Article type : Regular Article

Patterns of *Symbiodinium* (Dinophyceae) diversity and assemblages among diverse hosts and the coral reef environment of Lizard Island, Australia ¹

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¹ Received *date*. Accepted *date*.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jpy.12749

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Running title: *Symbiodinium* diversity in reef invertebrates

Editorial Responsibility: S. Lin (Associate Editor)

ABSTRACT

Large-scale environmental disturbances may impact both partners in coral host-*Symbiodinium* systems. Elucidation of the assembly patterns in such complex and interdependent communities may enable better prediction of environmental impacts across coral reef ecosystems. In this study, we investigated how the community composition and diversity of dinoflagellate symbionts in the genus *Symbiodinium* were distributed among 12 host species from six taxonomic orders (Actinaria, Alcyonacea, Miliolida, Porifera, Rhizostoma, Scleractinia) and in the reef water and sediments at Lizard Island, Great Barrier Reef before the 3rd Global Coral Bleaching Event. 454 pyrosequencing of the ITS2 region of *Symbiodinium* yielded 83 Operational Taxonomic Units (OTUs) at a 97% similarity cut-off. Approximately half of the *Symbiodinium* OTUs from reef water or sediments were also present in symbio. OTUs belonged to six clades (A-D, F-G), but community structure was uneven. The two most abundant OTUs (100% matches to types C1 and A3) comprised 91% of reads and OTU C1 was shared by all species. However, sequence-based analysis of these dominant OTUs revealed host species-specificity, suggesting that genetic similarity cut-offs of *Symbiodinium* ITS2 data sets need careful evaluation. Of the less abundant OTUs, roughly half occurred at only one site or in one

species and the background *Symbiodinium* communities were distinct between individual samples.

We conclude that sampling multiple host taxa with differing life history traits will be critical to fully understand the symbiont diversity of a given system and to predict coral ecosystem responses to environmental change and disturbance considering the differential stress response of the taxa within.

Keywords: 454 pyrosequencing; coral reef; Alcyonacea; Actinaria; Foraminifera; Scleractinia; symbiont diversity; *Tridacna*

Abbreviations: DGGE, Denaturing Gradient Gel Electrophoresis; ITS2, Internal Transcribed Spacer 2; NGS, Next-Generation Sequencing; OTU, Operational Taxonomic Unit, RFLP, Restriction Fragment Length Polymorphism; SSCP, Single-Strand Conformation Polymorphism

INTRODUCTION

Community and ecosystem ecology have historically focused on competition as a primary biotic driver in community assembly and in accounting for diversity at both the local and the regional scale. Aspects of microbial-level interactions, abundances, and distribution of species have only recently been incorporated into studies of community ecology (Leibold et al. 2004). Processes that occur within and between host species influence the ecological and evolutionary dynamics of both the endosymbionts and the host species themselves (Mihaljevic 2012, Thornhill et al. 2014, Bordenstein and Theis 2015). Studies of host-symbiont systems are vital to our broader understanding of ecological patterns, because these relationships are at the base of many important ecosystems. Our ability to predict recovery or resilience following disturbance events will be directly tied to this knowledge.

The relationship between hard corals and dinoflagellates of the genus *Symbiodinium* underpins the formation of coral reefs worldwide which are recognized as among the most threatened ecosystems (Hughes et al. 2003, Hoegh-Guldberg et al. 2007). In spite of the nutrient-poor waters in which they are generally found, coral reefs are among the most diverse ecosystems on the planet (Roberts et al. 2002). They are also among the most productive ecosystems, driven by the photosynthetic energy produced by *Symbiodinium* and due to the web of host-symbiont interactions they harbor (Muscatine and Porter 1977). While scleractinian coral-*Symbiodinium* interactions are thought to be the most important of these, and have been the most studied, a wide range of invertebrate and some protist hosts are also engaged in symbiotic relationships with dinoflagellates and depend on their algal partners (Lee 2011). Furthermore, free-living *Symbiodinium* in reef water and sediments represent a pool of diversity and potential for host colonization that is less well understood than the host-associated symbionts (Sweet 2014, Cunning et al. 2016).

Large scale environmental disturbances and stochastic events may impact both partners in coral or host-*Symbiodinium* systems, and elucidation of the patterns of assembly in such complex and interdependent communities can help us to understand these impacts across the reef system as a whole. It is critical to our understanding of these systems to determine the ways in which all of these groups assemble and govern the diversity of organisms both locally and regionally. Many important questions remain to be answered regarding host-*Symbiodinium* associations. For instance, can different host species or the surrounding environment act as symbiont sources at the local scale? Can hosts with differing phylogenetic, physiological or biochemical traits act as *Symbiodinium* refugia during environmental perturbations, i.e., how much diversity is shared between divergent host species? And how do interspecific host and symbiont interactions play out across spatial scales?

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Answers to these questions can only be addressed by first arriving at an understanding of symbiont community assembly patterns in space and several studies have begun to do so (LaJeunesse et al. 2003, Tonk et al. 2014). However, studies of this nature remain limited in number and have relied primarily on molecular methods such as Denaturing Gradient Gel Electrophoresis (DGGE). The sensitivity of this and other commonly used methods (i.e., DGGE, single-strand conformation polymorphism [SSCP], and restriction fragment length polymorphism [RFLP]) is limited and often only the most dominant symbiont type is detected (Loram et al. 2007, FitzPatrick et al. 2012). Use of these methods has provided an underestimation of the number of distinct symbiont types associated with a single hard coral species or other hosts (Silverstein et al. 2012). Mieog et al. (2007) demonstrated that DGGE is unable to identify rare ITS2 genotypes at frequencies below 10%. In contrast, the use of bacterial cloning leads to an overestimation of *Symbiodinium* diversity because of the multi-copy nature of the ITS2 marker region (Sampayo et al. 2007, Thornhill et al. 2007). Next Generation Sequencing (NGS), which can enable more exhaustive surveys, provides a much more sensitive measurement of genetic diversity, and can enable a view of rare or background community members (Quigley et al. 2014, Thomas et al. 2014, Ziegler et al. 2017). Similar to bacterial cloning, a large number of intragenomic ITS2 sequences are returned in NGS. To avoid overestimation of diversity, the current standard approach for analyzing NGS data sets includes clustering of sequences into Operational Taxonomic Units (OTUs) at 97% similarity, but it is unclear how well this approach performs in mixed community samples (Arif et al. 2014).

