Geographically driven differences in microbiomes of *Acropora cervicornis* originating from different regions of Florida’s Coral Reef

Sara D. Williams¹, J. Grace Klinges³, Samara Zinman¹, Abigail S. Clark⁴, Erich Bartels², Marina Villoch Diaz Maurino³ and Erinn M. Muller¹

¹ Mote Marine Laboratory, Sarasota, FL, United States of America
² Mote Marine Laboratory, Elizabeth Moore International Center for Coral Reef Research & Restoration, Summerland Key, FL, United States of America
³ Nova Southeastern University, Dania Beach, FL, United States of America
⁴ The College of the Florida Keys, Key West, FL, United States of America

ABSTRACT

Effective coral restoration must include comprehensive investigations of the targeted coral community that consider all aspects of the coral holobiont—the coral host, symbiotic algae, and microbiome. For example, the richness and composition of microorganisms associated with corals may be indicative of the corals’ health status and thus help guide restoration activities. Potential differences in microbiomes of restoration corals due to differences in host genetics, environmental condition, or geographic location, may then influence outplant success. The objective of the present study was to characterize and compare the microbiomes of apparently healthy *Acropora cervicornis* genotypes that were originally collected from environmentally distinct regions of Florida’s Coral Reef and sampled after residing within Mote Marine Laboratory’s *in situ* nursery near Looe Key, FL (USA) for multiple years. By using 16S rRNA high-throughput sequencing, we described the microbial communities of 74 *A. cervicornis* genotypes originating from the Lower Florida Keys (*n* = 40 genotypes), the Middle Florida Keys (*n* = 15 genotypes), and the Upper Florida Keys (*n* = 19 genotypes). Our findings demonstrated that the bacterial communities of *A. cervicornis* originating from the Lower Keys were significantly different from the bacterial communities of those originating from the Upper and Middle Keys even after these corals were held within the same common garden nursery for an average of 3.4 years. However, the bacterial communities of corals originating in the Upper Keys were not significantly different from those in the Middle Keys. The majority of the genotypes, regardless of collection region, were dominated by Alphaproteobacteria, namely an obligate intracellular parasite of the genus Ca. *Aquarickettsia*. Genotypes from the Upper and Middle Keys also had high relative abundances of *Spirochaeta* bacteria. Several genotypes originating from both the Lower and Upper Keys had lower abundances of *Aquarickettsia*, resulting in significantly higher species richness and diversity. Low abundance of *Aquarickettsia* has been previously identified as a signature of disease resistance. While the low-*Aquarickettsia* corals from both the Upper and Lower Keys had high abundances of an unclassified Proteobacteria, the genotypes in the Upper Keys were also dominated by *Spirochaeta*. The results of this study suggest that the abundance...
of *Aquarickettsia* and *Spirochaeta* may play an important role in distinguishing bacterial communities among *A. cervicornis* populations and compositional differences of these bacterial communities may be driven by regional processes that are influenced by both the environmental history and genetic relatedness of the host. Additionally, the high microbial diversity of low-*Aquarickettsia* genotypes may provide resilience to their hosts, and these genotypes may be a potential resource for restoration practices and management.

**Subjects** Conservation Biology, Ecology, Marine Biology, Microbiology  
**Keywords** Microbiome, *Acropora cervicornis*, Staghorn coral, *Aquarickettsia*, Coral Restoration, Florida’s Coral Reef, *Spirochaeta*

## INTRODUCTION

Caribbean populations of the staghorn coral *Acropora cervicornis* have exhibited marked declines since 1980 due to a multitude of stressors including infectious disease, poor water quality, high sea surface temperatures, and overfishing ([Acropora Biological Review Team, 2005](https://www.acropora.org/)). Once a predominant reef-building species of the Western Atlantic and Caribbean, this coral species has experienced a population reduction of nearly 95% and is now listed as critically endangered under the International Union of Conservation of Nature’s (IUCN) Red List ([Aronson & Precht, 2006](https://iucnredlist.org); [Acropora Biological Review Team, 2005](https://www.acropora.org/)). The decline of this species, in concurrence with its sister species, *A. palmata*, has contributed to a reduction in Caribbean coral reef cover of up to 80% ([Gardner et al., 2003](https://www.ipcc.es/)). Asexual propagation of *A. cervicornis*, for coral gardening purposes in both land and *in situ* nurseries, and restoration to *in situ* reef environments, referred to here as ‘outplanting’, is a commonly used conservation strategy on Florida’s Coral Reef ([Schopmeyer et al., 2017](https://www.acropora.org/); [Lirman et al., 2010](https://www.acropora.org/)) and throughout the greater Caribbean ([Mercado-Molina, Ruiz-Diaz & Sabat, 2015](https://www.acropora.org/); [Young, Schopmeyer & Lirman, 2012](https://www.acropora.org/)). Despite the overall success of outplanting ([Boström-Einarsson et al., 2020](https://www.acropora.org/); [Schopmeyer et al., 2017](https://www.acropora.org/)), environmental conditions have not improved and the ability for outplants to survive long-term is questionable ([Ware et al., 2020](https://www.acropora.org/); [van Woesik et al., 2021](https://www.acropora.org/)), therefore, restoration efforts must thoughtfully integrate genotypes most likely to succeed and survive.

The use of microsatellite loci to distinguish different genotypes (or asexual clones) of *Acropora cervicornis* has not only revealed the high level of genetic diversity of this species within the Caribbean, but has also identified variation in traits relevant to restoration success. *A. cervicornis* populations are extensively structured at regional scales on Florida’s Coral Reef (FCR), with high variation in genetic diversity among regions ([Drury, Manzello & Lirman, 2017](https://www.acropora.org/)). Significant differences in growth rates, frequency of branching, and calcification across coral genotypes have been documented in *Acropora* species ([Lirman et al., 2014](https://www.acropora.org/); [Kuffner et al., 2017](https://www.acropora.org/); [Lohr & Patterson, 2017](https://www.acropora.org/)). Specific genotypes have been identified that are more resilient to stressors, including thermal stress ([Drury et al., 2017](https://www.acropora.org/); [Lohr & Patterson, 2017](https://www.acropora.org/); [Ladd et al., 2017](https://www.acropora.org/)) and disease ([Libro & Vollmer, 2016](https://www.acropora.org/); [Muller, Bartels & Baums, 2018](https://www.acropora.org/)). The complex association of the coral microbiome with host
health state, however, precludes definitive association of preferential phenotypes with host traits alone. Symbiont identity plays an important role in determining thermotolerance (Swain et al., 2016; Cunning et al., 2015), disease response (Rouzé et al., 2016), and growth rates (Cunning et al., 2015) in Acropora species. Similarly, the bacterial communities harbored by corals perform numerous services for their hosts such as nutrient cycling and provide essential settlement cues (Lesser et al., 2007; Sharp, Distel & Paul, 2012; Peixoto et al., 2017). Certain coral-associated bacterial genera provide an essential first line of defense against pathogenic species, and therefore disease, through the production of antimicrobial compounds (Krediet et al., 2013; Glasl, Herndl & Frade, 2016). The diversity of the coral microbiome may also play a role in resilience to stressors, as higher microbial diversity may provide the coral with a greater arsenal of services to supplement coral metabolism and antibiotic defenses (Zilber-Rosenberg & Rosenberg, 2008; West et al., 2019).

Coral-associated microbes tend to vary by host species (Morrow et al., 2012; Littman et al., 2009; Rohwer et al., 2002), and within species, microbial community structure can be stable across broad geographic regions (Sunagawa, Woodley & Medina, 2010; Brener-Raffalli et al., 2018). Nonetheless, coral microbial communities respond to naturally-occurring spatiotemporal factors including depth (Glasl et al., 2017), predation by herbivores (Rice et al., 2019; Ezzat et al., 2020), and seasonality (Li et al., 2014; Ceh, Van Keulen & Bourne, 2011; Hong et al., 2009; Koren & Rosenberg, 2006). Further, the coral microbiome is responsive to environmental stressors including nutrient enrichment (Zaneveld et al., 2016; Wang et al., 2018), overfishing (Zaneveld et al., 2016; McDevitt-Irwin et al., 2017), macroalgal competition (Nugues et al., 2004; Vega Thurber et al., 2012; Pratte et al., 2018), and thermal stress (Bourne et al., 2008; Maher et al., 2019).

