THE POPULATION GENETIC STRUCTURE OF *ACROPORA PULCHRA* IN GUAM

BY

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ABSTRACT

Staghorn *Acropora* corals are ecologically important as locally dominant reef-builders, and habitat structurers for fishes and invertebrates. However, staghorn corals are also particularly susceptible to bleaching events, caused by warming sea surface temperatures that are increasing in frequency and severity. In Guam, staghorn corals suffered an estimated 50% loss spanning a three-year period marked by multiple bleaching events and extreme low tides (2013-2015). These declines have the potential to reduce genetic variation, thus impeding a species' ability to adapt to changing environmental conditions. In this study, we determine the presence of genetic structure among staghorn *Acropora pulchra* populations between the islands of Guam and Saipan, and among five populations around Guam. We analyzed genome-wide ddRADseq data, and genotyped 267 *A. pulchra* samples to assess levels of genotypic and genetic diversity, and to identify putative loci under selection. Our results reveal that *A. pulchra* in Guam are dominated by clonal genotypes suggesting significant asexual fragmentation as their predominant reproductive strategy. Further analyses indicate moderate but highly significant proportions of genetic diversity are partitioned between the islands of Guam and Saipan (1.6%; $p < 0.001$) and among Guam populations (1.2%; $p < 0.001$). Significant genetic differences are detected among Guam populations between northern (Urunao and West Agaña) and southern sites (Cocos), with the central population of Agat providing connectivity along the western coast. Intra-genomic analyses reveal minor signatures of positive selection, indicating limited population-specific adaptations. Moreover, we identify the dominant symbiont genus of *A. pulchra* in Guam to be *Cladocopium*, although several specimens in Urunao and Saipan associated predominantly with *Durusdinium*. The findings of this study suggest that management plans should protect Agat because it is central to gene flow, and promote Urunao corals because they are genotypically diverse with thermo-tolerant symbionts, to support

the recovery and resilience of Guam's ecologically important *A. pulchra*. Future restoration should incorporate more population genetic studies to promote genotypic, genetic and symbiont diversity in local coral nurseries.

Keywords: *population genetics, restoration, Guam, Micronesia, coral, reefs*

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Chapter 1 Introduction

Coral reefs worldwide are declining rapidly due to rises in seawater temperature, ocean acidification, and local anthropogenic stressors (Hoegh-Guldberg et al., 2007). Over the past three decades, dominant reef-building corals have faced huge losses, with one-third of reef corals being at risk of extinction (Carpenter et al., 2008; Mumby et al., 2008). Globally, coral reefs play vital functional roles contributing to economic growth, serving as coastal protection, providing habitats for various marine species, and sustaining cultural and traditional practices (Hicks, 2011).

Scleractinian corals occur in all the world's oceans and forge most of the framework of modern coral reefs. *Acropora* is a genus of stony corals belonging to the phylum Cnidaria, order Scleractinia, family Acroporidae. Globally, *Acropora* is the most abundant genus of branching corals with over 149 described species, whose geographic range spans the Indian ocean, the central Indo-Pacific, Australia, Southeast Asia, Japan, and the Caribbean Sea. However, its greatest diversity is found in the Great Barrier Reef and the Coral Triangle where they provide the bulk of the three-dimensional structure of reefs that support a diverse assemblage of reef-associated species (Wallace, 1999; Veron, 2000).

Staghorn *Acropora* are fast-growing, dominant reef-builders who thrive in sheltered areas due to their fragile, branching morphology. They are hermaphroditic broadcast spawners throughout their range (Baird et al., 2009). However, their local abundance and distribution on many reefs, such as the Caribbean, are largely a result of asexual fragmentation (Highsmith, 1982). Due to this heavy reliance on fragmentation with low sexual recruitment, staghorn *Acropora* species are particularly vulnerable to population declines with poor recovery rates over prolonged times (Tunnicliffe, 1981; Highsmith, 1982; Vargas-Angel et al., 2003). Although, *Acropora* can

recover on local scales via asexual fragmentation, recolonization of distant populations requires larval dispersal, which appears relatively limited (Highsmith, 1982).

Fast-growing, branching corals, such as *Acropora*, are particularly susceptible to bleaching events and are more impacted by thermal stress (Paulay, 1999; Baker et al., 2008), and prone to subsequent mortality (Marshall & Baird, 2000; Loya et al., 2001) in comparison to more massive corals. Coral bleaching is a breakdown of the relationship between coral host and its symbiotic algae, which provide nutrients, metabolic activity, and waste disposal for the coral host (Weis, 2008; Davy et al., 2012). Van Oppen et al. (2001), Ulstrup and van Oppen (2003) and others found *Acropora* species to harbor multiple clades of *Symbiodinium*, which have been suggested to play an interactive role in thermal adaptation (Baird et al., 2009).

In Guam, staghorn *Acropora* are locally-dominant back reef-building corals that form vital habitats for local fishes and invertebrates (Raymundo et al., 2017). Staghorn *Acropora* species mainly occur on shallow reef flats and lagoonal patch reefs throughout Guam (Raymundo et al., 2017). Burdick et al. (2008) identified four common staghorn species: *Acropora aspera*, *A. pulchra*, *A.* cf. *intermedia*, and *A. muricata*, and four rare species: *A. vaughani*, *A. teres*, *A. austera*, and *A. virgata* (Burdick et al., 2008). However, staghorn species affiliations and boundaries are poorly documented and currently remain somewhat unclear (Raymundo et al., 2017).

Guam's staghorn *Acropora* have been impacted by various threats including infectious disease (Myers & Raymundo, 2009), *Drupella* and *Acanthaster planci* predation and outbreaks (Burdick et al., 2008), and widespread coral bleaching (Raymundo et al., 2017). In 2013 and 2014, staghorn populations suffered an estimated 50% loss in coral cover over a three-year period (2013-2015), marked by consecutive bleaching events and extreme low tides (Reynolds et al., 2014; Raymundo et al., 2017). In Saipan, north of Guam within the Marianas Archipelago, there was

over a 90% loss of staghorn *Acropora* spp. in Saipan Lagoon (BECQ-DCRM, Long-Term Monitoring Program, unpublished data) due to the same consecutive annual bleaching events. Due to this recent and drastic decline, *Acropora* have become a target species for local conservation and restoration management.

To facilitate effective conservation and restoration of *Acropora*, it is vital to understand their population demography, structure, and dynamics (Meesters et al., 2001; Smith et al., 2005). Population genetic data connect evolutionary and ecological processes that are crucial in aiding management efforts for sustaining reef biodiversity and function (Vellend & Geber, 2005). Populations with higher genetic diversity are typically more resilient to disturbances due to their higher chance at possessing beneficial alleles that facilitate future adaptation and recovery (Hughes et al., 2008; Drury et al., 2016). More data considering factors that support *in situ* evolutionary processes and genetic diversity are thus needed for coral reef restoration and management (Drury et al., 2016). For example, if genetic diversity favorable for adaptive evolution can be identified and conserved, long-term implications can expand beyond the preservation of a species, and potentially impact biodiversity and ecosystem viability in the presence of disturbances (Bailey et al., 2009; Sgro et., al 2011).

In addition, genotypic diversity (i.e. the proportion of unique genotypes in a sample or population) is another valuable parameter for informing conservation and restoration management. Genotypically depauperate populations are anticipated to have low resilience in the face of abiotic and biotic disturbances (Lively et al., 1990; Reusch et al., 2005), especially disease epidemics (Vollmer & Kline, 2008). Low genotypic diversity is more common in areas of increased disturbances (Hunter, 1993; Coffroth & Lasker, 1998), and low population connectivity (Baums et al., 2006).

Population genetic studies have been conducted on various spatial scales to discern genetic connectivity of reefs in relation to biological and geographical factors. Over large spatial scales in the Caribbean, Baums et al. (2005) studied populations of threatened *A. palmata* and found two distinct populations in the eastern and western Caribbean, revealing little genetic exchange between the two regions. Later, Vollmer and Palumbi (2007) concluded that a closely related *Acropora* species, *A. cervicornis* similarly exhibits significant population genetic structure spanning the Caribbean, Bahamas, and Florida. Both Baums et al. (2005) and Vollmer and Palumbi (2007) reveal that there is insignificant genetic exchange for these two threated *Acropora* species across large spatial scales. Their findings suggested that surviving *Acropora* colonies should be marked as high conservation priority (Vollmer & Palumbi, 2007) with a need for more localized and smaller-scale marine reserves to mitigate *Acropora*'s poor replenishing ability over large distances (Baums et al., 2005).

In the Pacific, Wood et al. (2014) used seascape modeling to propose that the Micronesian islands act as a series of stepping stones connecting the Coral Triangle to the central Pacific. Later, Davies et al. (2015) confirmed this hypothesis by examining populations of *A. digitifera* and *A. hyacinthus* from Palau and the Marshal Islands. For both species they concluded that genetic divergence followed an isolation-by-distance pattern with genetic diversity decreasing with distance from the Coral Triangle (Davies et al., 2015). Interestingly, they found that *A. hyacinthus* was over two times more genetically structured than *A. digitifera* over large spatial scales, demonstrating how closely-related *Acropora* species have two significantly different divergence patterns despite their similar life history strategies (Davies et al., 2015).

Moreover, population genetic studies have also been conducted over small spatial scales. For example, Baums et al. (2010) confirmed and extended the results of Vollmer and Palumbi

(2007) in that they found *A. cervicornis* in the Florida Reef Tract (FRT) to have little population differentiation and no significant population structure. However, Drury et al. (2017) sampled over a greater range on the FRT, utilizing more advanced genetic markers, and found significant levels of population genetic structure across small spatial scales for *A. cervicornis*. In another study, Cros et al. (2016) found very little connectivity between populations of *A. hyacinthus* between Yap and Palau, and detected significant population structure within islands and between sample sites separated by as little as 5 km. Their findings suggested targeted management specific for environmental, ecological, and genetic differences among regions, and highlight the value of conserving surviving local populations (Cros et al., 2016).

In Guam, a previous study by Boulay (2016) examined the genetic connectivity and clonal structure of *A. pulchra* around Guam. Her findings indicate that *A. pulchra* is a highly asexual fragmenting species (Boulay, 2016). However, local populations are connected via larvae along the western coast of Guam, except for Cocos lagoon due to the presence of a dispersal barrier. Moreover, she identified the presence of two distinct populations: Western (Agat-Tanguisson) and Southern (Cocos lagoon-Achang) and concluded that populations should be managed on local scales aimed to maintain clone diversity (Boulay, 2016). However, she only sampled along the western coast of Guam, and employed a limited genetic approach using eight microsatellites (Boulay, 2016).

Ongoing, worldwide coral restoration efforts have taken advantage of branching coral's effective asexual reproduction and high growth rates (Young et al., 2012; Burns, 2018). Unlike sexual reproduction, which only occurs in April and May for *A. pulchra* in Guam (Lapacek, 2017), asexual fragmentation can occur year-round appealing more to management practices. Despite the potential benefits of fragmentation disadvantages must be considered, particularly the focus on

outplanting clonal populations versus genotypically rich populations. Therefore, it is vital to determine natural levels of genotypic diversity in populations to establish thresholds for the use of asexual fragmentation.

In conclusion, as the number and scope of reef restoration projects grow locally, thorough knowledge is needed about the extent of genetic diversity and connectivity and their role in prolonging, or re-establishing, new populations (Edwards & Clark, 1998; Edwards & Gomez, 2007). Population genetic studies can provide a framework to understand levels of sexual reproduction and connectivity to increase genetic diversity, which is necessary for adaptation (Sgrò et al., 2011; Oliver et al., 2015).

Statement of purpose

The decline of a dominant, habitat-defining coral species, *Acropora pulchra*, is well documented in Guam. Despite their ecological importance, little is known about their population genetic structure, making it challenging to develop effective management plans. I seek to determine whether population genetic structure exists for this dominant reef-building coral between Guam and Saipan, and within Guam. I tested two main hypotheses:

H1o There is no population genetic structure between Guam and Saipan.

H1a There is population genetic structure between Guam and Saipan.

H2o There is no population genetic structure among populations in Guam.

H2a There is population genetic structure among populations in Guam.

Chapter 2 Methods

Sample collection and processing

Acropora pulchra samples were collected from five locations around Guam between May 2018 and October 2019 (Figure 1, Table 1). The sampling site at Agat is an established National Park Historic Preservation area, for which required permits were obtained (Permit #: WAPA-2018-SCI-0010). Staghorn *Acropora* were sampled from depths between 1 and 5 m along a 500 m transect where one sample was collected every 10 m to minimize the collection of clonemates. However, the limited spatial extent of the remaining staghorn *Acropora* population at Togcha required haphazard sampling, deviating from normal transect sampling methods (Figure 1). Two neighboring, but separate sites were sampled at Cocos. Underwater photographs were taken of each sample for examination of individual morphology. Small nubbin samples were carefully removed with a wire cutter, placed in falcon tubes filled with seawater, and stored in chilled coolers during transport to the University of Guam (UOG) Marine Laboratory. Upon arrival, all tissue samples were transferred to cryovials, preserved in 95% ethanol and stored in a -20˚C freezer. A portion of each nubbin sample was bleached and catalogued in the UOG Biorepository for voucher purposes. In addition, forty-one *Acropora* samples were included that were collected over a wide spatial range in Saipan Lagoon by our collaborator, Dr. Lyza Johnston. In total, I obtained tissue samples from two hundred and sixty-seven (n=267) *Acropora* spp. that were used in further downstream processing (Table 1).

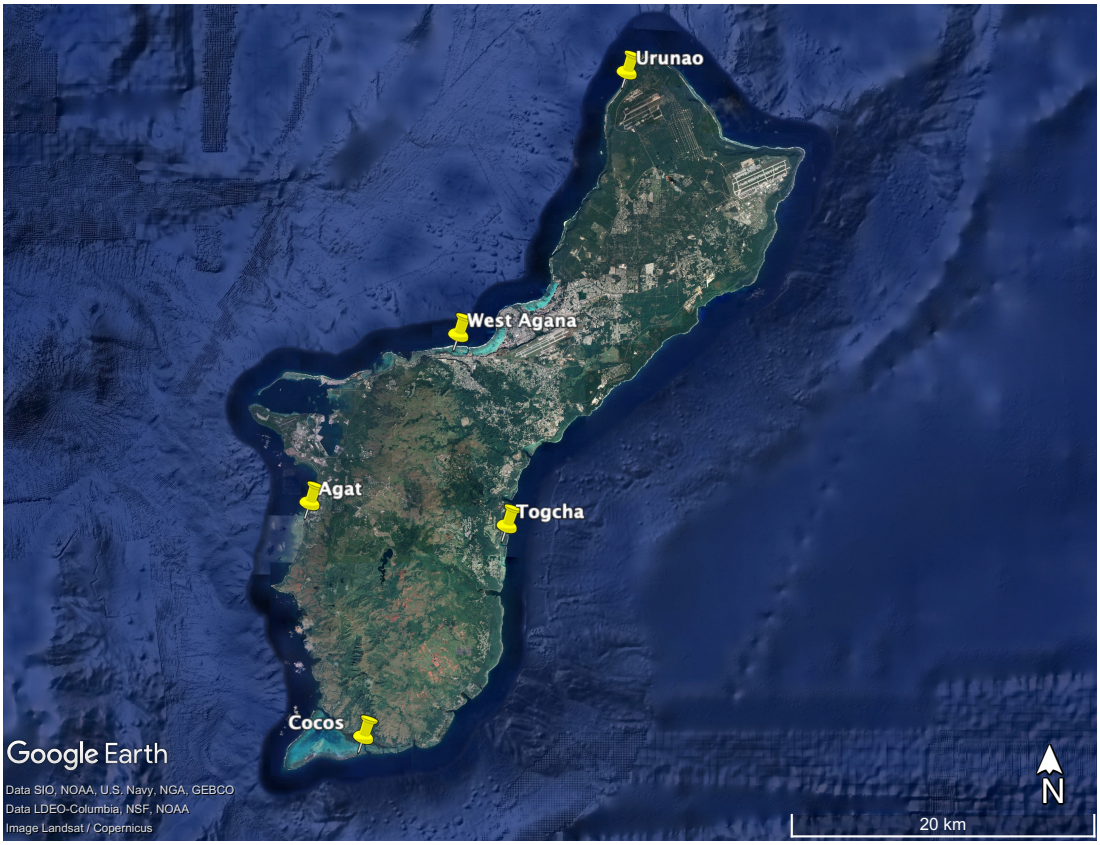

Figure 1. Location of the five sampling sites of *Acropora pulchra* in Guam

Table 1. *Acropora* samples collected across six sites in Guam and Saipan (n_c=267) and number of *A. pulchra* samples identified. GPS coordinates are in decimal degrees (WGS84). Two neighboring but separate sites were sampled in Cocos

Sampling site	Sampling date	Latitude	Longitude	*Acropora* samples collected	*A. pulchra* samples identified
Togcha	18 May 2018	13.36865	144.77541	21	21
Agat	11 July 2018	13.38322	144.65169	49	42
Urunao	23 October 2018	13.63672	144.84527	45	45
Cocos	14 December 2018	13.24589 13.25103	144.68489 144.67630	61	50
West Agaña	03 January 2019	13.47970	144.74590	50	50
Saipan	06-19 June 2019 11-22 July 2019	15.20568	145.74078	41	25

DNA extraction and ddRAD library preparation

Total genomic DNA was extracted using the DNAeasy Kit (Qiagen, Hildesheim, Germany) and the GenCatch Genomic DNA Extraction Kit (Epoch, Sugar Land, TX) following optimized manufacturer's protocols. The quantity of DNA was measured with a Qubit 3.0 dsDNA fluorometer (Thermo Fisher Scientific Inc., Waltham, MA).