Rare or background *Symbiodinium* taxa may prove important because they could potentially fulfill functions in symbiosis (Ziegler et al. 2018), as has been suggested for bacteria in the coral holobiont (Ainsworth et al. 2015) and for rare species in ecosystem functioning (Mouillot et al. 2013). In the case of *Symbiodinium* spp. these functions could include for example, synthesis of biochemical compounds, contribution to metabolic cycling and autotrophic energy production during periods of

environmental variability, or when environmental conditions exceed the optimum of the more abundant *Symbiodinium* types. Under changing environmental conditions, rare background types or free-living *Symbiodinium* may be able to replace the dominant symbiont and thus mediate the stress response in the holobiont (Buddemeier and Fautin 1993, Baker 2001, Boulotte et al. 2016) although many hosts show stable associations with their symbionts through time (Thornhill et al. 2006b, Stat et al. 2009). The *Symbiodinium* community may also revert to its original state after stress events (Goulet and Coffroth 2003, Hume et al. 2015) and more generally, it remains to be seen whether all *Symbiodinium*-harboring taxa may undergo shifts in the symbiont community upon stress (Ziegler et al. 2014). At the same time, emerging *Symbiodinium* types may also be inferior performers and may compromise the ecological performance of the holobiont, if they persist in the long term. For instance, the replacement of the corals' native *Symbiodinium* type with *Symbiodinium trenchii* led to decreased skeletal growth rates (Pettay et al. 2015), likely caused by lower transfer of energy from the non-native symbionts to the coral host (Little et al. 2004, Cantin et al. 2009). Moreover, some symbionts display opportunistic lifestyles that go beyond mutualism, as evidenced by two recently described *Symbiodinium* species, *Symbiodinium voratum* and *Symbiodinium necroappetens*. These species feed phagotrophically on bacteria and microalgae and emerged in diseased and damaged tissues (Jeong et al. 2012, Jeong et al. 2014, LaJeunesse et al. 2015). Recruited from the background community, *S. necroappetens* may dominate the *Symbiodinium* community of stressed coral hosts (*Orbicella* spp.) intermittently, underscoring the broad functional and ecological diversity found in the genus *Symbiodinium* (LaJeunesse et al. 2015). The application of high-resolution detection techniques will be essential to further understand the hidden diversity of *Symbiodinium* communities and to guide research on the function of the coral-*Symbiodinium* association.

Here we use 454 pyrosequencing techniques to investigate how genetic variants of dinoflagellate symbionts in the genus *Symbiodinium* are distributed across space in the environment and among different hosts at a local scale at the reefs surrounding Lizard Island in the Great Barrier Reef (GBR). Our samples were collected before the most recent Global Coral Bleaching Event in March 2017 that

severely affected the northern part of the GBR and that also devastated the reefs at the sampling sites (Hughes et al. 2017) and may be used as baseline data for future surveys of recovery dynamics.

More specifically, we ask: 1. What is the overall diversity of *Symbiodinium* found across different host species at Lizard Island? 2. Can the patterns of diversity be associated with specific traits of the host, such as phylogeny or horizontal vs. vertical symbiont transmission (i.e., the uptake of *Symbiodinium* into symbiont-free larvae each generation or the transfer of symbionts between generations)? 3. What is the turnover (i.e., beta diversity) of *Symbiodinium* across sites and taxa at Lizard Island? 4. What is the congruence between *Symbiodinium* communities in the environment and the host organisms? 5. Does the well-accepted 97 % cut-off for OTU clustering adequately describe the *Symbiodinium* diversity seen in these mixed host communities?

MATERIALS AND METHODS

Field collection

A total of 219 samples were collected in June 2013 at five sites around Lizard Island, which, together with its three smaller surrounding islands, belongs to the northern section of the GBR in Australia.

The Lizard Island Group is located about 30 km from the mainland and is thus considered a mid-shelf reef. In order to target a wide range of taxa representative of the host diversity found in association with *Symbiodinium*, we sampled 12 species belonging to five invertebrate and one protist order. The sampling set consisted of four species each in the orders Scleractinia (hard corals; *Acropora humilis*, *Pocillopora damicornis*, *Seriatopora hystrix*, *Stylophora pistillata*) and Alcyonacea (soft corals; *Bayerxenia* sp., *Isis* sp., *Lobophytum* sp., *Sarcophyton* sp.), two species in Actinaria (anemones; *Entacmaea quadricolor*, *Heteractis magnifica*), and one species each in Milioida (Foraminifera; *Marginopora vertebralis*) and Veneroida (giant clams; *Tridacna crocea*). Three replicate samples of each species were collected on snorkel between the water surface and 1.5 m depth at each of five

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sites (Coconut pass [-14.685° S, -145.467° W], Picnic beach [-14.697° S, -145.448° W], Loomis reef [-14.683° S, -145.449° W], Osprey reef [-14.668° S, -145.443° W], Mermaid cove [-14.646° S, -145.454° W]) around Lizard Island (Fig. 1). In addition, several unidentified sponge samples and one sample from the jellyfish *Cassiopea* sp. were obtained opportunistically. At the same time, three replicate samples of surface sediments (collected in 50 mL vials) and seawater from just above the reef (3 x 3 L) were taken at each site. A minimum distance of 5 m was maintained between sampled colonies of the same species to avoid sampling clones.

Sample processing and 454 sequencing

Samples were transported to Lizard Island Research Station. Water samples were filtered onto 0.22 µm Sterivex syringe filters (Millipore, Burlington, MA, USA), preserved in 200 µL sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris-HCl, pH 9.0, Giovannoni et al. [1990]), and frozen at -20°C. All tissue samples were rinsed with filtered seawater, preserved in sucrose lysis buffer, and frozen. DNA was extracted from tissue and sediment samples using the MoBio DNA extraction kit (MoBio, USA) following the manufacturer's protocol and frozen at -20°C.

DNA was extracted from water filters using an optimized phenol-chloroform method. Two volumes of 2X CTAB and 100 µg · mL⁻¹ proteinase K were added to the water filters, which were then incubated at 60°C for 1 h. SDS (2% final concentration) was added followed by a second 1 h incubation at 60°C. DNA was then extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), followed by two chloroform extractions. DNA was precipitated overnight after the addition of 0.1 volumes 3 M Na acetate and 2 volumes 95 % ethanol. DNA was centrifuged for 45 min at 14,000 rpm, washed with 70 % ethanol, dried by speed-vac, and resuspended in sterile Milli-Q water.