Florida’s Coral Reef has distinct spatial regions because of varying seawater circulation patterns due to the underlying geology that contribute to regional differences in environmental parameters and hardbottom communities (Jaap, 1984; Klein III & Orlando Jr, 1994; Murdoch & Aronson, 1999). While the reefs of the Upper Florida Keys (‘Upper Keys’) are fairly protected from the highly variable waters of the Gulf of Mexico and Florida Bay, reefs in the Middle and Lower Florida Keys (‘Middle Keys’ and ‘Lower Keys’, respectively) are subjected to more variable conditions due to passes and tidal channels that allow for increased water exchange (Klein III & Orlando Jr, 1994). The regional delineations of Upper, Middle, and Lower Keys are used by conservation managers and practitioners to account for spatial differences in monitoring and restoration activities. Given the differences in environmental conditions and coral community structure among the regions of FCR, microbiomes of corals used in restoration throughout the Keys may differ based on nursery, and later, outplanting location, resulting in possible differences in restoration success.

We previously characterized the microbiomes associated with different genotypes of A. cervicornis identified as susceptible or resistant to diseases (Klinges et al., 2020). We found that microbiomes of disease-susceptible genotypes collected from the Lower Keys were characterized by an overwhelming dominance of the bacterial species Candidatus Aquarickettsia rohweri (Klinges et al., 2020; Klinges et al., 2022). In contrast, disease-resistant genotypes were characterized by a more even and diverse microbiome, with low abundances of Ca. Aquarickettsia (hereafter, “Aquarickettsia”; Klinges et al., 2020).
Members of the genus *Aquarickettsia* are dominant in communities across many genotypes of *A. cervicornis* (*Rosales et al., 2019; Gignoux-Wolfsohn et al., 2020*) and a high abundance of its members are associated with increased disease prevalence, reduced coral growth, and increased tissue loss (*Zaneveld et al., 2016; Shaver et al., 2017*). *Ca. Aquarickettsia rohweri* was previously demonstrated to possess the genomic capacity to parasitize the coral holobiont for amino acids and ATP (*Klinges et al., 2019*) and responds positively to nutrient enrichment (*Shaver et al., 2017; Klinges et al., 2022*). Due to the apparent association of *Aquarickettsia* with disease-susceptible phenotypes, a broader assessment of the distribution of this bacterial genus across Floridian *Acropora* is necessary to examine its ubiquity and assess the contribution of *Aquarickettsia* to signatures of disease susceptibility and resistance. Comparison of acroporid microbiome composition across distinct geographic regions may allow for the identification of genotypes that are ideal candidates for restoration purposes, harboring low abundance of *Aquarickettsia* and therefore likely to be disease-resistant. Alternately, assessment of microbiomes from genotypes sourced from different regions of the Florida Keys may reveal that the relationship between *Aquarickettsia* and disease-response phenotype is limited to the Lower Keys.

Here, we examined differences in the microbial community composition of *Acropora cervicornis* genotypes originally collected from different regions of Florida’s Coral Reef and sampled after residing within Mote Marine Laboratory’s *in situ* coral nursery near Looe Key, FL (USA) for multiple years. Microbiomes of genotypes collected from the Upper, Middle, and Lower Keys were described using 16S rRNA high-throughput sequencing to characterize and compare the microbiomes of apparently healthy *A. cervicornis* residing within the coral nursery. We found that while the majority of *A. cervicornis* genotypes from all collection regions harbored microbiomes dominated by putative parasites of the genus *Aquarickettsia*, a subset of genotypes possessed a significantly different microbiome characterized by the dominance of either an unclassified sequence variant or the genus *Spirochaeta*. Additionally, genotypes from the Upper and Lower Keys retained a microbiome signature that was specific to the geographic region of origination, while genotypes from the Middle Keys were distinct from those originating from the Lower Keys, but not those from the Upper Keys. Our results provide key information on the diversity and potential geographical influences on the microbiomes of an important restoration species.

**MATERIALS & METHODS**

**Sample collection and processing**

In November-December 2019, *Acropora cervicornis* genotypes (*n = 74*) were collected from Mote Marine Laboratory’s *in-situ* nursery near Looe Key, FL (USA) in the Lower Keys and sampled for microbiome analysis. The Florida Keys National Marine Sanctuary authorized the use of nursery-grown corals under permit FKNMS-2015-163. These corals were curated for Mote’s coral restoration efforts throughout the Florida Keys and previously genotyped using both microsatellites (*Baums, Miller & Hellberg, 2005*) and the SNP chip platform (*Kitchen et al., 2020*). Of the 74 genotypes collected, 40 originated from the Lower Keys, 15 from the Middle Keys, and 19 from the Upper Keys (Fig. 1, Table S1). Genotypes from
the Lower Keys had been in the nursery for the longest time, spending 8.1 ± 3.2 years (mean ± S.E.) there before sampling (Table S1), whereas genotypes from the Middle and Upper Keys spent 3.4 ± 0.7 and 3.3 ± 0.8 years, respectively, in the nursery before sampling (Table S1; see Supplemental Methods & Results for additional temporal analyses). The A. cervicornis fragments, each ranging between 1.5 and three cm in length, were placed in individual 2-oz Whirl-Paks containing ambient seawater for transport (approximately 35 min) to Mote’s Elizabeth Moore International Center for Coral Reef Research and Restoration (Summerland Key, FL, USA). Once back at the lab, the seawater in each Whirl-Pak was discarded and the fragments were flash-frozen in a dewar containing liquid nitrogen for one minute. The frozen samples were then immediately stored at −80 °C until further processing.

DNA was isolated from the frozen coral fragments using DNeasy PowerSoil Kits (QIAGEN, Germantown, MD, USA) with modifications to the manufacturer’s protocol (Rosales et al., 2020). Using sterile tweezers, 4–5 polyps were removed from every coral and transferred to DNeasy PowerBead tubes. During polyp excision, the corals were kept on ice to prevent thawing. Following DNA extractions, a NanoDrop OneTM Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify DNA concentrations and purity.

The bacterial communities of each sample were determined using 16S rRNA Illumina sequencing on the MiSeq platform. DNA was sent to MR DNA (http://www.mrdnalab.com, Shallowater, TX, USA) for barcoding, amplification, and sequencing. Amplification of the 16S rRNA gene variable region (V4) was conducted using primers 515F (GTGCCAGCMGCCGCGGTAA; Original Earth Microbiome Project; Caporaso et al., 2011) and 806R (GGACTACVSGGGTATCTAAT; Archaea 806R; Takai & Horikoshi, 2000). Barcodes were on the forward primer. A polymerase chain reaction (PCR; 30 cycles)
was performed using the HotStarTaq Plus Master Mix Kit (QIAGEN, Germantown, MD, USA) under the following conditions: 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 53 °C for 40 s and 72 °C for 1 min, and by a final elongation step at 72 °C for 5 min. PCR products were checked on a 2% agarose gel to determine the success of amplification and the relative intensity of bands. Samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Agencourt Ampure XP beads (Beckman Coulter, CA, USA). Next, the pooled DNA library was generated using the Illumina TruSeq DNA library preparation protocol. Paired-end sequencing with a sequencing read length of 300 base pairs was performed at MR DNA using a single flow cell on a MiSeq following the manufacturer’s guidelines.

