Cryptic diversity and population connectivity of the coral-guard crab, *Trapezia bidentata*

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Dr. Sarah Lemer, Chair, Thesis Committee

Found throughout the Indo-Pacific, *Trapezia* crabs live in the interstitial spaces of coral colonies, assisting their host by removing sediments, deterring predators, and increasing survival rates of juvenile colonies. Due to their reliance on corals, they are susceptible to changing climates and thus it is important to evaluate their adaptive capacity. While previous studies have provided insight into the topology of the *Trapezia* phylogeny, the use of different single-gene markers resulted in variable resolutions. Additionally, several studies have indicated cryptic speciation within the species *Trapezia bidentata* and we intend to investigate both here. We use GRAS-Di sequencing to clarify *T. bidentata*'s position in the *Trapezia* phylogeny as well as assess the phylogeography and degree of connectivity between populations of *T. bidentata* throughout their geographic range. Further, we provide insight into the length of their pelagic larval phase through identifying population structure on the ocean and island-scale. Our phylogeographic analysis identified four subgroups within *T. bidentata* suggesting high divergence and potential for cryptic speciation. Significantly, one of these groups was found in the Marquesas Islands, a locality associated with high endemism. We also recover divergence

between the Indian and Pacific Oceans and admixture between the Central and West Pacific. On the island scale, we find minimal genetic structure suggesting a “long” pelagic larval phase and minimal impedance due to local biogeographic barriers. Overall, the greatest drivers of divergence within *T. bidentata* are currents and historic barriers to dispersal rather than geographic distance, with suggestions of fluctuation based on host availability. The broad geographic expanses attributed to each genetic cluster across a heterogeneous seascape indicate large gene pools and high adaptive potential.

Keywords: Trapezia*, population genetics, invertebrate genomics, coral reefs*

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Chapter 1: Introduction

Trapezia: Coral Exosymbionts

Coral reefs maintain the highest diversity of invertebrate symbioses of any other system (Castro 1988). The most well-known symbiosis is the relationship between corals and their endosymbiotic dinoflagellates (Blackall et al. 2015). However, many other important relationships exist between corals and exosymbiotic invertebrates (Przeslawski et al. 2008; Stella et al. 2011; Pisapia et al. 2020). These complex assemblages complement reef productivity via increasing coral host survival (Castro 1988; Leray et al. 2012; Rouzé et al. 2014) and yet, little is known about their susceptibility to climate change. Thus, it is important to better understand the vulnerability of these obligate exosymbionts by evaluating their biogeography, genetic diversity, and dispersal potential to determine their capacity to adapt to changing climates.

One symbiosis that has been gaining recognition is the relationship between the coral guard crab *Trapezia* and it's pocilloporid host. Found throughout the Indo-Pacific (Castro 2000), these mutualists live in the interstitial spaces of coral colonies and assist corals by removing sediments (Stewart et al. 2006), deterring crown-of-thorns seastar predation (Glynn 1980; Pratchett et al. 2001), and preventing incursion by corallivorous and mucus secreting snails (Shima, Osenberg & Stier, 2010; Stier et al. 2010). The crabs feed primarily on detritus, snail mucus, and other bacteria that reside or land on the coral tissues (Castro 1976) but will also consume the coral mucus itself or even zooplankton (Knudsen 1967; Shmuel et al. 2022). They also rely on the intricate branching of their host coral for protection from predators (Castro 2004). *Trapezia* crabs typically reside in heterosexual pairs of the same species alongside congenerics and are highly territorial toward conspecifics (Huber 1985; Castro 2004; Glynn 2013). Due to these aggressions, mated pairs must be established into the colony via rubbing chelipeds with resident crabs and similar interactions

with shrimp inhabitants (Huber 1985; Vannini 1985; Castro 2004; Glynn 2013). They are highly discriminatory between color morphs when choosing a mate, thus implying phenotypic assortative mating (Huber 1987). Diversity of crabs in a single colony tends to scale with the size of the coral, however, the largest crab species are often only found in large colonies while smaller species are typically found in smaller colonies as to avoid more effective competitors (Patton 1974; Preston 1973). Female *Trapezia* will brood eggs post-internal fertilization and release nauplius larvae into the water column as free-swimming plankton (Gotelli et al. 1985). Because of their planktonic life phase, they have a potentially broad dispersal range, however, the length of this free-swimming phase is unknown. Further, Richmond (1987) discussed the possibility of similar timing of larval settlement in *Trapezia* and their pocilloporid host due to parallels in genetic distance between Panama and Hawaii in both crab and coral populations described in Huber (1985), however, settlement triggers in *Trapezia* have not been identified. Because the larval phase of pocilloporid corals is measured, this observation could provide insight into the length of the pelagic phase of *Trapezia*.

Speciation in the genera is thus an intriguing question. Why do some species have incredibly broad geographic ranges while others are isolated (Castro 2004)? Could there be variation in the duration of their pelagic larval phases (Castro 2000)? Is assortative mating and discrimination between color morphs only designated to certain species (Huber 1987)? Do different species exhibit host preference that hasn't been observed due to unresolved species complexes within the *Pocillopora* genus? Could dispersal of some species have been opportunistic or due to climatic events? Are there cryptic species at these range limits that have yet evaded detection due to morphological likeness to other species? Further, the increasing occurrence of reef degradation and habitat fragmentation may have further isolated several reef-associated

species leading to decreased gene flow. How might habitat fragmentation impact *Trapezia* population structure on the island-scale?

While several species of *Trapezia* have been identified over the years, little is known about the diversity within each species and the structure of adjacent populations. On a broader scale, various reconstructions of the *Trapezia* phylogeny have hinted at the existence of cryptic species within several of the broadly dispersing clades (McKeon 2010; Rouzé et al. 2017; Canizales-Flores et al. 2020). Recognizing cryptic diversity is imperative to determining the vulnerability of species and the persistence of these mutualisms as different species experience different sensitivities to changing climates (Bickford et al. 2006). Through gaining an understanding of dispersal potential and cryptic speciation within the genera, we can infer their capacity to adapt to changing climates.

Diversity of *Trapezia*

Trapezia crabs have highly variable morphologies with a wide range of coloration patterns across species (fig. 1); however, they all share the characteristic flat carapace, which allows them to live in the interstitial spaces of coral colonies (Castro 2004). Species classification has changed for many *Trapezia* crabs over time due to disagreements in morphological assessments, however, a handful of genetic studies have sought to clarify these assignments. These studies have also tried to identify relationships between species, leading to several reconstructions of their phylogenetic tree (Lai et al. 2009; Rouzé et al. 2017; Pisapia et al. 2020). However, the phylogeny for *Trapezia* is not yet resolved as different, low resolution genetic markers lead to different tree topologies (Rouzé et al. 2017). Due to the limited genetic analysis of the genus, there is great potential for resolution via high-throughput sequencing both to clarify the phylogeny and investigate speciation events and finer population connectivity. Here we utilize Genotyping by Random Amplicon

Sequencing, Direct (GRAS-Di) to resolve uncertainties due to previously utilized genetic markers. GRAS-Di sequencing amplifies random sequences throughout the genome via PCR to provide ample genetic information (~9,000 SNPs) while also effectively handling degraded and low-quality DNA (Hosoya et al. 2019).

The diversity of these species is vital to resolve and maintain as different species provide different services to their host coral (McKeon et al. 2014) and as stresses increase, the diversity of species inhabiting one colony decreases (Stella et al. 2014). Further, it has been shown that variations in oceanographic influences, such as flow regime, impact the community composition of crustaceans even between adjacent coral colonies (Pisapia et al. 2020). As reefs degrade and coral coverage declines, host availability becomes limited and competition between conspecifics increases, leading to decreased densities and clutch sizes of obligate crabs (Stella et al. 2011a). This increase in competition may also change the population structure of species especially for less aggressive or resilient *Trapezia* species (Pisapia et al. 2020). Thus, it is even more imperative that cryptic diversity is defined, and the composition of these communities is maintained. For the purposes of this study, we will be focusing on *Trapezia bidentata*, a candidate of cryptic diversity.

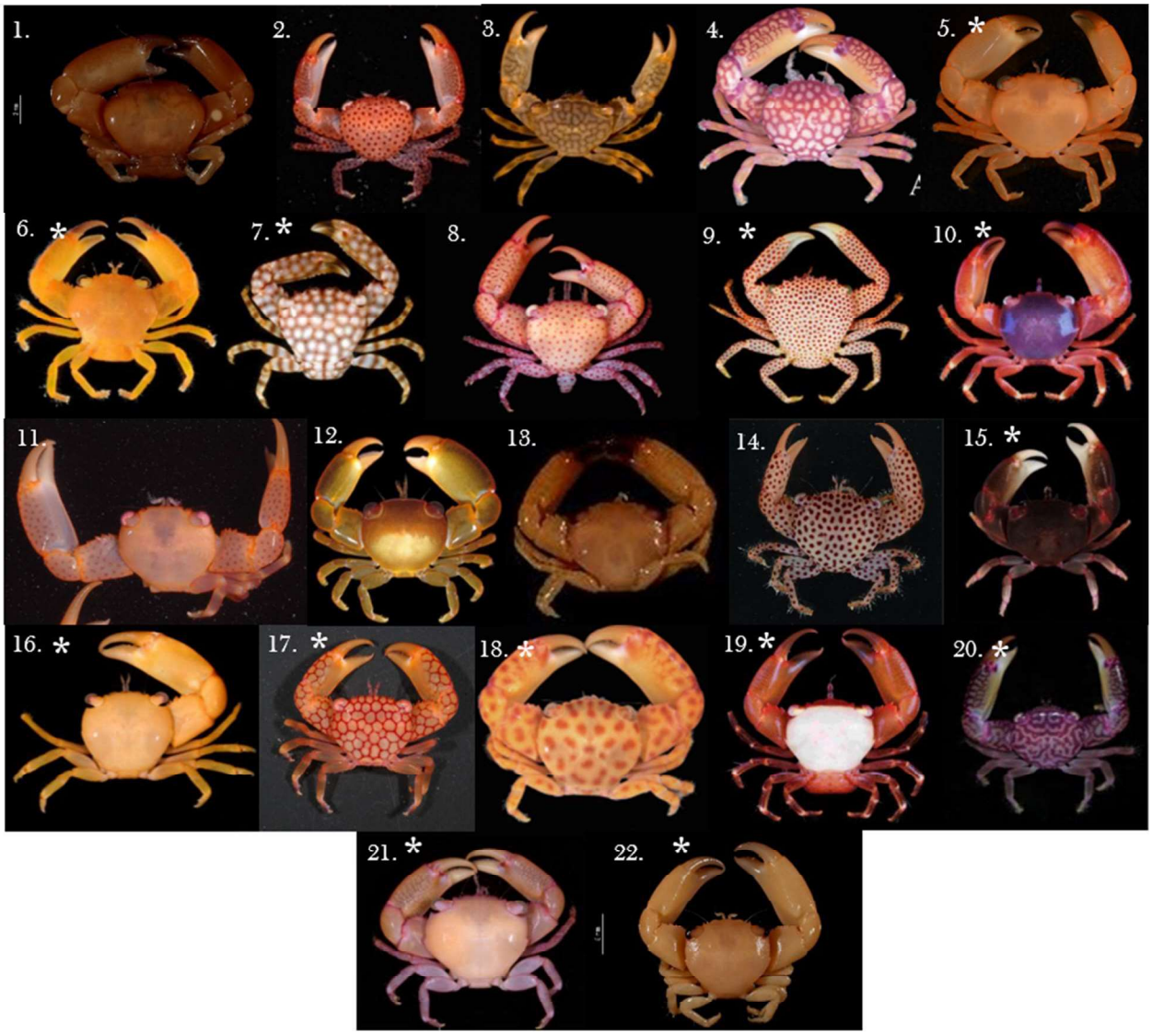

Figure 1. Example images of all 22 *Trapezia* species. Species found in Guam are indicated with *. 1) *T. globosa* 2) *T. bella* 3) *T. areolata* 4) *T. garthi* 5) *T. formosa** 6) *T. lutea** 7) *T. flavopunctata** 8) *T. richtersi* 9) *T. rufopunctata** 10) *T. cymodoce** 11) *T. punctimanus* 12) *T. cheni* 13) *T. corallina* 14) *T. tigrina* 15) *T. digitalis** 16) *T. bidentata** 17) *T. septata** 18) *T. intermedia** 19) *T. guttata** 20) *T. speciosa** 21) *T. serenei** 22) *T. plana**.
Photo credits: G. Paulay 2, 3, 6, 7, 8, 15, 19; A. Anker 2, 3, 6, 7, 8, 15, 19; Y. Chen 12; L. Flamme 1, 13, 22; T. Chan 10, 21; P. Castro 4; Poupin/Cléva 9; R. Lasley 5, 11, 14.

Trapezia bidentata, a case study.

Trapezia bidentata, previously *T. ferruginea*, is believed to have a broad range spanning the entire Indo-Pacific region with records of the species existing as far east as Baja, California and Panama and as far west as Madagascar and Saudi Arabia. Their broad geographic range

indicates a long-lived lineage with high dispersal potential; however, the timing of their speciation and the length of their free-swimming pelagic larval phase is unknown. Some even suggest that their extensive range may have been due to drifting pumice on the El Niño-southern oscillation events (Castro 1997). *Trapezia bidentata* has a characteristic orange carapace and black pinchers as well as diagnostic red dots on the terminal ends of their ambulatory legs (Castro 2004). It is classified as a medium-size species within *Trapezia* (Gotelli et al. 1985) and is thus effective at deterring *Culcita novaeguineae* corallivory according to a study by McKeon et al. (2014).

Host preference beyond family level of pocilloporids is not often observed in the genus, and *T. bidentata* is not an exception to this (McKeon 2010). Like all species of *Trapezia*, *T. bidentata* live primarily in mated pairs, however it is unknown how long these pairings are maintained as they have been documented to migrate frequently between coral colonies (Castro 1978; Gotelli et al. 1985). Studies detailing life history within the *Trapezia* genus are lacking, however, it has been presumed that, like other coral dwelling invertebrates, their metamorphosis is triggered by cues given off by their host coral (Pawlik 1992; Stella et al. 2011b). Further, their larvae are photopositive which plays a role in initiating long distance dispersal (Knudsen 1967).

A few studies have found hints of cryptic speciation between populations of *T. bidentata* in the Indian and Pacific Oceans including Rouzé et al. (2017) who analyzed two mitochondrial markers (COI and 16s) in populations of *T. bidentata* in the Reunion Islands and New Caledonia and McKeon (2010) who described the divergence between the Indian Ocean and Pacific with numerous sampling sites using a mitochondrial COI marker. Further, McKeon (2010) confirmed morphological distinction between the two potential allopatric sister taxa. Here we investigate this potential for cryptic speciation.

Trapezia bidentata phylogeography

There are several known biogeographical barriers in the Indo-Pacific that have been documented to influence species distributions over time (Crandall et al. 2019). In a study conducted by Keyse et al. (2018), it was discovered that the formation of the Sunda Shelf and Torres Strait drove early divergence between *Tridacna maxima* and *T. crocea*. The Sunda Shelf was exposed in the Pleistocene and prevented larval exchange between the Indian and Pacific Oceans while the Torres Strait was formerly a land bridge that prevented dispersal between the Gulf of Carpenteria and the Coral Sea (Keyse et al. 2018; fig. 2). Through this study we look at the genetic structure and connectivity of *T. bidentata* on multiple geographic and evolutionary scales to provide insight into their dispersal, oceanographic isolation, and identify potential cryptic species.

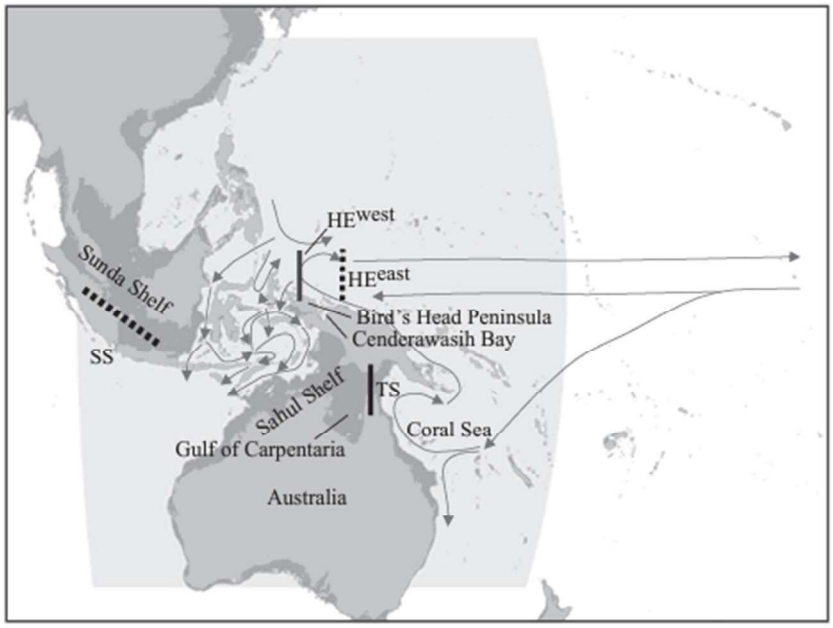

Figure 2. Map of the Indo-Australian archipelago. Present landmasses indicated by light grey, dark grey area represents land area at last glacial maximum, arrows represent ocean currents. Bold lines indicate barriers to dispersal including the Torres Strait (TS) and Halmahera Eddy (HE). The Sunda Shelf is indicated with a dashed line. Source: Keyse et al. 2018.

