Coral Reefs
https://doi.org/10.1007/s00338-019-01790-z

REPORT

Recruit symbiosis establishment and Symbiodiniaceae composition influenced by adult corals and reef sediment

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Received: 18 September 2018 / Accepted: 18 March 2019

Abstract For most reef-building corals, the establishment of symbiosis occurs via horizontal transmission, where juvenile coral recruits acquire their algal symbionts (family Symbiodiniaceae) from their surrounding environment post-settlement. This transmission strategy allows corals to interact with a diverse array of symbionts, potentially facilitating adaptation to the newly settled environment. We exposed aposymbiotic *Pseudodiploria strigosa* recruits from the Flower Garden Banks to natal reef sediment (C−S+), symbiotic adult coral fragments (C+S−), sediment and coral fragments (C+S+), or seawater controls (C−S−) and quantified rates of symbiont uptake and Symbiodiniaceae community composition within each recruit using metabarcoding of the ITS2 locus. The most rapid uptake was observed in C+S+ treatments, and this combination also led to the highest symbiont alpha diversity in recruits. While C−S+ treatments exhibited the next highest uptake rate, only one individual recruit successfully established symbiosis in the C+S− treatment, suggesting that sediment both serves as a direct symbiont source for coral recruits and promotes (or, potentially, mediates) transmission from adult coral colonies. In turn, presence of adult corals facilitated uptake from the sediment, perhaps via chemical signaling. Taken together, our results reinforce the key role of sediment in algal symbiont uptake by *P. strigosa* recruits and suggest that sediment plays a necessary, but perhaps not sufficient, role in the life cycle of algal Symbiodiniaceae symbionts.

Keywords Coral · Symbiosis · Symbiodiniaceae · Horizontal transmission · Sediment · Metabarcoding · ITS2

Introduction

Algal symbionts in the family Symbiodiniaceae are one of the most functionally and genetically diverse groups of endosymbionts across marine environments and are hosted by a variety of invertebrates ranging from cnidarians, to mollusks, to sponges (Baker 2003; Stat et al. 2006; LaJeunesse et al. 2018). In adult tropical reef-building corals, these algal symbionts supply photosynthetic products to the coral host in return for inorganic nutrients and a residence (Muscatine and Porter 1977; Muscatine and Cernichiari 1969; Trench and Blank 1987). Coral-associated algal symbionts have radiated into genetically divergent lineages, formerly known as clades (A thru I: Stat et al. 2012) and recently reclassified as separate genera (LaJeunesse et al. 2018), which exhibit extensive morphological and functional diversity. Some corals have been shown to harbor genetically diverse assemblage of symbiodiniaceans, and it has been suggested that this diversity can greatly impact the ecological function of the algal symbiont of a given coral host (e.g., Berkelmans and van Oppen 2006). It is also important to note that thermal tolerance

Topic Editor Morgan S. Pratchett

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00338-019-01790-z) contains supplementary material, which is available to authorized users.

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Published online: 23 March 2019

varies significantly within genera (Swain et al. 2016) and interactions between hosts and symbionts can also impact holobiont performance (Abrego et al. 2008; Cunning et al. 2015; Parkinson et al. 2015).

In general, corals algal symbionts are either maternally transmitted (vertical transmission) or obtained from their environment (horizontal transmission) (Harrison and Wallace 1990; Baird et al. 2009), although there is evidence that this dichotomy is not absolute (i.e., Byler et al. 2013). Corals that obtain their symbionts vertically are expected to host a lower diversity of symbiont types since this relationship is stable through time, facilitating the co-evolution of host-symbiont partners (Douglas 1998). On the other hand, horizontally transmitting species release aposymbiotic larvae that can travel great distances (Davies et al. 2015b; Baums et al. 2014; Rippe et al. 2017) and upon settlement these recruits are capable of establishing symbiosis with genetically diverse algal symbiont communities that do not necessarily reflect the symbiont community hosted by local conspecifics or parental colonies from their native environment (Coffroth et al. 2001; Weis et al. 2001; Little et al. 2004; Abrego et al. 2009b). However, as coral recruits mature, the hosted symbiont community generally becomes dominated by a single Symbiodiniaceae clone typical for the recruit's local environment and host species (reviewed in Thornhill et al. 2017). These host-specific associations with algal symbionts can be very strong, and in some coral species, this specificity has been suggested to be a genetically determined trait (Poland and Coffroth 2017). In addition, establishment of symbiosis with novel Symbiodiniaceae species as adults happens very rarely or never (Coffroth et al. 2010; LaJeunesse et al. 2010; Boulotte et al. 2016), suggesting that this initial acquisition of symbionts during recruitment represents a critical stage in coral-algal symbioses for horizontally transmitting coral hosts.

