THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF BIOLOGY

HOW DIVERSE ARE CORAL-BORING MUSSELS ACROSS THE PACIFIC?

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ABSTRACT

Coral reefs provide an important reservoir for biodiversity, and they are an integral part of the culture and economy of many island nations across the globe. Reefs form the foundation of islands inhabited by humans and protect the shoreline from erosion. The coral reefs also serve as important food sources. Nonetheless, climate change and pressure from invasive species are degrading them. For example, boring mussels in the genus *Lithophaga* infest *Porites* corals. The *Lithophaga* larvae are able to overcome the stinging cells that the corals use to defend themselves against invaders and bore into the coral skeleton. This destabilizes the coral colonies and can cause bioerosion. Certain species of *Porites* coral are more susceptible to the larvae, suggesting a species-specific interaction. Therefore, it is proposed here that mussel diversity in the Pacific Ocean follows a diversity gradient similar to that of the corals, where the highest diversity is found in the Indo-Pacific Region and gradually declines towards the east. In order to investigate this hypothesis, the cytochrome c oxidase I (CO1) region of mitochondrial DNA of the mussels was sequenced to look for a constant association between a dominant species of coral, *Porites lobata*, and a lesser species, *Porites evermanni*, and mussels across the Western, Central, and Eastern Pacific Ocean. Contrary to expectation, it was discovered that the mussels were actually of the genus *Leiosolenus* and not *Lithophaga*. Further, it was found that one species of mussel, *Leiosolenus hanleyanus* is predominately found in *Porites* corals across the Ocean. This is significant because the mussels and coral maintained that relationship through the Eastern Pacific Barrier, a 3000 km stretch of open ocean. This is the first report of *L. hanleyanus* east of the Central Pacific. Population genetic structure was similar in mussels and corals where the eastern populations were differentiated from other populations to the west.

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Chapter 1

Introduction

The Global Coral Crisis

Coral reefs are a very important ecosystem in that they house an amazing amount of biodiversity (Carpenter and Springer, 2005). It has been estimated that up to 1/3 of all marine species that have been described so far are found in coral reef ecosystems. The diversity found in these ecosystems is of paramount importance because diversity can provide ecosystem resilience and resistance and be a source for novel compounds relevant for medical research (Reaka-Kudla, 2001).

Coral reefs also provide a significant economic value. More than 100 of the world's 195 countries have coastlines lined with coral reefs (Moberg and Folke, 1999). In those countries, the majority of the population lives near the coast. In fact, 500 million people (8% of the global population) live within 100km of a coastline containing a coral reef (Bryant, et al. 1998). The ecosystems that comprise those regions (both marine and inland) provide about $25 trillion in economic value every year, which is about a quarter of the gross world product (Martínez, et al. 2007). Furthermore, the reefs serve as important source of food for the local populations as well, with many being a vital source of protein (Costanza, et al. 1997).

Coral reefs also serve as important geographical structures. Dozens of islands inhabited by humans across the globe have a coral reef foundation. For example, the island nation of Kiribati houses more than 100,000 people and is comprised of coral-based islands and atolls

(Donner and Weber, 2014). Another example is the Hawaiian Island Chain, where there are several inhabited atolls supported structurally by coral reefs (Grigg and Epp, 1989).

In addition to forming the foundation of several inhabited islands, coral reefs also function as wave breakers. These reefs are vital for protecting human settlements on smaller islands from violent storms. Unfortunately, many of these important reefs are on the decline due to a variety of environmental factors. One of the most important is increasing sea water temperatures that cause coral bleaching. Coral bleaching is a process where corals expel their microsymbionts due to heat stress, weakening the coral and exposing the chalky white aragonite skeleton beneath (Hoegh-Guldberg, 1999).

Alongside coral bleaching, reefs face other threats that result from global warming, such as sea level rise (Vermeer and Rahmstorf, 2009). Sea level rise has resulted in more nutrient runoff from the mainland leading to eutrophication (Nuttle and Portnoy, 1992). These factors also contribute to the destruction of coastal wetlands, which are important for the health of nearby reefs as well (Craft, et al. 2008).

Finally, corals face the threat of bioerosion as well. Many different organisms, ranging from tiny microbes (Chen, et al. 2013) to larger fish play a role in eroding away the aragonite skeleton of both dead and live corals (Boulay, et al. 2014). This bioerosion, alongside the factors above contribute to structural instability of the coral reefs, causing them to slowly crumble away (Woodroffe, 2008; Donner and Weber, 2014; Grigg and Epp, 1989). This thesis aims to further our understanding of the interaction between corals and coral-boring mussels in the Pacific Ocean in order to better understand the factors that lead to coral bioerosion in the Pacific Ocean.

Diversity Across the Pacific Ocean

It is well known that the Indo-Pacific Region contains an extremely high amount of biodiversity. This is owed to the fact that it is where the Indian and Pacific Oceans meet; therefore, diversity from both oceans is combined in this region (Bellwood and Hughes, 2001). The biodiversity allows the environment to better cope with environmental stressors, such as global warming or disease outbreaks, because there is more variety in the organisms in the environment. That variety increases the amount of adaptations that can be used to combat such threats (Carpenter and Springer, 2005). However, it has also been shown that the amount of biodiversity decreases along a west-east gradient across the Pacific Ocean. Therefore, less biodiversity is observed in the Eastern Pacific than the Western Pacific (Braby and Somero, 2006).

The Eastern pacific ocean is separated from the Central Pacific Islands by a 3000 km stretch of open water called the Eastern pacific barrier (Baums, et al. 2012). The lack of coral reef structures in the area between these two regions can make it difficult for larvae to traverse this barrier. This creates a genetic distinction between the populations of corals in the Western/Central Pacific and those in the Eastern Pacific (Baums, et al. 2012).

The Relationship Between Corals and Mussels

Mussels are an important biotic factor that can impact the health of reef-building corals (Glynn and Manzello, 2015). Mussels have a bipartite life-style. Adults release spawn into the water column where the larvae develop. After reaching competency, the larvae settle onto coral colonies. Mussel larvae are able to get past a complex set of coral defenses in order to access the

coral skeleton and begin creating a bore like the ones seen in Figure 1. First, the mussel larvae must be able to bypass the stinging cells called nematocysts on the exterior of the coral skeleton. Next, they must clear out an opening on the surface of the coral. Finally, the larvae proceed to dissolve the aragonite skeleton in order to begin digging their burrow (Scott and Risk, 1988).

