Spatial distribution of microbial communities among colonies and genotypes in nursery-reared *Acropora cervicornis*

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Background. The architecturally important coral species *Acropora cervicornis* and *A. palmata* were historically common in the Caribbean, but have declined precipitously since the early 1980s. Substantial resources are currently being dedicated to coral gardening and the subsequent outplanting of asexually reproduced colonies of *Acropora*, activities that provide abundant biomass for both restoration efforts and for experimental studies to better understand the ecology of these critically endangered coral species.

Methods. We characterized the bacterial and archaeal community composition of *A. cervicornis* corals in a Caribbean nursery to determine the heterogeneity of the microbiome within and among colonies. Samples were taken from three distinct locations (basal branch, intermediate branch, and branch tip) from colonies of three different coral genotypes.

Results. Overall, microbial community composition was similar among colonies due to high relative abundances of the Rickettsiales genus MD3-55 (*Candidatus* Aquarickettsia) in nearly all samples. While microbial communities were not different among locations within the same colony, they were significantly different between coral genotypes. These findings suggest that sampling from any one location on a coral host is likely to provide a representative sample of the microbial community for the entire colony. Our results also suggest that subtle differences in microbiome composition may be influenced by the coral host, where different coral genotypes host slightly different microbiomes. Finally, this study provides baseline data for future studies seeking to understand the microbiome of nursery-reared *A. cervicornis* and its roles in coral health, adaptability, and resilience.

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Abstract

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Introduction

Historically, *Acropora cervicornis* and *A. palmata* corals were the most abundant corals throughout the broader Caribbean region [1–3]. Since 1980, an estimated 97% of native populations of *Acropora* corals have diminished throughout the Caribbean [4], with large losses attributed to the microbially mediated white-band disease [5]. Both *A. cervicornis* and *A. palmata* are now listed as critically endangered by the IUCN [6] and threatened under the Endangered Species Act [7], providing a legal mandate to facilitate the recovery of these keystone species. Ecologically, repopulation of *Acropora* corals is critical for improving the health of entire reef systems because they contribute to reef accretion and provide structural complexity that serves as important habitat for fish and other reef organisms.

In response to the loss of coral reefs, organizations around the Caribbean have turned to coral gardening to replenish local populations of coral [8, 9]. For example, coral restoration projects have resulted in the outplanting of tens of thousands of nursery-reared *Acropora* corals on Florida reefs each year [10, 11]. These restoration efforts rely primarily on the asexual reproduction of these corals as small coral fragments grow at faster rates than larger colonies [12] and sequential fragmentation, which mimics natural breakage cycles, can provide more new coral colonies for restoration than could be produced through sexual reproduction in the same time frame. Fragmented corals rapidly grow in *in situ* ocean nurseries and are then outplanted back to reefs, a method that has proven to be highly effective for producing abundant *A. cervicornis* biomass. This improved growth and decreased mortality of *A. cervicornis* in ocean nurseries in comparison corals attached to reefs is due in part to reduced predation by the corallivorous snail *Coralliophila abbreviata* and the fireworm *Hermodice carunculata* [9]. In addition to the direct effects of predation on colony health, *C. abbreviata* is a known vector for white-band disease [13] and *H. carunculata* is a known vector for the coral pathogen *Vibrio shilohi* [14].

However, the survival of *Acropora* corals once placed back on the reef is often lower than desired given the resource-intensive nature of nursery production [15-17]. Survival of restored *Acropora* is also highly variable among sites [10, 18], suggesting that some environments may be better than others for long-term restoration efforts. To date, research has focused on optimizing out-planting techniques and measuring performance of different coral genotypes [12, 19–22], while few studies have examined the microbial community composition associated with the success of outplanted corals. Given the critical role of the microbiome in both the health and disease of corals [23-25], manipulation of the coral microbiome has recently been proposed as a way to increase the resilience of corals [26–28] and improve restoration efforts [29,30]. Several recent review papers [24, 26, 31-33] have detailed the potential roles that the microbiome plays

in the overall health of the coral holobiont, including protection against pathogens, tight recycling of nutrients within the holobiont, and nitrogen fixation, which benefits the photosynthetic dinoflagellate symbionts, and there is growing interest in designer microbes and probiotic strains to mitigate loss of coral reefs [26, 27, 34, 35].

Even as our ability to characterize coral microbial communities has become exponentially faster and cheaper due to technological advances in DNA sequencing, the availability of natural corals for study has drastically decreased. Leveraging access to abundant biomass in nursery-reared corals with known genotypes, we address two basic questions about the spatial distribution of coral microbial communities. First, we assess whether a single sample is representative of the microbiota across an entire colony, information that is useful for both researchers and resource managers that strive to reduce stressors to already threatened species. To date, only a handful of studies have assessed spatial distribution of the microbial communities within coral colonies. Spatial heterogeneity was observed using bacterial community fingerprinting methods in branching *Porites furcata* between branch tips and branch bases [36] and across mounding *Orbicella annularis* corals [37]. In contrast, pyrosequencing in *A. palmata* showed no statistical difference in bacterial communities from the top, underside, and base of the colonies, regions with distinct irradiance levels [38]. Second, we address whether the microbial community changes with coral genotype. Given the limited number of extant natural populations of *A. cervicornis* in many parts of the Caribbean, there are limited studies of its microbial community composition. Previous studies of natural populations have shown that the microbiota of *A. cervicornis* in Puerto Rico varied between deep and shallow sites [39] and that intercolony variation of *A. cervicornis* in Panama was weaker than seasonal variation [40]. However, variation of bacterial communities in *A. cervicornis* from Panama used in a disease transmission study demonstrated that colony had a stronger effect than collection site [41]. Here, we apply an extensive sampling scheme to *A. cervicornis* in ocean nurseries in the Cayman Islands to examine the spatial distribution and heterogeneity of *A. cervicornis* microbial communities.