The flexible symbioses of broadly dispersing, horizontally transmitting coral juveniles have been hypothesized to facilitate adaptation of the coral to environmental variation (Fournier 2013; van Oppen 2004; Sampayo et al. 2008), and indeed these associations have been implicated in local adaptation of the holobiont (Howells et al. 2013; Barfield et al. 2018). While much research has quantitatively described the genetic diversity of coral-Symbiodiniaceae symbioses across species and environments at the adult life stage, much less is known about adaptations and mechanisms that symbionts employ to ensure transmission to the next coral generation. One potential mechanism for establishing symbiosis is through infection from a nearby conspecific adult coral. Corals constantly expel photosynthetically active algal symbionts (Ralph et al. 2001; Hill and Ralph 2007). In theory, these cells could directly establish symbiosis with newly settled recruits. Alternatively, these expelled symbionts could colonize reef sediment, which could enable them to persist until the arrival of new recruits and Quigley et al. (2017) demonstrated that genetic diversity in reef sediment was four times higher than genetic diversity in coral recruits. Multiple studies have demonstrated that coral recruits are capable of establishing symbiosis in the presence of reef sediment (Adams et al. 2009; Cumbo et al. 2013; Nitschke et al. 2016); however, it remains unclear whether these sediment-derived symbionts are indeed ecologically important sources for coral symbiosis.

In this study, we first compared post-settlement symbiont uptake rates in the horizontally transmitting coral, *Pseudodiploria strigosa*, across multiple symbiont sources. *P. strigosa* recruits were placed in fully crossed treatments that included the presence of natal reef adult coral fragments (C+S−), natal reef sediment (C−S+), a combination of adult coral fragments and natal reef sediment (C+S+), and seawater controls (C−S−) to test which environment promoted the most efficient uptake. The diversity of these established symbiont assemblages was examined using metabarcoding of the Internal Transcribed Region 2 (ITS2), to characterize Symbiodiniaceae communities within each individual recruit, adult coral fragment, and population of conspecific adults on the native reef to explore how variation in symbiont communities among recruits correlates with the Symbiodiniaceae communities found within local coral hosts.

Materials and methods

Experimental methods

Coral spawning, larval rearing, and sediment and adult collections

During the annual coral spawning event at the Flower Garden Banks (FGB) on the evening of August 9th, 2012 at 21:15CDT (9 d after the full moon), gamete bundles from eight *Psuedodiploria strigosa* colonies were collected via scuba diving and spawning tents (Sharp et al. 2010). Gamete bundles were combined at the surface in a 14 L plastic tub filled with 1 µm filtered seawater (FSW) and left to cross-fertilize for 2 h. Excess sperm was then removed by rinsing embryos through 150 µm nylon mesh. Developing larvae were reared in 1 µm FSW in three replicate plastic culture vessels at a density of two larvae per ml. Larvae were transferred to the laboratory at the University of Texas at Austin (UT Austin) 1-d post-fertilization (dpf). Sediment collections were completed August 8th via scuba. One gallon ziplock bags were used for collections and all sediment (N = 6 ziplocks) was collected at

a depth of 23 m immediately below healthy coral colonies in a sand patch trying to increase the probability of algal symbiont reservoirs. Collections were targeted toward the surface sediment layer ($\sim$ top 10 cm), and this sediment at FGBNMS is assumed to be primarily coarse-grained calcium carbonate sediment, although grain size was not measured. Once at the surface, sediment was maintained in 1 μm filtered seawater with daily water changes. Sediment was transferred to UT Austin in ziplock bags in coolers. One large fragment of a single adult *Orbicella faveolata* was collected and maintained in the laboratory to serve as the adult coral source of algal symbionts. This adult source species was chosen because the original uptake experiment was meant to use *O. faveolata* larvae. Unfortunately, larval cultures from this species were lost before they could be settled on slides and we were only able to work with the remaining *P. strigosa* cultures. All collections were completed under the Flower Garden Banks National Marine Sanctuary (FGBNMS) permit #FGBNMS-2012-002.

