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Adaptation to reef habitats through selection on the coral animal and its associated microbiome

Short title: Local adaptation in corals

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Abstract

Spatially adjacent habitats on coral reefs can represent highly distinct environments, often harbouring different coral communities. Yet, certain coral species thrive across divergent environments. It is unknown whether the forces of selection are sufficiently strong to overcome the counteracting effects of the typically high gene flow over short distances, and for local adaptation to occur. We screened the coral genome (using restriction-site-associated sequencing [RAD-seq]), and characterized both the dinoflagellate photosymbiont and tissue-associated prokaryote microbiomes (using metabarcoding) of a reef flat and slope population of the reef-building coral, *Pocillopora damicornis*, at two locations on Heron Island in the southern Great Barrier Reef. Reef flat and slope populations were separated by <100 m horizontally and ~5 m vertically and the two study locations were separated by ~1 km. For the coral host, genetic divergence between habitats was much greater than between locations, suggesting limited gene flow between the flat and slope populations. Consistent with environmental selection, outlier loci primarily belonged to the conserved, minimal cellular stress response, likely reflecting adaptation to the different temperature and irradiance regimes on the reef flat and slope. Similarly, the prokaryote community differed across both habitat and, to a lesser extent, location, whereas the dinoflagellate photosymbionts differed by habitat but not location. The observed intra-specific diversity associated with divergent habitats supports that environmental adaptation involves multiple members of the coral holobiont. Adaptive alleles or microbial associations present in

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coral populations from the environmentally-variable reef flat may provide a source of adaptive variation for assisted evolution approaches, through assisted gene flow, artificial cross-breeding or probiotic inoculations, with the aim to increase climate resilience in the slope populations.

Introduction

Adaptation to local environmental conditions is a common phenomenon in both the terrestrial and the marine environment (Sanford & Kelly 2011; Shafer & Wolf 2013), including coral reef ecosystems (Bay & Palumbi 2014; Dixon *et al.* 2015; Edmunds *et al.* 2012). Local adaptation in reef-building corals has occurred over a range of spatial scales, i.e., between regions (Riegl *et al.* 2011), between adjacent seas (Howells *et al.* 2016), along latitudinal gradients (Berkelmans & van Oppen 2006), across the continental shelf (Berkelmans & van Oppen 2006), and between habitats on the same reef (Bay & Palumbi 2014). Compared to reef slope habitats, reef flat or back reef habitats are far more variable in temperature, light and partial pressure of carbon dioxide (pCO_2) (Albright *et al.* 2015; Ohde & van Woesik 1999; Price *et al.* 2012; Santos *et al.* 2011; Shaw *et al.* 2012). Corals from environmentally variable locations are generally better able to withstand stress through phenotypic plasticity (Barshis *et al.* 2013; Kenkel & Matz 2016; Oliver & Palumbi 2011) and have greater adaptive potential (McClanahan *et al.* 2007) compared to those that live in more stable environments. The potential of such populations to serve as a source for adaptive genetic variation for surrounding habitats remains poorly explored. Potentially, these populations can provide adaptive alleles that could be harnessed in assisted gene flow or selective breeding initiatives that aim to increase climate resilience in the receiving populations (Hoegh-Guldberg *et al.* 2008; van Oppen *et al.* 2017; van Oppen *et al.* 2015).

Reef-building corals harbour a diverse microbiome comprised of bacteria, archaea, dinoflagellate photosymbionts (*Symbiodinium* spp.), fungi, chlorophytes, viruses and other micro-organisms (Blackall *et al.* 2015). It is well known that the endosymbiotic *Symbiodinium* community plays an important role in coral nutrition and calcification, as well as the ability of corals to tolerate and recover from environmental stress (Bay *et al.* 2016; Berkelmans & van Oppen 2006), with not all *Symbiodinium* types providing the same benefits to the host (van Oppen *et al.* 2009). Prokaryotes

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are critical for nitrogen fixation, sulphur cycling, and coral host immunity (Bourne *et al.* 2016; Thompson *et al.* 2014). Differences in the prokaryotic community composition in conspecific corals from distinct environments (Glasl *et al.* 2017a; Hernandez-Agreda *et al.* 2016a) may be a signature of acclimatization and/or adaptation. Experimental heat stress induced changes in the bacterial community composition of *Acropora hyacinthus* colonies acclimatized to a relatively cooler and stable environment, but not in those that were acclimatized to a warmer and more variable environment, suggesting coral-associated bacteria may contribute to coral thermal tolerance in this species (Ziegler *et al.* 2017). Conversely, stable cnidarian-associated prokaryote communities under simulated environmental change (Webster *et al.* 2016) or in different habitats (Muller *et al.* 2016) have also been reported, and it is not yet clear to what extent the prokaryotic community contributes to the acclimatization/adaptation of corals to different reef environments.

The brooding coral, *Pocillopora damicornis*, is a common and widespread reef species that inhabits a wide range of reef habitats, including reef flats and deeper reef slopes. While recruitment in this species is often localized, it is supplemented by dispersal of short- and long-distance migrants (Torda *et al.* 2013a). It is not known whether the forces of selection on the reef flat versus slope can overcome the homogenizing effects of gene flow and lead to local adaptation between these spatially adjacent sites. Previous studies have demonstrated that conspecific coral populations which inhabit different habitats can be genetically distinct (Benzie *et al.* 1995; Bongaerts *et al.* 2015; Bongaerts *et al.* 2011; Bongaerts *et al.* 2010; Souter & Grahn 2008; Warner *et al.* 2015), but the underlying causes of these patterns have been difficult to interpret (Torda *et al.* 2013b). Further, the genomic pathways and loci under selection were not identified in these earlier studies (Bongaerts *et al.* 2013). Here, we examine the occurrence of local adaptation in multiple compartments of the *P. damicornis* holobiont, between the environmentally divergent, but spatially adjacent (<100 m) reef flat and reef slope habitats of *P. damicornis* at Heron Island in the southern Great Barrier Reef (GBR). Specifically, divergence was assessed at the level of (1) the coral host by using genome-wide single nucleotide polymorphism (SNP) analysis, (2) the associated photosynthetic endosymbionts (*Symbiodinium* spp.) by ribosomal DNA (rDNA) internal transcribed spacer 2 (ITS2) metabarcoding, and (3) tissue-associated prokaryote members of the microbiome by 16S ribosomal RNA (rRNA)

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metabarcoding. Our experimental design included two replicate locations, with 24 colonies sampled from each of the reef flat and slope sites at the two locations that are ~1 km apart, Coral Canyons and Coral Gardens.

Materials and Methods

Sample collection, local seawater temperatures and light levels

Fragments of 96 *P. damicornis* colonies (24 from each of four sites) were haphazardly collected between 27/02/2012 and 11/03/2012 from the reef flat (1-2 m depth) and slope (4-6 m depth) at two locations near the Heron Island Research Station on the Great Barrier Reef: "Coral Gardens" (flat: 23°26'48.50"S, 151°54'46.34" E; slope: 23°26'50.54"S, 151°54'45.46" E) and "Coral Canyons" (flat: 23°27'14.50"S, 151°55'34.55" E; slope: 23°27'18.80", 151°55'28.29" E) under Great Barrier Reef Marine Park Authority permit G12/34752.1. Samples were coded as follows: GF = Coral Gardens flat [samples 101-124], GS = Coral Gardens slope [samples 201-224], CF = Coral Canyons flat [samples 301-324], CS = Coral Canyons slope [samples 401-424]). Seawater temperature was monitored at the collection sites (~30 cm above the substratum) every 10 min between 03/03/2012 and 16/08/2013 using *in situ* temperature loggers (Sensus Ultra, ReefNet). The temperature logger failed at the Coral Gardens sites in August 2012 and, hence, no data are available from this site after this date. Photosynthetically active radiation (PAR) was measured underwater at the collection sites (~30 cm above the substratum) between 9 am and 3 pm from 05/03/2012 – 02/04/2012 and 25/03/2013 - 16/08/2013 using integrating light sensors (Odyssey Dataflow Systems). The light sensors were fitted with wipers designed to keep the instrument clean from unwanted deposits such as mud and bio-fouling during deployment, a setup prone to technical failure. Due to the short interval measured in 2012 these data were not included. Data were only collected for four months at the Canyons slope site in 2013. Profiles were indistinguishable between habitats when they could be compared and hence only the complete 2013 data series from the Garden location was displayed. Although not measured here, the location of the Garden reef was more exposed and it is likely the corals here would experience higher rates of water flow. Ocean chemistry was not measured, but pH and dissolved inorganic

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carbon, dissolved oxygen and other parameters have been found to vary on daily cycles in the Heron Island lagoon (Kline et al 2012; Santos et al 2011). We did not have access to equivalent data sets for the slope and, hence, do not discuss this further except to note that the reef flat sites were likely exposed to a range of variable environmental conditions.