In addition to barriers to dispersal shaping the distribution of the species, population structure can provide insight into the length of the pelagic larval phase and the degree of mixing between populations. In a study by Lal et al. (2017), they examined the distribution of the black-lipped pearl oyster, a species with a known long pelagic dispersal phase. They found high connectivity in the Pacific and genetic separation between the Indian and Pacific oceans. While here we seek the opposite, to assess connectivity in order to hint at the length of the pelagic larval phase, such studies suggest that a long pelagic larval phase would result in high connectivity across large expanses such as the Pacific Ocean, providing a basis of comparison and estimation of pelagic larval duration.

Trapezia bidentata diversity and demography

Investigating the diversity and demography of *T. bidentata* in different geographic locations can provide insight into how populations have changed over time and thus hint at how populations may change in the future. Here we look at the demographic history of two distinctive Pacific Islands, Guam and Mo'orea. The largest difference between these locations being the degree of isolation from both the equator and biodiversity hotspots. One of the most distinct climatic events within the last 50,000 years was the Last Glacial Maximum which drastically lowered sea level (Yokoyama et al. 2018). Because Guam exists near the equator and in close proximity to the Coral Triangle, it had the potential to recover from this event via rapid recolonization and it would have experienced early ice-melt whereas it would have taken Mo'orea much longer to achieve former population levels, if at all, due to its high degree of isolation. Another factor to consider is that Guam is on a plate margin and thus has experienced non-eustatic

shifts that a mid-Pacific plate island like Mo'orea would not have experienced (Mylroie et al. 2001). This could entail greater unexplained fluctuations in effective population size. While demography provides a broad view of past fluctuations in effective population size, measurements of diversity can help explain population dynamics on a much shorter scale. Both can feed into an assessment of the species in how changing climates have and will impact the future prospects of the species.

Island-scale population connectivity

While phylogeography can reveal cryptic diversity and alleviate uncertainty of species distributions, fine-scale connectivity can provide insight into small-scale barriers to dispersal and the impacts of habitat availability and adaptation to certain environmental conditions. Here we focus on population structure around Guam to assess potential physical barriers and investigate the impacts of environmental variability both in terms of location and pocilloporid coral coverage. As *Trapezia* are highly reliant on their host, it is likely that their distribution is correlated with the presence of pocilloporid corals. Further, the overall coral coverage of a site is likely to impact their ability to move between colonies and find mates (Castro 1978). Guam has also been impacted by multiple disturbances in the form of cyclones, crown-of-thorn seastar outbreaks, and bleaching events. Nearly half of the *Acropora* on Guam was lost between 2013-2014 and only 10% of the coral cover on the east side of the island remains (Burdick 2014). This decline in *Acropora* initiated a phase shift into a *Porites, Pocillopora,* and *Pavona* dominated reef, greatly increasing the coverage of pocilloporid corals (Heron et al. 2020).

However, while this is true, bleaching events have become more frequent and this domination of pocilloporids is not permanent. While smaller colonies are typically the target of

bleaching events, recent events in other places have disproportionately impacted larger colonies (Speare et al. 2021). As diversity of *Trapezia* tends to scale with colony size, frequent bleaching events can greatly reduce the diversity of resident populations (Preston 1973; Glynn 2013). It also has the potential of shifting the community structure within exosymbiont assemblages depending on the resistance or resilience of the inhabitants as some species may have been more susceptible to changes during coral decline. Reef degradation and high sedimentation due to sediment plumes are also common in Guam and may strengthen the barriers between subpopulations around the island (Minton et al. 2007; Burdick 2014). It is important to document the variation in connectivity among populations and whether habitat fragmentation and host availability negatively impacts larval dispersal and settlement.

OBJECTIVES & HYPOTHESES

Indo Pacific Phylogeography:

The first aim of my project is to analyze the broad phylogeography of *T. bidentata* and identify the presence of cryptic taxa. Further, we will identify geographical barriers that likely initiated these speciation events (Crandall et al. 2019). As implied by the incongruities within the current *Trapezia* phylogeny (Canizales-Flores et al. 2020; Rouzé 2017), I anticipate some level of cryptic speciation within *T. bidentata* in the Indo-Pacific. Additionally, we seek to clarify *T. bidentata*'s placement within the phylogeny of the *Trapezia* genus. As previous constructions of the phylogeny using single genes have varied according to the genetic marker used, I expect greater resolution provided by trees constructed from our SNP dataset.

Hypothesis #1

H_0: *T. bidentata* in the Indo-Pacific represents only one Molecular Operational Taxonomic Unit (MOTU).

H_1: *T. bidentata* in the Indo-Pacific represents more than one MOTU.

Population Genetics:

The second aim of my project is to conduct a population genetic analysis of a select number of localities around Guam. Localities will be distributed around Guam with emphasis on the four cardinal directions. Evidence of structure will allow us to infer barriers to dispersal such as habitat fragmentation or other various structures that may impede larval dispersal. From this data, we wish to infer dispersal patterns and provide insight into the length of *T. bidentata*'s pelagic larval phase.

In relation to population genetic analyses around Guam and the Mariana Islands investigating coral population dynamics, I predict that there is likely little genetic structure of *T. bidentata* around the island. Outside of a potential diverging current in the southeast part of the island and potential structuring between northern and southern populations and southern and western populations, no oceanographic barriers have been recorded as effective at impeding larval dispersal and while the peninsula on the west side of the island and the lagoon to the south seem likely candidates of dispersal barriers, the aforementioned studies showed that they did not obstruct larval mixing around the island (Tusso et al. 2016; Rios 2020; Townsend 2022).

<u>Hypothesis #2</u>

H_0: There is no detectable genetic structure of *T. bidentata* among sites around Guam.

H_1: There is detectable genetic structure of *T. bidentata* among sites around Guam.

The third aim of my project is to compare the genetic diversity and demographic history of Guam with that of Mo'orea. Due to Mo'orea's isolation from biodiversity hotspots and distance from the equator, it is likely that glaciation events more severely impacted the island's *T. bidentata* populations while populations in Guam were able to rapidly repopulate due to their proximity to the Coral Triangle. Further, this high degree of isolation likely led to lower levels of heterozygosity and higher levels of inbreeding due to the presence of fewer source populations in the Central Pacific. Thus, we predict that there will be higher genetic diversity and greater fluctuations in effective population size through time in Guam.

Hypothesis #3

H_0: There is no difference in effective population size between Guam and Mo'orea.

H_1: Effective population size is greater in Guam than in Mo'orea.

Chapter 2: Methods

Sample collection

To analyze the relationships between species within the *Trapezia* genus and clarify the placement of *T. bidentata*, we collected a total of 23 specimens from around Guam, representing 10 species. Collections were opportunistic and samples were obtained during our *T. bidentata* collections. To further supplement our tree, we gathered 21 samples of six species from the Florida and Paris museums. In total, the museum samples and our own collections represented 10 species in addition to our *T. bidentata* samples including: *T. lutea, T. punctimanus, T. digitalis, T. tigrina, T. speciosa, T. serenei, T. rufopunctata, T. flavopunctata, T. cymodoce,* and *T. formosa,* some of

which were previously misidentified. Samples were photographed, subsampled for DNA extraction, and collected samples were preserved in 95% EtOH.

To investigate the phylogeography of *T. bidentata* across the Indo-Pacific, we shipped in 61 samples from the Florida and Paris Museums representing 12 regions (fig. 3, Supplemental material Tables 1 and 2) including 20 samples from Mo'orea. Note that the samples we obtained from Mo'orea were not given a specified collection site, so we treated this sample set as a single population. Each sample was photographed and subsampled for DNA extraction.

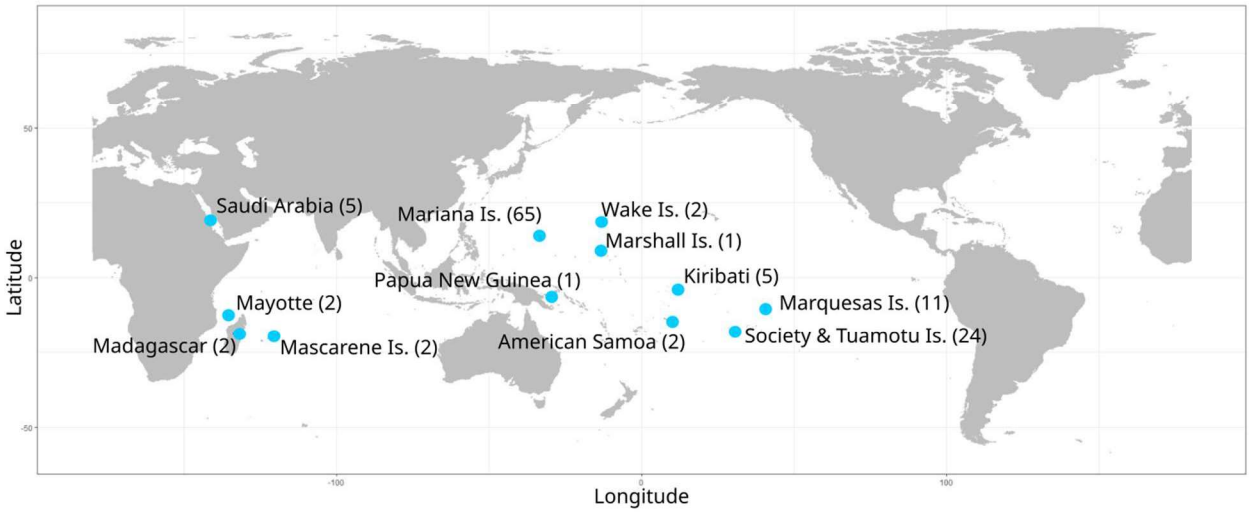

Figure 3. *T. bidentata* samples collected from throughout the Indo-Pacific. Samples were obtained from the Florida and Paris museums or personally collected (Guam).

To assess population structure and connectivity around Guam, we collected a total of 72 individuals from five different sites around the island (Table 2). These include Pago Bay (East), Luminao (West), Coco's Lagoon (South), Bile Bay (Southwest), and Urunao (Northwest) (fig. 4). The outer reef was sampled at Pago Bay and Bile Bay while Luminao and Urunao were sampled on the reef flat. Coco's Lagoon was sampled at the far left flat that extends from Coco's Island. For all sights, most collections occurred near the reef crest as that is where pocilloporid corals

were found. To catch the crabs, we used Eugenol (10% ethanol, 10% clove oil, and 80% seawater) to sedate the crabs before removing them from the coral with metallic forceps and placing them in a 50 mL falcon tube with seawater. Host coral colonies were then photographed next to the numbered falcon tube containing the resident crabs. Falcon tubes containing crabs were stored in a chilled cooler during transport to the University of Guam Marine Laboratory. Upon arrival at the lab, crabs were photographed, transferred to five mL cryovials with 95% ethanol, and stored in a -20^{o}C freezer until extraction.

Figure 4. Map of collection sites around Guam. Numbers in parentheses represent the total samples per locality. Blue dots represent personal collections, orange dots represent museum samples. Sites with fewer than six samples were not considered for downstream analysis.

Site characterization

To quantify pocilloporid coral density at our sites, we ran benthic coverage surveys for the four sites that qualified for downstream analysis (Luminao, Urunao, Pago Bay, and Bile Bay). The purpose of classifying various habitats around the island was to make conclusions regarding the influence of coral coverage and pocilloporid coral abundance on *T. bidentata*'s distribution. This may assist in understanding the potential population structure and genetic diversity around the island in our genetic analysis.

Surveys were conducted at Bile Bay, Urunao, Luminao, and Pago Bay (fig. 4). We laid three 50 m transects parallel to the shore at each site, then placed a 0.5x0.5 m quadrant every five m and took a photo. Image analyses were conducted via Coral Point Count v4.1 (Kohler & Gill 2006; fig. 5). 50 random points were selected per image and points that landed on a pocilloporid coral were counted. We then totaled the pocilloporid coral points and calculated the percentage out of the 1,500 total points per site and used it as a metric of pocilloporid density. A one-way ANOVA was used to test for significant differences between coverage at the four sites.

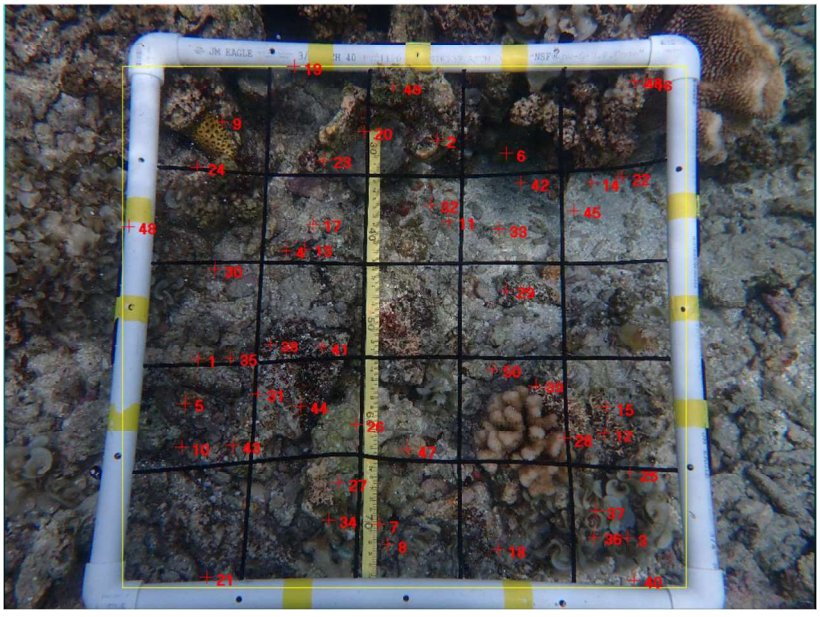

Figure 5. Example of benthic coverage survey analysis in Coral Point Count v.4.1. Site: Luminao, GU.

DNA Extraction, PCR, GRAS-Di library construction and sequencing

To extract DNA, we used the GenCatch Genomic DNA Extraction kit (EPOCH Life Sciences, Missouri City, TX), following the manufacturer's protocol, and eluted in 100 uL of nuclease-free water. We then measured DNA and RNA concentrations via high-sensitivity Qubit 3.0 fluorometer (Thermo Fisher Scientific Inc., Waltham, MA) assays. To construct a preliminary phylogeny and determine species identity genetically, we amplified the 16S rRNA gene via PCR following the protocols detailed in Rouzé et al. (2017) (universal primers: 16S: 16Sar/16Sbr (Palumbi 1996)). The PCR product was then shipped to EPOCH Life Sciences for Sanger sequencing. The raw sequence data was trimmed and visually inspected on Geneious Prime (2022.0.2). A MUSCLE alignment was then conducted in Geneious using eight iterations prior to RaxML tree construction (Stamatakis 2014) with 1,000 bootstrap replicates. Further, 16S sequences were BLASTed to both GeneBank and to Dr. Rob Lasley's unpublished sequence data of *Trapezia* to verify species identity. A subset of individuals per species and a subset of *T. bidentata* per locality, for a total of 180 samples (including 124 *T. bidentata*), were selected for Genotyping by Random Amplicon Sequencing-Direct (GRAS-Di) and shipped to the UC Davis Genome Center for library construction and sequencing.

Filtering and phylogenetic tree analysis:

Quality filtering, locus assembly of raw reeds, and genotyping were conducted in STACKS v2.60 (Catchen et al. 2011, 2013) with maximum-likelihood analyses. Parameters for STACKS were optimized using the methods detailed in Paris et al. (2017) to increase the number of Single Nucleotide Polymorphisms (SNPs) across sites and individuals (Supplemental Figure 4). Loci

assembly parameters were thus set to m=3, M=4, and n=5 to obtain 80% polymorphic sites and substantial coverage for all species. Using GenePop v1.2 (Raymond & Rousset 1995), we then removed individuals with fewer than 20% of the amplified SNPs. Selective SNPs were then filtered out via BayeScan v2.1 (Fol & Gaggiotti 2008) using default parameters and SNPs outside of Hardy-Weinberg Equilibrium were filtered out via dDocent (Puritz et al. 2014a, 2014b). A total of 169 samples represented by 3,316 loci and 11,534 SNPs were retained after filtering and the average coverage across all samples was 14.86X (SD = 23.28).