**Sequence data processing and analysis**

All data processing and analysis were performed in the program R (version 4.0.3, *R Development Core Team, 2019*). A total of 24,392,378 reads across 74 samples were processed using DADA2 (v1.16; *Callahan et al., 2016*) in R (see Table S2 for reads retained at each step). After quality plot inspection, forward and reverse reads were truncated to 210 base pairs at their 3’ end. Sequences were truncated at the first position where a quality score was ≤ 2. Reads with a total expected error of >2 or with the presence of Ns were discarded. This resulted in a total of 21,770,005 reads. An initial total of 4,434 amplicon sequence variants (ASVs) were inferred from unique reads and paired-end reads were subsequently merged. ASVs that did not match a target length of 250–255 (1,542 ASVs) were discarded. A total of 776 two-parent chimeras (bimeras) were removed and taxonomy was assigned at 100% sequence identity using the Silva reference database (v132) to preserve the high resolution of ASV data (*Quast et al., 2013*). An average of 81.45% of initial reads, corresponding to 2,216 ASVs, were retained through the quality filtering pipeline. The Silva taxonomic classification for the genus MD3-55 was changed to *Candidatus Aquarickettsia rohweri* (hereafter, “*Aquarickettsia*”) for congruence with current identifications in the literature (*Klinges et al., 2019*). The ASV table resulting from DADA2 processing was imported into *phyloseq* (v1.30.0) (*McMurdie & Holmes, 2013*). A total of 71 ASVs taxonomically identified as chloroplasts, mitochondria, or eukaryotic sequences were removed (corresponding to 17,363, 82, and 56 reads, respectively). Taxa with less than 10 reads in 10% of the samples were removed, equating to 755 ASVs (4,641 reads). Using alpha rarefaction curves in *phyloseq* (*Fig. S1*), samples were rarefied to a minimum sequence depth of 26,537 reads, which allowed for the inclusion of all samples while still maximizing sample diversity.

**Diversity and differential abundance analyses**

Non-metric Multidimensional Scaling analyses (nMDS) using the Bray-Curtis dissimilarity distances were performed to determine similarities in bacterial communities among samples due to collection region. Upon conducting the nMDS analysis, we visually identified 10 outliers. We then used the ‘OutlierDetection’ function (Outlier Detection package, *Tiwari & Kashikar, 2019*) to determine significant outliers out of all samples based on the euclidean
distance method. Relative abundance plots confirmed differences between the non-outlier and outlier samples. All further analyses were done separately for both the non-outliers and outlier sample groups. nMDS analyses were repeated separately for both groups. Differences in beta diversity among collection regions of both the non-outliers and outliers were also tested using the betadisper function (vegan package; Oksanen et al., 2020) to calculate the multivariate homogeneity of dispersion of the Bray-Curtis distances. Pairwise comparisons were made using Tukey post-hoc comparison tests.

Differences in bacterial communities among samples due to collection region were tested using a permutational multivariate analysis of variance, PERMANOVA, of the Bray-Curtis dissimilarity (vegan package; Oksanen et al., 2020) for the rarefied dataset. Multiple pairwise PERMANOVA tests were used to compare beta diversity between collection regions. P-values were adjusted using the Bonferroni correction.

Alpha diversity as a function of collection region was assessed for the rarefied dataset using species richness and the Shannon diversity metric. Normality conditions were tested using Shapiro–Wilks tests. We used Kruskal-Wallis rank sum tests to determine significant differences in alpha diversity metrics by region. Additional pairwise Kruskal-Wallis rank sum tests with Bonferroni-corrected P-values were used to determine significant differences between collection regions.

To determine differentially abundant microbial taxa as a function of collection region, we used the R package corncob (Martin, Witten & Willis, 2021). Corncob uses beta-binomial regression models and accounts for varying sequencing depth and within-sample correlations between taxa (Martin, Witten & Willis, 2021). Using the un-rarefied data, we built our models using region as the predictor variable for both the non-outlier and outlier datasets separately.

RESULTS

Microbiomes of Acropora cervicornis genotypes are characterized by differences in the relative abundance of the genus Aquarickettsia

Post-filtration, as described in the methods above, the dataset consisted of 1,195 ASVs, with a mean read depth per ASV of 1,643. The 16S rRNA sequences are available under BioProject ID PRJNA769275. Microbiomes of most genotypes were dominated by ASVs in the genus Ca. Aquarickettsia, except for genotypes identified as outliers due to the lower relative abundance of this taxon (Fig. 2). The average relative abundance of Aquarickettsia in non-outlier samples was 94.3 ± 5.51%, while in outlier samples it was 3.14 ± 4.83%. Of the Aquarickettsia ASVs, one strain in particular (ASV 1) dominated the microbiomes of most genotypes, accounting for approximately 90% or more of the relative abundances (Figs. S2 and S3). Many of the Lower Keys genotypes also had low relative abundances of bacteria in the genus Spirochaeta, which appeared in higher relative abundances in both the Middle and Upper Keys genotypes (Fig. 2). The average relative abundance of Spirochaeta in non-outlier samples was low and invariable, at 4.44 ± 5.72%, while in outlier samples it was highly variable at 43.2 ± 37.9% due to the low relative abundance of this genus in outlier samples from the Lower Keys, which were mostly dominated by an unclassified
proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.

**Bacterial diversity significantly differed among genotypes initially collected from different regions and was higher in genotypes not dominated by *Aquarickettsia***

nMDS analysis for the full dataset (Fig. 3A) confirmed the existence of possible outliers. We identified 10 significant outliers of the 74 genotypes sampled: four from the Lower Keys and six from the Upper Keys. There were no outliers among genotypes sampled from the Middle Keys. These outliers matched the observations made through visualization of the relative abundances –bacterial communities of the non-outlier genotypes from all regions were dominated by *Aquarickettsia* sp., while the microbiomes of the outlier genotypes were dominated by *Aquarickettsia* sp., unclassified proteobacteria (‘Unclassified_ASV_3’). According to NCBI’s basic local alignment tool (BLAST), this ASV is most similar to an uncultured bacterium clone p1BB03 sampled from A. palmata in Puerto Rico (E = 1e−127; 100% identity; GenBank EU861195.1). The outlier genotypes in the Upper Keys were dominated by bacteria in the genera of both the unclassified proteobacteria and Spirochaeta. The relative abundance of this unclassified ASV was 0.78 ± 0.96% in non-outlier samples, and 29.3 ± 20.5% in outlier samples.
genotypes were composed of many highly relatively abundant taxa (Fig. 2). All further analyses were performed on both the non-outlier, or high-\textit{Aquarickettsia}, and the outlier, or low-\textit{Aquarickettsia}, datasets.

Beta diversity analyses for both datasets determined significant differences among and between regions. nMDS analysis of the high-\textit{Aquarickettsia} genotypes (non-outlier samples) demonstrated similarities among fragments by collection region (Fig. 3B), and was supported by the betadisper analysis that determined significant differences between Lower and Upper Keys genotypes, but not between the Middle Keys and the other two regions (Fig. 4A). Bacterial communities of the high-\textit{Aquarickettsia} genotypes were significantly different by collection region (PERMANOVA, $df = 2$, $R^2 = 0.34$, $F = 0.05$, $P = 0.001$; similar results for the full dataset in Table S3). Multiple PERMANOVA tests for the high-\textit{Aquarickettsia} subset determined that the genotypes from the Lower Keys had significantly different microbial communities than both the Upper ($P = 0.003$) and Lower ($P = 0.003$) Keys; however, the microbial communities of the Upper and Middle Keys were not significantly different ($P = 0.555$; Table S3). The bacterial communities of the Lower and Upper Keys’ low-\textit{Aquarickettsia} genotypes (outlier samples) were significantly different as shown by the 95% confidence ellipses in the nMDS analysis (Fig. 3C), but this conclusion was not supported by the betadisper analysis due to a low sample size (Fig. 4B). However, the PERMANOVA did determine significant differences between Upper and Lower Keys low-\textit{Aquarickettsia} genotypes ($df = 1$, $R^2 = 0.39$, $F = 5.18$, $P = 0.012$, Table S3).

