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ORIGINAL ARTICLE

Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals

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Reef-building corals possess a range of acclimatisation and adaptation mechanisms to respond to seawater temperature increases. In some corals, thermal tolerance increases through community composition changes of their dinoflagellate endosymbionts (*Symbiodinium* spp.), but this mechanism is believed to be limited to the *Symbiodinium* types already present in the coral tissue acquired during early life stages. Compelling evidence for symbiont switching, that is, the acquisition of novel *Symbiodinium* types from the environment, by adult coral colonies, is currently lacking. Using deep sequencing analysis of *Symbiodinium* rDNA internal transcribed spacer 2 (ITS2) PCR amplicons from two pocilloporid coral species, we show evidence consistent with *de novo* acquisition of *Symbiodinium* types from the environment by adult corals following two consecutive bleaching events. Most of these newly detected symbionts remained in the rare biosphere (background types occurring below 1% relative abundance), but one novel type reached a relative abundance of ~33%. Two *de novo* acquired *Symbiodinium* types belong to the thermally resistant clade D, suggesting that this switching may have been driven by consecutive thermal bleaching events. Our results are particularly important given the maternal mode of *Symbiodinium* transmission in the study species, which generally results in high symbiont specificity. These findings will cause a paradigm shift in our understanding of coral-*Symbiodinium* symbiosis flexibility and mechanisms of environmental acclimatisation in corals.

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Introduction

The eukaryotic and prokaryotic microbial communities (that is, the microbiome) associated with animals and plants have essential roles in their health and functioning (McFall-Ngai *et al.*, 2013). Reef-building corals form symbioses with a wide range of microbial symbionts, including phototrophic dinoflagellates in the genus *Symbiodinium*. As the coral host depends on photosynthate for nutrition, a prolonged breakdown of the symbiosis (referred to as coral bleaching) often leads to coral death (Baker, 2003). Episodes of mass coral bleaching have increased in frequency and intensity due to climate change and have caused a substantial loss in coral cover in many coral reef regions over the last few decades (Hoegh-Guldberg, 1999; Hoegh-Guldberg *et al.*, 2007; De'ath *et al.*, 2012).

The role of *Symbiodinium* symbionts in acclimatisation of the coral holobiont to environmental changes has been extensively covered in the recent literature (Blackall *et al.*, 2015). The genus *Symbiodinium* is classified into nine phylogenetic clades (A through I) based on DNA sequence analysis, with a range of different types (putative species) within each clade (Pochon and Gates, 2010). *Symbiodinium* types can be transmitted directly from parent to offspring via eggs (vertical transmission) or aposymbiotic larvae/early recruits can acquire their symbionts from the environment (horizontal transmission) (Harrison and Wallace, 1990; van Oppen, 2001; Padilla-Gamino *et al.*, 2012). Different *Symbiodinium* types have distinct physiological optima and stress tolerance levels, which confer different

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phenotypes to their coral hosts. For instance, corals dominated by *Symbiodinium* clade D are generally more thermally tolerant compared with those predominantly associating with types in other clades (Berkelmans and van Oppen, 2006).

More than one *Symbiodinium* type can exist simultaneously within a single coral host (Mieog *et al*., 2007; Correa *et al*., 2009; Silverstein *et al*., 2012); these can occur in high abundance as 'dominant types' or in very low abundance known as 'background types', that is, the '*Symbiodinium* rare biosphere'. In other microbial ecosystems, the rare biosphere represents a low-abundance, high-diversity group (in terms of numbers of operational taxonomic units) representing $<1\%$ of relative abundance (Sogin *et al*., 2006; Reid and Buckley, 2009). Therefore, in the present study, all *Symbiodinium* types that occurred below this threshold were considered members of the '*Symbiodinium* rare biosphere'.