Fusion PCR was performed using dinoflagellate specific primers to amplify the ITS2 region of the rRNA gene (ITS-DINOforward; 5'GTGAATTGCAGAACTCCGTG3' and ITSs2rev2 5'CCTCCGCTTACTTATATGCTT3'; Stat et al. [2009]). Primers were designed so that the forward primer consisted of the Roche adapter A, followed by a 10 base error-correcting barcode for multiplexing (Hamady et al. 2008), and lastly the ITS-DINOforward primer. Fifty ng of DNA was used in a single step PCR performed in triplicate. The reactions contained 0.4 mM forward and reverse primer, 0.2 mM dNTP, 1 x GoTaqFlexi PCR buffer, 1.5 mM MgCl₂, 1.5 U Platinum Taq Polymerase, 1 x BSA, 32 mM KCl, and 2 µL of DNA template. The thermal cycler program consisted of a 7 min denaturing step at 95°C, followed by 30 cycles of 45 s at 95°C, 45 s at 52°C, 45 s at 72°C, and a 5 min final extension at 72°C. Successful PCR amplicons were purified using QiaQuick Gel Purification kits (Qiagen, USA) following specified protocols. PCR amplicons were quantified spectrophotometrically and combined in equimolar concentrations for sequencing. Sequencing template was quantitated fluorometrically using a picogreen dye kit, assayed for quality and fragment length on an Agilent bioanalyzer DNA chip for library preparation using Roche titanium reagents and titanium procedures. Up to 96 samples were run on one quarter region of a sequencing plate and all samples were sequenced on one plate in a single run. Pyrosequencing was performed on a Roche 454 FLX instrument following manufacturer's protocols.

Data processing

ITS2 region amplicons were denoised, screened for PCR artifacts and checked for chimeras using Ampliconnoise and Perseus (Quince et al. 2011). The ITS2 sequence data were analyzed using the QIIME analytical software package (v.1.8; Caporaso et al. 2010). The entire dataset was first clustered into operational taxonomic units (OTUs) at the 97 % similarity level using UCLUST (Edgar 2010) in the *pick_otus.py* script. The 97% level OTU cut-off has previously been shown to adequately capture genetic and clade-level diversity for mixed *Symbiodinium* spp. ITS2 communities (Arif et al.

2014). The most abundant sequence in each OTU was then picked as an OTU reference for downstream processing using the *pick_rep_set.py* script. Representative sequences were annotated taxonomically by conducting a local BLAST against an ITS2 reference database compiled in Arif et al. (2014). Annotations were manually checked with BLAST searches against the GenBank nr database. Generally, the annotations to the internal ITS2 reference database were of higher quality (e-values, query coverage, and identity) and manually annotated as in Arif et al. (2014). One exception was made for the fourth most abundant sequence, which was annotated as C21 in the internal database. A recent sequence submission to GenBank was a better match (100 %) and identified this sequence as C3nn (ACN KJ184190; Tonk et al. 2014). OTUs were designated as the closest identified ITS2 type, followed by the unique identifier of the representative OTU sequence. Subsequently, an OTU count table was created from the representative sequences, using the *make_otu_table.py* script and curated to filter out sequences that did not represent *Symbiodinium* spp. in the manual taxonomic annotation steps (Table S1 in the Supporting Information). A single 97%-level C1-like OTU comprised 55.6% of the entire sequencing dataset. As has been previously noted, a significant amount of ecological variation can exist within < 3% ITS2 variation among *Symbiodinium* spp. subclades (LaJeunesse and Thornhill 2011). To analyze intra-OTU ecological variation that may be obscured by using a 97% level cut-off, the sequences from the single dominant C1-like OTU were extracted from the dataset. Distinct sequences were manually curated with taxonomic assignments according to the Arif et al. (2014) database and compiled as a count table as above. The same procedure was repeated with the second most abundant OTU, which was annotated as *Symbiodinium* A3. Haplotypes were designated as the closest identified ITS2 type, followed by the unique identifier of the haplotype sequence.

Data analysis

A total of 180 samples were successfully amplified, consisting of 28 environmental samples and 152 samples from the different host species. Because the sequence reads obtained per host sample varied (range: 10 - 4339, mean: 1020, median: 795), each host sample was subsampled to 300 sequences using the command *single_rarefaction.py* in QIIME. This cut-off allowed us to retain a representative range of host samples (128/152) with reasonable sequencing depth. A Mantel test was run with Spearman rank correlations and 9,999 permutations using the RELATE function in PRIMER to evaluate a cut-off of 300 sequences with a cut-off of 500 ($p < 0.001$, Rho 0.968). Because a large proportion of the reads from environmental sediment and water samples were annotated as non-*Symbiodinium* sequences, the average read number of target sequences per environmental sample was comparatively low (range: 3 - 465, mean: 109, median: 50). A cut-off of 43 reads was chosen for subsampling of environmental samples to retain a sample set (16/28) representing the spatial structure of the biotic sampling approach for qualitative comparison.

The number of observed OTUs, alpha diversity from Shannon diversity index, and equitability (evenness) were calculated after multiple rarefaction with 1,000 iterations using *alpha_diversity.py* in QIIME for each species, all species at a site, and for the environmental samples. The Shannon diversity index incorporates evenness and species richness and approaches '1' when sequences are evenly distributed between OTUs and approaches '0' when all sequences belong to a single OTU. Equitability is a measure of the evenness of OTU frequencies and is defined as $\text{Shannon} / \log_2$ (number of OTUs; 1 = perfectly even, 0 = most uneven).

OTU turnover or beta diversity was calculated on subsampled host and environmental datasets using two approaches. Firstly, Bray Curtis distance matrices were calculated from the square root transformed subsampled OTU count tables, taking sequence abundance data into account. Secondly,

Binary Jaccard matrices were calculated from subsampled OTU tables of presence and absence only.

The distance matrices were used to calculate beta diversity within and between species and sites with the *make_distance_boxplots.py* command in QIIME. To further visualize patterns in the data, the distance matrices were used to plot samples using non-metric Multi-Dimensional Scaling (nMDS). A permutational analysis of variance (PERMANOVA) was run to test for differences in the OTU community between sites and species nested in sites, based on Bray Curtis and Binary Jaccard distances. For this analysis, unrestricted permutation of means, partial sum of squares, and 9,999 permutations with Monte Carlo correction were used in PRIMER (e-Primer, Plymouth, UK). Pairwise post-hoc comparisons were conducted where appropriate. In addition, the number of OTUs in each of the samples per site was compared with a one-factorial ANOVA ('site') and Fisher LSD post-hoc tests on square root transformed OTU counts with Statistica 10 (StatSoft Inc. 2011).

The variation among samples was further visualized separately for the dominant OTUs C1 and A3 that comprised the majority of the dataset using network diagrams. All distinct sequences from each OTU were used to create new count tables, which were imported into Cytoscape (Smoot et al. 2011) to visualize between-sample sharing of unique C1/A3 'haplotypes' (defined here as sharing 100 % sequence identity). The final network diagram was drawn with large nodes as samples, and unique C1 haplotypes as smaller nodes shared between samples, and organized using the spring-embedded algorithm.