There is clearly a very close relationship between the corals and the mussels. It has been shown that some coral species are more susceptible to invasion by a mussel larva, which suggests that the mussels are somewhat specialized to certain species of coral (Mokady, et al. 1991). Furthermore, the size and shape of the borehole varies based on the species of the host coral, not necessarily just by the species of mussel, due to differential success of the mussel in invading certain hosts versus others (Scott, 1988). An example of the variety seen in bore size is shown in Figure 2.

Invasion by a mussel is detrimental to the health of the coral host. The bore holes can destabilize the structural integrity of the colony and cause it to crumble (Namboothri and Fernando, 2012). Pieces of coral can also be broken off when fish try to prey on the mussels inside of the bores (Boulay, et al. 2014). Normally, the balance of erosion to skeleton construction is near equilibrium. However, due to other environmental stressors, primarily ocean acidification, the balance can be tilted towards erosion (Glynn and Manzello, 2015). Therefore, studying the interactions between mussels and corals can lead to a better understanding of bioerosion and reef health.

Goals of This Study

The main objective of this study was to examine in closer detail some of the patterns in the relationship between the coral-boring mussels of the Pacific Ocean and their hosts. The first objective was to identify the species of mussels invading one of the most important and widespread reef-building coral genus in the Pacific, *Porites* (Link, 1807). It is widely accepted that *Lithophaga* mussels comprise a large portion of the coral-boring mussels in the Pacific (Boulay, et al. 2014). However, other species such as *Leiosolenus* have been observed as coral-borers in the Pacific as well (Owada, 2006). Mussels were collected by sampling coral colonies in the genus *Porites* from across the Pacific Ocean (Palau, Jarvis Island, the Phoenix Islands, the Galapagos Islands). The Cytochrome c oxidase I (*COI*) gene region of the mitochondrial DNA of the mussels was sequenced and compared to publicly available sequences. The *COI* region was chosen because it is a region of high mutation and variation, even on the individual level (Folmer, et al. 2014). Phylogenetic trees were constructed to infer the evolutionary history of the mussels. It was hypothesized that most of the mussels sequenced will be from the genus *Lithophaga* (Boulay, et al. 2014; Owada, 2006).

The second objective was to unveil the specificity of the interaction between certain mussel species and their coral hosts. It was hypothesized that because of the complexity of the coral's defenses, there will be a species-specific interaction between mussels and coral hosts (Scott, 1988). This hypothesis was tested using a chi-square test with the first variable being the species of mussel and the second variable being the species of the host coral. The species of coral host was determined using available microsatellite genotyping data (Chan, 2019).

The third and final objective was to address the population genetics of the dominant coral boring mussel species across the Pacific Ocean. It was hypothesized that the mussels will follow

a similar pattern to the genetic structure of their host coral. In order to test this hypothesis, a one-way AMOVA test was used to examine the diversity of mussels from the Western, Central, and Eastern Pacific and compared to the published data of the coral host.

Chapter 2

Methods

Sample Collection

Boring mussels were collected from the Galapagos, Nikko Bay (Palau), and seven islands in the Phoenix Islands Protected Area (Kanton, Enderbury, Birnie, Rawaki, Manra, Orona, McKean, and Nikumaroro) between 2012 and 2017. SCUBA divers used a hammer and chisel to break open the coral skeleton around the burrowing mussel. Mussels were then extracted from the skeleton using forceps and placed in plastic bags filled with seawater. Once on shore, all samples were transferred to individual tubes filled with molecular grade 100% ethanol. In Palau, samples were preserved in dimethyl sulfoxide (DMSO). Figure 3 shows a general map of the sampling locations.

DNA Extraction

The mussels were taken out of frozen storage and allowed to equilibrate at room temperature. Approximately 25mg of each sample was then dissected out from the inside of the mussel and placed in 1.5ml microcentrifuge tubes (Qiagen, CA). The tubes were labelled in order to maintain the identity of each sample. The QIAGEN DNeasy kit was used to extract DNA following the manufacturer's protocol. First, 180µl of buffer and 20µl of proteinase K

(Qiagen, CA) were added to the tubes. The mixture was then vortexed and incubated in an Imperial III Incubator (Lab-line, IL) at 56°C overnight.

The samples were then removed and vortexed. 200µl each of buffer and ethanol were added to the tubes (Qiagen, CA) before they were vortexed again. The contents were then transferred over to pre-labelled centrifuge tubes (Qiagen, CA) and centrifuged in a microcentrifuge at 8,000rpm for 1 minute (Beckman-Coulter, CA). The contents were then washed with a buffer and centrifuged at 8,000rpm for one minute. The final wash was centrifuged at 14,000rpm for 3 minutes to dry the contents. 100µl of an elution buffer (Qiagen, CA) was then added directly to the filter and allowed to sit for 5 minutes. The contents were then centrifuged into labelled 1.5ml microcentrifuge tubes at 8,000rpm for 1 minute. The purity of the samples after this process was checked with a Spectrophotometer (Nanodrop, DE). The extracted DNA was frozen at -20C until further use.

DNA Amplification and Sequencing

The samples were removed were thawed and a Polymerase Chain Reaction was performed in order to amplify the extracted DNA. A sample protocol can be seen in Table 1. It consisted of 5µl of ammonia buffer (Bioline, TN), 5 µl of magnesium chloride (Bioline, TN), 4 µl of dNTPs (Takara, CA), 2.5µl of forward and reverse primers (Integrated DNA Technologies, IA), 1µl of BSA (New England Biolabs, MA), and 0.2µl of Taq polymerase (Bioline, TN) for every 1µl of sample DNA amplified. The samples were then placed in a Mastercycler Pro thermocycler (Eppendorf, NY). The samples were first heated to 95°C for 1 minute. Then they

were cycled between 40°C and 72°C 35 times followed by a final 7-minute period at 72°C before being held at 4°C until the samples were retrieved.