Double-digest restriction site-associated DNA (ddRAD) libraries were prepared following a modified protocol based on Peterson et al. (2012) and Combosch et al. (2017). Extracted DNA was digested using two high-fidelity restriction enzymes, PstI and MspI. Resulting fragments were ligated to custom P1 and P2 adaptors with sample-specific barcodes and primer annealing sites. Barcoded samples were pooled into libraries, and size-selected (320-420bp) with an E-Gel Size Select II Agarose Gel (Thermo Fisher Scientific Inc., Waltham, MA). Size-selected fragments were PCR-amplified using a high-fidelity polymerase and corresponding 5x buffer (New England Biolabs, Ipswich, MA) with primers containing additional indices. For PCR amplifications, between 15 and 22 PCR cycles (95°C for 30 s, 65°C for 30 s, 72°C for 60 s, with an initial denaturation step at 98°C for 30 s, and a final extension step at 72°C for 5 min) were used, depending on the concentrations of the resulting libraries. Between 2-10 separate PCR amplifications were set up per library and pooled subsequently to increase the diversity of sequencing pools.

Libraries were cleaned to remove adapters and primers using Agilent beads (Agilent Technologies, Santa Clara, CA) at a 1:0.6 library to beads ratio. Quality and quantity checks were performed on an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) and Qubit 3.0 (Thermo Fisher Scientific Inc., Waltham, MA), respectively. Lastly, libraries were single-end sequenced (120 bp) on an Illumina NextSeq500 Illumina (New England Biolabs, Ipswich, MA)

at the University of Guam Marine Laboratory. For a subset of samples (n=21), duplicated ddRAD libraries were generated to serve as technical replicates for downstream analysis.

Acropora species identification

To ensure that only *A. pulchra* samples would be used in this study, I used a recent phylogenetic analysis (Combosch, unpublished) that included ddRAD-Seq genotyping data for all the samples collected in this study (Figure 2). Phylogenetic analysis unambiguously identified two hundred and thirty-three (n=233) *A. pulchra* samples among the two hundred and sixty-seven (n=267) *Acropora* samples that were collected and genotyped for this study (Figure 2, Table 1). Only unambiguously identified *A. pulchra* samples (n=233) were used in subsequent population genetic analyses (Table 1).

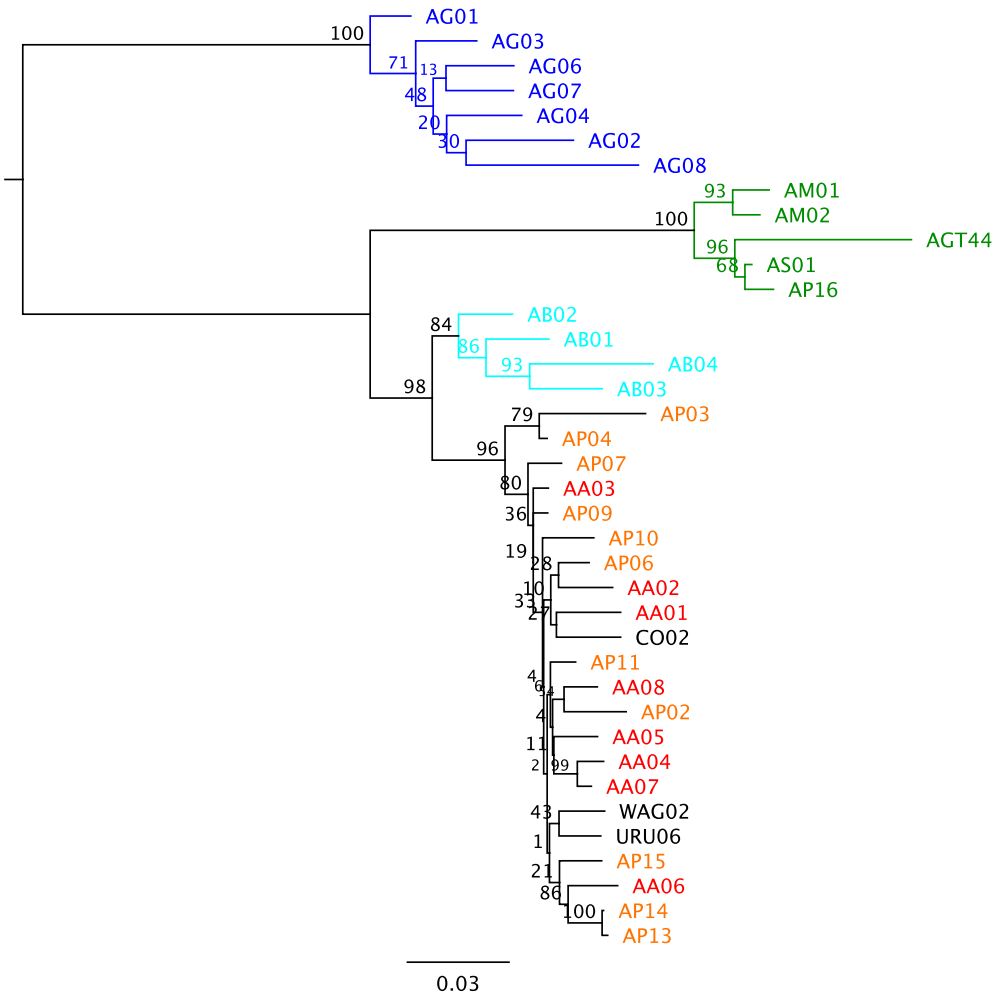

Figure 2. Phylogenetic tree by Combosch (unpublished) used for species delimitation of *Acropora.* Blue = *A. globiceps*, green = *A. muricata*, teal = *A. abrotanoides*, red/orange/black = *A. pulchra,* AA = *A. aspera*, AS = *A.* sp.)

Data curation and genotyping

Raw reads were quality-trimmed with TrimGalore 0.6.5 with default settings (Martin, 2011). All reads with an average quality score lower than 30 and shorter than 36 bp were discarded. Resulting reads were demultiplexed using a custom-made python3 script (identify_dbrs6.py, H. Weigand, personal communication) to remove reads of low quality, reads with uncalled bases, and reads without complete barcodes or restriction cut sites. Resulting reads were aligned to the *Acropora millepora* genome (Ying et al., 2019) using Bowtie2 v2.3.5 (Langmead & Salzberg,

2012), with default settings but excluding soft matches. Aligned reads were converted to bam files to only select primary reads, and sorted using SAMtools (Li et al., 2009).

Genotyping was performed using two separate approaches: ANGSD v0.93 (Korneliussen, et al., 2014) and STACKS v2.3 (Catchen et. al 2011, 2013). ANGSD utilizes genotype probabilities instead of inferred genotypes. This is useful for low coverage data due to the incorporation of genotype uncertainty (Korneliussen et al., 2014). ANGSD was run with the following filters: minimum mapping quality score of 20, minimum base call quality score of 30, a minimum allele frequency score of 0.05, a p-value of 2×10^{-6}, at least 50% of non-missing genotypes across samples, and a filter that retained only uniquely mapped reads. ANGSD genotype likelihood data was used for the following analyses: identity-by-state (IBS), principle coordinates analysis (PCoA), ngsAdmix, ngsRelate, and Thetastat.

For a subset of analyses that were unable to accommodate genotype probabilities, STACKS v2.3 was used to generate fixed genotype calls (Catchen et. al 2011, 2013). STACKS was used in the reference-based mode and single nucleotide polymorphisms (SNPs) were identified using a maximum-likelihood model (Catchen et. al 2011, 2013). In addition, the STACKS populations program was used to retain only loci that were present in 50% of all populations. STACKS genotype calls were used for the following analyses: Analysis of Molecular Variance (AMOVA), F_{ST}, and population genetic summary statistics.

Symbiodinium clade type determination

Methods for determining *Symbiodiniaceae* genera were adopted from Barfield et al. (2018). Quality filtered and trimmed ddRAD reads were mapped with Bowtie2 v2.3.5 with default settings excluding soft matches to transcriptomes of *Symbiodinium, Durusdinium, Cladocopium,* and *Breviolum* to determine the predominant clade in each sample. Transcriptomes for *Symbiodinium*

and *Breviolum* were acquired from Bayer et al. (2012), and transcriptomes for *Cladocopium* and *Durusdinium* were from Ladner et al. (2012). Resulting SAM files were used to calculate relative proportions of reads with highly unique matches, determined by a mapping quality of 40 or higher to each *Symbiodiniaceae* transcriptome, using a custom perl script zooxType.pl (https://github.com/z0on/).

Detection of clones and levels of relatedness

I began analyses by detecting clones present in the dataset, which is of high relevance to predict asexual versus sexual input among sampling locations. In addition, it is also crucial to remove clones prior to population genetic analyses to avoid distorting allele frequencies. However, it is difficult to distinguish clones because they are not completely identical due to sequencing error and/or sequencing randomness. Therefore, ANGSD was used to generate an identity-by-state (IBS) matrix following Manzello et al. (2018), and Barfield et al. (2018) using the R function hclust() and the method "average". To determine a clonality threshold for clones two approaches were used: a) technical replicates were used to determine a lower threshold and b) binned gap analysis (Table S1, Supp. Material) was used to compare levels of relatedness between virtually identical clonemates and unique genotypes.

The major advantage of ANGSD's IBS approach is its ability to reduce bias due to low and/or variable sequencing coverage by using genotype likelihoods. Results were displayed on a hierarchical clustering dendrogram whose branch lengths displayed levels of genetic similarity (Figure 3). Samples that exhibited lower or similar genetic distances as the clonality threshold were deemed as clones (Figure 3, Table S3, Supp. material). The dataset was subsequently pruned to leave only a single representative with the highest number of mapped reads from each clonal genotype for downstream population genetic analyses (Table 2). After removing clones and

technical replicates, ANGSD was re-run with the same filters to generate the final population genetics dataset.

To investigate how related samples were we used the ANGSD subprogram, NgsRelate to calculate pairwise relatedness (rab) based on genotype likelihoods and population allele frequencies (Korneliussen & Moltke, 2015). Rab values were computed with the entire dataset, including technical replicates and clones, and were also computed with the final population genetic dataset (i.e. excluding technical replicates and clones) to test the stringency of the clonality cutoff. Within the final dataset, resulting sample comparisons with rab > 0.1 were further assessed and compared within and between populations.

Population genetic analyses

To determine the presence of genetic structure between Saipan and Guam, and among Guam populations, I used covariance matrices for principle coordinate analysis (PCoA) via the ANGSD subprogram, ngsCovar and the R package, "vegan" with the constrained analysis of principal coordinates function following Barfield et al. (2020).

To further determine patterns of genetic structure, I used NgsAdmix (Skotte et al., 2013), which performs ADMIXTURE analysis on genotype likelihood data. The resulting bar charts were plotted in R, following Skotte et al. (2013) for genotypic cluster values K=1-6 to determine any detectable genome-wide *A. pulchra* admixture between Guam and Saipan populations and among Guam populations.

To assess the partitioning of genetic variation between islands, populations, and individuals, I used hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) in GenoDive (Meirmans & Van Tienderen, 2004) using an infinite allele model (IAM) and 999 permutations to assess its significance. To assess levels of population differentiation, pairwise

genetic differences (F_{ST}) between islands and populations were calculated using GenoDive (Meirmans & Van Tienderen, 2004) with subsequent sequential Bonferroni correction to adjust significance for multiple comparisons. Population genetic summary statistics were calculated in GenoDive to assess levels of genetic diversity (Meirmans & Van Tienderen, 2004).

F_{ST} outlier analyses

ddRAD loci putatively under selection were identified based on locus-population-specific differences in F_{ST} coefficients using the Bayesian program, BAYESCAN v2.1 (Foll & Gaggiotti, 2008). Final results were analyzed in R using BAYESCAN specific scripts (Foll & Gaggiotti, 2008). In addition, OutFLANK (Whitlock & Lotterhos, 2015) was used to identify candidate loci under selection by inferring the distribution of F_{ST} for loci not likely to be influenced by diversifying selection (Whitlock & Lotterhos, 2015). This approach is appealing due to its lower false positive rates (Whitlock & Lotterhos, 2015). Lastly, the ANGSD subprogram, ThetaStat, (Korneliussen et al., 2013), was used to detect other signatures of selection that include the estimation of different thetas (population-scaled mutation rate), and summaries of site frequency spectrum, such as Tajima's D. This approach accommodates the uncertainty of the genotype data due to varying sequencing depth across loci and samples.

Chapter 3 Results

Data summary and identification of genetic clones and relatedness

A total of 267 *Acropora pulchra* specimens were sequenced and produced more than 200 million raw reads overall (Table S2, Supp. material). Following quality filtering, only samples

with more than 5,000 reads were used, resulting in 188 *A. pulchra* samples that included 18 technical replicates (Table S2, Supp. material). Probabilistic genotype likelihoods generated with ANGSD resulted in 16,780 SNP loci genotyped in at least 50% of samples.

To identify clones, hierarchical clustering based on identity-by-state (IBS) distances were generated in ANGSD. The analysis revealed the presence of 132 clones, and 36 clonal genets (Figure 3, Table 2). Clones were only found within populations, suggesting clonality via fragmentation. There were differences in the number of clones and proportion of unique genotypes between sampling locations (Table 2). However, it is important to remember that I sampled Togcha haphazardly due to the low number of *Acropora* remaining, and Saipan was sampled by a collaborator (i.e. not following sampling protocol). Therefore, clonality results of Togcha and Saipan are not directly comparable with the four other Guam populations. For example, Saipan had the highest proportion of unique genotypes (N_g/N=0.85, Table 2) while Togcha had the lowest proportion of unique genotypes (N_g/N=0.10, Table 2), which is likely due to the differing sampling strategies employed at these sites (see Methods for details). Moreover, Togcha is composed of only two unrelated, clonal genotypes, of which one genet contained 19 ramets leading Togcha to have the largest average number of ramets per genet (Table 2).

