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ORIGINAL ARTICLE

Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*

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Reef-building corals form complex relationships with a range of microorganisms including bacteria, archaea, fungi and the unicellular microalgae of the genus *Symbiodinium*, which together form the coral holobiont. These symbionts are known to have both beneficial and deleterious effects on their coral host, but little is known about what the governing factors of these relationships are, or the interactions that exist between the different members of the holobiont and their environment. Here we used 16S ribosomal RNA gene amplicon sequencing to investigate how archaeal and bacterial communities associated with the widespread scleractinian coral *Seriatopora hystrix* are influenced by extrinsic (reef habitat and geographic location) and intrinsic (host genotype and *Symbiodinium* subclade) factors. Bacteria dominate the microbiome of *S. hystrix*, with members of the Alphaproteobacteria, Gammaproteobacteria and Bacteriodetes being the most predominant in all samples. The richness and evenness of these communities varied between reef habitats, but there was no significant difference between distinct coral host lineages or corals hosting distinct *Symbiodinium* subclades. The coral microbiomes correlated to reef habitat (depth) and geographic location, with a negative correlation between Alpha- and Gammaproteobacteria, driven by the key members of both groups (Rhodobacteraceae and Hahellaceae, respectively), which showed significant differences between location and depth. This study suggests that the control of microbial communities associated with the scleractinian coral *S. hystrix* is driven primarily by external environmental conditions rather than by those directly associated with the coral holobiont.
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Introduction

Reef-building corals form complex relationships with a range of different microbial partners, including bacteria, archaea and algae belonging to the genus *Symbiodinium,* which together form the coral holobiont. The genus *Symbiodinium* can be categorised into eight distinct phylogenetic clades (A–H) based on their ribosomal DNA sequence (ITS2), with five of these (A–D and F) found in association with the corals (Baker, 2003). A high degree of host specificity (LaJeunesse *et al.*, 2004, 2010), habitat partitioning and ecological diversification have been identified as controlling factors of the distribution of the different *Symbiodinium* clades (Douglas, 1998; LaJeunesse *et al.*, 2003; LaJeunesse, 2005; Stat *et al.*, 2008a; Bongaerts *et al.*, 2010, 2011b; LaJeunesse *et al.*, 2010; Silverstein *et al.*, 2011). To date the main role of *Symbiodinium* in the coral holobiont is thought to be through its involvement in its host's metabolism, including photosynthesis and nutrient cycling, which provides their hosts with up to 95% of their carbon requirements (Muscatine and Porter, 1977) and the cycling of nitrogenous compounds (Leggat *et al.*, 2007; Pernice *et al.*, 2012).

Coral-associated bacteria and archaea have been extensively studied for over four decades with thousands of species having been identified (Mouchka *et al.*, 2010). However, we are only just starting to investigate and understand the forces that govern their patterns of diversity, distribution and role in the coral holobiont ecosystem. Bacteria, archaea and viruses are ubiquitous in the marine system, and thrive in coral reef waters (Sorokin, 1973; Dinsdale *et al.*, 2008), but these communities

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have been shown to be distinct from those associated with the coral animal (Rohwer *et al.*, 2001, 2002; Cooney *et al.*, 2002; Frias-Lopez *et al.*, 2002; Guppy and Bythell, 2006; Santiago-Vazquez *et al.*, 2007; Kvennefors *et al.*, 2010; Sunagawa *et al.*, 2010; Chen *et al.*, 2011; Tremblay *et al.*, 2011; Ceh *et al.*, 2012; Schöttner *et al.*, 2012), suggesting that there is specific control of the microbiomes (associated bacterial and archaeal communities), by either biological or microenvironmental factors influenced by the host.

The complex physical colony structure of the coral host provides a multi-faceted habitat with different microbial communities occupying a range of niches within the surface mucus layer, tissue layers (Paul *et al.*, 1986; Ritchie and Smith, 1995b; Banin *et al.*, 2000; Johnston and Rohwer, 2007; Lesser *et al.*, 2007; Sharp *et al.*, 2010, 2012) and in the different zones of the colony (Rohwer *et al.*, 2002; Bourne and Munn, 2005). As with terrestrial ecosystems (Balser *et al.*, 2006), coral reef-associated microbes are thought to drive biochemical and ecological processes including nitrogen (Williams *et al.*, 1987; Shashar *et al.*, 1994; Ritchie and Smith, 1995b; Lesser *et al.*, 2004; Lesser *et al.*, 2007; Olson *et al.*, 2009; Kimes *et al.*, 2010; Lema *et al.*, 2012) and carbon cycling (Ducklow and Mitchell, 1979; Ritchie and Smith, 1995b; Wild *et al.*, 2004b, 2010b), allowing reefs to prosper in the oligotrophic waters of the tropics. In addition to their role in driving ecosystem function through nutrient cycling, microbes also have an important role in coral health. For example, they are able to contribute to pathogen inhibition through competition for space and nutrients, and the secretion of antibiotic substance (Koh, 1997; Castillo *et al.*, 2001; Ritchie, 2006; Rypien *et al.*, 2010; Sharp *et al.*, 2010; Kvennefors *et al.*, 2012), as well as potentially aiding reproduction and propagation (Apprill *et al.*, 2009; Sharp *et al.*, 2010, 2012). Less is known about the role of archaea associated with reef-building corals. Archaea associated with the coral tissues have been found to occur at varying cell densities (Wegley *et al.*, 2004), and with varied levels of diversity relative to bacterial communities (Wegley *et al.*, 2004, 2007; Siboni *et al.*, 2008; Lins-de-Barros *et al.*, 2010; Littman *et al.*, 2011), and in some cases have been found to be absent from coral colony tissues altogether (Yakimov *et al.*, 2006; Hansson *et al.*, 2009). Coral-associated archaeal communities are also independent of those in the water column, but in contrast to bacteria they are fairly cosmopolitan, displaying no evidence of species specificity. Despite their apparent generality it is thought that archaea still have an important role in the life of their host through nutrient cycling (Wegley *et al.*, 2004; Beman *et al.*, 2007; Siboni *et al.*, 2008, 2012).

