a better grasp on how fungi may function within the coral holobiont.

In Chapter 2, the slight differences in tanks (temperature and depth) showed no significant differences in the fungal composition of the corals. We also found in Chapter 3 on a much larger scale, with the fungal diversity in *Echinopora lamellosa* showing no significant differences between time points, reef sites or depths, and this echoes findings in other studies (Amend et al., 2012; Rabbani et al., 2021). However, this is different to the responses that have previously been reported for bacteria (Dunphy et al., 2019). It is notable that the results of this study are in contrast to those of some other studies examining fungi (Kusdianto et al., 2021). Such variability in findings suggests a complex system with several, possibly interacting factors playing a significant role in shaping the structure and distribution of fungal communities within corals. Currently, we simply do not have enough information to characterise the influences of these factors on the fungal communities. But Chapter 3 due to no significant change in fungal diversity across the different environmental gradients this supports the hypothesis that the host coral species may be having a stronger effect than external abiotic factors on community structure of fungi. As we saw the significant difference in fungal communities between host species in Chapter 2 as well as the consistency of the fungal communities across several external factors in Chapter 3. This hypothesis has also been suggested in coral associated bacterial communities (Chu and Vollmer, 2016; Dunphy et al., 2019; Engelen et al., 2018; Kusdianto et al., 2021; Voolstra and Ziegler, 2020).

In Chapter 2 we did find significant differences in fungal community structure between the mucus layer and the skeleton for the two *Porites* species. This suggests that there is a level of compartmentalization of fungi within corals, for which we found further evidence with the presence of limestone dwelling specialist fungi *Extremus adstrictus* (Owczarek-Kościelniak, 2020; Quaedvlieg et al., 2014) found only in the skeleton of *G. fascicularis*. This may mean that the function and preferences of the fungal species may determine where it is found within the coral compartments. Further, in both Chapter 2 and 3, samples taken of coral mucus were shown to have very similar fungal community structure with that of their surround environmental pools (water (Chapters 2 and 3) and sediment (Chapter 3)). This suggests that there is significant transfer of fungi between the surrounding environments and the coral mucus layer (Engelen et al., 2018; Glasl et al., 2016), helping to shape the coral associated fungal communities.

Finally, Chapter 3 ended by discussing the fungal taxa found throughout both studies which may well provide target species of interest for future research to further understand beneficial relationships between the fungi and its coral host. For example, those previously identified as beneficial plant endophytes such as *Meterhizium spp., D. crocea, V. victoriae,* and *Ch. globosum*. As well as saprophytes, pathogenic and endolithic fungi. Overall, this thesis demonstrates that corals harbour a wide range of fungal taxa with the potential for varying functionality. On this note, the fungal compositions found between the two studies was quite different, with only a few similar taxa shared across (including the previously mentioned *D. crocea*) the two studies. Interestingly, the diversity of fungal genera was higher in the in situ *E. lamellosa* samples, and this is something that has also been documented when comparing coral associated bacterial communities in situ vs ex situ (Röthig et al., 2017).

Future studies:

These studies have provided insights into the distribution and diversity of fungi within coral and how the fungal communities may respond to external factors and has also provoked a number of questions for further research. Firstly, the importance of understanding the effect that the coral host has on shaping the fungal communities and whether this casual influence may be the reason for variation in results across studies. This may also be due to studies being spread across a wide range of geo-spatial locations. To understand the relative importance of these potential influences, it would be beneficial to study as wide a range of coral species as possible in the future.

Chapter 3 also highlighted the importance of longer-term studies with multiple time points in order to establish if there are cycles in fungal community dynamics, or change that result in strong abiotic impacts. Another key suggestion for future research resulting from the findings of this thesis is the development of and understanding of the causes and dynamics of compartmentalization of fungi in corals. Future studies should, where possible, separate the compartments and treat them as individual, but connected, communities. Finally, there are still major challenges, with high proportions of the OTUs retrieved in coral-fungi studies being unidentified. As such, there really needs to be a big push to combine molecular studies with culturing to increase the number of fungal species being described. It is estimated that at today's rates of species identification, it will take between 750 and 1000 years to identify all of the worlds fungal species (Niskanen et al., 2023).

Conclusion:

In conclusion, these studies have derived much needed insight into the mysterious world of fungi within corals. Coral associated fungi appear compartmentalized, which hints at differing functional roles of fungi between compartments. Interestingly, the fungi described in this thesis did not respond as expected to external stressors, which suggests that the coral hosts may have a greater influence on structuring the fungal communities than environmental parameters (Chu and Vollmer, 2016; Dunphy et al., 2019; Engelen et al., 2018; Kusdianto et al., 2021; Voolstra and Ziegler, 2020). Finally, there is clear evidence for the role of coral mucus in the protection of corals from potential pathogenic fungi, as many fungi are either trapped or grow in the mucus but do not reach the coral tissue or skeleton.

Reference List:

Abdel-Azeem, A., 2019. Taxonomy and Biodiversity of the Genus Chaetomium in Different Habitats. pp. 3–77. https://doi.org/10.1007/978-3-030-31612-9_1

Ainsworth, T.D., Fordyce, A.J., Camp, E.F., 2017. The Other Microeukaryotes of the Coral Reef Microbiome. Trends in Microbiology 25, 980–991. https://doi.org/10.1016/j.tim.2017.06.007

Alain, K., Querellou, J., 2009. Cultivating the uncultured: limits, advances and future challenges. Extremophiles 13, 583–594. https://doi.org/10.1007/s00792-009-0261-3

Alker, A.P., Smith, G.W., Kim, K., 2001. Characterization of Aspergillus sydowii (Thom et Church), a fungal pathogen of Caribbean sea fan corals, in: Porter, J.W. (Ed.), The Ecology and Etiology of Newly Emerging Marine Diseases. Springer Netherlands, Dordrecht, pp. 105–111. https://doi.org/10.1007/978-94-017-3284-0_9

Amend, A., 2014. From Dandruff to Deep-Sea Vents: Malassezia-like Fungi Are Ecologically Hyper-diverse. PLOS Pathogens 10, e1004277. https://doi.org/10.1371/journal.ppat.1004277

Amend, A., Burgaud, G., Cunliffe, M., Edgcomb, V.P., Ettinger, C.L., Gutiérrez, M.H., Heitman, J., Hom, E.F.Y., Ianiri, G., Jones, A.C., Kagami, M., Picard, K.T., Quandt, C.A., Raghukumar, S., Riquelme, M., Stajich, J., Vargas-Muñiz, J., Walker, A.K., Yarden, O., Gladfelter, A.S., 2019. Fungi in the Marine Environment: Open Questions and Unsolved Problems. mBio 10, e01189-18. https://doi.org/10.1128/mBio.01189-18

Amend, A.S., Barshis, D.J., Oliver, T.A., 2012. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. ISME J 6, 1291–1301. https://doi.org/10.1038/ismej.2011.193

Apprill, A., 2017. Marine Animal Microbiomes: Toward Understanding Host–Microbiome Interactions in a Changing Ocean. Frontiers in Marine Science 4.