Alpha diversity was significantly different between high- and low-\textit{Aquarickettsia} genotypes (Richness $P = 16.7e-5$, $df = 1$, $X^2 = 14.173$; Shannon $P = 4.2e-7$, $df = 1$, $X^2 = 25.6$; Figs. 5A and 5B). Visualization of the relative abundances of bacteria genera

**Figure 3**  Non-metric multidimensional scaling analysis of the Bray–Curtis Distances grouping microbiome compositions by initial collection region. Non-metric multidimensional scaling analysis of the Bray–Curtis Distances grouping microbiome compositions by initial collection regions for (A) the full dataset (stress = 0.05), (B) the non-outlier samples (stress = 0.06), and (C) the outlier samples (stress = 0.09). Points represent samples, and the ellipses are 95% confidence ellipses for each collection region.
also supported the greater diversity in bacteria of the low-Aquarickettsia genotypes (Fig. 2). Species richness ($P = 2.7e-5$, $df = 2$, $X^2 = 21.03$; Fig. 5A) and Shannon diversity index ($P = 0.008$, $df = 2$, $X^2 = 9.55$; Fig. 5B) were both significantly different by collection region for the high-Aquarickettsia genotypes. Pairwise tests determined that only alpha diversity metrics of the high-Aquarickettsia genotypes from the Lower and Upper Keys were significantly different (Richness $P = 0.017$ and Shannon $P = 0.02$). Alpha diversity did not significantly differ between the Upper and Lower Keys low-Aquarickettsia genotypes (Richness $P = 0.2$, $df = 1$, $X^2 = 1.64$; Fig. 5A; Shannon $P = 0.4$, $df = 1$, $X^2 = 0.73$; Fig. 5B).
Aquarickettsia and Spirochaeta are differentially abundant taxa in A. cervicornis genotypes from different regions of the Florida Keys

Beta-binomial regression models from the corncob analysis determined ten significantly differentially abundant ASVs as a function of the original collection region for the high-Aquarickettsia genotypes (Fig. 6, Table S4). Only two ASVs (two and eight) in the Spirochaeta genus were significantly, relatively positively enriched in both the Middle and Upper Keys genotypes (Figs. 6A and 6B). Eight ASVs from four genera were significantly, relatively positively enriched in the Lower Keys, including five Aquarickettsia ASVs (ASV 1, 5, 14, 38, and 43). ASV 1, an Aquarickettsia sp., was highly relatively abundant in genotypes from all initial collection regions; however, it was more relatively abundant and less variable in genotypes from the Lower Keys (Fig. S3). Additional taxa significantly, relatively enriched in the high-Aquarickettsia genotypes from the Lower Keys include an ASV from the Helicobacteraceae family (ASV 10), an unclassified Proteobacteria ASV (ASV 12), and a Cetobacterium sp. (ASV 17). According to NCBI BLAST, Unclassified ASV 12 was most similar to an uncultured bacterium clone p1BB03 found in A. palmata from Puerto Rico (E =1e−117, 98.39% identity, GenBank EU861195.1), and also closely matched to an uncultured bacterium clone Acer JJ17 in A. cervicornis from Bocas del Toro, Panama (E =5e116, 97.98% identity, GenBank GU117990.1; Sunagawa, Woodley & Medina, 2010). Six ASVs were significantly differentially abundant between Lower and Upper Keys low-Aquarickettsia genotypes (Table S5 and Fig. S4). A Spirochaeta sp. (ASV 2), an Enterococcus sp. (ASV 9), unclassified ASV 21, a Cloacibacterium sp. (ASV 33), and ASV 35 in the family Microbacteriaceae were relatively positively enriched in the Upper Keys low-Aquarickettsia genotypes. Unclassified ASV 21 was most similar to an uncultured marine bacterium clone S82_61c03 found in the skeleton of Cladocora caespitosa in the Mediterranean Sea (E =3e−104, 94.49% identity, GenBank JQ235900.1; Meron et al., 2012). Only ASV 10 in the Helicobacteraceae family was relatively positively enriched in the Lower Keys low-Aquarickettsia genotypes.

DISCUSSION

We found significant differences in the microbial communities of A. cervicornis based on the initial collection region of the genotypes; however, the putative intracellular bacterial parasite, Ca. Aquarickettsia rohweri, dominated most genotypes sampled from all regions. It represented an average of 94.3 ± 5.51% of the microbiome in non-outlier, high-Aquarickettsia, genotypes (64 of 74 genotypes sampled). We found that a single ASV from this species was dominant in our samples; there was a 100% match to the published 16S rRNA sequence for the type species, strain acerv44, for which the complete genome is available (Klinges et al., 2019). This same strain appears to be dominant across samples of Acropora cervicornis throughout the Florida Keys and broader Caribbean region. The 16S rRNA sequence of this strain is identical to the dominant ASV in Florida A. cervicornis from Rosales et al. (2019) and to the second most dominant ASV in A. cervicornis from the Cayman Islands, which differed by a single nucleotide from the most dominant ASV in these corals (Miller et al., 2020). This sequence was furthermore found to be 99.18%
identical to the dominant Rickettsiales ASV in samples of *A. cervicornis* from Puerto Rico in Godoy-Vitorino et al. (2017). Members of *Aquarickettsia* recently identified in mucus of *A. cervicornis* from Mote’s *in situ* nursery and in outplants (Aguirre et al., 2022) ranged in identity to strain acerv44 from 94–100%. High abundance of this taxon has been proposed as a biomarker for disease susceptibility in *A. cervicornis*, as *Aquarickettsia* has been observed to dominate disease-susceptible genotypes while remaining relatively low in abundance within disease-resistant genotypes (Rosales et al., 2019; Klinges et al., 2020).
It is as of yet unknown how this taxon becomes dominant in microbiomes of *A. cervicornis*, as *Aquarickettsia* is notably absent in early life stages of this species, suggesting that the putative parasite is not inherited vertically (Baker et al., 2022). Genomes of this bacterial genus were found to cluster phylogenetically by collection region, rather than coral host, further supporting environmental acquisition (Baker et al., 2022). Low microbial diversity and single-taxon dominance such as observed in genotypes dominated by *Aquarickettsia* has been linked to disease in human systems, while conversely high diversity is proposed to support greater defenses against pathogens and contribute to host plasticity in the face of environmental change (West et al., 2019; Bourne, Morrow & Webster, 2016). The observed dominance of this taxon across many genotypes of *Acropora cervicornis* and the limited responsivity of microbiomes of this species to transplantation may be reflective of reduced fitness of Caribbean *Acropora* compared to non-Caribbean *Acropora*. Ziegler et al. (2019) found that *A. hemprichii* possessed a flexible, environmentally-responsive microbiome that may allow for greater adaptation to environmental change. Further study is necessary to characterize the effects of low microbial diversity on the health of Caribbean *Acropora* and to ascertain whether low microbial diversity signatures persist when these genotypes are restored to the reef.

ASVs in the genus *Spirochaeta*, two in the high-*Aquarickettsia* and one in the low-*Aquarickettsia* subsets, were significantly differentially abundant in genotypes from the Middle and Upper Keys when compared to the Lower Keys. ASVs in this genus were previously found in high abundances in *A. palmata* (Rosales et al., 2019). No *Spirochaeta* ASVs were found in relative abundances greater than 1% in 16 *A. cervicornis* genotypes from Mote’s *in situ* nursery sampled in 2015, 15 of which were again sampled in 2019 and studied here (all of which were originally collected from the Lower Keys; Klinges et al., 2020). Although *Spirochaeta* sp. are associated with nutrient cycling in a wide range of corals (Lawler et al., 2016; Van de Water et al., 2016; Park et al., 2021), *Spirochaeta* sp. in the microbiome of a disease susceptible *A. cervicornis* genotype did not respond to nutrient enrichment, while relative abundances of *Aquarickettsia* sp. significantly increased (Klinges et al., 2022). Lower abundance taxa that were still significantly relatively, positively enriched in the genotypes originally collected in the Lower Keys included ASVs in the Helicobacteraceae family, a *Cetobacterium* sp., and an unclassified Proteobacteria ASV similar to ASVs found previously in other *Acropora* sp. samples. Members of the Helicobacteraceae family were enriched in *A. cervicornis* with white band disease in Panama (Gignoux-Wolfsohn, Aronson & Vollmer, 2017; Gignoux-Wolfsohn et al., 2020).