Materials & Methods

Sample Collection. Field collections were authorized by permit from the Department of Environment on behalf of the National Conservation Council, Cayman Islands to CM and TF (December 1, 2017 to December 1, 2018). Coral colonies for this study were sampled at the Integrated Coral Observation Network Reef Nursery (-80° 3’ 36” N, 19° 41’ 60” E) operated by the Central Caribbean Marine Institute (CCMI) in Little Cayman. The nursery uses a coral gardening technique [11, 12] on PVC trees and frames to cultivate *A. cervicornis* at 18 meters depth, under conditions of natural currents, temperatures, and light. Three previously identified coral genotypes determined via genotyping-by-sequencing [42], herein referred to as green (G), red (R), and yellow (Y) genotypes, were sampled from the nursery. Colonies of these genotypes were added to the CCMI nursery in 2012 as fragments from local populations. Each colony was fragmented from a larger coral colony in 2016, approximately one and a half years prior to

sampling. Colonies used in this study had no apparent signs of disease or illness at the time of sample collection. Colonies observed 10 months after sampling showed no signs of disease or distress (Figure 1).

All *in situ* collection was completed on December 8, 2017 by AAUS-certified science divers. The coral microbial community was sampled from three colonies each of three coral genotypes. All 9 colonies hung within 1 meter of each other on the same frame structure (Figure 1). On each colony, three replicate samples were collected from each of the following locations: basal branches (1°), intermediate branches (2°), and apical colony tips (3°), for a total of 81 coral surface samples. After brief agitation by a needleless syringe, the surface mucus layer and coral tissue was aspirated with a sterile 60-ml syringe. Collections were transported back to the CCMI laboratory within the hour. Syringes were positioned vertically for about five minutes to allow the coral sample material to settle at the tip of the syringe. Coral mucus and tissues were transferred to sterile 2-ml cryotubes, centrifuged at 13,000 rpm for 5 minutes, and the saltwater supernatant was decanted. Lastly, 5 volumes of RNAlater (Ambion, Austin, TX) to 1 volume sample pellet was added to the cryotube. Samples were incubated at 4°C for 12 hours and then stored at -20°C until nucleic acid extraction.

V4 Amplicon Library Preparation. DNA was extracted from up to 0.5 ml of mucus and tissue using a DNeasy Powersoil Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions, including bead beating for 10 minutes. As extraction controls were not collected when samples were processed in early 2018, we acknowledge the potential for contamination from lab reagents [43], either in the extraction kit or in subsequent PCR and cleanup reagents. The processing of extraction controls has since become standard practice in our lab beginning in mid-2018. After DNA extraction, guidelines of the Earth Microbiome Project 16S Illumina Amplicon Sequencing Protocol [44] were followed for amplicon sequencing preparation using the 2015-current primer set with barcoded forward primer 515FB [45] and reverse primer 806RB [46]. targeting Bacteria and Archaea. The V4 region of the 16S rRNA gene was amplified in three replicate 25-µl reactions for each coral sample using Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA), 3% dimethyl sulfide, 0.25 µM of each primer, and roughly 10 ng of DNA template. PCR cycling was as follows (35 cycles): 45 seconds at 94°C denaturation, 60 seconds at 50°C annealing, 90 seconds at 72°C extension. Initial denaturation and final extension were for 3 minutes at 94°C and 10 minutes at 72°C, respectively. Negative PCR controls were examined by gel electrophoresis on ethidium bromide-stained 1% agarose gels to ensure no contamination. Triplicate PCR products were then combined and purified using a MinElute PCR Purification Kit (Qiagen). Cleaned amplicon library concentrations were determined with a Nanodrop 1000. A total of 240 ng of each amplicon library was pooled for sequencing using an Illumina MiSeq with paired 150-bp reads (v.2 cycle format) at the University of Florida Interdisciplinary Center for Biotechnology Research.

Analysis of V4 Amplicon Libraries. Illumina adapters and primers were removed using cutadapt v. 1.8.1 [47]. Additional processing and analysis of amplicon libraries was completed in R v. 3.5.1. Quality filtering, error estimation, merging of reads, dereplication, removal of chimeras, and selection of amplicon sequence variants (ASVs) were performed with DADA2 v. 1.10.0 [48] as in [49], using the filtering parameters truncLen=c(150,150), maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE to remove all sequences with ambiguous basecalls and phiX contamination. Taxonomy was assigned in DADA2 to ASVs using the SILVA reference dataset v. 132 [50]. Sequences that could not be assigned as bacteria or archaea and sequences identified as chloroplasts or mitochondria were removed from further analysis.

