#### **REPORT**



# Characterization of a thermally tolerant *Orbicella faveolata* reef in Abaco, The Bahamas

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Abstract Increased ocean temperatures from anthropogenic climate change induce coral bleaching, the breakdown of symbioses between corals and photosynthetic dinoflagellates. However, some corals thrive in marginal, warm environments that exceed typical bleaching thresholds. Their survival may be mediated by specific genes within the coral host, association with heat-tolerant algal symbionts, and/or distinct bacterial communities. At Mermaid Reef in Great Abaco, The Bahamas, Orbicella faveolata colonies did not bleach during a warming event that reached 33.0 °C, while at Sandy Cay Reef (~ 18 km south), which reached only 32.0 °C, O. faveolata bleached extensively. To investigate abiotic and biotic factors contributing to Mermaid Reef's higher thermal tolerance, we compared temperature, depth, and coral composition at each site and used microsatellite genotyping, quantitative PCR, and 16S rRNA metabarcoding to examine host

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genotype diversity, Symbiodiniaceae composition, and bacterial communities in O. faveolata. All O. faveolata colonies at the tolerant Mermaid Reef were clonemates and hosted exclusively *Durusdinium* symbionts, while colonies at Sandy Cay Reef comprised diverse genotypes and hosted varying proportions of four Symbiodiniaceae genera, which were primarily structured by depth. Mermaid Reef colonies also tended to have higher bacterial family richness than Sandy Cay Reef. These findings suggest that shallow, warm environments like Mermaid Reef may select for few, putatively heat-tolerant genotypes of corals and symbionts, and that while warming may greatly reduce genetic diversity, certain individuals may thrive. Such individuals existing today can provide valuable biological insights and resources for intervention conservation aimed at boosting reef resilience under climate change.

**Keywords** Coral bleaching · Scleractinia · Symbiodiniaceae · Microsatellite markers · 16S metabarcoding

## Introduction

Increasing ocean temperature resulting from anthropogenic climate change is the primary driver of ongoing coral reef decline (Hoegh-Guldberg et al. 2007; Hughes et al. 2018). Exposure to temperatures only ~ 1 °C higher than historical maxima can induce thermal bleaching—the breakdown of coral symbioses with photosynthetic dinoflagellates—which can lead to mass coral mortality and subsequent reef ecosystem degradation (Jokiel and Coles 1990; Glynn 1993; Baker et al. 2008). Coral cover on Caribbean reefs has already declined by more than 80% since 1970 (Gardner et al. 2003), and the majority of reefs



globally are projected to experience annual severe bleaching by the mid-2050s (van Hooidonk et al. 2013, 2015).

Despite the negative impacts of ocean warming on reefs around the world, some corals and reefs thrive in marginal, warm-water environments that resemble future climate predictions. For example, corals inhabiting a tide pool in American Samoa with high temperature variability tolerate temperatures up to 35 °C at peak afternoon heat throughout the summer (Palumbi et al. 2014). Despite exposure to extreme temperature, these corals demonstrate increased growth rates and symbiont photosynthesis compared to colonies in neighboring tide pools with exposure to less extreme temperatures. Thermotolerant coral populations have also been characterized in New Caledonia, withstanding temperatures upwards of 33 °C (Camp et al. 2017), as well as the Arabian Sea and Persian Gulf, surviving maximum seasonal temperatures of 36 °C and daily fluctuations of 9 °C (Coles 1997, 2003; Hume et al. 2013; Kinsman 1964; Riegl et al. 2011).

The ability of corals to inhabit these extreme environments may be attributed to multiple adaptive mechanisms within the coral holobiont—the animal host and its associated eukaryotic and prokaryotic microbiota (Rohwer et al. 2002). In the coral animal, certain transcription factors, heat shock proteins, and cell signaling proteins may increase heat tolerance (Palumbi et al. 2014), and higher expression of these genes (Barshis et al. 2013), or plasticity in their expression (Kenkel and Matz 2016), may be a signature of adaptation to warmer environments. Indeed, the evolution of heat tolerance likely involves many loci throughout the coral genome (Bay and Palumbi 2014; Dixon et al. 2015; Dziedzic et al. 2019), with certain coral individuals being more heat-tolerant than others (Baird et al. 2009; Kayousi et al. 2015; Cunning et al. 2016).