DNA extractions, RAD-seq library preparation and sequencing

Total genomic DNA (gDNA) was extracted from ethanol-preserved specimens (n = 96) using a salt-precipitation method including both RNAse A and Proteinase K treatment (van Oppen *et al.* 2011). Total gDNA was normalised to around 75 ng.μL^{-1} by fluorometry using the Quant-It PicoGreen dsDNA quantification kit (Thermo Fisher), and by also assessing the high molecular weight (HMW) band against an uncut lambda DNA standard on agarose gels. DNA purity was determined spectrophotometrically from Nanodrop 1000 (Thermo Scientific) 260/230 and 260/280 nm absorbance ratios. RAD-seq library construction and sequencing was conducted at Floragenex Inc. (Eugene, USA) following the protocol described by Baird *et al.* (2008). In short, gDNA was digested using the SbfI restriction endonuclease (6 bp recognition sequence TGCAGG), and "barcoded" by ligating sequencing adapters with a set of index nucleotides. Reactions were pooled and sheared, after which they were size-selected and taken through several purification steps. RAD-seq fragment libraries were sequenced using single read 100 bp chemistry on the Illumina HiSeq 2000 platform using the manufacturer's recommended protocol, with a subset of the samples sequenced across multiple runs to increase coverage. RAD-seq data are available as fastq files on NCBI Sequence Read Archive (accession number PRJNA450871), and VCF files on Dryad (doi:10.5061/dryad.j09c05c).

Coral ID assay

All collected colonies were of the *P. damicornis* morphology (Schmidt-Roach *et al.* 2013), and their species ID was confirmed by a molecular assay. The assay is based on PCR amplification of a portion of the mitochondrial putative control region, which shows consistent and easily

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identifiable fragment size differences after Alu1 restriction enzyme digestion of the amplicons (Torda *et al.* 2013c).

Identification of coral host loci

Clustering of RAD-seq loci and SNP variant calling was carried out using PyRAD v3.0.6 (Eaton 2014) to allow for indel variation (common in marine invertebrates), using a minimum coverage of 6, a clustering threshold of 88%, and a maximum of 4 low-quality sites in reads (using a PHRED threshold of 20). Further parsing and analyses were carried out using custom Python scripts (available through https://github.com/pimbongaerts/radseq) unless otherwise indicated. One representative sequence was then extracted for each RAD-seq locus to identify only those loci belonging to the coral host. Putative coral host loci that both mapped (using BWA; Li & Durbin 2009) and matched (using BLASTn; max. e-value of 10^{-15}) against at least one of three *P. damicornis* transcriptome references (Lundgren *et al.* 2013; Mayfield *et al.* 2014; Traylor-Knowles *et al.* 2011) were retained. Remaining *Symbiodinium* contamination was identified using a BLASTn comparison (max. e-value of 10^{-15}) against genomes of *Symbiodinium* B1 (Shoguchi *et al.* 2013) and *Symbiodinium* C1 (Liu *et al.* 2017) and transcriptomes of C and D types (Ladner *et al.* 2012; Levin *et al.* 2016).

QC of SNPs and identification of clones

Given a slight overrepresentation of SNPs toward the end of reads, RAD-seq loci were truncated to 80 bp. Remaining SNPs were evaluated for significant deviations from Hardy-Weinberg equilibrium using Arlequin (arlecore v3.5.2.2; Excoffier & Lischer 2010), and loci with SNPs that either deviated (excess or deficit) in at least three populations or had a significant excess in at least one population (to filter out potential paralogous loci) were removed. Genetic similarity (Hamming-based) was calculated between all individuals, and those with >94% similarity were considered clone mates based on a distinct break in the frequency distribution of similarities (using the "vcf_clone_detect" script). Two separate data sets were then compiled: one with clones and one without clones. In both datasets, only SNPs with a minimum representation of 30% in

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each population were retained in the data sets, and minor alleles that occurred as singleton or doubleton were removed (to reduce elimination of non-erroneous minor alleles through a frequency filter).

Outlier analyses and putatively neutral SNPs

Initially, outliers were identified for the overall dataset (considering only biallelic SNPs) and for each pairwise population comparison using both Lositan (Antao *et al.* 2008) and BayeScan (Foll 2012), with a threshold of 0.99 and a false discovery rate (FDR) of 0.1. To conservatively identify between-habitat outlier SNPs, i.e., SNPs that are under selection from different environmental conditions in the two habitats, we selected those that met all of the following criteria: (1) identified by both Bayescan and Fdist as "overall" outliers, (2) identified by Fdist as outliers in at least two cross-habitat comparisons, and (3) never identified by Fdist as outliers in within-habitat, between-location comparisons (i.e., between locations). This strategy provides a high level of confidence in the identified loci being under selection due to habitat-specific differences. Loci containing outliers were annotated by BLASTx search of the corresponding transcriptome contig to ZoophyteBase (Dunlap *et al.* 2013). This database was used because it has good annotations.

To identify a subset of putatively neutral SNPs, the outlier analyses were repeated using a cut-off of 0.95 (lower) and a FDR of 0.1 (the same), and retaining SNPs that were never identified in any of the overall analyses. Again, separate data sets were compiled for those with clones and without clones.

Genetic structure

Genetic clustering and potential admixture was assessed using the non-clonal version of both the overall and "neutral" datasets using the "structure_mp" script (Bongaerts *et al.* 2017) for STRUCTURE (Pritchard *et al.* 2000). STRUCTURE was run for a K ranging from 2 to 6 using

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the admixture model and correlated allele frequencies with no priors, an MCMC burn-in of 100,000 chains and 50,000 replications. Ten independent replicates were run for each K, using a different subset of SNPs for each replicate (one per locus, sampled with replacement), with results summarized in CLUMPP (Jakobsson & Rosenberg 2007), and estimators for the most likely K calculated as in Puechmaille (2016) and Evanno et al. (2005). Pairwise genetic distances (F_{ST}) were calculated in the "adegenet" R package (Jombart & Ahmed 2011).

Prokaryote community composition

DNA was diluted tenfold with MilliQ water prior to PCR. PCR was carried out in Applied Biosystems 2720 thermal cyclers under the following cycling conditions: 1 cycle at 95°C for 15 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s; and one final extension cycle of 72°C for 7 min. Triplicate 15 µL reactions were set up for each sample. Each reaction consisted of 7.5 µL Qiagen Multiplex PCR mastermix reagent, one µL of template DNA, and 4 pmol of each of 16S rRNA gene V4 515F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA-3'; Apprill *et al.* 2015) and 806Rb (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACNVGGGTWTCTAAT-3'; Parada *et al.* 2015) primer. The underlined segments represent Illumina adapters (Illumina, San Diego, CA, USA). Three µL of PCR product were run on a 2.5% agarose, 0.5x TBE gel to confirm successful amplification. Ten µL from each of the triplicate PCRs for each sample were pooled and sent to a sequencing service provider (Ramaciotti Centre for Genomics the University of New South Wales) for PCR purification, amplicon library construction and Illumina MiSeq analysis. Sequencing was conducted using Illumina MiSeq 300 bp paired-end chemistry. Retrieved raw reads plus respective metadata have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA380169. Demultiplexed reads were analysed in QIIME (version 1.9.0) (Caporaso *et al.* 2010). Paired-end reads were merged into contigs, quality trimmed using default thresholds (and a minimum Phred quality score of 25) and primers removed. Chimeric sequences were removed using usearch61 (Edgar 2010) as implemented in