Apprill, A., Weber, L.G., Santoro, A.E., 2016. Distinguishing between Microbial Habitats Unravels Ecological Complexity in Coral Microbiomes. mSystems 1, 10.1128/msystems.00143-16. https://doi.org/10.1128/msystems.00143-16

Averill, C., Bhatnagar, J.M., Dietze, M.C., Pearse, W.D., Kivlin, S.N., 2019. Global imprint of mycorrhizal fungi on whole-plant nutrient economics. Proceedings of the National Academy of Sciences 116, 23163–23168. https://doi.org/10.1073/pnas.1906655116

Averill, C., Turner, B.L., Finzi, A.C., 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505, 543–545. https://doi.org/10.1038/nature12901

Bahram, M., Netherway, T., 2022. Fungi as mediators linking organisms and ecosystems. FEMS Microbiology Reviews 46, fuab058. https://doi.org/10.1093/femsre/fuab058

Bar-On, Y.M., Phillips, R., Milo, R., 2018. The biomass distribution on Earth. Proceedings of the National Academy of Sciences 115, 6506–6511. https://doi.org/10.1073/pnas.1711842115

Bashir, U., Mushatq, S., Akhtar, N., 2014. First report of Alternaria metachromatica from Pakistan causing leaf spot of tomato. Pakistan Journal of Agricultural Sciences 51, 315–318.

Bensch, K., Braun, U., Groenewald, J.Z., Crous, P.W., 2012. The genus Cladosporium. Studies in Mycology, The genus 72, 1–401. https://doi.org/10.3114/sim0003

Biagi, E., Caroselli, E., Barone, M., Pezzimenti, M., Teixido, N., Soverini, M., Rampelli, S., Turroni, S., Gambi, M.C., Brigidi, P., Goffredo, S., Candela, M., 2020. Patterns in microbiome composition differ with ocean acidification in anatomic compartments of the Mediterranean coral Astroides calycularis living at CO2 vents. Science of The Total Environment 724, 138048. https://doi.org/10.1016/j.scitotenv.2020.138048

Blackall, L.L., Wilson, B., van Oppen, M.J.H., 2015. Coral—the world's most diverse symbiotic ecosystem. Molecular Ecology 24, 5330–5347. https://doi.org/10.1111/mec.13400

Bonacolta, A.M., Weiler, B.A., Porta-Fitó, T., Sweet, M., Keeling, P., del Campo, J., 2023. Beyond the Symbiodiniaceae: diversity and role of microeukaryotic coral symbionts. Coral Reefs 42, 567–577. https://doi.org/10.1007/s00338-023-02352-0

Bonifaz, A., Badali, H., de Hoog, G.S., Cruz, M., Araiza, J., Cruz, M.A., Fierro, L., Ponce, R.M., 2008. Tinea nigra by Hortaea werneckii, a report of 22 cases from Mexico. Studies in Mycology, Black fungal extremes 61, 77–82. https://doi.org/10.3114/sim.2008.61.07

Bonthond, G., Merselis, D.G., Dougan, K.E., Graff, T., Todd, W., Fourqurean, J.W., Rodriguez-Lanetty, M., 2018. Inter-domain microbial diversity within the coral holobiont Siderastrea siderea from two depth habitats. PeerJ 6, e4323. https://doi.org/10.7717/peerj.4323

Bourne, D., Iida, Y., Uthicke, S., Smith-Keune, C., 2008. Changes in coral-associated microbial communities during a bleaching event. ISME J 2, 350–363. https://doi.org/10.1038/ismej.2007.112

Brener-Raffalli, K., Clerissi, C., Vidal-Dupiol, J., Adjeroud, M., Bonhomme, F., Pratlong, M., Aurelle, D., Mitta, G., Toulza, E., 2018. Thermal regime and host clade, rather than geography, drive Symbiodinium and bacterial assemblages in the scleractinian coral Pocillopora damicornis sensu lato. Microbiome 6, 39. https://doi.org/10.1186/s40168-018-0423-6

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Chu, N.D., Vollmer, S.V., 2016. Caribbean corals house shared and host-specific microbial symbionts over time and space. Environmental Microbiology Reports 8, 493–500. https://doi.org/10.1111/1758-2229.12412

Coleine, C., Stajich, J.E., de los Ríos, A., Selbmann, L., 2021. Beyond the extremes: Rocks as ultimate refuge for fungi in drylands. Mycologia 113, 108–133. https://doi.org/10.1080/00275514.2020.1816761

Colin, P.L., Johnston, T.M.S., 2020. Measuring Temperature in Coral Reef Environments: Experience, Lessons, and Results from Palau. Journal of Marine Science and Engineering 8, 680. https://doi.org/10.3390/jmse8090680

Díaz-Almeyda, E.M., Prada, C., Ohdera, A.H., Moran, H., Civitello, D.J., Iglesias-Prieto, R., Carlo, T.A., LaJeunesse, T.C., Medina, M., 2017. Intraspecific and interspecific variation in thermotolerance and photoacclimation in Symbiodinium dinoflagellates. Proceedings of the Royal Society B: Biological Sciences 284, 20171767. https://doi.org/10.1098/rspb.2017.1767

Duc, P.M., Hatai, K., Kurata, O., Tensha, K., Yoshitaka, U., Yaguchi, T., Udagawa, S.-I., 2009. Fungal Infection of Mantis Shrimp (Oratosquilla oratoria) Caused by Two Anamorphic Fungi Found in Japan. Mycopathologia 167, 229–247. https://doi.org/10.1007/s11046-008-9174-4

Dunphy, C.M., Gouhier, T.C., Chu, N.D., Vollmer, S.V., 2019. Structure and stability of the coral microbiome in space and time. Sci Rep 9, 6785. https://doi.org/10.1038/s41598-019-43268-6

Engelen, A.H., Aires, T., Vermeij, M.J.A., Herndl, G.J., Serrão, E.A., Frade, P.R., 2018. Host Differentiation and Compartmentalization of Microbial Communities in the Azooxanthellate Cupcorals Tubastrea coccinea and Rhizopsammia goesi in the Caribbean. Frontiers in Marine Science 5.

Ettinger, C.L., Eisen, J.A., 2019. Characterization of the Mycobiome of the Seagrass, Zostera marina, Reveals Putative Associations With Marine Chytrids. Frontiers in Microbiology 10.

Ferguson, B.A., Dreisbach, T.A., Parks, C.G., Filip, G.M., Schmitt, C.L., 2003. Coarse-scale population structure of pathogenic Armillaria species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. Can. J. For. Res. 33, 612–623. https://doi.org/10.1139/x03-065

Fifer, J.E., Bui, V., Berg, J.T., Kriefall, N., Klepac, C., Bentlage, B., Davies, S.W., 2022. Microbiome Structuring Within a Coral Colony and Along a Sedimentation Gradient. Frontiers in Marine Science 8.