In addition to the coral genotype, other factors that significantly influence thermal tolerance of the holobiont are the identity and abundance of symbiotic microbiota (Glynn et al. 2001; Berkelmans and van Oppen 2006; Cunning and Baker 2014). Corals host an array of Bacteria and Archaea (Rohwer et al. 2002; Kellogg 2004), viruses (Wilson et al. 2005; Nguyen-Kim et al. 2014), fungi (Bentis et al. 2000; Amend et al. 2012), and endosymbiotic photosynthetic dinoflagellates from the family Symbiodiniaceae. Of the several genera that comprise the Symbiodiniaceae, Symbiodinium, Breviolum, Cladocopium, and Durusdinium (formerly clades A, B, C, and D; LaJeunesse et al. 2018) are most commonly observed in association with Caribbean corals. The important reef-builder *Orbi*cella faveolata hosts species from all four genera (Rowan and Knowlton 1995; Kemp et al. 2015), and these tend to vary in thermal tolerance, with its Durusdinium and Symbiodinium partners having the highest thermal thresholds,

followed by *Breviolum*, and then *Cladocopium* (Rowan et al. 1997; LaJeunesse et al. 2009; Kennedy et al. 2016). Indeed, corals hosting species of *Durusdinium* may gain an additional  $\sim 1$ –1.5 °C in thermal tolerance (Berkelmans and van Oppen 2006; Silverstein et al. 2015).

In addition to the Symbiodiniaceae, corals associate with diverse bacteria that play important functional roles in nutrient cycling, protection from pathogens, and possibly thermal tolerance (reviewed by van Oppen and Blackall 2019). Ziegler et al. (2017) identified distinct bacterial communities in corals with differing thermal sensitivities, but whether there is a causal relationship between bacteria and heat tolerance remains to be determined. Nevertheless, the thermal tolerance of coral holobionts is a complex trait that may be linked to the coral animal itself, its Symbiodiniaceae community, and/or other members of its microbiome. Studying corals in extreme environments with unusually high thermal tolerance represents an opportunity to test the relative contributions of these factors that may mediate the future survival of coral reefs.

Unusually high thermal tolerance was observed at a reef comprised primarily of *O. faveolata* in Great Abaco, The Bahamas. From May to September 2015, Mermaid Reef experienced a warming event with temperatures reaching 33.0 °C, dramatically exceeding the local bleaching threshold of 30.4 °C (i.e., MMM + 1 °C, from Pathfinder v5.0 1982-2008 climatology), yet no bleaching was observed. In contrast, Sandy Cay Reef, a more diverse reef ~ 18 km further south, experienced maximum temperatures of only 32.0 °C, but extensive bleaching of *O. faveolata* was observed. The higher thermal tolerance of corals at Mermaid Reef indicates there is something distinct about the biology of these corals allowing them to survive in this extreme environment.

To characterize this unusual coral reef, temperature and benthic composition data were collected, as well as genetic samples from *O. faveolata* colonies at both tolerant Mermaid Reef and sensitive Sandy Cay Reef. To understand what biological factors may be contributing to the higher thermal tolerance of Mermaid Reef corals, these samples were used to analyze the diversity of (1) host genotypes, (2) Symbiodiniaceae communities, and (3) bacterial microbiomes associated with the colonies in each location. The survival strategies exhibited at Mermaid Reef and other extreme reefs may provide insight into how corals can adapt or acclimatize to changes in the environment.



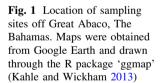
#### Materials and methods

#### Site characterization and coral sampling

Benthic community and coral composition surveys of both sites were conducted on January 24, 2019, at Mermaid Reef (Fig. 1; 26° 33′ 12.90″ N 77° 03′ 12.66″ W) and May 26, 2019, at Sandy Cay Reef (Fig. 1; 26° 23′ 55.90 ″N 76° 59′ 20.50″ W) following the Atlantic and Gulf Rapid Reef Assessment (AGRRA) protocols. Benthic community composition was surveyed along 10-m transects, five at Mermaid Reef and four at Sandy Cay Reef. Corals were measured to determine diameter, height, and width of each colony with averaged values per site used for analysis.