After the PCR reaction had been completed, the samples were each run through a 50ml 1% agarose gel electrophoresis to ensure that the PCR worked properly (Bio-Rad, CA). 4µl of each sample was mixed with 1.5µl of loading dye and run through a gel for 35 minutes at 100V. DNA hyperladder I was used as the DNA ladder for these gels (Bioline, TN). The gels were imaged using a universal imaging hood (Bio-Rad, CA). The samples were then cleaned using an Exo-Sap reaction consisting of 5µl PCR product and 2µl of Exo-Sap. Exo-Sap is a mixture of 30µl of $1\mu M^{-1}$ shrimp alkaline phosphate, 15µl of $20\mu M^{-1}$ exonuclease 1, and 15µl of water (New England Biolabs, MA). Samples were then sent to the PSU Genomics Core Facility for Sanger Sequencing.

DNA Analysis

Sequences were trimmed to remove primers using CodonCode software (CodonCode Corporation, MA). Then, BLAST (Altschul, et al. 1990) was used to identify appropriate reference sequences for the alignment in the NCBI genbank database. Four reference sequences were obtained and added to the alignment. The sample sequences, along with the four references were aligned using CodonCode software (CodonCode Corporation, MA).

Tree Building

Once the alignment was completed, the sequences were imported into MEGA (Kumar, et al. 2018) for tree building. Two different trees were constructed using the Tamaura-Nei

substitution model (Tamura and Nei, 1993). This substitution model was determined to be the best fit for the data by running a jModel Test (Posada, 2008). A maximum parsimony tree was constructed with 500 bootstrap samples and condensed so that only branches with over a 50% likelihood were maintained. The same process was followed for a maximum likelihood tree, again with 500 bootstrap samples.

Phylogenetic Network Construction

The data was imported into the program SplitsTree4 (Huson and Bryant, 2006) via a fasta file. From there, a Neighbor Network was created using 1000 bootstrapped samples. All of the samples minus the three reference outgroups were included. Networks both including and excluding the samples from the Eastern Pacific were constructed to increase resolution on the Western and Central Pacific Networks.

Analysis of Molecular Variance, Haplotype Diversity, and Tajima's D Tests

Once the sequences were properly aligned, a program called DNA Sequence Polymorphism (Barcelona, Spain) was used to extrapolate haplotype information from the sequences. An analysis of molecular variance was then performed using the program Arlequin (Excoffier and Schneider, 2005). All 104 samples were assigned to three different groups based on their origin: Palau (Western Pacific), Jarvis Island/The Phoenix Islands (Central Pacific), and The Galapagos Islands (Eastern Pacific). The analysis then compared the three groups amongst each other using 1,000 permutations. The differences amongst populations were then calculated

by measuring pairwise FST values and performing a Tajima's-D Neutrality Test through Arlequin (Excoffier and Schneider, 2005) with 100 permutations at a significance level of 0.05.

Chapter 3

Results

Constructed Trees

The trees help to demonstrate that an overwhelming majority of the species that were sequenced were *Leiosolenus hanleyanus*. Figure 4 shows a maximum likelihood tree while Figure 5 shows a maximum parsimony tree. Both trees returned fairly similar evolutionary histories. In each tree, 102 mussels were identified as *L. hanlyeanus*. They were all very closely related and any branches beyond the branch with *L. hanelyeanus* were condensed when the trees were scaled so that only branches with a significance of over 50% were included. The next most closely related individual (17_1076) was from the Western Pacific. Two of the other samples seemed to be quite different from *L. hanleyanus* and the other reference sequences that were included (*Leiosolenus mucronatus* and *Leiosolenus lima*). One sequence that differed was from the Western Pacific (17_1077) while the other was from the Eastern Pacific (12_1529). However, the BLAST results on NCBI's website suggested that they are still in the *Leiosolenus* genus. Sample 17_1077 had a 93% match with *L. lima* while 12_1529 had a 94% match with *L. mucronatus*.

The main difference between the trees was centered around the divergence of 17_1077 and 12_1259 from the rest of the references. Figure 4 suggests that 17_1077 diverged more recently from *L. lima* than the other species in the *Leiosolenus* genus. Figure 5 contradicts this by showing that 17_1077 may have diverged from *L. lima* around the same time as the other

species. With respect to 12_1259, Figure 5 suggested that it split with *L. hanleyanus* around the same time as *L. mucronatus*. However, Figure 4 showed that it diverged earlier.

From this information, in accordance with the information on the host coral species contained in Table 2, it was determined that *L. hanleyanus* was the main bioeroder in the Pacific in the two well-sampled corals species, *Porites evermanni* and *Porites lobata*.

Population Statistics

The Analysis of molecular variance showed that the populations in the three different locations are significantly different from another. There was an average percent difference of 18.29% between the regions and the Fixation Index was 0.18 ($p=0.035$). Additionally, the population-specific FST values were calculated, with the Western and Central Pacific populations ($FST_{West}=0.14$; $FST_{Central}=0.20$) showing more diversity than the corals in the Eastern Pacific ($FST_{East}=0.04$). These results are summarized in Table 3.

When comparing the pairwise FST values between two of the populations, the Eastern Pacific population showed the most difference from the other two regions. The Western and Central Pacific had a fairly low pairwise FST value ($FST_{West/Central}=0.04$; $p=0.018$), signifying relatively higher levels of gene flow. However, the Eastern Pacific was highly differentiated from the mussels in the Central Pacific ($FST_{Central/East}=0.73$; $p=0.000$). The comparison between the Western and Eastern populations did not yield significant results ($p=0.090$), possibly because of the smaller sample size in each population ($N_{West}=23$; $N_{East}=5$).

Table 5 contains the results of the Tajima's-D Neutrality Test. While the sample size seems to be too small to infer any difference from neutrality for the populations in the Eastern

Pacific, neutrality could be rejected for both the Central (D = -2.325; p=0.000) and Western (D = -1.893; p=0.016) mussel populations at a 0.05 significance level. Therefore, it can be inferred that these populations were undergoing selection and/or that the populations sizes were not stable.