Symbiont uptake experimental design

On August 14th, 2012 (5 dpf), twelve (20 L) experimental tanks were filled with artificial seawater (Instant Ocean, Blacksburg, VA, USA) and 800 ml of 1 μm filtered FGB water in order to "prime" experimental tanks with microbes (< 1 μm) that might play an important role in coral husbandry. Tanks were randomly assigned to one of four treatments (n = 3/treatment): 1. FGB natal reef sediment only (C−S+), 2. *Orbicella faveolata* coral host fragment only (C+S−), 3. FGB natal reef sediment and *O. faveolata* coral host fragment (C+S+), and 4. Seawater control (C−S−) (Supplemental Figure S1). For tanks containing sediment, all sediment collections were placed in an extra empty tank for mixing to reduce variation in sediment across tanks, and then a layer of sediment ($\sim$ 3 cm deep) was added to each tank. Tanks were maintained at identical salinity (35.5 ppt) and temperature (as measured by hobo data loggers: 25.5–28.5 °C, Supplemental Figure S2) throughout the uptake experiment. Four dpf, thousands of competent *P. strigosa* larvae were placed in sterile plastic dishes filled with artificial seawater (Instant Ocean, Blacksburg, VA, USA) and conditioned glass slides. Autoclaved, finely ground FGB crustose coralline algae (CCA) was added to slides to induce settlement (as per Davies et al. (2014, 2015a)) and larvae were given 4 d in dark conditions to metamorphose. Four days later (8 dpf) plastic dishes were cleaned, and settlement conditions were replicated with new larvae to maximize recruitment rates per slide.

On August 21st (12 dpf), slides with settled *P. strigosa* recruits were randomly placed into each treatment tank (n = 3 slides per tank; Supplemental Figure S1). Symbiont uptake was visually assessed using a fluorescent stereomicroscope MZ-FL-III (Leica, Bannockburn, IL, USA) equipped with F/R double-bandpass filter (Chroma no. 51004v2). Recruits were considered as having established symbiosis when individual algal symbiont cells were obvious in recruit tentacles (Fig. 1a, b). Recruits were surveyed daily from August 22–28th (13–19 dpf), after which surveys were completed every 3 d. Uptake was continually monitored until October 16th (68 dpf) when final counts were completed due to algal overgrowth beginning to affect juvenile coral survival. Individuals successfully infected with algal symbionts were then individually collected immediately after this final uptake timepoint (68 dpf) using sterile razor blades, preserved in 95% EtOH and stored at − 20 °C until processing.

Symbiont genotyping

Symbiont DNA was isolated from individual recruits using a DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Recruits were disrupted by micropestle for 5 min using an aliquot of Lysing Matrix A (MP Biomedicals). Symbiont DNA was isolated from adult corals following Davies et al. (2013).

The ITS2 region was amplified via PCR using the forward primer *its-dino* (5′ GTGAATTGCAGAACTCCGTG 3′) and the reverse primer *its2rev2* (5′ CCTCCGCTTA CTTATATGCTT 3′) (Pochon et al. 2001), following the protocols described in Kenkel et al. (2013) and Quigley et al. (2014), using 2 μL of template DNA of unknown concentration. Briefly, amplifications were verified on agarose gels following 21 cycles and additional cycles were added as necessary to achieve a faint band (to reduce PCR biases) when 3 μL of product was loaded on a 1% agarose gel and run for 15 min at 180 V (Supplemental Figure 3A). Cycle numbers ranged from 26 to 41 across samples (Supplemental Table S1); however, several samples were amplified to 42 cycles along with no-template negative controls to assure that results at high cycle numbers were not due to contamination (Supplemental Figure 3B). These "cycle-check" PCRs were performed on a Tetrad 2 Peltier Thermal Cycler (Bio-Rad) using the following conditions: 94 °C for 5 min, followed by 21 cycles of 94 °C for 15 s, 59 °C for 30 s and 72 °C for 30 s and a final extension of 10 min at 72 °C. Once optimal cycle numbers were obtained, all samples were re-amplified to their previously specified cycle number and verified on a gel to test for equivalent band intensity across samples (Supplemental Figure 3A).