In April 2019, a series of 6700 high-resolution photographs were taken to compile an orthomosaic image of Mermaid Reef (Fig. 2). Images were obtained using a Nikon D850 camera with a 35-mm lens in a Sea and Sea underwater housing. To capture images, a diver swam parallel transects back and forth across Mermaid Reef in a north-south orientation followed by parallel transects across the entire reef in an east-west orientation with the camera capturing images at 1-s intervals. GPS coordinates were taken for 38 positions along the reef, including temporary markers that were captured in images, and permanent tags of corals that were sampled. The images were compiled into a georeferenced orthomosaic image using Agisoft Photoscan software (Agisoft 2016).

Tissue samples from colonies of *O. faveolata* were collected from tolerant Mermaid Reef (n = 40) and sensitive Sandy Cay Reef (n = 30) between January 21 and 24, 2019. Coral colonies were selected opportunistically, and tissue clippings ( $\sim 1 \text{ cm}^2$ ) were taken by SCUBA divers from the top of each colony with toenail clippers, and the



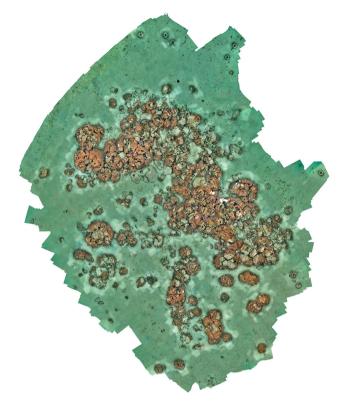


Fig. 2 Orthomosaic of Mermaid Reef. Total area of the orthomosaic is approximately 900  $\ensuremath{\text{m}}^2$ 

depth of each sampled colony was recorded. Samples were divided in half and preserved in two replicate microcentrifuge tubes with 500  $\mu$ L DNA buffer (5 M NaCl, 0.5 M EDTA) with 1% sodium dodecyl sulfate, and incubated for one hour at 65 °C. One replicate of each sample was transported to the John G. Shedd Aquarium (CITES Export





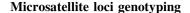
Permit 2019/18), and the other to Middlebury College for genetic analyses.

HOBO Pendant (Onset Corporation, Model Number: UA-002-08, 64 K) data loggers were deployed from May 08 to October 15, 2015. One data logger was deployed at each reef at a depth of 2.5 m at Mermaid Reef and 3.3 m at Sandy Cay Reef. Temperature and light intensity (lum/ft²) were recorded at 15-min intervals throughout deployment.

#### Symbiodiniaceae community analysis

Genomic DNA from each coral sample was extracted using a CTAB-chloroform protocol (https://doi.org/10.17504/ protocols.io.dyq7vv). Samples were assayed using qPCR targeting loci specific to O. faveolata and each of four symbiont genera (Symbiodinium, Breviolum, Cladocopium, and Durusdinium). Assays for a single-copy O. faveolata gene locus (Severance et al. 2004; Cunning et al. 2015) were carried out in 12.5 µL reactions with PowerUp SYBR Green Master Mix (Thermo Fisher Scientific), 900 nM (5'-TCACTTTCGCAGAGCAATGG-3'), Orb-sc9F 900 nM Orb-sc9R (5'-GGCAATGTTTGTACCCACGAT-3'), and 1 µL DNA template. Symbiodiniaceae assays were carried out in 10 µL reactions using primers and probes targeting genus-specific actin loci (Table S1), and Cladocopium and Durusdinium assays were multiplexed. All Symbiodiniaceae assays included 5 µL of TaqMan Genotyping Master Mix (Thermo Fisher Scientific) and 1 µL of DNA template. Positive and negative controls were run alongside samples on each plate. All reactions were performed on a QuantStudio 3 Real-Time PCR system (Life Technologies, Foster City, CA, USA) with the following thermal cycling conditions: initial incubation at 50 °C for 2 min, denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 10 s and annealing at 60 °C for 1 min. Resulting cycle threshold  $(C_T)$  values at a set threshold of  $\Delta R_n = 0.01$  were used for data analysis (Cunning et al. 2015). Two technical replicates indicated amplification of target DNA.