Evidence to date suggests that the control of coral-associated bacterial and archaeal communities may be a result of multiple factors and at different spatial scales, however, not all studies are in agreement as to the primary controlling factor. Environmental factors such as temperature and nutrients, which may differ between location and season, have been found to significantly influence the specificity of bacterial–coral associations (Guppy and Bythell, 2006; Klaus *et al.*, 2007; Hong *et al.*, 2009; Littman *et al.*, 2009b; Schöttner *et al.*, 2012). In addition, species-specific control where bacterial communities were specifically associated with corals of the same species across distinct geographical regions at different times has been seen (Frias-Lopez *et al.*, 2002; Rohwer *et al.*, 2002; Kvennefors *et al.*, 2010). It is thought that the control of associated microbial communities at this level is due to the differences in coral species-specific mucus properties (Ducklow and Mitchell, 1979; Meikle, *et al.*, 1988; Ritchie and Smith, 2004; Wild *et al.*, 2004a, 2010a; Ritchie, 2006; Tremblay *et al.*, 2011; Schöttner *et al.*, 2012), which in turn is strongly linked to physiological differences between coral species. Nonetheless, it remains unclear whether the specificity of microbial communities extends to the host intraspecific level (differences in the microbiomes of closely related coral lineages) and whether the observed microbiome differences are influenced by the type of *Symbiodinium* present in the coral (which also contributes substantially to the metabolic makeup of the mucus (Kellogg and Patton, 1983; Crossland, 1987; Anthony and Fabricius, 2000).

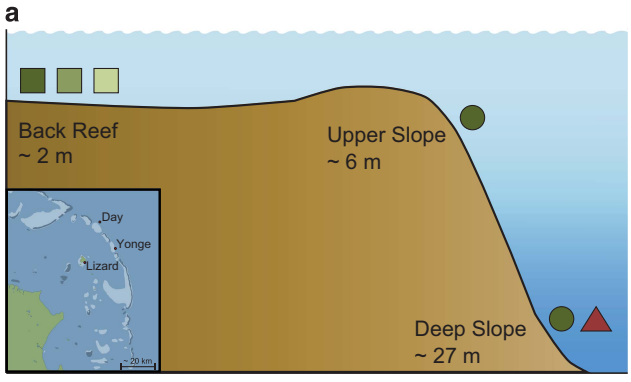
Here, the microbiomes of the scleractinian coral *Seriatopora hystrix* were assessed in a multi-factorial design, testing for differences related to coral host genotype (based on the putative control region of the coral mitochondrial DNA), *Symbiodinium* subclade genotype (based on the ITS2 region of the algal ribosomal DNA), reef habitat (depth) and geographic location. Samples were collected from three habitats ('Back Reef', 'Upper Slope' and 'Deep Slope') and at three locations on the Northern Great Barrier Reef (Yonge Reef, Day Reef and Lizard Island). Host-symbiont assemblages were genotyped in a previous study (Bongaerts *et al.*, 2010), representing a total of five distinct host–*Symbiodinium* combinations that allowed the assessment of the individual effects of host and *Symbiodinium* genotype (one host genotype associated with three different *Symbiodinium* subclades and one *Symbiodinium* subclade found across two different host genotypes). Similarly, although these host–*Symbiodinium* genotypes were partitioned across habitats, genotype–environment 'mismatches' (for example, a 'shallow' genotype occurring in the 'Deep Slope' habitat) allowed us to assess the individual effects of host–*Symbiodinium* genotype and environment. Microbial communities were characterised using 16S ribosomal RNA gene amplicon sequencing, and the individual contributions to richness and evenness were assessed across distinct host lineages, symbiont genotypes, reef habitats and geographic locations.