RESULTS

A total of 186,419 ITS2 quality-filtered, denoised sequence reads (mean length = 325 bp) were obtained from 180 total samples. A large proportion of the filtered OTUs were non-*Symbiodinium* organisms that were prevalent mostly in the water and sediment samples. After manual curation

and removal of non-*Symbiodinium* sequences 156,115 ITS2 sequences remained. Removal of singletons (OTUs with 1 sequence read) reduced the final number of sequences to 155,601; and these were clustered into 83 *Symbiodinium* OTUs at 97% similarity.

To include the highest possible fraction of samples in the analysis, subsampling of sequences was conducted to a cut-off of 300 sequences. Rarefaction analyses showed that this was sufficient to detect most of the diversity (Fig. S1 in the Supporting Information). Good's coverage was above 0.99 for 126 of 128 subsampled biotic samples, and 0.983 and 0.984 for the two remaining samples (mean: 0.996, SD: 0.003). Good's coverage was lower in sediment (mean: 0.971, SD: 0.024) and water (mean: 0.965, SD: 0.018) than in biotic data.

***Symbiodinium* diversity and patterns in host-symbiont association**

48 OTUs were detected in our biotic dataset after subsampling, including 8 OTUs from clade A (17%), 1 OTU from clade B (2%), 36 OTUs from clade C (75%), 1 OTU from clade D (2%), and 2 OTUs from clade G (4%). Twenty-nine of the OTUs were found in only one species and 29 OTUs were found at only one site. These OTUs were not identical, though largely overlapping (27 OTUs only found in one species at one site). 76% of subsampled reads belonged to the OTU C1_796, of which the most abundant sequence was a 100% match to *Symbiodinium goreau* (sensu C1; ACN AF333515; LaJeunesse 2001). C1_796 was the only OTU shared between all species (Fig. 2a). All four hard and soft coral species, the foraminifera and the sponge sample carried OTU C1_796 as their most abundant OTU (Table 1). *Tridacna crocea* and *Cassiopea* sp. were dominated by OTU A3_302 (ACN AF333507; LaJeunesse 2001). OTU A3_302 was the second most abundant OTU (15 %) and it was present in all species with the exception of *Heteractis magnifica*, which was dominated by OTU C3nn_1031 (ACN KJ184190; Tonk et al. 2014; Table 1). In the species *Entacmaea quadricolor* OTU

C42.2_1367 (ACN KF134173; Pontasch et al. 2014) and C1_796 occurred in almost equal abundances (51 and 47%, respectively). However, all but one of the samples were dominated by only one of the OTUs. The only clade D OTU (D5_677) was found in two samples of the soft coral *Isis* sp., in which it occurred at equal abundance with OTU C1_796 (Fig. 2a).

OTU community composition differed significantly between species (PERMANOVA, $F_{50, 78} = 13$, $p < 0.001$). These differences were driven by the divergence in the association with the main *Symbiodinium* OTUs, as can be deduced from the nMDS plot using Bray Curtis distances, i.e., taking OTU abundance into account (Fig. 3a). In this plot, the species with main *Symbiodinium* OTUs other than OTU C1_796 (*E. quadricolor*, *T. crocea*, *H. magnifica*, *Cassiopea* sp.) were separated from those dominated by OTU C1_796. Analyses of Binary Jaccard distances revealed a more even spread of samples (PERMANOVA, $F_{50, 78} = 2$, $p < 0.001$), representing the between-species differences in the occurrence of distinct OTU combinations.

Most individual hosts harbored two to three OTUs per sample, while the overall diversity found within each host species was higher (with the exception of *H. magnifica*; Table 1). Samples from *A. humilis* harbored the most OTUs per sample (mean 4.57, max. 9) and a total of 18 OTUs were found in association with *A. humilis* (Table 1). These findings were also reflected in the alpha- and beta-diversity indices: Shannon's diversity index was comparably high in *A. humilis*, but also in *Isis* sp. and the sponge sample (Table 1). For *A. humilis* and *Isis* sp. beta diversity according to Bray Curtis distances was also high, signifying differences in the OTU community composition between samples (Table 1). Alpha- and beta-diversity indices of *P. damicornis*, *S. pistillata*, and *Lobophytum* sp. were low and in contrast to those of *A. humilis* (Table 1). All other host species displayed intermediate alpha- and beta-diversity values.

***Symbiodinium* in the environment**

Twenty-four *Symbiodinium* OTUs were found in the subsampled data from the environment, including 10 OTUs from clade A (42%), 1 OTU from clade B (4%), 8 OTUs from clade C (33%), 1 OTU from clade D (4%), 1 OTU from clade F (4%), and 3 OTUs from clade G (13%). Sediment samples contained *Symbiodinium* OTUs from clade A – C while water samples were more diverse containing OTUs from clade A – D, F, and G. Five OTUs were found in both seawater and sediment samples (Fig. S2 in the Supporting Information). As with the biotic samples, OTU C1_796 was most abundant (71.2%) and it was found in every sample. OTU A3_302 was second most abundant (17.6%), while all other OTUs were each less abundant than 5%, and the least abundant six OTUs together contributed less than 1% of sequence counts. Of the rare environmental OTUs, 14 were only found at one site (Fig. S2). Roughly half of the encountered OTUs (11 of 24) in the environment were also present in symbiosis (A3_1278, A3_302, B36_356, C1_796, C1#_797, C1.8_763, C3w_1030, C42.2_1367, D5_677, G3_802, G3_860).

Spatial structure of *Symbiodinium* communities

Four OTUs (A3_302, B36_356, C1_796, C1c.C45_1398) were encountered at all sites and because of its dominance in the biotic data, OTU C1_796 was the most abundant OTU across sites (Table 2).

Given the dominance of a few common OTUs at all sites, Bray Curtis distances were not significantly different between sites (PERMANOVA, $F_{4, 78} = 1.3$, $p > 0.05$). However, presence and absence of OTUs was different between sites (PERMANOVA, $F_{4, 78} = 5.5$, $p < 0.001$; Fig. 3). This pattern was driven by the lagoon sites, Coconut Pass and Picnic Beach, which were different from all other sites (pairwise $t = 1.9 - 2.8$, $p < 0.005$ and pairwise $t = 1.7 - 2$, $p < 0.05$, respectively, with the exception of Picnic beach to Loomis reef at the edge of the lagoon pairwise $t = 1.2$, $p > 0.05$), while they were not significantly different from each other (pairwise $t = 1.1$, $p > 0.05$). The sites outside the lagoon

(Mermaid Cove, Osprey Reef) and at the edge of the lagoon (Loomis Reef) were not different from each other (pairwise $t = 1 - 1.6$, $p > 0.05$). The lagoon sites had the highest number of OTUs, and OTU communities were more evenly distributed at these than at the other sites (Table 2). In line with this, samples at Coconut Pass and Picnic Beach had significantly higher numbers of OTUs than those at Osprey Reef and Loomis Reef (ANOVA, $F_{4, 119} = 7.4$, $p < 0.001$). Samples at Mermaid Cove had intermediate numbers of OTUs that were not significantly different from those at Osprey Reef and Picnic Beach ($p > 0.05$), but significantly lower compared to those from Coconut Pass ($p = 0.01$) and higher compared to Loomis Reef ($p < 0.05$). In contrast, the beta-diversity between species at each site was similar (Table 2).