Data were imported into R v3.6.0 (R Core Team 2018) using the steponeR package (Cunning 2018). Symbiont-to-host cell ratios for each algal symbiont genus were calculated adjusting for differences in target locus copy number, DNA extraction efficiency, and probe fluorescence intensity following Cunning and Baker (2013) and converted to relative abundance within each sample. The dominant symbiont in each sample was assigned as the most abundant genus. Figures were created with ggplot2 (Wickham 2016) and igraph (Csardi et al. 2006) packages.



Genomic DNA was extracted using the Qiagen DNeasy Power Soil Kit according to manufacturer's instructions. A subset of twelve *O. faveolata* colonies from Mermaid Reef and twelve colonies from Sandy Cay Reef were genotyped at six neutral microsatellite loci (multiplexes "A" and "B") following Davies et al. (2013). Microsatellite fragments were run on an ABI 3500 Genetic Analyzer (Applied Biosystems), allele peaks were called in Geneious v9 (Geneious, New Zealand), and clonality was determined in GenoDive v3 (Meirmans and Van Tienderen 2004). Colonies sharing identical alleles at all loci were deemed to be clonemates (probability of identity:  $1.6 \times 10^{-6}$ ).

## Microbial DNA processing, sequencing, and analysis

DNA from a subset of six O. faveolata colonies from tolerant Mermaid Reef and six colonies from sensitive Sandy Cay Reef were submitted to the University of Vermont's Vermont Integrative Genomics Resource (VIGR) for standard V4 16S rRNA Illumina amplicon library preparation with the Nextflex Rapid DNA-Seq kit (PerkinElmer Applied Genomics) with the Earth Microbiome primers 515F and 806R. Amplicon libraries were sequenced using Illumina MiSeq PE250 chemistry. Due to challenges with the reverse reads, only forward reads were used in subsequent analyses. Forward reads were filtered, trimmed, and dereplicated with dada2 v 1.3.5 (Callahan et al. 2016). After error learning, the central dada algorithm was applied to assign amplicon sequence variants (ASVs), after which chimeric ASVs were removed. Taxonomy was assigned to genus level in dada2 using the silva nr v132 training set (Quast et al. 2013). ASVs were passed off to phyloseq v 1.30.0 (McMurdie and Holmes 2013). Data were visualized using ggplot2 (v. 3.2.1) (Wickham 2016). Finally, DivNet (v. 0.3.1) and breakaway (v 4.6.14) were used to statistically estimate microbial diversity metrics (Willis and Bunge 2015; Willis et al. 2017).

## **Results**

## Site characterization

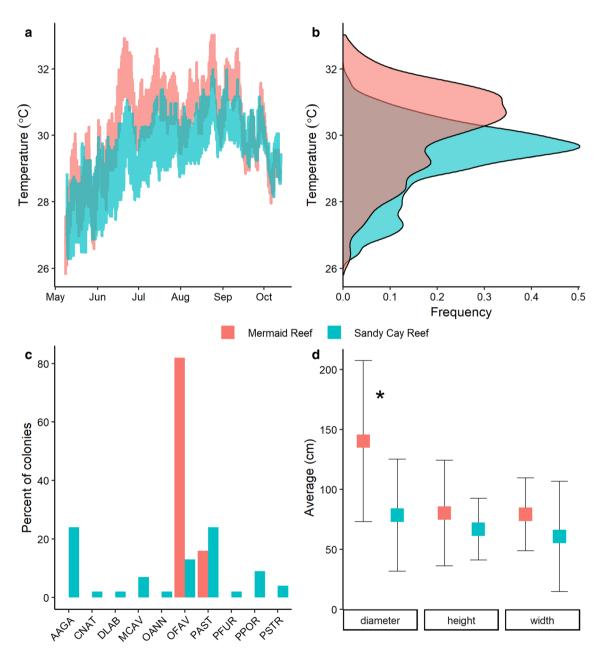
The two reefs experienced different temperature distributions during summer/fall 2015 (Fig. 3ab; t test, p < 0.001). Waters around tolerant Mermaid Reef were an average 0.8 °C warmer than around sensitive Sandy Cay Reef throughout the six-month period. The maximum temperature at Mermaid Reef (33.0 °C) was 1.0 °C warmer than Sandy Cay Reef (32.0 °C). Mermaid Reef also experienced