Phylogenetic Networks

Figures 6 and 7 show the proposed phylogenetic networks of the sequences obtained based on 1000 bootstrap samples. In Figure 6, only the samples from the Western and Central Pacific were included in order to gain a higher amount of resolution since the Galapagos samples from the eastern Pacific were so distantly related. Blue circles are samples from the Western Pacific, red circles are from the Central Pacific, and green squares represent nodes where samples from both populations were observed. The large green square to the left of the figure is where the reference sequence for *L. hanleyanus* was located, along with 33 other samples from both the Western and Central Pacific. The moderate amount of branching demonstrates that there was differentiation between the populations.

Figure 7 includes the 5 samples from the Eastern Pacific in the analysis to show the stark contrast from the Western and Central Populations. Beyond the genetic difference between the populations, this figure also demonstrates that the populations in the Western and Central Pacific contained more genetic diversity than the population in the Eastern Pacific. In fact, the distance between nodes of the populations in the Eastern Pacific were so small that all four appear as only one node in the Figure.

Chapter 4

Discussion

Identification of Mussels

The BLAST results show that an overwhelming majority of the species examined were only from one species. However, contrary to expectations, this species was not a member of the genus *Lithophaga* (Distel, 2000). Therefore, the first hypothesis was rejected. Instead the sequences assigned to the genus *Leiosolenus*. *Leiosolenus* mussels also bore into corals and are detrimental to the structural integrity of the coral colony. *Leiosolenus* are morphologically similar to *Lithophaga*, with only minor differences seen between the two genera (Owada, 2006). In fact, originally the two genera were combined in the same genus (Molluscabase, 2019). However, they were eventually separated based on genetic differences and the composition of the bores that each one creates. *Leiosolenus* mussels have a chalky coating that covers their boreholes, while *Lithophaga* do not. This chalky substrate can also manifest itself on the shells of the *Leiosolenus* themselves, creating deposits on top of their shell (Kleemann and Maestrati, 2012).

While both mussel genera occupy the same niche, they are quite different genetically (Liu, et al. 2018). It is believed that this similarity is not due to a close evolutionary history, but rather because of convergent evolution (Owada, 2006). In fact, I could not align any of the *Lithophaga* reference sequences with a *Leiosolenus* sequence as a comparison because they were too different.

Some *Leiosolenus* species are an invasive in that they have traveled with their invasive coral host to new areas (Vinagre et al. 2018). It has been shown that since the age of exploration,

many different marine species have been dispersed across the globe in the ballast water of ships and on ship hulls (Vinagre, et al. 2018). *L. aristatus* is suspected of having invaded the Mediterranean coast of Israel this way (Galil 2007). Recent invasion would provide an explanation for the expansion of the known range for *L. hanleyanus* from the Red Sea and Indo-West Pacific to the Central and Eastern Pacific reported here (see below).

Mussel Specialization and Diversity

Contrary to the second hypothesis, the host coral data suggests that one mussel species invades several species of coral. This would mean that the mussels are able to bypass the defenses of many different species of corals instead of being specific to just one. While this initially appears contradictory to much of the literature currently on this subject (Boulay, et al. 2014), it could again be explained by the possibility of these mussels being an invasive species and natural selection has not had enough time to create defense mechanisms in the hosts. Nevertheless, some studies have shown differences in the health and success of a mussel once it is attached to a certain host. Mussels that are attached to certain species of coral hosts grow to bigger sizes than mussels on other hosts (Scott, 1988).

A search of the World Register of Marine Species (WoRMS) database was undertaken to examine the current reported locations of *L. hanleyanus*. While *Leiosolenus* mussels have been found in the Pacific for a long time, the species identified in this study, *L. hanleyanus*, has only been reported thus far from the Red Sea, Southeastern Mediterranean Sea (invasive), the Indo-West Pacific and Australia (Molluscabase, 2019). This could be explained in two ways: the corals have existed in the region for a long time and have just not been recorded, or that they

have recently migrated into the Central and Eastern Pacific. Regardless, this is the first report of *L. hanleyanus* from the Central and Eastern Pacific.

The data does not contradict the possibility that the mussels were invasive, at least in the Eastern Pacific (Fig 7). An examination of the phylogenetic networks shows that the mussels were much less differentiated in the Eastern Pacific than in the Western or Central Pacific. This would indicate that the population had not been there for a long time, and thus had not experienced much differentiation from the original founding population of mussels.

Furthermore, there seems to be a barrier to gene flow between the populations of the Western and Central Pacific and the Eastern Pacific. Given the low FST value when comparing the Central to the Western Pacific, and the comparatively higher FST when compared to the Eastern Pacific, it can be inferred that the population in the East was quite different from the other populations in the Pacific. A barrier to gene flow in the Eastern Pacific has been previously described for other species, mainly corals (Baums, et al. 2012), so it would not be surprising to see a similar effect here as well. This would again point to an acute introduction of *L. hanleyanus* to the Eastern Pacific resulting from human activity. The founder effect stemming from a single introduction and a subsequent barrier to gene flow between the Eastern Pacific and the rest of the Pacific would explain the observed differences between the populations. Clearly, this interpretation is limited by the small samples from the Eastern Pacific but is intriguing enough to warrant further study. Examination of museum collections from the Eastern Pacific may provide clues as to whether *L. hanleyanus* may be a recent invader.

Conclusions and Future Directions

This study showed that there is still a lot to understand about the interactions between coral-boring mussels and the host corals that they infect. Firstly, that there are several species of mussels that can infect coral hosts: both *Lithophaga* and *Leiosolenus*. However, despite the multitude of species, the data did not show that the mussels were selective about which coral host that they choose to infect, possibly due to a lack of defenses against the recent introduction of an invasive species. Finally, it was shown that the mussels, while drastically different in the Eastern Pacific, are less diverse on an individual basis. This could point to recent introduction to the ecosystem by human activity.

However, this study was not without its flaws. First and foremost, it could be biased by the uneven sample size among regions. Only 104 samples were used, with the vast majority (76) coming from the Central Pacific. In addition to that, only 5 samples came from the Eastern Pacific. This could make it hard to examine the diversity across the Pacific with such a large portion of the samples coming from one area. An avenue for further research could be gathering a larger sample size from the Eastern Pacific and comparing their diversity to other populations in the Pacific Ocean.