Specificity of hosts with *Symbiodinium* haplotypes

All haplotype analyses were conducted on the 101 samples that contained more than 300 sequence reads within the OTU C1_796. This OTU consisted of 271 unique sequences representing 84,873 reads (non-subsampled). The subsampled data set contained 82 distinct sequences ('haplotypes'), the most abundant of these (C1_h79; 38.7% of all reads) was a 100% match with C1 (ACN AF333515; LaJeunesse 2001), and an additional nine low abundance (< 0.5% of all reads) haplotypes were annotated to C1. Other abundant haplotypes were 100% matches with ITS2 *Symbiodinium* genotypes C8 (ACN EU808003; Sampayo et al. 2007), C120 (ACN HM185737; Bongaerts et al. 2010), C3 (ACN AF333516; LaJeunesse 2001), C1d (ACN FN298461; Pochon et al. 2010), and C64 (ACN FM877432; Pochon et al. 2010). The sum of the reads from the 55 least abundant sequences together made up less than 1% of all reads.

The associations of distinct haplotype sequences within the OTU C1_796 was species specific (Fig. 2b), but the main haplotype within the OTU (C1_h79) was still shared by many species (Fig. 4). In addition to C1_h79, samples from *S. pistillata* and *A. humilis* were consistently associated with two

other ubiquitous haplotypes, i.e., C8_h111 and C8_h239. In contrast, the majority of the remaining haplotypes were found in association with only a few host species, with whom they clustered separately from other species. For instance, most samples from *S. hystrix* associated with haplotype C120_h23, *P. damicornis* with C1d_h102, *Bayerxenia* sp. with C64_h237 and C7_h28, *M. vertebralis* with C3_h167, and *Lobophytum* sp. and *Sarcophyton* sp. with C71j_h37. All four samples from *Isis* sp. included in this analysis clustered separately, because the haplotypes they were associated with (C1c.C45_h44, C1c.C45_h208, C42.2_h67) were not shared with any other species.

Seventeen samples contained > 300 sequences belonging to the second most abundant OTU A3_302. These included all 15 samples of *T. crocea*, and one sample of *M. vertebralis* and *Cassiopeia* sp. each. Overall, OTU A3_302 consisted of 15 unique sequences representing 51,295 reads. The most abundant sequence represented 75% of all reads, while the least abundant 11 sequences together contributed less than 1% of all reads. All sequences were annotated most closely to A3 (ACN AF333507; LaJeunesse 2001), with the most abundant sequence being a 100 % match. In addition, the representative sequences of six other OTUs (A3_412, A3_413, A3_475, A3_611, A3_1263, A3_1278) best matched to *Symbiodinium* A3 as well, but together they only made up 22 reads over all samples.

DISCUSSION

What is the overall diversity of *Symbiodinium* found across different host species at Lizard Island?

The use of 454 pyrosequencing techniques elucidated how genetic variants of dinoflagellate symbionts of the genus *Symbiodinium* were distributed among different hosts and across space at a local scale on reefs surrounding Lizard Island, GBR. These samples were collected before the most recent Global Coral Bleaching Event in March 2017 that severely affected the northern part of the

GBR and that also devastated the reefs at Lizard Island (Hughes et al. 2017) and therefore represent valuable baseline data against which recovering reef communities can be compared. Applying NGS to this multispecies sampling framework, we detected 83 OTUs distinguished at the 97% level. In a study designed to focus on only the dominant *Symbiodinium* sequences, Tonk et al. (2014) observed 13 ITS2 *Symbiodinium* types in the same set of species, at mid-shelf locations of the GBR including LI. In the Western Indian Ocean five times as many host genera (75) were sampled to detect a similar number of *Symbiodinium* types as subsampled OTUs (48) in our study (LaJeunesse et al. 2004b, LaJeunesse et al. 2010). The comparably low values in other studies are likely explained by the use of more traditional methods for *Symbiodinium* typing such as DGGE, which usually focus on only the most abundant *Symbiodinium* types. This is because detection thresholds of less abundant genotypes are comparably high for DGGE (around 10% depending on type; Mieog et al. 2007).

The number of OTUs encountered at Lizard Island by this study is more similar to other studies that used next-generation sequencing techniques. For example, Thomas et al. (2014) encountered 33 OTUs at 97% level in 11 species belonging to the genus *Acropora* in Western Australia using 454 NGS. A single *Symbiodinium* OTU dominated the community assemblage and drove the diversity pattern observed around Lizard Island. This pattern is comparable to the data set from Western Australia (Thomas et al. 2014), and reinforces what is already known from using techniques with higher detection thresholds (LaJeunesse et al. 2004b, LaJeunesse 2005, Tonk et al. 2014). Given the greater sensitivity of NGS techniques, what insights do higher resolution techniques offer on *Symbiodinium* diversity assemblages?

The sensitivity to detect different *Symbiodinium* OTUs in environmental samples is increased 10,000-fold using NGS (Thomas et al. 2014), hence an obvious advantage is the resolution of those *Symbiodinium* variants present at cryptic abundances. The investigation of diversity in *Symbiodinium* from our data revealed three main patterns. Firstly, the diversity of OTUs in each sample was greater

than previously estimated. While the association with multiple symbionts in a species can be considered a given (e.g., Silverstein et al. 2012), we showed for the first time, that every individual sample is associated with at least two symbiont OTUs. Secondly, each sample hosted multiple symbionts, but the total number of symbionts for each species exceeded the mean number of OTUs per sample. In fact, individual samples from each species were quite distinct from their conspecifics, which indicates that high sample replication within each taxon is required in future studies on *Symbiodinium* diversity. Lastly, the differences between samples lay primarily in the background, low abundance diversity, rather than in the association with an exclusive main *Symbiodinium* OTU. The lack of specificity among the low abundance OTUs and hosts warrants further inspection.

Can the patterns of diversity be associated with specific traits of the host, such as host phylogeny or symbiont transmission mode?