Frenken, T., Alacid, E., Berger, S.A., Bourne, E.C., Gerphagnon, M., Grossart, H.-P., Gsell, A.S., Ibelings, B.W., Kagami, M., Küpper, F.C., Letcher, P.M., Loyau, A., Miki, T., Nejstgaard, J.C., Rasconi, S., Reñé, A., Rohrlack, T., Rojas-Jimenez, K., Schmeller, D.S., Scholz, B., Seto, K., Sime-Ngando, T., Sukenik, A., Van de Waal, D.B., Van den Wyngaert, S., Van Donk, E., Wolinska, J., Wurzbacher, C., Agha, R., 2017. Integrating chytrid fungal parasites into plankton ecology: research gaps and needs. Environmental Microbiology 19, 3802–3822. https://doi.org/10.1111/1462-2920.13827

Galand, P.E., Ruscheweyh, H.-J., Salazar, G., Hochart, C., Henry, N., Hume, B.C.C., Oliveira, P.H., Perdereau, A., Labadie, K., Belser, C., Boissin, E., Romac, S., Poulain, J., Bourdin, G., Iwankow, G., Moulin, C., Armstrong, E.J., Paz-García, D.A., Ziegler, M., Agostini, S., Banaigs, B., Boss, E., Bowler, C., de Vargas, C., Douville, E., Flores, M., Forcioli, D., Furla, P., Gilson, E., Lombard, F., Pesant, S., Reynaud, S., Thomas, O.P., Troublé, R., Zoccola, D., Voolstra, C.R., Thurber, R.V., Sunagawa, S., Wincker, P., Allemand, D., Planes, S., 2023. Diversity of the Pacific Ocean coral reef microbiome. Nat Commun 14, 3039. https://doi.org/10.1038/s41467-023-38500-x

Galloway, S., Work, T., Bochsler, V., Harley, R., Kramarsky-Winters, E., McLaughlin, S., Meteyer, C., Morado, J., Nicholson, J., Parnell, P., Peters, E., Reynolds, T., Rotstein, D., Sileo, L., Woodley, C., 2007. Coral Disease and Health Workshop: Coral Histopathology II. NOAA Technical Memorandum NOS NCCOS 56 and NOAA Technical Memorandum CRCP 4 National Oceanic and Atmospheric Administration, Silver Spring, MD.

Gardner, S.G., Camp, E.F., Smith, D.J., Kahlke, T., Osman, E.O., Gendron, G., Hume, B.C.C., Pogoreutz, C., Voolstra, C.R., Suggett, D.J., 2019. Coral microbiome diversity reflects mass coral bleaching susceptibility during the 2016 El Niño heat wave. Ecology and Evolution 9, 938–956. https://doi.org/10.1002/ece3.4662

Glasl, B., Herndl, G.J., Frade, P.R., 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. ISME J 10, 2280–2292. https://doi.org/10.1038/ismej.2016.9

Góes-Neto, A., Marcelino, V.R., Verbruggen, H., da Silva, F.F., Badotti, F., 2020. Biodiversity of endolithic fungi in coral skeletons and other reef substrates revealed with 18S rDNA metabarcoding. Coral Reefs 39, 229–238. https://doi.org/10.1007/s00338-019-01880-y

Golubic, S., Radtke, G., Campion-Alsumard, T.L., 2005. Endolithic fungi in marine ecosystems. Trends in Microbiology 13, 229–235. https://doi.org/10.1016/j.tim.2005.03.007

Grégoire, V., Schmacka, F., Coffroth, M.A., Karsten, U., 2017. Photophysiological and thermal tolerance of various genotypes of the coral endosymbiont Symbiodinium sp. (Dinophyceae). J Appl Phycol 29, 1893–1905. https://doi.org/10.1007/s10811-017-1127-1

Grottoli, A.G., Dalcin Martins, P., Wilkins, M.J., Johnston, M.D., Warner, M.E., Cai, W.-J., Melman, T.F., Hoadley, K.D., Pettay, D.T., Levas, S., Schoepf, V., 2018. Coral physiology and microbiome dynamics under combined warming and ocean acidification. PLoS One 13, e0191156. https://doi.org/10.1371/journal.pone.0191156

Hassan, S., Ishaq, M., Zhang, W., Jin, H.-Z., 2020. An Overview of the Mechanisms of Marine Fungi-Derived Anti-Inflammatory and Anti-Tumor Agents and their Novel Role in Drug Targeting. Current Pharmaceutical Design 26. https://doi.org/10.2174/1381612826666200728142244

Hawksworth, D.L., Lücking, R., 2017. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiology Spectrum 5, 10.1128/microbiolspec.funk-0052–2016. https://doi.org/10.1128/microbiolspec.funk-0052-2016

Huckabone, S.E., Gulland, F.M.D., Johnson, S.M., Colegrove, K.M., Dodd, E.M., Pappagianis, D., Dunkin, R.C., Casper, D., Carlson, E.L., Sykes, J.E., Meyer, W., Miller, M.A., 2015. Coccidiodomycosis and other systemic mycoses of marine mammals stranding along the central California, USA coast: 1998-2012. Journal of Wildlife Diseases 51, 295–308. https://doi.org/10.7589/2014-06-143

Huggins, J.L., Garner, M.M., Raverty, S.A., Lambourn, D.M., Norman, S.A., Rhodes, L.D., Gaydos, J.K., Olson, J.K., Haulena, M., Hanson, M.B., 2020. The Emergence of Mucormycosis in Free-Ranging Marine Mammals of the Pacific Northwest. Frontiers in Marine Science 7.

Hughes, D.P., Andersen, S.B., Hywel-Jones, N.L., Himaman, W., Billen, J., Boomsma, J.J., 2011. Behavioral mechanisms and morphological symptoms of zombie ants dying from fungal infection. BMC Ecol 11, 13. https://doi.org/10.1186/1472-6785-11-13

Humphreys, C.P., Franks, P.J., Rees, M., Bidartondo, M.I., Leake, J.R., Beerling, D.J., 2010. Mutualistic mycorrhiza-like symbiosis in the most ancient group of land plants. Nat Commun 1, 103. https://doi.org/10.1038/ncomms1105

Jones, E.B.G., 2011. Fifty years of marine mycology. Fungal Diversity 50, 73–112. https://doi.org/10.1007/s13225-011-0119-8

Jones, E.B.G., Pang, K.-L., Abdel-Wahab, M.A., Scholz, B., Hyde, K.D., Boekhout, T., Ebel, R., Rateb, M.E., Henderson, L., Sakayaroj, J., Suetrong, S., Dayarathne, M.C., Kumar, V., Raghukumar, S., Sridhar, K.R., Bahkali, A.H.A., Gleason, F.H., Norphanphoun, C., 2019. An online resource for marine fungi. Fungal Diversity 96, 347–433. https://doi.org/10.1007/s13225-019-00426-5

Jones, E.B.G., Ramakrishna, S., Vikineswary, S., Das, D., Bahkali, A.H., Guo, S.-Y., Pang, K.-L., 2022. How Do Fungi Survive in the Sea and Respond to Climate Change? Journal of Fungi 8, 291. https://doi.org/10.3390/jof8030291

Jones, E.B.G., Suetrong, S., Sakayaroj, J., Bahkali, A.H., Abdel-Wahab, M.A., Boekhout, T., Pang, K.-L., 2015. Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. Fungal Diversity 73, 1–72. https://doi.org/10.1007/s13225-015-0339-4

Kaewkrajay, C., Chanmethakul, T., Limtong, S., 2020. Assessment of Diversity of Culturable Marine Yeasts Associated with Corals and Zoanthids in the Gulf of Thailand, South China Sea. Microorganisms 8, 474. https://doi.org/10.3390/microorganisms8040474

Kannan, B., Ramasamy, K.P., Manohar, C.-S., Meena, R., 2017. Diversity and decolorization potential of fungi isolated from the coral reef regions off Kavaratti, India. Indian Journal of Geo-Marine Sciences 46, 497–503.