Part of the reason that the same mussel species was observed could be that all of the mussels besides two were sampled from the same genus of coral. It could be that mussels are not species-specific, but are genus-specific. It would be another interesting route going forward to investigate mussel samples taken from a wide variety of coral species in order to see if some differences are observed.

To conclude, this study has provided insight on the interaction between corals and coral-boring mussels. The main takeaway is that these mussels appear able to parasitize many

different species of coral. Additionally, the mussels themselves could be from a number of different genera, with *Leiosolenus* being observed in this study and *Lithophaga* in others (Distel, 2000; Boulay, et al. 2014). It is even possible that some of these species are invaders that have arrived via the ballast water from other parts of the world. Therefore, it is important to understand the diversity of mussels that are present in coral reef ecosystems. This will help to decrease stress in coral populations that are already under pressure from climate change. Ideally, this will contribute to conserving both the biodiversity, and economic stability, that these coral reefs provide.

Appendix A

List of Figures

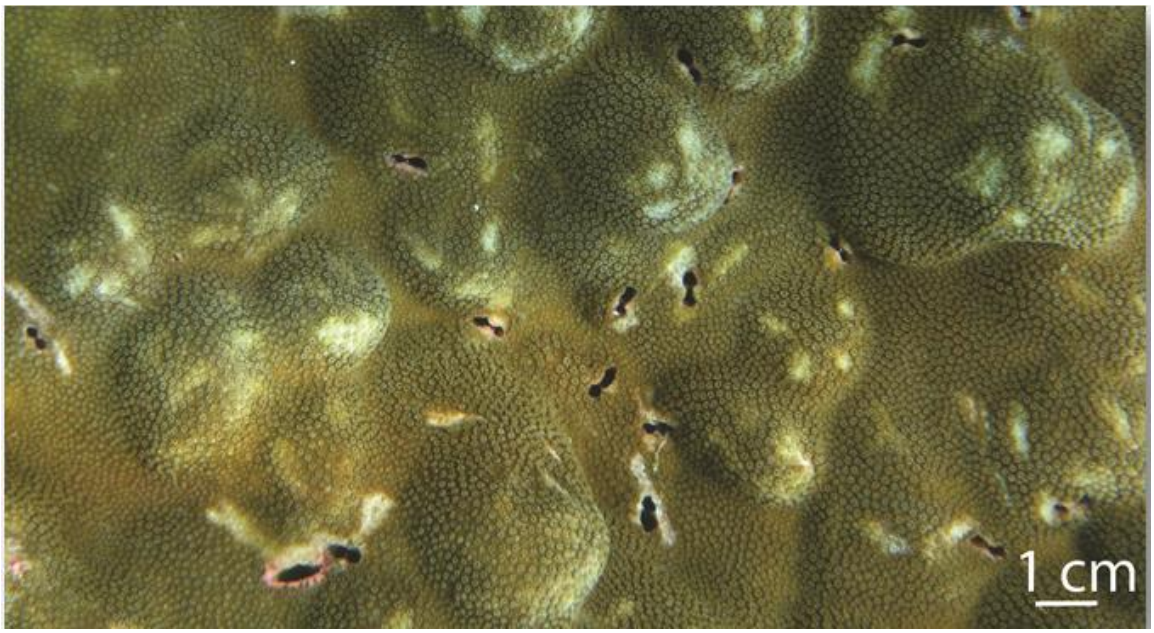

Figure 1. Close-Up of Boring Marks

Figure 2. Differently-Sized Boring Marks

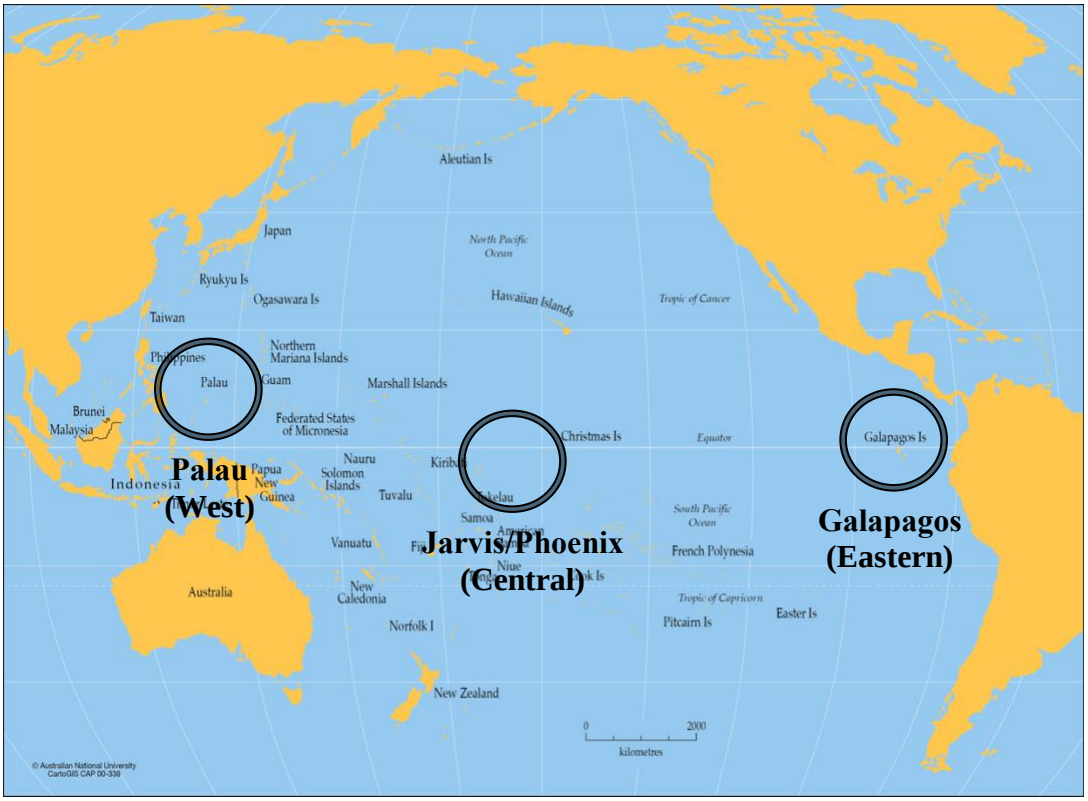

Figure 3. Sampling Locations (Palau=23, Jarvis/Phoenix=76, Galapagos=5)