Several genotypes in the Upper and Lower Keys had distinct microbiomes, as apparent from the nMDS and outlier detection analysis, and were termed “low-*Aquarickettsia*” because of their high bacterial diversity and low abundances of *Aquarickettsia* ASVs. These low-*Aquarickettsia* genotypes had significantly higher richness and Shannon diversity than the high-*Aquarickettsia* genotypes. Like those of the high-*Aquarickettsia* genotypes, the microbial communities of the low-*Aquarickettsia* genotypes significantly differed by initial collection region. According to the differential abundance analysis, these differences were due to six ASVs. These included two ASVs that were significantly, relatively differentially abundant in the high-*Aquarickettsia* genotypes: a *Spirochaeta* ASV was positively enriched...
in the Upper Keys while a Helicobacteraceae ASV was positively enriched in the Lower Keys’ low-\textit{Aquarickettsia} genotypes. The high bacterial diversity of these low-\textit{Aquarickettsia} \textit{A. cervicornis} genotypes is indicative of potential disease resistance, as it has been proposed that the comparatively high diversity in these genotypes may occlude niche space that could otherwise be infiltrated by opportunistic species (Klinges et al., 2020). Our analysis included 15 out of 16 genotypes used in this previous study of coral fragments collected in 2015 (Klinges et al., 2020). However, microbiome analysis of collections from 2019 (the present study) show that genotypes M-L-3 and M-L-7 (genotypes 3 and 7 in Klinges et al., 2020), while found to be comparatively low in \textit{Aquarickettsia} at both timepoints, now are dominated by \textit{Spirochaeta} and an unclassified ASV, respectively, rather than dominated by no single taxon as found in 2015 samples of these genotypes. This reduction in community evenness from 2015 to 2019 may reflect either a reduction in capacity for disease resistance resulting from a decrease in microbial diversity over time, or instead the development of a new symbiotic relationship with \textit{Spirochaeta} and an unclassified species that may confer unknown benefits to these genotypes of \textit{A. cervicornis} lacking Ca. \textit{Aquarickettsia} rohweri.

Coral host-microbe interactions are influenced by environmental factors that vary over both space and time (Dinsdale et al., 2008; Dunphy et al., 2019), even over small spatial scales (Wainwright et al., 2019). Microbiomes of both high and low-\textit{Aquarickettsia} genotypes significantly differed by initial collection region. Pairwise comparisons of diversity, both alpha and beta, of the high-\textit{Aquarickettsia} genotypes indicated that the differences by initial collection region were driven by significant differences between genotypes from the Upper and Lower Keys. Interestingly, the microbial communities of the genotypes initially collected from the Middle Keys were more similar to the Upper Keys than to the Lower Keys. This pattern of dissimilarity in \textit{A. cervicornis} microbiomes across the Florida Keys supports the existence of geographic influences on host-microbe interactions, which may be retained for years after relocation. The distinct spatial regions of FCR experience pronounced environmental differences (Jaap, 1984; Klein III & Orlando Jr, 1994; Murdoch & Aronson, 1999) that likely play a role in structuring the differences in the microbiomes seen here. However, \textit{A. cervicornis} from the different regions are also genetically distinct (Drury, Manzello & Lirman, 2017), thus it is difficult to tease apart the role of host genetics from that of the environment when comparing the microbiomes of \textit{A. cervicornis} across geographical gradients in the Florida Keys. Regardless, genotype, environmental factors (Drury, Manzello & Lirman, 2017), and microbes (Mao-Jones et al., 2010; Ritchie, 2011) have considerable influence on the health and survival of their hosts, therefore geographical differences in initial collection region should be considered by managers and practitioners when determining outplanting strategies, even on a regional (i.e., Florida Keys) scale.

The rate at which the coral microbiome responds to changing environments may play a role in coral resilience. Rapid shifts in microbiome structure in response to environmental shifts may reflect a highly adaptive microbiome that may lead to host environmental flexibility, but is at a higher risk for the loss of beneficial species as well as opportunists (Ziegler et al., 2019). In contrast, corals that possess an inflexible microbiome may be slow to respond to environmental cues, but may preserve relationships with essential members
of the microbiome that perform key functions (Ziegler et al., 2019). Interestingly, although the genotypes originally collected from the Middle and Upper Keys had been located in Mote’s in situ nursery in the Lower Keys for an average of 3.4 years, their microbiomes still held geographic markers, suggesting the potential for retaining microbiome signatures over time. Host identity is known to have greater control on microbiome composition than temporal differences (Dunphy et al., 2019), however, there is still evidence of temporal variability in coral microbiomes (Epstein et al., 2019). Stability of coral microbiomes over time or when transplanted is known to be a species-specific response (Roitman et al., 2020; Deignan & McDougald, 2021; Dunphy, Vollmer & Gouhier, 2021; Strudwick et al., 2022).

In a previous study of microbiome shifts resulting from translocation, a non-Caribbean Acroporid coral, A. hemprichii, experienced significant microbiome restructuring when moved across sites in the Red Sea (Ziegler et al., 2019). In contrast, Dunphy, Vollmer & Gouhier (2021) found that A. cervicornis in Panama had highly stable microbiomes during a reciprocal field transplant experiment that also perturbed the microbiome using an antibiotic. As we did not sample the genotypes studied here before they were moved to Mote’s nursery, we do not know the structure of their microbiomes at the time of collection or soon after translocation. Further research is therefore needed to understand how microbiomes of restored corals may change through time—when moved from the reef to a nursery, transferred among different nurseries, or outplanted from a nursery back onto the reef. The presence of geographic signatures of microbiome composition in our study suggest that Floridian genotypes of Acropora cervicornis may possess a microbiome signature associated with geographic region of origin, which may result in preserved relationships with potential symbionts.

CONCLUSIONS

The present study characterized the microbiomes of apparently healthy Acropora cervicornis genotypes that originated from different regions across Florida’s Coral Reef and have been housed in Mote Marine Laboratory’s in situ nursery near Looe Key, FL (USA) for multiple years. We found significant differences in bacterial diversity of the genotypes driven by the initial collection region, even after the corals had been kept under common garden conditions for multiple years. These differences in microbial communities due to geographical origin most likely reflect the genetic relatedness and environmental history of the genotypes sampled. Our results suggest that the abundance of two key bacterial taxa, Ca. Aquarickettsia and Spirochaeta, may play an important role in distinguishing bacterial communities among A. cervicornis populations. Most genotypes were dominated by Aquarickettsia sp., a presumptive bacterial parasite of A. cervicornis that is associated with disease susceptibility (Klinges et al., 2019; Klinges et al., 2020). Furthermore, several genotypes originally collected from both the Lower and Upper Keys had higher bacterial diversity due to lower abundances of Aquarickettsia sp. These low-Aquarickettsia genotypes may be a potential resource for restoration initiatives since populations that are more likely to succeed and survive in stressful environments should be thoughtfully targeted for restoration efforts.
ACKNOWLEDGEMENTS

We would like to thank Chelsea Petrik for help with sample collection.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Funding for this project was supported by a National Fish and Wildlife Foundation Award (#62145), NSF CAREER Award (#1452538-OCE), NSF Biological Oceanography Award (#1923836), and by the Arthur Vining Davis Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
National Fish and Wildlife Foundation Award: #62145.
NSF CAREER Award: #1452538-OCE.
NSF Biological Oceanography Award: #1923836.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

• Sara D. Williams conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
• J. Grace Klinges conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
• Samara Zinman analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
• Abigail S. Clark conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
• Erich Bartels performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
• Marina Villoch Diaz Maurino performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
• Erinn M. Muller conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:
The 16S rRNA sequences are available at NCBI: PRJNA769275.
**Data Availability**

The following information was supplied regarding data availability:

The data and code are available at GitHub: https://github.com/MoteCHaDlab/FLKeys_AcerMicrobiome_geographicaldifferences.