Kassam, R., Yadav, J., Jaiswal, N., Chatterjee, M., Hada, A., Chawla, G., Kamil, D., Rao, U., 2022. Identification and potential utility of Metarhizium anisopliae (ITCC9014) for the management of root-knot nematode, Meloidogyne incognita. Indian Phytopathology 75, 875–881. https://doi.org/10.1007/s42360-022-00498-5

Kemp, D.W., Hernandez-Pech, X., Iglesias-Prieto, R., Fitt, W.K., Schmidt, G.W., 2014. Community dynamics and physiology of Symbiodinium spp. before, during, and after a coral bleaching event. Limnology and Oceanography 59, 788–797. https://doi.org/10.4319/lo.2014.59.3.0788

Kim, K., Drew Harvell, C., 2004. The Rise and Fall of a Six-Year Coral-Fungal Epizootic. The American Naturalist. https://doi.org/10.1086/424609

Kobmoo, N., Mongkolsamrit, S., Wutikhun, T., Tasanathai, K., Khonsanit, A., Thanakitpipattana, D., Luangsa-Ard, J.J., 2015. New species of Ophiocordyceps unilateralis, an ubiquitous pathogen of ants from Thailand. Fungal Biology 119, 44–52. https://doi.org/10.1016/j.funbio.2014.10.008

Kong, X., Ma, X., Xie, Y., Cai, S., Zhu, T., Gu, Q., Li, D., 2013. Aromatic polyketides from a sponge-derived fungus Metarhizium anisopliae mxh-99 and their antitubercular activities. Arch. Pharm. Res. 36, 739–744. https://doi.org/10.1007/s12272-013-0077-7

Krcmery, V., Jr, Mateicka, F., Grausova, S., Kunova, A., Hanzen, J., 1999. Invasive infections due to Clavispora lusitaniae. FEMS Immunology & Medical Microbiology 23, 75–78. https://doi.org/10.1111/j.1574-695X.1999.tb01719.x

Kusdianto, H., Kullapanich, C., Palasuk, M., Jandang, S., Pattaragulwanit, K., Ouazzani, J., Chavanich, S., Viyakarn, V., Somboonna, N., 2021. Microbiomes of Healthy and Bleached Corals During a 2016 Thermal Bleaching Event in the Upper Gulf of Thailand. Frontiers in Marine Science 8.

Le Campion-Alsumard, T., Golubic, S., Priess, K., 1995. Fungi in corals: symbiosis or disease? Interaction between polyps and fungi causes pearl-like skeleton biomineralization. Marine Ecology Progress Series 117, 137–147.

Li, J., Zhong, M., Lei, X., Xiao, S., Li, Z., 2014. Diversity and antibacterial activities of culturable fungi associated with coral Porites pukoensis. World J Microbiol Biotechnol 30, 2551–2558. https://doi.org/10.1007/s11274-014-1701-5

Liu, C., Cui, Y., Li, X., Yao, M., 2021. microeco: an R package for data mining in microbial community ecology. FEMS Microbiology Ecology 97, fiaa255. https://doi.org/10.1093/femsec/fiaa255

Maire, J., Buerger, P., Chan, W.Y., Deore, P., Dungan, A.M., Nitschke, M.R., van Oppen, M.J.H., 2022. Effects of Ocean Warming on the Underexplored Members of the Coral Microbiome. Integrative and Comparative Biology 62, 1700–1709. https://doi.org/10.1093/icb/icac005

Marchetta, A., Gerrits van den Ende, B., Al-Hatmi, A.M.S., Hagen, F., Zalar, P., Sudhadham, M., Gunde-Cimerman, N., Urzì, C., De Hoog, S., De Leo, F., 2018. Global Molecular Diversity of the Halotolerant Fungus Hortaea werneckii. Life 8, 31. https://doi.org/10.3390/life8030031

McLachlan, R.H., Price, J.T., Solomon, S.L., Grottoli, A.G., 2020. Thirty years of coral heat-stress experiments: a review of methods. Coral Reefs 39, 885–902. https://doi.org/10.1007/s00338-020-01931-9

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Morris, L.A., Voolstra, C.R., Quigley, K.M., Bourne, D.G., Bay, L.K., 2019. Nutrient Availability and Metabolism Affect the Stability of Coral–Symbiodiniaceae Symbioses. Trends in Microbiology 27, 678–689. https://doi.org/10.1016/j.tim.2019.03.004

Mouton, M., Postma, F., Wilsenach, J., Botha, A., 2012. Diversity and Characterization of Culturable Fungi from Marine Sediment Collected from St. Helena Bay, South Africa. Microb Ecol 64, 311–319. https://doi.org/10.1007/s00248-012-0035-9

Nagano, Y., Nagahama, T., 2012. Fungal diversity in deep-sea extreme environments. Fungal Ecology, Fungi in Extreme Environments 5, 463–471. https://doi.org/10.1016/j.funeco.2012.01.004

Nelson, C.E., Goldberg, S.J., Wegley Kelly, L., Haas, A.F., Smith, J.E., Rohwer, F., Carlson, C.A., 2013. Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. ISME J 7, 962–979. https://doi.org/10.1038/ismej.2012.161

Netherway, T., Bengtsson, J., Krab, E.J., Bahram, M., 2021. Biotic interactions with mycorrhizal systems as extended nutrient acquisition strategies shaping forest soil communities and functions. Basic and Applied Ecology 50, 25–42. https://doi.org/10.1016/j.baae.2020.10.002

Nian, L., Xie, Y., Zhang, H., Wang, M., Yuan, B., Cheng, S., Cao, C., 2023. Vishniacozyma victoriae: An endophytic antagonist yeast of kiwifruit with biocontrol effect to Botrytis cinerea. Food Chemistry 411, 135442. https://doi.org/10.1016/j.foodchem.2023.135442

Niskanen, T., Lücking, R., Dahlberg, A., Gaya, E., Suz, L.M., Mikryukov, V., Liimatainen, K., Druzhinina, I., Westrip, J.R.S., Mueller, G.M., Martins-Cunha, K., Kirk, P., Tedersoo, L., Antonelli, A., 2023. Pushing the Frontiers of Biodiversity Research: Unveiling the Global Diversity, Distribution, and Conservation of Fungi. Annual Review of Environment and Resources 48, 149–176. https://doi.org/10.1146/annurev-environ-112621-090937

Nogueira, O.M.N., Bernal, S.P.F., Peres, C.K., Boroski, M., Passarini, M.R.Z., 2024. Isolation of marine-derived filamentous fungi and their potential application for bioremediation process. Braz J Microbiol. https://doi.org/10.1007/s42770-024-01536-2

Oksanen, J., Kindt, R., Legendre, P., Hara, B., Simpson, G., Solymos, P., Henry, M., Stevens, H., Maintainer, H., Oksanen@oulu, jari, 2009. The vegan Package.