Aside from Saipan, the highest proportion of unique genotypes was found in Urunao, where more than half of all samples constitute unique genotypes (Table 2). In contrast, when excluding Togcha, the highest number and proportion of clones was detected in Cocos, which also had the lowest number of unique genotypes (N_g=7, Table 2). Proportions of unique genotypes (Table 2) increased along a north to south gradient, of which northern Urunao had the highest proportion of unique genotypes (N_g/N = 0.59), followed by West Agaña (N_g/N = 0.44), Agat (N_g/N = 0.33), and southernmost Cocos (N_g/N = 0.27)

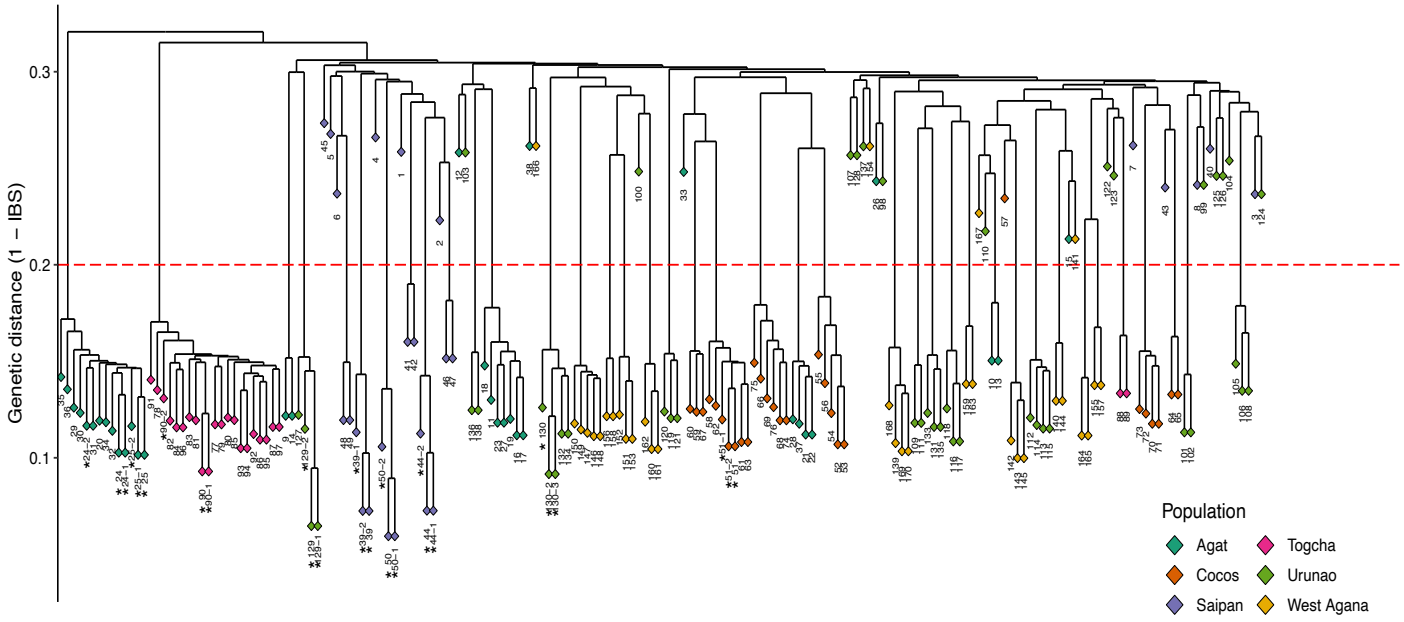

Figure 3. Hierarchical cluster dendrogram based on pairwise identity-by-state (IBS) values from ANGSD for 188 samples, including 18 technical replicates, indicated by asterisks (*). Numbers correspond to sample ID in Table S3, Supp. material. Gap analysis and technical replicates were used to determine a threshold (indicated by the dashed red line) to distinguish clones (below threshold) from unique genotypes (above threshold)

The dataset was pruned to leave only a single best sequenced ramet per genet. After the removal of genetic clones, and technical replicates, a total of 74 samples (n=74) with unique genotypes comprised the final population genetic dataset. ANGSD was re-run with the same filters on the final dataset (n=74), and generated a total of 19,940 SNP loci, genotyped in at least 50% of all samples (see Methods for details). For a subset of analyses, fixed genotypes were required and generated using STACKS across a minimum of 50% of samples within the final population genetic dataset (see Methods for details). In total, 11,490 RAD loci and 25,820 SNPs were obtained for these analyses.

Table 2. Clonality statistics for all six sampling sites. Note: genets are the number of clones as determined by hierarchical clustering. Number of observed genets/number of samples (N_g/N) is the genet-ramet ratio and serves as a diversity statistic. Togcha and Saipan were sampled differently, therefore results are not directly comparable

Population	Number of samples (N)	Number of clonal genets (G)	Average ramet per clonal genet (R/G)	Number of unique genotypes (N_g)	Proportion of unique genotypes (N_g/N)
Cocos	26	6	4	7	0.27
Agat	30	5	5	10	0.33
W. Agaña	32	10	3	14	0.44
Urunao	41	10	3	24	0.59
Togcha	21	2	11	2	0.10
Saipan	20	3	2	17	0.85
TOTAL	170	36	4	74	0.44

NgsRelate was used to determine how related samples are and to further support the clonality cutoff. With the entire dataset (n=188), the average pairwise relatedness was high overall (rab=0.8), mostly due to the presence of numerous clones and technical replicates. With the final population genetic dataset (n=74), the average pairwise relatedness was significantly reduced (rab=0.1), tentatively supporting the clonality cutoff. Several second-degree relative pairs (rab>0.1, i.e. second cousins) were identified both within and between Saipan and Guam populations (Table 3a-b). In total, 29 relative pairs with rab>0.1 were discovered within populations and 28 relative pairs were found between populations (Table 3a-b). Within populations, Cocos has the largest proportion of relative pairs found (6.1%, Table 3a). Between Saipan and Guam populations, a total of five second degree relative pairs were discovered: four between Saipan and Urunao and one between Saipan and Agat (Table 3a-b). Among Guam populations, the northern sites of Urunao and West Agaña shared the greatest number of second-degree relatives as did the southern populations of Cocos and Agat (Table 3).

Table 3. a) Proportions of relative pairs **b)** number of relative pairs (rab > 0.1) within (above diagonal) and between (below diagonal) populations, compared to the total number of comparisons possible. Color scale corresponds to low (red) vs. high (green) values

3a	Cocos	Agat	W. Agaña	Urunao	Togcha	Saipan
Cocos	6.1%					
Agat	10.0%	1.0%				
West Agaña	1.0%	1.4%	1.5%			
Urunao	0.0%	2.5%	1.8%	1.2%		
Togcha	0.0%	5.0%	0.0%	0.0%	0.0%	
Saipan	0.0%	0.6%	0.0%	1.0%	0.0%	5.2%

3b	Cocos	Agat	W. Agaña	Urunao	Togcha	Saipan
Cocos	3/49					
Agat	7/70	1/100				
West Agaña	1/98	2/140	3/196			
Urunao	0/168	6/240	6/336	7/576		
Togcha	0/14	1/20	0/28	0/48	0/1	
Saipan	0/119	1/170	0/238	4/408	0/34	15/289

Population structure and genetic differentiation

GenoDive was used on STACKS genotype calls to calculate various population genetic statistics for all populations but Togcha (due to the small number of unique genotypes, n=2). Results showed relatively consistent patterns of genetic diversity between Guam and Saipan (Table 4). Among Guam populations, similar patterns of genetic diversity were also detected (Table 4). For example, while the number of alleles per population is heavily driven by the number of samples per population and thus hardly comparable, the extremely consistent number of effective alleles testifies to the overall comparable allelic diversity across populations (Table 4). Heterozygosity was also generally consistent across populations with slightly less observed than expected, indicating moderate inbreeding (Table 4). Perhaps most interesting is the slightly elevated inbreeding level in Agat (Table 4).

Table 4. Population genetic summary statistics calculated for each island (bottom) and population (top). Togcha was not include in this analysis due to the low number of unique genotypes (n=2)

Population	Number of samples	Number of alleles	Effective num. of alleles	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Inbreeding coefficient (G_{is})
Cocos	7	1.41	1.15	0.101	0.110	0.086
Agat	10	1.48	1.16	0.099	0.112	0.115
West Agaña	14	1.55	1.15	0.098	0.109	0.096
Urunao	24	1.62	1.15	0.095	0.104	0.087
Guam	57	1.84	1.14	0.097	0.109	0.112
Saipan	17	1.61	1.16	0.100	0.113	0.115

Principle coordinate analysis (PCoA) based on covariance matrices were generated with the ANGSD subprogram, ngsCovar. Results revealed the presence of genetic structure between the islands of Guam and Saipan, with each island clustering separately along axis 1 in the PCoA (Figure 4). Populations within Guam displayed a general separation between the northern populations of Urunao and West Agaña, and the southernmost population of Cocos with Agat overlapping both of these genetic clusters (Figure 4). It is interesting to note that the two Togcha samples appeared more closely related to the northern genetic cluster of Urunao and West Agaña (Figure 4).

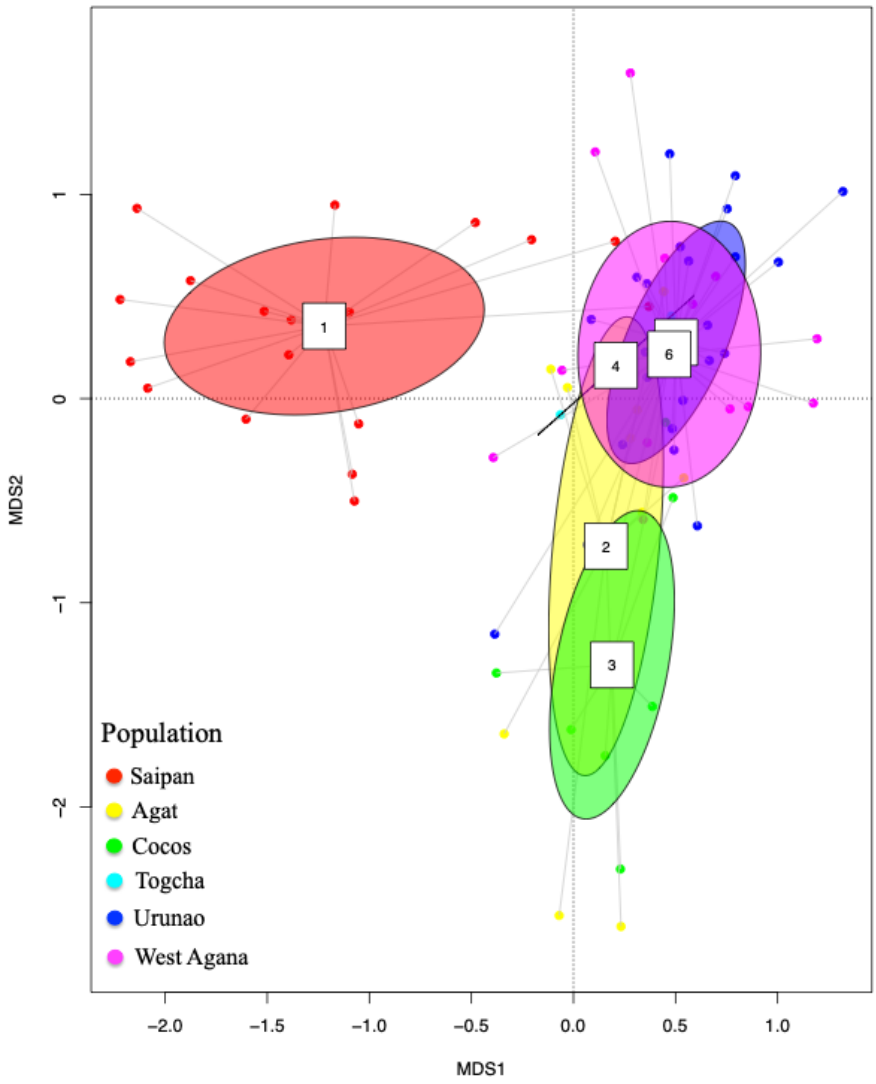

Figure 4. Principal coordinate analysis (PCoA) based on covariance matrices generated by the ANGSD subprogram ngsCovar and the R package "vegan" with constrained analysis of principal coordinates. 1- Saipan, 2- Agat, 3 – Cocos, 4 – Togcha, 5 – Urunao, 6 – West Agaña

Admixture analyses were conducted with NGSAdmix for K=2 to K= 6. Visual inspections indicated that K=2 was most meaningful (Figure S1a, Supp. material). Admixture proportions (K=2, Figure 5) showed that Saipan samples were dominated by the red cluster. The majority of Saipan samples (10 out of 17) showed 100% genetic affiliation with the red cluster and all samples had significant portions of red ancestry (Figure 5). In contrast, Guam samples were predominantly

affiliated with the green cluster with several samples showing 100% affiliation with the green cluster (Figure 5). This pattern indicates a gradual distinction between samples from Guam and Saipan, which is consistent with the PCoA (Figure 4). Interestingly, there seemed to be more green admixture in Saipan than red admixture in Guam (Figure 5).

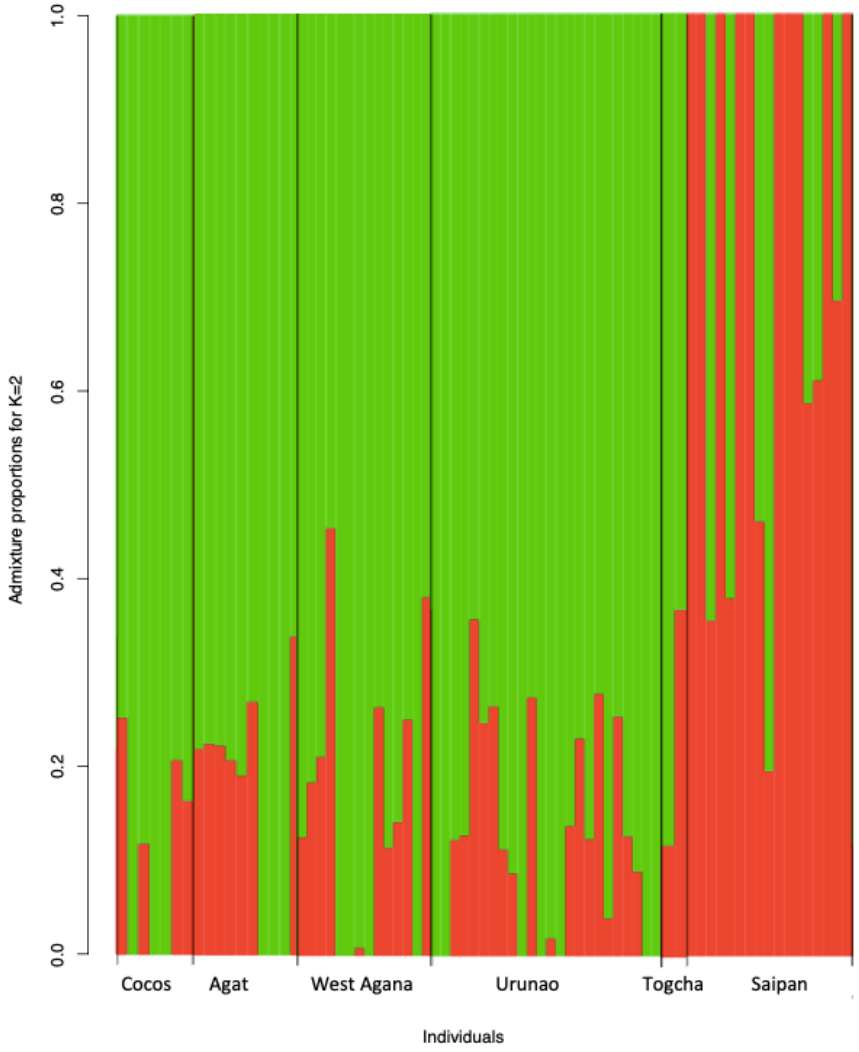

Figure 5. Admixture analysis showing separation of individuals into two genetic clusters, red and green. Each vertical bar represents an individual for which the proportion of red and green indicate the ancestry or assignment to each genetic cluster

Further admixture analyses among Guam populations indicated no significant separation of genetic variation between sites when analyzed for two or more admixture clusters (Figure S1b, Supp. material).

Hierarchical AMOVA analysis, conducted with GenoDive based on STACKS v2.3 output, indicated moderate but highly significant proportions of genetic variation partitioned between islands (1.6%; $p < 0.001$) and among populations within islands (1.2%; $p < 0.001$). All pairwise F_{ST} comparisons among populations were significant, except for Cocos *vs*. Agat (Table 5). F_{ST} between islands was fairly small (F_{ST}=0.023), but significant ($p < 0.05$) (Table 5). Saipan and Urunao had the lowest inter-island F_{ST} (F_{ST}=0.023), while Saipan and West Agaña had the highest F_{ST} (F_{ST} =0.028), both of which proved significant ($p < 0.05$) (Table 5). Low levels of pairwise F_{ST} were found between Urunao *vs.* West Agaña (F_{ST} =0.008) (Table 5), further supporting their genetic overlap in the PCoA (Figure 4) and results from NgsRelate (Table 3). In addition, pairwise comparisons between Agat *vs*. Urunao (F_{ST}=0.011) and West Agaña (F_{ST}=0.017) were also reduced but significant (Table 5). On the other hand, genetic differentiation was greatest between Cocos *vs*. West Agaña (F_{ST}=0.020) and Urunao (F_{ST}=0.019) (Table 5), which affirms the genetic differentiation between Cocos and the two northern population, which were clearly separated on the PCoA as well (Figure 4). Togcha was excluded from these analyses due to its extremely low number of unique genotypes (n=2).

Table 5. Pairwise F_{ST} values calculated by GenoDive between islands and between populations. All comparisons in bold have a significant p value ($p = 0.05$) determined after Bonferroni correction. Color scale corresponds to low (red) vs. high (green) values

	Cocos	Agat	West Agaña	Urunao	Guam
Agat	-0.004				
West Agaña	**0.020**	**0.017**			
Urunao	**0.019**	**0.011**	**0.008**		
Saipan	**0.026**	**0.025**	**0.028**	**0.023**	**0.023**

Loci under selection

Neutrality statistics performed with the ANGSD subprogram, ThetaStat, revealed that the majority of loci were under mild balancing selection (Table 6, Table S4, Supp. material). However, several populations such as Saipan, Urunao, and Agat had a few sites under putative positive selection (Table 6, Table S4, Supp. material).

