Population Genetics of Gastropods at Hydrothermal Vents at the Mata Volcanoes

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## Abstract

Gastropods *Ifremeria nautilei* and *Alviniconcha boucheti* were collected at deep-sea hydrothermal vents near Tonga with the goal of developing a better understanding of the gene flow between vent communities. Mitochondrial DNA was extracted from multiple samples and a fragment of the cytochrome oxidase I (COI) mitochondrial gene was amplified using different primers. Data obtained from sequencing was used to form a phylogenetic tree verifying that the *I. nautilei* and *A. boucheti* samples obtained were the same species as those previously sequenced. Results from AMOVA and nested clade analyses showed that there was no significant genetic structure of the gastropod populations in regard to depth, vent location, and Northern vs. Southern populations. These results support the hypothesis that there is high gene flow between hydrothermal vent communities. Further research could be done with microsatellites and a larger sample size in order to further investigate the results obtained in this study.

## Introduction

Hydrothermal vents lie thousands of meters below the surface of the ocean with no light penetration but are teeming with life. The vents release chemicals and minerals that chemosynthetic bacteria utilize to form the basis for most life found around the vents. Both *Ifremeria nautlilei* and *Alviniconcha boucheti* live in symbiotic relationships with chemosynthetic bacteria. The gastropods provide the bacteria with a place to live and in return, the chemosynthetic bacteria give the gastropods the nutrition they need to survive (Thaler et al., 2010). Due to this, similar symbiotic relationships are observed in almost every active vent ecological community (Amend, 2004).

Hydrothermal vents appear and disappear relatively frequently, which means that for species to survive, they must have a wide larval dispersal. *I. nautilei* has a distinct larval form known as Warén's larva that is free-swimming, which allows the larvae to travel to new hydrothermal vents (Reynolds et al., 2010). It is unknown what type of larvae the *Alviniconcha* genus produces, but they are thought to be planktotrophic larvae (Warén and Bouchet, 1993). These planktotrophic larvae, which must feed on plankton within their immediate vicinity after being released, are able to travel long distances. The *Alviniconcha* genus's planktotrophic larvae allow the young snails to disperse to other hydrothermal vents, similar to *I. nautilei*. The ability of the larvae to travel long distances to other vents is what allows different populations of snails to interact.

The Mata Volcanoes are a group of underwater volcanoes that allow for the emergence of hydrothermal vents. There are nine distinct volcanoes located in the South Pacific at varying depths in the Northeast region of the Lau Basin. They range in depth from 1800 meters to 2700 meters (Clague et al., 2011). These volcanoes are all active and relatively young. Found at the Mata Volcanoes is an abundance of life, consisting of chemosynthetic bacteria and invertebrates. Shrimp and gastropods were discovered in abundance at some of the volcanoes, and of the gastropods, there were two prominent species found, the *Ifremeria nautilei* and *Alviniconcha boucheti.* Many of the organisms found at these vents are endemic to vent ecosystems. Previous studies have been done on these organisms in Manus, North Fiji, and Lau basins. Thaler et al. (2011) found no population structure between the different basins in a population genetics study utilizing mitochondrial and microsatellite markers. Additionally, two main haplotype groups were discovered within the *Ifremeria nautilei* (Thaler et al., 2011).

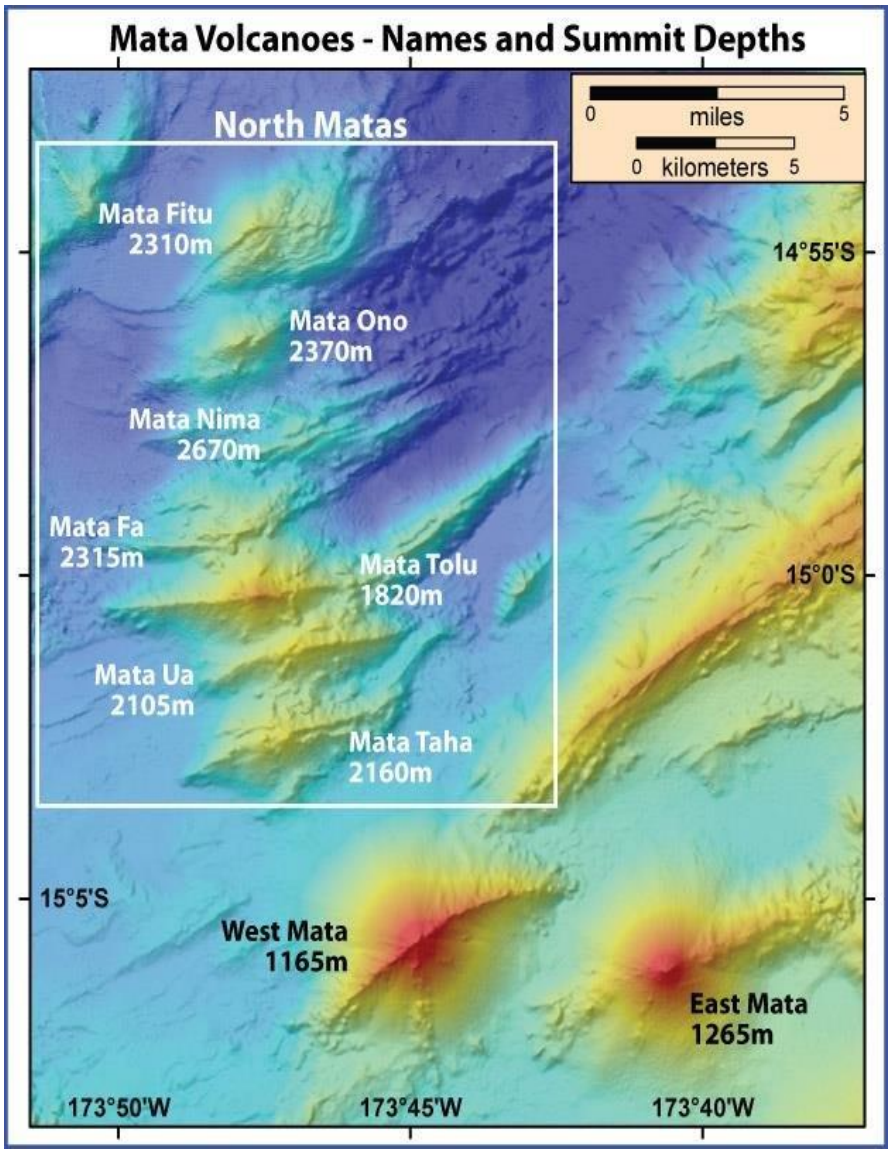


Figure 1: Volcanoes in North Mata Range

Both *A. boucheti* and *I. nautilei* are in the phylum Mollusca, class Gastropoda, family Provannidae. The family Provannidae are classified by having a conical shell that is 6-15 millimeters in length. They also have reduced eyes near the outside of the cephalic tentacles, a paired jaw, a stomach with a developed style sac and gastric shield with a simple chamber, and digestive glands that open to the stomach. The females have gonopericardial ducts while the males have an aphallate with dimorphic sperm (Warén and Ponders, 1991). The *Alviniconcha* genus has six different species identified which are *A. adamantis, A. boucheti, A hessleri, A. kojimai, A. marisindica,* and *A. strummeri* (S.B Johnson et al., 2014, Okutani and Ohta, 1988). The *Ifremeria* genus only has one identified species which is *I. nautilei* (Bouchet and Warén, 1991). *Ifrmeria* and *Alviniconcha* are endemic to hydrothermal vents, meaning that vent sites are the only places you can find these species in the world.