Orsi, W., Biddle, J.F., Edgcomb, V., 2013. Deep Sequencing of Subseafloor Eukaryotic rRNA Reveals Active Fungi across Marine Subsurface Provinces. PLOS ONE 8, e56335. https://doi.org/10.1371/journal.pone.0056335

Owczarek-Kościelniak, M., 2020. Extremus adstrictus from a dolomite wall in Poland: the first report outside Mallorca. | Plant & Fungal Systematics | EBSCOhost [WWW Document]. https://doi.org/10.35535/pfsyst-2020-0034

Paço, A., Duarte, K., da Costa, J.P., Santos, P.S.M., Pereira, R., Pereira, M.E., Freitas, A.C., Duarte, A.C., Rocha-Santos, T.A.P., 2017. Biodegradation of polyethylene microplastics by the marine fungus Zalerion maritimum. Science of The Total Environment 586, 10–15. https://doi.org/10.1016/j.scitotenv.2017.02.017

Peixoto, R.S., Rosado, P.M., Leite, D.C. de A., Rosado, A.S., Bourne, D.G., 2017. Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. Frontiers in Microbiology 8.

Peixoto, R.S., Sweet, M., Villela, H.D.M., Cardoso, P., Thomas, T., Voolstra, C.R., Høj, L., Bourne, D.G., 2021. Coral Probiotics: Premise, Promise, Prospects. Annual Review of Animal Biosciences 9, 265–288. https://doi.org/10.1146/annurev-animal-090120-115444

Pernice, M., Raina, J.-B., Rädecker, N., Cárdenas, A., Pogoreutz, C., Voolstra, C.R., 2020. Down to the bone: the role of overlooked endolithic microbiomes in reef coral health. ISME J 14, 325–334. https://doi.org/10.1038/s41396-019-0548-z

Phukhamsakda, C., Nilsson, R.H., Bhunjun, C.S., de Farias, A.R.G., Sun, Y.-R., Wijesinghe, S.N., Raza, M., Bao, D.-F., Lu, L., Tibpromma, S., Dong, W., Tennakoon, D.S., Tian, X.-G., Xiong, Y.-R., Karunarathna, S.C., Cai, L., Luo, Z.-L., Wang, Y., Manawasinghe, I.S., Camporesi, E., Kirk, P.M., Promputtha, I., Kuo, C.-H., Su, H.-Y., Doilom, M., Li, Y., Fu, Y.-P., Hyde, K.D., 2022. The numbers of fungi: contributions from traditional taxonomic studies and challenges of metabarcoding. Fungal Diversity 114, 327–386. https://doi.org/10.1007/s13225-022-00502-3

Pichler, G., Muggia, L., Carniel, F.C., Grube, M., Kranner, I., 2023. How to build a lichen: from metabolite release to symbiotic interplay. New Phytologist 238, 1362–1378. https://doi.org/10.1111/nph.18780

Pogoreutz, C., Rädecker, N., Cárdenas, A., Gärdes, A., Wild, C., Voolstra, C.R., 2018. Dominance of Endozoicomonas bacteria throughout coral bleaching and mortality suggests structural inflexibility of the Pocillopora verrucosa microbiome. Ecology and Evolution 8, 2240–2252. https://doi.org/10.1002/ece3.3830

Pootakham, W., Mhuantong, W., Putchim, L., Yoocha, T., Sonthirod, C., Kongkachana, W., Sangsrakru, D., Naktang, C., Jomchai, N., Thongtham, N., Tangphatsornruang, S., 2018. Dynamics of coral-associated microbiomes during a thermal bleaching event. MicrobiologyOpen 7, e00604. https://doi.org/10.1002/mbo3.604

Puntin, G., Craggs, J., Hayden, R., Engelhardt, K.E., McIlroy, S., Sweet, M., Baker, D.M., Ziegler, M., 2023. The reef-building coral Galaxea fascicularis: a new model system for coral symbiosis research. Coral Reefs 42, 239–252. https://doi.org/10.1007/s00338-022-02334-8

Quaedvlieg, W., Binder, M., Groenewald, J.Z., Summerell, B.A., Carnegie, A.J., Burgess, T.I., Crous, P.W., 2014. Introducing the Consolidated Species Concept to resolve species in the Teratosphaeriaceae. Persoonia 33, 1–40. https://doi.org/10.3767/003158514X681981

Rabbani, G., Huang, D., Wainwright, B.J., 2021. The mycobiome of Pocillopora acuta in Singapore. Coral Reefs 40, 1419–1427. https://doi.org/10.1007/s00338-021-02152-4

Ramos-Flores, T., 1983. Lower marine fungus associated with black line disease in star corals (montastrea annularis, e. & s.). The Biological Bulletin 165, 429–435. https://doi.org/10.2307/1541208

Roik, A., Reverter, M., Pogoreutz, C., 2022. A roadmap to understanding diversity and function of coral reef-associated fungi. FEMS Microbiology Reviews 46, fuac028. https://doi.org/10.1093/femsre/fuac028

Ros, M., Camp, E.F., Hughes, D.J., Crosswell, J.R., Warner, M.E., Leggat, W.P., Suggett, D.J., 2020. Unlocking the black-box of inorganic carbon-uptake and utilization strategies among coral endosymbionts (Symbiodiniaceae). Limnology and Oceanography 65, 1747–1763. https://doi.org/10.1002/lno.11416

Rosa, L.H., Pinto, O.H.B., Šantl-Temkiv, T., Convey, P., Carvalho-Silva, M., Rosa, C.A., Câmara, P.E.A.S., 2020. DNA metabarcoding of fungal diversity in air and snow of Livingston Island, South Shetland Islands, Antarctica. Sci Rep 10, 21793. https://doi.org/10.1038/s41598-020-78630-6

Rosendahl, S., 2008. Communities, populations and individuals of arbuscular mycorrhizal fungi. New Phytologist 178, 253–266. https://doi.org/10.1111/j.1469-8137.2008.02378.x

Röthig, T., Roik, A., Yum, L., Voolstra, C., 2017. Distinct Bacterial Microbiomes Associate with the Deep-Sea Coral Eguchipsammia fistula from the Red Sea and from Aquaria Settings. Frontiers in Marine Science 4, 259. https://doi.org/10.3389/fmars.2017.00259

Rueden, C.T., Schindelin, J., Hiner, M.C., DeZonia, B.E., Walter, A.E., Arena, E.T., Eliceiri, K.W., 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18, 529. https://doi.org/10.1186/s12859-017-1934-z