The capacity of reef-building corals to host different symbionts (symbiotic flexibility) suggests two potential adaptive mechanisms to environmental changes: symbiont 'shuffling' and 'switching' (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004). Some corals have been shown to resist and/or recover from thermal stress through changes in the relative abundance of *Symbiodinium* types that constitute the *in hospite* community, that is, symbiont shuffling (Baker *et al*., 2004; Rowan, 2004). This acclimatisation response is well documented (Baker *et al*., 2004; Chen *et al*., 2005; Berkelmans and van Oppen, 2006; Jones *et al*., 2008; Baskett *et al*., 2009), but is believed to be limited to the *Symbiodinium* types acquired vertically or horizontally in early life stages. Symbiont 'switching' refers to a change in the *in hospite Symbiodinium* community due to the uptake of new *Symbiodinium* types from the environment, potentially from the water column and sediments (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004). Preliminary studies have indicated that adult corals are unable to form stable symbioses with exogenous algal symbionts; therefore, this mechanism is believed to occur only during a relatively short period of the coral larval and early juvenile life stages (Goulet and Coffroth, 2003; Little *et al*., 2004; Coffroth *et al*., 2010).

Testing of this hypothesis has been hampered, however, by the use of genetic methods that lack sensitivity to detect *Symbiodinium* types that occur below 5–10% of total relative abundance. Here, we challenge this notion by exploring the *Symbiodinium* rare biosphere using next-generation sequencing, a cost-effective, high-throughput method that has been recently shown to accurately detect low-abundance *Symbiodinium* types (Quigley *et al*., 2014; Thomas *et al*., 2014; Arif *et al*., 2014; Green *et al*., 2014; Edmunds *et al*., 2014b). We assess *Symbiodinium* communities in a time-series sample set to investigate (1) the cryptic diversity of the *Symbiodinium* rare biosphere within two common pocilloporid species; (2) possible changes within the *Symbiodinium* community over a period of time that spans two successive bleaching events; and (3) whether *Symbiodinium* shuffling and/or switching has occurred in pocilloporid corals from a subtropical reef at Lord Howe Island (LHI), eastern Australia.

Materials and methods

Study location

Lord Howe Island is located 600 km off the east coast of northern New South Wales, Australia, in a dynamic oceanographic transitional region at latitude 31.5 °S and longitude 159.0 °E (Harriott *et al*., 1995). LHI is the world's southernmost true lagoonal coral reef, which was inscribed on the UNESCO World Heritage list in 1982 and classified as a Marine Park in 2002 (Hutton and Harrison, 2004). This isolated island supports unique benthic reef assemblages resulting from a biogeographical overlap of tropical, subtropical and temperate marine species, which accounts for the high species diversity present (Harriott *et al*., 1995). Although located over 1000 km from the southernmost regions of the Great Barrier Reef, approximately 100 scleractinian coral species have been reported to occur on its fringing reefs and on rocky substrate in deeper waters, providing habitat for many threatened and protected marine species (Harriott *et al*., 1995, P Harrison, unpublished data). The occurrence of tropical coral species at LHI results from the influence of the East Australian Current that flows southwards from the Great Barrier Reef, enabling the migration of some tropical marine species further south (Harriott *et al*., 1995; Ayre and Hughes, 2004; Noreen *et al*., 2009).

Sample collection and DNA extraction

During the 2010 and 2011 austral summers, abnormally high sea surface temperatures, high light penetration and calm seas resulted in the first recorded extensive and severe coral bleaching at LHI (Harrison *et al*., 2011; Dalton and Carroll, 2011). Up to 95% of the coral community in the lagoon displayed variable bleaching with 41% and 56% mortality occurring in *Pocillopora damicornis* and *Stylophora pistillata,* respectively; two species that dominate the LHI lagoonal coral community (Harriott *et al*., 1995; Dalton and Carroll, 2011).

Two hundred coral fragments were collected (*P. damicornis* $n=110$; *S. pistillata* $n=90$) from two locations within the lagoon (Comet's Hole and North Bay Wreck) over a 2-year period: 2 and 6 months after the first bleaching event in 2010, during the second bleaching event in 2011, and 18 months afterwards in 2012. All samples were fixed in absolute ethanol and DNA was extracted following

Wayne's method with slight modifications (Lundgren *et al.*, 2013).