Additionally, Bayescan was used for outlier loci detection in pairwise population comparisons and identified 28 outliers total between Saipan and Guam, with Saipan *vs.* Urunao having the highest number of outliers between individual populations (Table 7). Among Guam populations, 32 outlier loci were identified in total with the highest number of outliers found between the southern and northern ends of the island, Cocos *vs.* Urunao, respectively (Table 7).

Lastly, no outliers were detected with OutFLANK.

Table 6. Tajima's D neutrality test statistics indicating the number of sites with Tajima's D > 2 corresponding to sites under balancing selection and Tajima's D < -1 corresponding to sites under putative positive selection

Population	**Total number of sites**	**Sites under putative balancing selection** (Theta >2)	**Sites under putative positive selection** (Theta < -1)
Cocos	1,370	67	0
Agat	1,280	107	4
West Agaña	1,095	239	0
Urunao	1,163	286	6
Togcha	2,205	8	0
Guam	1,094	690	11
Saipan	1,102	115	9

Table 7. Number of outliers found between populations as detected by Bayescan. Color scale corresponds to low (red) vs. high (green) values

	Cocos	Agat	West Agaña	Urunao
Agat	0			
West Agaña	7	7		
Urunao	11	4	0	
Togcha	0	0	0	3
Saipan	2	6	9	11

Algal symbiont characterization

In total, more than 10,000 reads aligned to the four symbiont genomes across the final population genetic dataset (n=74; on average, 36 reads per sample). Overall, most Guam individuals predominantly hosted *Cladocopium* while Saipan individuals predominantly hosted *Durusdinium* (Figure 6, Table 8). The distribution among populations was noteworthy and non-random. For a majority of Guam samples (72%), the dominant symbiont genus was *Cladocopium*, suggesting this symbiont to be the dominant algal symbiont genus hosted by *A. pulchra* in Guam (Figure 6, Table 8). However, the northernmost Guam population of Urunao greatly differed from its Guam counterparts and predominantly harbored symbiont genus, *Durusdinium* (64%) over *Cladocopium* (36%) (Figure 6, Table 8). Interestingly, West Agaña, the population closest to Urunao with overall genetic similarity (Figure 4, Table 5) was dominated by *Cladocopium* but also had a single sample that hosted only *Durusdinium*. Small proportions of *Breviolum* (ranging from 0.2%-2.0%) were found in individuals from Togcha, West Agaña, and Agat (Figure 6).

For almost all Saipan samples (88%) the dominant symbiont genus was *Durusdinium*, with two individuals showing complete affiliation with *Cladocopium*, and two individuals showing small affiliations with *Symbiodinium* (0.3%) and *Breviolum* (0.2%) (Figure 6, Table 8). Interestingly, some individuals were seen to have affiliations with different symbiont types (Figure 6, Table 8). Comparisons of symbionts between clonemates indicated that most clonemates hosted the same predominant symbiont genus (Figure S2a-f). However, in clonal genets from Agat, Cocos, and Urunao, individual ramets were found to surprisingly host differing symbiont types (Figure S2-b, S2-c, S2-e).

Pairwise F_{ST} between individuals who predominantly hosted *Cladocopium* over *Durusdinium* revealed significantly low levels of differentiation (F_{ST} =0.004; $p < 0.006$). In fact,

F_{ST} between individuals with *Cladocopium vs. Durusdinium* was lower than all but one population pair (Cocos vs Agat, F_{ST}=-0.004), which was previously reported. Additionally, Bayescan detected no outliers between these two groups.

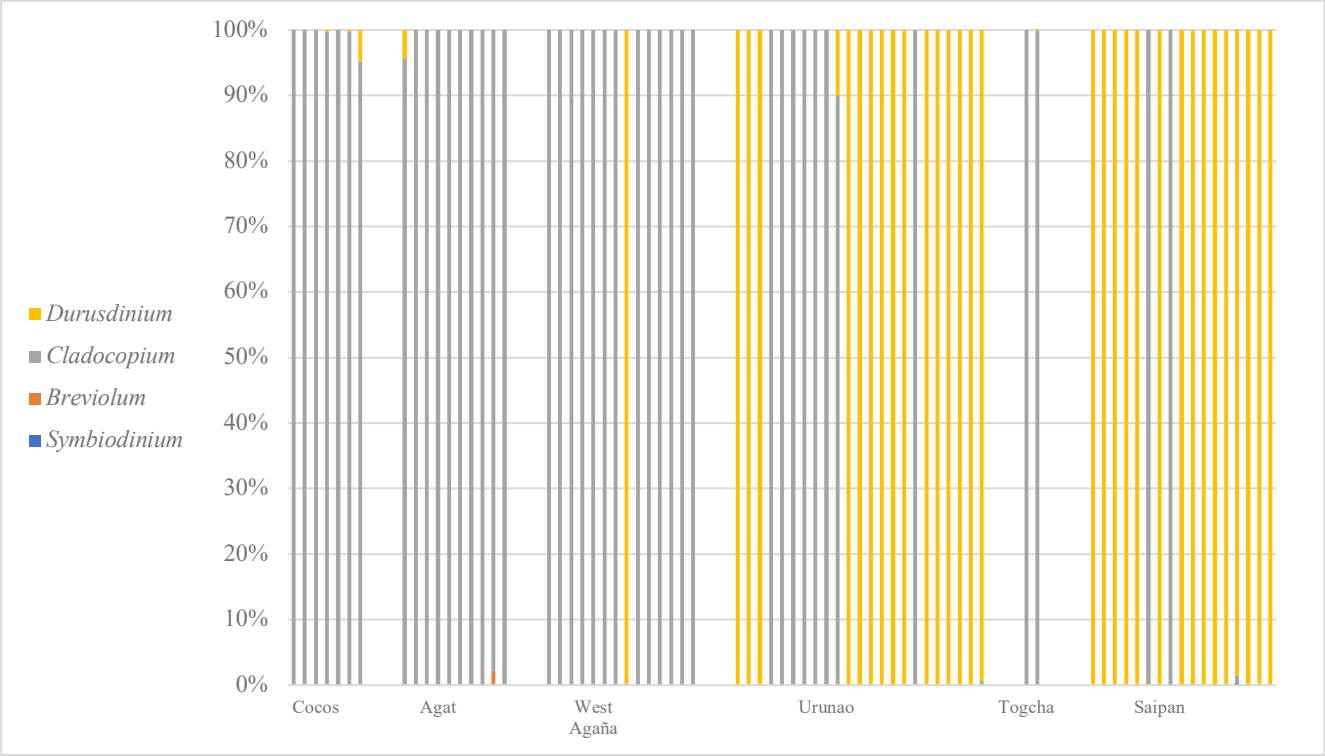

Figure 6. Bar plot representing the relative proportions of ddRAD reads producing highly unique matches to transcriptomes of four different genera of algal symbionts, *Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durusdinium* (formerly Clades A-D, respectively)

Table 8. Table indicating the presence or absence of symbionts in respective number of individuals seen in each population

	Cocos	Agat	West Agaña	Urunao	Togcha	Saipan
Durusdinium	0.8%	0.4%	7.1%	65.6%	99.9%	88.1%
Cladocopium	99.2%	99.4%	92.9%	34.4%	<0.1%	11.8%
Breviolum		0.2%	<0.1%		<0.1%	<0.1%
Symbiodinium						<0.1%

Chapter 4 Discussion

Analyses revealed that staghorn *Acropora pulchra* in Guam rely predominantly on asexual fragmentation to maintain large population sizes, but also exhibit patterns indicating ongoing sexual reproduction. Between Guam and Saipan, and around Guam, partitioning of genetic variation among populations proved small, but highly significant. In Guam, two separate population genetic clusters were found between northern and southern localities, which are connected by the central Agat population. Pairwise genetic distances and relatedness were observed to roughly correspond with geographic patterns between sites. Basic population genetic parameters proved relatively consistent across populations. Only a handful of loci appeared to be under putative positive selection. In addition, a majority of *A. pulchra* were found to predominantly associate with *Cladocopium*, but numerous samples in Saipan and Urunao were found to predominantly harbor *Durusdinium.*

Clonality, relatedness, and intra-population patterns

I found *Acropora pulchra* populations in Guam to be dominated by clonal genotypes. Overall, almost half of the samples were members of a clonal genet and when removing all but one ramet per genet, the final population genetic dataset was reduced to 44% (i.e. 56% of samples were removed). Importantly, I detected high levels of clonality even while ensuring samples were separated by ~10 m distance (see Methods).

In this study, clones were only present within populations suggesting significant local fragmentation. This is commonly the case for staghorn *Acropora* corals, which have been shown to propagate widely via asexual fragmentation (Tunnicliffe, 1981; Highsmith, 1982). For example, Boulay (2016) found no single multi locus genotype (MLG) of *A. pulchra* shared between sites in

Guam. Barfield et al. (2018) similarly used hierarchical clustering to identify *A. hyacinthus* clones in Yap, and found clones present only within the same site. Instances of asexual reproduction in *A. pulchra* were detected in all sites (Figure 3, Table 2), which is consistent with previous reproductive studies of Guam's *Acropora* corals (Birkeland, 1997; Boulay, 2016; Lapacek, 2017). For example, Lapacek (2017) concluded different species of *Acropora* in Guam have overall low reproductive outputs. Notably, *A. pulchra* and *A. acuminata* (previously reported as *A. intermedia*) exhibited low fecundity suggesting a heavy reliance on asexual reproduction (Lapacek, 2017).

Clonal indices can be used as direct measures of the contribution asexual reproduction has on a population (Arnaud-Haond & Belkhir, 2007). Clonality levels were highest within Togcha (Table 2), where I found only two unique, unrelated genotypes, of which 90% of ramets belonged to a single clonal group. However, it is important to note that I sampled this population haphazardly over smaller spatial distances between samples due to the low number of *Acropora* remaining. Togcha, the only east coast population, occurring along a reef crest is subjected to strong, turbulent currents. The northeast trade winds surrounding Guam cause the predominant swell to come from the northeast making Togcha experience higher wave exposure compared to its western counterparts (Emery, 1962). Turbulent waves can increase the incidence of coral dislodgement, potentially resulting in high levels of asexual fragmentation (Tunnicliffe, 1981). In addition, disruptive wave action also threatens a coral's optimal reproductive size, further comprising their sexual reproductivity. Thus, this high-energy population seems to maintain itself almost exclusively by asexual fragmentation.

Agat was found to have the largest spatial extent between ramets of the same genet (~200 m distance). Previous studies found ramets of the same genet in *A. cervicornis* (Drury et al., 2019) and *A. palmata* (Baums et al., 2006) spread over distances of 20 m in Florida, and 75 m in the Caribbean, respectively. Therefore, the spatial extent of clones I observed here is significantly

larger than previously observed. Arnaud-Haond and Belkhir (2007) described how inferences can be made on the history of clonal growth and competitive interactions among clones based on their spatial positions. Based on the relatively large spatial area occupied by clonemates in Agat, this suggests a long history of clonal lineages, and somewhat weak competitive interactions among clones in Agat (Arnaud-Haond & Belkhir, 2007).

Previous studies have found that when corals are stressed, reproductive capacity is one of the first processes to be compromised (Ward et al., 2000; Baird & Marshall, 2002). From 2013-2017, Guam suffered four successive bleaching events that were accompanied by extreme low tides and disease outbreaks, which resulted in the decline of 53% of staghorns around Guam (Raymundo et al., 2017, 2019). In 2015, Raymundo et al. (2017) assessed and quantified the extent of bleaching mortality for four (Agat, Cocos, Togcha, and West Agaña) out of the five studied Guam populations. The mean estimated mortality of the sampling locations in 2015 were all significant and ranged from: 25% at Agat, 30% at Cocos, 55% at West Agaña, and 65% at Togcha (Raymundo et al., 2017). It is interesting that Togcha suffered the highest bleaching mortality out of the sampling sites while currently showing the highest incidence of clonality (Table 2). Thus, I hypothesize that Togcha's high bleaching susceptibility may be a consequence of its high clonality rates (Edmunds, 1994). Additionally, Togcha in particular suffered from extreme thermal stress (Raymundo et al., 2017), which may have disrupted its ability to sexually reproduce. As previously mentioned, Lapacek (2017) found that most of Guam's staghorn *Acropora* species displayed low reproductive output, which may be attributed to the aftermath of bleaching and extreme low tide events. However, Togcha is also the only east coast population and the shallowest population (together with Urunao) so environmental differences likely contributed as well. Further investigation is needed to determine whether clonality levels were a direct result of recent bleaching events or determined more by varying environmental conditions.

Over the last several decades, coral larval recruitment has continued to decline across the Mariana Islands in comparison to other Pacific islands (Birkeland & Randall, 1981; Neudecker, 1981; Minton & Lundgren, 2006). With the high capacity for asexual reproduction in *Acropora*, some corals may rely more on fragmentation versus the more energetically demanding sexual reproduction. However, a better understanding of *A. pulchra*'s reproductive output and its variation in response to local stressors is important for restoration.

Population structure of *A. pulchra*

Inter-island

One overarching result of several analyses presented here was the significant population structure between the islands of Guam and Saipan (Table 5, Figure 4). Only one other study has previously assessed genetic differences among Marianas Islands: Boulay (2016) also found significant genetic structure between Guam and Saipan (F_{ST} = 0.032 - 0.147). Other studies used very different methods to assess inter-island connectivity. For example, Kendall and Poti (2015) used oceanographic modelling and computer simulations to examine the transport pathways of marine larvae around the Marianas Archipelago. They observed highly variable currents passing from Guam to Saipan, and predicted coral larvae originating from the Mariana Islands are likely swept westward due to the dominant North Equatorial Current (Figure 7), or may be locally retained due to the existence of local leeward eddies (Suntsov & Domokos, 2013; Kendall & Poti, 2015). In addition, they found a clear breakpoint in connectivity between Guam and Rota for larvae with a 12-20 day pelagic larval duration (PLD). The maximum competency period of *A. pulchra* is 14 days with settlement often occurring 10 days after fertilization (Baird, 2009), which would allow for occasional direct larval exchange between Guam and Saipan (Kendall & Poti, 2015).

These findings are perfectly consistent with the results (Table 3a-b, Figure 4), and I therefore assume limited gene flow between islands.

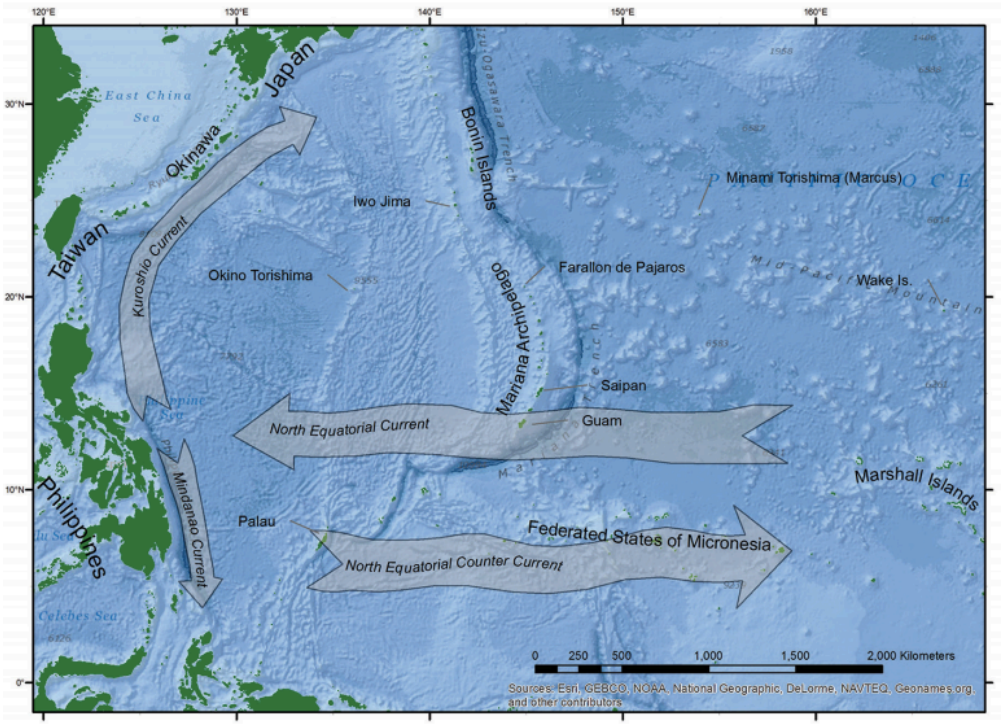

Figure 7. Major current patterns in the northwest Pacific. Source: Kendall and Poti (2015)

However, I also found indications of ongoing larval exchange between Guam and Saipan. Several pairs of closely related individuals across islands, mainly between Saipan and Urunao, were discovered (Table 3a-b). In addition, I observed broad overlap in admixture between Saipan and Guam populations with a higher incidence of green (Guam) admixture in Saipan in comparison to red (Saipan) admixture in Guam (Figure 5). This might indicate higher gene flow northward, from Guam to Saipan than vice versa. Moreover, the North Equatorial Current surrounding Guam (Figure 7) has a dominant northwestward flow (Uda, 1970), which can potentially facilitate larval transport from Guam towards Saipan.