The community assemblage of *Symbiodinium* spp. encountered across all sampling sites around Lizard Island in this study largely reflects large-scale biogeographic patterns and is in accordance with assemblages of the southern (LaJeunesse et al. 2003), central (LaJeunesse et al. 2004b), and northern (Tonk et al. 2014) GBR. *Symbiodinium* C1 was dominant across all sampling sites around Lizard Island, but not in all host species; *T. crocea* and *Cassiopea* sp. were both dominated by *Symbiodinium* A3, which is found less frequently in the region, but was reported from these hosts before (LaJeunesse 2001, LaJeunesse 2002, LaJeunesse et al. 2004b). The only host species that was sometimes dominated by another type (*Symbiodinium* C42.2) was *E. quadricolor*, for which a latitudinal shift of *Symbiodinium* types from C25 and C3-variants in the south to C42.2. and C1 in the north of the GBR is congruent with our data (Pontasch et al. 2014).

The overall diversity of *Symbiodinium* spp. in *A. humilis* was markedly higher than in any of the other hard corals. This may be related to the fact that it represents the only hard coral species with horizontal symbiont transmission we sampled (Baird et al. 2009). Horizontal symbiont transmission has previously been suggested to increase host-symbiont recombination frequency and decrease host-symbiont specificity (LaJeunesse et al. 2004a, Stat et al. 2008). The initial infection with *Symbiodinium* in coral larvae is relatively unspecific, but followed by the development of a more specific host-symbiont association during the coral host's adult life (Abrego et al. 2009). Our findings in turn highlight that this seeming specificity/ fidelity is accompanied by a plethora of *Symbiodinium* OTUs at background abundances.

Samples from the genus *Acropora* were also the most diverse in *Symbiodinium* OTUs compared to all other (non-hard coral) taxa. However, this list also contains genera, known to release symbiont-free gametes, including *Sarcophyton* (Benayahu and Loya 1986, Barneah et al. 2004), *Xenia/Bayerxenia* (Barneah et al. 2004), *Tridacna* (Mies et al. 2012), and *Marginopora* (Lee 2006, during their rare sexual reproduction events; Fay et al. 2009). This in turn indicates that the transmission mode of *Symbiodinium* between generations cannot be the only determinant influencing the specificity of the host.

What is the turnover (i.e., beta diversity) of *Symbiodinium* across sites and taxa at Lizard Island?

While the spatial pattern in OTU diversity was mainly driven by symbiotic community patterns, i.e., by the host species encountered at each site, the OTU turnover was comparably high with 27 of 48 (56%) OTUs only found at one site and in one host species. Despite the predominance of one OTU, the high turnover can thus be explained by the remainder of the OTUs, which were rare and therefore not shared between many samples or hosts. 76% of sequences belonged to OTU C1_796

and 15% to OTU A3_302, so that all other remaining OTUs together comprised only 9% of sequence reads. This has important implications for biodiversity estimates, because it illustrates that diversity in these systems is strongly linked to host diversity, and sampling only one or few host species, even when highly replicated, will not reveal the full diversity in the *Symbiodinium* system. Single host studies will not allow us to predict changes in the host-symbiont response to climate change, and thus, examining host species with differing internal environments and life history traits is critical.

Interestingly, *Symbiodinium* community diversity was different between the sites around Lizard Island, with the two most exposed sites (Coconut Pass and Picnic Beach) displaying higher diversity and evenness than the other more sheltered sites. While different patterns in diversity of *Symbiodinium* spp. have been linked to the diversity of the host species and the evolutionary rates of diversification in ocean basins (Lajeunesse 2005), the causes for differences in the diversity between sites on a local scale remain poorly understood (Done 1982, Wismer et al. 2009). Several possible scenarios may explain the observed differences. For instance, when the sites are grouped according to their exposure to the prevailing southeasterly winds, the two most exposed sites are also the most diverse. On the one hand, these sites may benefit from the supply of non-resident *Symbiodinium* types with water currents. On the other hand, assuming the exposed sites also experience more variable environmental conditions, a larger genetic pool of *Symbiodinium* may be an adjustment to cope with this variability (Thornhill et al. 2006b). Furthermore, the sampling sites may vary in their history of disturbances, such as bleaching events, cyclone damage, and predation by crown-of-thorn starfish, which may have shifted the baseline at some of the sites.

What is the congruence between *Symbiodinium* communities in the environment and the host organisms?

Despite the potential importance of the pool of free-living *Symbiodinium* spp. to the resilience of reefs, these *Symbiodinium* communities in the water column and sediment are a largely overlooked topic in coral reef research. Few previous studies attempted to relate environmental *Symbiodinium* communities to those in the host species, for example by exposing aposymbiotic recruits to the natural reef environment (Coffroth et al. 2006, Thornhill et al. 2006a). Only two studies directly compared free-living and host-associated *Symbiodinium*, with one of these limited to a single host species and to the detection of the most abundant types using DGGE (Sweet 2014) and the other using a NGS-framework and multispecies approach focusing on hard corals (Cunning et al. 2016). Thus the present study represents a more comprehensive account of *Symbiodinium* diversity in diverse host species and the surrounding coral reef environment.

The diversity of *Symbiodinium* in the environment was higher compared to the host samples, and this difference persisted despite the lower cut-off for subsampling of sequence reads in the environmental samples. The density of *Symbiodinium* cells in reef water (up to 80 cells · mL⁻¹) and sediments (1,000 - 4,000 cells · mL⁻¹) around Lizard Island is relatively high (Littman et al. 2008), and together with the presented data suggests a substantial pool of *Symbiodinium* diversity in the coral reef environment.

We detected a wide phylogenetic range of *Symbiodinium* from six clades in the environmental samples (A - D, F, and G); these largely reflect the diversity of environmental samples observed in other regions. For instance, clades A, C, and E were detected in water and sediments at Heron Island (Sweet 2014) and clades A - D, F, G, and H in the Caribbean and Pacific (Coffroth et al. 2006, Manning and Gates 2008, Takabayashi et al. 2012, Cunning et al. 2016).

The dominant OTUs found in the environment were also most abundant in the host species, and more generally roughly half of environmental *Symbiodinium* OTUs were also recovered in symbio. The congruence between endosymbiotic and environmental *Symbiodinium* communities on Lizard Island supports similar findings from Heron Island in the GBR, Hawaii, and Florida (Takabayashi et al. 2012, Sweet 2014), but less symbionts were shared between hard corals and the environment at American Samoa (Cunning et al. 2016). Generally, this pattern suggests the possibility of recolonization of the resident *Symbiodinium* types from the environment back into a bleached host, corresponding to the initial uptake of symbionts of aposymbiotic recruits upon exposure to reef water and sediments (Coffroth et al. 2006, Adams et al. 2009). Given the potential importance of recolonization processes during the stress response of coral reef organisms, further investigation of the relationship between host-associated and free-living *Symbiodinium* assemblages is needed.