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QIIME, with both de novo and reference based detection, and a total of 1,240,976 reads (mean length 203 bp ± 38 SD) included in the analyses. Short reads representing coral mitochondrial 16S or *Symbiodinium* chloroplast 16S reads were removed. Clustering was based on 97% similarity cut-off value and no singletons. Sequences were aligned and classified against the SILVA database (release 123) (Pruesse *et al.* 2007). Chloroplast-assigned reads were removed, as well as rare OTUs (i.e., comprising <0.005% of all sequences), often associated with spurious reads (Bokulich *et al.* 2013). All alpha- and beta-diversity analyses of microbial communities were based on relative abundances at OTU level (97% similarity) and were performed in R (R_Development_Core_Team 2013) using packages 'vegan', 'labdsv', 'MASS', 'cluster', 'indicspecies', 'permute', 'dplyr' and 'tidyr'. Firstly, 800 sequences were randomly picked from each sample in order to correct for different sequencing depths; sequencing produced many host 16S mitochondrial reads, as well as 16S chloroplast reads that were removed before analyses, resulting in a low number of prokaryote reads for some samples. Rarefaction curves indicated that even with only 800 sequences, saturation was approached in almost all samples (data not shown). OTU richness and Shannon-Weaver diversity for the different sampling groups were compared by Analysis of Variance (ANOVA). To determine whether habitat and location drive the variation in community assemblage, these factors were included in a Canonical Correspondence Analysis (CCA), which applied an automatic backward and forward model selection tool for constrained ordination methods ('ordistep', based on AIC index, with a maximum of 200 permutations) before final significance was determined by an ANOVA-like permutation test based on 9999 permutations. To visualize variation in community composition among habitats and locations, a non-metric multidimensional scaling ordination (nMDS, after 9999 permutations) was run based on Bray-Curtis dissimilarities of relative abundance data. Differences in community structure were tested by analysis of similarity (ANOSIM; 9999 permutations) and by permutational multivariate analysis of variance (PERMANOVA, 9999 permutations) after confirming for homogeneity of multivariate dispersion using a measure of betadispersion (PERMDISP). The OTUs significantly associated with the distinct levels of significant community drivers were detected by Indicator Values (IndVal) analysis, which resolves the taxonomic groups with the highest probability of specificity and fidelity to a particular sampling group.

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Symbiodinium community composition

PCR and sequencing proceeded as described for the prokaryotic community, with the exception that primers targeting the *Symbiodinium* nuclear DNA ribosomal partial 5.8S, the entire ITS2 and the partial 28S gene (itsD 5′-GTGAATTGCAGAACTCCGTG-3' and its2rev2 5′-CCTCCGCTTACTTATATGCTT-3′; Pochon *et al.* 2001) were used in place of the prokaryote 16S V4 primers. Retrieved raw reads plus respective metadata have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA380172. Read assembly, denoising and chimera removal were performed in QIIME (version 1.9.0) (Caporaso *et al.* 2010) as described above. Only assembled reads for which both forward and reverse primers were found were kept in the analysis, comprising a total of 3,453,257 reads with a mean length of 300 bp (±11 SD). To assign taxonomic identity to sequences, we employed a three-step clustering approach in QIIME. Firstly, we performed a rough clustering at 80% similarity cut-off against representative sequences of each recognized *Symbiodinium* clade (A, B, C, D, E, F, G, H and I) retrieved from a database compiled by Arif *et al.* (2014). This database includes 433 distinct ITS2 types taken from the GeoSymbio (Franklin *et al.* 2012), Scott Santos' (www.auburn.edu/~santosr/sequencedatasets.htm) and Todd LaJeunesse's SD2-GED (https://131.204.120.103/srsantos/symbiodinium/sd2_ged/database/views.php) databases. This allowed removing non-*Symbiodinium* reads (such as sequencing errors or reads belonging to other dinoflagellate taxa) and assigning 3,449,467 *Symbiodinium* ITS2 sequences to their respective clades. Secondly, clade-assigned sequences were clustered at 97% similarity cut-off (shown to cluster ITS2 intragenomic variants into a single OTU; Arif *et al.* 2014) against all ITS2 types available in the Arif database for that particular clade using a closed-reference approach. Finally, the failed assignments of the second step (i.e., not assigned to the reference database) were clustered at 97% similarity with an open-reference OTU picking method using usearch61 (Edgar 2010). Resulting OTU tables were then merged into a single summary file representing all ITS2 types found (across all *Symbiodinium* clades), from which singletons and rare OTUs (i.e., comprising <0.005% of all sequences) were removed. All further statistical analyses were performed on *Symbiodinium* OTUs at 97% similarity following the approach described above for

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the prokaryotic community after rarefying abundance distributions to 20,000 *Symbiodinium* reads per sample (i.e., the lowest number of reads among the samples). Effects of host genetic structure on *Symbiodinium* communities was tested by PERMANOVA, ANOSIM, and CCA as explained above for the prokaryote 16S rRNA gene data.

Results

Environmental variables

In situ measurements at the sites of collection over an 18 months period showed that while the mean seawater temperatures were similar between the reef flat and slope sites, temperature ranges were much larger on the flat compared to the slope (annual range flat: 15.8 - 33.2°C; slope: 18.7° - 28.0°C; Fig. 1A and Supplementary Fig. S1). Photosynthetically active radiation (PAR) varied between the flat and the slope sites and was approximately three times higher and four times more variable on the flat compared to the slope (daily PAR between 9 am and 3 pm at flat: 150 - 1,400 µmol photons.m^{-2}.s^{-1}, and slope ~100 - 400 µmol photons.m^{-2}.s^{-1}) (Fig. 1B). Mean of PAR over the period March-August 2013 was 781, 743 and 84 µmol photons.m^{-2}.s^{-1} at Gardens reef flat, Canyons reef flat and Gardens reef slope, respectively.

Coral ID

All samples, except GS203 and GS221, were successfully PCR amplified and RE digested using the protocol of Torda *et al.* (2013c). All of these were confirmed to be *P. damicornis*, although GS209 showed a restriction digestion pattern suggestive of incomplete digestion, and could therefore not be confidently identified.

Coral host genetic partitioning

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Sequencing of the RAD-seq libraries resulted in an average of 3.6 million reads per sample. A total of 23,350 SNPs in 5,063 RAD-seq loci were identified across 94 *P. damicornis* individuals (two samples failed). We only retained the 2,091 loci that mapped to publicly available *Pocillopora* transcriptomes, and that did not have matches against available *Symbiodinium* genomes and transcriptomes. Additional filtering, as described in the Material and Methods section, reduced this data set to 2,268 SNPs across 890 *Pocillopora* transcriptome loci. We identified 10 sets of highly similar individuals (>94% allelic similarity), likely representing clones. Eight pairs occurred at the same site, and one individual of each clone-pair was removed from the data set (GF105, GF108, GF112, GF120, GS204, GS207, GS209, CS409) as well as three individuals from a clone-set (CS412, CS420, CS423). Where clone mates occurred at different collection sites, both individuals were retained within the data set (CF301 and GF115, CS407 and GS213, GS211 and CS420). This resulted in a non-clonal dataset of 83 individuals.

Strong genetic partitioning occurred between habitats rather than between locations (Figs. 2A and B). Under the assumption of two genetic clusters (K=2), the Bayesian model-based clustering analysis implemented in STRUCTURE (Fig. 2A) showed a partitioning between reef flat versus slope populations for both the overall and "neutral" datasets, with a small number of individuals with mixed probability of assignment to both genetic clusters, particularly in the reef flat sites. Additional structuring within habitats became apparent for K=3 (identified as "optimal K" using both estimator methods for the "neutral" dataset), however for replicate runs this structure occurred alternatively in the flat and slope habitat. At K=4 (Fig. 2A), the substructure was identified in both habitats. Although STRUCTURE results for the overall dataset and "neutral" SNPs (2,084 SNPs) were similar, the "neutral" run only identified two admixed individuals: one on the reef flat and one on the reef slope of Coral Gardens (Supplementary Fig. S2). We did not observe any evidence for cross-habitat migrants, and the general lack of admixture (except for the two individuals mentioned above) is indicative of limited gene flow between habitats. Both the genetic partitioning between habitats and further differentiation within habitats were confirmed by a Principal Component Analysis (PCA) (Fig. 2B). Global pairwise F_{ST} distances (i.e., genetic differentiation based on all SNPs) between habitats were roughly double that between locations (Coral Canyons and Coral Gardens) (Supplementary Table S1).