Intra-island

Overall, there was moderate genetic structure between Guam populations (Figure 4, Table 5). PCoA results indicate separate northern and southern population clusters, inter-connected by the population of Agat (Figure 4). I detected the greatest number of second-degree relatives between Cocos and Agat and between Urunao and West Agaña, all of which displayed a genetic overlap on the PCoA and share the closest geographic proximity (Table 3a-b, Figure 4, Figure 1). The lowest pairwise genetic distance was observed between West Agaña and Urunao (F_{ST}=0.008), further supporting their connectivity (Table 5, Figure 4). Pairwise F_{ST} values between southernmost Cocos, and the northwestern sites of West Agaña and Urunao was higher than other comparisons, which may indicate geographically limited larval dispersal over kilometer scales between populations (Table 5). At the entrance to Apra Harbor, current patterns between Agat and West Agaña (sites E-F, Figure 8), reveal a predominant southwestward directionality of currents while currents around Agat (site G, Figure 8), have a predominant northern flow, which may allow for larvae intermixing at these sites (Wolanski et al., 2003).

Additionally, the PCoA identified the southernmost site, Cocos, as clearly distinct from the northwestern populations of West Agaña and Urunao (Figure 4). This may be due to the presence of smaller coastal eddies with a southward current near Cocos that disrupts larvae dispersal towards the northern part of Guam (Wolanski et al., 2003). Moreover, no relative pairs were detected between Cocos and Urunao (Table 3a-b), which share the largest geographic distance between sites (Figure 1). Interestingly, I detected a single second-degree relative between Cocos and West Agaña (Table 3a-b). This may be due to the divergence of westward and northward currents between West Agaña and Cocos (Figure 8), or may be due to occasional storm events, which might facilitate occasional larval mixing.

PCoA results further suggest Agat is central to gene flow along the western coast of Guam, connecting the two separate northern and southern population clusters (Figure 4). This could be due to Agat acting as source of larvae that disperse north and south, or Agat acting as a sink for larvae from the north and south that settle in Agat. Oceanographic analysis by Wolanski et al. (2003) revealed a net northwestward current around Guam. The predominant directionality of currents around West Agaña (site D, Figure 8) is westward, where they converge near the entrance to Apra Harbor (sites E-F, Figure 8) with northward currents coming from Agat and Cocos (Wolanski et al., 2003). This convergence may allow for the intermixing of larvae along the western coast of Guam with Agat central to gene flow. Additionally, in southwestern Guam, Wolanski et al. (2003) discovered energetic, cyclonic eddies that showed a complete rotation in 4-5 days. These eddies may carry larvae throughout the island or return them to their natal reefs, such as those seen in fish eggs and coral larvae in Barbados, West Indies (Lee et al., 1994) and the Florida Keys (Cowen et al., 2000).

Furthermore, I identified the highest number of second-degree relatives between Agat and other populations, particularly with Cocos and Urunao, but also with isolated Togcha and Saipan, consistent with the theory that Agat is a central stepping-stone population for gene flow between *A. pulchra* populations (Table 3a-b). Oceanographic and population genetic analyses do not clearly indicate whether Agat acts more like a sink or source of larvae. In fact, it may be neither, and might instead serve as an area for migration between sites.

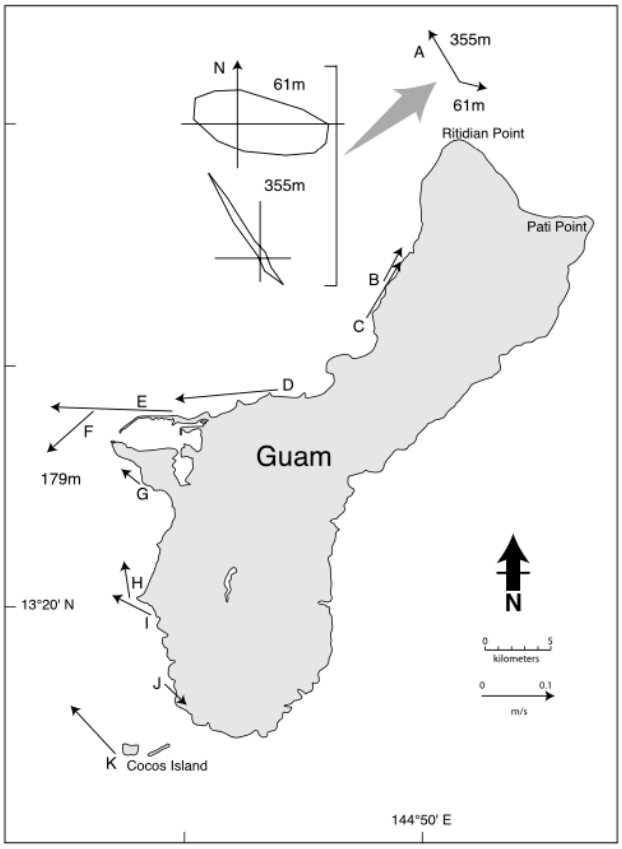

Figure 8. Net currents shown as arrows around Guam. The inset shows the directional histograms of currents. Source: Wolanski et al. (2003)

Population genetic statistics

Overall, population genetic statistics proved to be consistent across populations, indicating very little differences in genetic diversity among populations. For example, results revealed virtually identical levels of observed and expected heterozygosity in Saipan and Guam (H_o= 0.100 vs. 0.097, H_e= 0.113 vs. 0.109, Table 4) and the number of effective alleles (1.16 vs 1.14) (Table 4). In previous studies of multi locus genotype (MLG) data from *A. millepora* in the GBR, van Oppen et al. (2011) found surprisingly similar levels of genetic diversity (estimated by allelic richness) between populations that have and have not undergone recent major disturbances, such as bleaching mortality. They suggested this finding was due to recovery through both new recruitment and possibly tissue regrowth that materialized quickly (van Oppen et al., 2011).

Additionally, Hemond and Vollmer (2010) compared levels of nucleotide and haplotype diversity in *A. cervicornis* between Florida and across the greater Caribbean (Vollmer & Palumbi, 2007). Comparisons revealed Florida had higher levels of standing genetic diversity than the greater Caribbean (Vollmer & Palumbi, 2007), suggesting *A. cervicornis* in Florida comprise a unique population that should be treated as a distinct management unit for conservation (Hemond & Vollmer, 2010). However, Hemond and Vollmer (2010) pose that historical recruitment from the Western Caribbean and other regions is one possible explanation for the relatively high diversity in Florida.

The most interesting difference I observed was the slightly elevated levels of inbreeding in Agat (G_{is}=0.115, Table 4). One possible explanation is the convergence of larvae from northern and southern populations in Agat. However, none of the admixture analyses indicated the presence of distinct genetic clusters in this population so it is not entirely clear what caused this pattern.

Symbiont characterization

This study revealed the presence of four different symbiont genera among *A. pulchra* populations in Guam and Saipan. Interestingly, *A. pulchra* photosymbiont associations were highly specific and notably non-random among locations (Figure 6, Table 8). The majority of *A. pulchra* in Guam contained the heat-sensitive *Cladocopium* (Figure 6, Table 8), which may explain the high bleaching mortality observed by Raymundo et al. (2017). In contrast, individuals from Saipan predominantly hosted the more thermo-tolerant *Durusdinium*, which may explain their surprisingly low bleaching mortality during a recent bleaching Alert 2 from June to August 2020 (Johnston, personal communication). Therefore, differences in symbiont composition may influence bleaching sensitivities of *A. pulchra*.

van Oppen et al. (2001) and Ulstrup and van Oppen (2003) found some *Acropora* species harbor multiple clades of *Symbiodinium*, which has been suggested to play an interactive role in thermal adaptation (Baird et al., 2009). In addition, Barfield et al. (2018) found that genetically identical symbionts in the same host species stemming from different thermal environments displayed differences in host gene expression. Thus, I hypothesize that since populations of Togcha, West Agaña, Cocos and Agat all predominantly harbor the same symbiont, *Cladocopium*, differences in environmental conditions, such as water temperature (Raymundo et al., 2017) and water motion (Fifer et al., in review) at these sites may also influence their different bleaching susceptibilities.

Corals have demonstrated the ability to respond to changing environmental conditions at both the colony and population level (Coles & Jokiel, 1978; Brown et al., 2002). Most noticeably, they survived warming episodes, which warrants the supposition that corals bear adaptive mechanisms (Pandolfi, 1996, 1999; Hughes et al., 2003). Corals have shown adaptive qualities through some species' ability to harbor different symbionts, resulting in different thermal tolerances (Berkelmans & van Oppen, 2006). The most striking finding was that *A. pulchra* from Urunao predominantly harbored *Durusdinium* instead of the more commonly found *Cladocopium* (Figure 6, Table 8). *Durusdinium*, in particular, has shown to increase the bleaching thresholds of coral holobionts and is often present in high temperature environments and on reefs with high water motion (Oliver & Palumbi, 2009, 2011; Stat & Gates, 2011; Ladner et al., 2012).

The presence of *Durusdinium* in Urunao may be an adaptation specific to this population's environment (Figure 6, Table 8). Urunao is the northernmost and shallowest site (Figure 1), rendering it the most thermally variable environment along with observed aerial exposure. Thus, corals present in this site may possess higher inclinations to harbor *Durusdinium* (Oliver and

Palumbi, 2009, 2011; Stat and Gates, 2011; Ladner et al., 2012). Although hardly anything is known about Urunao's population history, spatial extent, previous bleaching patterns, and the variability in environmental conditions, its geology is unique for it occurs on detrital limestone plateau with freshwater seeps, unlike other sites.

Cooke et al. (2020) found *A. tenuis* colonies along the GBR to differ in symbiont affiliations as a result of differing environmental conditions among reefs. Populations in Guam are subject to differing environmental conditions, especially between northern and southern sites, however only a small amount of loci were found to be under selection (Table 6-7). Previous studies have shown that the presence of stress-tolerant populations may improve adaptive capabilities and could fuel adaptation through natural or assisted gene flow (Dixon et al., 2015; Anthony et al., 2017; Morikawa et al., 2019; Schoepf et al., 2019). It is important to note that Urunao may be further threatened due to unfolding, nearby military buildup, which includes the construction of a new military complex, and a live firing range that extends right across this valued population. Therefore, an urgent and better understanding of the environmental influence on these staghorns is vital to unravel the causes for its distinctiveness among Guam populations.

Local genetic adaptation and physiological acclimatization have proven to result in increased thermotolerance across diverse assemblages of reef-building corals in American Samoa (Bay & Palumbi, 2014), the GBR (Dixon et al., 2015), and the Florida Keys (Kenkel et al., 2013). In addition, *Symbiodiniaceae* within the same genus have shown local adaptions to differing environments (Oliver & Palumbi, 2011; Baums et al., 2014; Levin et al., 2016). Therefore, further investigation into what factors contribute to multiple combinations of host and *Symbiodiniaceae* across *A. pulchra* populations around Guam are needed to uncover the unknown mechanisms at play.

Clonality and symbionts

Of particular interest in this context is the presence of different symbionts among clonemates (ramets) within the same genet (Figure S2a-f, Supp. material). Results reveal that the majority of *A. pulchra* clonemates hosted the same symbiont (Figure S2a-f, Supp. material). However, some ramets of the same genet hosted different types and proportions of symbionts (Figure S2a-f, Supp. material). For example, Urunao-08, and Urunao-11 had 100% affiliation with *Cladocopium*, while clonemate Urunao-09 had only a 20% affiliation with *Cladocopium* and an 80% affiliation with *Durusdinium* (Figure S2e, Supp. material). Clonemates, Cocos-48 and Cocos50-51 exhibited 100% affiliation with *Cladocopium*, while Cocos-47 showed 95% association with *Cladocopium* and 5% association with *Durusdinium* (Figure S2c, Supp. material). Like many reef-building corals, *Acropora* frequently reproduce via fragmentation, sometimes resulting in the formation of large, clonal stands (Baums et al., 2006; Foster et al., 2007; Williams et al., 2014). These clonal colonies often harbor the same host-symbiont combinations (Baums et al., 2014; Manzello et al., 2019). However, a previous study on sea anemone, *Anemonia viridis*, revealed greater similarities in host symbiont communities among genets than among ramets, demonstrating a wide range of symbiotic associations and suggesting a capacity for horizontal acquisition in *A. virdis* (Porro et al., 2020).

Overall, almost all ramets of the same genet contained the same proportion of the dominant symbiont type, except for one out of five clonal genets in Agat, two out of six clonal genets in Cocos, and four out of ten clonal genets in Urunao (Figure S1a-f, Supp. material). Before fragmenting, all clonemates presumably hosted the same symbiont so these results indicate an interesting relative switch, which could be a consequence of recent bleaching (Buddemeir & Fautin, 1993; Raymundo et al., 2017).

Abrego et al. (2008) found that interactions between the coral host and their symbionts alter the overall holobiont thermal physiology. Kenkel et al. (2013) found substantial differences in phenotypic responses to thermal stress in *P. astreoides* with the same symbiont less than 10 km apart along the Florida Keys. Similarly, along the Florida reef tract, Durante et al. (2019), found phenotypic variation in stress responses within genets of *A. palmata* that harbored a single strain of *Symbiodinium*. Moreover, Cooke et al. (2020) found differences in symbiont associations of *A. tenuis* at local scales where almost all samples were dominated by *Cladocopium* while a single sample showed a significant association with *Durusdinium*. Cooke et al. (2020) hypothesized water quality to be a main driver of differing symbiont types in a single coral species. This hypothesis may explain Urunao's uniqueness in symbiont types due to more pristine water quality present at this site versus other Guam locations. Moreover, this begs the question as to what mechanisms account for such symbiont variability in ramets of the same genet at Agat, Cocos, and Urunao (Figure S2a-f, Supp. material). A significant amount of intra-genet variation in symbionts and bleaching susceptibility observed in this study remains to be explained. However, I hypothesize that differing environmental conditions allow for the shuffling of symbionts at local scales, even among clonemates (Figure S2a-f, Supp. material), which may be a response to recent bleaching events (Buddemeir & Fautin, 1993; Raymundo et al., 2017).

Implications for management

The incorporation of these population genetic findings into management plans can provide local coral reef managers the genetic insight to further promote the survivorship of *A. pulchra* in Guam. The findings in this study highlight that management plans for *A. pulchra* should safeguard Agat, which is central to gene flow (Figure 4, Table 3a-b), and protect Urunao with presumably

higher sexual reproduction and genotypic diversity (Table 2). Management efforts should also treat Cocos as a separate population due to its genetic separation from other Guam populations, most notably the populations along the northwestern coast (Figure 4). In addition, I recommend future nursery and transplanting efforts include fragments from Urunao due to its high genotypic diversity (Table 2), symbiotic association with thermotolerant *Durusdinium* (Figure 6, Table 8), and the possibility that this population has adapted and acclimatized to particularly harsh environmental conditions (Table 6-7).

Drury and Lirman (2017) highlight the importance of using genetically and genotypically diverse stocks in coral reef restoration to enhance the functional role of biodiversity. I further advocate for the promotion of biodiversity in coral nurseries since it has been proven that more diverse systems have higher levels of survivorship, resilience, and adaptive capabilities to endure disturbances that are predicted to transpire (Jump et al., 2009). I suggest further population genetic studies focus on other *Acropora* species and incorporate these findings into future local restoration management.

Conclusions

As Guam's coral reefs continue to face challenges posed by ongoing climate change, genetic information can shed invaluable insight in predicting how local reefs will respond. This study demonstrates the utility of genome-wide analysis generated using a double-digest RAD approach to quantify the genetic structure of *Acropora pulchra* between Saipan and Guam and among local Guam populations. These results have important management implications that include promoting the further genetic exchange along the west side of Guam, localizing management strategies, and safeguarding populations with increased sexual capacity and

genotypic diversity. This study is the first to encompass a wide geographic range of *A. pulchra* in Guam to include a population on the east, and a newly discovered northern population. We were the first to use genome-wide genotype likelihood data produced by ANGSD to detect previously unknown signatures of selection between populations. Lastly, this study is the first to characterize symbiont compositions of *A. pulchra* in Guam.