The mitochondrial genome is often used when looking at the population genetics of different species. Mitochondrial DNA is maternally inherited, has high mutation rates, and has a significantly shorter genome than nuclear DNA (Raupach et al., 2015). This enables the easy replication and identification of mutations in mitochondrial genes. A molecular marker often used in evolutionary studies is the cytochrome oxidase I (COI) mitochondrial gene. The gene is relatively short, which allows for easy collection and analysis. Additionally, the COI gene evolves in a relatively short amount of time, has no introns, many copies in each cell, and haploid inheritance with no recombination (Raupach et al., 2015). These factors make the COI gene an optimal molecular marker for evolutionary studies. The COI gene has been effectively used in gastropod studies to measure the relatedness between different gastropod populations at hydrothermal vents (Thaler et al., 2011 and Suzuki et al., 2006).

Microsatellites are tandem repeats of 1-6 nucleotides found in the nuclear genomes of most taxa (Selkoe and Toonen, 2006). The DNA surrounding the microsatellite locus is known as the flanking region and is usually conserved across organisms of the same species. This enables primers to be easily made to bind to the flanking region and guide in PCR. The high mutation rates of microsatellites enable the study of allelic diversity on relatively short ecological timescales (Selkoe and Toonen, 2006). This study aims to use microsatellites as an additional method of studying gene flow across populations. Using microsatellites could show more mutations than in the mitochondrial genome and provide a better understanding of the genetic diversity within vent communities.

## Materials and Methods

Sample collection:

The gastropod samples were collected on the vessel *R/V Falkor* during the 2009 expedition to Samoa and the Mata Volcanoes, FK171110: Underwater Fire. Specifically, the remotely operated vehicle (ROV) SuBastian was utilized to collect the specimens from the hydrothermal vents. Samples were collected using either a multi-chamber suction sampler or scoop nets. While at sea, the samples were preserved by being frozen directly at -80°C or stored first in 95% EtOH or RNALater and then frozen. When they arrived at the lab, samples were kept at -80°C for long-term storage.

DNA processing:

In the lab, DNA extractions were performed on the samples using the DNEasy Blood & Tissue Kit from Qiagen (Hilden, Germany), according to manufacturer's protocols with the following modifications: when eluting the DNA, 100 microliters of buffer AE was used, then the elution step was repeated with an additional 100 microliters of buffer AE. Additionally, during the final collection step, the samples were centrifuged at 13,200 rpm instead of 14,000 rpm. The DNA extractions were kept in 1.5 mL microcentrifuge tubes and stored at -20°C for short term-storage and -80°C for long term-storage. After the DNA extractions were completed, the samples were run on gel electrophoresis to check for the presence of DNA. The gel was made with 1.5% agarose gel, 1X TBE buffer, and ethidium bromide. The concentrations of the samples were checked with a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, Waltham, MA).

The extractions were first diluted to a concentration of 10-12 ng/µL using MilliQ water. Molecular markers were amplified using Polymerase Chain Reaction (PCR), which contained 5 µL of 5X PCR Buffer, 2.5 mM of MgCl2, 0.5 µM of each primer, 0.2 mM of DNTP, 2 µL of extracted DNA template, and 0.2 µL of Taq polymerase. MilliQ water was added to bring the final concentration of each reaction to 25 µL. A fragment of the COI mitochondrial gene from *I. nautilei* and *A. boucheti* samples was amplified with PCR using jgHCO/LCO (Geller et al., 2013) and COIB/6 (Suzuki et al., 2006) primers. Additionally, the *Alviniconcha* sp. Samples

were amplified using HCO/LCO (Folmer, 1994) and dgHCO/LCO (Meyer, 2003) primers. Specific PCR conditions are listed in Table 1. After the PCR, the samples were run through 1.5% agarose gels in gel electrophoresis to check for the presence of DNA, and bands were ranked on a scale of bright, medium, dull, or no band. If there was a band present, the sample was purified using the Wizard PCR Preps DNA Purification System (Promega, Madison, WI), according to the manufacturer's protocol. The QIAquick PCR Purification kit (Qiagen, Hilden, Germany) was also used to purify the PCR products, according to the manufacturer's protocols.

After the PCR products were purified, they were run through gel electrophoresis to check for the presence of a band, indicating a sufficient amount of DNA to sequence. The purified PCR products that contained a band were sent out for bi-directional Sanger sequencing (Eurofins Genomics, Louisville, KY).

Table 1: PCR Conditions for Mitochondrial Primers

| Forward Primer | Reverse Primer | Initial denaturation step | Denaturation step | Annealing step | Extension step | # of cycles | Final extension | |
|---|---|---|---|---|---|---|---|---|
| HCO | LCO | 95°C for 2:00 | 94°C for 0:45 | 42°C for 0:45 | 72°C for 1:30 | Repeat 2-4 35x | 72°C for 5:00 | End |
| jgHCO | jgLCO | 94°C for 2:00 | 94°C for 1:00 | 48°C for 1:00 | 72°C for 1:00 | Repeat 2-4 30x | 72°C for 5:00 | End |
| dgHCO | dgLCO | 95°C for 2:00 | 94°C for 0:45 | 40°C for 0:45 | 72°C for 1:30 | Repeat 2-4 35x | 72°C for 5:00 | End |
| COIB | COI6 | 94°C for 1:00 | 92°C for 0:40 | 50°C for 1:00 | 72°C for 1:30 | Repeat 2-4 30x | | End |

Genetic analysis:

The sequencing results were received from Eurofins as chromatogram files and downloaded into CodonCode Aligner (CodonCode Corporation, Centerville, MA) where messy ends were trimmed, ambiguous base calls were determined by eye, and forward and reverse sequence strands of the same sample were formed into contigs. These contigs were then aligned with CLUSTAL. Sequences were translated into amino acid sequences to confirm the absence of

stop codons and pseudogenes. The completed sequences were checked against sequences in GenBank using a BLAST search to check for highly similar sequences already present in the database. A new alignment was then created that included the matching BLAST results and representative outgroups. These were then exported as a FASTA file. New alignments were made in MEGA11 (Tamura and Stecher, 2021) and alignments were made using MUSCLE within MEGA. MEGA11 was then used to create a Neighbor-Joining tree using the Kimura 2-P model of evolution and 500 bootstraps. The tree was then rooted from the outgroup. The distance between group means was computed on MEGA by splitting the samples into groups based on the phylogenetic tree. The between group mean distance was calculated to ensure that each of the different groups was the same species. A value that is under 6.98% indicates that they are the same species (Borges et al., 2016).