The 16S rRNA sequences are available at NCBI: PRJNA769275.

**Supplemental Information**

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13574#supplemental-information.

**REFERENCES**

_Acropora_ Biological Review Team. 2005. Atlantic _Acropora_ status review: report to national marine fisheries service.

Aguirre EG, Million WC, Bartels E, Krediet CJ, Kenkel CD. 2022. Host-specific epibiomes of distinct _Acropora cervicornis_ genotypes persist after field transplantation. _Coral Reefs_ 41(2):265–276 DOI 10.1007/s00338-022-02218-x.

Aronson RB, Precht WF. 2006. Conservation, precaution, and Caribbean reefs. _Coral Reefs_ 25(3):441–450 DOI 10.1007/s00338-006-0122-9.

Baker LJ, Reich HG, Kitchen SA, Grace Klinges J, Koch HR, Baums IB, Muller EM, Thurber RV. 2022. The coral symbiont Candidatus Aquarickettsia is variably abundant in threatened Caribbean acroporids and transmitted horizontally. _ISME Journal_ 16:400–411 DOI 10.1038/s41396-021-01077-8.

Baums IB, Miller MW, Hellberg ME. 2005. Regionally isolated populations of an imperiled Caribbean coral, _Acropora palmata_. _Molecular Ecology_ 14(5):1377–1390 DOI 10.1111/j.1365-294X.2005.02489.x.

Boström-Einarsson L, Babcock RC, Bayraktarov E, Ceccarelli D, Cook N, Ferse SCA, Hancock B, Harrison P, Hein M, Shaver E, Smith A, Suggett D, Stewart-Sinclair PJ, Vardi T, McLeod IM. 2020. Coral restoration—A systematic review of current methods, successes, failures and future directions. _PLOS ONE_ 15:e0226631 DOI 10.1371/journal.pone.0226631.

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

Bourne DG, Morrow KM, Webster NS. 2016. Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. _Annual Review of Microbiology_ 70:317–340 DOI 10.1146/annurev-micro-102215-095440.

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

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. _Nature Methods_ 13:581–583 DOI 10.1038/nmeth.3869.
Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America* **108**(Supplement 1):4516–4522 DOI 10.1073/pnas.1000880107.

Ceh J, Van Keulen M, Bourne DG. 2011. Coral-associated bacterial communities on Ningaloo Reef, Western Australia. *FEMS Microbiology Ecology* **75**:134–144 DOI 10.1111/j.1574-6941.2010.00986.x.

Cunning R, Gillette P, Capo T, Galvez K, Baker AC. 2015. Growth tradeoffs associated with thermotolerant symbionts in the coral *Pocillopora damicornis* are lost in warmer oceans. *Coral Reefs* **34**(1):155–160 DOI 10.1007/s00338-014-1216-4.

Deignan LK, McDougald D. 2021. Differential response of the microbiome of *Pocillopora acuta* to reciprocal transplantation within Singapore. *Microbial Ecology* **83**(3):608–618 DOI 10.1007/s00248-021-01793-w.

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L. 2008. Microbial ecology of four coral atolls in the northern line Islands. *PLOS ONE* **3**(2):e1584 DOI 10.1371/journal.pone.0001584.

Drury C, Manzello D, Lirman D. 2017. Genotype and local environment dynamically influence growth, disturbance response and survivorship in the threatened coral, *Acropora cervicornis*. *PLOS ONE* **12**(3):e0174000 DOI 10.1371/journal.pone.0174000.

Drury C, Schopmeyer S, Goergen E, Bartels E, Nedimyer K, Johnson M, Maxwell K, Galvan V, Manfrino C, Lirman D. 2017. Genomic patterns in *Acropora cervicornis* show extensive population structure and variable genetic diversity. *Ecology and Evolution* **7**(16):6188–6200 DOI 10.1002/ece3.3184.

Dunphy CM, Gouhier TC, Chu ND, Vollmer SV. 2019. Structure and stability of the coral microbiome in space and time. *Scientific Reports* **9**(1):6785 DOI 10.1038/s41598-019-43268-6.

Dunphy CM, Vollmer SV, Gouhier TC. 2021. Host–microbial systems as glass cannons: explaining microbiome stability in corals exposed to extrinsic perturbations. *Journal of Animal Ecology* **90**(5):1044–1057 DOI 10.1111/1365-2656.13466.

Epstein HE, Hillary AS, Neal EC, Veronique Mocellin JL, Torda G, Van Oppen MJH. 2019. Temporal variation in the microbiome of acropora coral species does not reflect seasonality. *Frontiers in Microbiology* **10**:1775 DOI 10.3389/fmicb.2019.01775.

Ezzat L, Lamy T, Maher RL, Munsterman KS, Landfield KM, Schmeltzer ER, Clements CS, Vega Thurber RL, Burkepile DE. 2020. Parrotfish predation drives distinct microbial communities in reef-building corals. *Animal Microbiome* **2**:5 DOI 10.1186/s42523-020-0024-0.

Gardner TA, Côté IM, Gill JA, Grant A, Watkinson AR. 2003. Long-term region-wide declines in caribbean corals. *Science* **301**(5635):958–960 DOI 10.1126/science.1086050.

Gignoux-Wolffson SA, Aronson FM, Vollmer SV. 2017. Complex interactions between potentially pathogenic, opportunistic, and resident bacteria emerge during infection on a reef-building coral. *FEMS Microbiology Ecology* **93**(7):fix080 DOI 10.1093/femsec/fix080.
Gignoux-Wolfsohn S, Precht W, Peters E, Gintert B, Kaufman L. 2020. Ecology, histopathology, and microbial ecology of a white-band disease outbreak in the threatened staghorn coral Acropora cervicornis. Diseases of Aquatic Organisms 137:217–237 DOI 10.3354/dao03441.

Glasl B, Bongaerts P, Elisabeth NH, Hoegh-Guldberg O, Herndl GJ, Frade PR. 2017. Microbiome variation in corals with distinct depth distribution ranges across a shallow–mesophotic gradient (15–85 m). Coral Reefs 36:447–452 DOI 10.1007/s00338-016-1517-x.

Glasl B, Herndl GJ, Frade PR. 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. The ISME Journal 10:2280–2292 DOI 10.1038/ismej.2016.9.

Godoy-Vitorino F, Ruiz-Diaz CP, Rivera-Seda A, Ramirez-Lugo JS, Toledo-Hernández C. 2017. The microbial biosphere of the coral Acropora cervicornis in Northeastern Puerto Rico. PeerJ 5:e3717 DOI 10.7717/peerj.3717.

Hong M-J, Yu Y-T, Chen CA, Chiang P-W, Tang S-L. 2009. Influence of species specificity and other factors on bacteria associated with the coral Stylophora pistillata in Taiwan. Applied and Environmental Microbiology 75:7797–7806 DOI 10.1128/AEM.01418-09.

Jaap WC. 1984. Ecology of the south Florida coral reefs: a community profile (No. FWS/OBS-82/08; MMS-84-0038). Florida Dept. of Natural Resources, St. Petersburg (USA). Marine Research Lab.