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Supplemental Materials

Supplementary Table 1. Binned gap analysis results for the number of counts of pairwise relationships per category. Highlighted in yellow is the chosen clonality threshold.

Threshold categories	Count of pairwise relationships
<0.1	3
0.1-0.11	1
0.11-0.12	0
0.12-0.13	3
0.13-0.14	16
0.14-0.15	116
0.15-0.16	207
0.16-0.17	75
0.17-0.18	32
0.18-0.19	10
0.19-0.2	5
0.2-0.21	0
0.21-0.22	4
0.22-0.23	8
0.23-0.24	11
0.24-0.25	23
0.25-0.26	87
0.26-0.27	198
0.27-0.28	467
0.28-0.29	1314
0.29-0.30	3160
>0.3	12038

Supplementary Table 2. Samples, number of raw ddRAD reads, and number of mapped reads

Sample	Raw reads	Mapped coral reads
AAA01.1	2,221,990	265,312
AAA02.1	547,568	119,770
AAA03.1	137,956	11,411
AAA04.1	1,005,026	118,544
AAA05.1	971,016	66,827
AAA06.1	2,451,556	171,435
AAA07.1	274,095	21,207
AAA08.1	678,070	64,799
ABB01.1	51,747	8,008

ABB02.1	72,694	4,316
ABB03.1	2,362,656	223,772
ABB04.1	990,191	83,710
AGG01.1	1,446,135	114,097
AGG02.1	5,565,502	353,431
AGG03.1	865,034	36,106
AGG04.1	263,921	30,852
AGG05.1	121	34
AGG06.1	4,924,027	324,114
AGG07.1	232,818	14,711
AGG08.1	4,752,687	253,405
AMM01.1	744,042	70,947
AMM02.1	464,384	22,609
AMM03.1	12,881	845
APP01.1	41,023	611
APP02.1	786,922	215,447
APP03.1	482,441	9,378
APP04.1	1,057,377	80,614
APP05.1	875,955	40,752
APP06.1	340,346	138,928
APP06.2.1	180,185	110,873
APP07.1	318,487	39,265
APP08.1	410	187
APP09.1	18,502	3,304
APP10.1	251,895	85,643
APP10.1.1	308,381	36,732
APP11.1	99,298	7,508
APP12.1	31,957	6,462
APP13.1	398,663	72,812
APP14.1	305,244	58,758
APP15.1	103,490	4,684
APP16.1	522,972	514,746
APP16.2	751,966	283,195
ASS01.1	199,358	75,407
ASS02.1	93,685	38,710
AGT01.1	637,595	25,333
AGT02.1	7,413	788
AGT04.1	32,359	13,239
AGT05.1	342	136

AGT06.1	1,166	502
AGT07.1	80,276	22,167
AGT08.1	3,663	1,377
AGT09.1	1,536,385	359,050
AGT10.1	6,824,658	186,998
AGT11.1	68,056	20,221
AGT13.1	17,310,677	8,274
AGT14.1	195,369	53,477
AGT15.1	2,653,439	485,185
AGT16.1	280,119	30,249
AGT17.1	55,487	13,917
AGT18.1	907,196	230,065
AGT19.1	1,096	414
AGT20.1	54,574	540
AGT21.1	447,721	124,120
AGT22.1	321,919	43,680
AGT23.1	133,685	34,039
AGT24.1	375,565	114,060
AGT24.2.1	328,429	98,821
AGT25.1.1	447,153	138,095
AGT25.2.1	279,235	84,850
AGT26.1	122,342	40,283
AGT27.1	71,803	27,174
AGT28.1	979,470	156,040
AGT29.1	205,539	82,661
AGT30.1	366,771	137,889
AGT31.1	630,468	221,220
AGT32.1	2,466,704	692,144
AGT33.1	740,218	139,948
AGT34.1	697,836	118,660
AGT35.1	15,440	7,952
AGT36.1	33,064	16,799
AGT37.1	47,600	14,646
AGT38.1	255,716	60,664
AGT39.1	202	138
AGT40.1	4,312	1,879
AGT41	55,630	1,037
AGT42.1	35,248	361
AGT43.1	15,961	4,999

AGT44.1	425,625	123,359
AGT45.1	8,246	1,796
AGT46.1	451,146	115,393
AGT47	4,711	181
AGT48.1	8,880	399
AGT49.1	30,053	800
COC01.1	204,956	158,471
COC02.1	6,736,816	204,705
COC03.1	102,058	32,007
COC04.1	30,942	6,624
COC05.1	76,009	33,706
COC06.1	35,594	15,999
COC07.1	308,662	68,552
COC08.1	149,175	38,152
COC09.1	180,617	44,788
COC10.1	1,120,849	72,798
COC11.1	3,015,027	173,528
COC12.1	55,392	25,201
COC13.1	5,363,531	295,465
COC14.1	152,648	112686
COC14.2	40,757	423,531
COC15.1	346	37
COC16.1	50	14
COC17.1	37,679	177
COC18.1	67,630	1,606
COC19.1	2,785	1,684
COC20.1	318,329	6,538
COC21.1	42,649	2,038
COC22.1	732	155
COC23.1	18,667	4,636
COC24.1	80,240	20,554
COC25.1	1,547,984	51,105
COC26.1	3,244,837	66,659
COC27.1	499	153
COC29.1	211,603	3,735
COC31.1	2,441,896	38,714
COC32	243	46
COC34.1	35,858	631
COC35.1	18,160	431

COC37.1	1,476,989	28,457
COC38.1	7,231,301	86,908
COC39	48	14
COC40.1	78,597	9,631
COC41.1	323	106
COC42.1	646	262
COC43.1	46,260	542
COC44	6,104	701
COC45.1	9,161	1,406
COC46	21,225	112
COC47.1	250,452	90,479
COC48.1	67,494	27,462
COC49.1	3,274	678
COC50.1	19,958	10,794
COC51.1	37,070	13,756
COC52.1	99,944	20,365
COC53.1	14,890	6,220
COC54.1	23,496	7,194
COC65.1	520	126
COC66	135	28
COC67.1	731,837	120,673
COC68.1	53,299	20,660
COC69.1	1,763,805	340,241
COC70.1	1,438,708	144,917
COC71.1	3,301,276	230,493
TOG01.1	350,142	136,063
TOG02.1	416,075	18,530
TOG03.1	140,874	57,403
TOG04.1	257,792	103,220
TOG05.1	509,387	89,614
TOG06.1	221,766	14,597
TOG07.1	699,376	99,061
TOG08.1	244,096	88,601
TOG09.1	2,159,627	90,235
TOG10.1	6,927,653	315,392
TOG11.1	197,486	51,095
TOG12.1	379,016	96,804
TOG13.1	142,712	15,906
TOG.14.1	548,406	213,109

TOG14.2	20,468	12,551
TOG15.1	69,837	25,769
TOG16.1	962,856	73,953
TOG17.1	2,061,536	172,684
TOG18.1	3,004,777	229,241
TOG19.1	4,950,981	432,475
TOG20.1	118,050	30,546
TOG21.1	1,700,538	483,325
URU01.1	550,476	198,134
URU02.1	25,866	11,005
URU03.1	159,785	59,025
URU04.1	432,848	152,603
URU05.1	783,539	136,531
URU06.1	3,171,173	316,944
URU07.1	2,151,632	438,682
URU08.1	539,148	62,691
URU09.1	148,341	43,867
URU10.1	150,572	42,286
URU11.1	49,405	18,252
URU12.1	35,375	14,616
URU13.1	20,351	4,038
URU14.1	13,310	5,262
URU15.1	920,652	174,938
URU16.1	7,075	915
URU17.1	116,643	22,639
URU18.1	100,519	38,077
URU19.1	344,021	132,367
URU20.1	316,911	124,624
URU21.1	37,092	18,406
URU22.1	35,559	18,205
URU23.1	191,498	36,957
URU24.1	1,082,025	184,218
URU25.1	275,625	49,888
URU26.1	814,073	196,606
URU27.1	612,519	138,902
URU28.1	374,093	124,468
URU29.1	58,790	10,328
URU30.1	210,636	42,787
URU31.1	242,260	47,033

URU32.1	99,165	17,132
URU33.1	2,815,267	121,913
URU34.1	47,934	153,638
URU34.2	78,453	23,997
URU35.1	83,847	37,968
URU35.2	246,701	12,437
URU36.1	121,473	27,548
URU37.1	1,001,580	127,395
URU38.1	215,814	33,040
URU39.1	970,193	141,933
URU40.1	161,259	36,969
URU41.1	76,489	22,171
URU45.1	6,815	2,169
URU48.1	631,226	156,273
URU49.1	57,485	15,962
URU50.1	15,335	4,938
WAG01.1	51,984	1,548
WAG02.1	2,171,631	65,271
WAG03.1	9,122	2,757
WAG04.1	6,698	2,174
WAG05.1	574	51
WAG06.1	28,370	8,239
WAG07.1	15,856	3,107
WAG08.1	2,322,636	19,332
WAG09.1	1,435,590	27,512
WAG11.1	1,294,219	19,320
WAG13.1	292,997	10,924
WAG14.1	2,670,254	84,111
WAG15.1	1,672	400
WAG16.1	995,122	237,368
WAG17.1	552,038	58,825
WAG18.1	519	52
WAG19.1	613,367	154,979
WAG20.1	303,024	82,401
WAG21.1	277,072	75,373
WAG22.1	21,121	1,241
WAG23.1	2,226,878	567,027
WAG24.1	443,389	83,167
WAG25.1	590,990	132,975

WAG27.1	3,422	1,303
WAG28.1	634,818	146,058
WAG30.1	74,492	26,373
WAG31.1	1,700,764	397,574
WAG32.1	5,581,046	198,705
WAG33.1	3,788	1,323
WAG34.1	2,203,622	42,775
WAG35.1	465	239
WAG36.1	11,456	3,193
WAG37.1	7,294	3,556
WAG38.1	178,194	70,115
WAG39.1	852,080	115,241
WAG40.1	849,606	125,623
WAG41.1	377,796	87,922
WAG42.1	249,900	93,400
WAG43.1	86	26
WAG44.1	1,931,628	58,852
WAG45.1	33,576	6,420
WAG46.1	112,861	15,341
WAG47.1	538,589	11,060
WAG48.1	64,595	9,246
WAG49.1	77,274	30,289
WAG50.1	14,514	5,366
AVERAGE	750,724	79,198
MEDIAN	202,157	35,073
MIN	48	14
MAX	17,310,677	692,144

Supplementary Table 3. List of sample name, sample ID, and population designation for clonal IBS analysis

Sample name	Sample ID	Population
AAA01.1.sorted.bam	1	Saipan
AAA02.1.sorted.bam	2	Saipan
AAA03.1.sorted.bam	3	Saipan
AAA04.1.sorted.bam	4	Saipan
AAA05.1.sorted.bam	5	Saipan
AAA06.1.sorted.bam	6	Saipan
AAA07.1.sorted.bam	7	Saipan

AAA08.1.sorted.bam	8	Saipan
AGT01.1.sorted.bam	9	Agat
AGT04.1.sorted.bam	10	Agat
AGT07.1.sorted.bam	11	Agat
AGT09.1.sorted.bam	12	Agat
AGT10.1.sorted.bam	13	Agat
AGT11.1.sorted.bam	14	Agat
AGT13.1.sorted.bam	15	Agat
AGT14.1.sorted.bam	16	Agat
AGT15.1.sorted.bam	17	Agat
AGT16.1.sorted.bam	18	Agat
AGT17.1.sorted.bam	19	Agat
AGT18.1.sorted.bam	20	Agat
AGT21.1.sorted.bam	21	Agat
AGT22.1.sorted.bam	22	Agat
AGT23.1.sorted.bam	23	Agat
AGT24.1_R1.TR.sorted.bam	24	Agat
AGT24.1.sorted.bam	24-1	Agat
AGT24.2_R1.TR.sorted.bam	24-2	Agat
AGT25.1_R1.TR.sorted.bam	25-1	Agat
AGT25.1.sorted.bam	25	Agat
AGT25.2_R1.TR.sorted.bam	25-2	Agat
AGT26.1.sorted.bam	26	Agat
AGT27.1.sorted.bam	27	Agat
AGT28.1.sorted.bam	28	Agat
AGT29.1.sorted.bam	29	Agat
AGT30.1.sorted.bam	30	Agat
AGT31.1.sorted.bam	31	Agat
AGT32.1.sorted.bam	32	Agat
AGT33.1.sorted.bam	33	Agat
AGT34.1.sorted.bam	34	Agat
AGT35.1.sorted.bam	35	Agat
AGT36.1.sorted.bam	36	Agat
AGT37.1.sorted.bam	37	Agat
AGT38.1.sorted.bam	38	Agat
AP10.1_R1.TR.sorted.bam	39-1	Saipan
AP10.2_R1.TR.sorted.bam	39-2	Saipan

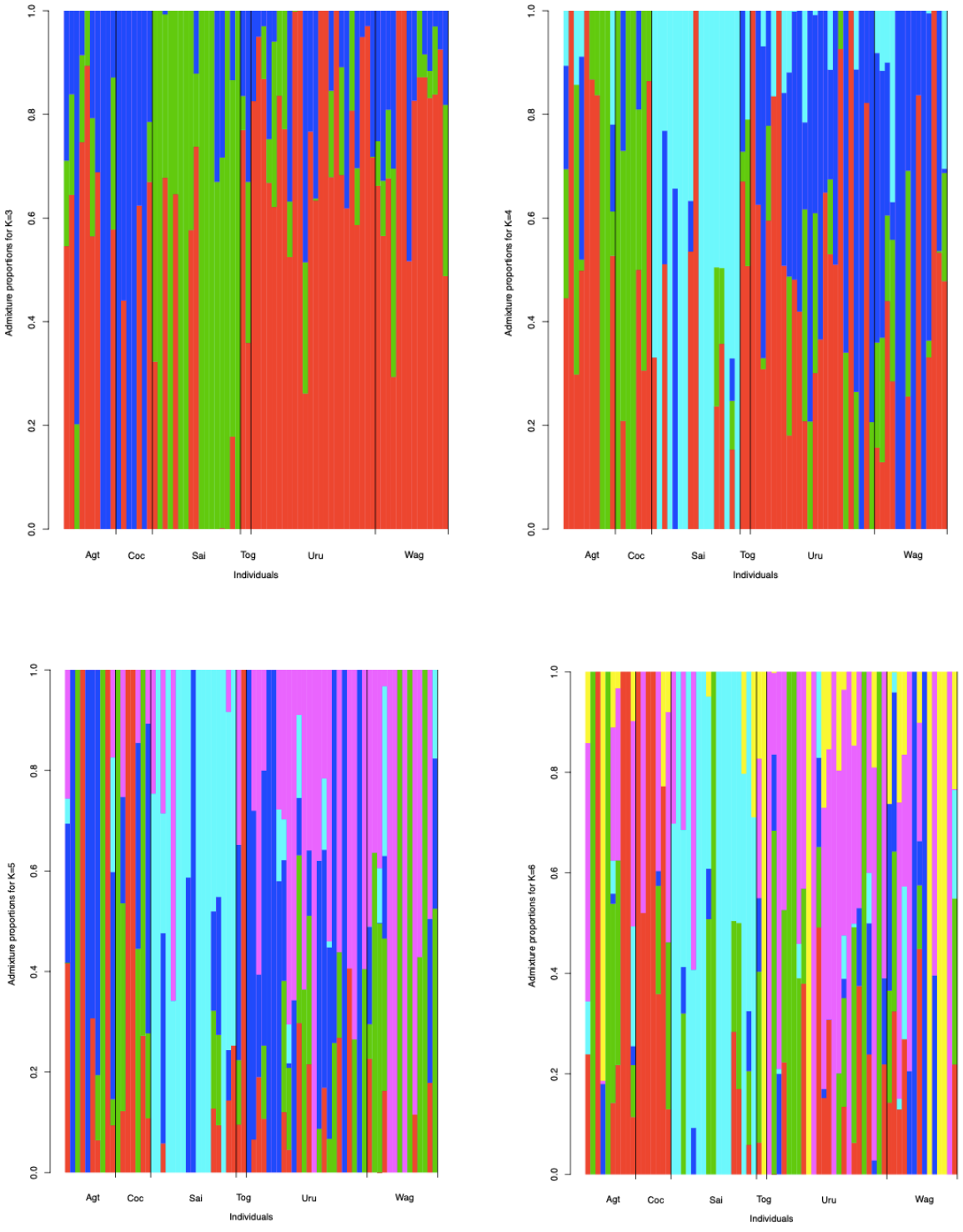
APP02.1.sorted.bam	40	Saipan
APP03.1.sorted.bam	41	Saipan
APP04.1.sorted.bam	42	Saipan
APP05.1.sorted.bam	43	Saipan
APP06.1.sorted.bam	44	Saipan
APP06.1.TR.sorted.bam	44-1	Saipan
APP06.2.TR.sorted.bam	44-2	Saipan
APP07.1.sorted.bam	45	Saipan
APP10.1.sorted.bam	39	Saipan
APP11.1.sorted.bam	46	Saipan
APP12.1.sorted.bam	47	Saipan
APP13.1.sorted.bam	48	Saipan
APP14.1.sorted.bam	49	Saipan
ASS02.1.sorted.bam	50	Saipan
ASS02.2_R1.TR.sorted.bam	50-1	Saipan
ASS02.3_R1.TR.sorted.bam	50-2	Saipan
CO14.1_R1.TR.sorted.bam	51-1	Cocos
CO14.2_R1.TR.sorted.bam	51-2	Cocos
COC01.1.sorted.bam	52	Cocos
COC02.1.sorted.bam	53	Cocos
COC03.1.sorted.bam	54	Cocos
COC04.1.sorted.bam	55	Cocos
COC05.1.sorted.bam	56	Cocos
COC06.1.sorted.bam	57	Cocos
COC08.1.sorted.bam	58	Cocos
COC09.1.sorted.bam	59	Cocos
COC10.1.sorted.bam	60	Cocos
COC11.1.sorted.bam	61	Cocos
COC12.1.sorted.bam	62	Cocos
COC13.1.sorted.bam	63	Cocos
COC14.1.sorted.bam	51	Cocos
COC20.1.sorted.bam	64	Cocos
COC24.1.sorted.bam	65	Cocos
COC26.1.sorted.bam	66	Cocos
COC37.1.sorted.bam	67	Cocos
COC38.1.sorted.bam	68	Cocos
COC40.1.sorted.bam	69	Cocos