The ASV and taxonomy tables, along with associated sample metadata were imported into phyloseq v. 1.26.0 [51] for community analysis. ASVs with counts across all samples of less than 5 were removed from the analysis. ASVs with zero counts were transformed using the count zero multiplicative method with the zCompositions package v. 1.1.2 in R [52]. The zero-replaced read counts were transformed with the centered log-ratio transformation and the Aitchison distance metric was calculated with CoDaSeq v. 0.99.2 [53]. Principal components analysis of the Aitchison distance was performed with the package prcomp in R and plotted with ggplot2 v. 3.1.0 [54]. Analysis of similarities (ANOSIM) was performed on the Aitchison distance using the R package vegan v. 2.5-3 [55]. Differential abundance of microbial families among coral genotypes was determined with Analysis of Composition of Microbiomes (ANCOM) [56] as in [49], using an ANOVA significance level of 0.05 and removing families with zero counts in 90% or more of samples. Only families detected in at least 70% of samples were reported. All metadata and R scripts used for analysis are available in Github (github.com/meyermicrobiolab/Microbiomes-of-nursery-reared-Acropora-cervicornis).

Results

A total of 81 amplicon libraries were analyzed from nine separate coral colonies representing three genotypes of *A. cervicornis*. After quality control, libraries contained an average of 77,923 reads and ranged from 432-184,540 reads (Table S1). Sequencing reads with primers and adapters removed are available at NCBI's Sequence Read Archive under BioProject PRJNA495377. Overall, nursery-reared *A. cervicornis* microbial communities were similar within colonies and did not differ statistically by branch location (ANOSIM $R = 0.024$, $p = 0.057$). Microbial community composition varied by both coral genotype (ANOSIM $R = 0.199$, $p = 0.001$) and by colony (ANOSIM $R = 0.264$, $p = 0.001$), although the effect size of each was relatively small. Microbial communities of yellow colonies appeared more distinct from microbial communities of the red and green coral genotypes (Figure 2).

The most abundant bacterial orders detected were Rickettsiales, Synechococcales, Vibrionales, and an unclassified order of Alphaproteobacteria (Figure 3). Both Rickettsiales and Synechococcales were detected in most samples. Diverse ASVs classified as Vibrionales were

common and most abundant in the yellow genotype colonies. One abundant ASV from the unclassified order of Alphaproteobacteria common in corals of the green genotype was an exact match to an unpublished clone library sequence from *A. palmata* in Puerto Rico (GenBank Accession EU853842) and 99.6% similar to a clone sequence (GenBank Accession AY323179) from healthy *A. palmata* in Barbados [57].

There were two samples with very distinct community structure: one sample from a basal branch (1°) on a red genotype colony and one sample from an apical tip (3°) on a yellow genotype colony (Figure 3, panels A, C). The unique sample taken from a red colony had a relative abundance of 38% for a single Alteromonadales ASV classified as *Algicola*, which was an exact match to a clone library sequence from a diseased *Pavona duerdeni* coral in Thailand (GenBank Accession KC527315) [58] as well as to several other marine sources. The unique sample taken from a yellow colony also had high relative abundances of two Alteromonadales ASVs, which were different from the predominant Alteromonadales ASV in the red colony. The first was an ASV classified as *Thalassotalea* (16% relative abundance) that was an exact match to the type strain *Thalassotalea euphylliae* str. Eup-16 isolated from the coral *Euphyllia glabrescens* (GenBank Accession NR_153727.1) [59] and to clone libraries from the coral *Galaxea fascicularis* (GenBank Accession KU354186), crustose coralline algae (GenBank Accession JQ178640.1) [60], and the sponge *Diacarnus erythraeanus* (GenBank Accession HM854473.1) [61]. The second common Alteromonadales ASV in the red colony was an unclassified genus of Alteromonadaceae (11% relative abundance) that was an exact match to several clone library sequences from corals, including *Gorgonia ventalina* (GenBank Accession GU118325) [62], *Porites lutea* (GenBank Accession KF179648) [63], *Acropora pruinosa* (GenBank Accession JQ347406), and a coral-encrusting sponge (GenBank Accession HM593584) [64]. The unique yellow colony sample was also distinguished by the high relative abundance (27%) of one Opitutales ASV classified as *Coraliomargarita* and was 98% similar to clone library sequences from the corals *Porites lutea* (GenBank Accession KF179779) [63] and *Favia* corals with Black Band Disease (GenBank Accession GU472376).

By far, the most abundant bacterial order in nearly all of the samples from this study was Rickettsiales. In total, there were 11 ASVs classified as Rickettsiales, including five ASVs classified as Rickettsiales genus MD3-55, one ASV classified as Rickettsiales family S25-593, and five ASVs classified as Rickettsiaceae. The two most abundant ASVs were both classified as genus MD3-55 and together these two ASVs averaged a relative abundance of 75% in all samples. The remaining 9 Rickettsiales ASVs collectively had an average relative abundance well below 1%. The two most abundant MD3-55 ASVs (hereafter, ASV1 and ASV2, respectively) differed by a single nucleotide. ASV1 differed by one nucleotide in the V4 region of the 16S ribosomal RNA gene from the metagenome-assembled genome (GenBank Accession NZ_RXFM01000000) of an MD3-55 population from *A. cervicornis* collected in the Florida keys named *Candidatus* Aquarickettsia rohweri strain a_cerv_44 [65]. ASV2 was an exact match

in the V4 region to the metagenome-assembled genome. According to BLASTn results, both abundant Rickettsiales ASVs in all samples were also 99% - 100% identical to a clone library sequence from healthy Caribbean *Orbicella faveolata* (GenBank Accession JQ516457) [66].