Phylogenetic reconstruction of 12 *Trapezia* species was performed using IQTREE 1.6.12 (Kalyaanamoorthy et al. 2017, Hoang et al. 2018) with an initial model test prior to running the tree with the optimal GTR+F+I+G4 model, 1,000 bootstrap replicates, and 10,000 iterations. Additional trees were constructed with RAxML version 8.2.12 (Stamatakis 2014), with a GTRCAT model (without correction for rate heterogeneity) and 1,000 rapid bootstrap replicates (Supplementary Figure 1). After initial tree construction, we separated the *T. bidentata* samples to run population genetic analyses.

Phylogeography and population genetics analyses:

A total of 124 individuals (fig. 3), including 53 samples collected from Guam (fig. 5), were used in our population genetic analysis of *T. bidentata*. For this analysis, we retained only the first SNP of each locus with STACKS using a maximum-likelihood model (Catchen et al. 2011, 2013) to minimize the effect of linkage disequilibrium. Admixture analyses were performed via STRUCTURE 2.3.4 (Pritchard, Stephens & Donnelly 2000) to visualize fine-scale genotypic variation throughout the Indo Pacific. For every STRUCTURE analysis, 150,000 replicates were run after a burnin period of 25,000 and eight K clusters were tested. Results were gathered and

assessed via Structure Harvester (Earl & vonHoldt 2012) and visualized with CLUMPP v 1.1.2 (Jakobsson & Rosenberg 2007) and DISTRUCT v 1.1 (Pritchard et al. 2000a; Falush et al. 2003).

We measured Kimura's two-parameter genetic distance (K2P) between the four genetic groups identified by the STRUCTURE analyses via MEGA 11 (Tamura et al. 2021). K2P distances were calculated using 1,000 bootstrap replicates, uniform rates, and gap/missing pairwise deletion. A principal component analysis (PCA) was also constructed of the full *T. bidentata* dataset using the package ade4 and dudi.pca (Dray and Dufour 2007) with implementation of fviz_pca_ind from the factoextra package for better visualization (Lê & Husson 2008). We then calculated pairwise F_{ST} values between each of the genetic clusters identified by the STRUCTURE analyses using the STAMPP package in R. P-values were calculated around a 95% confidence interval with 1,000 bootstrap iterations. Further, we also calculated H_O, H_S, and F_{IS} for each genetic cluster using the adegenet (Jombart 2008) and hierfstat (Goudet 2004) packages in R.

To determine whether there was substructure within the major four clades identified by both the phylogenetic and STRUCTURE analyses, we split the data into four individual sub-datasets (one per genetic cluster). We then re-ran STRUCTURE, constructed PCAs, and calculated diversity indices, as described above, for each sub-dataset.

To analyze the population structure and genetic diversity around Guam, we constructed a PCA plot and conducted a STRUCTURE analysis, as described above, for all samples. We then selected sites with more than six samples (Bile Bay, Urunao, Luminao, and Pago Bay) to calculate pairwise F_{ST} values and run relatedness analyses via relatedness2 in vcftools (Danecek et al. 2011).

Next, we compared genetic diversity in Guam with that of Mo'orea. Since our sampling of Guam and Mo'orea were uneven, we first took a random subset of 20 samples from Guam to equate the population sizes and avoid effects of sampling bias in our population genetic summary

statistics. To select samples, we used a random number generator. We then calculated H_O, H_S, and F_{IS} for each island using adegenet and hierfstat in R. We then ran a demography analysis via STAIRWAYPLOT2 (Liu & Fu, 2015, 2020) with 1,000 iterations, utilizing easySFS (script available here: https://isaacovercast.github.io) to create a folded SFS file from our vcf STACKS output. For this analysis, we assigned a mutation rate of 2.6E-9 to *T. bidentata* loci according to measurements of *Alpheus* snapping shrimp in Selliman (2021). We also approximated the generation time of *Trapezia* to be approximately three years, equating the study of a closely related genus, Xanthidae (Knudsen 1960).

Morphometric analysis:

Lastly, to quantitatively compare morphology across the four genetic clusters, we ran a morphometric analysis of 11 similarly sized specimens from each cluster. We used ImageJ to measure the length (from frontal region to before the abdomen) and width (between outer edge of eye sockets) of the carapace and then ran a one-way ANOVA to test for significance between clusters (Schneider et al. 2012). In addition to sorting by cluster, we also tested for significant differences between sexes as sexual dimorphism is typically found in crustaceans (Rufino et al. 2004; Josileen 2011; Hajjej et al. 2016).

Chapter 3: Results

Trapezia Phylogenetic analyses

According to our 16S genotyping analysis, several *T. bidentata* museum specimens were misidentified including three samples from French Polynesia that identified as *T. punctimanus*, one Kiribati sample that identified as *T. tigrina*, one French Polynesia sample that identified as *T.*

serenei, and two Kiribati and one Papua New Guinea sample that identified as *T. cymodoce*. Fortunately, while detracting from our collection of *T. bidentata*, we were able to supplement our phylogeny with these species and clarify their identification for the Florida Museum.

Delineation between species was strongly supported with 100% bootstrap support (BS) for all nodes (fig. 6). Both the IQTREE and RaxML trees displayed the same topology and clarified the relationship between most species as well as the position of *T. bidentata* in relation to other *Trapezia*. Previously, the position of *T. bidentata* varied according to the genetic marker used to construct the phylogeny (Rouzé et al. 2017). Here, we recovered *T. bidentata* as a sister species to the clade containing *T. lutea*, *T. cymodoce, T. punctimanus* and *T. guttata*, with full nodal support (100% BS). *Trapezia lutea* and *T. cymodoce* were sister species, and so were *T. punctimanus* and *T. guttata*, with full nodal support (100% BS). Similar to previous constructions of the *Trapezia* phylogeny, *T. rufopunctata* and *T. flavopunctata* appeared to be sister species and distantly related to the rest of the genus. This clade is placed at the base of the *Trapezia* tree with full nodal support (100% BS). Finally, the only non-resolved position was the placement of *T. digitalis*. In the IQTREE, it was placed as sister to the clade formed by *T. tigrina, T. serenei, T. speciosa* and *T. formosa*, but with low nodal support (62% BS). However, in the RaxML tree *T. digitalis* was recovered as sister to most *Trapezia* species with the exception of *T. rufopunctata* and *T. flavopunctata*, with full nodal support (100% BS; fig. 6; Supplementary Figure 1).

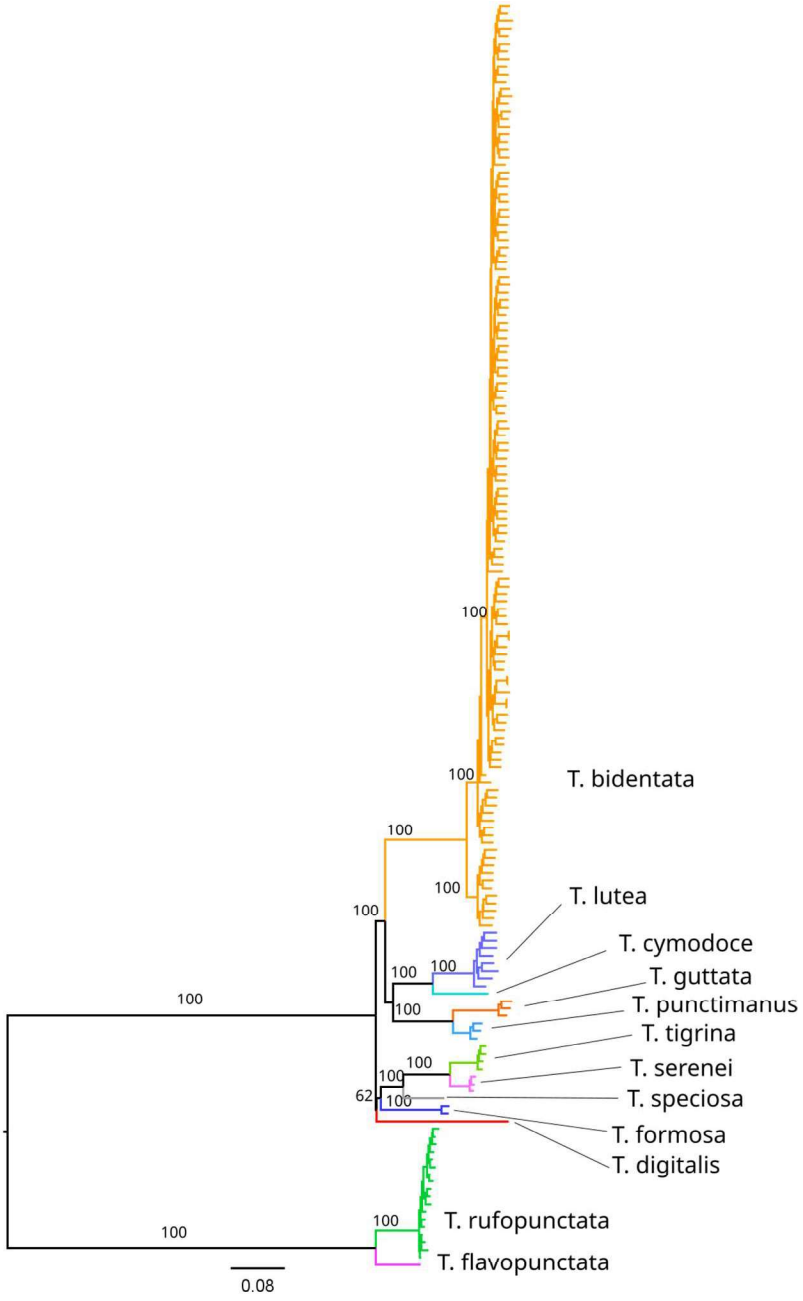

Figure 6. Phylogenetic tree of all species constructed in IQTREE using GRAS-Di data. Bootstrap values are indicated on the branches.

Trapezia bidentata phylogeographic analyses

Phylogeography analyses conducted for *T. bidentata* clearly supported a genetic separation between *T. bidentata* found in the Indian Ocean and those in the Pacific Ocean, with

strong nodal support (100% BS). We also found evidence of divergence between the clade containing individuals from the Marquesas islands and the clade containing the rest of the Pacific samples (96% BS). Finally, we observe that the remaining Pacific individuals were further divided in two clades (100% BS); a Western Pacific clade with individuals from the Mariana, Marshall, Wake, Phoenix and Line islands, and the North coast of Papua New Guinea; and an Central Pacific clade with individuals from French Polynesia (excluding Marquesas) Kiribati, Wake Island, and American Samoa (fig. 7, 8). The STRUCTURE analysis indicated that the most likely number of genetic clusters was K=4, thus supporting the four clades obtained with the phylogenetic analyses (fig. 7, 8; Supplemental Figure 4). The STRUCTURE analysis further revealed that there is little to no admixture between the Indian Ocean and the Marquesas clades with the other clades. However, STRUCTURE did detect a gradient of admixture from the West Pacific clade into the Central Pacific clade: all but two Central Pacific individuals showed signs of admixture from the West Pacific, while only 24 West Pacific individuals (out of 76) showed signs of admixture from the Central Pacific. The same four distinct clades and the admixture between West and Central Pacific clades were also recovered in the PCA analysis (fig. 8).

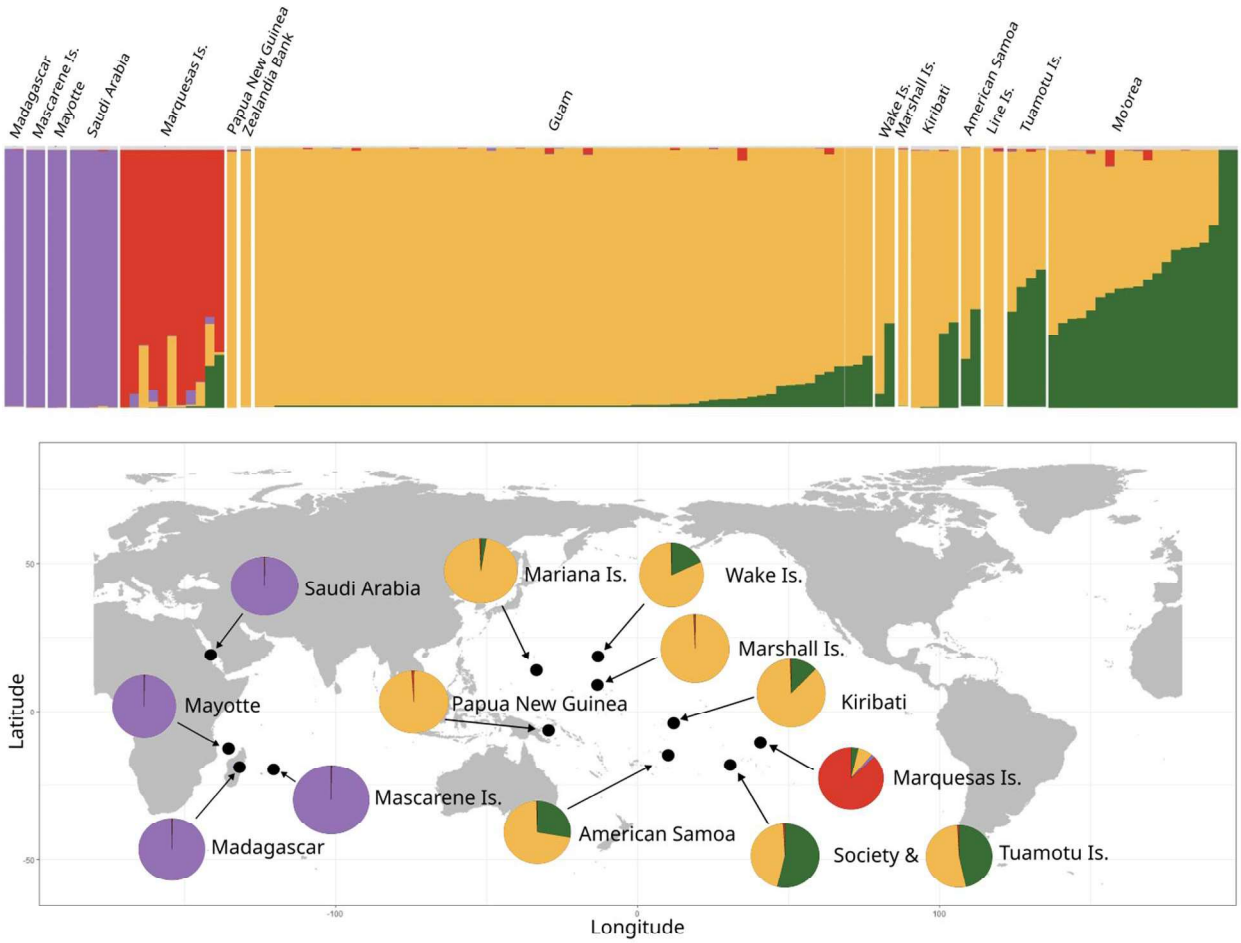

Figure 7. STRUCTURE analysis of *T. bidentata* (K=4; see Supplemental Figure 4). Map of localities with pie charts indicating admixture proportions. Colors indicate genetic clusters (purple = Indian Ocean clade, yellow = West Pacific clade, green = French Polynesia clade, red = Marquesas Islands clade).

To detect the presence of genetic structure within each genetic clade, we conducted a STRUCTURE analysis (fig. 8). Within the West Pacific clade (K=3) there was admixture throughout all localities with no indication of structure (fig. 8). In the Central Pacific clade (K=3), we identified four individual outliers in Mo'orea with no evidence of structure elsewhere in the group. It is important to note that individuals were assigned to the Central or West Pacific based on the clade they fell into and that some localities occur in both clusters. The Marquesas Islands clade (K=3) showed some structure between Hiva Oa and Eiao with the rest of the archipelago while the other islands were similar genetically. Within the Indian Ocean clade (K=2), there were

two groups, one in the Red Sea and the other in the Southwest Indian Ocean with minimal admixture between the groups.