Amplification of the internal transcribed spacer 2 region and preparation for Roche 454 targeted amplicon sequencing

The *Symbiodinium* nuclear DNA ribosomal internal transcribed spacer 2 (ITS2) region was amplified by PCR using the specific forward 5′-GTGAATTGC AGAACTCCGTG-3′ and reverse 5′-CCTCCGCTTAC TTATATGCTT-3′ primers, which further contained a known 10-bp tag (identifier) allowing the identification of amplicons from different samples after pooling for 454 sequencing. Each 25-µl PCR contained 1 µl of 1/100 diluted DNA template (from 20 to 50 ng μl^{-1}), 12.5 µl of Taq HotStart mix (Bioline, Eveleigh, NSW, Australia), 2 µl of 2 µM of each forward and reverse primer and 9.5 µl of DNAse-free water. PCR was performed using the following conditions: 95 °C for 5 min, followed by 30 cycles of 30 s at 95 °C, 40 s at 52 °C and 30 s at 72 °C, with a final extension at 72 °C for 3 min. PCR products were run on a 1% TAE-agarose gel, excised and purified using an in-house method before a second PCR step. For this second PCR, each 35 µl PCR contained 1 µl of purified PCR template, 12.5 µl of Taq HotStart mix (Bioline), 2 µl of 2 µM of each forward and reverse primer and 20.5 µl of DNAse-free water. PCR was performed using the following conditions: 95 °C for 10 min, followed by 10 cycles of 30 s at 95 °C, 40 s at 52 °C and 1 min at 72 °C, with a final extension at 72 °C for 10 min. PCR products were purified using Sephadex G-50 Columns (Sigma, Castle Hill, NSW, Australia). To ensure good coverage per sample, up to 44 PCR products per quarter of plate were pooled. Pooled samples were sent to an external sequencing provider (Macrogen, Seoul, Republic of Korea) for 454 targeted amplicon sequencing using Roche GS FLX Technology.

Bioinformatic analysis of 454 sequencing output

The raw 454 sequencing reads were demultiplexed and denoised using QIIME (Caporaso *et al.*, 2010). First, demultiplexing and quality control were performed which included filtering sequences with short reads (<150 bp), low read quality (<20), sequences with >6 ambiguous base calls and sequences that imperfectly matched the priming and the barcoding site. After each read was assigned to one barcode and the reverse primer truncated, sequences were denoised in order to remove noise (errors) generated by the amplification and sequencing process (Reeder and Knight, 2010).

To assign an identity to each read, SymTyper (www.symtyper.com, M Belcaid in review, see Edmunds *et al.*, 2014a; Cunning *et al.*, 2015), a Symbiodinium-specific bioinformatics pipeline was used. Type level assignment was completed in SymTyper using BLAST against a *Symbiodinium* reference ITS2 database (www.symtyper.com) that classified sequences into six categories: (1) Multiple Hits—alignment with equal similarity and length to multiple target sequences; (2) Perfect—unambiguous alignment to only one sequence in the reference database (for example, 100% similarity to 96% of the length of the target); (3) Unique—alignment of >97% similarity over 96% of the target length; (4) New—no alignment to a single target with >97% similarity over 96% of the length of the target; (5) ShortNew—alignment with high similarity to a reference sequence according to the dynamic similarity threshold (1); and (6) Short—does not meet minimum similarity and length requirements (for example, <90% similarity to <96% of the length of the target). To confidently resolve subtypes for reads that are shorter than the reference sequences, SymTyper dynamically adjusts the minimum similarity threshold required to accept a hit such that, as the query length decreases to 90%, the percent similarity required to accept a hit increases. In addition to satisfying the dynamic similarity threshold for short reads, a best hit is also required to be unique (highest raw bit score) for the hit to be valid. The minimum required similarity threshold is computed as follows:

$$\text{Required similarity} = 100 - \frac{C - \min_c}{1 - \min_c} \times (100 - \min_s)$$

where C is the actual coverage fraction of the query and the hit sequences; $\min_c$ is the minimum accepted coverage fraction between the query and the hit sequences and $\min_s$ is the minimum similarity threshold between the query and the hit sequences.