The distribution of the five Rickettsiales genus MD3-55 ASVs was strikingly different across the three coral genotypes (Figure 4). Only ASV1 was detected in all samples from the three green genotype colonies and all samples from one red genotype colony. The other two red colonies were predominately ASV2, along with lower levels of ASV3 and ASV4. All five of the MD3-55 ASVs were detected in the yellow colonies, which contained predominantly ASV2. To determine the consistency of this result, we repeated the entire analysis pipeline six times to calculate the relative abundance of the five MD3-55 ASVs across all samples and achieved identical results.

Given the high relative abundance of just two Rickettsiales ASVs across nearly all samples, the differences in microbial community composition among coral genotypes were based on microbes with low relative abundances. Sixteen microbial families were differentially abundant across coral genotypes (Figure 5). The largest difference among coral genotypes was in an unclassified family of Alphaproteobacteria (6 ASVs), corresponding to the unclassified order of Alphaproteobacteria that was common in green colonies (Figure 3). This Alphaproteobacteria family was most abundant in green colonies and least abundant in red colonies (Figure 5). As described above, the most abundant ASV in this family matched sequences previously detected in coral microbial communities. In contrast, the family Francisellaceae (4 ASVs) was most abundant in red colonies and least abundant in green colonies. The most abundant Francisellaceae ASV was 99.6% similar to four clone library sequences (GenBank Accessions FJ202895, FJ202734, FJ202359, FJ202230) and more than 98% similar to twenty additional clone library sequences from the same study on Caribbean *Orbicella faveolata* corals [67].

The microbial community composition of yellow colonies appeared distinct from both red and green colonies (Figure 2), which appears to be driven primarily by four differentially abundant bacterial families that were detected in higher abundances in the yellow colonies compared to red and green. These include Desulfobacteraceae, Pirellulaceae, Puniceicoccaceae, and Xenococcaceae (Figure 5). A total of four Desulfobacteraceae ASVs were detected in this study. The most abundant Desulfobacteraceae ASV was an exact match to clone library sequences from several coastal marsh habitats, including a clone library sequence (KF513059) associated with the “pink berry” consortia of sulfate-reducing bacteria and sulfur-oxidizing bacteria [68]. A total of two Pirellulaceae ASVs (phylum Planctomycetes) were detected at low abundance in most samples and these sequences matched clone library sequences from a variety of marine habitats. There were eight Puniceicoccaceae ASVs (phylum Verrucomicrobia) and the most abundant were classified as *Coraliomargarita* as discussed above and *Lentimonas*. The most abundant *Lentimonas* ASV was an exact match to many clone library sequences from marine habitats, including an unpublished sequence (MF039937) from the sponge *Theonella*. A total of four

Xenococcaceae ASVs (phylum Cyanobacteria) were detected and the most abundant was almost entirely absent from red and green colonies. This ASV was classified as the genus Xenococcus PCC-7305 and was an exact match to clone library sequences from stromatolites in the Bahamas (EU249096) [69] and several unpublished clone library sequences from ooilitic sands in the Bahamas (JX255853, JX255856, JX255863, JX255892, JX504367, JX504398).

Discussion

By characterizing the microbial community composition of nine samples per coral colony, we have demonstrated that sampling from one location on an *A. cervicornis* coral colony is likely to provide a representative sample of the microbial community for the entire colony. This has important implications for researchers and resource managers who are concerned with how many samples are appropriate to take per colony and how to minimize sampling to reduce stress to colonies. It is also important to note that even with the heavy sampling that was performed here, namely nine samples taken in one day from a basketball-sized colony, no visible stress to the colonies was discernable. These colonies were observed during regular maintenance of the *in situ* nursery and roughly a year after sampling, all of the colonies were thriving. Our finding that one sample is likely representative of the whole colony is consistent with a similar analysis of *A. palmata* colonies that demonstrated microbial communities were not different between the topside of the wide branches of elkhorn coral and the more shaded underside of branches and colony bases [38]. Thus, for Caribbean acroporid corals, it appears that microbial community composition is relatively uniform at the colony scale. Since both of these coral species are critically endangered, this means that sampling for microbiota can be minimized to reduce impact to the coral host. However, this remains to be tested more broadly across different coral species, and especially in wild populations when available, rather than the relatively sheltered nursery-reared colonies sampled here.

These results seemingly conflict with earlier work using fingerprinting methods that detected spatial heterogeneity within colonies of *Porites* and *Orbicella* corals [36, 37]. It is possible that other coral groups exhibit more heterogeneity across colonies, however, this conflict may also be an artefact of the different methods used and the increased sample sizes of our study. For example, Rohwer *et al*. [36] used T-RFLP on a total of eight samples and Daniels *et al.* [37] used ARISA on a total of seventeen samples. While we did detect differences among samples (and a few samples were clearly very distinct), overall the differences among colonies and among genotypes were stronger than the differences within colonies. Therefore, heterogeneity does exist in the microbial communities across the surface of the coral colony regardless of coral species, but these differences are likely much smaller than differences among colonies.