COC47.1.sorted.bam	70	Cocos
COC48.1.sorted.bam	71	Cocos
COC50.1.sorted.bam	72	Cocos
COC51.1.sorted.bam	73	Cocos
COC52.1.sorted.bam	74	Cocos
COC53.1.sorted.bam	75	Cocos
COC54.1.sorted.bam	76	Cocos
TOG01.1.sorted.bam	77	Togcha
TOG02.1.sorted.bam	78	Togcha
TOG03.1.sorted.bam	79	Togcha
TOG04.1.sorted.bam	80	Togcha
TOG05.1.sorted.bam	81	Togcha
TOG06.1.sorted.bam	82	Togcha
TOG07.1.sorted.bam	83	Togcha
TOG08.1.sorted.bam	84	Togcha
TOG09.1.sorted.bam	85	Togcha
TOG10.1.sorted.bam	86	Togcha
TOG11.1.sorted.bam	87	Togcha
TOG12.1.sorted.bam	88	Togcha
TOG13.1.sorted.bam	89	Togcha
TOG14.1.sorted.bam	90	Togcha
TOG14.1.TR.sorted.bam	90-1	Togcha
TOG14.2.TR.sorted.bam	90-2	Togcha
TOG15.1.sorted.bam	91	Togcha
TOG16.1.sorted.bam	92	Togcha
TOG17.1.sorted.bam	93	Togcha
TOG18.1.sorted.bam	94	Togcha
TOG19.1.sorted.bam	95	Togcha
TOG20.1.sorted.bam	96	Togcha
TOG21.1.sorted.bam	97	Togcha
URU01.1.sorted.bam	98	Urunao
URU02.1.sorted.bam	99	Urunao
URU03.1.sorted.bam	100	Urunao
URU04.1.sorted.bam	101	Urunao
URU05.1.sorted.bam	102	Urunao
URU06.1.sorted.bam	103	Urunao
URU07.1.sorted.bam	104	Urunao

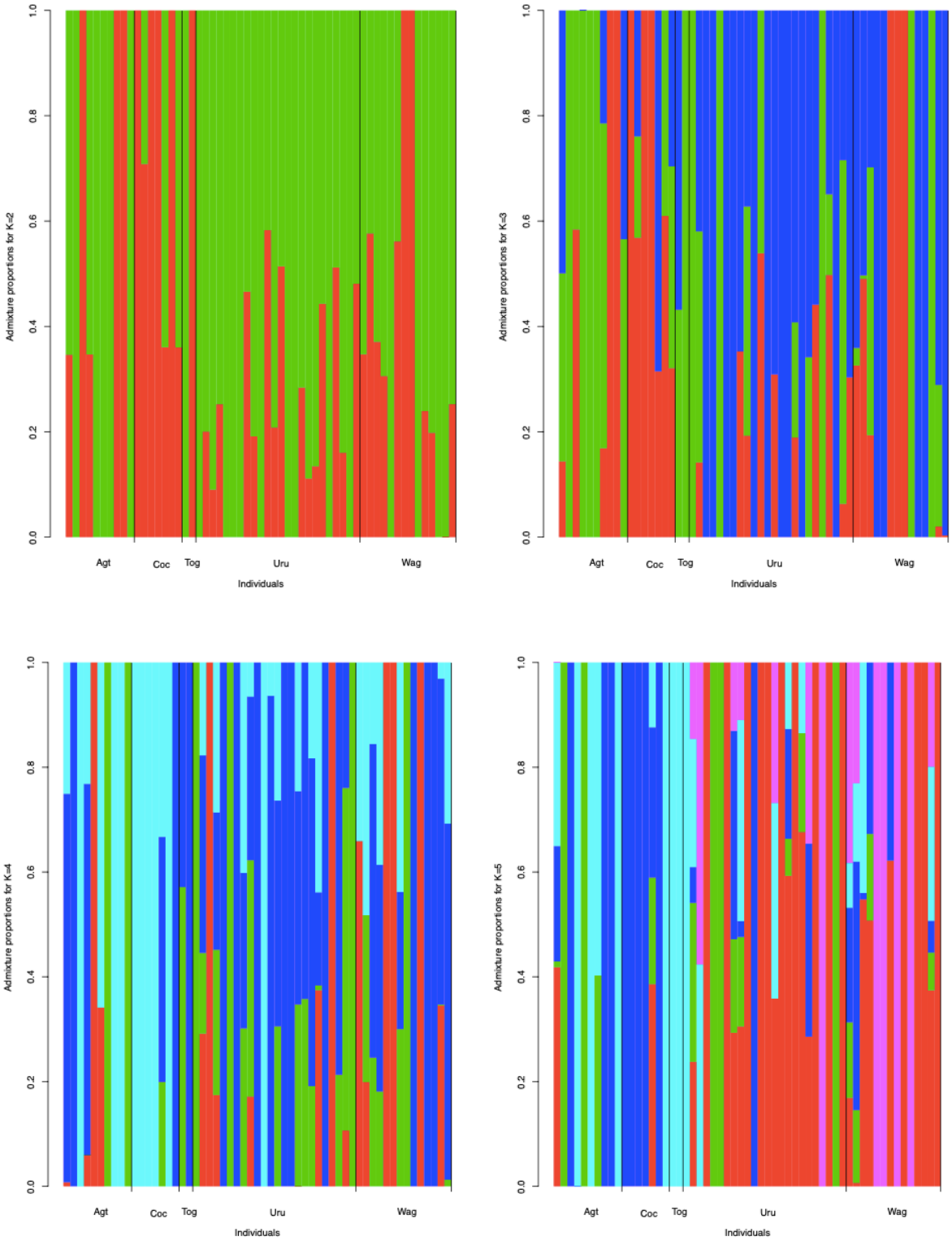
URU08.1.sorted.bam	105	Urunao
URU09.1.sorted.bam	106	Urunao
URU10.1.sorted.bam	107	Urunao
URU11.1.sorted.bam	108	Urunao
URU12.1.sorted.bam	109	Urunao
URU14.1.sorted.bam	110	Urunao
URU15.1.sorted.bam	111	Urunao
URU17.1.sorted.bam	112	Urunao
URU18.1.sorted.bam	113	Urunao
URU19.1.sorted.bam	114	Urunao
URU20.1.sorted.bam	115	Urunao
URU21.1.sorted.bam	116	Urunao
URU22.1.sorted.bam	117	Urunao
URU23.1.sorted.bam	118	Urunao
URU24.1.sorted.bam	119	Urunao
URU25.1.sorted.bam	120	Urunao
URU26.1.sorted.bam	121	Urunao
URU27.1.sorted.bam	122	Urunao
URU28.1.sorted.bam	123	Urunao
URU29.1.sorted.bam	124	Urunao
URU30.1.sorted.bam	125	Urunao
URU31.1.sorted.bam	126	Urunao
URU32.1.sorted.bam	127	Urunao
URU33.1.sorted.bam	128	Urunao
URU34.1.sorted.bam	129	Urunao
URU34.1.TR.sorted.bam	129-1	Urunao
URU34.2.TR.sorted.bam	129-2	Urunao
URU35.1_R1.TR.sorted.bam	130	Urunao
URU35.1.sorted.bam	130-2	Urunao
URU35.2_R1.TR.sorted.bam	130-3	Urunao
URU36.1.sorted.bam	131	Urunao
URU37.1.sorted.bam	132	Urunao
URU38.1.sorted.bam	133	Urunao
URU39.1.sorted.bam	134	Urunao
URU40.1.sorted.bam	135	Urunao
URU41.1.sorted.bam	136	Urunao
URU48.1.sorted.bam	137	Urunao

URU49.1.sorted.bam	138	Urunao
WAG02.1.sorted.bam	139	West Agana
WAG06.1.sorted.bam	140	West Agana
WAG08.1.sorted.bam	141	West Agana
WAG09.1.sorted.bam	142	West Agana
WAG11.1.sorted.bam	143	West Agana
WAG13.1.sorted.bam	144	West Agana
WAG14.1.sorted.bam	145	West Agana
WAG16.1.sorted.bam	146	West Agana
WAG17.1.sorted.bam	147	West Agana
WAG19.1.sorted.bam	148	West Agana
WAG20.1.sorted.bam	149	West Agana
WAG21.1.sorted.bam	150	West Agana
WAG23.1.sorted.bam	151	West Agana
WAG24.1.sorted.bam	152	West Agana
WAG25.1.sorted.bam	153	West Agana
WAG28.1.sorted.bam	154	West Agana
WAG30.1.sorted.bam	155	West Agana
WAG31.1.sorted.bam	156	West Agana
WAG32.1.sorted.bam	157	West Agana
WAG34.1.sorted.bam	158	West Agana
WAG38.1.sorted.bam	159	West Agana
WAG39.1.sorted.bam	160	West Agana
WAG40.1.sorted.bam	161	West Agana
WAG41.1.sorted.bam	162	West Agana
WAG42.1.sorted.bam	163	West Agana
WAG44.1.sorted.bam	164	West Agana
WAG45.1.sorted.bam	165	West Agana
WAG46.1.sorted.bam	166	West Agana
WAG47.1.sorted.bam	167	West Agana
WAG48.1.sorted.bam	168	West Agana
WAG49.1.sorted.bam	169	West Agana
WAG50.1.sorted.bam	170	West Agana

a.


Admixture proportions for K=3
Admixture proportions for K=4
Admixture proportions for K=5
Admixture proportions for K=6
0.0
0.2
0.4
0.6
0.8
1.0
Agt
Coc
Sai
Tog
Uru
Wag
Individuals

b.



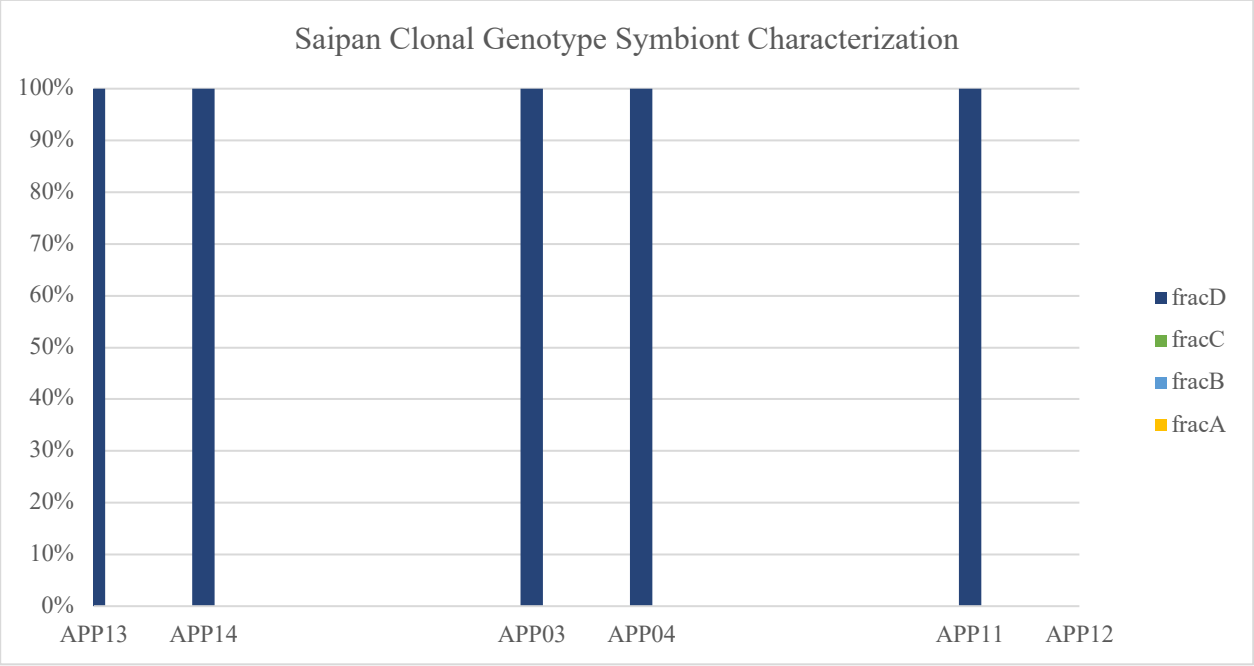
Supplementary Figure 1 a Admixture analysis for six sampling locations showing separation of individuals into three to six (K=3-6) genetic clusters. Each vertical bar represents a separate individual, in

which the proportion of each color indicates the percent ancestry or assignment to each genetic cluster. **b** Admixture analysis for five Guam locations showing separation of individuals into three to five (K=3-5,) genetic clusters. Each vertical bar represents a separate individual where the proportion of each color indicates the percent ancestry or assignment to each genetic cluster

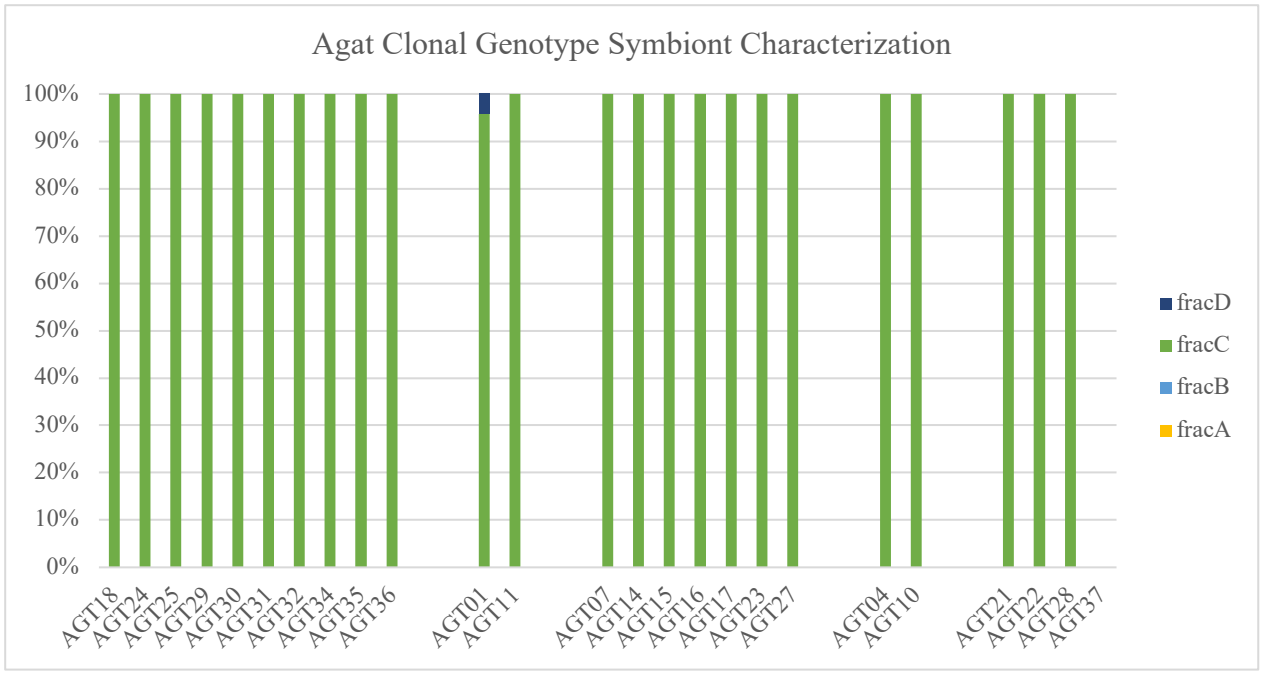
Supplementary Table 4. Tajima's D values calculated using ANGSD subprogram, ThetaStat on the final population genetic dataset (n = 74)