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Our conservative outlier analysis, which only included SNPs that were outliers in both replicate slope - flat comparisons and were not identified as outliers between Coral Canyons and Coral Gardens, revealed 16 outlier SNPs in 13 loci (Fig. 3). The loci were mapped back to the longer contigs in the publicly available *Pocillopora* transcriptomes and those contigs were annotated using BLASTx in ZoophyteBase (Dunlap *et al.* 2013). Ten of the outlier loci had a robust annotation based on e-values ($<10^{-8}$) and bit scores (Table 1), four of which match genes involved in the innate immune response, four are environmental stress response genes, one is involved in lipid metabolism and one plays a role in DNA processing. Battenin is a protein encoded by the *CLN3* gene that is involved in the lysosome system. Lysosomes are membrane-bound organelles that contain hydrolytic enzymes, including lysozyme, which is regarded as one of the key innate immune effector molecules against bacteria by causing bacterial lysis. NF-κβ is a 'master' immunity regulator (Davy *et al.* 2012). The NF-κβ inhibitor-like protein 2 is a transcription factor that regulates the innate immune response; it acts as negative regulator of the Toll-like receptor and interferon-regulatory factor signaling pathways. As such, it contributes to the negative regulation of transcriptional activation of *NF-κβ* target genes in response to endogenous proinflammatory stimuli. Cannabinoid receptor 2 is involved in the release of nitric oxide (NO). NO is a direct signaling molecule between *Symbiodinium* and coral host that may initiate a bleaching cascade (Weis 2008). Histamine receptor H2 is an integral membrane protein and mediates the actions of histamine; histamine is involved in local immune responses to foreign pathogens. Ubiquitin-protein ligases (*E3, UBR3, UBR5, EDD1*) and ubiquitin thioesterase (*CYLD, USLP2*) have functions in the ubiquitin system which is responsible for the selective degradation of proteins. ApaH, bis (5'-nucleosyl)-tetraphosphatase hydrolyzes diadenosine 5',5'''-P1,P4-tetraphosphate, a regulatory metabolite of stress conditions, to yield ADP.

Coral tissue-associated prokaryotes

PCR using primers targeting the 16S rRNA V4 region yielded two amplicons (~330 and ~410 bp in size) in three of the four populations, and only the shorter product in the reef slope population of Coral Gardens. Sequence analysis indicated this shorter product was of coral mtDNA origin,

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therefore, no prokaryote 16S rRNA gene data were obtained for this population. Further, the prokaryote communities in the water column above the sampled coral colonies were not examined and it can therefore not be ruled out that coral-associated communities were shaped by differences in the communities present in the water column in the two habitats. We consider this explanation unlikely given that connectivity among water-borne microbiomes separated by <100 m distance is likely high (Frade *et al.* 2016; Martiny *et al.* 2006). Nevertheless, the differences in prokaryote community composition between reef flat and slope habitats we describe below need to be taken with some caution. Samples from the remaining three sites yielded between 120 and 20,187 prokaryotic reads. Samples with <800 reads (five samples) were removed, leaving 35 flat and 20 slope samples with a mean number of reads of 7,348 ± 4630 (SD) and a median of 6,691 for further analyses. Taxonomic assignment (see Supplementary Fig. S3 for relative abundances) revealed that operational taxonomic unit (OTU) diversity was low (~27 OTUs per sample, range = 13-55) compared to other studies that show 100s-1000s of OTUs per colony (Blackall *et al.* 2015; Bourne *et al.* 2016; Hernandez-Agreda *et al.* 2016a). Rarefaction at 800 reads per sample will have contributed to the low diversity observed. Further, excessive production of mucus during collection in the field would have diluted the mucus-associated microbial community, and EtOH preservation of the samples removed some of the coral mucus where a taxonomically diverse community of prokaryotes resides. Hence, the OTUs identified (n = 200) here are likely associated with the coral tissue.

The reef flat was significantly less rich in OTUs than the reef slope (observed OTU richness of 23 ± 6 and 34 ± 9, respectively; $F_{(53,1)} = 32.88$, $p<0.001$), and also less diverse (Shannon-Weaver diversity of 1.18 ± 0.41 and 1.64 ± 0.26 for reef flat and reef slope, respectively; $F_{(53,1)} = 20.95$, $p<0.001$), consistent with previous findings for the related coral species *Seriatopora hystrix* (Pantos *et al.* 2015). When non-rarefied data were used, the number of OTUs resolved increased to 38 ± 9 for the flat, and 78 ± 11 for the slope, an approximately two-fold increase in richness compared to the rarefied data. Shannon diversity was stable at 1.19 ± 0.40 and 1.67 ± 0.26, respectively for flat and slope, showing that by rarefying we only excluded very rare members of the microbiome (likely with uneven distributions). No differences in alpha-diversity were found between locations.

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As with the host genome data, ordination analysis of the prokaryotic community assemblages (beta-diversity) showed a clear division between habitats (i.e., reef flat and slope) at the Coral Canyons where this could be assessed (Fig. 4; Supplementary Figs. S4A - D). Differences between reef habitats were statistically supported by two distinct approaches (PERMANOVA pseudo $F_{(53,1)} = 34.402$; $p<0.001$; and ANOSIM statistic $R = 0.6042$, $p<0.001$). Constrained beta-diversity comparisons via Canonical Component Analysis (CCA) showed that both habitat ($F_{(53,1)}=6.5572$, $p<0.001$) and location ($F_{(53,1)}=1.6006$, $p=0.0323$) were significant drivers of prokaryotic community composition. Non-overlapping distributions of reef flat samples belonging to the two distinct locations in the non-metric multidimensional scaling (nMDS) ordination (Fig. 4) supports that in addition to partitioning between habitats, differences in prokaryote communities exist between the locations: Coral Canyons and Coral Gardens. The non-homogeneous multivariate dispersion verified among reef locations (PERMDISP $F_{(53,1)} = 13.466$; $p<0.001$) precluded any further statistical comparison based on dissimilarity distributions. Host population genetic structure (based on K = 4 in the STRUCTURE analyses) within habitats did not explain any additional variation (Supplementary Figs. S4C, D).

The difference in prokaryotic community composition between the reef flat and slope populations of *P. damicornis* was driven by a dominance of *Endozoicomonas* sp. (Gammaprotobacteria, Oceanospirales, Hahellaceae) on the flat, while the slope population showed co-dominance of *Endozoicomonas* sp. and *Brevibacterium* sp. (Actinobacteria, Micrococcales, Brevibacteriaceae). Other taxa were present mostly below 1% relative abundance (Archaea <0.1% relative abundance) (Supplementary Fig. S3). Twenty-three OTUs were significantly associated with a particular habitat (Fig. 5), ten of which belonged to *Brevibacterium* sp. and which were significant indicators (Glasl *et al.* 2017b) of the reef slope community. The most abundant of these OTUs had an affinity to *Brevibacterium marinum* (AM421807; Lee 2008), reaching on average 28.5% of the reads retrieved from the reef slope samples, compared to only 2.2% on the flat. This was also the most dominant OTU within the genus, with an average relative abundance of >96% of the *Brevibacterium* sp. reads across habitats and locations (Supplementary Fig. S4A). Two low-abundance *Endozoicomonas*-affiliated OTUs were also identified as indicators for the reef slope, despite the fact that *Endozoicomonas* spp. were overall more abundant on the flat. The

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most dominant *Endozoicomonas* OTU (KC669021; Bayer *et al.* 2013) was ubiquitous and represents >73% of the reads within the genus across all samples (Supplementary Fig. S4B). Other indicator taxa for the reef slope (Fig. 5) included a *Nevskia* sp. (Gammaproteobacteria, Xanthomonadales, Nevskiaceae), *Halomonas salina* (Gammaproteobacteria, Oceanospirillales, Halomonadaceae), a *Bradythizobium* sp. (Alphaproteobacteria, Rhizobiales, Bradyrhizobiaceae), a *Dietzia* sp. (Actinobacteria, Corynebacteriales, Corynebacteriaceae) and a *Corynebacterium* sp. (Actinobacteria, Corynebacteriales, Corynebacteriaceae). The only OTU pinpointed as a significant indicator for the reef flat was a *Tenacibaculum* sp. (Bacteroidetes, Flavobacteriales, Flavobacteriaceae).