Each PCR was cleaned using a PCR clean-up kit (Fermentas) following the manufacturer's instructions, measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific) and diluted to 10 ng μL^{-1}. This product was

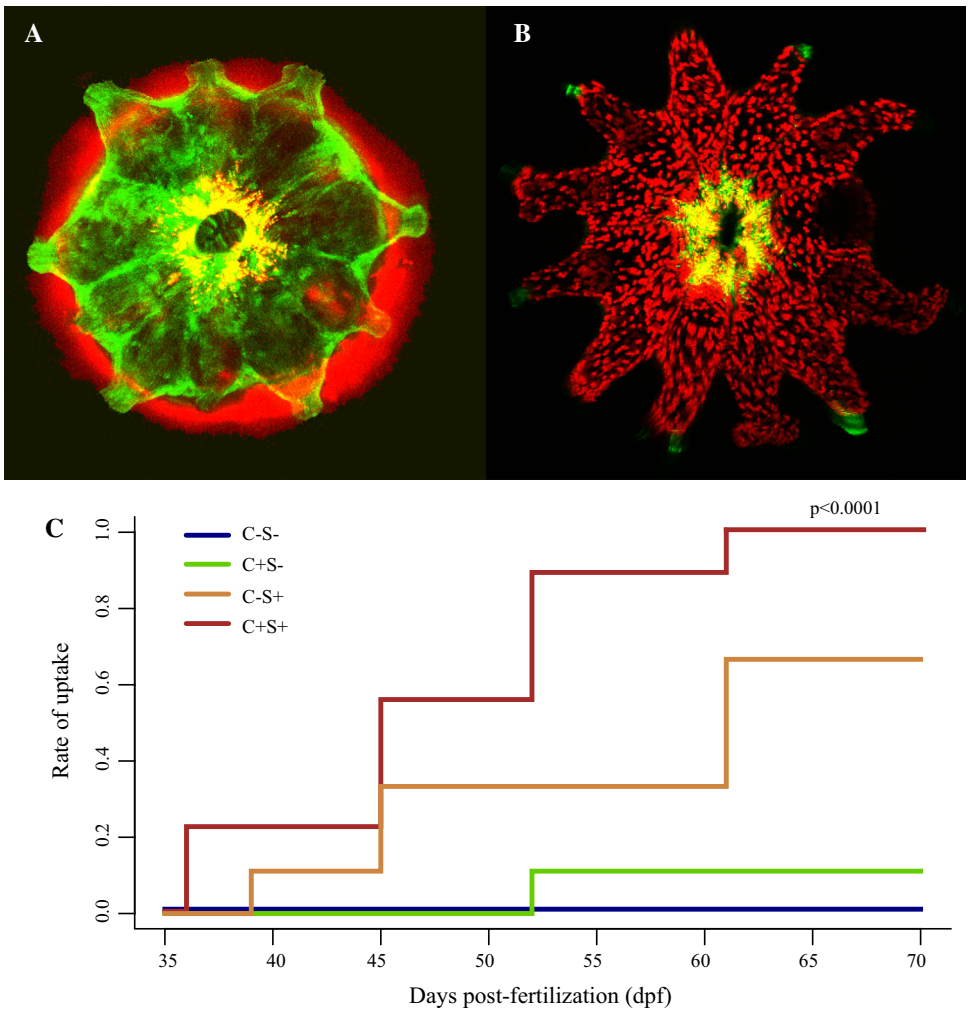

Fig. 1 Algal symbiont uptake rates in *Pseudodiploria strigosa* recruits. Single *P. strigosa* recruit under confocal microscopy showing **a** no algal symbiont uptake and **b** symbiosis with the algal symbiont demonstrating the clear phenotypic differences in recruit uptake using fluorescence microscopy. Green fluorescence is the innate fluorescence from the green fluorescent protein from the coral recruit and the red color is chlorophyll fluorescence, which can be seen surrounding the recruit in **a** (turf algae) and as discrete algal cells in **b**. **c** Mean cumulative uptake rates of algal symbionts in *P. strigosa* recruits through time demonstrating the proportion of recruits that established symbiosis through time (*dpf* days post-fertilization) across the four experimental uptake treatments. *P* value corresponds to cox-proportional hazards model indicating significant differences in uptake rate. C−S+ = FGB natal reef sediment only, C+S− = *Orbicella faveolata* coral host fragment only, C+S+ = FGB natal reef sediment and *O. faveolata* coral host fragment, and C−S− = seawater control

then used as template for an additional PCR step used to incorporate 454-RAPID primers and barcodes to each sample. Each PCR contained 0.33 µM B-Rapid ITS2-forward primer (Br-ITS2-F: 5′ CCTATCCCCTGTGTGC CTTGAGAGACGHC + GTGAATTGCAGAACTCCGT G 3′) in addition to 0.33 µM of unique A-Rapid-reverse primer containing an 8-bp barcode for subsequent sample identification (e.g., Ar-ITS2-R-16: 5′ CCATCT-CATCCCTGCGT GTCTCCGACGACT + **TGTAGCG C** + CCTCCGCTTACTTATATGCTT 3′, barcode sequence in bold). Each sample was uniquely barcoded.

Amplifications were visualized on a gel and based on visually assessed band intensities varying amounts of each barcoded sample were pooled for 454 sequencing. This pooled sample was cleaned via ethanol precipitation and re-suspended in 25 µL milli-Q water. 10 µL of this cleaned product was run on a 1% agarose gel stained with SYBR Green (Invitrogen) for 45 min at 100 V. The gel was visualized on a blue-light box, and the target band was excised using a sterile razor blade and placed in 25 µL milli-Q water for overnight incubation at 4 °C. The resulting supernatant was then submitted for 454 sequencing at the Genome Sequencing and Analysis Facility at the University of Texas at Austin. Raw sff files were uploaded to Sequence Read Archive (SRA) Accession Number SRP144167.