Unlike the *Symbiodinium* community found in the exclusive host environment, a variety of planktonic and benthic eukaryotes (e.g., diatoms, fungi, and other dinoflagellates) dominate free-living microbial communities. This poses a challenge in the molecular detection of *Symbiodinium* from environmental samples and may result in the yield of low numbers of target sequences as was the case in this study. For instance, no *Symbiodinium* ITS2 sequence was amplified in water samples in a study conducted at Hawaii, and in the successful amplification of 3 out of 18 sediment samples from the same study only five *Symbiodinium* types were recovered using the Cp23S HVR marker (Pochon et al. 2010). Similarly, using an 18S marker on water and sediment samples, only 0.01% of PCR amplified sequences were annotated as *Symbiodinium* (Littman et al. 2010). Hence, one persisting challenge in the study of environmental *Symbiodinium* diversity seems to be the selection of specific primers for PCR amplification. In addition, alternative sampling and preservation techniques such as size fractionation could be explored to increase the yield of target *Symbiodinium* sequences.

Does the well-accepted 97% cut-off for OTU clustering adequately describe the diversity seen in mixed host communities?

The intragenomic variability of the ITS2 region is structured by one dominant sequence and a number of less abundant ITS2 sequence haplotypes, for which numbers vary with *Symbiodinium* genotype (Lajeunesse 2005, Thornhill et al. 2007). Intragenomic variance of the ITS2 region was investigated in several recent studies using NGS on isoclonal cultures of *Symbiodinium*, and a similarity cut-off of 97% was recommended for clustering of *Symbiodinium* ITS2 sequences in an OTU-based framework to avoid overestimation of *Symbiodinium* diversity (Arif et al. 2014, Green et al. 2014, Quigley et al. 2014, Thomas et al. 2014). This is the same cut-off commonly used for 16S-based analysis of prokaryotic communities (Stackebrandt and Goebel 1994, Schloss and Handelsman 2006). Until now, the application of the 97% threshold to *Symbiodinium* ITS2 data has never been tested extensively in a biological multi-species framework, which is critical to our understanding of diversity patterns in mixed host- and symbiont species assemblages. Our dataset with OTUs clustered at 97% similarity level was dominated by a single OTU C1 (76% of sequence reads). OTU C1 contained 271 unique sequences, of which the most frequent (37%) was accompanied by several other abundant haplotype sequences, which each represented 100% matches to other previously described ITS2 types. These distinct sequences showed discrete clustering with specific host species, indicating that if rigorously applied, a cut-off of 97% for *Symbiodinium* OTU analysis will likely miss a potentially important part of the diversity in mixed host-*Symbiodinium* pairings.

The challenge in determining a meaningful cut-off for OTU-based analysis of ITS2 diversity that reflects *Symbiodinium* genotypic diversity lies in the divergence of this region between lineages of *Symbiodinium*. For instance, *Symbiodinium* type C1 stands at the base of a diverse lineage, formed through a series of adaptive radiation events (Lajeunesse 2005). Within this lineage, single point mutations in the ITS2 marker represent completely different ecological properties (Hume et al.

2015), deeming an OTU-based approach unsuitable to detect ecological units. On the other hand the second most abundant OTU, *Symbiodinium* A3, contained less than a tenth of the sequence variance of C1 within the 97% cut-off, which is in agreement with previous cloning-based comparisons of ITS2 intragenomic variance between cultures of *Symbiodinium* types A3 and C1 (Thornhill et al. 2007). Further, all haplotype sequences matched best to the *Symbiodinium* A3 type sequence and additionally, several other OTUs with best matches to *Symbiodinium* A3 were encountered, indicating that the intragenomic variance of ITS2 type A3 may exceed the 97% threshold. In conclusion, previous studies found the 97% cut-off to be adequate for some *Symbiodinium* lineages, while our data suggest that this cut-off might be too low (e.g., type C1) or too high (e.g., type A3) for others. Within-sample OTU clustering in contrast to across-sample clustering, as recently introduced by Cunning et al. (2017), may address some of these challenges, but only in cases where the most abundant ITS2 sequences are distinct. Given the differences in intra- and intergenomic variance of the ITS2 marker, we advocate a multi-phased approach, in which initial haplotype screening (introduced by Smith et al. 2017) should be combined with OTU-based clustering to reveal all levels of genetic variance in mixed-community data sets.

Conclusions

In this study we investigated how the patterns in community composition and diversity of the dinoflagellate *Symbiodinium* are distributed across environmental space and among six different taxonomic orders of host species at a local scale in the GBR to understand the drivers of the symbiotic community assemblage. Our data show that the *Symbiodinium* community is dominated by two abundant OTUs, even though the communities in individual samples were quite distinct from each other. Furthermore, sequence-based patterns revealed some degree of host species-specificity, suggesting that genetic similarity cut-offs for the analysis of *Symbiodinium* ITS2 data sets need careful evaluation. We conclude that sampling multiple host taxa with differing internal

environments will be critical to fully understand the symbiont diversity of a given system, and to predict changes of this system considering the differential stress response of the taxa within. Our study also highlights a great overlap in the occurrence of *Symbiodinium* between the environment and the host species, which has been a largely overlooked component of coral reef systems. This aspect warrants more attention in future studies and the application of NGS techniques will enhance the understanding of host-symbiont assembly patterns in coral reefs and guide research on the functional diversity of the coral-*Symbiodinium* association.

ACKNOWLEDGEMENTS

We are grateful to Lizard Island Research Station staff for their support. Samples were collected under Great Barrier Reef Marine Parks permit # G13/36006.1. The trip to Lizard Island and the molecular work were funded by the UNM Honors College Research Institute, Sonnet and Ian McKinnon and by a UNM Research Allocations Grant. Research reported in this publication was supported by the National Institute of General medical Sciences of the National Institutes of Health under Award Number P30GM110907. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. We thank the UNM undergraduate course participants for assistance with sample collection and the reviewers and editor for their comments that helped to improve this manuscript.

DATA ACCESSIBILITY

Sequences determined in this study have been deposited in the NCBI Sequence Read Archive under accession no. PRJNA413992.

AUTHOR CONTRIBUTIONS

US, CTV, ES, and MZ designed the study, MZ, ES, and US collected the samples, ES conducted lab work, MZ and DC conducted data processing, MZ conducted data analysis, CTV and US contributed reagents, MZ and US wrote the manuscript with contributions from ES, DC, and CTV.

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TABLES

Table 1: Summary of sequence analyses of OTUs clustered at 97% similarity level per host species and most abundant haplotype sequences within the most abundant OTU C1_796.