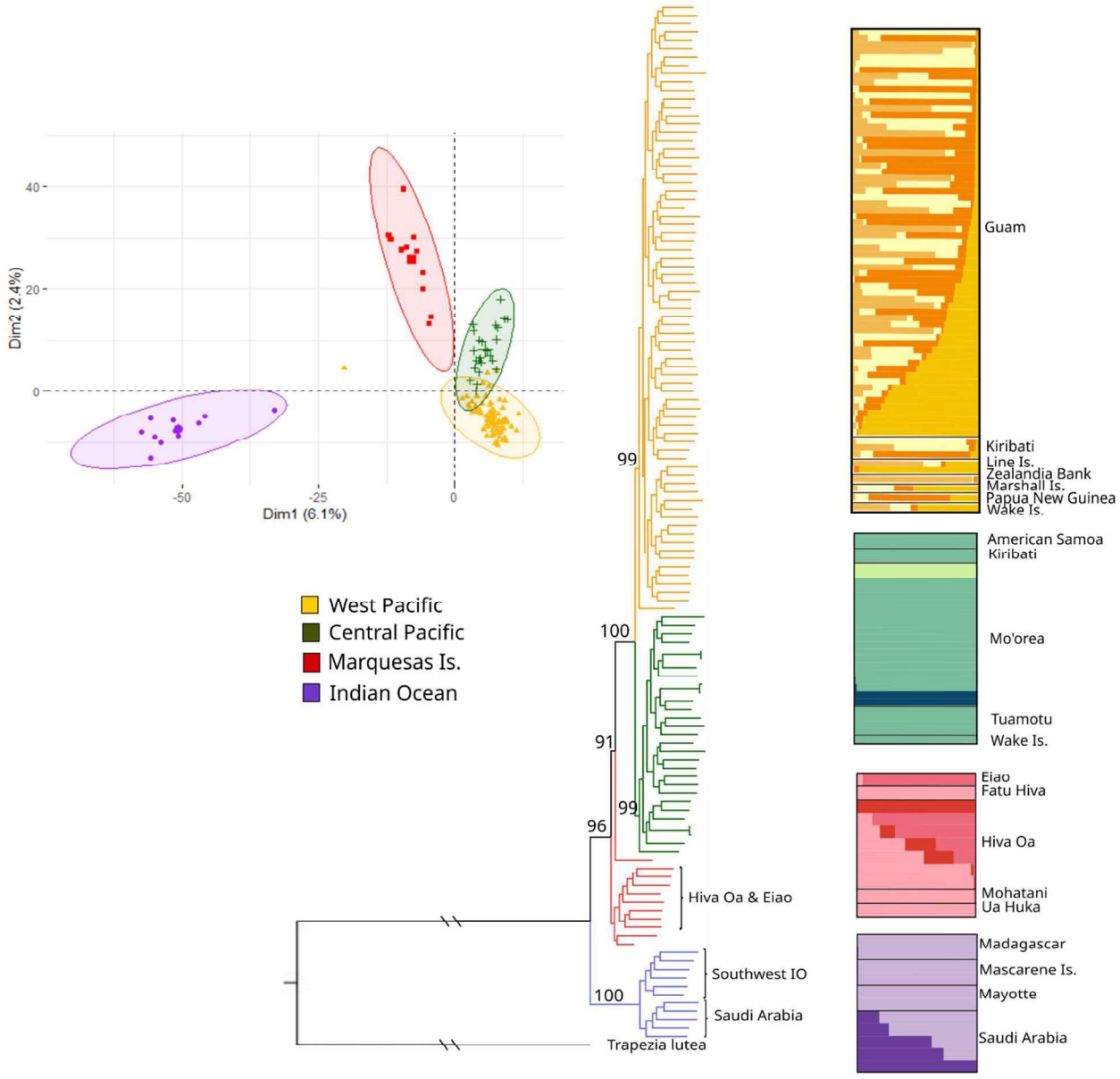

Figure 8. PCA and IQTREE defining the four genetic clusters within *T. bidentata* as indicated by STRUCTURE analysis. STRUCTURE within each defined cluster is shown to the right of the IQTREE. Unless specified in figure, localities within genetic clusters are mixed within IQTREE and do not form distinct clades.

Pairwise genetic distances estimated between clades with K2P and F_{ST} values were all significant (P-value < 0.001) and showed similar results (tables 1, 2). As expected, the highest pairwise K2P and F_{ST} values were recovered between the Indian Ocean clade and all others (K2P ranging from 0.03 to 0.04 and F_{ST} ranging from 0.22 to 0.27). The lowest genetic distances were recovered between the West and Central Pacific clades (F_{ST} = 0.03, not as clear with K2P) supporting the STRUCTURE analyses. Despite the Central Pacific clade containing all other Polynesian samples, the Marquesas clade showed equal pairwise genetic distance with the West (K2P = 0.02 and F_{ST}=0.1) and Central Pacific clades (K2P = 0.02 and F_{ST}=0.1).

Table 1. K2P distances (lower diagonal) calculated between each of the 4 genetic clusters. Pairwise Fst (upper diagonal) calculated between each of the 4 genetic clusters. Significant values are indicated in bold P-value < 0.001).

	Indian Ocean	West Pacific	Marquesas	Central Pacific
Indian Ocean	-	**0.25**	**0.22**	**0.27**
West Pacific	**0.04**	-	**0.10**	**0.03**
Marquesas	**0.04**	**0.02**	-	**0.10**
Central Pacific	**0.03**	**0.02**	**0.02**	-

Genetic diversity analyses revealed that all four clades had similar observed heterozygosity indices ranging from 0.21 to 0.29 (Central Pacific and Marquesas Islands, respectively; Table 3) and inbreeding coefficients (F_{IS}), ranging from 0.11 to 0.21 (Marquesas Islands and Indian Ocean, respectively).

Table 2. Population genetic summary statistics calculated for each of the 4 genetic clusters.

Population	Number of samples	Number of polymorphic sites	Observed heterozygosity (*Ho*)	Expected heterozygosity (*Hs*)	Inbreeding coefficient (F_{IS})
Indian Ocean	11	1271	0.26	0.32	0.21
Marquesas Is.	11	1823	0.29	0.33	0.11
Central Pacific	29	2324	0.21	0.25	0.16
West Pacific	73	2120	0.24	0.28	0.14

Morphologically, there are some notable differences between the clades (fig. 9). Please note that, due to limited sample size for some of the groups, these observations are not statistically supported, and are thus purely personal observations. First, samples from the Indian Ocean tended to be much larger, broader, and slightly more globose than samples from the Pacific (inclusive of French Polynesia and the Marquesas Islands). They also typically had broader claws and the indentations in their frontal region tend to be smoother with sharper spikes on either side of their eye sockets.

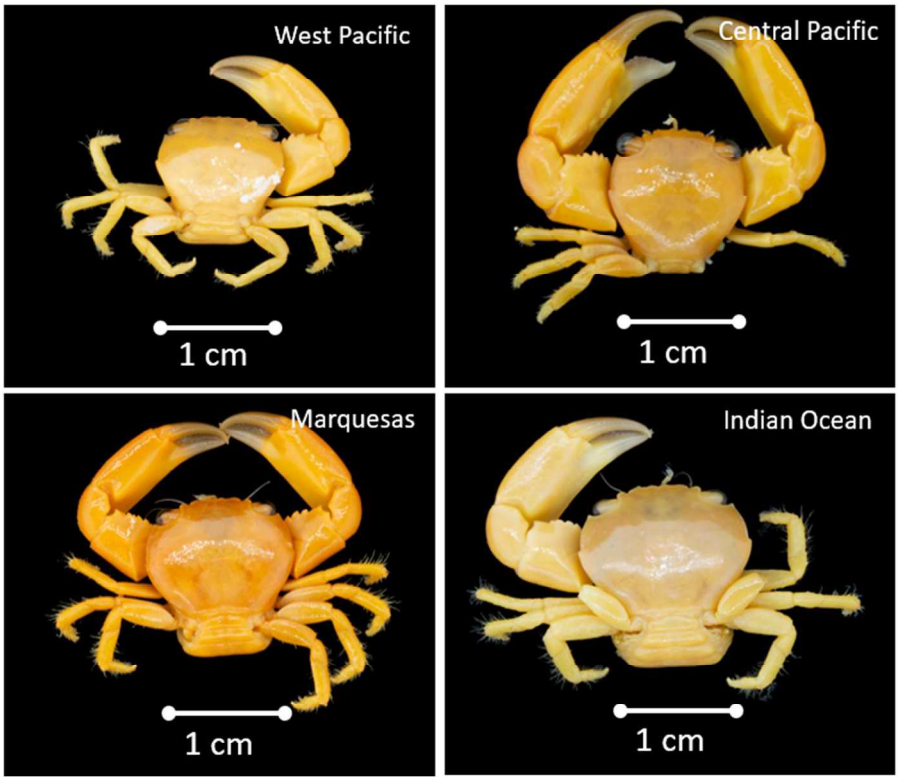

Figure 9. Images of specimens representing the 4 different genetic clusters. Scale bars are 1 cm and aligned so that crab size is represented correctly.

However, despite appearances, there are no quantitatively significant differences in carapace measurements across the four clades (p-value = 0.07 and 0.441 for males and females, respectively). Measurements between sexes were also not significant (p-value = 0.589; fig. 11).

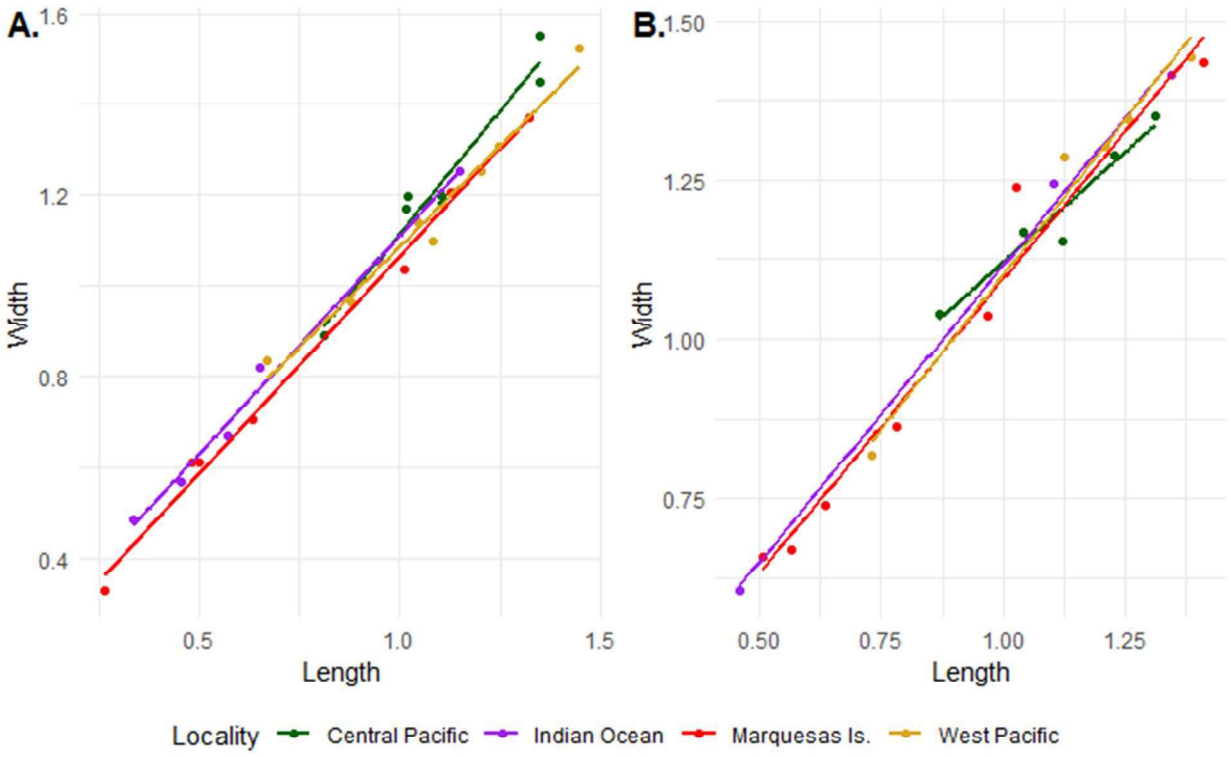

Figure 10. Length over width of carapace in centimeters for A) male and B) female crabs in the four genetic clusters.

Additional STRUCTURE and PCA analyses conducted within each of the four genetic clusters showed that each cluster varied in their amount of sub-structure. The Indian Ocean clade showed a clear separation between the Red Sea and Southwest Indian Ocean individuals (fig. 8 and 12C). The Marquesas clade showed sub-structure between islands with Hiva Oa being the most distinct from the neighboring islands (fig. 12D), however this may be an artifact of most of our samples coming from Hiva Oa (seven out of 11). The West Pacific clade had no specific structure as all samples tended to cluster together (fig. 12A). In the Central Pacific clade, a few individuals from Mo'orea and Kiribati appear to be genetically distinct from the rest of the Central Pacific, however, they still reside within the Central Pacific clade in our phylogeny (fig. 8, 12B).

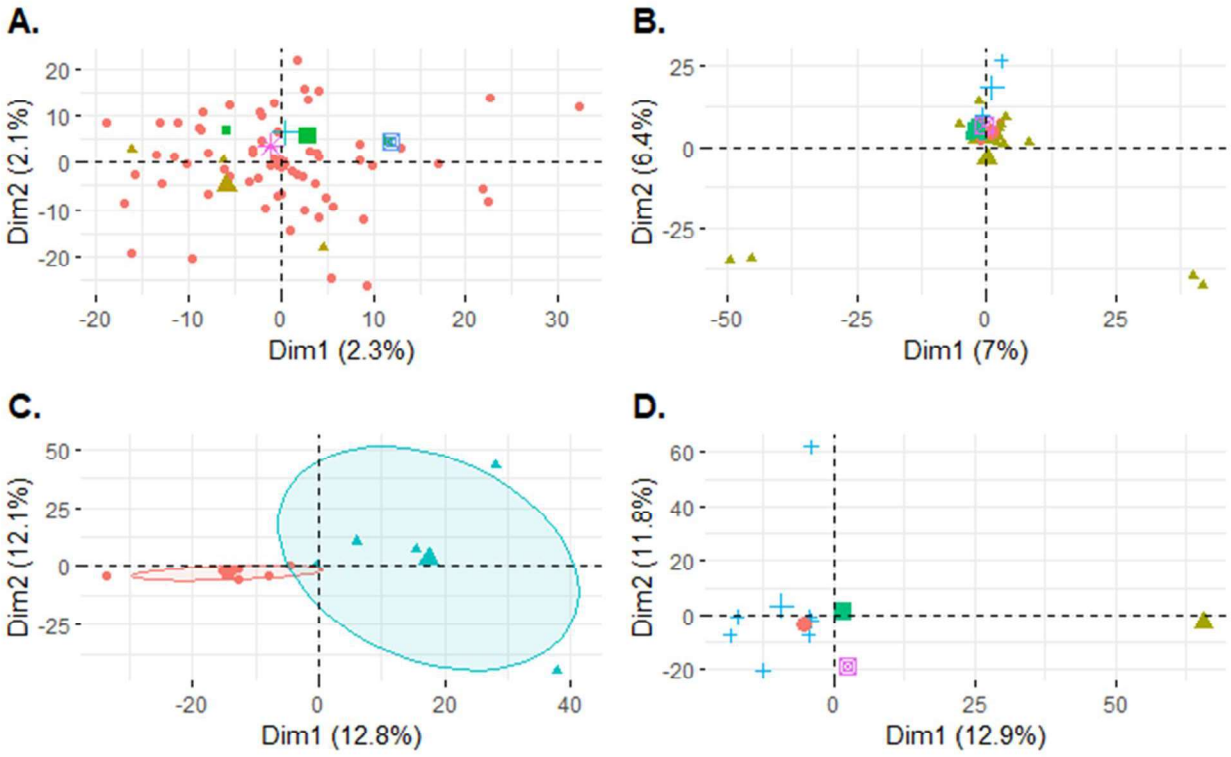

Figure 11. Principal Component Analyses (PCA) of the populations within the 4 genetic clusters: A) West Pacific (red = Guam, olive = Kiribati, green = Line Islands, blue = Marshall Islands, dark blue = Papua New Guinea, pink = Wake Island), B) Central Pacific (olive = Mo'orea, green = Tuamotu, red = American Samoa, blue = Kiribati, pink = Wake Island) , C) Indian Ocean (red = Saudi Arabia, blue = Madagascar, Mayotte, and Mascarene Islands), D) Marquesas Islands (blue = Hiva Oa, red = Eiao, green = Mohatani, pink = Ua Huku, olive = Fatu Hiva).

Trapezia bidentata population genetics in Guam:

The 16S genotyping analyses conducted on all samples collected in Guam, revealed that only 16 out of 37 and nine out of 23 specimens collected in Urunao and Bile's Bay, respectively, were *T. bidentata*, the remaining specimens were *T. lutea* or *T. formosa* unfortunately mis-identified as *T. bidentata.* Further, eight samples from Urunao and one sample from Bile Bay were removed due to poor sequencing quality. While sample sizes for both Urunao and Bile Bay were small, recent studies have shown that with high-throughput sequencing datasets, only six to eight samples are required for accurate measurements of population allele frequencies (Li et al. 2020). With that in mind, we removed all sites that had fewer than six individuals from our population

genetic analyses, retaining four populations: Bile Bay (eight samples), Luminao (20 samples), Urunao (eight samples), and Pago Bay (22 samples). However, it should be noted that the allowance of allele frequency estimations of small populations is debated and the results here for both Bile Bay and Urunao diversity indices and F_{ST} should be interpreted with caution.