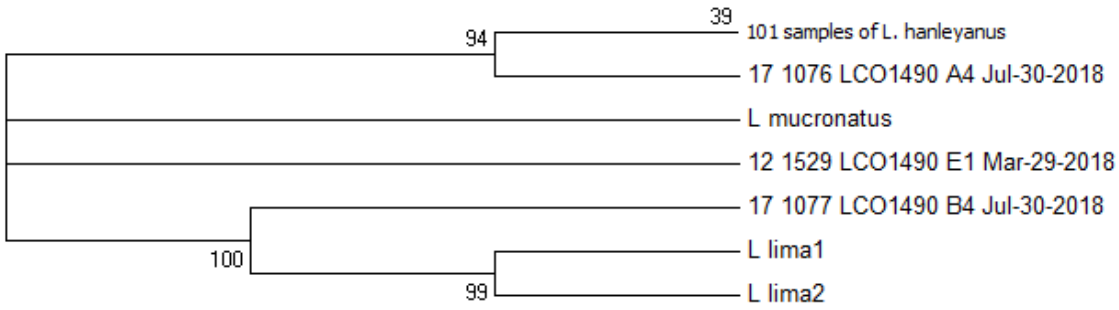

Figure 4. Maximum Likelihood Tree. References denoted by their species name. Sequenced samples listed as numbers representing year and location sampled. 500 bootstrap samples.

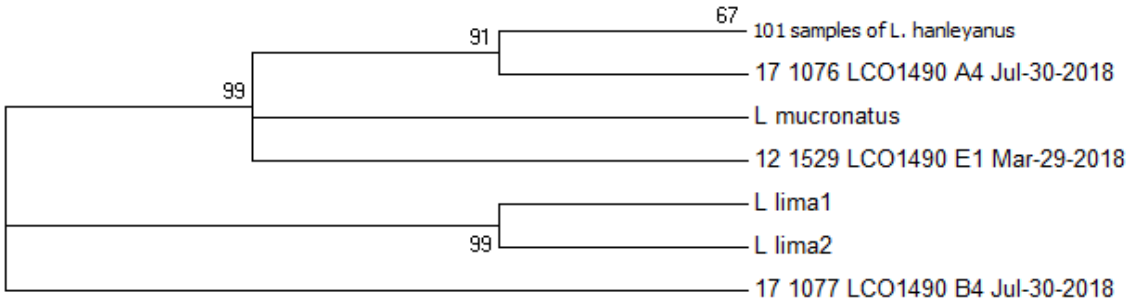

Figure 5. Maximum Parsimony Tree. References denoted by their species name. Sequenced samples listed as numbers representing year and location sampled. 500 bootstrap samples.

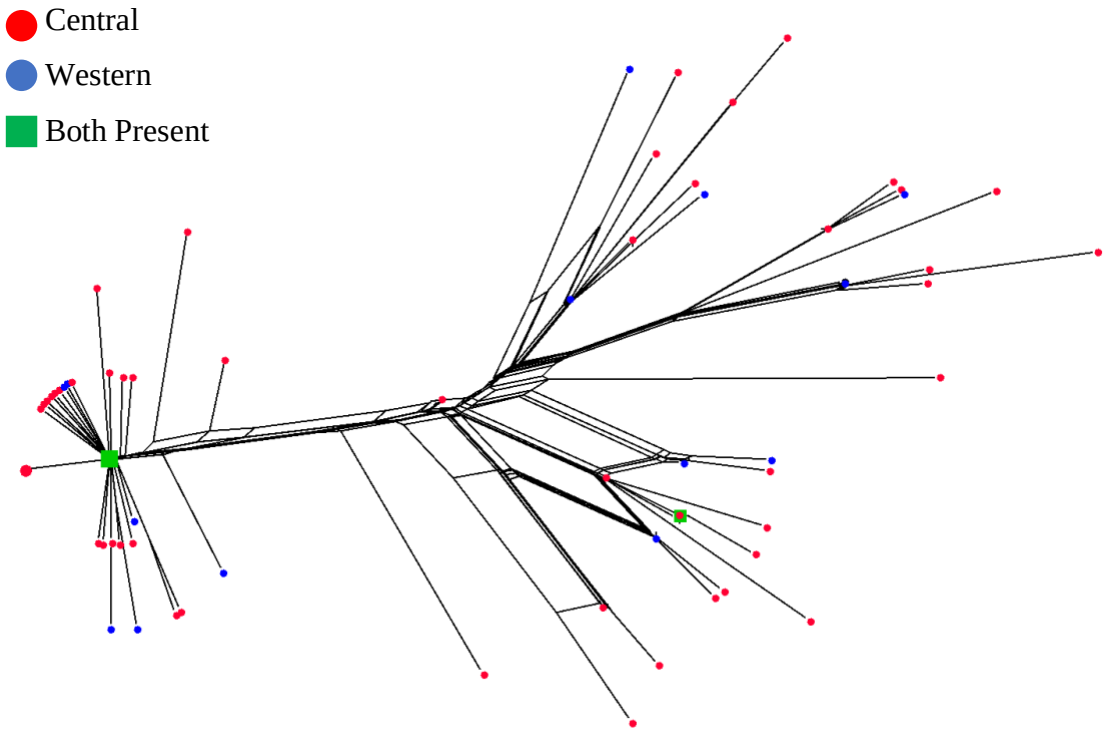

Figure 6. Phylogenetic Network for Western and Central Pacific

Figure 7. Phylogenetic Network Including Eastern Pacific

Appendix B

List of Tables

Table 1. Sample PCR Protocol

Reagent	Stock [ ]	Company	uL	Master Mix
Water			28.8	489.6
Buffer	10xNH4 PCR Rxn Buffer	Bioline	5	85
dNTP	2.5 mM	Takara	4	68
MgCl2	25 mM	Bioline	5	85
F	10 uM	IDT	2.5	42.5
R	10 uM	IDT	2.5	42.5
BSA	10mg/ml	NEB	1	17
Taq	5U/ul	Bioline	0.2	3.4
DNA			1	
Total:			50	833