Santoro, E.P., Borges, R.M., Espinoza, J.L., Freire, M., Messias, C.S.M.A., Villela, H.D.M., Pereira, L.M., Vilela, C.L.S., Rosado, J.G., Cardoso, P.M., Rosado, P.M., Assis, J.M., Duarte, G.A.S., Perna, G., Rosado, A.S., Macrae, A., Dupont, C.L., Nelson, K.E., Sweet, M.J., Voolstra, C.R., Peixoto, R.S., 2021. Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. Sci. Adv. 7, eabg3088. https://doi.org/10.1126/sciadv.abg3088

Schultz, J., Modolon, F., Rosado, A.S., Voolstra, C.R., Sweet, M., Peixoto, R.S., 2022. Methods and Strategies to Uncover Coral-Associated Microbial Dark Matter. mSystems 7, e00367-22. https://doi.org/10.1128/msystems.00367-22

Sen, K., Sen, B., Wang, G., 2022. Diversity, Abundance, and Ecological Roles of Planktonic Fungi in Marine Environments. Journal of Fungi 8, 491. https://doi.org/10.3390/jof8050491

Shashar, N., Cohen, Y., Loya, Y., 1993. Extreme Diel Fluctuations of Oxygen in Diffusive Boundary Layers Surrounding Stony Corals. The Biological Bulletin 185, 455–461. https://doi.org/10.2307/1542485

Sheridan, C., Kramarsky-Winter, E., Sweet, M., Kushmaro, A., Leal, M.C., 2013. Diseases in coral aquaculture: causes, implications and preventions. Aquaculture 396–399, 124–135. https://doi.org/10.1016/j.aquaculture.2013.02.037

Sime - Ngando, T., 2012. Phytoplankton Chytridiomycosis: Fungal Parasites of Phytoplankton and Their Imprints on the Food Web Dynamics. Frontiers in Microbiology 3.

Simon, J.-C., Marchesi, J.R., Mougel, C., Selosse, M.-A., 2019. Host-microbiota interactions: from holobiont theory to analysis. Microbiome 7, 5. https://doi.org/10.1186/s40168-019-0619-4

Spribille, T., Resl, P., Stanton, D.E., Tagirdzhanova, G., 2022. Evolutionary biology of lichen symbioses. New Phytologist 234, 1566–1582. https://doi.org/10.1111/nph.18048

St. Leger, R.J., Wang, J.B., 2020. Metarhizium: jack of all trades, master of many. Open Biology 10, 200307. https://doi.org/10.1098/rsob.200307

Steinbach, R.M., El Baidouri, F., Mitchison-Field, L.M.Y., Lim, F.Y., Ekena, J., Vogt, E.J.D., Gladfelter, A., Theberge, A.B., Amend, A.S., 2023. Malassezia is widespread and has undescribed diversity in the marine environment. Fungal Ecology 65, 101273. https://doi.org/10.1016/j.funeco.2023.101273

Strake, D.T., Jaap, W.C., Truby, E., Reese, R., 1988. FUNGAL FILAMENTS IN MILLEPORA COMPLANATA Lamarck, 1816 (Cnidaria: Hydrozoa) AFTER MASS EXPULSION OF ZOOXANTHELLAE. Florida Scientist 51, 184–188.

Sullivan, C.F., Parker, B.L., Skinner, M., 2022. A Review of Commercial Metarhizium- and Beauveria-Based Biopesticides for the Biological Control of Ticks in the USA. Insects 13, 260. https://doi.org/10.3390/insects13030260

Sun, F., Yang, H., Zhang, X., Tan, F., Shi, Q., 2022. Response characteristics of bacterial communities in multiple coral genera at the early stages of coral bleaching during El Niño. Ecological Indicators 144, 109569. https://doi.org/10.1016/j.ecolind.2022.109569

Sweet, M., Burn, D., Croquer, A., Leary, P., 2013. Characterisation of the Bacterial and Fungal Communities Associated with Different Lesion Sizes of Dark Spot Syndrome Occurring in the Coral Stephanocoenia intersepta. PLOS ONE 8, e62580. https://doi.org/10.1371/journal.pone.0062580

Sweet, M.J., Croquer, A., Bythell, J.C., 2011. Bacterial assemblages differ between compartments within the coral holobiont. Coral Reefs 30, 39–52. https://doi.org/10.1007/s00338-010-0695-1

Takashima, M., Deak, T., Nakase, T., 2001. Emendation of Dioszegia with redescription of Dioszegia hungarica and two new combinations, Dioszegia aurantiaca and Dioszegia crocea. J Gen Appl Microbiol 47, 75–84. https://doi.org/10.2323/jgam.47.75

Takashima, M., Manabe, R., Ohkuma, M., 2019. Draft Genome Sequences of Basidiomycetous Epiphytic Phylloplane Yeast Type Strains Dioszegia crocea JCM 2961 and Dioszegia aurantiaca JCM 2956. Microbiology Resource Announcements 8, 10.1128/mra.01727-18. https://doi.org/10.1128/mra.01727-18

Tambutté, E., Allemand, D., Zoccola, D., Meibom, A., Lotto, S., Caminiti, N., Tambutté, S., 2007. Observations of the tissue-skeleton interface in the scleractinian coral Stylophora pistillata. Coral Reefs 26, 517–529. https://doi.org/10.1007/s00338-007-0263-5

Taylor, J.D., Cunliffe, M., 2016. Multi-year assessment of coastal planktonic fungi reveals environmental drivers of diversity and abundance. ISME J 10, 2118–2128. https://doi.org/10.1038/ismej.2016.24

Tedersoo, L., Anslan, S., Bahram, M., Drenkhan, R., Pritsch, K., Buegger, F., Padari, A., Hagh-Doust, N., Mikryukov, V., Gohar, D., Amiri, R., Hiiesalu, I., Lutter, R., Rosenvald, R., Rähn, E., Adamson, K., Drenkhan, T., Tullus, H., Jürimaa, K., Sibul, I., Otsing, E., Põlme, S., Metslaid, M., Loit, K., Agan, A., Puusepp, R., Varik, I., Kõljalg, U., Abarenkov, K., 2020. Regional-Scale In-Depth Analysis of Soil Fungal Diversity Reveals Strong pH and Plant Species Effects in Northern Europe. Frontiers in Microbiology 11.

Tisthammer, K.H., Cobian, G.M., Amend, A.S., 2016a. Global biogeography of marine fungi is shaped by the environment. Fungal Ecology, Aquatic Fungi 19, 39–46. https://doi.org/10.1016/j.funeco.2015.09.003

Tisthammer, K.H., Cobian, G.M., Amend, A.S., 2016b. Global biogeography of marine fungi is shaped by the environment. Fungal Ecology, Aquatic Fungi 19, 39–46. https://doi.org/10.1016/j.funeco.2015.09.003

Tong, H., Cai, L., Zhou, G., Zhang, W., Huang, H., Qian, P.-Y., 2020. Correlations Between Prokaryotic Microbes and Stress-Resistant Algae in Different Corals Subjected to Environmental Stress in Hong Kong. Frontiers in Microbiology 11.