Pairwise F_{ST} were low but significant for every pairwise comparison between all four sites. The highest pairwise F_{ST} value was found between Pago and Bile Bay (F_{ST}= 0.013; P-value < 0.001) and the lowest between Pago Bay and Luminao (F_{ST}= 0.002; P-value = 0.03; table 3).

Table 3. Pairwise F_{ST} values between sites around Guam. All significant values are indicated in bold (P-value < 0.05). Values significant with a P-value<0.01 are indicated with an *.

	Bile Bay	**Luminao**	**Pago Bay**	**Urunao**
Bile Bay	**-**			
Luminao	**0.008***	–		
Pago Bay	**0.013**	**0.002**	–	
Urunao	**0.007**	**0.006***	0.003	**-**

Accordingly, both the PCA and STRUCTURE analyses were not able to discriminate between sampling sites, reflecting the low amount of genetic structure detected with the pairwise F_{ST} analysis (fig. 13).

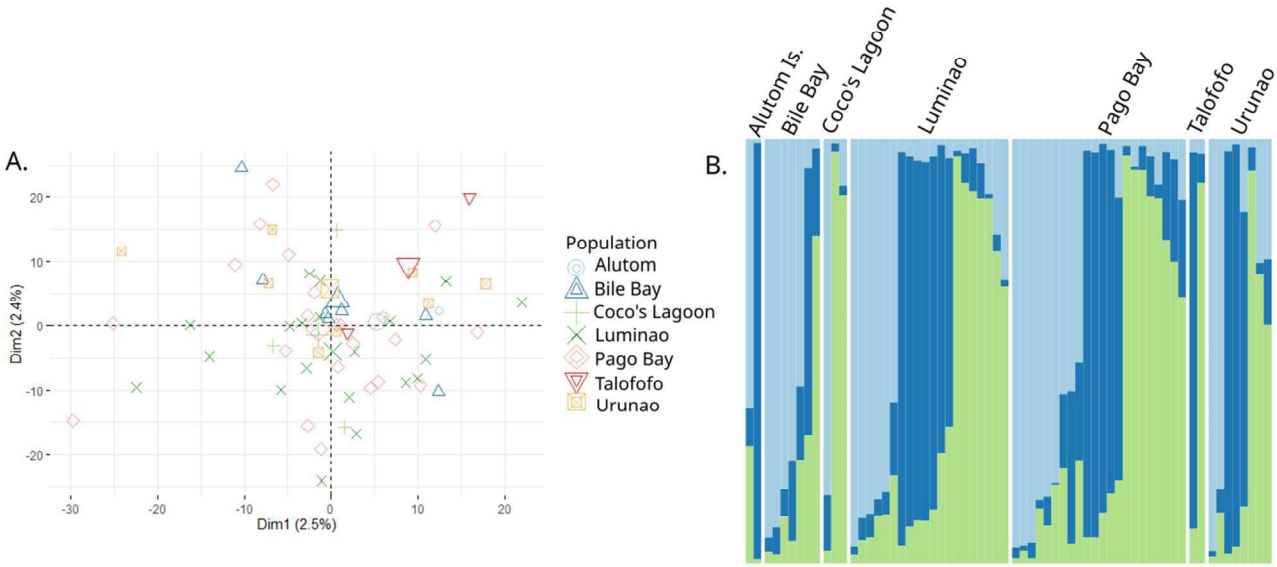

Figure 12. A) PCA and B) STRUCTURE of *T. bidentata* at sites around Guam. K=3 for STRUCTURE analysis.

Overall, genetic diversity statistics did not vary greatly between sites. Heterozygosity (H_O) ranged from 0.25 to 0.26 (Luminao and Pago Bay, respectively) and Inbreeding (F_{IS}) ranged from 0.14 to 0.15 (Pago Bay and Luminao, respectively; table 4).

Table 4. Population genetic summary statistics calculated for each of the sampling sites around Guam.

Population	Number of samples	Number of polymorphic sites	Observed heterozygosity (H_O)	Expected heterozygosity (H_S)	Inbreeding coefficient (F_{IS})
Bile Bay	8	1964	0.31	0.36	0.15
Luminao	20	2110	0.25	0.30	0.15
Pago Bay	22	2035	0.26	0.30	0.14
Urunao	8	2017	0.29	0.33	0.12

The relatedness analysis confirmed that individuals within each population around the island were not related to each other, as represented by negative measures of relatedness (fig. 15).

Further, sites were characterized according to *Pocillopora* coverage ranging from 1.3% to 8.7% (Bile Bay and Luminao, respectively; fig. 15). Coverages were significantly different ($p < 0.05$).

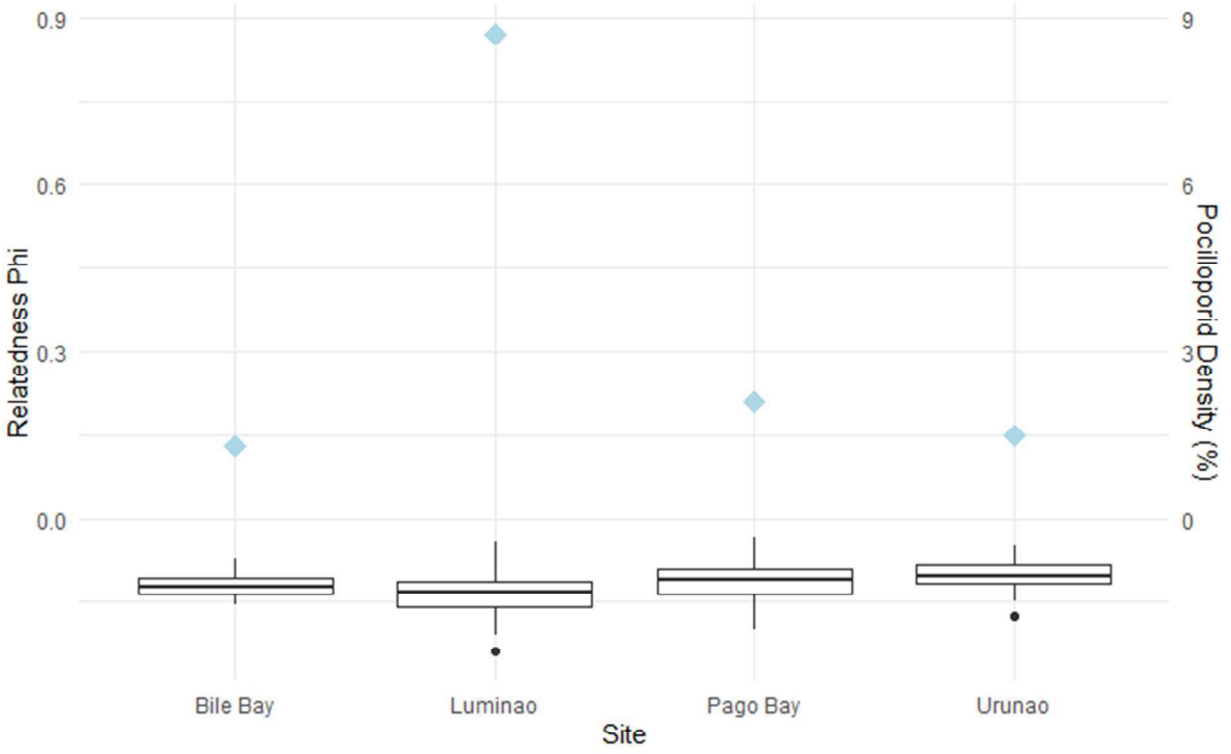

Figure 13. Relatedness analysis within sites around Guam. The y-axis (left) shows the relatedness phi value, the y-axis (right) shows pocilloporid density (%) as measured in our CPC analysis, and the x-axis shows the different sites around Guam. Box plots represent relatedness values and standard deviation and light blue diamonds represent the average pocilloporid density (%) at each site.

Guam vs. Mo'orea:

To estimate diversity indices of Guam and Mo'orea populations, we first subsampled our Guam populations to match the population size of Mo'orea (20 samples) and compared both islands as whole populations. From our genetic summary statistics, we can conclude that both islands harbor similar amounts of genetic diversity (table 5).

Table 5. Population genetic summary statistics for Guam and Mo'orea.

Population	Number of samples	Number of polymorphic sites	Observed heterozygosity (H_o)	Expected heterozygosity (H_s)	Inbreeding coefficient (F_{is})
Guam	20	2074	0.25	0.29	0.14
Mo'orea	20	2037	0.25	0.30	0.17

To assess the demographic history of the Guam and Mo'orea populations, we ran Stairway Plots. 104 sequences and 1318 shared SNPs were retained for Guam and 30 sequences, and 1110 shared SNPs were retained for Mo'orea. The current effective population size (*N*e) of Guam appears to be higher than Mo'orea's (18K vs. 4K, respectively). Both plots showed a general decline in effective population size through time, however, Guam's *N*e appeared bimodal while Mo'orea's *N*e displayed a more steady and gradual decline through time (fig. 15). Both populations' *N*e reached a peak of 150K and remained stable between 200,000 and 100,000 years ago. Mo'orea's *N*e decreased around 6,000 years ago until reaching today's *N*e of 4K about 300 years ago. Guam's *N*e decreased first around 50,000 years ago to 40K before increasing to 75K around 1,500 years ago and eventually experienced a sharp and fast decrease around 400 years ago to reach today's *N*e of 18K.

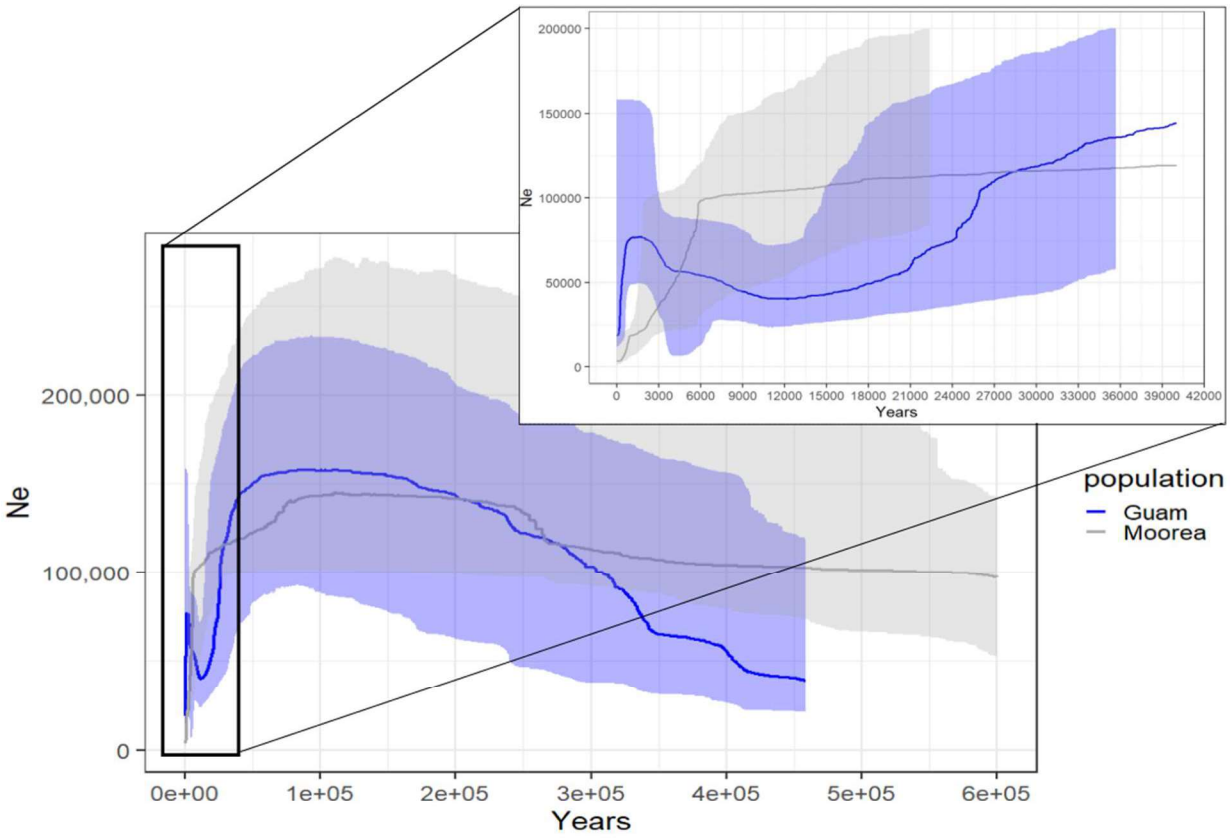

Figure 14. Stairway Plot of both Guam (blue) and Mo'orea (grey). The y-axis represents effective population size and the x-axis represents time in years. Present day indicated on the far left. The ribbon represents the 95% (CI 95%). Zoom window shows the last 40K years in greater detail.

Chapter 4: Discussion

Overall, this study clarified the phylogenetic position of *T. bidentata* and the relationships between 11 species of *Trapezia*. Within *T. bidentata*, we found structure on the global scale, but not on the island scale. There were four distinct genetic clusters throughout the geographic range of *T. bidentata* including the Indian Ocean, Central and West Pacific, and the Marquesas Islands with admixture between the Central and West Pacific. There was expected divergence between the north and south Indian Ocean samples and little structure within any other clade. When comparing Mo'orea to Guam, we found that they were very similar in terms of population structure and diversity. Our large data set is able to tell a more complete story using thousands of SNPs across

the entire genome. This kind of extensive dataset can help disentangle phylogenies and provide greater insight into population history, structure and adaptability.

A clarified phylogeny of *Trapezia*:

Over the years, there has been debate over the phylogenetic relationships of *Trapezia* species and studies using one or a few genetic markers obtain conflicting tree topologies. It is an especially intriguing topic as several sister morphospecies have striking variations in color pattern without being greatly distinctive genetically (McKeon 2010). Further, it has also been proposed that color pattern is one of the first characters to change during speciation events within the genus (Knowlton 1986). In this study we clarify the backbone of the *Trapezia* tree as well as several interspecies relationships within the genus. It is important to note, however, that our current phylogeny does not include all species within the genus and thus some parts of the tree remain unresolved. Further, while there are fossils of extinct *Trapezia* species, none of them are dated and so we cannot speak on the evolutionary time scale of the speciation events. Lastly, our tree currently does not have an appropriate outgroup, so we used the most distant clade within the genus, *T. rufopunctata* and *T. flavopunctata*, to root our tree, similar to previous and unpublished phylogenetic studies (McKeon 2010; G. Paulay personal communication).

Regarding the placement of individual species within our phylogeny, the configuration varies quite drastically from previous studies. For instance, while *T. bidentata* formerly appeared sister to *T. septata* (Rouzé et al. 2017 with CO1) or to *T. cymodoce* and *T. lutea* (Rouzé et al. 2017 with 16s) or to *T. septata* and *T. guttata* (Canizales-Flores et al. 2020 with CO1), here it is recovered as sister to several species including *T. lutea, T. cymodoce, T. punctimanus,* and *T. guttata* (100% BS). *Trapezia lutea* and *T. cymodoce* are sister species across all current

phylogenies, and our tree also supports this relationship (Rouzé et al. 2017; Canizales-Flores et al. 2020). *Trapezia serenei, T. tigrina,* and *T. speciosa* retain their relationship as presented in Rouzé et al. (2017)'s CO1 tree, however, our tree provides much higher support (68% vs. 100% BS). *Trapezia guttata*'s placement used to vary greatly depending on the marker used, for example in Rouzé et al. (2017)'s 16s tree it appears as sister to the rest of the genus, but in Rouzé et al. (2017) and Canizales-Flores (2020)'s CO1 trees it appears as sister to *T. punctimanus* and *T. rufopunctata* or as sister to *T. septata,* respectively. Our tree clarifies its position as sister to *T. punctimanus* (100% BS). Finally, *T. digitalis* appears as sister to the other species of *Trapezia*, except for *T. rufopunctata* and *T. flavopunctata,* while previously it had never been incorporated into a phylogeny.

Trapezia rufopunctata and *T. flavopunctata* are supported as a genetically distant sister taxa to all other *Trapezia* in all our analyses as previously found in McKeon (2010). In their dissertation, McKeon also considered assigning them to a potential sub-genus due to their distance and distinct morphological characteristics from the rest of the genus. Further, because we sampled an ample amount of *T. rufopunctata* compared to other species, we were able to see that they express minimal genetic structure between populations from the Indian and Pacific Oceans while most other *Trapezia* species display some level of genetic structure, detectable with single markers, across the two oceans (Rouzé et al. 2017). This may suggest that *T. rufopunctata* larvae have a longer pelagic larval duration than other species or can potentially tolerate harsher environments and are thus able to be more ubiquitous than other species. Additionally, *T. rufopunctata* and *T. flavopunctata,* while expressing the same overall coverage as the other species (~14X), these distant taxa share few loci with the rest of the genus (Supplementary Figure 3).