Short sequences were removed from the analysis, while New sequences were manually compared using nucleotide BLAST in NCBI and reported to the clade level only (that is, LHI_C.XX). Sequences with Multiple hits were placed in a phylogenetic tree, assigned to the most recent common ancestor node and reported to the clade level with a node ID (that is, C_I:52). The raw sequences have been deposited in NCBI under accession number PRJNA311610.

Statistical analysis

All statistical analyses were conducted using PRIMER v.6 software (http://www.primer-e.com). To compare the genetic structure of *Symbiodinium* in *P. damicornis* and *S. pistillata*, the log-transformed abundance of the *Symbiodinium* types was compared for each pair of samples using the Bray-Curtis similarity coefficient. A non-metric multidimensional scaling ordination diagram was produced using the AVERAGE function to visualise the relationship of *Symbiodinium* communities within *P. damicornis* and *S. pistillata* over time and between sites. To test for significant spatial and temporal partitioning of *Symbiodinium* communities within hosts, a PERMANOVA test was performed with 'host', 'time' and 'site' as fixed factors, using type III sums of squares and unrestricted

permutation of raw data. A *post hoc* pair-wise comparisons test among all pairs of levels of 'host × time × site' factor was used to identify where these significant differences occurred. The Similarity Percentages (SIMPER) test was used to identify *Symbiodinium* types contributing towards dissimilarity using a 90% cutoff for low contributions of selected variables between groups. Shannon diversity (H') and species richness (S) indices were calculated and plotted in SPSS (http://www-01.ibm.com/software/au/analytics/spss).

Results

Symbiodinium diversity within Pocillopora damicornis and Stylophora pistillata using next-generation sequencing

The deep sequencing analysis of *Symbiodinium* rDNA ITS2 PCR amplicons, yielding 5115 ± 189 s.e.m. and 4730 ± 440 s.e.m. reads per coral colony (see Supplementary Figure S4) for *P. damicornis* and *S. pistillata*, respectively, revealed a total of 258 *Symbiodinium* types belonging to clades A, B, C, D, F and G. Among these, 51 were previously known sequence types while 207 were undescribed *Symbiodinium* ITS2 sequences. All members newly discovered here (with the exception of a few C types) formed part of the *Symbiodinium* rare biosphere. A mean of 11 ± 0.17 and 10 ± 1.95 s.d. *Symbiodinium* types were simultaneously hosted in each *P. damicornis* and *S. pistillata* colony, respectively.

We acknowledge that the multiple-copy nature of the ITS2 region, which may result in pseudogenes or numerous low-abundant functional variants (Thornhill *et al*., 2007; Sampayo *et al*., 2009; LaJeunesse and Thornhill, 2011; Arif *et al*., 2014), can affect the interpretation of next-generation sequencing data as an individual sequence does not necessarily represent an individual biological entity (Stat *et al*., 2011). However, while not designed as a species delineation taxonomic approach, the data presented here represent a robust comparative approach to assess genetic variation and new genetic variants in the assemblage of *Symbiodinium* sequences throughout two consecutive bleaching events.

Symbiodinium community changes throughout two consecutive bleaching events

After the first bleaching event in 2010 and during the second bleaching event in 2011, the two coral species were associated with the dominant type C_I:52, representing an average of 99.3% of the total *Symbiodinium* abundance in *P. damicornis* and 96.2% in *S. pistillata* (Figures 1a and b). Although no changes within dominant types were observed during this period, PERMANOVA tests revealed significant spatial and temporal partitioning of *Symbiodinium* communities throughout the two thermal stress periods (PERMANOVA $P = 0.001$, Supplementary Table S1). Indeed, a few shuffling events occurred within the *Symbiodinium* rare biosphere between May 2010 and September 2010 and between September 2010 and March 2011 (Figures 1a and b). Several instances of new appearances of *Symbiodinium* types previously not observed in the coral tissues were also recorded, which we interpret as *de novo* uptake (that is, switching events). These new acquisitions resulted in new members in the rare biosphere (Figures 1a and b, and see Supplementary Figures S1 and S2 for the complete list of all *Symbiodinium* rare biosphere members detected).