on average 4.7 times higher light during the first week of logger deployment (Figure S1).

AGRRA surveys in 2019 revealed that tolerant Mermaid Reef featured low species diversity: 82% of colonies surveyed were *O. faveolata*, while 16% were *Porites astreoides* (Fig. 3c). Sensitive Sandy Cay Reef was more diverse: 10 coral species were observed, with the most abundant being *Agaricia agaricites* (24%), *P. astreoides* 

(24%), and *O. faveolata* (13%). Benthic community composition was similar at the two sites in terms of live coral cover with 50.6% at Mermaid Reef and 50.9% at Sandy Cay Reef. Corals at Mermaid Reef were shallower (1.2 to 2.6 m), whereas corals at Sandy Cay Reef occurred from 2.6 to 7.6 m depth. On average, the diameter of *O. faveolata* colonies was greater at Mermaid Reef compared to Sandy Cay Reef (140.4  $\pm$  67.1 cm vs. 78.6  $\pm$  46.8 cm;



**Fig. 3** Temperature and coral composition of Mermaid Reef (pink) and Sandy Cay Reef (blue). **a** Temperature data from May to October 2015 recorded by HOBO pendants. **b** Distribution of temperature values between both sites. **c** Composition of coral species of each reef: AAGA = Agaricia agaricites, CNAT = Colpophyllia natans, DLAB = Diploria labyrinthiformis, MCAV = Montastraea

cavernosa, OANN = Orbicella annularis, OFAV = Orbicella faveolata, PAST = Porites astreoides, PFUR = Porites furcata, PPOR = Porites porites, PSTR = Pseudodiploria strigosa. **d** Average diameter, height, and width of *O. faveolata* colonies surveyed at each site. Asterisks indicate a significant difference at p < 0.05



p < 0.05) (mean  $\pm$  SD). A larger average width (79.3  $\pm$  30.4 cm vs.  $60.8 \pm 46.0$  cm) and height (80.4  $\pm$  44.0 cm vs.  $66.9 \pm 25.6$  cm; Fig. 3d) were also recorded, but these were not significantly different.

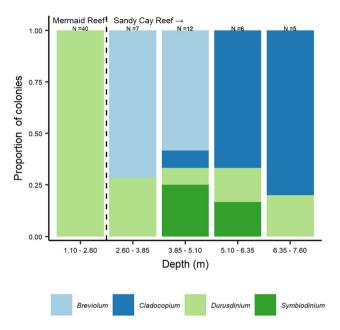
## Host microsatellite genotyping

Microsatellite genotyping of *O. faveolata* identified all twelve colonies from tolerant Mermaid Reef to be clonemates, exhibiting identical alleles at all six loci tested (Fig. 4a, Table S2). Of the twelve samples collected from sensitive Sandy Cay Reef, two were identified as clonemates, while all other colonies were genetically distinct. There was no overlap in genotypes from Mermaid Reef and Sandy Cay Reef.

#### Symbiodiniaceae composition

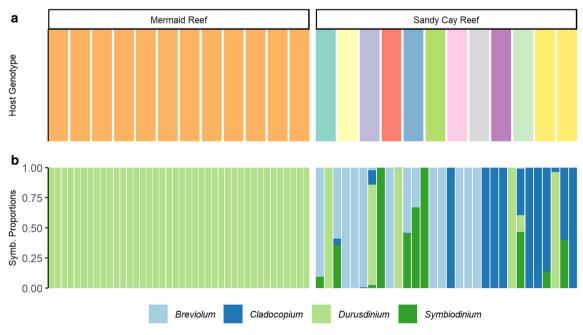
Durusdinium was the only Symbiodiniaceae genus detected in O. faveolata colonies at tolerant Mermaid Reef (Fig. 4b). In contrast, all four Symbiodiniaceae genera (Symbiodinium, Breviolum, Cladocopium, and Durusdinium) were detected in varying proportions at sensitive Sandy Cay Reef (Cladocopium was present in 60% of samples, Breviolum in 50%, Symbiodinium in 33%, and Durusdinium in 20%).