Materials and methods

Sample collection and surveys

Corals were identified as *Seriatopora hystrix* based on characters described by Veron (2000) and Veron and Pichot (1976). Small fragments of *Seriatopora* colonies were collected as described in Bongaerts *et al.* (2010) from three different habitats (Figure 1): the 'Back Reef' (2 m depth ± 1 m), 'Upper Slope' (6 m depth ± 1 m) and 'Deep Slope' (27 m depth ± 2 m) at two reef locations, Yonge Reef (14°36′59.9″S; 145°38′11.1″E) and Day Reef (14°28′28.4″S;145°32′19.1″E) along the continental shelf edge of the Great Barrier Reef, and from a 'Back Reef' habitat (2 m ± 1 m) at Lizard Island (14°41′39.1″S; 145°27′58.2″E). All sampled colonies were separated by at least 3 m in order to minimise the inclusion of potential clone mates due to fragmentation. Coral tissue was separated from the coral skeleton with a modified air gun attached to a SCUBA cylinder and subsequently stored in 20% dimethyl sulfoxide preservation buffer and kept at −20 °C until further processing.

Photographs were taken of random colonies in the three depth habitats at the time of sampling at Yonge Reef in 2008 ($n = 40$) and again during the 'Catlin Seaview Survey' in 2012 when both Yonge and Day Reef were revisited ($n = 44$ and $n = 45$, respectively), and visually assessed for level of pigmentation ('pigmented' vs 'pale').



b

LOCATION	HABITAT	HOST-SYMBIODINIUM ASSEMBLAGE
Lizard Island	Back Reef	
Day Reef	Back Reef	
	Upper Slope	
	Deep Slope	* *
Yonge Reef	Back Reef	
	Upper Slope	
	Deep Slope	* *

HostB HostU HostD1 HostD3 HostD2

C120 C120a C1m-aa C3n-t C3-ff

Figure 1 Study locations, habitats and *Seriatopora hystrix* coral–*Symbiodinium* assemblages. (**a**) Studied coral host–*Symbiodinium* assemblages across the three different habitat types: 'Back Reef', 'Upper Slope' and 'Deep Slope'. Inset: map of the three study locations on the northern Great Barrier Reef: Lizard Island, Yonge Reef and Day Reef. (**b**) Diversity and distribution of host and *Symbiodinium* genotypes across the three habitats and locations. Symbol shape refers to host genotype, and colour refers to symbiont genotype. Asterisk indicates assemblages not included in this study.

Host–Symbiodinium *genotyping and sample selection*

Genotyping of the corals and associated *Symbiodinium* was performed on total genomic DNA extracts as described in Bongaerts *et al.* (2010). Here, we selected 11 different combinations of host genotype, *Symbiodinium* genotype, reef habitat (depth) and location for which triplicate samples (each from a different coral colony) were available (Figure 1b). Five different host–*Symbiodinium* genotype combinations were assessed, representing three different host genotypes (mtDNA genotypes: B, U and D1) and four different *Symbiodinium* ITS2 subclades (C120, C120a, C1m-aa and C3n-t; Figure 1b). Although combinations were partitioned across habitats, there was some overlap in genotypes (that is, HostU:C120 occurring in the 'Upper Slope' and 'Deep Slope' habitats) which allowed us to test for the effect of habitat independent of genotype. Similarly, in the 'Back Reef' habitat a single host genotype with three different *Symbiodinium* types were found at Day Reef, allowing us to test for the independent effect of *Symbiodinium* type.

Analysis of bacterial and archaeal community composition by amplicon sequencing

Primers broadly targeting all bacteria and archaea containing the Roche 454 adaptor ligated at the 5′ end were used to amplify the V6–V8 region of the 16S ribosomal RNA gene: pyroLSSU803F 5′-TTAGAK ACCCBNGTAGTC-3′ and pyroLSSU1392R 5′-ACGG GCGGTGWGTRC-3′. Amplification reaction mixture (50 μl) included: ×10 PCR buffer (5 μl); 1 μl dNTPs (10 mM each); 4 μl MgCl (25 mM); 1 μl each primer (10 μM); 0.2 μl Taq DNA Polymerase (Thermo Fisher Scientific, Scoresby, VIC, Australia); 1.5 μl bovine serum albumin; ~20 ng DNA template; and made up to 50 μl with RNA/DNA-free water. Reaction conditions were as follows: an initial denaturation was carried out at 95 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 30 sec; annealing at 55 °C for 30 sec; and primer extension at 74 °C for 30 sec. This was followed by a final extension step at 74 °C for 10 min. Amplicon size section was carried out using Pippin Prep System (Sage Science, Beverly, MA, USA) before sequencing on a Roche 454 GS FLX (Roche, Basel, Switzerland) at the Australian Centre for Ecogenomics, University of Queensland, Australia. The sequence data set was deposited in the NCBI Sequence Read Archive (SRA) database (accession numbers: SRR1664591–SRR1664608, SRR1664610–SRR1664614 and SRR1664616–SRR1664625).