Population	Average	Median	Min	Max	Number of sites <-1	Number of sites >2
Saipan	0.990	1.13	-1.89	3.03	9	115
Agat	1.06	1.23	-1.51	2.71	4	107
Cocos	1.10	1.27	-0.923	2.43	0	67
Togcha	0.647	0.658	-0.747	2.16	0	8
Urunao	1.27	1.45	-1.42	3.19	6	286
West Agana	1.29	1.43	-0.868	2.99	0	239

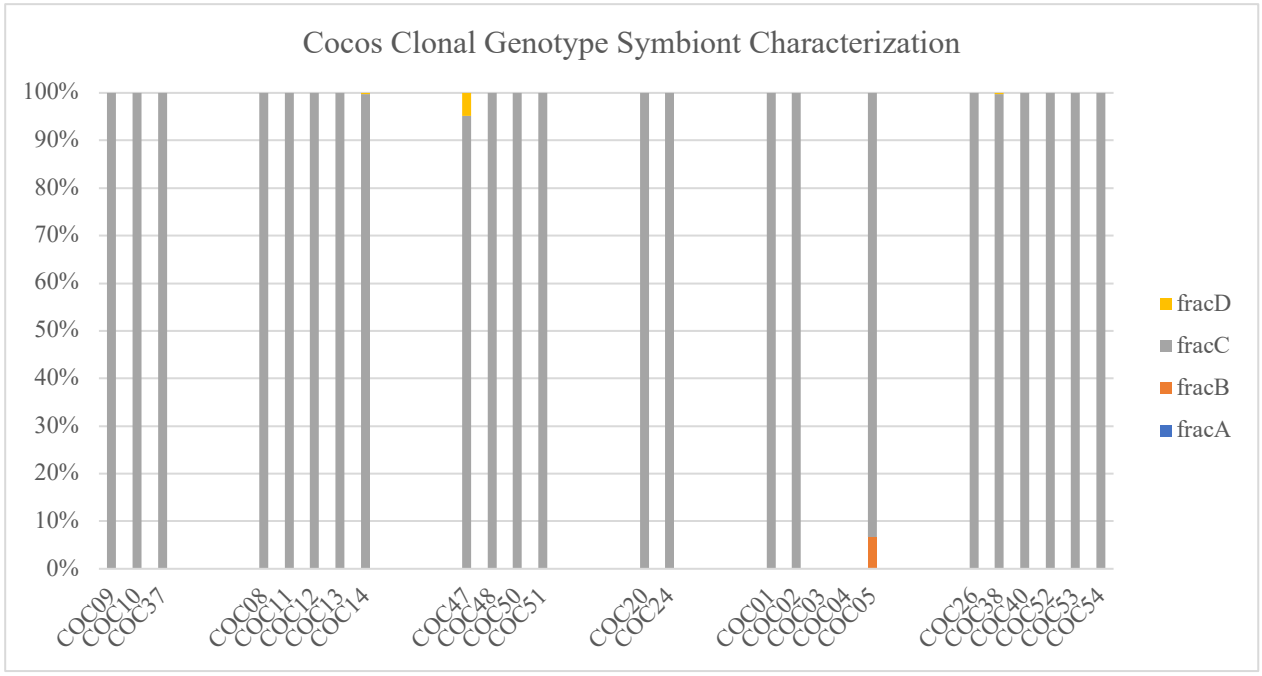
a.



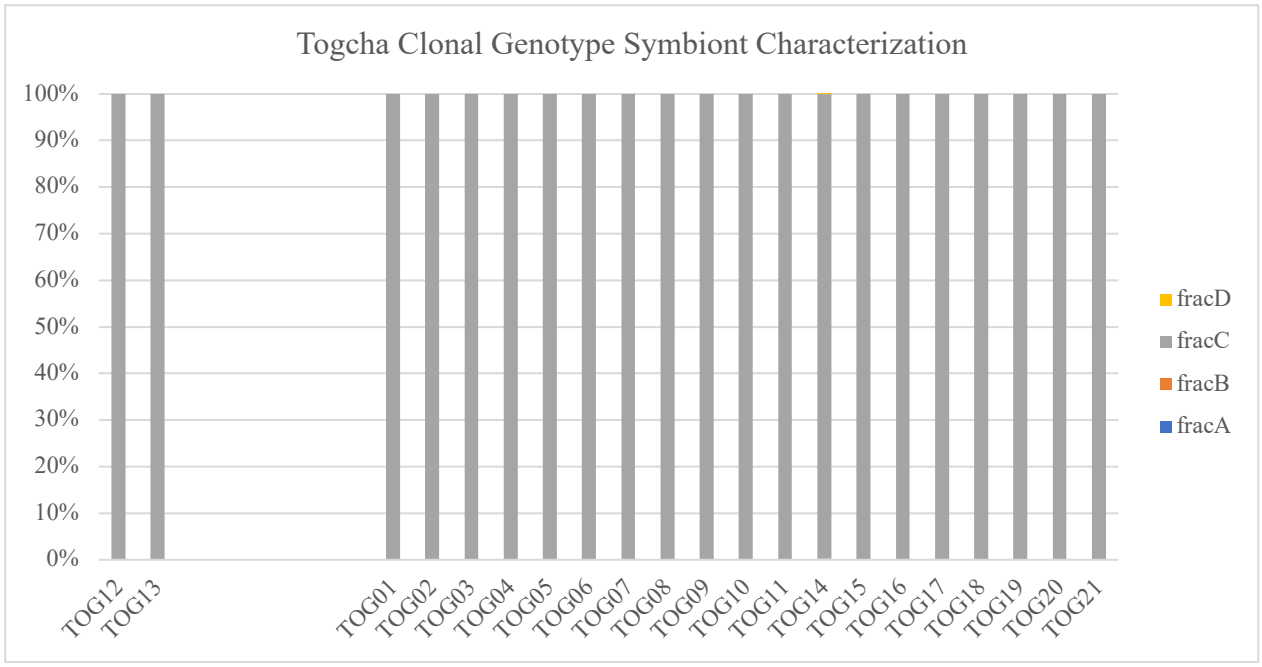
b.


Agat Clonal Genotype Symbiont Characterization
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
AGT18
AGT24
AGT25
AGT29
AGT30
AGT31
AGT32
AGT34
AGT35
AGT36
AGT01
AGT11
AGT07
AGT14
AGT15
AGT16
AGT17
AGT23
AGT27
AGT04
AGT10
AGT21
AGT22
AGT28
AGT37
fracD
fracC
fracB
fracA

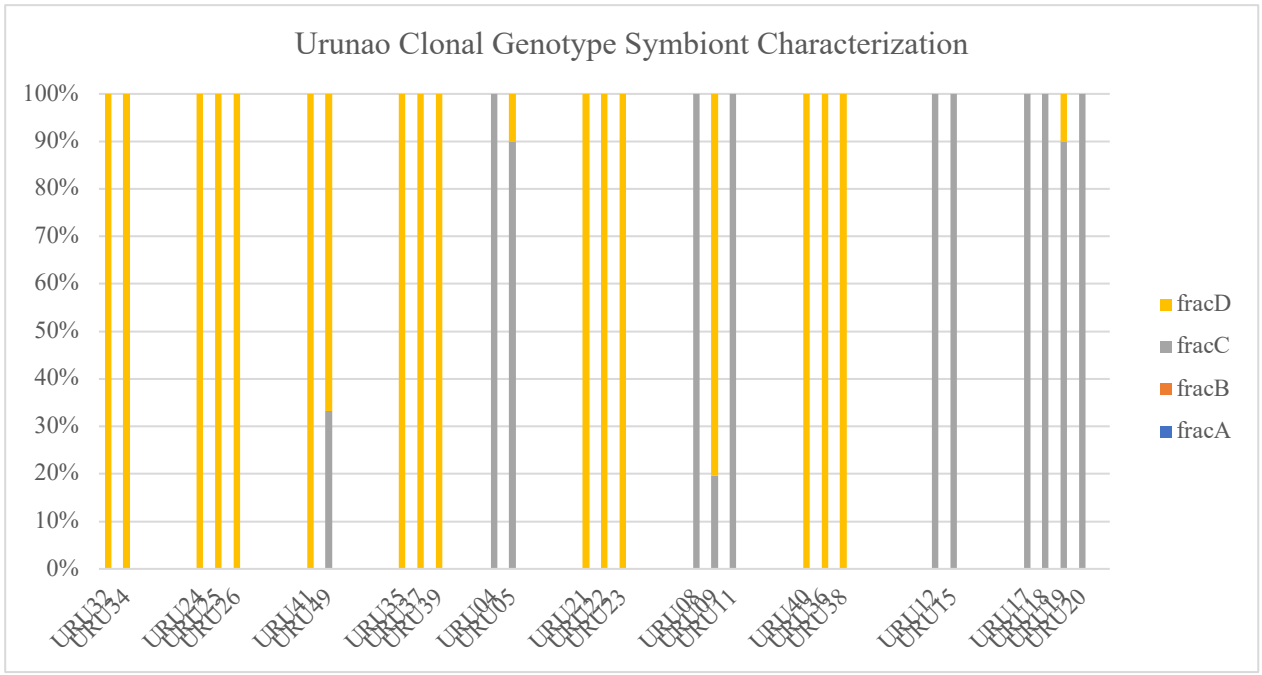
c.


Cocos Clonal Genotype Symbiont Characterization
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
COC09
COC10
COC37
COC08
COC11
COC12
COC13
COC14
COC47
COC48
COC50
COC51
COC20
COC24
COC01
COC02
COC03
COC04
COC05
COC26
COC38
COC40
COC52
COC53
COC54
fracD
fracC
fracB
fracA

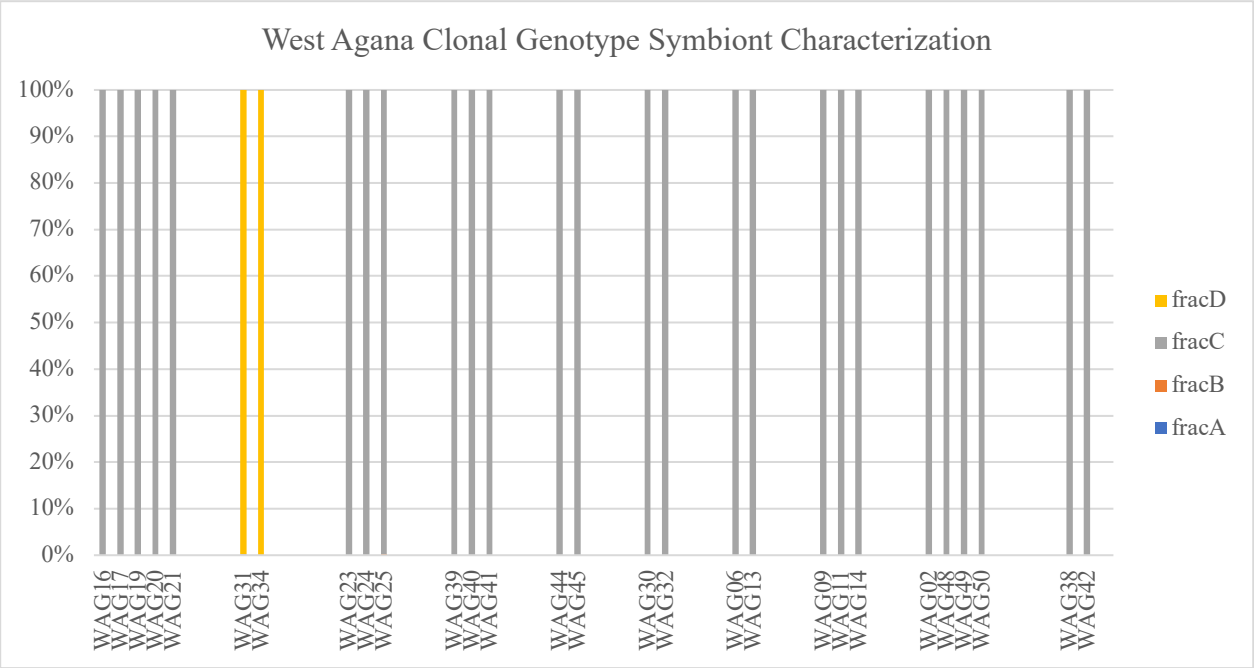
d.


Togcha Clonal Genotype Symbiont Characterization
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
TOG12
TOG13
TOG01
TOG02
TOG03
TOG04
TOG05
TOG06
TOG07
TOG08
TOG09
TOG10
TOG11
TOG14
TOG15
TOG16
TOG17
TOG18
TOG19
TOG20
TOG21
fracD
fracC
fracB
fracA

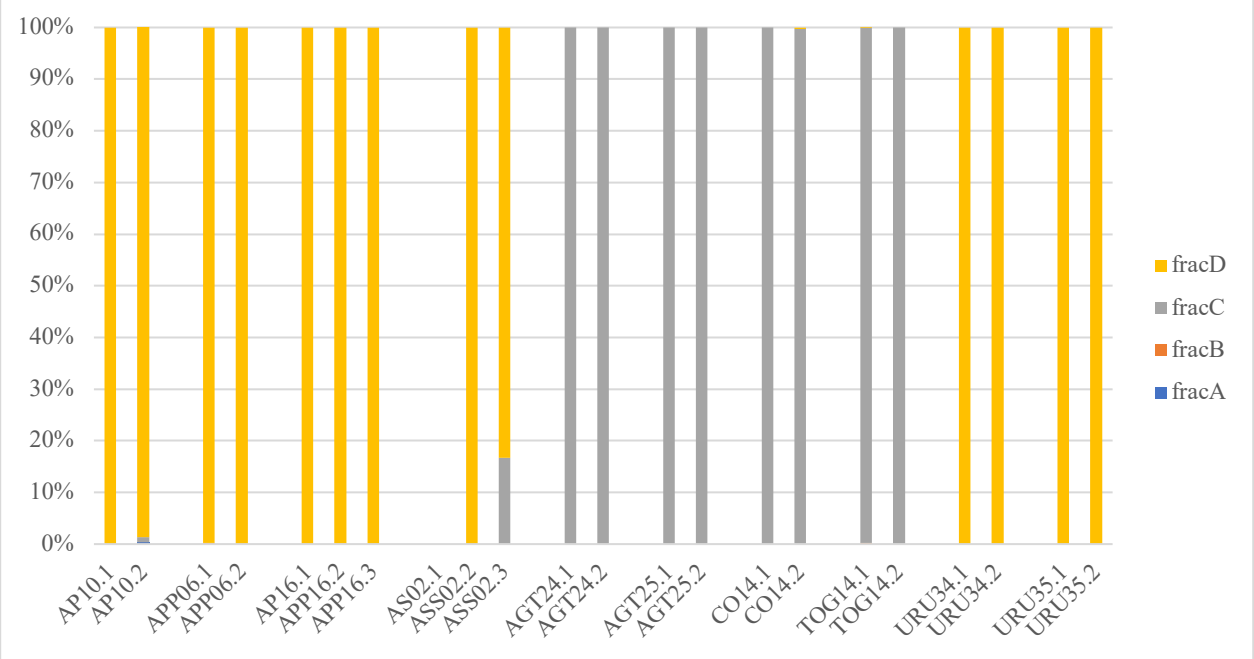
e.


Urunao Clonal Genotype Symbiont Characterization
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
URU34
URU24
URU25
URU26
URU41
URU49
URU35
URU37
URU39
URU04
URU05
URU21
URU22
URU23
URU08
URU09
URU11
URU40
URU36
URU38
URU12
URU15
URU17
URU18
URU19
URU20
fracD
fracC
fracB
fracA

f.



Supplementary Figure 2a-f. Bar plot representing the relative proportions of each population and their clonal genotypes (separated by column spaces) producing highly unique matches to transcriptomes of four different genera of algal symbionts, *Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durusdinium* (formerly Clades A-D, respectively)



Supplementary Figure 3. Bar plot representing the relative proportions of each population and their technical replicates (separated by column spaces and indicated by ".1-.3") producing highly unique matches to transcriptomes of four different genera of algal symbionts, *Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durusdinium* (formerly Clades A-D, respectively)