Thermocycler:

95C	40C	72C	72C	4C
1min	1min	1.5min	7min	hold

Table 2. List of Samples, Region, and Host Coral Species

Sample#	Region	Coral Host
12_1336	Galapagos	
12_1376	Galapagos	
12_1529	Galapagos	
12_1533	Galapagos	
12_1562	Galapagos	
15_1131	Jarvis/Pheonix	
15_1132	Jarvis/Pheonix	
15_1133	Jarvis/Pheonix	
15_1134	Jarvis/Pheonix	
15_1135	Jarvis/Pheonix	
15_1136	Jarvis/Pheonix	
15_1137	Jarvis/Pheonix	P. lobata
15_1138	Jarvis/Pheonix	P. lobata
15_1139	Jarvis/Pheonix	P. lobata
15_1140	Jarvis/Pheonix	P. lobata
15_1143	Jarvis/Pheonix	P. lobata
15_1144	Jarvis/Pheonix	P. lobata
15_1145	Jarvis/Pheonix	P. evermanni
15_1146	Jarvis/Pheonix	P. evermanni
15_1147	Jarvis/Pheonix	P. lobata
15_1148	Jarvis/Pheonix	P. lobata
15_1149	Jarvis/Pheonix	P. lobata
15_1150	Jarvis/Pheonix	P. lobata
15_1151	Jarvis/Pheonix	P. lobata
15_1152	Jarvis/Pheonix	P. lobata
15_1153	Jarvis/Pheonix	P. lobata
15_1154	Jarvis/Pheonix	Porites spp.
15_1155	Jarvis/Pheonix	Porites spp.
15_1156	Jarvis/Pheonix	P. lobata
15_1157	Jarvis/Pheonix	P. lobata
15_1158	Jarvis/Pheonix	P. lobata
15_1159	Jarvis/Pheonix	P. lobata
15_1160	Jarvis/Pheonix	P. lobata
15_1161	Jarvis/Pheonix	P. lobata
15_1162	Jarvis/Pheonix	non-porites
15_1163	Jarvis/Pheonix	non-porites
15_1164	Jarvis/Pheonix	P. lobata
15_1165	Jarvis/Pheonix	P. lobata
15_1166	Jarvis/Pheonix	P. lobata
15_1167	Jarvis/Pheonix	P. lobata
15_1168	Jarvis/Pheonix	P. lobata
15_1169	Jarvis/Pheonix	P. evermanni
15_1170	Jarvis/Pheonix	P. evermanni
15_1171	Jarvis/Pheonix	P. lobata
15_1176	Jarvis/Pheonix	P. evermanni
15_1177	Jarvis/Pheonix	P. evermanni
15_1178	Jarvis/Pheonix	P. evermanni
15_1179	Jarvis/Pheonix	P. evermanni
15_1180	Jarvis/Pheonix	P. evermanni
15_1181	Jarvis/Pheonix	P. evermanni
15_1182	Jarvis/Pheonix	P. evermanni
15_1183	Jarvis/Pheonix	P. evermanni
15_1188	Jarvis/Pheonix	P. lobata
15_1189	Jarvis/Pheonix	P. lobata
15_1190	Jarvis/Pheonix	P. lobata
15_1202	Jarvis/Pheonix	P. lobata
15_1203	Jarvis/Pheonix	P. lobata
15_1204	Jarvis/Pheonix	P. lobata
15_1216	Jarvis/Pheonix	P. lobata
15_1217	Jarvis/Pheonix	P. lobata
15_1218	Jarvis/Pheonix	P. lobata
15_1229	Jarvis/Pheonix	P. lobata
15_1230	Jarvis/Pheonix	P. lobata
15_1231	Jarvis/Pheonix	P. lobata
15_1233	Jarvis/Pheonix	P. lobata
15_1234	Jarvis/Pheonix	P. lobata
15_1235	Jarvis/Pheonix	P. lobata
15_1246	Jarvis/Pheonix	P. lobata
15_1247	Jarvis/Pheonix	P. lobata
15_1248	Jarvis/Pheonix	P. lobata
15_1257	Jarvis/Pheonix	Porites spp.
15_1258	Jarvis/Pheonix	Porites spp.
15_1259	Jarvis/Pheonix	Porites spp.
15_1260	Jarvis/Pheonix	Porites spp.
15_1269	Jarvis/Pheonix	P. lobata
15_1270	Jarvis/Pheonix	P. lobata
15_1271	Jarvis/Pheonix	P. lobata
15_1272	Jarvis/Pheonix	P. lobata
15_1273	Jarvis/Pheonix	P. lobata
15_1276	Jarvis/Pheonix	P. evermanni
15_1277	Jarvis/Pheonix	P. evermanni
15_1278	Jarvis/Pheonix	P. evermanni
15_1279	Jarvis/Pheonix	P. evermanni
15_1280	Jarvis/Pheonix	P. evermanni
15_1298	Jarvis/Pheonix	
15_1300	Jarvis/Pheonix	
17_1001	Palau	
17_1003	Palau	
17_1005	Palau	P. lobata
17_1007	Palau	P. lobata
17_1009	Palau	P. lobata
17_1011	Palau	P. lobata
17_1013	Palau	P. lobata
17_1015	Palau	P. lobata
17_1017	Palau	P. lobata
17_1019	Palau	P. lobata
17_1021	Palau	P. lobata
17_1023	Palau	P. lobata
17_1056	Palau	P. lobata
17_1060	Palau	P. lobata
17_1064	Palau	P. lobata
17_1066	Palau	P. lobata
17_1068	Palau	P. lobata
17_1071	Palau	P. lobata
17_1074	Palau	P. lobata
17_1076	Palau	P. lobata
17_1077	Palau	P. lobata
17_1080	Palau	P. lobata
17_1082	Palau	P. lobata