In order to determine whether there was any genetic structure between the gastropod populations collected at different hydrothermal vents, haplotype networks were created using TCS 1.21 (Clement et al., 2000). The haplotype networks were then modified using TCS-Beautifier (dos Santos et al., 2016) to make the size of the haplotypes proportional to frequency and indicate geographic location by color. A nested clade analysis was performed using ANeCA 1.2 (Panchal, 2007). Analysis of molecular variance (AMOVA) testing for population structure was also performed using Arlequin 3.5 (Excoffier and Lischer, 2010). The following comparisons were made: by geography where populations were organized by volcano and compared against each other, by northern vs. southern populations, and by depth (1800, 2300, and 2600 meter intervals). A mantel test was also performed to test for isolation by distance (Excoffier and Lischer, 2010).

Microsatellites:

Six different primer pairs were obtained from ThermoFisher Scientific (Waltham, MA) for microsatellite markers identified by Thaler et al. (2010). Three primers (Ifr78, Ifr94, and Ifr 103) were chosen based on their ability to cross amplify with *A. boucheti*. The remaining three primers (Ifr 40, Ifr43, and Ifr 93) were utilized due to their allele size ranges (Thaler et al., 2010). The primers were mapped on Multiplex Manager 1.2 (Holleley and Geerts, 2018) for the

identification of appropriate primer pair combinations. Once obtained, the labeled primers were wrapped in tinfoil and the entire package was placed in a -20 °C freezer.

Primer stocks were made with the unlabeled primers in order to determine if the sequences would amplify and to determine the best conditions for amplification in a multiplex PCR. A gradient PCR was performed to determine the best annealing temperature for *I. nautilei* (61℃) and *A. boucheti* (53℃). After confirming that the unlabeled primers worked for both species, the labeled primers were utilized. Molecular markers were amplified in multiple PCR's using 12.5 μL of 2X Type-It Multiplex PCR Master Mix, 2.5 μL of 10X primer mix (2 μM of each primer), 2 μL of template DNA, and 8 μL of RNase free water. Specific PCR conditions are listed in Tables 2 and 3. The products of all Multiplex PCR reactions with labeled primers were stored in a container covered with tinfoil at -20℃.

Table 2: Multiplex PCR conditions for microsatellite marker amplification for *I. nautilei.*

| Initial denaturation step | Denaturation step | Annealing step | Extension step | # of cycles | Final extension | Initial denaturation step |
|---|---|---|---|---|---|---|
| 95℃ for 5:00 | 95℃ for 0:30 | 61℃ for 1:30 | 72℃ for 0:30 | Repeat 2-4 30x | 60℃ for 30:00 | End |

Table 3: Multiplex PCR conditions for microsatellite marker amplification for *A. boucheti*

| Initial denaturation step | Denaturation step | Annealing step | Extension step | # of cycles | Final extension | Initial denaturation step |
|---|---|---|---|---|---|---|
| 95℃ for 5:00 | 95℃ for 0:30 | 53℃ for 1:30 | 72℃ for 0:30 | Repeat 2-4 24x | 60℃ for 30:00 | End |

**Results**

A total of 17 *A. boucheti* and 57 *I. nautilei* samples were used in the present study. *A. boucheti* samples were collected from 4 sites ranging in depths of 1813-2360 m. *I. nautilei* samples were collected from 7 sites ranging in depths of 1813-2623 m (Table 4 and Figure 2).

Table 4: Metadata for *A. boucheti and I. nautilei* samples used in this study.

| **Site** | **Dive** | **Station** | **Latitude_South** | **Longitude_West** | **Depth (m)** | ***A. boucheti* Samples** | ***I. nautilei* Samples** |
|---|---|---|---|---|---|---|---|
| Mata Ua | S089 | 25 m NE of WP3 | -15.01773560 | -173.78851680 | 2359 | 4 | 24 |
| Mata Tolu | S091 | Low Smoker | -15.00463940 | -173.79273930 | 1823 | 0 | 7 |
| Mata Tolu | S094 | Snail Alcove | -15.00710620 | -173.79273300 | 1813 | 5 | 9 |
| Mata Fitu | S097 | at Eiffel Tower | -14.91347600 | -173.77908400 | 2623 | 0 | 2 |
| Mata Ua | S100 | Big Smoke | -15.01675310 | -173.78596890 | 2318 | 5 | 2 |
| Mata Ono | S101 | Dark Castle Vent | -14.94057800 | -173.79955800 | 2360 | 0 | 6 |
| Mata Ono | S102 | Rock Star | -14.94053600 | -173.79937500 | 2360 | 3 | 7 |

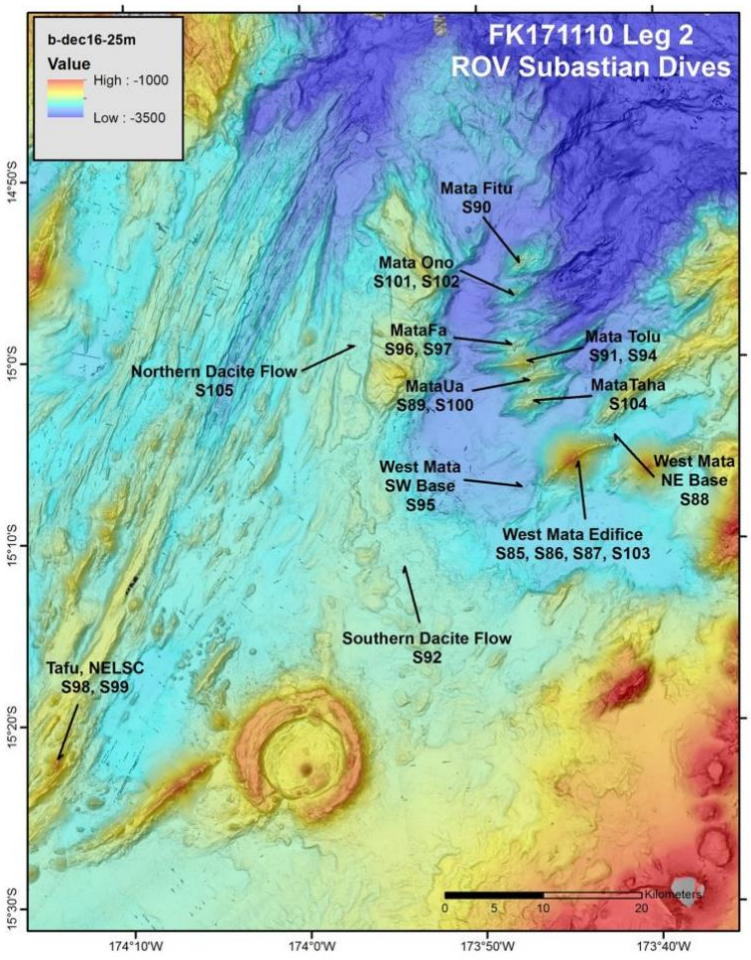


Figure 2: Map showing the location of each ROV dive where samples used in this study were collected.

*A. boucheti* analyses

The phylogenetic analysis confirmed that 16 samples were *A. boucheti*, but also revealed that one of the specimens is *Alviniconcha kojimai* (Figure 3). Table 5 confirms that the individuals within the different clades are the same species as the levels of sequence divergence are under 6.98%. The tree does not appear to show any pattern by dive or location in which some samples are more closely related. An alignment of COI sequences was created 1214 bp in length for *A. boucheti* and 1073 bp in length for *I. nautilei.*

A haplotype network was also created with haplotypes shared across the different volcanoes (Figure 4). The nested clade analysis found no significant results for this cladogram.