In contrast, 18 months after the second bleaching event (September 2012), significant changes in the *Symbiodinium* community composition harboured by the two coral species were observed, with changes occurring in both dominant types and within the rare biosphere (Figure 2; non-metric multidimensional scaling *post hoc* test $P = 0.001$, Supplementary Tables S3 and S4). Shuffling of type C_I:53, which previously belonged to the rare biosphere (0.03% of the total abundance in 2011), resulted in a mean relative abundance of 47% in 2012, while abundance of the previously dominant C_I:52 was significantly reduced following the second bleaching event (Figures 1a and b). Furthermore, in five *P. damicornis* samples, there was uptake of exogenous *Symbiodinium* LHI_C.28, which reached a mean relative abundance of ~33% in these samples (Supplementary Figure S3), and ~7% mean relative abundance when averaged across the 20 sampled colonies (Figure 1a). Additionally, the relative abundance of the previously dominant C_I:52 type declined to ~4% (Supplementary Figure S3). Of particular importance, our results suggest the *de novo* acquisition of two members of clade D, types D_I:6 and D1.12 in both coral species. These D types were the most abundant types within the rare biosphere in September 2012 (Figures 1a and b).

Temporal changes in the *Symbiodinium* community composition were mostly due to members in the rare biosphere. The SIMPER test revealed that the two co-dominant types C_I:52 and C_I:53 found in association with both *P. damicornis* and *S. pistillata* in September 2012, explained only 4.52% and 11.47% of the dissimilarity in the *Symbiodinium* community composition respectively. More than 80% of the dissimilarity between the disturbance period '2010–2011' and the recovery period in 2012 were explained by the rare biosphere (Supplementary Table S2). In addition, the *Symbiodinium* community diversity (using Shannon's index) was 10 times higher in September 2012 than previous (Figure 2 and Supplementary Table S5).

Discussion

Extraordinary Symbiodinium diversity and symbiotic flexibility in LHI reef-building corals

In both coral species, the deep sequencing analysis revealed an extraordinary diversity within the