Sequence data processing and statistical analysis

Sequences were quality filtered and dereplicated with the QIIME script split_libraries.py with the homopolymer filter deactivated (Caporaso *et al.*, 2010) and then checked for chimeras against the

2012 edition of the Greengenes database (DeSantis *et al.*, 2006) with UCHIME ver. 3.0.617 (Edgar *et al.*, 2011) as previously described (Dennis *et al.*, 2013). Homopolymer errors were corrected with Acacia (Bragg *et al.*, 2012). Sequences were then subjected to the following procedures with QIIME scripts at the default settings. (i) Sequences were clustered at 97% similarity using UCLUST ver. 3.0.617 (Edgar *et al.*, 2011), (ii) cluster representatives were selected, (iii) greengenes taxonomy (2012 edition) was assigned to the cluster representatives by BLAST and (iv) tables with the abundance of different operational taxonomic units (OTUs) and their taxonomic assignments in each sample were generated. All reads matching eukaryotes or chloroplasts were removed and the number of reads was normalised to 1900 per sample. The mean number of OTUs (observed richness) and Simpson diversity index values (Simpson, 1949) corresponding to 1900 sequences per sample were calculated with QIIME. Generalised linear modelling was used to assess whether variation in observed richness and Simpson diversity index values could be explained by any of our treatments (that is, reef, depth, host genotype and *Symbiodinium* subclade). Differences in the composition of microbial communities between samples were assessed using permutational multivariate analysis of variance (PERMANOVA). Richness and evenness were compared using Tukey's Honestly Significant Difference (HSD) tests. All analyses were implemented with R version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria). Sequences were aligned against the greenegenes nucleotide database (version September 2014) in ARB using PyNAST (Ludwig *et al.*, 2004). Neighbour-joining maximum likelihood phylogenetic trees were constructed in FastTree v2.1.7 (Lawrence Berkeley National Lab, Berkeley, CA, USA).

Results and Discussion

Three representative samples (one per colony) of each combination at each site were amplified and sequenced, producing an average of 1900 reads per sample. The alpha diversity of the microbial communities as described by the Simpson Index and OTU richness associated with the *Seriatopora hystrix* colonies demonstrated a wide range of variation (Figure 2 and Supplementary Table S1) with the Simpson's index ranging from 0.265 to 0.988, and up to 705 OTUs. Archaeal populations comprised $<1.5\%$ relative abundance of coral microbiomes in all samples, with most OTUs belonging to the *Crenarchaeota*. The bacterial communities were dominated by members of the Alpha- and Gammaproteobacteria at all locations and depths as reported in other studies (Klaus *et al.*, 2007; Littman *et al.*, 2009a, b; Olson *et al.*, 2009; Kvennefors *et al.*, 2012). In particular, members of the alphaproteobacterial family Rhodobacteraceae and gammaproteobacterial genus *Endozoicomonas* were the most abundant OTUs. Phylogenetic analysis showed that the most abundant *Endozoicomonas* OTU clustered (Figure 3a) with sequences from cultivated and uncultivated species previously found in eight other scleractinian corals (Kvennefors *et al.*, 2010; Sunagawa *et al.*, 2010; Yang *et al.*, 2010; Speck and Donachie, 2012; Apprill *et al.*, 2013; Bayer *et al.*, 2013; Jessen *et al.*, 2013), as well as soft corals, sea slugs, sea anemones and sea cucumbers (Kurahashi and Yokota, 2007; Schuett *et al.*, 2007). Bayer *et al.* (2013) identified *Endozoicomonas* cells in high densities within the tissues of the coral *Stylophora pistillata* where it formed aggregations in close proximity to the endosymbiotic *Symbiodinium* in the endoderm, resembling the ovoid bacterial clusters found in other studies (Peters, 1984; Santavy and Peters, 1997; Ainsworth and Hoegh-Guldberg, 2009). Due to their high densities and intimate relationship with the coral tissues and algal endosymbionts it is thought that they may provide an advantage to their host's health through the production of antimicrobial compounds (Ritchie, 2006). Principal component analysis identified 17 OTUs, in addition to the *Endozoicomonas* OTUs (OTU 69 and 71), which were responsible for driving the differences in microbial communities associated with the different samples. These other OTUs were related to members of the *Rhodobacterales* and *Vibrionales*, several members of which have previously been identified as putative pathogens, or associated with disease lesions of scleractinian corals and other marine invertebrates (Imai *et al.*, 2006; Koren and Rosenberg, 2008; Becker *et al.*, 2009; Sekar *et al.*, 2009; Sunagawa *et al.*, 2009; Case *et al.*, 2011; Fernandes *et al.*, 2011; Figure 3b). OTUs from these groups were also found to have a highly significant negative correlation to *Endozoicomonas*. For example, the *Endozoicomonas* OTU 69 demonstrated a very strong negative correlation to OTU 25 ($r = -0.73$, $P < 0.01$), with moderate-to-strong negative correlations to OTUs 51, 30, 28 and 21 ($r = -0.58$, -0.39, -0.61 and -0.5 respectively, $P < 0.05$).