Combined data from both sites revealed patterns in symbiont dominance influenced by depth (Fig. 5).



**Fig. 5** Proportion of colonies with the dominant Symbiodiniaceae genus at a given depth. Mermaid Reef samples were all located within 1.1–2.6 m (left of the dashed line). Sandy Cay Reef samples were divided among 1.25 m increments (to the right of the dashed line). Number of samples per depth interval is indicated at the top of each bar

Durusdinium dominated the majority of corals less than 3 m deep (including all colonies at Mermaid Reef), but also dominated 10–25% of corals in other depth intervals at Sandy Cay Reef. *Breviolum* was dominant in 63.2% of



**Fig. 4** Host genotype diversity and Symbiodiniaceae composition of colonies from Mermaid Reef and Sandy Cay. **a** Microsatellite genotyping of *Orbicella faveolata* hosts (n = 12 at each study site). Unique colors represent genetically distinct colonies. **b** Composition

of Symbiodiniaceae in colonies at each study site (n = 40 at Mermaid Reef; n = 30 at Sandy Cay Reef). Individual bars represent a single sample ordered left to right from least to greatest depth

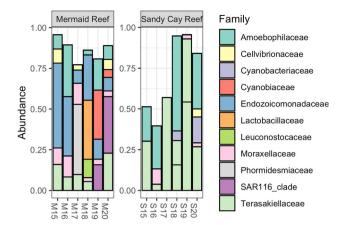


corals from 2.60 to 5.10 m and *Symbiodinium* was dominant in 22.2% of corals from 3.85–6.35 m. The majority of samples deeper than 5.10 m were dominated by *Cladocopium* (66.7%). With respect to mixed communities (including dominant and sub-dominant symbionts), Sandy Cay Reef colonies hosted either one (n = 17), two (n = 9), three (n = 2), or four genera (n = 2) in varying proportions; we visualized these combinations using network analysis (Fig. 6). Genera most frequently occurring alone within a colony were *Breviolum* (in n = 8 colonies) and *Cladocopium* (n = 7), while *Symbiodinium* and *Durusdinium* only occurred alone once each, and were more frequently found in mixed communities with other genera.

## Microbial community analysis

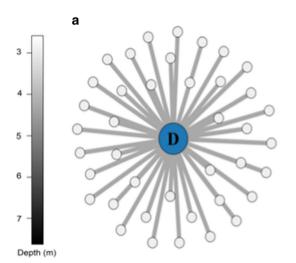
Initially 816 microbial taxa were assigned to dada2 amplicon sequence variants (ASVs) across the twelve samples from both reefs. However, after filtering out ASVs that matched to mitochondria, chloroplast, and Suessiaceae algal sequences, there were 87 total taxa represented across the samples. This filtering step reduced the median number of bacterial reads per sample to 104. The most abundant families of bacteria associated with colonies at tolerant Mermaid Reef were Amoebophilaceae, Endozoicomonadaceae, Moraxellaceae, Phormidesmiaceae, Lactobacillaceae, Leuconostocaceae, Cyanobiaceae, Cellvibrionaceae, SAR116 clade, and Terasakiellaceae (Fig. 7). The sensitive Sandy Cay reef had fewer abundant families, but included Amoebophilaceae, Terasakiellaceae, Phormidesmiaceae, and Cellvibrionaceae.