Strikingly, the most abundantly detected sequences in this study belong to the Rickettsiales genus MD3-55. This is consistent with earlier work on natural populations of *A. cervicornis*, *A. palmata*, and *A. prolifera* at sites in Puerto Rico, Panama, and the Florida Keys [70]. Casas *et al*.

[70] found high relative abundances of coral-associated Rickettsiales in clone libraries of near full-length 16S rRNA genes in both *A. cervicornis* and *A. prolifera* but were only able to detect Rickettsiales using a targeted nested-PCR approach in *A. palmata*. Rickettsiales were also detected at low levels in natural populations of *A. cervicornis* and *A. palmata* in Panama using metabarcoding of the V6 hypervariable region [40]. More recent studies using metabarcoding of the V4 hypervariable region have detected very high relative abundances of Rickettsiales genus MD3-55 in natural populations of *A. cervicornis* in Puerto Rico [39] and in nursery-reared *A. cervicornis* (but not in *A. palmata*) from the Florida Keys [71]. In Florida nursery-reared *A. cervicornis*, experimental nutrient enrichment substantially increased the relative abundance of one Operational Taxonomic Unit of Rickettsiales [72]. Together, these results suggest that the Rickettsiales genus MD3-55 is common in Caribbean *A. cervicornis* and less common in *A. palmata*. The results also suggest that the predominance of MD3-55 may be impacted by the choice of PCR primer (V6 versus V4, for example) or that variation in the abundance of the presumably intracellular MD3-55 may be influenced by environmental conditions.

The role of the Rickettsiales genus MD3-55 in the health of acroporid corals is unclear. Here, we detected high relative abundances of this genus in all samples from nursery-reared colonies that exhibited no signs of disease in the year and a half prior to sampling and more than a year after sampling. However, this group was originally suspected to play a role in coral disease when intracellular bacterial aggregates were detected in histological slides of *Acropora* tissues with white-band disease (WBD) [73]. Subsequently, Rickettisales-like organisms, which can be detected by Geimsa stain, were detectable inside mucocytes of both healthy *A. cervicornis* and in WBD tissues [74, 75]. The presence of Rickettsiales in association with WBD in *A. cervicornis* has been confirmed by molecular methods, however, the relative abundance of Rickettsiales at times increased with the disease [76], and at times did not increase with the disease [77]. The recent recovery of a metagenome-assembled genome of the dominant MD3-55 strain detected by Casas *et al*. [70] and the widespread detection of MD3-55 sequences in both environmental samples and coral-associated microbial community datasets [65] have shed new light on the role of this apparently ubiquitous intracellular invertebrate symbiont.

The representative genome of MD3-55 from Caribbean *A. cervicornis*, dubbed *Candidatus* Aquarickettsia rohweri, has a reduced genome size, limited capacity for the biosynthesis of sugars and amino acids, and genes encoding nucleotide transport proteins, all of which are reflective of its intracellular, host-dependent lifestyle [65]. The *Candidatus* Aquarickettsia rohweri genome also has genes to sense extracellular nitrate levels, despite lacking genes to metabolize nitrogen. It has been hypothesized that *Candidatus* Aquarickettsia rohweri responds to environmental nitrate enrichment with increased growth, which saps the host of energy and makes it more susceptible to diseases like WBD [65]. However, to date strong empirical evidence is lacking for the connection between abundance of members of the genus MD3-55 (*Candidatus* Aquarickettsia) and quantifiable disease signs. This may, in part, be due to biases in

the production of 16S rRNA gene amplicon libraries which may not correspond to true biological abundances *in situ* or it may reflect the indirect relationship between nutrient enrichment, abundance of MD3-55, and signs of disease in which a certain threshold of MD3-55 must be reached before host health is negatively affected.

Alternatively, there is the possibility that not all strains of MD3-55 are equally harmful to the host. Here, we detected two predominant strains of MD3-55 that varied across coral genotypes in addition to three minor strains. This is consistent with recent work demonstrating that while the genus *Endozoicomonas* was predominant among *Acropora tenuis* bacterial communities, individual ASVs of *Endozoicomonas* were highly host genotype-specific [76]. Similarly, *Endozoicomonas* strains were identified as sporadic symbionts in the Pacific Line Islands [78], where *Endozoicomonas* was found on *Porites lutea* from only one island out of six islands sampled. However, further study is required to determine if these strains have genomic differences between strains that result in functional differences that affect coral health.

Conclusions

We examined the heterogeneity of microbial communities within and among coral colonies and found that while some heterogeneity exists within samples from the same coral colony, only differences among coral colonies and genotypes were statistically significant. However, the effect size was small, as almost every sample had high relative abundances of sequences classified as the Rickettsiales genus MD3-55 (*Candidatus* Aquarickettsia rohweri). To date, this presumably parasitic intracellular bacterium has been associated with white-band disease, although direct evidence of a negative relationship between genus MD3-55 and its coral hosts is lacking. Here, high relative abundances of MD3-55 were found in corals showing no signs of diseases over more than 2 years of monitoring. In addition, we detected a striking pattern of unique amplicon sequence variants of MD3-55 associated with different coral genotypes, suggesting that these strains may have functional differences selected by the host. Future work incorporating both physiological and genomic analysis of these bacteria is required to fully elucidate the role of MD3-55 bacteria in *A. cervicornis* corals.