Intracellular photosymbionts in the genus Symbiodinium

Eighty-nine samples across the four sampling sites yielded between 21,179 and 56,014 *Symbiodinium* ITS2 reads, with a mean number of reads of 37,806 ± 7567 (SD) and a median of 37,417. These corresponded to 55 distinct OTUs (at the 97% sequence similarity cut-off), including 45 clade C types and one clade B type assigned from the Arif et al. database (Arif *et al.* 2014), and nine new OTUs (Fig. 6). Compared to the reef slope, the reef flat showed higher *Symbiodinium* richness (44 ±-2 against 35 ± 5 OTUs; $F_{(87,1)} = 100.7$, $p<0.001$) and Shannon-Weaver diversity (1.35 +- 0.09 against 0.75 ± 0.22; $F_{(87,1)} = 291.9$, $p<0.001$). ITS2 sequence types C33 and C42 were dominant on the reef slope and reef flat, respectively, together with other sequence types with matches to C1, C1.8 and C23 (Fig. 6). It should be noted that our approach could not distinguish C42 from C42a (as they have identical sequences in the Arif *et al.* database for the region sequenced in this study) or C33 from C33a (as divergence is too low to be captured by the similarity cut-off value applied). However, when clustering was performed at a 99% sequence similarity cut-off, the majority of C33-associated sequences were reassigned to C33a, with only a minor fraction (5-15%) of those sequences assigned to C33. Because sequences types C33a, C33 and C42 have previously been identified in *P. damicornis* colonies from the slope at a Heron Island reef nearby our collection sites (Sampayo *et al.* 2007), we assume the same *Symbiodinium* types are present in the colonies from Coral Canyons and Coral

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Gardens. Differences in *Symbiodinium* community structure between the reef flat and slope (Fig. 6) were confirmed by PERMANOVA (pseudo $F_{(87,1)} = 807.16$; $p<0.001$) and ANOSIM statistic ($R = 0.9817$, $p<0.001$) after verifying for homogeneous multivariate dispersions between the two habitats. Constrained beta-diversity comparisons (via CCA) showed that only habitat ($F_{(85,1)}=215.7771$, $p<0.001$) was a significant driver of *Symbiodinium* community assemblage. Location was not a significant structuring factor in *Symbiodinium* alpha-diversity or community structure parameters, with most samples within each habitat having near identical relative abundances of *Symbiodinium* sequences. Host population genetic structure within sites also did not explain variation in the *Symbiodinium* communities, and there was no correlation between *Symbiodinium* and prokaryote community composition (Fig. S5).

Discussion

This study demonstrates genome-wide differentiation between adjacent populations of the reef-building coral, *Pocillopora damicornis*, which inhabit the reef flat and slope at Heron Island in the southern GBR. Of the 2,268 SNPs included in the analyses, ~0.7% were conservatively identified as outliers between the flat and slope habitats, indicating the importance of environmental selection on the coral host genome. Community composition of intracellular photosymbionts (*Symbiodinium* spp.) and coral tissue-associated prokaryotes also differed significantly between the reef flat and slope populations of *P. damicornis*. Few previous studies have examined divergence between habitats in the coral host genome (rather than host genotype based on one or a few neutral markers) simultaneously with the associated *Symbiodinium* communities (Barshis *et al.* 2013; Bongaerts *et al.* 2017); only two have examined all three compartments (coral host, *Symbiodinium* and prokaryotes) at the same time, but no functional analysis of loci under selection was conducted (Gonzalez-Zapata *et al.* 2018; Pantos *et al.* 2015). Our novel findings indicate that local adaptation to the distinct reef habitats, flat and slope, has occurred through selection on at least three members of the coral holobiont: the coral host, its *Symbiodinium* and the tissue-associated prokaryotes.

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Signatures of local adaptation in the coral genome

The majority of the outlier genes identified for *P. damicornis* from the reef flat and slope at Heron Island are involved in immune and environmental stress responses (Table 1). High temperature and light levels, as well as a number of other stressors, cause an increased generation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) by the coral photosymbionts, *Symbiodinium* spp., and to some extent by the coral host mitochondria (Lesser 2006; Lesser 2011; Weis *et al.* 2008). High ROS levels can stimulate innate immune-like pathways in the coral host (Hawkins & Davy 2012; Perez & Weis 2006) that are part of the conserved minimal cellular stress response (Kültz 2005). Certain components of the stress response are evolutionarily highly conserved and recruited by a number of different stressors (Kültz 2003). For example, in marine oysters it has been demonstrated that both biotic (pathogens) and abiotic (e.g., acidified water) stressors affect mitochondrial function, the cytoskeleton, energy production and ROS generation. These stressors cause the recruitment of the same cellular pathways (Anderson *et al.* 2015) involving genes encoding antioxidant enzymes (e.g., superoxide dismutases - *SODs*), chaperones (e.g., heat shock proteins - *HSPs*), removal of macromolecular debris generated by stress (ubiquitin/proteasome pathway), the *NF-κβ* pathway and genes involved in apoptosis and cytoskeleton remodelling (Kültz 2003). Transcriptome analysis in the damselfish, *Acanthochromis polyacanthus*, showed immune- and stress-response as well as metabolic genes underpin transgenerational acclimation to elevated temperature (Veilleux *et al.* 2015). The coral heat-stress response is also known to involve chaperones, such as heat shock proteins, and the ubiquitin system (Csaszar *et al.* 2009; Desalvo *et al.* 2008; DeSalvo *et al.* 2010; Maor-Landaw *et al.* 2014; Voolstra *et al.* 2009). Further, a growing body of evidence supports local adaptation to different environmental factors, including temperature, involves selection on genes in the ubiquitin system (Barshis *et al.* 2010; Bay & Palumbi 2014; Jin *et al.* 2016; Lundgren *et al.* 2013; Thomas *et al.* 2017). Our results on *P. damicornis* are consistent with previous findings and highlight the prevalent role of the ubiquitin system in the coral acclimatization and adaptive responses to environmental differences.

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Selection on coral-associated microbial communities

While we provide compelling evidence for local environmental selection on the coral host genome, the differences in the community composition of *Symbiodinium* and prokaryotes hosted by slope and flat coral populations suggest the coral microbiome also plays important roles in local adaptation and the ability of corals to adjust to environmental differences and change. *Symbiodinium* communities associated with *P. damicornis* colonies located between 3 and 19 m depth at Heron Island have previously been assessed using Denaturing Gradient Gel Electrophoresis analysis of the ITS2 region (Sampayo *et al.* 2007) and sequence analysis of a the ITS2 and other regions (Sampayo *et al.* 2009). However, this earlier study only included samples from the reef slope and no reef flat colonies were examined. The spatially closest sampling location in these previous studies to our reef locations is Harry's Bommie, ~600 m away from Coral Canyons. The *P. damicornis* colonies at Harry's Bommie were dominated by a combination of ITS2 sequence types C33 and C33a, consistent with our results for the slope populations. Sequence types C42 and C1 were also present in our and the previous studies, and, as expected for the higher resolution amplicon next generation sequencing method, we observed additional sequence types previously reported from cloning methods (Sampayo *et al.* 2009; Fig. 6). These co-occurring sequence types likely represent single but distinct *Symbiodinium* types in *P. damicornis* colonies on the reef flat versus the slope. Conspecific coral colonies that are dominated by distinct *Symbiodinium* types can differ considerably in physiological performance and environmental tolerance (Blackall *et al.* 2015; van Oppen *et al.* 2009), and we hypothesize that adaptation to the reef flat and slope environments by *P. damicornis* at Heron Island is partly contingent on the distinct *Symbiodinium* communities harboured.