Tsuji, M., Vincent, W.F., Tanabe, Y., Uchida, M., 2022. Glacier Retreat Results in Loss of Fungal Diversity. Sustainability 14, 1617. https://doi.org/10.3390/su14031617

Velez, P., Salcedo, D.L., Espinosa-Asuar, L., Gasca-Pineda, J., Hernandez-Monroy, A., Soto, L.A., 2022. Fungal Diversity in Sediments From Deep-Sea Extreme Ecosystems: Insights Into Low- and High-Temperature Hydrothermal Vents, and an Oxygen Minimum Zone in the Southern Gulf of California, Mexico. Frontiers in Marine Science 9.

Vicente, V.A., Orélis-Ribeiro, R., Najafzadeh, M.J., Sun, J., Guerra, R.S., Miesch, S., Ostrensky, A., Meis, J.F., Klaassen, C.H., de Hoog, G.S., Boeger, W.A., 2012. Black yeast-like fungi associated with Lethargic Crab Disease (LCD) in the mangrove-land crab, Ucides cordatus (Ocypodidae). Veterinary Microbiology 158, 109–122. https://doi.org/10.1016/j.vetmic.2012.01.031

Videira, S.I.R., Groenewald, J.Z., Verkley, G.J.M., Braun, U., Crous, P.W., 2015. The rise of Ramularia from the Mycosphaerella labyrinth. Fungal Biology 119, 823–843. https://doi.org/10.1016/j.funbio.2015.06.003

Voolstra, C.R., Ziegler, M., 2020. Adapting with Microbial Help: Microbiome Flexibility Facilitates Rapid Responses to Environmental Change. BioEssays 42, 2000004. https://doi.org/10.1002/bies.202000004

Wang, L., Shantz, A.A., Payet, J.P., Sharpton, T.J., Foster, A., Burkepile, D.E., Vega Thurber, R., 2018. Corals and Their Microbiomes Are Differentially Affected by Exposure to Elevated Nutrients and a Natural Thermal Anomaly. Frontiers in Marine Science 5.

Wang, W., Liao, Y., Tang, C., Huang, X., Luo, Z., Chen, J., Cai, P., 2017. Cytotoxic and Antibacterial Compounds from the Coral-Derived Fungus Aspergillus tritici SP2-8-1. Marine Drugs 15, 348. https://doi.org/10.3390/md15110348

Wegley, L., Edwards, R., Rodriguez-Brito, B., Liu, H., Rohwer, F., 2007. Metagenomic analysis of the microbial community associated with the coral Porites astreoides. Environmental Microbiology 9, 2707–2719. https://doi.org/10.1111/j.1462-2920.2007.01383.x

Wickham, H., 2016. ggplot2, Use R! Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4

Work, T., Meteyer, C., 2014. To Understand Coral Disease, Look at Coral Cells. EcoHealth 11, 610–618. https://doi.org/10.1007/s10393-014-0931-1

Work, T.M., Aeby, G.S., Hughen, K.A., 2016. Gross and Microscopic Lesions in Corals from Micronesia. Vet Pathol 53, 153–162. https://doi.org/10.1177/0300985815571669

Wright, R.M., Strader, M.E., Genuise, H.M., Matz, M., 2019. Effects of thermal stress on amount, composition, and antibacterial properties of coral mucus. PeerJ 7, e6849. https://doi.org/10.7717/peerj.6849

Wu, B., Hussain, M., Zhang, W., Stadler, M., Liu, X., Xiang, M., 2019. Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. Mycology 10, 127–140. https://doi.org/10.1080/21501203.2019.1614106

Xu, L., Meng, W., Cao, C., Wang, J., Shan, W., Wang, Q., 2015. Antibacterial and Antifungal Compounds from Marine Fungi. Marine Drugs 13, 3479–3513. https://doi.org/10.3390/md13063479

Xu, W., Gong, L., Pang, K.-L., Luo, Z.-H., 2018. Fungal diversity in deep-sea sediments of a hydrothermal vent system in the Southwest Indian Ridge. Deep Sea Research Part I: Oceanographic Research Papers 131, 16–26. https://doi.org/10.1016/j.dsr.2017.11.001

Yao, G.-S., Ma, Z.-L., Zheng, Y.-Y., Lv, L., Mao, J.-Q., Wang, C.-Y., 2022. Bioactive Alkaloids from the Marine-Derived Fungus Metarhizium sp. P2100. J Fungi (Basel) 8, 1218. https://doi.org/10.3390/jof8111218

Yarden, O., Ainsworth, T.D., Roff, G., Leggat, W., Fine, M., Hoegh-Guldberg, O., 2007. Increased Prevalence of Ubiquitous Ascomycetes in an Acropoid Coral (Acropora formosa) Exhibiting Symptoms of Brown Band Syndrome and Skeletal Eroding Band Disease. Applied and Environmental Microbiology 73, 2755–2757. https://doi.org/10.1128/AEM.02738-06

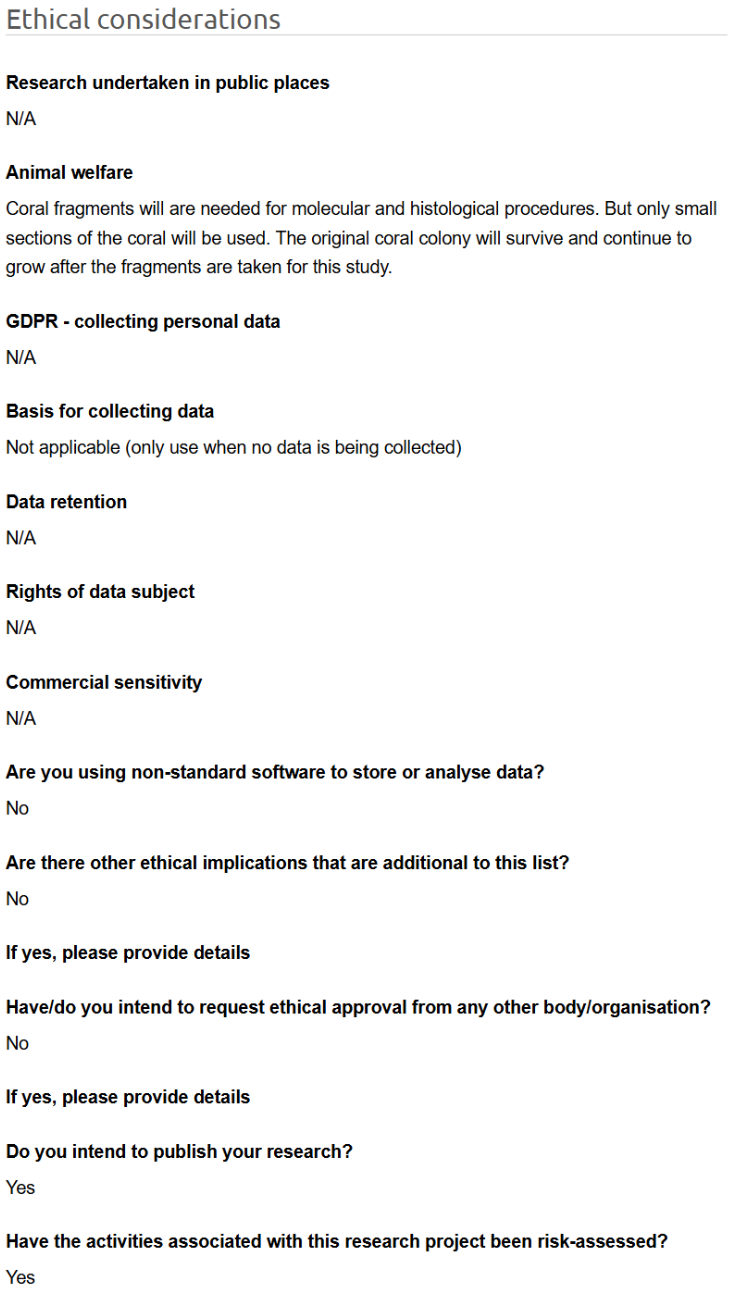
Zabel, R.A., Morrell, J.J., 2020. Chapter Three - The characteristics and classification of fungi and bacteria, in: Zabel, R.A., Morrell, J.J. (Eds.), Wood Microbiology (Second Edition). Academic Press, San Diego, pp. 55–98. https://doi.org/10.1016/B978-0-12-819465-2.00003-6