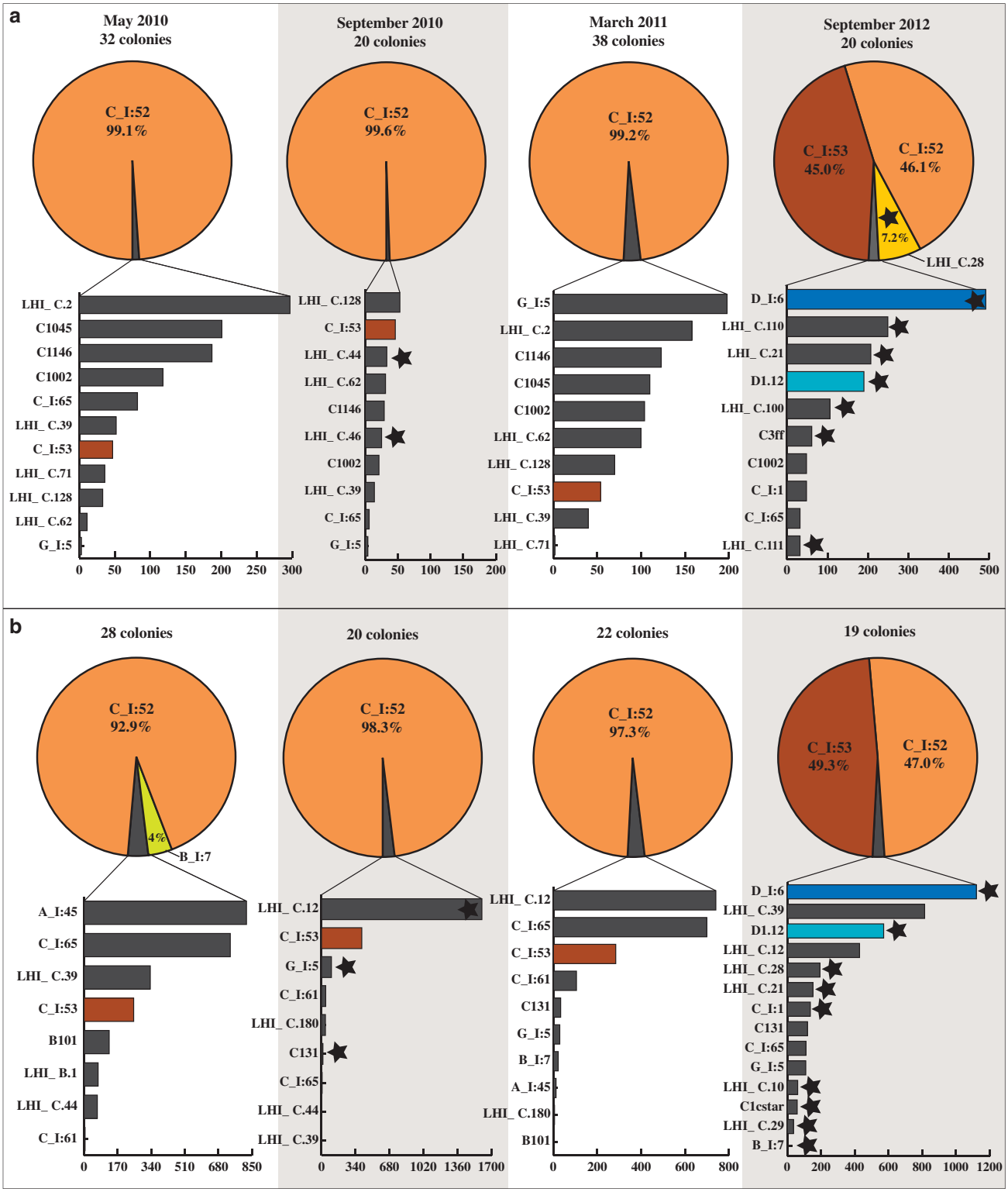

Figure 1 Summary of *Symbiodinium* diversity in *Pocillopora damicornis* (**a**) and *Stylophora pistillata* (**b**) from four collection periods spanning May 2010 to September 2012. Pie charts represent the mean relative abundances of *Symbiodinium* types across all sampled colonies detected at each time point. Types that are dominant at any of the time points are represented in orange, brown or yellow; and types belonging to the rare biosphere throughout the sampling period are represented in grey. Bar graphs represent the abundances (expressed in number of sequencing reads) of *Symbiodinium* types in the rare biosphere only; and black stars represent a switching event. A switching event was deemed to occur when, during any one sampling a type was detected among multiple samples, but was absent from previous sampling times among any sample. Note that only the most abundant types and the ones that shuffled or switched are shown in this figure. See Supplementary Figures S1 and S2 for the complete figure of all the *Symbiodinium* background types detected for each period of time.