Community composition of prokaryotes associated with the *P. damicornis* tissues differed between the slope and flat at the location where this could be assessed, with one and 22 indicator OTUs being identified for the flat and slope respectively. Additional genetic structure was present between locations but this divergence was smaller than that between habitats. Other prokaryotic taxa associated with the coral surface mucus layer may also differ between the two

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habitats, but these organisms were not examined here. The corals inhabiting the reef flat were dominated by *Endozoicomonas* spp. (Gammaprotobacteria, Oceanospirales, Hahellaceae), while the slope population showed co-dominance of *Endozoicomonas* sp. and *Brevibacterium* sp. (Actinobacteria, Micrococcales, Brevibacteriaceae). *Endozoicomonas* spp. are commonly associated with reef-building corals, and can aggregate inside the tissue of corals in the family *Pocilloporidae* (Bayer *et al.* 2013; Blackall *et al.* 2015; Morrow *et al.* 2015; Morrow *et al.* 2012; Neave *et al.* 2016a; Neave *et al.* 2016b; Pantos *et al.* 2015). The most dominant *Endozoicomonas* OTU matches an OTU associated with another pocilloporid, *Stylophora pistillata*, from the Red Sea (Bayer *et al.* 2013). Another OTU is a perfect match to an *Endozoicomonas* discovered in *P. damicornis* colonies from the Red Sea (KC668747; Bayer *et al.* 2013). These findings are concordant with recent observations of strong specificity of certain *Endozoicomonas* OTUs in *Pocillopora verrucosa*, although the authors argued that this may be related to their broadcast spawning behaviour (Neave *et al.* 2016b); *P. damicornis* is primarily an asexual brooder, but has been observed to also release gametes (Schmidt-Roach *et al.* 2012). *Brevibacterium* spp. are less well known from corals. Nevertheless, *Brevibacterium aureum* was present at a mean relative abundance of ~31% in all *Porites lobata* colonies sampled from the Persian/Arabian Gulf (Hadaidi *et al.* 2017). *Brevibacterium* was also found to be the dominant taxon among culturable bacteria isolated from the mucus and tissue of three coral species from the Arabian Gulf (Mahmoud & Kalendar 2016).

Our findings of strong habitat differentiation in the prokaryotic communities of a depth generalist (*P. damicornis* occurs from the reef flat down to ~55 m of depth; Bridge *et al.* 2012) align with those of Glasl *et al.* (2017a), who concluded that corals with a relatively broad depth distribution show structuring in tissue-associated prokaryotic communities across their depth range. Environmental factors can drive coral-associated prokaryotic community composition (Hernandez-Agreda *et al.* 2016b; Littman *et al.* 2009; Pantos *et al.* 2015), and may explain the patterns observed in *P. damicornis* at Heron Island. Alternatively, the differences in prokaryote community composition between the flat and slope populations may be caused by both the coral host and the *Symbiodinium* photosymbiont having undergone divergence and niche diversification (Bongaerts *et al.* 2015; Bongaerts *et al.* 2013; Bongaerts *et al.* 2010). This could

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in turn has led to the existence of particular holobiont microniches to which at least a small fraction of the microbial community is adapted (Glasl *et al.* 2017a). The challenge now is to determine how the different prokaryote communities affect the functioning of the coral holobiont across these different habitats, and the extent to which adaptation of the coral host and its microbiome are linked.

Implications for human intervention strategies that aim to enhance coral climate resilience

The GBR off the east coast of Australia spans 14 degrees of latitude and a mean sea surface temperature (SST) gradient of ~3°C, hence, corals in the northern GBR have evolved to cope with higher temperatures compared to their southern counterparts (Berkelmans 2002; Berkelmans & Willis 1999; Howells *et al.* 2013). Translocation of corals from warm to cooler locations (i.e., assisted gene flow) has been suggested as a strategy to increase thermal tolerance in the cooler locations (Hoegh-Guldberg *et al.* 2008; Weeks *et al.* 2011), especially if the translocated colonies breed with the corals native to the cooler but warming environment (Dixon *et al.* 2015; van Oppen *et al.* 2014). When corals from the northern (warmer) and central (cooler) GBR were crossed in the laboratory, their regional hybrid larval offspring showed higher heat tolerance under laboratory conditions than purebreds from the cooler location, especially if the mother was sourced from the warmer location (Dixon *et al.* 2015). These results indicate that north to south translocation along the GBR may be a valuable strategy to assist southern GBR coral populations to adapt to a warming ocean. A common concern regarding translocations between different locales is the risk of introducing pathogens or parasites from the donor into the receiving population, negatively affecting the health of the latter (Laikre *et al.* 2010). Reduced resistance of migrants to local pathogens has also been reported (Grady *et al.* 2015; Van der Putten 2012). Further, translocated adult colonies may be maladapted to other environmental conditions at the new locations and may not survive to reproduce (Howells *et al.* 2013). A safer alternative may therefore be to harness the adaptive variation contained in distinct temperature habitats on the same reef. Our results demonstrate that coral populations from the reef flat and slope can be genetically divergent and adapted to their local environments. The limited gene flow between

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slope and flat populations is likely due to maladaptation to environmental conditions rather than physical restrictions to larval dispersal given the small spatial distances between habitats (<100 m) (Marshall *et al.* 2010). Assuming these populations are still cross-fertile, artificial *ex-situ* habitat-crosses may provide an avenue to breed increased thermal tolerance into conspecific slope populations, the reef habitat where coral cover tends to be highest. Inoculations with microbiomes (probiotics) (Damjanovic *et al.* 2017; Peixoto *et al.* 2017) isolated from the reef flat corals may be another avenue that can be explored to increase thermal resilience in the slope populations.

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Data Accessibility

RADseq data are available as fastq files on NCBI Sequence Read Archive (accession number PRJNA450871), and VCF files on Dryad (doi:10.5061/dryad.j09c05c). Prokaryote community raw reads plus respective metadata have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA380169. Raw reads for the *Symbiodinium* community data plus respective metadata have been deposited in the NCBI Sequence Read Archive under BioProject accession number PRJNA380172.

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946 Author Contributions

947 M.v.O. and L.B. conceived and designed the study, conducted fieldwork and collected samples;
948 L.P. conducted all laboratory work; P.B., P.F., S.B., H.N. and M.v.O. performed statistical
949 analyses and interpreted the results; M.v.O., P.B., P.F. and L.B. wrote the manuscript and all
950 authors edited various versions of the manuscript.

951 Figure Captions:

952

953 Figure 1: *In situ* measurement of environmental variables. (A) Temperature in degrees Celcius
954 over two years. The habitat temperatures were indistinguishable between the two locations and
955 only Coral Gardens is displayed (Slope in blue and Flat in red). (B) PAR in µmol photons.m^{-2}.s^{-1}
956 measured over eight months in 2013. The light levels in habitats were indistinguishable between
957 locations and only Coral Gardens is displayed (Slope in blue and Flat in red).

958 Figure 2: Coral host genetic structure based on all SNP loci. (A) STRUCTURE results at K=2
959 and K=4 with individuals on the x-axis (sorted by population) and assignment probability on the
960 y-axis. (B) PCA. Colors in (B) indicate the cluster assignment in (A). Grey color indicates
961 individuals that had a STRUCTURE assignment of <0.8. Circles indicate reef flat sites, triangles
962 indicate reef slope sites as shown in (A).

963 Figure 3: Coral host genetic differentiation and outlier loci. (A) Genetic differentiation versus
964 expected heterozygosity, with a histogram on the y-axis showing the F_{ST} frequency distribution
965 for all SNPs. Colors indicate the different outlier categories (as described in the text). (B)
966 Genotype frequencies of the 16 habitat-outlier SNPs, with the hues indicating the frequency of
967 homozygote (ref = reference allele, alt = alternate allele) and heterozygote (ref/alt) genotypes.

968 Figure 4: Ordination analysis of coral tissue-associated prokaryote beta-diversity based on 16S
969 rRNA gene sequence data (nMDS plot) in the two reef flat and the one reef slope populations.
970 CF= Coral Canyons Reef Flat, CS=Coral Canyons Reef Slope, GF= Coral Gardens Reef Flat.