No clear patterns emerged between reefs in the Bray-Curtis nonmetric multidimensional scaling plot when

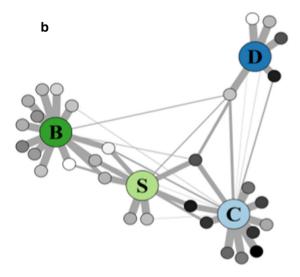


**Fig. 7** Abundant bacterial taxa from Mermaid Reef and Sandy Cay Reef. The twenty most abundant bacterial families assigned to amplicon sequence variants between Mermaid Reef (M15–M20) and Sandy Cay Reef (S15–S20). Less abundant families make up the remaining percent abundance

considering all bacterial ASVs (Fig. S2). Breakaway diversity estimates based on the number of observed families ranged from 6 to 13 at tolerant Mermaid Reef, and 3 to 16 at sensitive Sandy Cay Reef (Table S3). The mean family-level diversity, where errors in the estimates could be predicted for all samples, at Mermaid Reef (n = 6) was  $\sim 10$  families, and there was a trend that Sandy Cay Reef (n = 6) had lower family diversity by  $\sim 3$  families (p = 0.045; Table S3).



**Fig. 6** Network analysis of Symbiodiniaceae composition of *Orbicella faveolata* from **a** Mermaid Reef and **b** Sandy Cay Reef. Larger colored nodes represent Symbiodiniaceae genera: B = *Breviolum*, C = *Cladocopium*, D = *Durusdinium*, S = *Symbiodinium*. Smaller



nodes represent individual coral samples with grayscale corresponding to depth. Thickness of lines connecting corals to Symbiodiniaceae genera indicate the relative amount of each genus in the sample



#### Discussion

Our characterization of tolerant Mermaid Reef reveals distinct abiotic and biotic conditions that may underlie its observed thermal tolerance and ability to survive in marginal conditions. Mermaid Reef experiences warmer and more variable temperatures than nearby Sandy Cay Reef and could also experience higher turbidity as a result of its closer proximity to a developed shoreline. However, the shallower depth of corals at Mermaid Reef and higher documented light intensities indicate that these corals also experience extreme irradiance. Exposure to chronic extreme temperatures and higher irradiances has potentially shaped distinct patterns in the genetic diversity of *O. faveolata* corals and their symbionts, providing insight into multiple mechanisms for coral survival in extreme environments.

The genetic diversity of O. faveolata at Mermaid Reef was very low: 100% of sampled colonies were clonemates at Mermaid Reef, whereas only 16% of sampled colonies were clonemates at sensitive Sandy Cay Reef. Additionally, AGRRA survey found only O. faveolata and a small proportion of P. astreoides colonies at Mermaid Reef. The detection of only one genotype and overall low coral species diversity suggests that the entire population of O. faveolata at Mermaid Reef could be the product of asexual propagation from one or few colonization events by unusually stress-tolerant individuals, similar to other marginal reefs (e.g., Baums et al. 2014). However, the large size of the O. faveolata colonies at Mermaid Reef indicates they are well established. The extreme environment may select against migration into the area by new O. faveolata genotypes and may have eliminated sensitive colonies in the past. A full genomic analysis may reveal the functional basis for how this stress-tolerant genotype differs from O. faveolata genotypes at nearby Sandy Cay Reef.

The diversity of Symbiodiniaceae at Mermaid Reef was also greatly reduced compared to Sandy Cay Reef. Mermaid Reef colonies exclusively hosted Durusdinium, a genus associated with stress tolerance (LaJeunesse et al. 2010b, 2014). At Sandy Cay Reef, symbiont communities were primarily dominated by Cladocopium and Breviolum, but were also highly variable in their composition. This variability was strongly related to depth, indicating that environmental parameters, and light in particular, may mediate symbiont competition in O. faveolata (McIlroy et al. 2019). The depth distributions were consistent with previous work showing Cladocopium dominating deeper areas with low irradiance, and Symbiodinium and Breviolum dominating shallower, light exposed areas (Kemp et al. 2015). Durusdinium is often found in environments that are shallow and turbid (LaJeunesse et al. 2010a; Keshavmurthy

et al. 2014) with high temperature variability (Carballo-Bolaños et al. 2019). *Durusdinium* is also found more commonly in shallower colonies of *Montipora capitata* in Hawaii, while *Cladocopium* dominates these corals at deeper depths, presumably due to gradients in irradiance (Innis et al. 2018). Irradiance could explain the depth distribution of Symbiodiniaceae observed here across both reefs (Fig. 5), although since all the corals were shallow at Mermaid Reef, differences in the temperature regime or other environmental factors at this site could also explain the competitive dominance of *Durusdinium*.