Effect of eukaryotic holobiont members on associated microbial communities

Microbes are highly sensitive to their chemical microenvironment. Due to this and their intimate relationship with the coral tissues it was expected that community structure would change with host and *Symbiodinium* genotype and the associated physiological differences as seen in previous studies (Ikeda and Miyachi, 1995; Ritchie and Smith, 1995a, b; Ritchie, 2006; Littman *et al.*, 2009a; Raina *et al.*, 2010; Tremblay *et al.*, 2011). However, neither host nor *Symbiodinium* differences were found to influence the associated microbial communities. The microbial communities associated with the dominant host–*Symbiodinium* assemblages of 'Upper Slope'

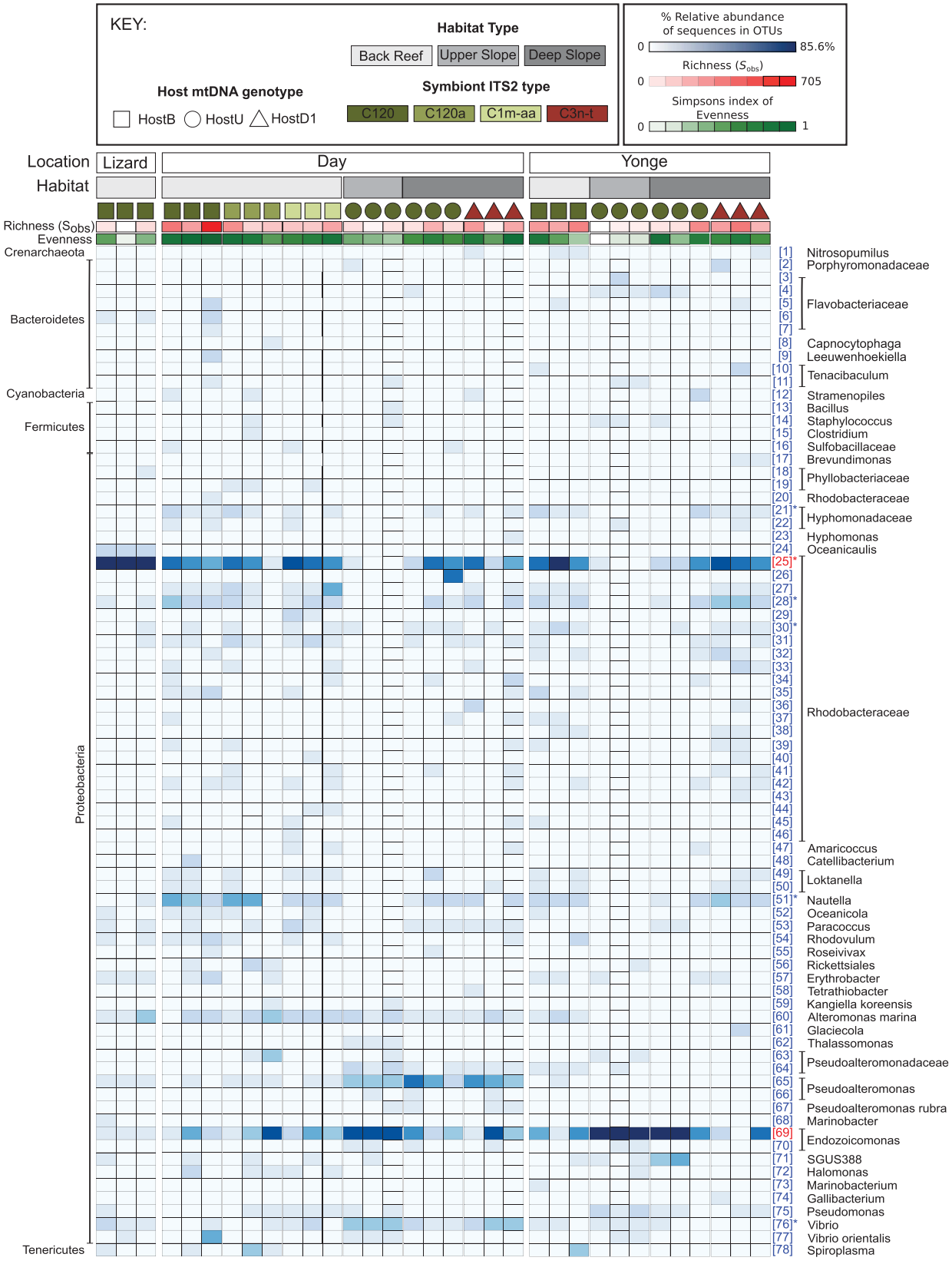


Figure 2 Heatmap summarising the alpha diversity and population abundances of dominant bacterial and archaeal OTUs (those present at >1% relative abundance) associated with *Seriatopora hystrix* colonies from Day Reef, Yonge Reef and Lizard Island Reef with different host–*Symbiodinium* genotype combinations. A total of three samples per location habitat and host–*Symdiodinium* genotype combination were analysed. Symbol shape refers to host genotype, and colour refers to symbiont genotype.