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Figure 5: Bubble plots of significant indicator prokaryotic OTUs in the two habitats, reef flat and reef slope. Bubble sizes are representative of average relative abundance across all samples of each habitat. Codes "d", "p", "c", "o", "f", "g" and "sp" represent OTU taxonomic affiliation at domain, phylum, class, order, family, genus and species level, respectively. Entries between brackets represent NCBI Accession Numbers for known representative sequences of each OTU (or "new" in case no match was found above 97% similarity in the SILVA database)

Figure 6: Relative abundance of the ten most abundant *Symbiodinium* ITS2 sequence types (plus 'others' shown as one category) at the two reef flat and two slope sites. CF= Coral Canyons Reef Flat, CS= Coral Canyons Reef Slope, GF= Coral Gardens Reef Flat, GS= Coral Gardens Reef Slope.

Table 1: Annotation of outlier loci based on BLASTx in ZoophyteBase. Dark grey background indicates genes involved in the immune response, light grey background indicates genes involved in environmental stress responses, white background indicates 'other'.

Contig name	**Annotation of top BLASTx hit**	**Putative function (KEGG)**	**E-value**	**Bit score**	**Query cover (%)**
427	Battenin (BTS, CLN3)	Cellular Processes: Lysosome system	$3.5e^{-46}$	161.4	50.1
2762[a]	5-hydroxytryptamine receptor 7	Environmental Information Processing: Ligand-receptor interaction	$1.0e^{-80}$	260.4	97.1

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4435	HRH2, histamine receptor H2	Environmental Information Processing: Ligand-receptor interaction, involved in local immune responses to foreign pathogens	$9.3e^{-11}$	58.9	27.9
4491	NF-κß inhibitor-like protein 2	Genetic information processing: Transcription factor in innate immune response	$2.2e^{-24}$	83.2	11.9
3837	apaH, bis(5'-nucleosyl)-tetraphosphatase	Metabolism: regulatory metabolite of stress conditions	$2.8e^{-9}$	56.2	62.4
804	Ubiquitin-protein ligase (UBR3, E3)	Genetic information processing: Ubiquitin system	$9.5e^{-58}$	200.7	89.1
28	Ubiquitin-protein ligase (EDD1, UBR5, E3)	Genetic information processing: Ubiquitin system	0	1004.2	39.2
2255	Ubiquitin thioesterase (CYLD,	Metabolism:	$8.0e^{-8}$	53.1	6.6

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	USLP2)	Ubiquitin system			
1133	ATP-dependent DNA helicase (PIF1)	Genetic information processing: DNA replication proteins	9.9 e^{-58}	206.8	13.37
3046	Ectonucleotide pyrophosphatase/phosphodiesterase family member 6 (ENPP6)	Metabolism: Lipid Metabolism	0	562.0	89.8

[a]Second best match was to cannaboid receptor 2 (E-value $1.0e^{-26}$, bit score 112.5, query cover 76.3

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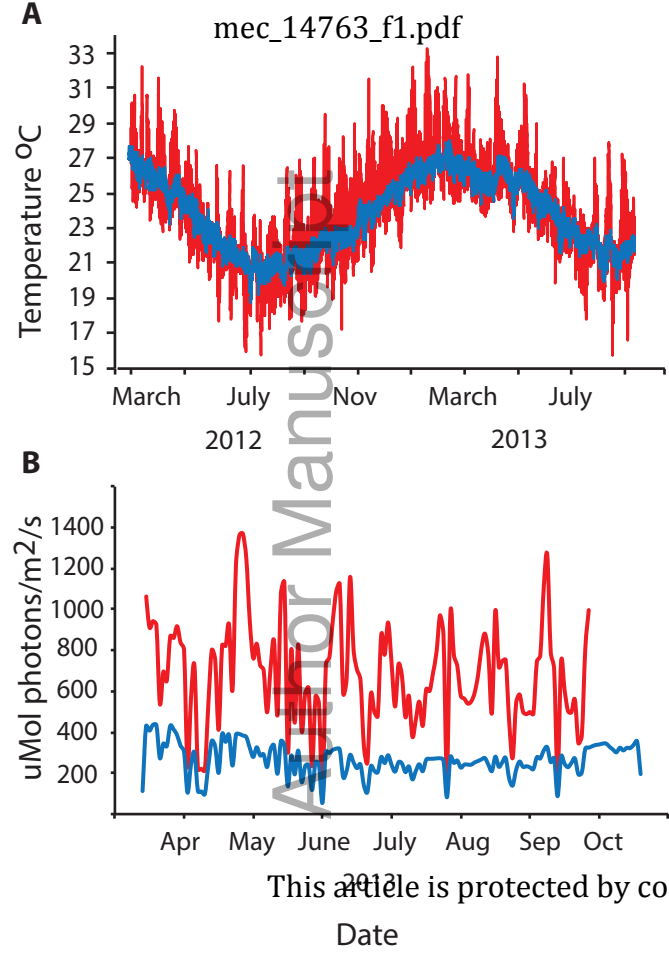

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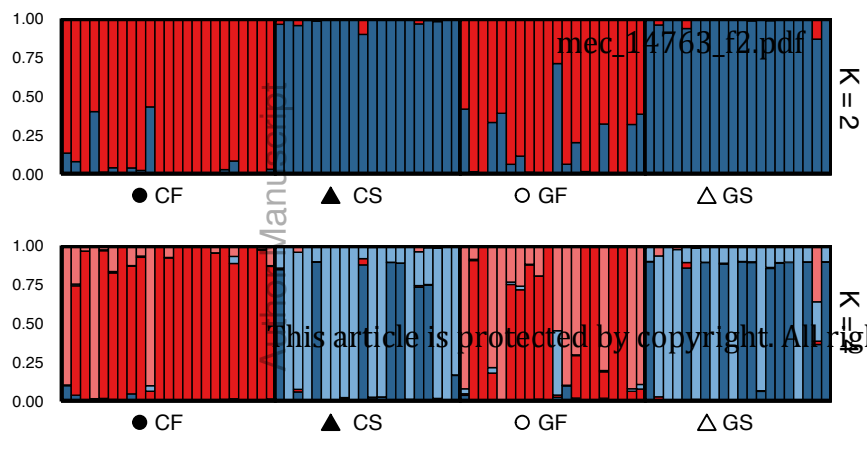

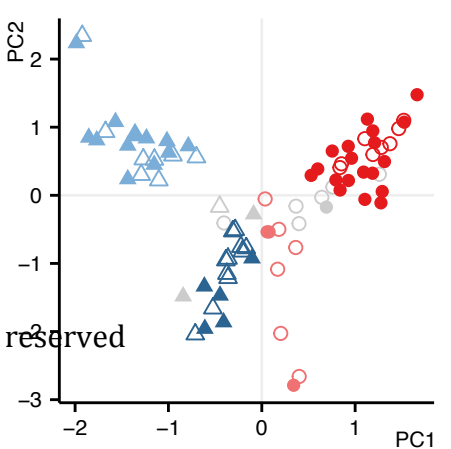

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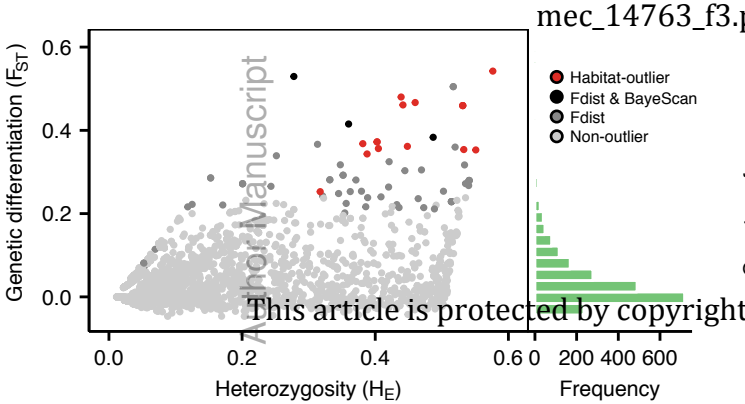