Bacterial communities were composed of families previously described in Orbicella spp. and other corals. Amoebophilaceae, with members hypothesized as symbionts of Symbiodiniaceae or other protists, and Endozoicomonadaceae have been documented in a range of coral hosts (Ziegler et al. 2016; Sharp et al. 2017). In Porites astreoides, Endozoicomonadacea are associated with healthy mucus microbiota, and their loss is linked to decreased resilience under pathogen stress (Glasl et al. 2016). The Terasakiellaceae family was present in almost all of the corals sampled, and highly abundant in the Sandy Cay Reef samples. This family has not been described in other Orbicella species, and their environmental role is understudied, but they have been described in other marine invertebrates including Paragorgia arborea and two marine sponges, and in the case of the P. arborea, it is posited that these taxa are involved in nitrogen metabolism (Weiler et al. 2018; Sacristán-Soriano et al. 2019). Lactobacillaceae were noted among the taxa enriched in healthy microbiomes of Orbicella spp. and Indo-Pacific corals as compared to those afflicted with White Plague disease (Roder et al. 2014). While many of these bacterial families have not been explicitly linked to thermal tolerance, it is possible that they are common members of healthy coral microbiomes that confer a wide range of resilience. Although only moderately significant, microbial diversity was marginally greater at tolerant Mermaid Reef, despite a more homogenous host population. This could relate to the environment and/or to chronic stress at the site (Zaneveld et al. 2017; Ahmed et al. 2019).

Higher thermal tolerance from association with thermally tolerant symbionts in some corals is often accompanied by physiological tradeoffs, such as reduced growth (Jones and Berkelmans 2010, Little et al. 2004), disease resistance (Merselis et al. 2018), or fecundity (Jones and Berkelmans 2011). While we were unable to assess potential tradeoffs directly at Mermaid Reef, anecdotal observations suggest there may be differences in reproduction. Observers in the water on August 20–21, 2019, witnessed no spawning at tolerant Mermaid Reef, while an estimated ~ 50% of *O. faveolata* colonies monitored at sensitive Sandy Cay Reef spawned (M. Miller, pers.



commun.). Regionally, *O. faveolata* also spawned on the same days in the Florida Keys and Biscayne National Park. While corals at Mermaid Reef could have spawned outside the short window of surveillance, these observations suggest possible spawning failure, or at least asynchrony, which could reflect the genetic differentiation of these host corals. Further evaluation of growth and reproduction in corals on extreme reefs will provide valuable insight into the broader ecology of future reefs.

Our results suggest that as Caribbean reefs warm, O. faveolata communities will be characterized by the presence of particular stress-tolerant coral genotypes and/or heat-tolerant Symbiodiniaceae. Susceptible corals and symbionts may be outcompeted and extirpated from future reefs, leading to reductions in genetic diversity. The identification of extreme reefs and stress-tolerant genotypes, such as those found at Mermaid Reef, is of high research priority. Other marginal locations may also have been colonized by rare, stress-tolerant individuals, and sufficient replication across these reefs may identify common patterns of selection as well as unique genomic solutions for surviving in such conditions. Corals living at Mermaid Reef and other extreme environments could be utilized in restoration through propagation in nurseries, and for selective breeding. Further study of corals at these locations may help us better understand acclimatization and adaptation to ongoing climate change.

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#### Compliance with ethical standard

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

**Data availability** Symbiodiniaceae qPCR data, environmental data, and R code for analysis are available at github.com/kparker96/Sandy\_Mermaid, and are archived at Zenodo (Parker and Cunning 2020). Microbial raw data are available through the NCBI SRA via accession number PRJNA591009, with R code for analysis at github.com/eme47/CoralMicrobiome.

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