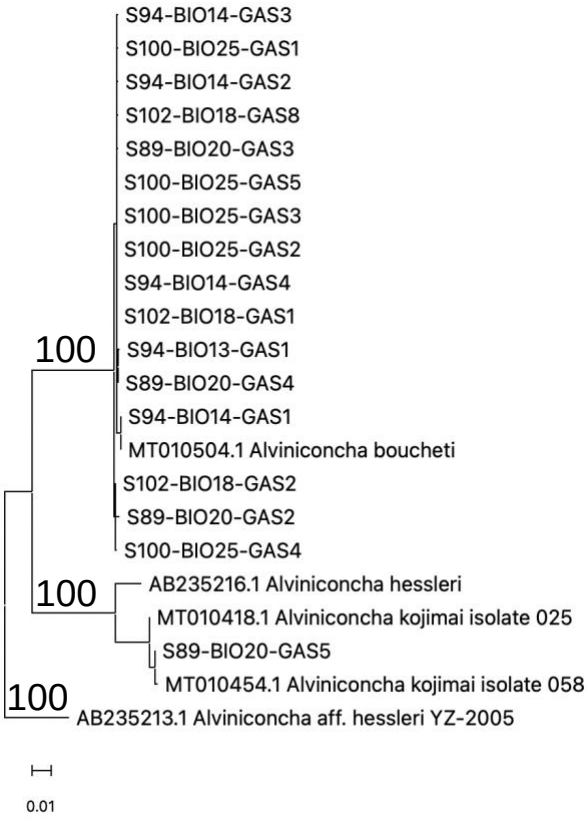


Figure 3: Neighbor-joining phylogenetic tree for *Alviniconcha* sp. samples used in this study. *A. hessleri* was used as the outgroup. The tree with the bootstrap values shows the support for each node and how likely they are to be grouped in the same way again with 100 being confident in placement.

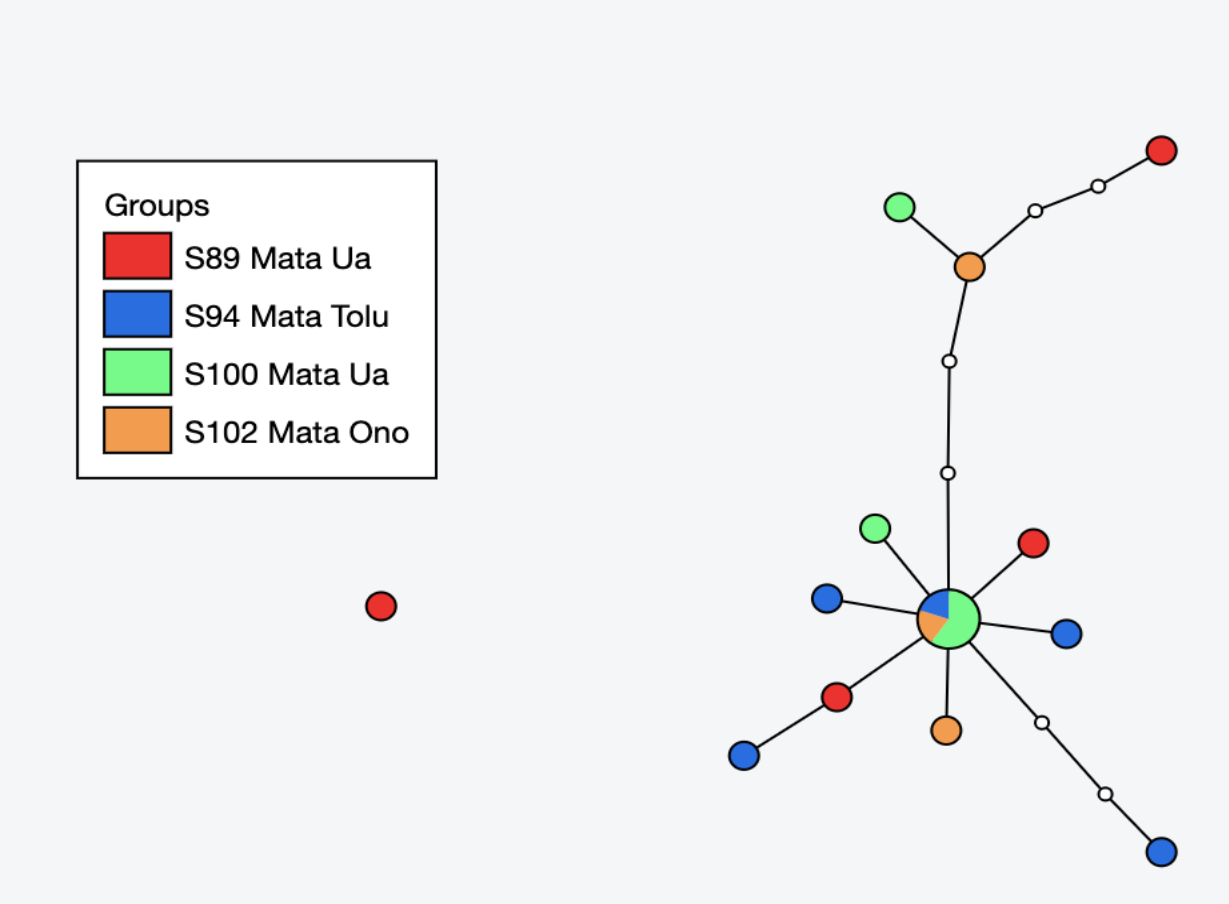


Figure 4: Haplotype Network for *Alviniconcha* sp. samples. The colors of the nodes indicate the dive/volcano and the size of the circles is proportional to the number of individuals within each given haplotype. Each step represents a single mutation. The ancestral node is depicted by the largest circle. The disconnected red sample is the *A. kojimai* sample.

Table 5: Within mean group distance for *Alviniconcha* sp. A value under 0.0698 indicates the samples in each group are the same species.

| Groups | distance |
|---|---|
| *A. boucheti* | 0.00 |
| *A. kojimai* | 0.02 |
| Outgroup | n/c |

The AMOVA was performed for the *A. boucheti* samples collected at different volcanoes [Mata Ua (S89 and S100) versus Mata Tolu (S94) versus Mata Ono (S102)], depths [1813 m (S94) versus >2320 m (S89, S100, S102)], and Northern vs. Southern volcanoes [Northern (S102) versus Southern (S89, S94, S100)]. All comparisons had p-values greater than 0.05, so the null hypothesis that there is no significant genetic structure between the different populations and samples compared is accepted (Table 6). In Table 7 the results from Tajima's D neutrality test are shown and all of the P-values are greater than 0.05, which leads to the acceptance of the null hypothesis and that there is no significant change in the population. However, in the second trial of Tajima's D test, the P-values for the S94 and S100 populations are approaching significance, meaning that those populations may be under selection.