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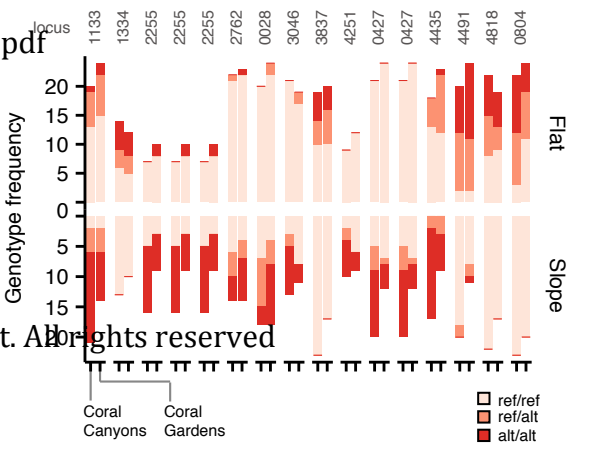

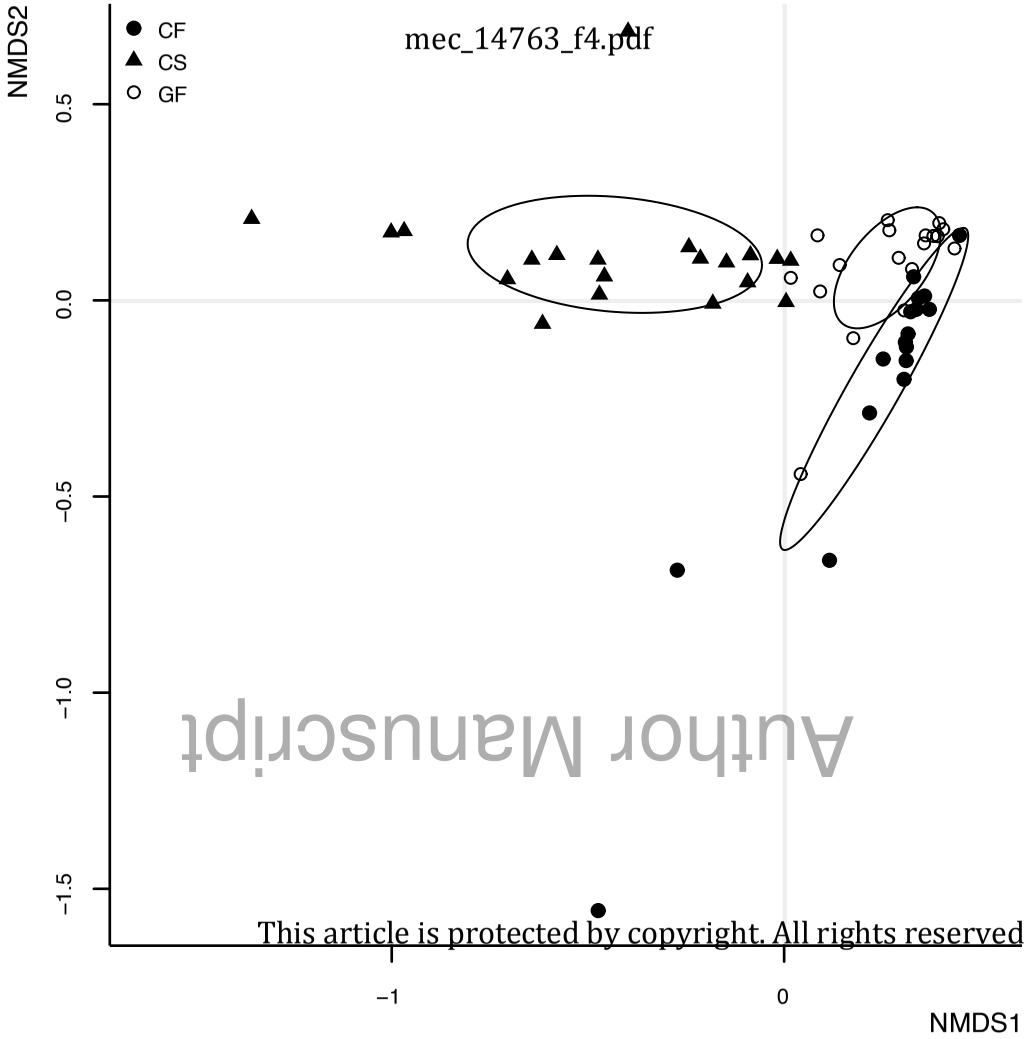

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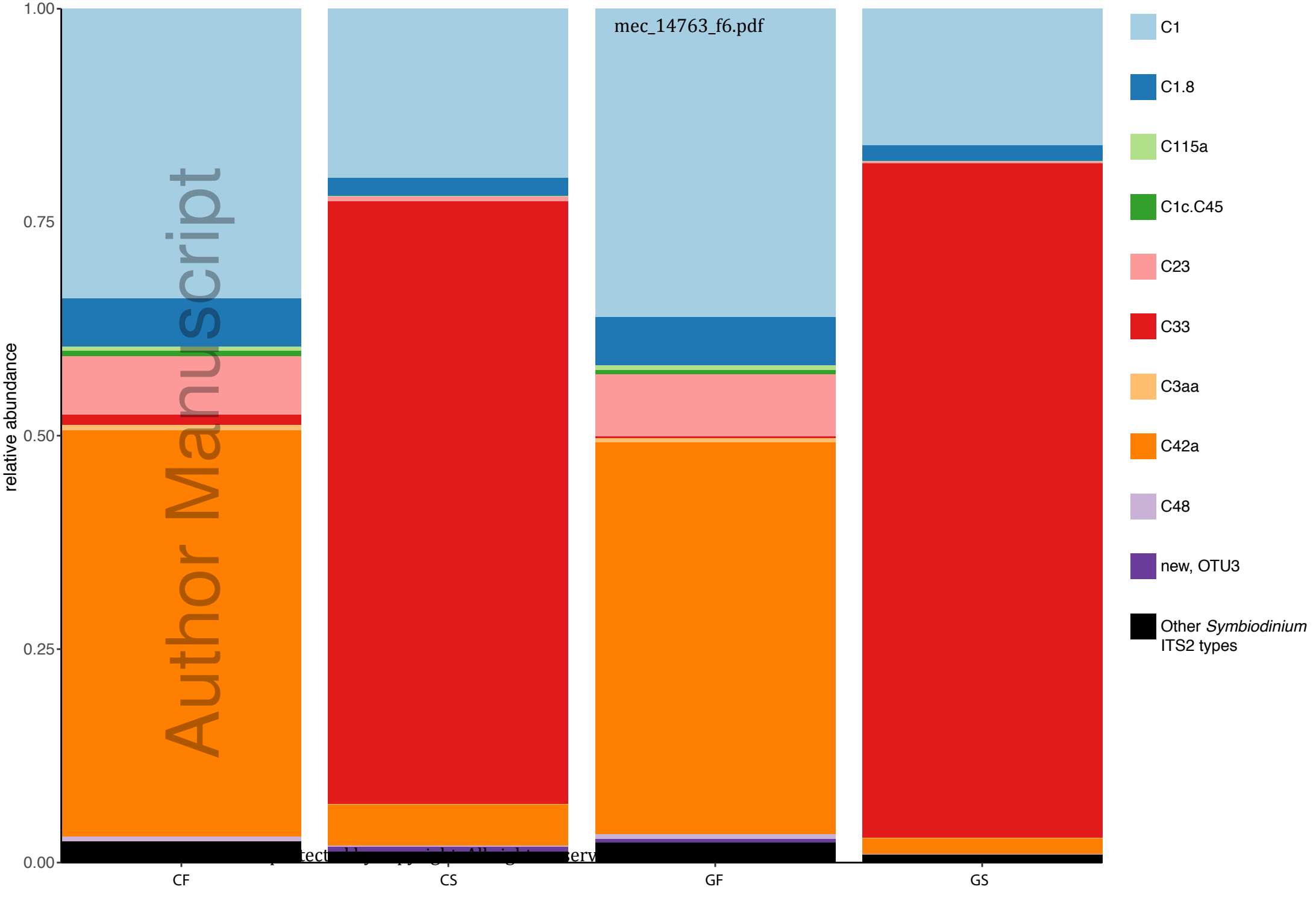

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