Kitchen SA, Von Kuster G, Kuntz KLV, Reich HG, Miller W, Griffin S, Fogarty ND, Baums IB. 2020. STAGdb: a 30K SNP genotyping array and science gateway for Acropora corals and their dinoflagellate symbionts. Scientific Reports 10:12488 DOI 10.1038/s41598-020-69101-z.

Klein III CJ, Orlando Jr SP. 1994. A spatial framework for water-quality management in the Florida Keys National Marine Sanctuary. Bulletin of Marine Science 54(3):1036–1044.

Klinges G, Maher RL, Thurber RLV, Muller EM. 2020. Parasitic ‘Candidatus Aquarickettsia rohweri’ is a marker of disease susceptibility in Acropora cervicornis but is lost during thermal stress. Environmental Microbiology 22:5341–5355 DOI 10.1111/1462-2920.15245.

Klinges JG, Patel SH, Duke WC, Muller EM, Vega Thurber RL. 2022. Phosphate enrichment induces increased dominance of the parasite Aquarickettsia in the coral Acropora cervicornis. FEMS Microbiology Ecology 98(2):fiac013 DOI 10.1093/femsec/fiac013.

Klinges JG, Rosales SM, McMinds R, Shaver EC, Shantz AA, Peters EC, Eitel M, Wörheide G, Sharp KH, Burkepile DE, Silliman BR. 2019. Phylogenetic, genomic, and biogeographic characterization of a novel and ubiquitous marine invertebrate-associated Rickettsiales parasite, Candidatus Aquarickettsia rohweri, gen. nov. sp. nov. The ISME Journal 13(12):2938–2953 DOI 10.1038/s41396-019-0482-0.
Koren O, Rosenberg E. 2006. Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. Applied and Environmental Microbiology 72:5254–5259 DOI 10.1128/AEM.00554-06.

Krediet CJ, Ritchie KB, Paul VJ, Teplitski M. 2013. Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proceedings of the Royal Society B: Biological Sciences 280:20122328 DOI 10.1098/rspb.2012.2328.

Kuffner IB, Bartels E, Stathakopoulos A, Enochs IC, Kolodziej G, Toth LT, Manzello DP. 2017. Plasticity in skeletal characteristics of nursery-raised staghorn coral, Acropora cervicornis. Coral Reefs 36:679–684 DOI 10.1007/s00338-017-1560-2.

Ladd MC, Shantz AA, Bartels E, Burkepile DE. 2017. Thermal stress reveals a genotype-specific tradeoff between growth and tissue loss in restored Acropora cervicornis. Marine Ecology Progress Series 572:129–139 DOI 10.3354/meps12169.

Lawler SN, Kellogg CA, France SC, Clostio RW, Brooke SD, Ross SW. 2016. Coral-associated bacterial diversity is conserved across two deep-sea Anthothela species. Frontiers in Microbiology 7:458 DOI 10.3389/fmicb.2016.00458.

Lesser MP, Falcón LI, Rodríguez-Román A, Enriquez S, Hoegh-Guldberg O, Iglesias-Prieto R. 2007. Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral Montastraea cavernosa. Marine Ecology Progress Series 346:143–152 DOI 10.3354/meps07008.

Li J, Chen Q, Long L-J, Dong J-D, Yang J, Zhang S. 2014. Bacterial dynamics within the mucus, tissue and skeleton of the coral Porites lutea during different seasons. Scientific Reports 4:7320 DOI 10.1038/srep07320.

Libro S, Vollmer SV. 2016. Genetic signature of resistance to white band disease in the caribbean staghorn coral Acropora cervicornis. PLOS ONE 11:e0146636 DOI 10.1371/journal.pone.0146636.

Lirman D, Schopmeyer S, Galvan V, Drury C, Baker AC, Baums IB. 2014. Growth dynamics of the threatened caribbean staghorn coral Acropora cervicornis: influence of host genotype, symbiont identity, colony size, and environmental setting. PLOS ONE 9(9):e107253 DOI 10.1371/journal.pone.0107253.

Lirman D, Thyberg T, Herlan J, Hill C, Young-Lahiff C, Schopmeyer S, Huntington B, Santos R, Drury C. 2010. Propagation of the threatened staghorn coral Acropora cervicornis: methods to minimize the impacts of fragment collection and maximize production. Coral Reefs 29:729–735 DOI 10.1007/s00338-010-0621-6.

Littman RA, Willis BL, Pfeffer C, Bourne DG. 2009. Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. FEMS Microbiology Ecology 68:152–163 DOI 10.1111/j.1574-6941.2009.00666.x.

Lohr KE, Patterson JT. 2017. Intraspecific variation in phenotype among nursery-reared staghorn coral Acropora cervicornis (Lamarck, 1816). Journal of Experimental Marine Biology and Ecology 486:87–92 DOI 10.1016/j.jembe.2016.10.005.

Maher RL, Rice MM, McMinds R, Burkepile DE, Vega Thurber R. 2019. Multiple stressors interact primarily through antagonism to drive changes in the coral microbiome. Scientific Reports 9(1):6834 DOI 10.1038/s41598-019-43274-8.
Mao-Jones J, Ritchie KB, Jones LE, Ellner SP. 2010. How microbial community composition regulates coral disease development. *PLOS Biology* 8:e1000345 DOI 10.1371/journal.pbio.1000345.

Martin BD, Witten D, Willis AD. 2021. corncob: count regression for correlated observations with the beta-binomial. R package version 0.2.0. Available at https://CRAN.R-project.org/package=corncob.

McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL. 2017. Responses of coral-associated bacterial communities to local and global stressors. *Frontiers in Marine Science* 4:262 DOI 10.3389/fmars.2017.00262.

McMurdie PJ, Holmes S. 2013. *phyloseq*: an r package for reproducible interactive analysis and graphics of microbiome census data. *PLOS ONE* 8(4):e61217 DOI 10.1371/journal.pone.0061217.

Mercado-Molina AE, Ruiz-Diaz CP, Sabat AM. 2015. Demographics and dynamics of two restored populations of the threatened reef-building coral *Acropora cervicornis*. *Journal for Nature Conservation* 24:17–23 DOI 10.1016/j.jnc.2015.01.001.

Meron D, Rodolfo-Metalpa R,Cunning R, Baker AC, Fine M, Banin E. 2012. Changes in coral microbial communities in response to a natural pH gradient. *The ISME Journal* 6(9):1775–1785 DOI 10.1038/ismej.2012.19.

Miller N, Maneval P, Manfrino C, Frazer TK, Meyer JL. 2020. Spatial distribution of microbial communities among colonies and genotypes in nursery-reared *Acropora cervicornis*. *PeerJ* 8:e9635 DOI 10.7717/peerj.9635.

Morrow KM, Moss AG, Chadwick NE, Liles MR. 2012. Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Applied Environmental Microbiology* 78:6438–6449 DOI 10.1128/AEM.01162-12.

Muller EM, Bartels E, Baums IB. 2018. Bleaching causes loss of disease resistance within the threatened coral species *Acropora cervicornis*. *Elife* 7:e35066 DOI 10.7554/eLife.35066.

Murdoch TJ, Aronson RB. 1999. Scale-dependent spatial variability of coral assemblages along the Florida Reef Tract. *Coral Reefs* 18(4):341–351 DOI 10.1007/s003380050210.

Nugues MM, Smith GW, Van Hooidonk RJ, Seabra MI, Bak RPM. 2004. Algal contact as a trigger for coral disease. *Ecology Letters* 7:919–923 DOI 10.1111/j.1461-0248.2004.00651.x.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O’Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2020. vegan: Community Ecology Package. R package version 2.5-7. Available at https://CRAN.R-project.org/package=vegan.

Park JS, Han J, Suh SS, Kim HJ, Lee TK, Jung SW. 2021. Characterization of bacterial community structure in two alcyonacean soft corals (*Litophyton sp.* and *Sinularia sp.*) from Chuuk, Micronesia. *Coral Reefs* Epub ahead of print 2021 14 September DOI 10.1007/s00338-021-02176-w.