Phylogeography of *Trapezia bidentata* reveals deep genetic divergence and putative cryptic species

Within *T. bidentata*, we found evidence of four distinct genetic clusters: in the Indian Ocean (Saudi Arabia, Madagascar, Mayotte, and Mascarene Islands), West Pacific (Wake Island, Kiribati, Mariana Islands, Marshall Islands, and Papua New Guinea), Central Pacific (French Polynesia, American Samoa, Kiribati, and Wake Island), and the Marquesas Islands supported by all our analyses. This is in support of our first hypothesis of potential cryptic speciation within *T. bidentata*. We do not, however, detect morphological variation between groups, despite previous records of such, and thus we do not describe any of the four clades as new species here. This is not to say there is no morphological variation as it is possible that there may be significant morphometric measurements with a larger sample set of crabs within the same size-class.

The greatest divergence within *T. bidentata* is the separation between the Indian and Pacific Oceans. Previous studies reporting similar discontinuities between the two ocean basins have traced the separation back to historical biogeographic barriers at the time of the Last Glacial Maximum. During glaciation, sea levels dropped drastically, exposing submerged land and creating barriers to dispersal for several marine organisms (Keyse et al. 2018, DeBoer et al. 2014, Nelson et al. 2000). Two prominent historical biogeographic barriers are the Torres Strait and the Sunda Shelf. The Torres Strait was once a land bridge that connected southern Papua New Guinea and northeastern Australia, separating the Gulf of Carpentaria and the Coral Sea. The land bridge formed during the Pleistocene and submerged as sea levels rose, however, it remained intact for ~80% of the last 250 kyr (Voris 2000). The Sunda Shelf is the shallow reservoir connecting the countries of Southeast Asia. Similar to the Torres Strait, as sea level dropped, this land was exposed, reducing flow between the Indian and Pacific Oceans (Voris 2000). As sea levels rose,

connection between the oceans opened and allopatrically separated species regained contact (Crandall et al. 2008). This temporary restriction of flow between the oceans has led to several genetic divergences between marine organisms that disperse as planktonic larvae including populations of *Tridacna* clams, the false clownfish *Amphiprion ocellaris*, and the brooding coral *Seriatopora hystrix* (van der Ven et al. 2021, Keyse et al. 2018, DeBoer et al. 2014, Nelson et al. 2000). The lack of mixing between the Indian and Pacific Ocean clades of *T. bidentata,* supported by our STRUCTURE analysis, aids in the conclusion that *T. bidentata* was also impacted by these historic barriers. Further, the lack of admixture between the groups thus suggests potential allopatric speciation and that these populations have been separate for an extensive period of time. Additionally, variation in color patterns between these geographically separated groups were identified by McKeon (2010). While we cannot support his conclusion without examining fresh specimens, we can contribute observations of morphological differences that suggest these groups are actually two separate species.

Our data also reports limited connectivity between the Red Sea and southern Indian Ocean as represented in our PCA and STRUCTURE analyses. A study by Farhadi et al. (2017), found that the population of spiny lobster, *Panulirus homarus*, in the northwest Indian Ocean was similarly genetically isolated and proposed the cause to be the Arabian Sea Gyre, local eddies, and east-west monsoon currents (Farhardi et al. 2013). They suggested that local eddies within the Arabian Sea likely impede larval transport to the east while seasonal interactions between the South Equatorial Current, the Somali Current, and the Equatorial Countercurrent further drive separation between northern populations and those south of Tanzania (Farhardi et al. 2017; fig. 16). With a long pelagic larval phase, upwards of six months, and a broad geographic range throughout the Indo-Pacific, *P. homarus* likely experiences similar, if not higher, dispersal abilities

to *T. bidentata*. Further, seasonal cold-water upwelling events may impede sensitive larvae along with high levels of salinity and temperature that are harbored in the Red Sea (Torquato et al. 2019, DiBattista et al. 2016). While our STRUCTURE analysis shows signs of admixture between populations in the northern and southern Indian Ocean, the barriers described above likely hinder constant intermixing between populations. Admixture between populations is likely dependent on seasonality and influenced by monsoon intensity. Further, the great distance between the Red Sea and Madagascar likely perpetuates genetic isolation between populations.

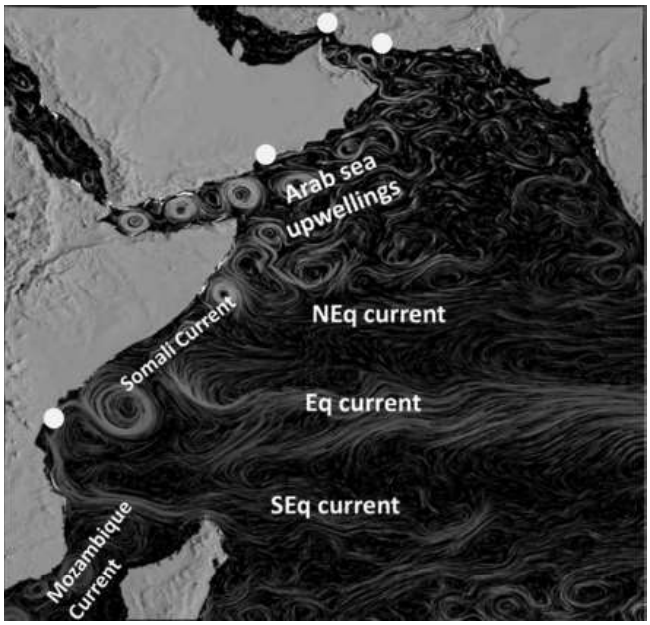

Figure 15. Map of currents between the northern and southern sites in the Indian Ocean. Source: Farhardi et al. 2013.

Within the Pacific Ocean, we also found three distinct genetic clusters: the Marquesas Islands, and the Central and West Pacific. In particular, the Marquesas Islands were highly genetically distinct from neighboring populations as displayed in all our analyses. Although thus far undetected in *T. bidentata*, this divergence is concordant with extensive reports of endemism within the archipelago (Randall 2001, Gaither et al. 2010, Szabó et al. 2014). Marine endemism in

the Marquesas Islands is largely influenced by geographic isolation and unique sea temperatures driven by equatorial upwelling, ENSO events (Randall 2001), as well as the South Equatorial and Marquesas Counter Currents impeding dispersal from the east (Randall 2001; Lemer et al. 2014). The closest neighboring islands are the Tuamotu Archipelago (500 km to the West) and the Galapagos Islands (3700 km to the East) (Randall 2001). Further, reef systems throughout the Marquesas archipelago are patchy, scattered, and low in diversity and lack any form of barrier reef (Randall 2001, Fey et al. 2020).

While most studies regarding endemism at this locality widely focus on reef fish populations, a small collection of studies speak of genetic divergence in species with a long pelagic dispersal phase such as the black-lipped pearl oyster *Pinctata margaritifera* (15-30 day dispersal; Thomas et al. 2014; Lemer et al. 2014; Reisser et al. 2019). Here we identified minimal admixture between the Marquesas Islands and any other population. It is thus likely that, despite its long pelagic larval phase, *T. bidentata* is also victim to the various environmental factors influencing genetic divergence in the archipelago. Further, the minimal coral coverage and lack of barrier reefs may help explain the fine-scale structure we observe within the island system. Like *P. margaritifera*, *T. bidentata* not only has a long dispersal phase, but also must find suitable habitat to settle (Lal et al. 2017; Thomas et al. 2014) which may further deplete population size and decrease connectivity throughout the archipelago. Additionally, harsh currents and the lack of protection in the form of a barrier reef may create a difficult environment to take up residence (Lemer et al. 2014), leading to greater mortality during early settlement stages.

Apart from the Marquesas Islands, *T. bidentata* in the Pacific Ocean make up two distinct groups: the Central and West. While other genetic clusters within our study can be drawn back to historical, oceanographic, or biophysical barriers, the incomplete delineation between the Central

and West Pacific in *T. bidentata* is likely attributed to geographic distance and habitat heterogeneity. In a study by Lal et al. (2017), they found that while currents greatly stimulated population connectivity across the Pacific Ocean, regional circulation patterns and extensive geographic distances along with varying habitat geomorphology, helped maintain population structure throughout the ocean basin. As our sampling sites within the Pacific Ocean cover an expanse of 9,100 km between our two furthest sites (Mariana Islands and Tuamotu Archipelago), it is thus reasonable to believe that mixing between these distant populations is infrequent. Further, reef status, both in terms of degradation and species composition, have fluctuated differently throughout the Pacific over time, driven by regional influences such as storms and anthropogenic impacts, among other factors (Bruno & Selig 2007). Due to *T. bidentata*'s reliance on a specific host, it is likely that their ability to colonize certain localities fluctuated with host availability.

With admixture throughout the Pacific Ocean, it is logical to conclude that *T. bidentata* has not been present long enough to diversify throughout the region, is not impeded by present barriers, and that the larval phase of *T. bidentata* is likely long to have sustained minimal structuring over thousands of years. Significantly, this would imply that *T. bidentata* has an expansive gene pool with highly connected populations across greatly heterogenous environments. From this we can infer that they have a high adaptive capacity, however, considering their dependence on a susceptible host, they remain at risk to changing climates and rising sea temperatures.

Island-scale connectivity:

Overall, there was minimal genetic structure among populations on Guam. The greatest amount of structure was found between the eastern and southern sites of the island while there was low structure between the East and West implying that mixing between sites around the island was

not equal. Wolanski et al. (2003) found that the North Equatorial Current creates several eddies around the northern point of Guam and overall that the energetic nature of eddies around Guam enable larvae to return to their natal sites (fig. 18). This strong net northward current may increase mixing around the northern point of the island, increasing population connectivity between the East and West sites while directing larvae away from the south. Additionally, because there is a lagoon at the southern tip of the island, larval transport may be impeded due to decreased wave action.

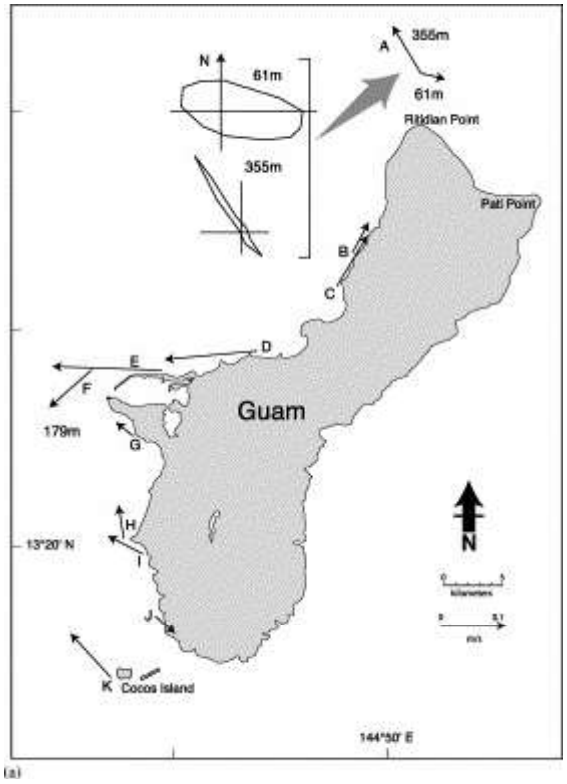

Figure 16. Net currents around Guam with an inset showing the directional histogram of currents. Source: Wolanski et al. 2003.

Furthermore, genetic diversity statistics did not vary greatly between sites and individuals within each site were not related. This supports that the larval phase of *T. bidentata* is sufficiently long to enable transport away from their natal site, despite potential retention driven by local eddies. Additionally, *Trapezia* are highly reliant on their host coral, however, while this appears

to impact species presence (personal observation), it does not seem to impact relatedness between individuals or population genetic diversity levels; most likely because *T. bidentata* lack host preference beyond the family level (McKeon 2010).

Diversity and Demography of two Pacific Islands:

In this study we see that, while Guam is much larger and likely plays host to a larger population, Mo'orea surprisingly maintains a similar level of diversity in its populations. Considering the extensive lagoonal system that surrounds the island (fig. 18), it is possible that there is similar amounts of available habitat for *Trapezia* in Mo'orea than in Guam, so despite the size discrepancy between the islands, they could be host to the same size of populations. Further, this difference in diversity is potentially due to variability in pocilloporid coral coverage around the islands and the size of the colonies available to exosymbiotic invertebrates. While both reef systems have experienced various stressors, their fluctuations in community composition have varied greatly (Adjeroud et al. 2018; Heron et al. 2020; Speare et al. 2021). However, despite it being likely that host availability played a great role in the diversity levels we are seeing, it is important to note that the samples from Mo'orea were sampled roughly 16 years prior to our sample collection around Guam. It is thus probable that events during the time in between collections could have altered the community composition and diversity of *T. bidentata*.

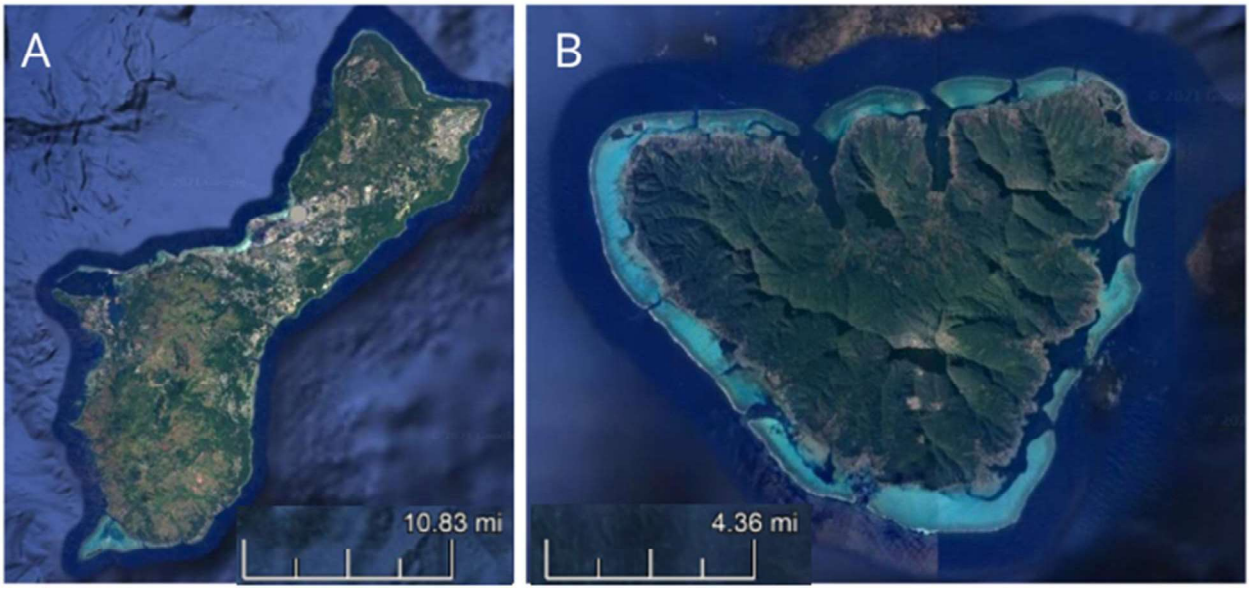

Figure 17. Images of A) Guam and B) Mo'orea. Source: Google Earth Pro.

The demographic history of *T. bidentata* varies quite drastically between the two islands. While Guam appears to fluctuate in effective population size, Mo'orea steadily declined to its present-day size. Firstly, Guam's populations experience a continual decline from 50,000 to 20,000 years ago transitioning from consecutive thermal maxima to the Last Glacial Maximum as temperature and sea levels fell, which occurred at the end of the Pleistocene into the Holocene, and then experienced a resurgence ~10,000 years ago as the glaciers receded, sea levels rose, and ocean temperatures increased again (Yokoyama et al. 2018). This rapid increase in effective population size could potentially be due to its proximity to the coral triangle biodiversity hotspot and to the equator where the ice would have thawed first. This decline, however, is absent from Mo'orea's demographic history, potentially due to the deep lagoonal system that surrounds the island. It is possible that enough water remained within the lagoon to support coral populations, or that some corals remained on the outer reefs, albeit shifted downslope (Paulay 1990). Because pocilloporid corals live within the top 60 m (Hibbert et al. 2018), they would need to rapidly

proliferate and grow to gradually move down-slope as sea-levels fell. As pocilloporids are fast-growing corals and broadcast spawners (Hibbert et al. 2018), they were likely able to compete with the depressing sea-levels and even maintain invertebrate assemblages during this time. Further, recolonization of these depleted lagoonal systems is thought to have happened rapidly in organisms with long pelagic larval phases (Paulay 1990). Secondly, the gradual decline of Mo'orea's *Trapezia* populations between 6,000 to 200 years ago could be in part due to anthropogenic impacts during the Holocene (Adjeroud et al. 2009, Stella et al. 2011a, Peters et al. 2015, Pratchett et al. 2018). However, according to Stevenson et al. (2017), the first signs of human presence on the island occurred around 1060–980 cal. yr. BP, thus this decline was seemingly initiated by a separate influence. It could be due to rising sea levels as current sea level was reached around this time or environmental changes that occurred early in the Holocene (Hallmann et al. 2020). Because Mo'orea is isolated from biodiversity hotspots, it is likely that *Trapezia* were not able to replenish populations devastated by reef exploitation as they may have temporarily on Guam. Guam's population drastically declined around 400 years ago, this could be linked to reef degradation associated with human impacts becoming overwhelming and habitat availability diminishing significantly.