Statistical analyses

All analyses were completed in the R statistical environment (R Core Team 2017), and scripts are available at http://github.com/NicolaKriefall/sym_uptake. Numbers of recruits that established symbiosis with algal symbionts, which were measured as binary variables of recruit successes and failures, were fit to a Cox's proportional hazards regression model. Rates of symbiont uptake by coral recruits were compared using the package *Survival* (Therneau and Lumley 2015). A cumulative incidence curve was generated from this model, and an ANOVA test was run to test for significant differences in uptake rates. To assess differences between pairs of treatments, the analysis was run pairwise for adult host fragment, natal reef sediment, and adult host fragment and natal reef sediment treatments. In addition, to determine whether there were synergistic effects of the host and sediment treatments, we tested for a significant interaction between these treatments. We used a Cox's proportional hazards model and to evaluate this interaction, we reformulated the model with two separate factors (host and sediment), and fitted the model with these factors and their interaction as predictors.

To determine the community composition of Symbiodiniaceae in each coral recruit, 454 sequencing data were analyzed using the package *dada2* (Callahan et al. 2016). First, 454 pyrosequencing files were converted to FASTQ format using the package *R453Plus1Toolbox* (Klein et al. 2011) as *dada2* only processes FASTQ files (Callahan et al. 2016). Subsequently, the *dada2* tutorial at https://benjjeb.github.io/dada2/tutorial.html was followed with modifications for ITS2 as outlined in Kenkel & Bay (2018). FASTQ files were filtered and trimmed using the *filterAndTrim()* defaults with the following exceptions: the first 20 bp of each sequence were truncated to remove ITS2 primers, the final length was truncated to 300 bp as determined by sequence quality profiles and we allowed for a maximum of one expected error, which is a conservative value (Callahan et al. 2016). Next, sequencing error rate calculation and de-replication were carried out following *dada2* standards (Callahan et al. 2016). Sequence variants were then inferred using the core sample inference algorithm, "BAND_SIZE" was set to 32 instead of the default of 16 as per author recommendations for ITS2 data rather than the default 16S data, and an Amplicon Sequence Variant (ASV) table was produced (Callahan et al. 2016). One chimeric sequence was identified and removed from the ASV file using the *removeBimeraDenovo()* function in *dada2* (Callahan et al. 2016).