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Figure 1

Nursery-reared *Acropora cervicornis* sampled in December 2017 for microbial community composition.

A) Three colonies each of red (R), green (G), and yellow (Y) coral genotypes were sampled. B) On each colony, three replicate samples were collected on basal branches (1°), intermediate branches (2°), and apical colony tips (3°). C) The same colonies photographed in October 2018 showed rapid growth and no signs of disease. *Acropora cervicornis* icon courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

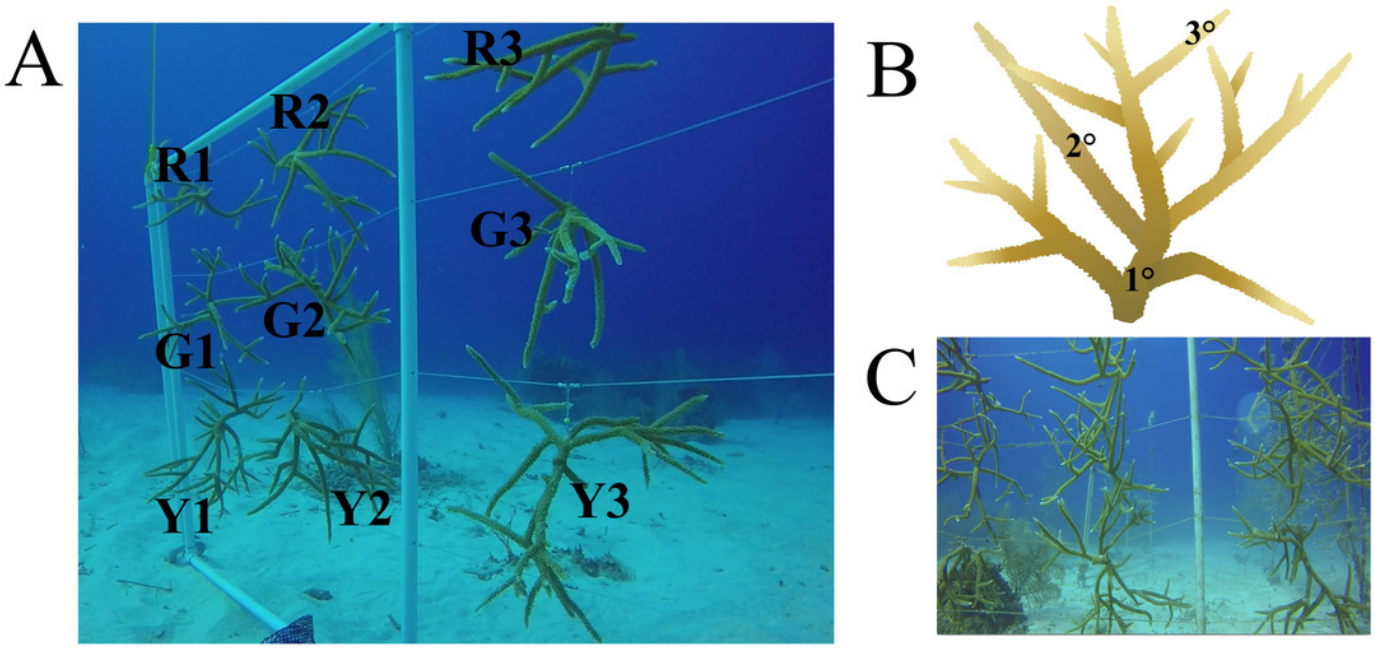

Figure 2

Principal Component Analysis of the Aitchison distance between microbial communities from green (G), red (R), and yellow (Y) genotypes of nursery-reared *Acropora cervicornis*.

Location of samples within individual colonies are indicated by symbol shape. Principal Component 1 (PC1) explained 24% of the variation among communities and Principal Component 2 (PC2) explained 8% of the variation among communities.