Zhang, T., Fei Wang, N., Qin Zhang, Y., Yu Liu, H., Yan Yu, L., 2015. Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). Sci Rep 5, 14524. https://doi.org/10.1038/srep14524

Ziegler, M., Grupstra, C.G.B., Barreto, M.M., Eaton, M., BaOmar, J., Zubier, K., Al-Sofyani, A., Turki, A.J., Ormond, R., Voolstra, C.R., 2019. Coral bacterial community structure responds to environmental change in a host-specific manner. Nat Commun 10, 3092. https://doi.org/10.1038/s41467-019-10969-5

Appendix

Appendices 1.1: Ethics Application:



Ethical considerations

Research undertaken in public places

N/A

Animal welfare

Coral fragments will are needed for molecular and histological procedures. But only small sections of the coral will be used. The original coral colony will survive and continue to grow after the fragments are taken for this study.

GDPR - collecting personal data

N/A

Basis for collecting data

Not applicable (only use when no data is being collected)

Data retention

N/A

Rights of data subject

N/A

Commercial sensitivity

N/A

Are you using non-standard software to store or analyse data?

No

Are there other ethical implications that are additional to this list?

No

If yes, please provide details

Have/do you intend to request ethical approval from any other body/organisation?

No

If yes, please provide details

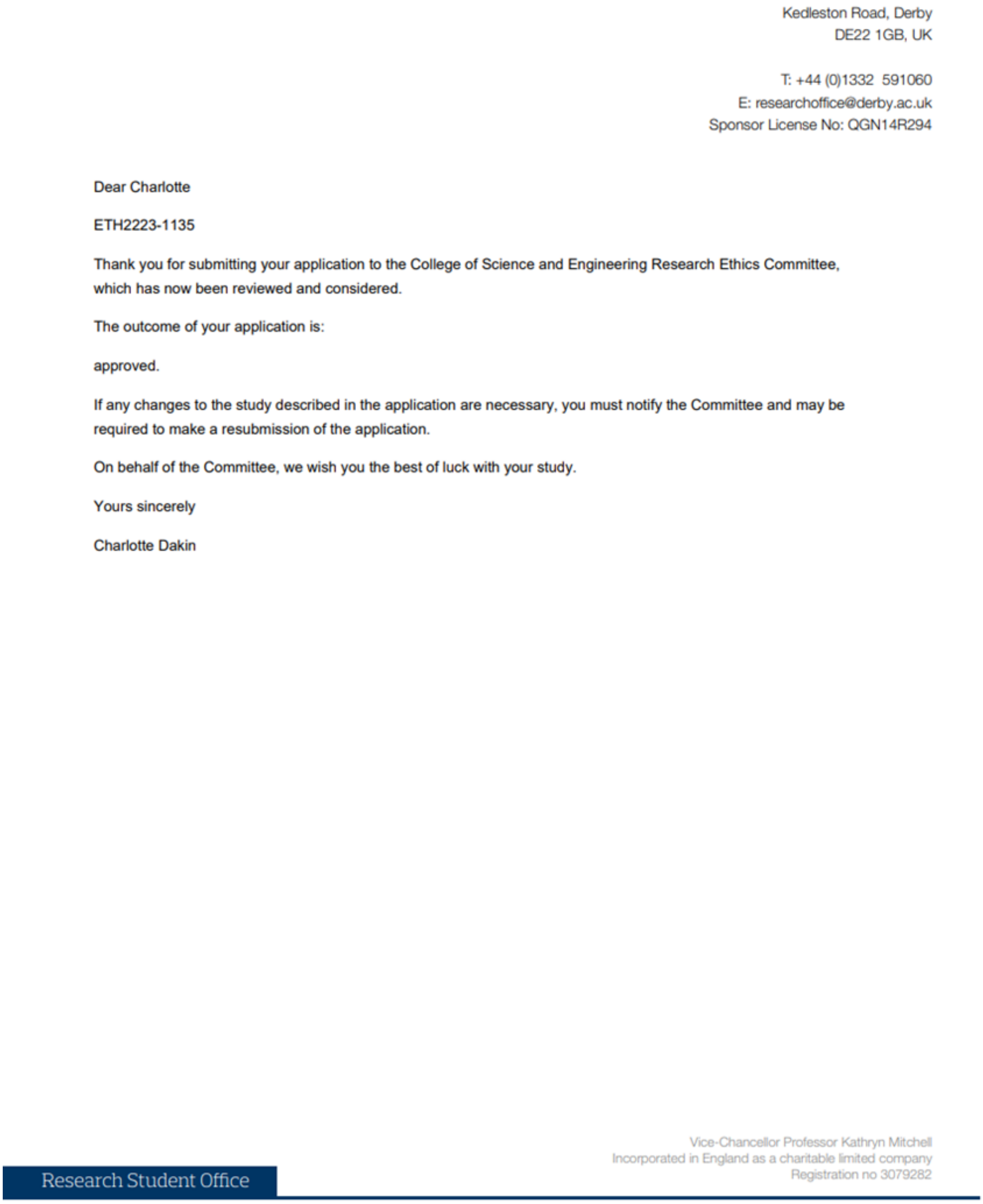
Do you intend to publish your research?

Yes

Have the activities associated with this research project been risk-assessed?

Yes

Appendices 1.2: Ethics Approval:



Kedleston Road, Derby
DE22 1GB, UK

T: +44 (0)1332 591060
E: researchoffice@derby.ac.uk
Sponsor License No: QGN14R294

Dear Charlotte

ETH2223-1135

Thank you for submitting your application to the College of Science and Engineering Research Ethics Committee, which has now been reviewed and considered.

The outcome of your application is:

approved.

If any changes to the study described in the application are necessary, you must notify the Committee and may be required to make a resubmission of the application.

On behalf of the Committee, we wish you the best of luck with your study.

Yours sincerely

Charlotte Dakin

Vice-Chancellor Professor Kathryn Mitchell
Incorporated in England as a charitable limited company
Registration no 3079282

Research Student Office