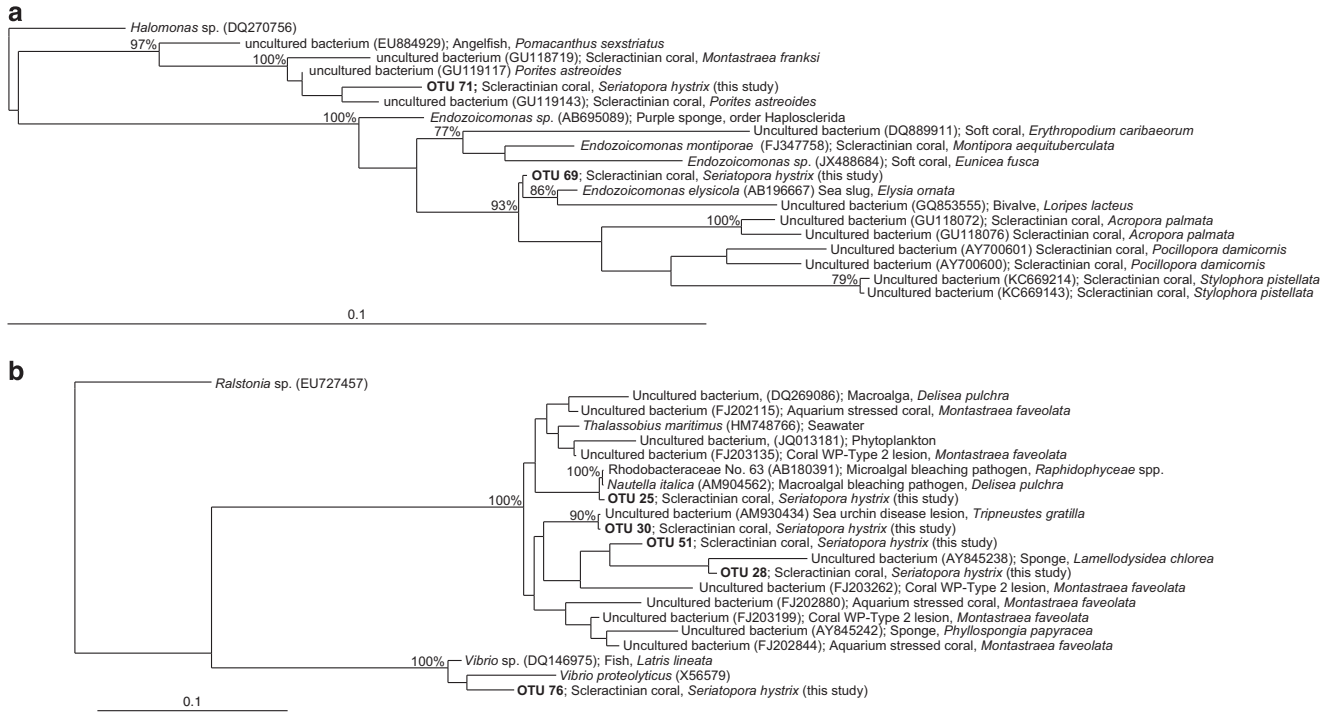


Figure 3 Phylogenetic trees, based on 16S ribosomal RNA gene sequences, showing the positions of OTUs identified associated with *Seriatopora hystrix* colonies relative to previously identified ribotypes obtained from public databases. (**a**) Position of OTUs 69 and 71 relative to members of the genus *Endozoicomonas* identified associated with other marine organisms including six Scleractinian corals. Scale bar represents 0.1 changes per nucleotide. (**b**) Position of OTUs 25, 28, 30, 51 and 76 relative to previously identified marine pathogens. Scale bar represents 0.1 changes per nucleotide. GenBank accession numbers are shown in parentheses. Numbers at nodes are bootstrap percentages (of 100 resampled data sets); only $\geqslant 75\%$ are shown.

and 'Deep Slope' habitats (HostU:C120 and HostD1:C3n-t, respectively) were not found to be significantly different when co-occurring at the same depth (that is, HostU:C120 occurs in low abundances in the 'Deep Slope' habitat). This, despite the strong physiological differences observed for these host–*Symbiodinium* assemblages, even when growing in the same habitat (Bongaerts *et al*., 2011a). The 'Back Reef' coral communities of Day Reef were made up of three different host–*Symbiodinium* genotype assemblages, consisting of the same host genotype (HostU) and three independent algal subclades (C120, C120a and C1m-aa). Comparison of the three assemblage types demonstrated also no difference in community structure with *Symbiodinium* genotype. The close genetic relationship of the three subclades present within the 'Back Reef' corals at Lizard Island (Bongaerts *et al*., 2011a) may mean that they have similar physiologies and consequently do not produce significantly different photosynthetic products, although identification of differences in photosynthate composition at the subclade level remains to be carried out.

Effect of reef location on coral-associated microbial communities

Ribbon reefs at the edge of the continental shelf such as Yonge and Day Reef experience substantially different environment conditions (for example, temperature and nutrients) to mid-shelf reefs like Lizard Island due to the direct exposure to deep oceanic waters (Berkelmans *et al*., 2004; De'ath and Fabricius, 2010; Fabricius *et al*., 2014). These environmental differences may have a strong influence on the coral-associated microbial communities at each location. The microbial communities associated with *S. hystrix* colonies from the 'Back Reef' habitat (HostB:C120) were significantly different at the three locations (PERMANOVA, $F_{2,6} = 2.48$, $P < 0.005$). Lizard Island 'Back Reef' communities were dominated by members of the Alphaproteobacteria (OTUs 24 and 25, Figure 2), while Yonge Reef 'Back Reef' communities had a greater relative abundance of members of the gammaproteobacterial genus *Endozoicomonas* (OTU 69, Figure 2). The microbial communities associated with the 'Back Reef' colonies at Day Reef shared similarities with both Yonge and Lizard Island Reefs with higher relative abundance of members of both the Rhodobacteraceae and *Endozoicomonas* as well as a high relative abundance of other Alphaproteobacteria. Significant differences also existed in the alpha diversity between the different 'Back Reef' habitats (one-way analysis of variance: S_{obs}: $F_{2,6} = 9.022$, $P < 0.05$; Simpson Diversity: $F_{2,6} = 9.022$, $P < 0.01$). The richness and evenness (Supplementary Table S1 and Supplementary Figure S1) of microbial communities from the 'Back Reef' of Lizard Island reef were significantly lower than those of Day Reef