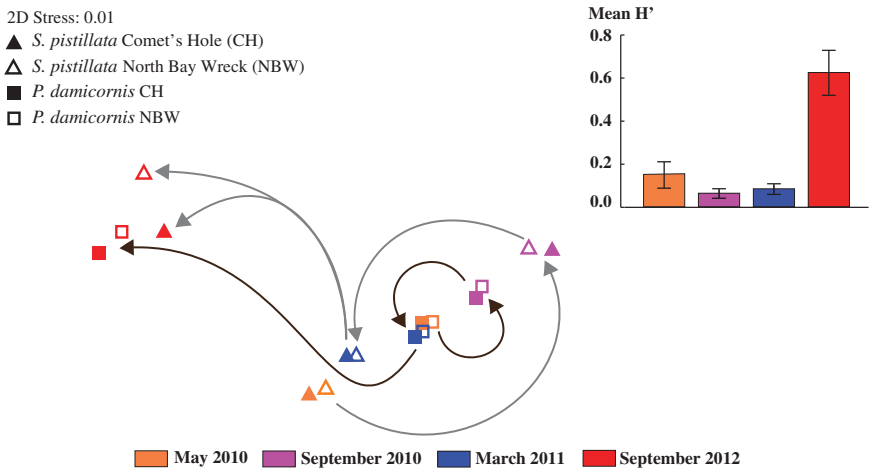

Figure 2 Non-metric multidimensional scaling ordination (nMDS) representing the *Symbiodinium* genetic structure from the resemblance matrix of *Pocillopora damicornis* and *Stylophora pistillata* centroids belonging to samples collected from 2010 to 2012. The nMDS showed a temporal partitioning within hosts of the *Symbiodinium* types divided into two distinct groups: May 2010, September 2010 and March 2011 are clustered together while September 2012 is widely separated. This highlights a substantial change in the structure of *Symbiodinium* assemblage 18 months after the second bleaching event. The bar chart represents the average of Shannon diversity over time within the two Pocilloporidae coral species. Error bars represent 95% confidence interval (CI).

Symbiodinium community. In fact, the diversity reported here is almost five times greater than that reported in other recent next-generation sequencing studies on *Symbiodinium* diversity (Quigley *et al.*, 2014; Thomas *et al.*, 2014; Arif *et al.*, 2014; Green *et al.*, 2014; Edmunds *et al.*, 2014b). For example, a study on *Acropora* coral species (Thomas *et al.*, 2014) at another high latitude reef (Abrolhos Island, Western Australia) found a Shannon diversity of 0.145 (vs 0.620 at LHI in September 2012). The high *Symbiodinium* diversity as well as the endemicity of LHI coral-algal symbioses (mostly composed of previously undescribed ITS2 *Symbiodinium* types) support previous studies showing that the LHI *Symbiodinium* community is genetically and physiologically distinct (Wicks, 2009; Wicks *et al.*, 2010; Noreen *et al.*, 2015).

Our results highlight a high level of symbiont diversity within LHI subtropical corals, with a mean of 11 symbiont types per coral host. Although only *Symbiodinium* belonging to clade C have been previously detected in LHI corals using a gel electrophoresis-based method (Wicks *et al.*, 2010), here we detected *Symbiodinium* types from clades A, B, C, D, F and G. The association of *Symbiodinium* clade B with *S. pistillata* and clade G with both *P. damicornis* and *S. pistillata* found here have not been previously observed.

Nevertheless, the majority of the symbionts detected here were members of *Symbiodinium* clade C, which explains the high level of specificity to clade C reported previously (Wicks *et al.*, 2010). Further research is needed to investigate whether different *Symbiodinium* clade C types simultaneously hosted by a single colony can provide different physiological performance and potentially enable acclimatisation, as previously suggested for clade C types in Caribbean corals (Sampayo *et al.*, 2008).

Temperature anomalies may drive fine-scale changes within the Symbiodinium community

During the two bleaching events, we did not observe any changes within dominant types; however, the *Symbiodinium* rare biosphere showed a dynamic pattern where both shuffling and switching events were quite common during thermal stress and recovery periods (Supplementary Figures S1 and S2). For instance, we observed the new appearance of 104 and 80 *Symbiodinium* types in *P. damicornis* and *S. pistillata*, respectively, over all sampling periods. The substantial changes observed in the *Symbiodinium* community of both coral species following each of the two bleaching events suggest that environmental disturbance drives symbiont community changes in LHI corals (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004; Berkelmans and van Oppen, 2006; Jones *et al.*, 2008; Silverstein *et al.*, 2015) and that symbiotic associations in species that show maternal symbiont transmission are more flexible than previously thought. This concurs with a recent study showing that corals that are sensitive to environmental conditions display high intra- and inter-species flexibility (Putnam *et al.*, 2012).

Interestingly, 18 months after the two bleaching events, the recovered coral colonies harboured a completely different *Symbiodinium* assemblage with new dominant and background types. We hypothesise that the newly acquired dominant *Symbiodinium* type (LHI_C.28), and the type that was already present in the rare biosphere at the first sampling time point (C_I:53), may be better adapted to cope with temperature anomalies and the potentially altered environmental conditions following such events. Notably, we observed a switching event to *Symbiodinium* clade D and 90% of the *Symbiodinium* rare biosphere members were also

newly acquired in 2012, which may provide more options to cope with future bleaching events. These findings overthrow the notion that the period for uptake of algal endosymbionts is narrow and only limited to early life stages in these reef-building corals.