There is also potentially great variability in the composition of pocilloporid coral communities on either island. As mentioned earlier, differences in dominant coral species after coral die-offs may have led to different sizes of pocilloporid colonies present for symbiotic invertebrates to colonize. Further, fluctuations in species composition due to various stressors and the differences in source population availability to the two islands could greatly impact their ability to host a variety of species. While we are unable to see these events in our analyses for Mo'orea as the samples were collected before the die-off occurred, it drives further questions of the status

of populations today and if this radical decline and successive proliferation of pocilloporid corals impacted the persistence of the invertebrates they play host to or otherwise changed regional connectivity. On a broader scale, it encourages greater observation of populations far from biodiversity hotspots due to their inability to be rapidly recolonized. As we venture further into the Anthropocene, these distant populations may be at the greatest risk of isolation, heightened inbreeding, and deleterious mutations that follow species range limits (Fifer et al. 2022).

Conclusion

Overall, the lack of population structure on the island scale and minimal genetic structure within the three Pacific Ocean groups suggests that *T. bidentata* has an extensive pelagic larval phase, allowing it to travel great distances and mix with neighboring populations. This ability of an otherwise sedentary organism to disperse throughout the Indo-Pacific with little impedance creates an expansive gene pool, greatly increasing its adaptive capacity. Further, with low admixture between the four genetic groups and the greatest drivers of divergence being currents and historical barriers to dispersal, it is logical to assume that allopatry is the primary method of speciation within *Trapezia*. It is thus important to investigate and demystify other cryptics within this genus and to increase the number of genetic studies in this system.

The present study provides greater insight into the phylogeography and population genetics of *T. bidentata* throughout its geographic range. It also helps to clarify the phylogenetic tree of several species within the genus using a highly supported SNP data set. Overall, the contribution of high-throughput genetic analysis is an exemplary tool in delineating species and for providing a more in-depth view of population connectivity and dynamics. Further, this study reveals several

unanswered questions about this genus and supports it as a remarkable system of crustacean phylogenetics.

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Supplementary Materials

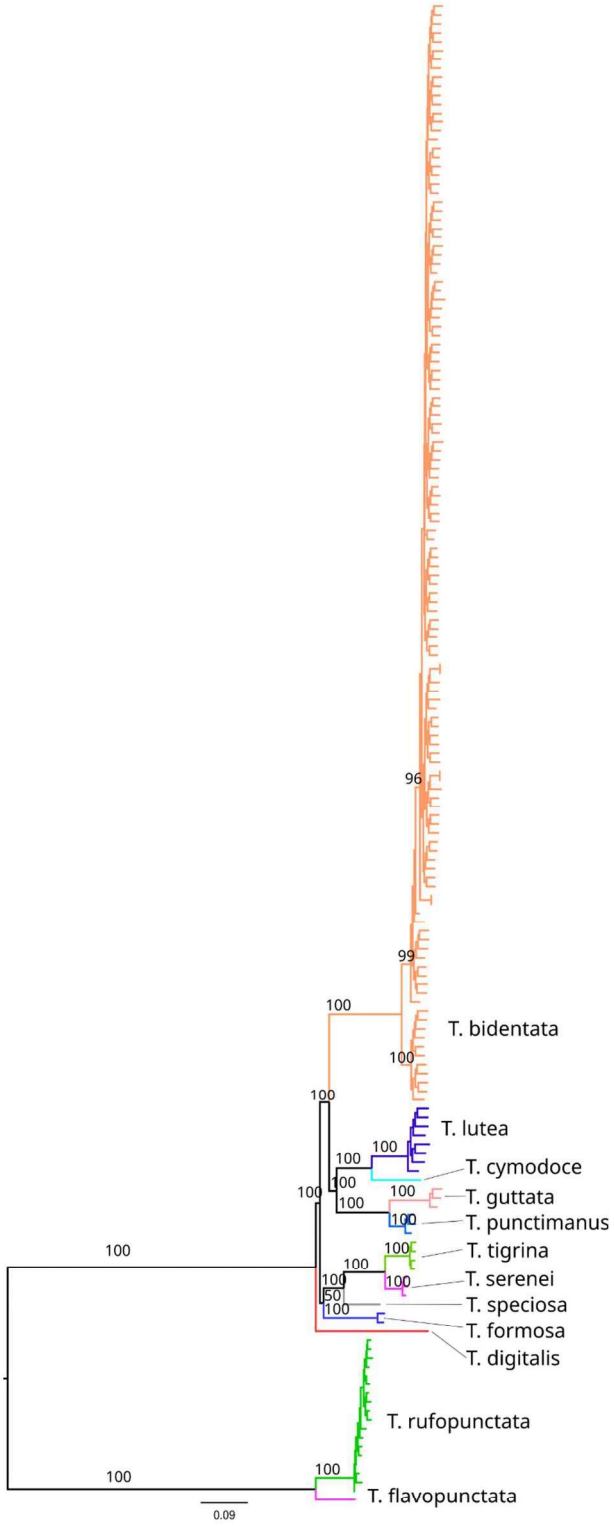


Figure 1. Phylogenetic tree of all species constructed in RAxML using GRAS-Di data.

Supplementary Table 1. List of samples obtained from the Florida and Paris Museums.

Museum	Museum ID	#	Genus	Species	Country	State	County	Locality	Collectors	Collection Date
Florida	10208	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Maharepa, Stier's Pocillopora site	McKeon, Christian	5/31/2006
Florida	10215	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Maharepa, Stier's Pocillopora site	McKeon, Christian	5/31/2006
Florida	10219	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Moorea	McKeon, Christian	5/31/2006
Florida	10222	3	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Moorea	McKeon, Christian	5/31/2006
Florida	10232	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Sea's Locality	McKeon, Christian	7/31/2006
Florida	10233	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Sea's Locality	McKeon, Christian	7/31/2006
Florida	10240	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Barrier Locality	McKeon, Christian	5/31/2006
Florida	10241	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Barrier Locality	McKeon, Christian	5/31/2006

Florida	10274	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Maharepa, Stier's Pocillopora site	McKeon, Christian	5/31/2006
Florida	10292	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Maharepa, Stier's Pocillopora site	McKeon, Christian	5/31/2006
Florida	11104	2	Trapezia	bidentata	USA	Line Islands	Palmyra Atoll	Ssw side of Atoll	Knowlton, Nancy; Paulay, Gustav	8/18/2005
Florida	12932	1	Trapezia	bidentata	Mascarene Islands	Reunion Island		Saint-Gilles, Passe de l'Hermitage	Bruggemann, Henrich; Hubert, Nicolas; Michonneau, Francois; Paulay, Gustav	8/17/2007
Florida	13100	1	Trapezia	bidentata	Mascarene Islands	Reunion Island		Saint-Gilles, Passe de l'Hermitage	Bruggemann, Henrich; Hubert, Nicolas; Michonneau, Francois; Paulay, Gustav	8/17/2007
Florida	13382	2	Trapezia	bidentata	Mariana Islands	Guam	Guam Island	Talafofo, in front of Jeff's Tavern	Michonneau, François	3/21/2008
Florida	14515	1	Trapezia	bidentata	Madagascar			Nosy Be, reef slope just S of CNRO	Boissin, E.; Michonneau, F.; Hoareau, T.; Werner, T.	5/19/2008
Florida	14516	1	Trapezia	bidentata	Madagascar			Nosy Be, reef slope just S of CNRO	Boissin, E.; Michonneau, F.; Hoareau, T.; Werner, T.	5/19/2008

Florida	14689	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Barrier Locality	McKeon, Christian	5/31/2006
Florida	14697	8	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Barrier Locality	McKeon, Christian	5/31/2006
Florida	17902	1	Trapezia	bidentata	Republic of the Marshall Islands	Marshall Islands	Majuro Atoll	Laura, ocean side, N end of the island	Michonneau, François; Kim, Sun	4/4/2008
Florida	17904	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	off Floating Hotel West Side	McKeon, Christian	5/31/2006
Florida	17918	1	Trapezia	bidentata	Mariana Islands	Guam	Guam Island	Alutom Islet, NE Side	Conley, H.	6/6/2002
Florida	18043	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	S of Vaiare Pass	Meyer, Christopher; McKeon, S.; Paulay, Gustav; Moore, Jenna	10/27/2008
Florida	18368	1	Trapezia	rufopunctata	French Polynesia	Society Islands	Moorea Island	Moorea		9/30/2008
Florida	18485	1	Trapezia	bidentata	French Polynesia	Tuamotu Archipelago	Makemo Atoll	Mekemo	Bacchet, Philippe; Letourneaux, John	3/31/2009
Florida	18489	1	Trapezia	bidentata	French Polynesia	Tuamotu Archipelago	Makemo Atoll	Mekemo	Bacchet, Philippe; Letourneaux, John	3/31/2009
Florida	19921	1	Trapezia	rufopunctata	Taiwan			Nanwan Bay	McKeon, Sea	7/31/2009

Florida	2957	1	Trapezia	bidentata	Mariana Islands	Guam	Guam Island	Alutom Islet, N and NE Side	Conley, H.	6/10/2002
Florida	29900	1	Trapezia	rufopunctata	French Polynesia	Marquesas Islands	Nuku Hiva Island	Haahopu Bay, isolated rock 100 meters offshore, W side of island	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	11/22/2011
Florida	29968	1	Trapezia	bidentata	French Polynesia	Marquesas Islands	Eiao Island	bay N of Vaituhu Bay	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	11/24/2011
Florida	30149	4	Trapezia	bidentata	French Polynesia	Marquesas Islands	Hiva Oa Island	Matafenua, second bay N, W side of island	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	12/2/2011
Florida	30155	1	Trapezia	rufopunctata	French Polynesia	Marquesas Islands	Hiva Oa Island	Matafenua, NW side of island	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	12/1/2011
Florida	30170	1	Trapezia	bidentata	French Polynesia	Marquesas Islands	Fatu Hiva Island	Mahitoa Point, SW side of island	Evans, Nathaniel; Andrefouet, Serge;	12/3/2011

									Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	
Florida	30219	1	Trapezia	rufopunctata	French Polynesia	Marquesas Islands	Tahuata Island	Anaete Bay, S side of bay, W side of island	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	12/5/2011
Florida	30266	3	Trapezia	bidentata	French Polynesia	Marquesas Islands	Hiva Oa Island	S of Kiukiu, W side of island	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	12/8/2011
Florida	35233	1	Trapezia	rufopunctata	Japan	Ryukyu Islands	Okinawa Island	Okinawa, Horseshoe Manza	Evans, Nathaniel; Michonneau, Francois; Schils, Tom; Devilliers, Amanda; Uyeno, Daisuke	7/2/2010
Florida	35649	1	Trapezia	bidentata	French Polynesia	Marquesas Islands	Ua Huka Island	islet W of Kohai, N of Haunanu.	Evans, Nathaniel; Andrefouet, Serge; Benzoni, Francesca; Menou, Jean-Louis; Olivirio, Marco; Payri, Claude	11/29/2011
Florida	36473	1	Trapezia	bidentata	Saudi Arabia			Farasan Banks, Shi'b Ammar	Anker, Arthur; Norby, Patrick; Paulay, Gustav	3/3/2013

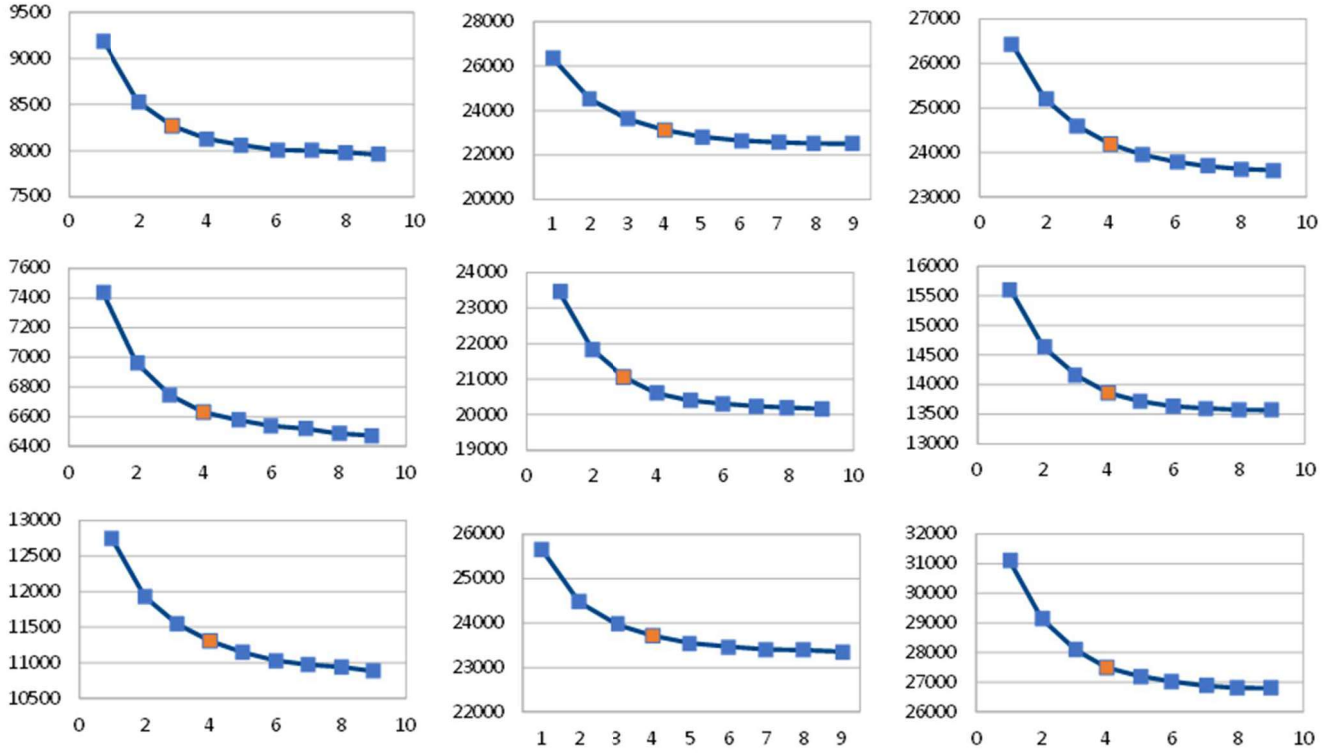
Florida	37912	1	Trapezia	rufopunctata	French Polynesia	Marquesas Islands	Hatutu Island	Hatutu	White, Jada	9/17/2008
Florida	38167	2	Trapezia	bidentata	Saudi Arabia			Gulf of Aqaba, Joey's Shipwreck Bay	Paulay, Gustav; Uyeno, Daisuke	10/1/2013
Florida	38640	1	Trapezia	bidentata	Kiribati	Line Islands	Millenniu m	Millennium Island, tent site	Michonneau, François; Knowlton, Nancy	11/6/2013
Florida	40719	4	Trapezia	bidentata	Kiribati	Line Islands	Malden Island	Malden Island, tent site	Michonneau, Francois; Leray, Matthieu; Knowlton, Nancy	11/1/2013
Florida	40844	1	Trapezia	bidentata	Kiribati	Line Islands	Millenniu m	Millennium Island	Michonneau, Francois; Leray, Matthieu; Knowlton, Nancy	11/8/2013
Florida	42301	1	Trapezia	rufopunctata	French Polynesia	Marquesas Islands	Mohotani Island	Mohotani	White, Jada	10/17/2008
Florida	46807	1	Trapezia	rufopunctata	New Caledonia	Province Sud		Mbéré barrier reef, N of Dumbea Commune Pass	Paulay, Gustav; Uyeno, Daisuke; Moroz, Leonid	11/8/2017
Florida	48426	1	Trapezia	bidentata	Saudi Arabia			Al Qunfudhah, Dorish Island	Uyeno, Daisuke; Lasley, Robert; Moore, Jenna	10/16/2014