(Tukey's HSD *post hoc*: S_{obs} $P<0.05$; Simpsons Diversity: $P<0.01$), and the evenness was significantly lower to those of Yonge Reef (Tukey's HSD *post hoc*: Simpson's diversity: $P<0.05$). These differences may be due to varying levels of habitat heterogeneity of the mid- and outer-shelf reefs. Heterogeneity has been shown to be one of the main determinants of community structure (Jiang and Patel, 2008). It may therefore be expected that the higher levels of disturbance and extremes of conditions such as temperature and solar irradiance (Bongaerts *et al.*, 2011a) experienced in 'Back Reef' environments may lead to specialisation of communities and reduced diversity (Buckling *et al.*, 2000; Berga *et al.*, 2012). Day and Yonge Reefs however may not be exposed to the same levels of extremes as Lizard Island due to their proximity to the shelf edge and exposure to oceanic waters, which is also reflected in the differential bleaching susceptibility of reefs depending on the shelf position (Berkelmans and Oliver, 1999).

As no effect of host-*Symbiodinium* genotype was found within 'Deep Slope' habitats (that is, between HostU:C120 and HostD1:C3n-t genotypes), samples from these habitats were grouped and compared between locations (that is, Day and Yonge Reef). A significant difference (PERMANOVA, $F_{1,9}=2.58$, $P<0.05$) was found between 'Deep Slope' coral microbiomes from Yonge Reef and Day Reef, but no difference was found in their alpha diversity. 'Deep Slope' communities were dominated by Alphaproteobacteria with differences driven by a greater prevalence of members of the Gammaproteobacteria, with Vibrionaceae and Alteromonadaceae associated with the Day Reef corals than those from Yonge Reef, which had a greater occurrence of Pseudoalteromonadaceae (Figure 2). In contrast, for the 'Upper Slope' habitat (HostU:C120 genotype), no significant difference was found in the microbial communities of Day and Yonge Reef, which were dominated by *Endozoicomonas* (Figure 2, OTU 69).

Previous studies have also found that geographical differences exist between the microbial communities associated with corals (Rohwer *et al.*, 2002; Guppy and Bythell, 2006; Klaus *et al.*, 2007; Littman *et al.*, 2009b; Chen *et al.*, 2011; Schöttner *et al.*, 2012). The potential pathogen-dominated communities associated with 'Back Reef' colonies at Lizard Island may be due to their closer proximity to human influences (Dinsdale *et al.*, 2008; Furby *et al.*, 2014) and reduced influence of oceanic waters due to their position in the mid-shelf. Although Yonge and Day Reef 'Deep Slope' coral microbiomes possessed the same group of Rhodobacteraceae related to potential pathogens, those of the Day Reef had a greater abundance of *Endozoicomonas* (Figure 2, OTU 69). At the time the samples were collected (October 2008) it was found that in contrast to the shallow habitats, deep reef habitats (~27 m depth) experienced a more constant environmental state, in terms of more consistent and lower levels of irradiance due to light attenuation through the water column and reduced thermal variability compared with the diurnal fluctuations experienced in the 'Back Reef' habitats (although in summer, the 'Deep Reef' habitat can experience cold-water influxes; Bongaerts *et al.*, 2011a). It would also be expected that the Deep Reef sites would also experience reduced wave stress in comparison with the shallow habitats. These more homogeneous and stable conditions may allow location-specific factors not measured as a part of this study to have a role in shaping the microbial communities, resulting in differences between communities associated with deep-water *S. hystrix* colonies.

Effect of depth habitat on associated microbial communities

S. hystrix colonies with the HostU:C120 genotype can be found at both the 'Upper Slope' and 'Deep Slope' habitats (albeit in low abundance in the latter) of Day and Yonge Reef, and their microbial communities were found to be significantly different between depths (PERMANOVA, $F_{1,9}=4.398$, $P<0.001$). The community alpha diversity also differed significantly with depth, with 'Deep Slope' *S. hystrix* microbiomes being considerably more diverse and even than those of the 'Upper Slope' colonies (one-way analysis of variance: richness $F_{2,9}=5.325$, $P<0.05$; evenness $F_{2,9}=13.77$, $P<0.05$), matching the higher genotypic diversity observed for coral–*Symbiodinium* assemblages in this habitat (Bongaerts *et al.*, 2010). Owing to the absence of identical host–*Symbiodinium* assemblages at all depth habitats, it was not possible to compare the microbial communities from 'Back Reef' corals with those of the 'Upper Slope' and 'Deep Slope' habitats, without it being confounded by coral–*Symbiodinium* genotype.