To assign taxonomy of the resulting ASV table, the *AssignTaxonomy()* function in *dada2* was utilized, which follows a naïve Bayesian classifier method with reference sequences (Callahan et al. 2016). For reference sequences, we modified the GeoSymbio database of ITS2 types from Franklin et al. (2012) by expanding the header for each sequence (i.e., "A1" to "Symbiodinium; Clade A; A1") as *phyloseq* requires this taxonomic information (McMurdie and Holmes 2013). Following Kenkel and Bay (2018), we accepted taxonomic assignments with bootstrap confidence values of 5/100, as we were targeting clade/genera-level community differences rather than species-specific accuracy. Only the first 46 sequences in the ASV table were used for all subsequent analyses and visualizations given that the remainder of the ASV sequences (47–119) had less than 76 counts total.

The package *phyloseq* (McMurdie and Holmes 2013) was then used to create barplots to visualize and sort relative abundances of different Symbiodiniaceae ITS2-types. Cumulative counts across ITS2-types within a sample were then log-normalized following Green et al. (2014) and *pheatmap* (Kolde 2015) was used to visualize algal symbiont differences across recruits and adults. *Phyloseq* was also used to construct an alpha diversity plot using Simpson and Shannon diversity controlling for effect of sample size. As differences in PCR cycle number could impact among-sample estimates of diversity, we quantified the relationship between number of amplification cycles and subsequent Simpson and Shannon diversity within-sample. There was no relationship between cycle number and Simpson diversity ($r2 = 0.032$, $p = 0.178$). A positive relationship was detected between cycle number and Shannon diversity ($r2 = 0.174$, $p = 0.016$); however, there was no significant interaction between cycle number and tank treatment ($p > 0.05$), suggesting that differences in Shannon diversity across tank treatments are not driven by PCR cycle numbers (Supplementary Figure 3C, D). All raw sequence numbers through *dada2* filtering steps can be found in (Supplemental Table 2).

Lastly, a phylogenetic tree was constructed to visualize algal symbiont relatedness and illustrate accuracy of taxonomic assignments. Among ASVs aligning to the identical ITS2-type in the GeoSymbio database, the ASV with the highest counts was used as the reference ASV for that ITS2 type. These reference ASV sequences have been deposited in GenBank (Accession #SUB4526136) and were combined into a FASTA file along with matching Symbiodiniaceae species reference sequences from the Franklin et al. (2012) GeoSymbio database and one additional reference sequence of Symbiodiniaceae species B1 previously obtained from the FGB in Green et al. (2014). This FASTA file was uploaded to www.phylogeny.fr using the "One Click Mode" (Dereeper et al. 2008). In brief, Multiple Sequence Comparison by Log-Expectation aligned sequences, Gblocks selected conserved sequences, phyML and the approximate Likelihood-Ratio Test (aLRT)

assigned phylogeny and bootstrap values were determined based on the maximum likelihood model, and finally the TreeDyn function in Phylogeny.fr visualized the tree (Dereeper et al. 2008, 2010). The Newick output of the constructed tree was visualized using R package *ggtree* (Yu et al. 2017). The tree construction was carried out separately for all unique *Symbiodinium* (formerly clade A) and *Brevolium* (formerly clade B) species to better visualize within-genera differences on the phylogenetic tree (LaJeunesse et al. 2018).

Results

Coral recruit symbiont uptake

Initial symbiont uptake by *P. strigosa* recruits was not observed until 36 d post-fertilization (dpf), which was 18 d after recruits were added to uptake treatments. Uptake was confirmed by assessing chlorophyll fluorescence (Fig. 1a, b). The first recruits to exhibit uptake were in FGB natal reef sediment and *O. faveolata* coral host fragment treatments (C+S+) (Fig. 1c). Additionally, slides in C+S+ treatments were the only slides on which 100% of recruits successfully established symbiosis by the end of the experiment (68 dpf; 56 d after being placed in uptake treatments). The FGB natal reef sediment (C−S+) treatment was the second to exhibit uptake (Fig. 1c); however, significantly fewer recruits acquired symbionts when compared to C+S+ treatments (Wald's $p < 0.05$). *Orbicella faveolata* coral host fragment (C+S−) treatments exhibited the slowest uptake rates (Fig. 1c), and final uptake proportions in this treatment were significantly lower than C+S+ treatments (Wald's $p < 0.01$) and C−S+ treatments (Wald's $p < 0.05$). As expected, recruits in seawater control treatments (C−S−) exhibited no uptake (Fig. 1c).