Florida	48433	1	Trapezia	bidentata	Saudi Arabia			Al Qunfudhah, Dorish Island	Uyeno, Daisuke; Lasley, Robert; Moore, Jenna	10/16/2014
Florida	50808	1	Trapezia	bidentata	Kiribati	Phoenix Islands	Kanton Island	Kanton Island, North of channel	Lasley, Robert	9/7/2015
Florida	51560		Trapezia	bidentata	Kiribati	Phoenix Islands	Nikumaro ro Island	Nikumaroro Island, Nai'a Point	Lasley, Robert	9/23/2015
Florida	6099	1	Trapezia	bidentata	Mariana Islands	CNMI	Zealandia Bank	N/A		8/25/2003
Florida	8435	2	Trapezia	bidentata	Wake Island			N/A	Bonito, V.	11/13/2005
Florida	9281	2	Trapezia	bidentata	American Samoa	Samoa Islands	Ofu Island	N/A	McKeon, Sea	2/26/2007
Florida	9301	1	Trapezia	rufopunctata	American Samoa	Samoa Islands	Ofu Island	N/A	McKeon, Sea	2/26/2007
Florida	9490	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Temae; ENE Shore of Moorea	Malay, Maria Celia; Emmanuelli, Esther	5/31/2006
Florida	9594	2	Trapezia	bidentata	French Polynesia	Tuamotu Archipelago	Fakarava Atoll	E of The N Pass of Fakarava Atoll; SE of Mfaka-6; closer to Lighthouse	Malay, Maria Celia	6/9/2006

Florida	9784	1	Trapezia	bidentata	French Polynesia	Society Islands	Moorea Island	Fore Reef NE of Tareu Pass	McKeon, Christian; Meyer, Christopher; Paulay, Gustav	7/24/2006
Paris	2009-1042	1	Trapezia	rufopunctata	MAYOTTE				J.-M. Bouchard, V. Dinhut, J. Dumas	11/4/2009
Paris	2009-1045	1	Trapezia	rufopunctata	MAYOTTE				J.-M. Bouchard, V. Dinhut, J. Dumas	11/14/2009
Paris	2009-940	1	Trapezia	bidentata	MAYOTTE				J.-M. Bouchard, V. Dinhut, J. Dumas	11/13/2009
Paris	2009-943	1	Trapezia	bidentata	MAYOTTE				J.-M. Bouchard, V. Dinhut, J. Dumas	11/13/2009
Paris	2011-8989	1	Trapezia	bidentata	ARCHIPEL DES MARQUISES				AAMP	1/19/2012
Paris	2013-11648	1	Trapezia	bidentata	PAPUA NEW GUINEA					12/10/2012
Paris	2013-11782	1	Trapezia	rufopunctata	PACIFIQUE ORIENTAL					
Paris	2013-337	1	Trapezia	bidentata	PACIFIQUE ORIENTAL					

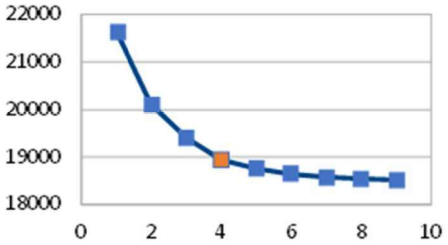
Supplementary Figure 2. STACKS optimization based on Paris et al. (2017). Trial sample sets were chosen randomly using a random number generator. Numbers in top charts are the number of SNPs retained. Orange points are selected SNP values on the charts.

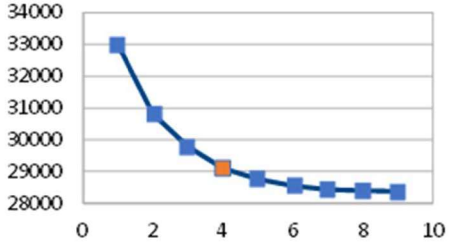
	Set 1			Set 2			Set 3		
	I.SA4	WP.FPMo6	WP.GuamB12	WP.GuamL16	WP.FPMo21	WP.Tai1_R	I.Aus1_R	WP.FPMo14	WP.GuamL20
M1	9182	7441	12745	26354	23457	25665	26438	15605	31126
M2	8531	6964	11931	24505	21837	24471	25201	14637	29130
M3	**8269**	6744	11544	23619	**21059**	23975	24584	14164	28117
M4	8128	**6629**	**11306**	**23121**	20603	**23713**	**24186**	**13860**	**27493**
M5	8061	6577	11146	22823	20401	23549	23948	13718	27204
M6	8009	6537	11032	22654	20309	23466	23788	13633	27022
M7	8000	6518	10972	22576	20241	23409	23692	13602	26879
M8	7982	6485	10942	22521	20203	23389	23630	13576	26808
M9	7958	6471	10884	22507	20163	23356	23598	13565	26794

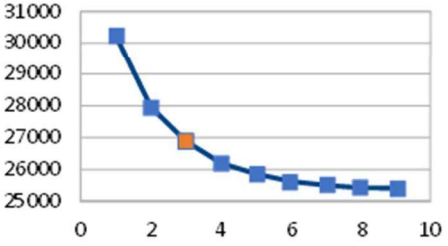


Set 4

	WP.FPMo22	WP.GuamP9	WP.GuamP13
M1	21639	32984	30232
M2	20098	30812	27948
M3	19397	29784	**26881**
M4	**18936**	**29099**	26198
M5	18754	28754	25857
M6	18639	28548	25618
M7	18573	28432	25506
M8	18540	28399	25422
M9	18512	28371	25410

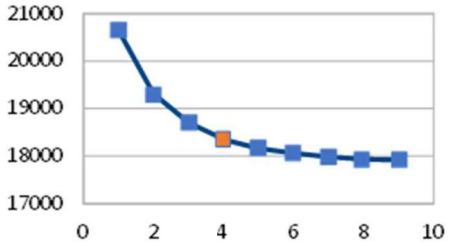


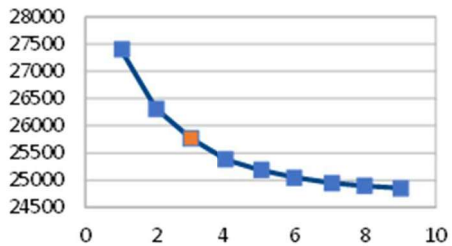


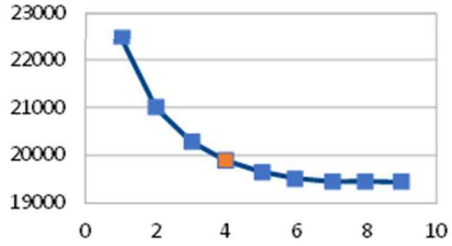


Set 5

WP.FPMo11	WP.GuamB13	WP.GuamU16
20654	27409	22490
19304	26314	21002
18697	**25765**	20291
18353	25383	**19892**
18166	25184	19658
18062	25048	19517
17986	24952	19453
17938	24895	19455
17920	24855	19441







Optimal: M=4

while set to r = 0.8

Optimal: n=5

n3 = 350 loci; 5203 SNPs

n4 = 393 loci, 6241 SNPs

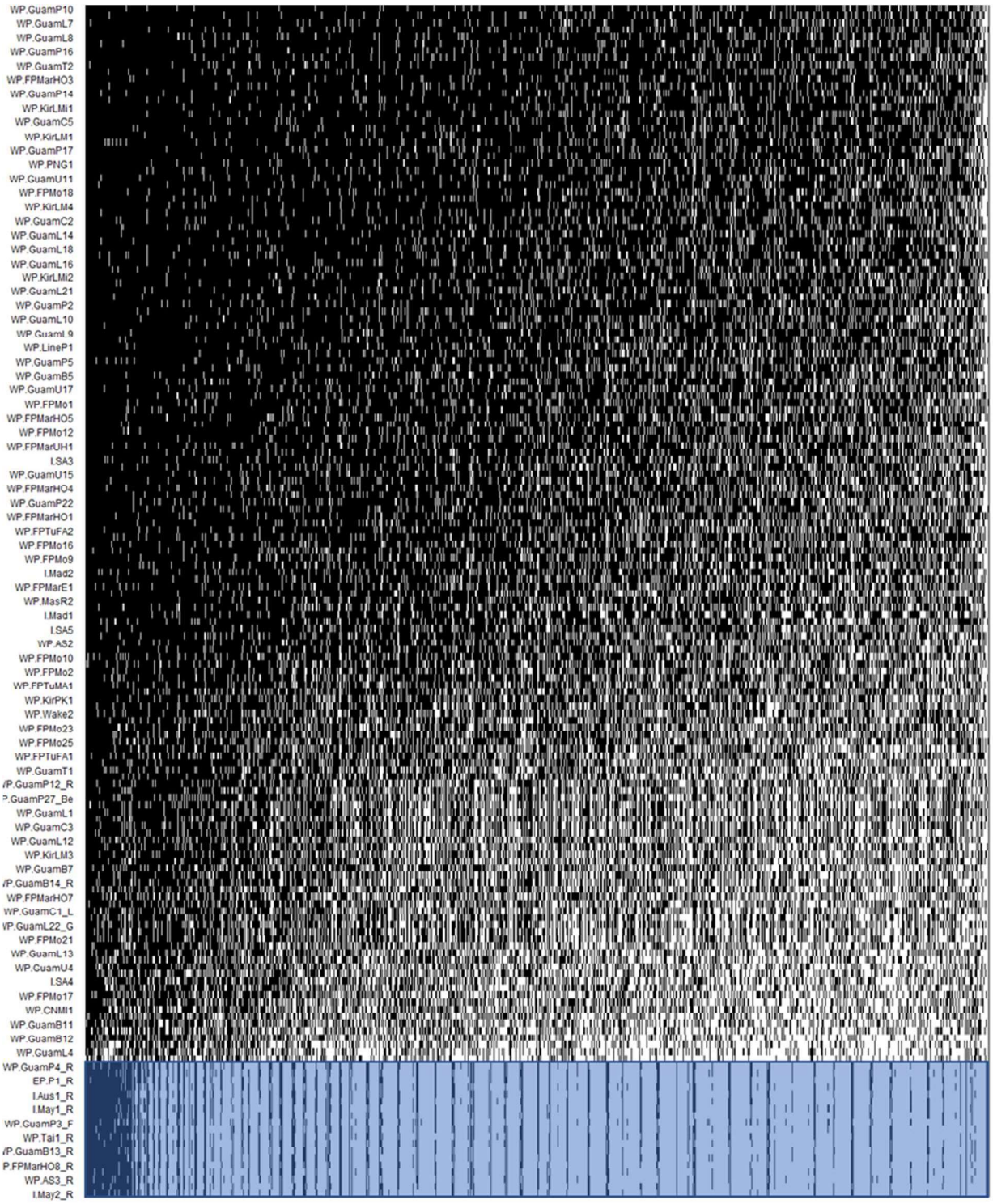
Optimal r=0.5

n5 = 410 loci, 6595 SNPs

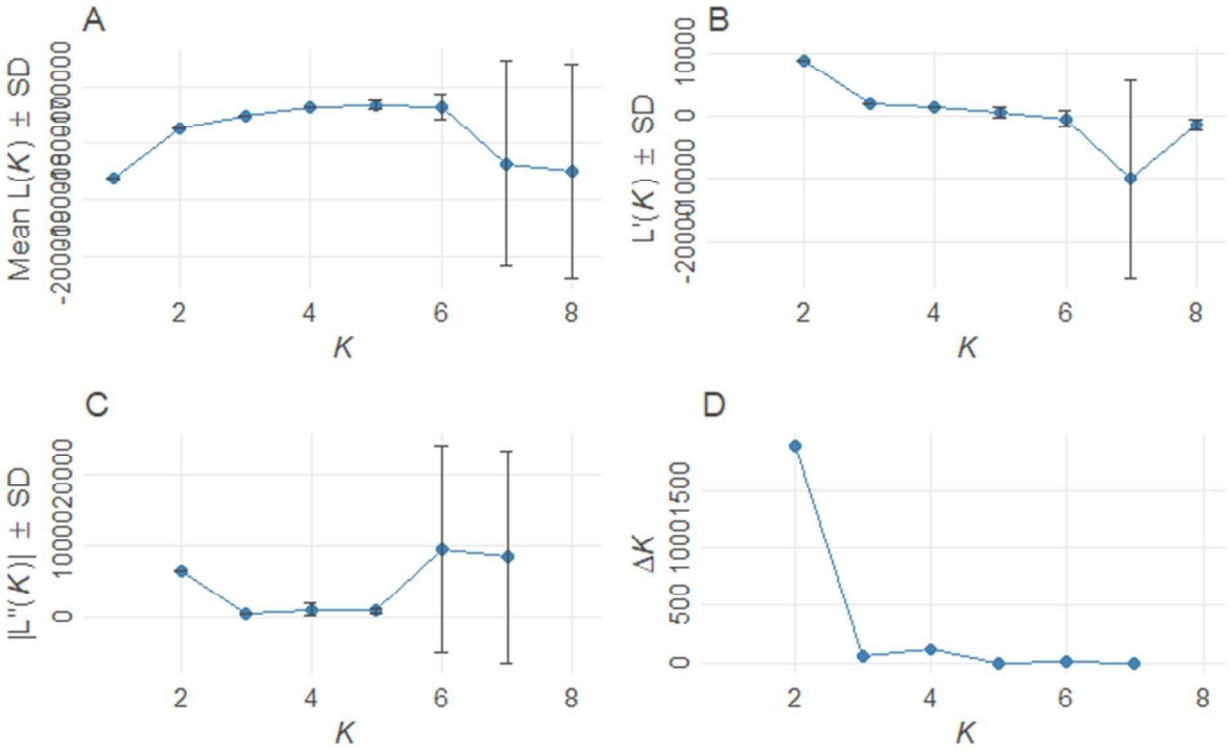
Populations Test

min maf	Retained Ind	Loci	Allelers
0.1	170	6386	12772
0.05	**169**	**11534**	**23068**
0.005	150	48265	96530

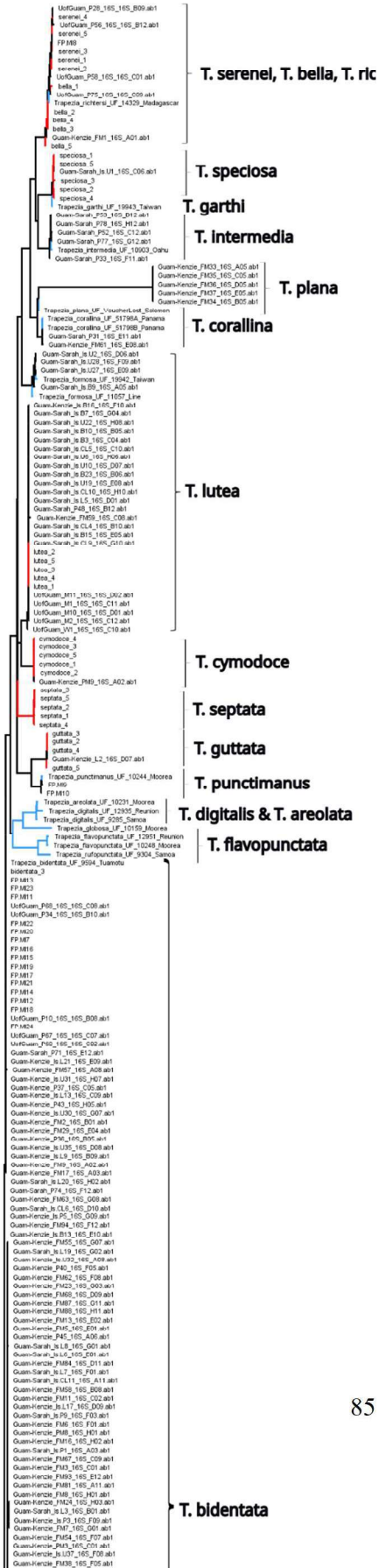
Supplementary Figure 3. Matrix representing sample coverage. Black = locus present, white = locus absent. Blue box highlights *T. rufopunctata* and *T. flavopunctata*. Source code: de Medeiros BA. (2019) Matrix Condenser v.1.0. Available at: https://github.com/brunoasm/matrix_condenser/



Supplemental Figure 4. EvannoMethod results for choosing K for STRUCTURE plot of all *Trapezia bidentata*. Here we selected K=4.



Supplemental Figure 5. 16S Tree constructed with RAXmL via the CIPRES gateway to check species identity against GenBank sequences. Blue lines indicate sequences provided by Dr. Rob Lasley, red lines are sequences from GenBank and black lines are my sequences.




guttata_1
bidentata_2
bidentata_1
Tetralia_cavimana_UF_7659_Oman
Tetralia_nigrolineata_UF_25115_Heron
Tetralia_brengalae_UF_23137_Taiwan
Tetraloides_nigrifrons_UF_18089_Moorea
Tetralia sp.
0.3

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