Table 6: Results from the AMOVA and Mantel test between separate volcanoes, depth, and North vs. South for *A. boucheti*. The P-values are shown and if $p < 0.05$, then the null hypothesis is rejected.

| Populations Tested | Among Groups | Among Populations Within Groups | Within Groups | Mantel Test |
|---|---|---|---|---|
| S89 and S100 vs. S94 vs. S102 (Volcanoes) | 0.68035 | 0.13490 | 0.26979 | 0.28446 |
| S94 vs. S89, S100 and S102 (1800 m vs 2300 m) | 0.33627 | 0.12805 | 0.30205 | 0.28055 |
| S102 vs. S89, S94 and S100 (N vs. S) | 0.28250 | 0.05963 | 1.0000 | 0.29130 |

Table 7: Results from Tajima's D test of neutrality P-values. When $p > 0.05$, the null hypothesis that there is no significant change in the populations is accepted.

| | S89 | S94 | S100 | S102 | Mean |
|---|---|---|---|---|---|
| Tajima's D P-value | 0.18000 | 0.04800 | 0.06800 | 1.00000 | 0.32400 |

*Ifremeria nautilei* analyses

The phylogenetic tree for the *I. nautilei* (Figure 5) reveals that all the samples are confirmed to be *I. nautilei*. They are all genetically similar, with sequence divergences under the 6.98% threshold typical for gastropod species (Borges et al. 2016), while the *A. hessleri* outgroup is more distant and genetically different. There is also the haplotype network for the *I. nautilei* (Figure 6) that was created, which shows the samples stemming from one center. The haplotypes do not cluster geographically, indicating that there is high gene flow and the haplotypes are dispersed throughout the different locations. Nested clade analysis found no significant results for this cladogram.

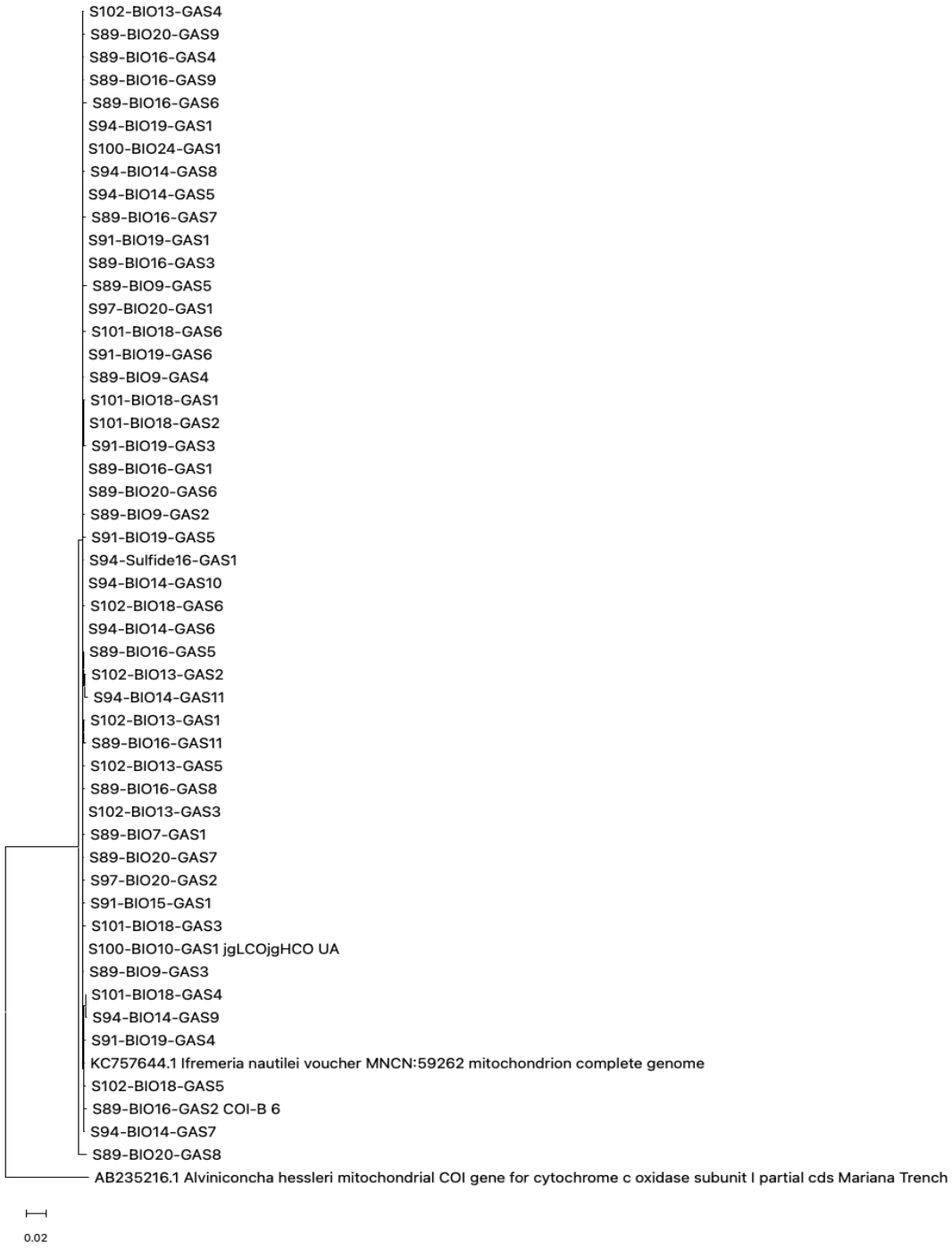


Figure 5: Neighbor-joining phylogenetic tree for *I. nautilei* samples used in this study with *Alviniconhca* as an outgroup.