Table 3. AMOVA Results

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AMOVA design and results :
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Reference: Weir, B.S. and Cockerham, C.C. 1984.
           Excoffier, L., Smouse, P., and Quattro, J. 1992.
           Weir, B. S., 1996.
```

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	2	54.935	1.14178 Va	18.29
Within populations	104	530.523	5.10119 Vb	81.71
Total	106	585.458	6.24297	

Fixation Index FST : 0.18289

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Population specific FST indices
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Pop#	Name	FST
1	Palau	0.14281
2	Jarvis_Phoenix	0.19753
3	Galapagos	0.04372

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Table 4. Pairwise Haplotype Diversity Results with p-values

FST Values	West	Central	East
West	0		
Central	0.04	0	
East	0.37	0.73	0

P-values	West	Central	East
West	*		
Central	0.02±0.01	*	
East	0.10±0.03	0.00±0.00	*

Table 5. Tajima's D Test Results

Tajima's D Test	East	Central	West	Mean
Sample Size	5	76	23	34.67
S	86.00	105.00	141.00	116.75
Pi	86.00	6.52	20.24	48.77
Tajima's D	0.00	-2.33	-1.89	-0.75
p-value	1.00	0.00	0.02	0.47

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Academic Vita of Tanner Quiggle

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EDUCATION

The Pennsylvania State University, University Park, PA
Expected Graduation: May, 2019
Schreyer Honors Scholar
Bachelor of Science, Biology
Bachelor of Arts, Spanish

IES Abroad Center, Madrid, Spain
15 credits, 5 classes
Courses taught by professors from La Universidad Complutense de Madrid

WORK EXPERIENCE

Cranberry Township Engineering Department - Intern (May 2016 - Aug 2016)

- Logged and mapped MS4 outfalls for Stormwater Management Program
- Performed a traffic study to improve stoplight efficiency during peak traffic hours
- Located Best Management Practices to ensure proper maintenance was performed
- Exposed to the planning process for new township development projects

UPMC Hillman Cancer Center Radiation Oncology Department - Researcher (May 2017– Aug 2017)

- Investigated the effectiveness of low-level laser therapy for head and neck cancer patients
- Organized a cohort of 31 patients using data collected from clinical trials
- Compared the experimental group to a control using variables such as quality of life scores, toxicity assessments, and pain scales
- Submitted an abstract that is being reviewed for publication

Asociación de Celíacos y Sensibles al Gluten - Research Intern (Sept 2017- Dec 2017)

- Created and maintained a patient database with over 1,500 patients
- Organized excel spreadsheets of all patients with documented diagnostic test results
- Published an article, written in Spanish, in the March 2018 edition of "Sin Gluten" magazine on Irritable Bowel Syndrome
- Attended an accredited class about the diagnosis of gluten sensitivity diseases
- Conducted all conversations in Spanish, formal and informal

RESEARCH EXPERIENCE

Brain, Language, and Computation Lab - Undergraduate Research Assistant (Jan 2016 - Present)

- Study the Effects of Native Language on Cognitive Processes
- Help develop a series of texts to stimulate the lingual part of the subject's brain
- Analyze fMRI scans of the subjects to determine localized brain activity
- Use Python coding to obtain pure data and results
- Role expanded to include additional research independence

Coral Sustainability and Evolution Lab - Undergraduate Research Assistant (Sept 2016 - Present)

- Study ocean acidification, coral bleaching, and genetic diversity in the Pacific Ocean
- Working on a Thesis involving the genetic diversity of mussels across the Pacific Ocean
- Perform techniques such as PCR, gel electrophoresis, and gene sequencing to obtain results
- Managing the pH, salinity, temperature, CO_2 levels, and cleanliness of two aquatic systems
- Observe how the symbionts living within the coral react to CO_2 treatments
- Feed, rotate, and clean corals on a weekly basis

PRESENTATIONS AND PUBLICATIONS

Publications

- "Síndrome del Intestino Irritable: Una Enfermedad, Causas Multiples" (Quiggle, 2018)
    - Article in "Sin Gluten", a Spanish scientific magazine

Acknowledgements

- "Speaking two Languages in America: A semantic space analysis of how presidential candidates and their supporters represent abstract political concepts differently" (Li, Schloss, and Follmer, 2017)

VOLUNTEER EXPERIENCE

Miracle League of Southwestern PA - Player Buddy (Apr 2009 - Present)

- Instruct children with disabilities on how to play baseball
- Play with the same child since I first joined (9 years total)

Scholars Helping Scholars Program - Member (Sept 2015 - Present)

- Participate in a pilot program to assist scholars with mental health issues

Real Colegio Nuestra Señora De Loreto - Volunteer English Teacher (Sept 2017 - Dec 2017)

- Taught English to 1st and 4th grade students
- Lectured in Spanish to 1st graders and in English to 4th graders
- Combined lesson material with American games to create a new learning experience

ACTIVITIES

NCAA Division I Varsity Track and Field/Cross Country (Aug 2015 – Aug 2016)

- Competed in Cross Country, Indoor Track and Field, and Outdoor Track and Field
- Member of a Top-25 nationally ranked team
- Averaged 80 miles of running in 25 hours of practice time per week
- Participated in outreach programs for the American Cancer Society and American Red Cross

Penn State Club Quidditch Team (Sept 2016 – Present)

- Team Captain
- Fundraising Chairperson
- Participate in every official tournament since joining the team
- Travel to compete in Regional and National Championships

HONORS, AWARDS, AND RESEARCH GRANTS

- Eberly College of Science Research Grant
    - Awarded $1,000 to support Honors Thesis on Genetic Diversity of *Lithophaga* mussels in the Pacific Ocean
- Winner of the Best Design Process Award for the Freshman EDSGN 100 Project, Spring 2016

    - A campus-wide engineering competition involving all freshman engineers
- High School Valedictorian (#1 out of 581 students)
- Golden Key International Honor Society
- Alpha Epsilon Delta (Health Pre-Professional Honor Society)
- Phi Eta Sigma (Freshman Academic Honor Society)