Role and importance of members of the 'Symbiodinium rare biosphere'

There is increasing evidence to suggest that members of *Symbiodinium* clade D can confer enhanced thermal tolerance to the coral holobiont compared with other clades (Stat and Gates, 2011). Repopulation of recovering bleached coral hosts with clade D types has been reported as a survival mechanism for elevated sea temperatures (Chen *et al.*, 2005; Berkelmans and van Oppen, 2006; Mieog *et al.*, 2007; Jones *et al.*, 2008; Stat *et al.*, 2013; Silverstein *et al.*, 2015). This mechanism has, however, been primarily attributed to shuffling of *Symbiodinium* D pre-existing in the rare biosphere rather than *de novo* acquisition. Although the newly acquired D types in LHI corals occurred at low relative abundance in our results, studies on other microbial communities have demonstrated that rare species can be metabolically very active (Campbell *et al.*, 2011; Logares *et al.*, 2014). It has also been shown that rare functionally important species can become dominant to maintain the integrity of functional processes when environmental conditions change (Shade *et al.*, 2014). Moreover, a network theoretic modelling approach on coral-*Symbiodinium* associations under climate change (Fabina *et al.*, 2013) predicts that both elevated symbiont diversity and types occurring at low abundance, which provide redundant or complementary symbiotic function, can significantly increase community stability in response to environmental change. Hence, following these predictions, our results indicate that the switch to clade D in the *Symbiodinium* rare biosphere and the increase in symbiont diversity documented here in LHI corals may enhance the ability of these corals to resist and/or recover from future bleaching events.

The repopulation with previously undetectable clade D was also documented in an experimental study following two induced bleaching events (Silverstein *et al.*, 2015). Even though the source of these newly dominant types could not be identified (from the rare biosphere or from the environment), the authors found an increase in the host thermotolerance and concluded that members of the *Symbiodinium* rare biosphere can be critical components of coral recovery (Silverstein *et al.*, 2015). Similarly, the newly acquired *Symbiodinium* clade D documented here could increase their hosts' thermotolerance during future bleaching events.

It is now well-established that the rare biosphere has significant ecological roles in ecosystems such as diazotrophic bacteria in seawater, bacterial and archaeal ammonia oxidisers in soils, methanogens in intestines (Shade *et al.*, 2014), marine planktonic microeukaryotes in the ocean (Logares *et al.*, 2014) and, our findings suggest the same is true for reef-building corals.

Implications of symbiont switching for reef-building coral community structure

Climate change is responsible for changes in species composition and population structure (Ateweberhan *et al.*, 2013). In coral reef ecosystems, in particular, the general trend is the loss of stress-sensitive coral species and replacement by stress-tolerant species that survive the disturbance. For example, a study conducted over a 14-year period that included two thermal stress events (in 1998 and 2001) at the high latitude reef of Sesoko Island (Okinawa, Japan) reported a complete change in the coral community structure (van Woesik *et al.*, 2011). The stress-sensitive branching pocilloporid corals were replaced by stress-tolerant massive corals such as poritids and brain corals. Our study suggests that symbiont switching to more thermally tolerant symbionts in the two pocilloporid coral species has the potential to assist the persistence of these environmentally-sensitive coral species over time. Given that the frequency of thermal stress events is predicted to increase (IPCC 2014), these findings have important implications for predicting coral assemblage recovery after mass bleaching events and will also help to refine evolutionary models that predict the future of coral reefs.

Conflict of Interest

The authors declare no conflict of interest.

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Author contributions

SJD, MJHvO, AGC and PLH designed the project. SJD and AGC collected the coral samples from 2010 to 2012 at Lord Howe Island. NMB and LMP carried out the DNA extraction, ITS2 amplification and samples preparation for 454 amplicon sequencing. HMP and NMB performed the bioinformatics analysis of 454 sequencing output. NMB analysed the results. NMB and MJHvO wrote the manuscript. All co-authors edited the manuscript before submission.

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