Order	Species	n	# of QCed sequences	# of sites	# of OTUs	# of OTUs per sample	dominant OTU	dominant haplotype in OTU C1_796	Shannon	Equitability	Bray Curtis distance	Binary Jaccard dist.
Scleractinia	<i>Acropora humilis</i>	7	5,645	4	18	4.57 (1.90)	C1	C8/C1	0.55 (0.39)	0.26 (0.18)	0.38 (0.40)	0.77 (0.12)
	<i>Pocillopora damicornis</i>	14	11,561	5	13	2.79 (1.53)	C1	C1d/C1/C42a	0.11 (0.12)	0.07 (0.05)	0.02 (0.02)	0.57 (0.22)
	<i>Seriatopora hystrix</i>	13	9,217	5	15	3.07 (1.38)	C1	C120/ C1	0.30 (0.33)	0.17 (0.18)	0.09 (0.08)	0.72 (0.15)
	<i>Stylophora pistillata</i>	15	13,655	5	10	2.47 (1.19)	C1	C8/C1	0.11 (0.10)	0.07 (0.06)	0.03 (0.03)	0.63 (0.18)
Alcyonacea	<i>Bayerxenia</i> sp.	7	5,348	3	8	2.72 (1.25)	C1	C64/C7/C8	0.27 (0.35)	0.14 (0.16)	0.11 (0.11)	0.62 (0.15)
	<i>Isis</i> sp.	6	5,336	3	5	2.50 (1.05)	C1	C1/C8/C1c.C45	0.52 (0.65)	0.28 (0.33)	0.31 (0.27)	0.67 (0.13)
	<i>Lobophytum</i> sp.	14	12,482	5	11	2.29 (1.20)	C1	C1/C71j	0.13 (0.10)	0.09 (0.06)	0.02 (0.02)	0.49 (0.26)
	<i>Sarcophyton</i> sp.	14	10,703	5	8	2.50 (1.45)	C1	C1/C71j	0.21 (0.20)	0.14 (0.13)	0.05 (0.05)	0.58 (0.18)
Actinaria	<i>Entacmaea quadricolor</i>	10	15,981	4	10	2.80 (1.32)	C42.2 /C1	C42.2	0.32 (0.29)	0.22 (0.25)	0.55 (0.41)	0.61 (0.18)
	<i>Heteractis magnifica</i>	2	4,489	2	3	2.50 (0.71)	C3nn	C3	0.28 (0.05)	0.25 (0.02)	0.02	0.33
Veneroida	<i>Tridacna crocea</i>	15	47,938	5	6	2.20 (0.78)	A3	C9/C66	0.22 (0.18)	0.19 (0.17)	0.05 (0.04)	0.34 (0.27)
Miliolida	<i>Marginopora vertebralis</i>	9	6,605	4	10	2.89 (1.05)	C1	C3	0.46 (0.43)	0.27 (0.22)	0.26 (0.23)	0.58 (0.12)

Rhizostomae	<i>Cassiopea</i> sp.	1	2,605	1	2	2.00	A3	C21/C3/ C8	0.30	0.29		
Porifera		1	975	1	3	3.00	C1	C8/C1	0.72	0.37		
ALL GENERA		128	152,540	5	48	2.70 (1.33)	C1				0.40 (0.41)	0.66 (0.19)
Sediment		8	2,131	5	11	3.50 (1.41)	C1	C8/C1	0.95 (0.34)	0.59 (0.16)	0.27 (0.18)	0.52 (0.18)
Seawater		8	930	3	18	4.13 (1.46)	C1	C1/C15	0.84 (0.42)	0.44 (0.16)	0.32 (0.16)	0.77 (0.08)

Table 2: Summary of sequence analyses of OTUs clustered at 97% similarity level for the five sampling sites summarized for all host species found at the site excluding environmental samples.

Site	# of quality filtered sequence reads	# of host species	# of OTU	# of OTU per sample	dominant OTU	Shannon	equitability	Bray Curtis distance	Binary Jaccard distance
Coconut Pass	33,969	10	24	3.87 (1.84)	C1	0.57 (0.38)	0.31 (0.19)	0.41 (0.32)	0.56 (0.18)
Picnic Beach	34,911	10	18	3.14 (1.64)	C1	0.28 (0.29)	0.16 (0.14)	0.31 (0.37)	0.64 (0.20)
Loomis Reef	27,454	8	14	2.10 (1.22)	C1	0.06 (0.07)	0.05 (0.07)	0.34 (0.46)	0.63 (0.27)
Osprey Reef	26,806	10	15	2.04 (0.69)	C1	0.08 (0.05)	0.06 (0.04)	0.42 (0.48)	0.65 (0.18)
Mermaid Cove	29,400	11	16	2.71 (1.27)	C1	0.20 (0.19)	0.15 (0.20)	0.32 (0.42)	0.60 (0.21)
ALL	152,540	11	48	2.86 (1.57)	C1	0.26 (0.31)	0.17 (0.18)	0.38 (0.40)	0.66 (0.19)

FIGURE LEGENDS

Figure 1: Sampling sites 1-5 around Lizard Island. 1: Coconut Pass (-14.685 °S, -145.467 °W), 2: Picnic Beach (-14.697 °S, -145.448 °W), 3: Loomis Reef (-14.683 °S, -145.449 °W), 4: Osprey Reef (-14.668 °S, -145.443 °W), 5: Mermaid Cove (-14.646 °S, -145.454 °W). Dashed lines represent reefs; the white star marks the location of Lizard Island Research Station (LIRS). Map courtesy of LIRS.

Figure 2: Distribution of *Symbiodinium* OTUs (a) and *Symbiodinium* haplotype sequences within the most abundant OTU C1_796 (b) in each host genus per site at Lizard Island, Great Barrier Reef (n = 3). Site designations 1: Coconut Pass, 2: Picnic Beach, 3: Loomis Reef, 4: Osprey Reef, 5: Mermaid Cove

Figure 3: Ordination (nMDS) of *Symbiodinium* OTU community over species (a) and sites (b) based on Bray Curtis (a) and Binary Jaccard distances (b).

Figure 4: Network analysis of haplotype sequences annotated to *Symbiodinium* OTU C1_796 for their occurrence in different host species, which are represented by symbols. Unique sequences are represented by nodes the most prominent of which are labeled with their closest ITS2 annotations.

SUPPORTING INFORMATION

Table S1 *Symbiodinium* OTU abundance counts over samples with annotation and ITS2 reference sequence for each OTU.

Figure S1: Rarefaction curves of *Symbiodinium* ITS2-based OTUs from all organisms, water, and sediment samples collected at Lizard Island, Australia.

Figure S2: *Symbiodinium* OTUs at each of five sampling sites around Lizard Island present in environmental samples taken from the water column and sediments, OTUs marked with an asterisk were also found in the host species.