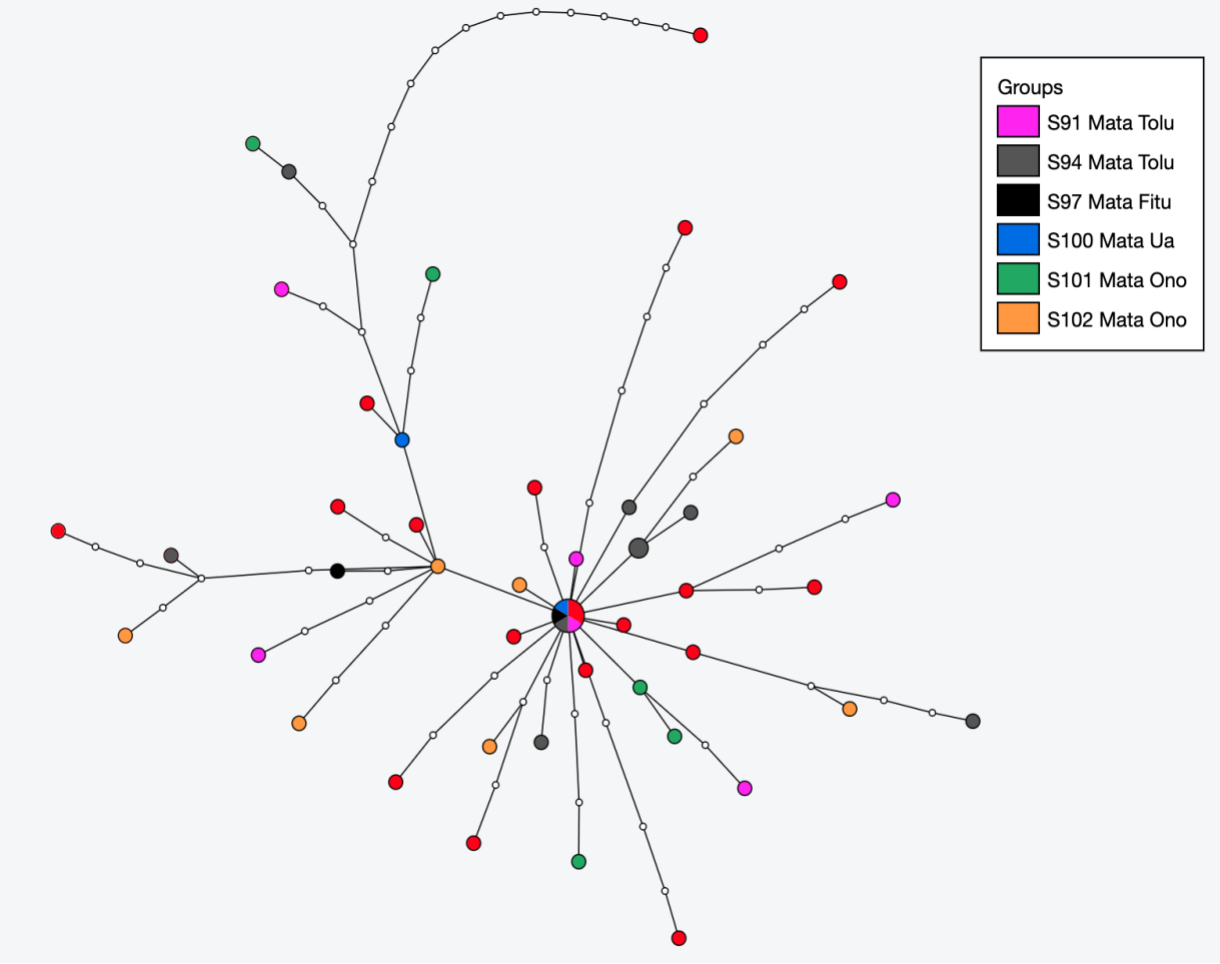


Figure 6: Haplotype network for *I. nautilei* samples with the colors of the nodes representing the dive/site and the size of the circles is proportional to the number of individuals within each given haplotype. Each step represents a single mutation. The ancestral node is depicted by the largest circle.

An AMOVA was run between the *I. nautilei* samples at different volcanoes, different depths, and Northern vs. Southern volcanoes (Table 8). The AMOVA revealed no significant difference between the samples in any of the comparisons as $p>0.05$. The mantel test (Table 8) that assesses isolation by distance was also not significant because $p>0.05$. Tajima's D neutrality test (Table 9) was performed on the *I. nautilei* samples. Only the S89 dive/Mata Ua population had a significant P-value below 0.05, indicating that the population may be under selection.

Table 8: Results from the AMOVA and Mantel test between separate volcanoes, depth, and North vs. South for *I. nautilei*. The P-values are shown and if $P < 0.05$ then the null hypothesis is rejected.

| Populations Tested | Among Groups | Among Populations Within Groups | Within Groups | Mantel Test |
|---|---|---|---|---|
| S97 vs. S101 and S102 vs. S91 and S94 vs. S89 and S100 | 0.69110 | 0.92962 | 0.99902 | 0.294000 |
| S97 vs. S89, S100, S101 and S102 vs. S91 and S94 | 0.81036 | 0.96872 | 0.99609 | 0.298000 |
| S97, S101, and S102 vs. S89, S91, S94, and S100 (N vs. S) | .99511 | .98827 | .76540 | 0.285000 |

Table 9: Results from Tajima's D test of neutrality P-values. When $P > 0.05$, the null hypothesis that there is no significant change in the populations is accepted.

| | S89 | S91 | S94 | S97 | S100 | S101 | S102 | Mean |
|---|---|---|---|---|---|---|---|---|
| Tajima's D P-value | 0.00000 | 0.08000 | 0.06600 | 1.00000 | 1.0000 | 0.4820 | 0.12500 | 0.39329 |

Microsatellites:

The primers were tested to ensure that they work. For the *I. nautilei* samples, both Primer Stock One (Ifr40, Ifr43, Ifr93, and Ifr94) and Primer Stock Two (Ifr78 and Ifr103) amplified the appropriate number of bands in the expected base-pair length regions (Ifr40: 200-220bp, Ifr43:

160-200 bp, Ifr93: 250-275bp, Ifr94: 260-270bp) (Figure 7). For *A. boucheti*, no samples were amplified using Primer Stock One but both primers from Primer Stock Two successfully amplified the appropriate number of bands in the expected base-pair length regions (Ifr78: 240-250bp and Ifr103: 225-360bp) (Figure 8).

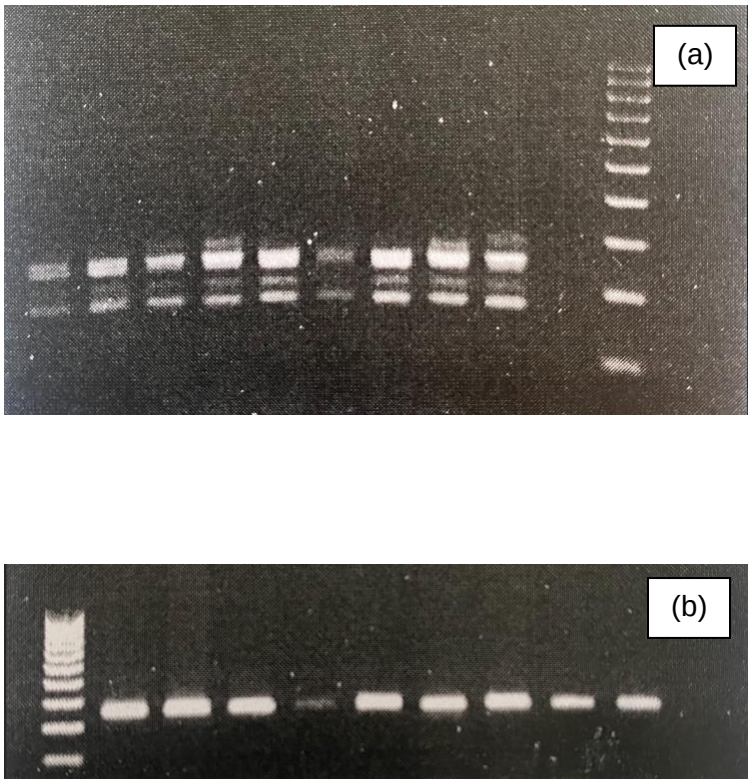


Figure 7: Images of multiplex PCR products from *I. nautilei* samples of Primer Stock One (a) and Primer Stock Two (b) showing bands from each of the primers. Four distinct bands can be seen in the top gel indicating that each primer amplified the DNA successfully. Minor separation of two bands can be seen in the bottom gel. A 100bp ladder is included in the right-most lane.