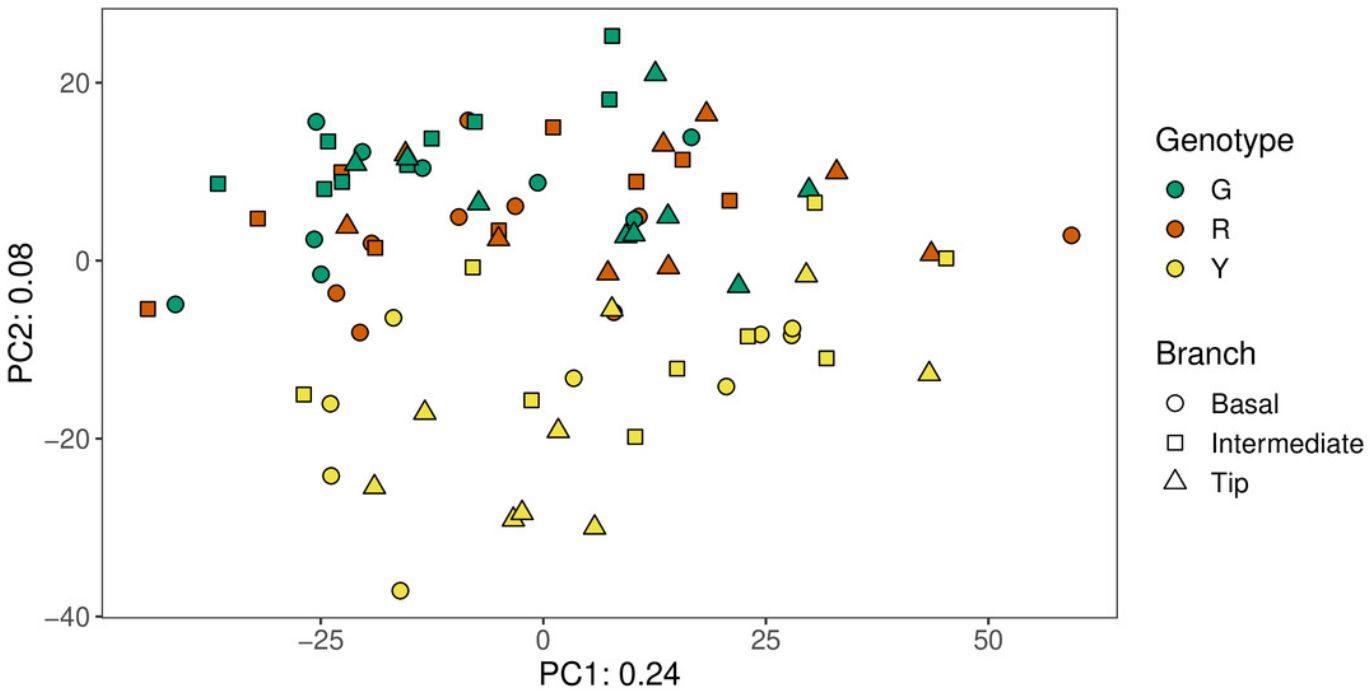

Figure 3

Relative abundance of amplicon sequence variants of the V4 region of 16S rRNA genes in nursery-reared *Acropora cervicornis*, colored by bacterial order.

Samples are grouped by coral genotype: (A) green (G), (B) red (R), and (C) yellow (Y) and by location within the colony: basal branches (1°), intermediate branches (2°), and apical colony tips (3°).

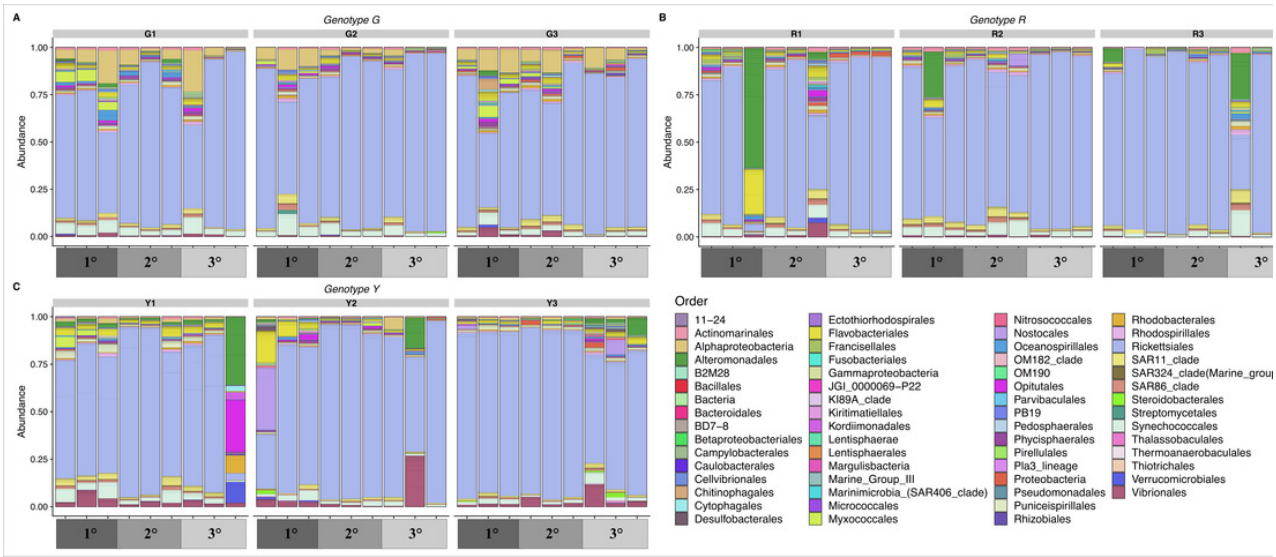
A
Genotype G
G1
G2
G3
B
Genotype R
R1
R2
R3
C
Genotype Y
Y1
Y2
Y3
Abundance
1.00
0.75
0.50
0.25
0.00
1°
2°
3°
Order
11–24
Actinomarinales
Alphaproteobacteria
Alteromonadales
B2M28
Bacillales
Bacteria
Bacteroidales
BD7–8
Betaproteobacteriales
Campylobacterales
Caulobacterales
Cellvibrionales
Chitinophagales
Cytophagales
Desulfobacterales
Ectothiorhodospirales
Flavobacteriales
Francisellales
Fusobacteriales
Gammaproteobacteria
JGI_0000069–P22
KI89A_clade
Kiritimatiellales
Kordiimonadales
Lentisphaerae
Lentisphaerales
Margulisbacteria
Marine_Group_III
Marinimicrobia_(SAR406_clade)
Micrococcales
Myxococcales
Nitrosococcales
Nostocales
Oceanospirillales
OM182_clade
OM190
Opitutales
Parvibaculales
PB19
Pedosphaerales
Phycisphaerales
Pirellulales
Pla3_lineage
Proteobacteria
Pseudomonadales
Puniceispirillales
Rhizobiales
Rhodobacterales
Rhodospirillales
Rickettsiales
SAR11_clade
SAR324_clade(Marine_grou
SAR86_clade
Steroidobacterales
Streptomycetales
Synechococcales
Thalassobaculales
Thermoanaerobaculales
Thiotrichales
Verrucomicrobiales
Vibrionales