Microbial communities in the 'Upper Slope' were dominated by ribotypes related to *Endozoicomonas* (OTU 69 and 71, Figure 2), whereas microbial communities in the 'Deep Slope' are characterised by members of the Rhodobacteraceae (for example, OTU 25, 28 and 51; Figure 2). Of particular interest are OTU 25 and 51 which are closely related to known algal pathogens that cause bleaching and mortality of both macro- and microalgae (Imai *et al.*, 2006; Case *et al.*, 2011; Fernandes *et al.*, 2011). Colonies of both the 'Back Reef' and 'Deep Slope' habitats at all locations were observed to be paler in colour than those of the 'Upper Slope' habitats (Supplementary Figure S2). Physiological parameters for the corals of the three depth habitats at Yonge Reef measured in a previous study found that they also had corresponding lower concentrations of areal Chlorophyll a and lower net photosynthesis (Bongaerts *et al.*, 2011a). OTU 25 and 51 are also closely related to bacteria identified, associated with the disease lesions of White Plague Type II

in the reef-building coral *Montastraea faveolata* (Sunagawa *et al.*, 2009), which is characterised by an advancing bleached zone in front of the lesion boundary during a disease outbreak following a mass bleaching event (Richardson *et al.*, 1998). The presence of potential pathogens and presentation of similar associated symptoms suggests that the health state of the 'Deep Slope' and 'Back Reef' colonies may be sub-optimal.

Although it is not possible at this stage to determine the depth-related factors responsible for the differences in microbial communities of 'Deep Slope' and 'Upper Slope' *S. hystrix* colonies, one explanation may be the influence of differing light levels on photosynthate production (Crossland, 1987) and the sensitivity of microbial communities to their chemical microenvironment (Ritchie and Smith, 1995a, b; Ritchie, 2006; Raina *et al.*, 2010). The lipid content of coral tissues is mainly derived from photosynthesis by the *Symbiodinium* (Kellogg and Patton, 1983; Harland *et al.*, 1993) which varies with light levels, and therefore also depth (Oku *et al.*, 2003). This photosynthetic source is assimilated into mucus by the host and contains high concentrations of proteins, polysaccharides and lipids, making it an ideal environment for microbes (Ducklow and Mitchell, 1979; Ferrier-Pages *et al.*, 2000; Wild *et al.*, 2004b). Differences in photosynthesis due to depth or photosynthetic potential due to the presence of photosymbiont pathogens may therefore result in changes in the condition of the coral tissues and surface mucus layer, consequently further influencing other members of the associated microbial communities (Crossland, 1987; Benlloch *et al.*, 1995; Ikeda and Miyachi, 1995; Schäfer *et al.*, 2001; Cooper *et al.*, 2011; Tremblay *et al.*, 2011; Nelson *et al.*, 2013). This reduced function in photosynthesis in 'Back Reef' and 'Deep Slope' colonies, coupled with differing light levels would therefore result in a difference in the availability of carbon and other metabolites (Crossland, 1987; Ikeda and Miyachi, 1995; Oku *et al.*, 2003) to the associated microbial communities. The coral microbiomes may therefore be subject to similar environmental factors such as temperature and irradiance that result in depth specificity seen in *Symbiodinium*-type zonation (Baker, 2003; Stat *et al.*, 2008b).

Conclusion

It is well established that diverse microbial communities inhabit adult corals (Williams *et al.*, 1987; Santavy, 1995; Bythell *et al.*, 2002; Lesser *et al.*, 2004) and have a key role in the health of their host (Koh, 1997; Castillo *et al.*, 2001; Ritchie, 2006; Kvennefors *et al.*, 2010; Rypien *et al.*, 2010; Sharp *et al.*, 2010). However, there is continuing debate as to which factors predominantly influence these communities. This is the first study to examine the differences in the composition of bacterial and archaeal communities associated with a scleractinian coral with respect to both the host coral genotype, their *Symbiodinium* subclade and their habitat type (location and depth). The results of this study supports those of others that habitat is the overall controlling factor of the coral microbiome and not the other main members of the holobiont. However, it must be considered that teasing apart physiological factors such as photosynthesis from environmental ones is hard as they often go hand-in-hand. Distinct changes with depth from *Endozoicomonas*-dominated communities to a community dominated by potential pathogens were also observed. Although it is beyond the scope of this study to determine the mechanisms behind these changes, it provides further evidence that members of the *Endozoicomonas* provide a beneficial function to the coral holobiont (Bourne *et al.*, 2008; Ainsworth and Hoegh-Guldberg, 2009; Kvennefors *et al.*, 2012; Bayer *et al.*, 2013). Their high densities and close physical relationship to the *Symbiodinium* within the coral endoderm identified in previous studies and the strong negative correlation to the occurrence of bleaching pathogens and associated visible signs of coral bleaching found here suggests that they may be providing direct protection to the photosymbionts. Coupled with their intimate relationship with corals worldwide, this bacterial endosymbiont appears to be a vital member of the coral holobiont, possibly coevolving with the coral host and *Symbiodinium*, making them integral to the success of the coral holobiont system, and therefore warrants further investigation.

Conflict of Interest

The authors declare no conflict of interest.

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