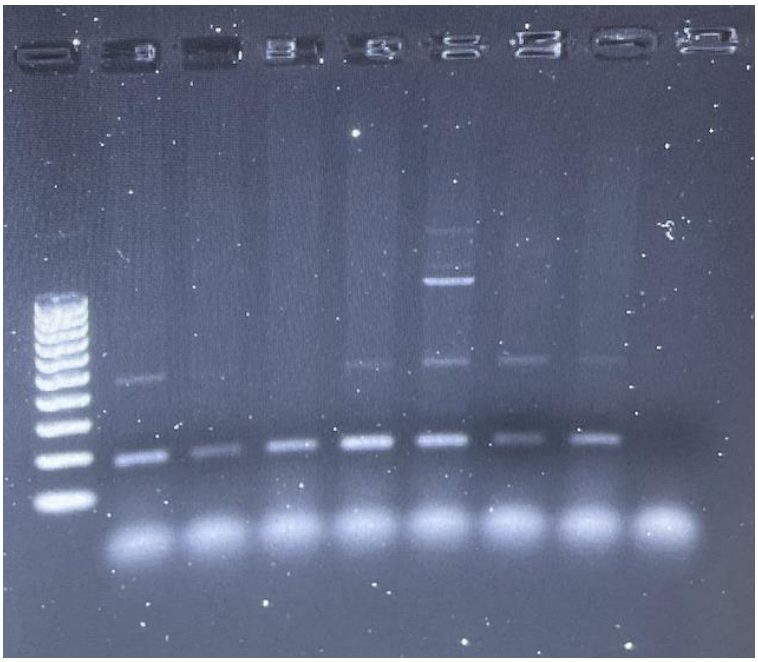

Figure 8: Images of gels from PCR products of *A. boucheti* samples with Primer Stock Two showing bands from each of the primers. A 100bp ladder is included in the left-most lane.

**Discussion**

This study confirmed the identity of the two main species of gastropods found at the hydrothermal vents at the Mata Volcanoes as *I. nautilei* and *A. boucheti*. There were fifty-seven *I. nautilei* samples, sixteen *A. boucheti* samples, and one *A. kojimai* sample in this study. All population genetic analyses revealed that there is no significant population structure between any of the gastropod populations, including comparisons of populations by volcanoes, depth, and northern vs. southern populations (Tables 6 and 8). These results are not surprising due to the ephemeral nature of hydrothermal vents and how frequently they can appear and disappear, making it necessary for populations of vent-associated fauna to have larvae capable of wide dispersal in order to survive.

The Tajima's D test of neutrality measures genetic diversity and can reveal whether populations are currently under selection. The Tajima's D test revealed that for the *A. boucheti*, there were no populations under selection. However, the Mata Tolu population sampled at the "Snail Alcove" station of dive S94 had a p-value of 0.04800 (Table 7), which is significant,

indicating that this population is currently undergoing selection. For the *I. nautilei*, the Tajima's D value for the same S94 population is approaching significance, indicating that the population may be under selection (Table 9). Similarly, the *I. nautilei* samples collected from that same S94 dive had a p-value of 0.06600, indicating that this population may be undergoing selection as well. This reveals that there may have been an event at this volcano that led to the gastropod populations undergoing selection. The *A. boucheti* samples from the "Big Smoke" station of dive S100 had a p-value of 0.06800, which is approaching significance indicating that the population may be undergoing selection as well. For the *I. nautilei* the samples collected from the S89 dive at the station "25 m NE of WP3" had a p-value of 0.00000, indicating that the population is undergoing selection. The *I. nautilei* samples from the S91 dive at the "Lower Smoke" station had a p-value of 0.08000, indicating that this population may be undergoing selection.

The phylogenetic analysis for the *Alviniconcha sp.* (Figure 3) helped identify one of the samples as an *A. kojimai* and confirmed that the rest were *A. boucheti*. There was no significant result from the nested clade analysis, indicating that there is high gene flow between different volcanoes and little genetic structure within these populations. This can be seen in the haplotype network (Figure 4) as the samples from the same dives/volcanoes, indicated by color, are not clustered together. This shows that the common haplotypes are spread throughout the different locations.

The *I. nautilei* phylogenetic analysis (Figure 5) confirms that all the collected samples were *I. nautilei*. Similar to the *A. boucheti*, there was no significant result from the nested clade analysis, indicating that there is high gene flow between the different populations. The haplotype network (Figure 6) also shows that the samples collected from the same dive/volcano are not clustered together. This indicates high gene flow within populations, similar to the results obtained from the *Alviniconcha* sp. haplotype network. However, the *I. nautilei* haplotype network is much more reticulated and shows haplotypes that have many more mutations than that seen in the *Alviniconcha* sp. haplotype network. This may indicate that the *I. nautilei* populations may have diversified more than the *A. boucheti* populations. However, this may be a sampling artifact as there were more than three times as many samples of *I. nautilei* as there were *A. boucheti*.

The result of high gene flow and little population structure is similar to the data obtained in previous studies. Thaler et al. (2011) found a similar pattern of high gene flow in populations

of *I. nautilei* within the Manus Basin of the West Pacific. Additionally, no genetic differentiation was observed between *I. nautilei* samples collected from the North Fiji and Lau Basins. A study performed by Beedessee et al. (2013) in the Central Indian ridge revealed no genetic differentiation between vent endemic organisms *A. rodriguezensis*, *R. kairei*, *Alviniconcha sp.* type 3, and scaly-foot gastropod. The study also found that high connectivity was observed in slow and intermediate (<60 mm/year) spreading ridge systems. On the other hand, slow and ultraslow-spreading vent systems had higher variability in chemical and physical vent activities which provided increased niches for vent organisms (Beedessee et al., 2013).

Regarding microsatellites, it has been confirmed that the chosen primers work as expected. The data obtained through microsatellite fragment analysis may help to reveal genetic structure that could not be seen through the mitochondrial analyses performed in this study.

Future studies would include using the higher resolution microsatellite markers to further study the population structure of these communities. In addition, sampling size may have been a limiting factor in this study and more samples may be needed in order to obtain a more accurate understanding of gene flow within these hydrothermal vent communities. The data collected in this study, especially with the *A. boucheti*, may not be as accurate due to the low number of samples. Increasing the sample size would result in a better representation of each of the populations.

The work done in this study is important in understanding the complex relationships that exist within hydrothermal vent communities. In recent years, there has been increased interest in mining the area around vents for precious metals (Thaler et al., 2011). The genetic data acquired from organisms on these vents can be used to determine if mining or future eruptions have the opportunity to destroy unique species. Although this study concluded high gene flow between vents, it is important to gather more data from different sites before commercial mining operations commence.

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References


Amend, Jan P., et al. “Microbially Mediated Sulfur-Redox: Energetics in Marine Hydrothermal Vent Systems.” *Sulfur Biogeochemistry - Past and Present*, vol. 379, 1 Jan. 2004, doi:10.1130/0-8137-2379-5.17.