Figure 4

Proportion of amplicon sequence variants classified as the Rickettsiales genus MD3-55 relative to all ASVs in the communities in colonies of green (G), red (R), and yellow (Y) coral genotypes of nursery-reared *Acropora cervicornis*.

Each bar represents one sample, with a total of nine samples per coral colony.

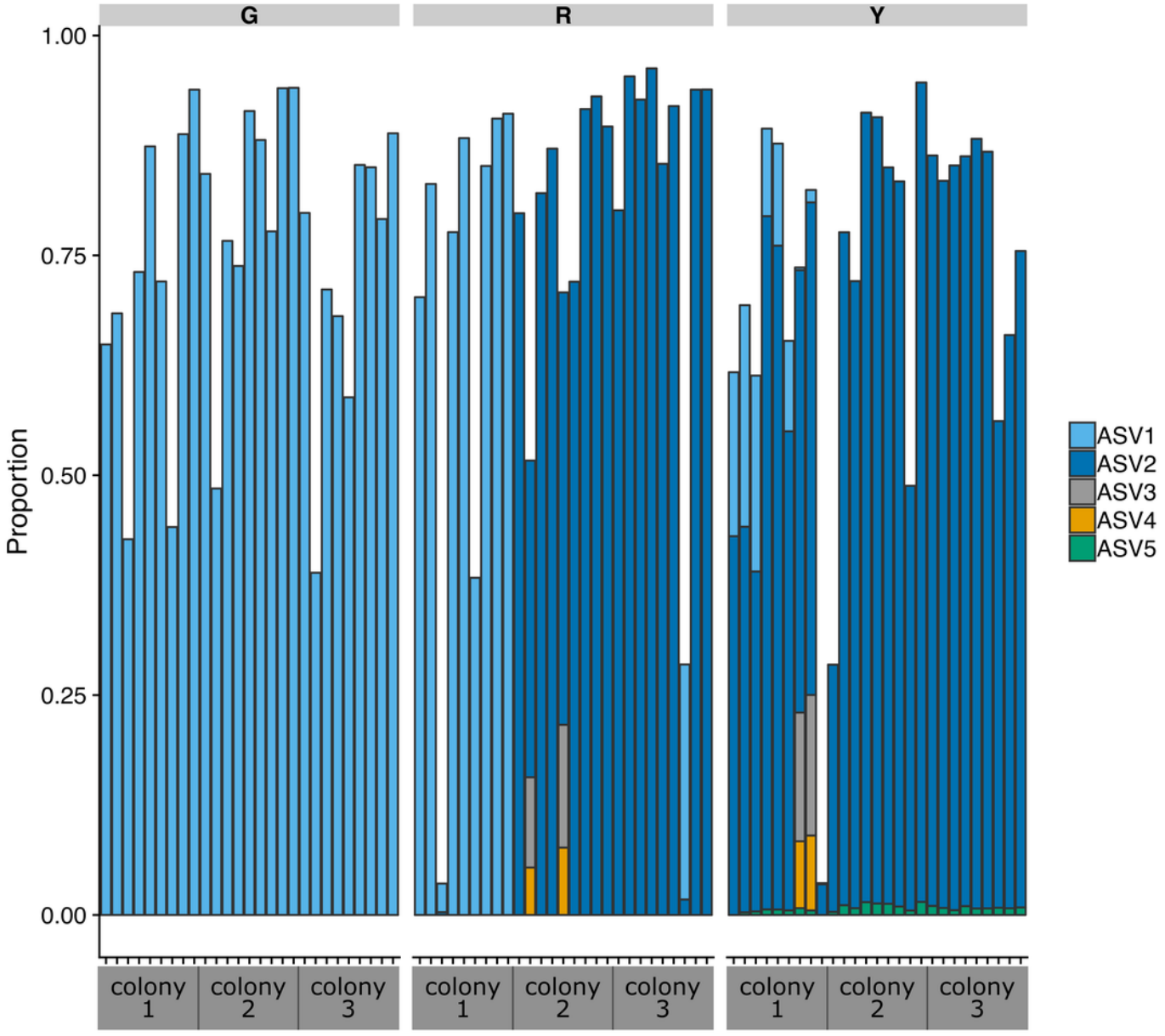

Figure 5

Relative abundance of amplicon sequence variants in differentially abundant families between colonies of green (G), red (R), and yellow (Y) coral genotypes of nursery-reared *Acropora cervicornis*.

To increase separation of very small values, relative abundances are plotted on a log scale, with 0.001 added to every value to avoid taking the log of zero.