Peixoto RS, Rosado PM, Leite DCDA, Rosado AS, Bourne DG. 2017. Beneficial microorganisms for corals (BMC): proposed mechanisms for coral health and resilience. *Frontiers in Microbiology* 8:341 DOI 10.3389/fmicb.2017.00341.
Pratte ZA, Longo GO, Burns AS, Hay ME, Stewart FJ. 2018. Contact with turf algae alters the coral microbiome: contact versus systemic impacts. *Coral Reefs* 37:1–13 DOI 10.1007/s00338-017-1615-4.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Oliver Glöckner F. 2013. The silva ribosomal rna gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41(D1):D590–D596.

R Development Core Team. 2019. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rice MM, Maher RL, Thurber RV, Burkepile DE. 2019. Different nitrogen sources speed recovery from corallivory and uniquely alter the microbiome of a reef-building coral. *PeerJ* 7:e8056 DOI 10.7717/peerj.8056.

Ritchie KB. 2011. In: Rosenberg E, Gophna U, eds. *Bacterial symbionts of corals and symbiodinium*. Berlin, Heidelberg: Springer, 139–150.

Rohwer F, Seguritan V, Azam F, Knowlton N. 2002. Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series* 243:1–10 DOI 10.3354/meps243001.

Roitman S, López-Londoño T, Joseph Pollock F, Ritchie KB, Galindo-Martínez CT, Gómez-Campo K, González-Guerrero LA, Pizarro V, López-Victoria M, Iglesias-Prieto R, Medina M. 2020. Surviving marginalized reefs: assessing the implications of the microbiome on coral physiology and survivorship. *Coral Reefs* 39(3):795–807 DOI 10.1007/s00338-020-01951-5.

Rosales SM, Clark AS, Huebner LK, Ruzicka RR, Muller EM. 2020. *Rhodobacterales* and *Rhizobiales* are associated with Stony Coral Tissue Loss Disease lesions and its suspected sources of transmission. *Frontiers in Microbiology* 11:681 DOI 10.3389/fmicb.2020.00681.

Rosales SM, Miller MW, Williams DE, Traylor-Knowles N, Young B, Serrano XM. 2019. Microbiome differences in disease-resistant vs. susceptible *Acropora* corals subjected to disease challenge assays. *Scientific Reports* 9:18279 DOI 10.1038/s41598-019-54855-y.

Rouzé H, Lecellier G, Saulnier D, Berteaux-Lecellier V. 2016. Symbiodinium clades A and D differentially predispose *Acropora cytherea* to disease and *Vibrio spp.* colonization. *Ecology and Evolution* 6:560–572 DOI 10.1002/ece3.1895.

Schopmeyer SA, Lirman D, Bartels E, Gilliam DS, Goergen EA, Griffin SP, Johnson ME, Lustic C, Maxwell K, Walter CS. 2017. Regional restoration benchmarks for *Acropora cervicornis*. *Coral Reefs* 36:1047–1057 DOI 10.1007/s00338-017-1596-3.

Sharp KH, Distel D, Paul VJ. 2012. Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral *Porites astreoides*. *The ISME Journal* 6:790–801 DOI 10.1038/ismej.2011.144.

Shaver EC, Shantz AA, McMinds R, Burkepile DE, Vega Thurber RL, Silliman BR. 2017. Effects of predation and nutrient enrichment on the success and microbiome of a foundational coral. *Ecology* 98:830–839 DOI 10.1002/ecy.1709.
Strudwick P, Seymour J, Camp EF, Edmondson J, Haydon T, Howlett L, Le Reun N, Siboni N, Suggett DJ. 2022. Impacts of nursery-based propagation and outplanting on coral-associated bacterial communities. Coral Reefs 41:95–112 DOI 10.1007/s00338-021-02207-6.

Sunagawa S, Woodley CM, Medina M. 2010. Threatened corals provide underexplored microbial habitats. PLOS ONE 5:e9554 DOI 10.1371/journal.pone.0009554.

Swain TD, Vega-Perkins JB, Oestreich WK, Triebold C, DuBois E, Henss J, Baird A, Siple M, Backman V, Marcelino L. 2016. Coral bleaching response index: a new tool to standardize and compare susceptibility to thermal bleaching. Global Change Biology 22:2475–2488 DOI 10.1111/gcb.13276.

Takai K, Horikoshi K. 2000. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Applied Environmental Microbiology 66:5066–5072 DOI 10.1128/AEM.66.11.5066-5072.2000.

Thurber RV, Burkepile DE, Correa AMS, Thurber AR, Shantz AA, Welsh R, Pritchard C, Rosales S. 2012. Macroalgae decrease growth and alter microbial community structure of the reef-building coral, Porites astreoides. PLOS ONE 7:e44246 DOI 10.1371/journal.pone.0044246.

Tiwari V, Kashikar A. 2019. OutlierDetection: outlier detection. R package version 0.1.1. Available at https://CRAN.R-project.org/package=OutlierDetection.

Van de Water JA, Melkonian R, Junca H, Voolstra CR, Reynaud S, Allemand D, Ferrier-Páges C. 2016. Spirochaetes dominate the microbial community associated with the red coral Corallium rubrum on a broad geographic scale. Scientific Reports 6(1):1–7 DOI 10.1038/s41598-016-0001-8.

Van Woesik R, Banister RB, Bartels E, Gilliam DS, Goergen EA, Lustic C, Maxwell K, Moura A, Muller EM, Schopmeyer S, Winters RS. 2021. Differential survival of nursery-reared Acropora cervicornis outplants along the Florida reef tract. Restoration Ecology 29(1):e13302 DOI 10.1111/rec.13302.

Wainwright BJ, Afiq-Rosli L, Zahn GL, Huang D. 2019. Characterisation of coral-associated bacterial communities in an urbanised marine environment shows strong divergence over small geographic scales. Coral Reefs 38(6):1097–1106 DOI 10.1007/s00338-019-01837-1.

Wang L, Shantz AA, Payet JP, Sharpton TJ, Foster A, Burkepile DE, Vega Thurber R. 2018. Corals and their microbiomes are differentially affected by exposure to elevated nutrients and a natural thermal anomaly. Marine Science 5:101 DOI 10.3389/fmars.2018.00101.

Ware M, Garfield EN, Nedimyer K, Levy J, Kaufman L, Precht W, Winters RS, Miller SL. 2020. Survivorship and growth in staghorn coral (Acropora cervicornis) outplanting projects in the Florida Keys National Marine Sanctuary. PLOS ONE 15:e0231817 DOI 10.1371/journal.pone.0231817.

West AG, Waite DW, Deines P, Bourne DG, Digby A, McKenzie VJ, Taylor MW. 2019. The microbiome in threatened species conservation. Biological Conservation 229:85–98 DOI 10.1016/j.biocon.2018.11.016.
Young C, Schopmeyer S, Lirman D. 2012. A Review of reef restoration and coral propagation using the threatened genus *Acropora* in the Caribbean and western Atlantic. *Bulletin of Marine Science* **88**:1075–1098 DOI 10.5343/bms.2011.1143.

Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, McMinds R, Payet JP, Welsh R, Correa AMS, Lemoine NP, Rosales S, Fuchs C, Maynard JA, Thurber RV. 2016. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nature Communications* **7**:11833 DOI 10.1038/ncomms11833.

Ziegler M, Grupstra CGB, Barreto MM, Eaton M, BaOmar J, Zubier K, Al-Sofyani A, Turki AJ, Ormond R, Voolstra CR. 2019. Coral bacterial community structure responds to environmental change in a host-specific manner. *Nature Communications* **10**:3092 DOI 10.1038/s41467-019-10969-5.

Zilber-Rosenberg I, Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Review* **32**:723–735 DOI 10.1111/j.1574-6976.2008.00123.x.