Beedessee, G. et al. "High Connectivity of Animal Populations in Deep-Sea Hydrothermal Vent Fields in the Central Indian Ridge Relevant to Its Geological Setting." *PLoS One*, vol. 8, no. 12, 2013, p. e81570, doi:10.1371/journal.pone.0081570.

Borges, Luísa M., et al. “With a Little Help from DNA Barcoding: Investigating the Diversity of Gastropoda from the Portuguese Coast.” *Scientific Reports*, vol. 6, no. 1, 15 Feb. 2016, doi:10.1038/srep20226.

Clague, David A., et al. “Volcanic Morphology of West Mata Volcano, Ne Lau Basin, Based on High-Resolution Bathymetry and Depth Changes.” *Geochemistry, Geophysics, Geosystems*, vol. 12, no. 11, 12 Nov. 2011, doi:10.1029/2011gc003791.

Clement, M., et al. “TCS: A Computer Program to Estimate Gene Genealogies.” *Molecular Ecology*, vol. 9, no. 10, Oct. 2000, pp. 1657–1659., doi:10.1046/j.1365-294x.2000.01020.x.

Excoffier, L. and H. E. Lischer. "Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows." *Mol Ecol Resour*, vol. 10, no. 3, 2010, pp. 564-7, doi:10.1111/j.1755-0998.2010.02847.x.

Folmer, O. et al. "Dna Primers for Amplification of Mitochondrial Cytochrome C Oxidase Subunit I from Diverse Metazoan Invertebrates." *Molecular marine biology and biotechnology*, vol. 3, no. 5, 1994, pp. 294-99, MEDLINE with Full Text.

Geller, J. et al. "Redesign of Pcr Primers for Mitochondrial Cytochrome C Oxidase Subunit I for Marine Invertebrates and Application in All-Taxa Biotic Surveys." *Mol Ecol Resour*, vol. 13, no. 5, 2013, pp. 851-61, doi:10.1111/1755-0998.12138.

Holleley, C. E. and P. G. Geerts. "Multiplex Manager 1.0: A Cross-Platform Computer Program That Plans and Optimizes Multiplex Pcr." *Biotechniques*, vol. 46, no. 7, 2009, pp. 511-7, doi:10.2144/000113156.

Johnson, Shannon B., et al. “Molecular Taxonomy and Naming of Five Cryptic Species Of Alviniconcha Snails (Gastropoda: Abyssochrysoidea) from Hydrothermal Vents.” *Systematics and Biodiversity*, vol. 13, no. 3, 3 Dec. 2014, pp. 278–295., doi:10.1080/14772000.2014.970673.

Kojima, S. et al. "Genetic Differentiation of Populations of a Hydrothermal Vent-Endemic Gastropod, Ifremeria Nautilei, between the North Fiji Basin and the Manus Basin Revealed by Nucleotide Sequences of Mitochondrial Dna." *Zoolog Sci*, vol. 17, no. 8, 2000, pp. 1167-74, doi:10.2108/zsj.17.1167.

Meyer, Christopher P. "Molecular Systematics of Cowries (Gastropoda: Cypraeidae) and Diversification Patterns in the Tropics." *Biological Journal of the Linnean Society*, vol. 79, no. 3, 2003, pp. 401–459., doi:10.1046/j.1095-8312.2003.00197.x.

Múrias dos Santos, António, et al. "TcsBU: A Tool to Extend TCS Network Layout and Visualization." *Bioinformatics*, vol. 32, no. 4, 2016, pp. 627–628., doi:10.1093/bioinformatics/btv636.

Okutani, T., & Ohta, S. (1988). A new gastropod mollusk associated with hydrothermal vents in the Mariana Back-Arc Basin, Western Pacific. *Venus*, *47*, 1–10.

Osca, D. et al. "The Mitochondrial Genome of Ifremeria Nautilei and the Phylogenetic Position of the Enigmatic Deep-Sea Abyssochrysoidea (Mollusca: Gastropoda)." *Gene*, vol. 547, no. 2, 2014, pp. 257-66, doi:10.1016/j.gene.2014.06.040.

Panchal, M. "The Automation of Nested Clade Phylogeographic Analysis." *Bioinformatics*, vol. 23, no. 4, 2007, pp. 509-10, doi:10.1093/bioinformatics/btl614.

Panchal, M. and M. A. Beaumont. "The Automation and Evaluation of Nested Clade Phylogeographic Analysis." *Evolution*, vol. 61, no. 6, 2007, pp. 1466-80, doi:10.1111/j.1558-5646.2007.00124.x.

Raupach, M. J. et al. "The Application of Dna Barcodes for the Identification of Marine Crustaceans from the North Sea and Adjacent Regions." *PLoS One*, vol. 10, no. 9, 2015, p. e0139421, doi:10.1371/journal.pone.0139421.

Reynolds, K. C. et al. "New Molluscan Larval Form: Brooding and Development in a Hydrothermal Vent Gastropod, Ifremeria Nautilei (Provannidae)." *Biol Bull*, vol. 219, no. 1, 2010, pp. 7-11, doi:10.1086/BBLv219n1p7.

Selkoe, K. A. and R. J. Toonen. "Microsatellites for Ecologists: A Practical Guide to Using and Evaluating Microsatellite Markers." *Ecol Lett*, vol. 9, no. 5, 2006, pp. 615-29, doi:10.1111/j.1461-0248.2006.00889.x.

Suzuki, Y. et al. "Host-Symbiont Relationships in Hydrothermal Vent Gastropods of the Genus Alviniconcha from the Southwest Pacific." *Appl Environ Microbiol*, vol. 72, no. 2, 2006, pp. 1388-93, doi:10.1128/AEM.72.2.1388-1393.2006.

Tamura, K. et al. "Mega11: Molecular Evolutionary Genetics Analysis Version 11." *Mol Biol Evol*, vol. 38, no. 7, 2021, pp. 3022-27, doi:10.1093/molbev/msab120.

Thaler, A. D., et al. “Characterization of 12 Polymorphic Microsatellite Loci in IFREMERIA Nautilei, a Chemoautotrophic Gastropod from Deep-Sea Hydrothermal Vents.” *Conservation Genetics Resources*, vol. 2, no. 1, 2010, pp. 101–103., doi:10.1007/s12686-010-9174-9.

Thaler, A. D. et al. "The Spatial Scale of Genetic Subdivision in Populations of Ifremeria Nautilei, a Hydrothermal-Vent Gastropod from the Southwest Pacific." *BMC Evol Biol*, vol. 11, 2011, p. 372, doi:10.1186/1471-2148-11-372.

Waren, Anders, and Philippe Bouchet. “New Records, Species, Genera, and a New Family of Gastropods from Hydrothermal Vents and Hydrocarbon Seeps*.” *Zoologica Scripta*, vol. 22, no. 1, 1993, pp. 1–90., doi:10.1111/j.1463-6409.1993.tb00342.x.

Waren, Anders, and Winston F. Ponder. “New Species, Anatomy, and Systematic Position of the Hydrothermal Vent and Hydrocarbon Seep Gastropod Family Provannidae Fam.n. (Caenogastropoda).” *Zoologica Scripta*, vol. 20, no. 1, 1991, pp. 27–56., doi:10.1111/j.1463-6409.1991.tb00273.x.