The likelihood of symbiont uptake by *P. strigosa* recruits was significantly affected by experimental treatment ($\chi^2 = 30.779$ and $p < 0.0001$). Hazard ratios from the Cox's proportional hazards model demonstrated that uptake in the C−S+ treatment was significantly lower than the C+S+ treatment (0.294, CI: 0.097, 0.893), but higher than C+S− treatments (0.034, CI: 0.004, 0.283), suggesting that the presence of sediment increased the probability of symbiont acquisition in *P. strigosa* recruits. When the Cox's proportional hazards model was reformulated to test for the effect of host, sediment and the interaction between these factors, we found that the interaction term was statistically significant ($p = 0.0218$) and that coral recruits exhibited a 5.7-fold increase in uptake of algal symbionts, demonstrating synergistic effects of coral hosts and sediment.

Symbiodiniaceae genetic diversity

To compare Symbiodiniaceae diversity among individual infected recruits across treatments, a total of 42 corals were successfully genotyped using 454 metabarcoding of the ITS2 locus (Supplemental Table S2). Thirty of these samples were individual recruits from experimental treatment tanks, six were *O. faveolata* host fragments from experimental treatment tanks and six were native *P. strigosa* adults collected from the east Flower Garden Banks (FGB) (Supplemental Table S1). A total of 67,027 raw reads were generated, 55,589 of which were left after adaptor trimming, quality filtering, and discarding reads shorter than 300 bp using the statistical package *dada2*. Recruit 2B1 was excluded from statistical analysis due to low number of remaining reads. Number of filter-passing reads in retained samples ranged from 589 to 4341 with an average of 1264 reads (Supplemental Table S2).

The dominant ITS2 type in adult *P. strigosa* was Symbiodiniaceae B1 (genus *Breviolum* (LaJeunesse et al. 2018), representing nearly 100% of sequences retrieved from *P. strigosa* colonies (i.e., they exclusively hosted B1, Fig. 2). B1 was also the dominant Symbiodiniaceae reference sequence in *O. faveolata* adults (98.7–100%), but this species also associated with background levels of B10 (up to 1.3%, Fig. 2).

Notably, the average proportion of B1 in juvenile *P. strigosa* recruits was 8.9% (i.e., were background) and only a single recruit from the C+S+ treatment was dominated by B1 (Fig. 2a). In general, the majority of sequences observed in coral recruits were not detected at any level in adult fragments (Fig. 2a). Still, the two most common symbiont ITS2 types among recruits did belong to the genus *Breviolum*; however, they were B2 (average proportion of 36.6% in recruits) and B3 (average proportion of 43.5% in recruits). *P. strigosa* recruits also established symbiosis with a wider diversity of symbionts compared to adult samples (A1.1, A2, A3, A4, A4a, A4.3, B1, B10, B2, B19, B3; Figs. 2 and 3a). B2 was observed at higher abundances in C−S+ treatments, comprising an average proportion of 87.8% in each individual recruit, while B3 was common in treatments that included adult coral fragments (C+S− and C+S+). B3 represented 93.3% of sequences in C+S− treatment; however, these values were derived from a single recruit. In C+S+ treatments, B3 was present at higher average proportions (53.3%) when compared to B2 (24.1%) (Figs. 2, 3a).

Shannon and Simpson alpha diversity were calculated for each treatment and a one-way ANOVA tested for diversity differences across symbiont source treatments. Both diversity measures indicated that alpha diversity varied significantly across source treatments (Simpson: $F = 15.77$, $p < 0.0001$; Shannon: $F = 11.01$, $p < 0.0001$;

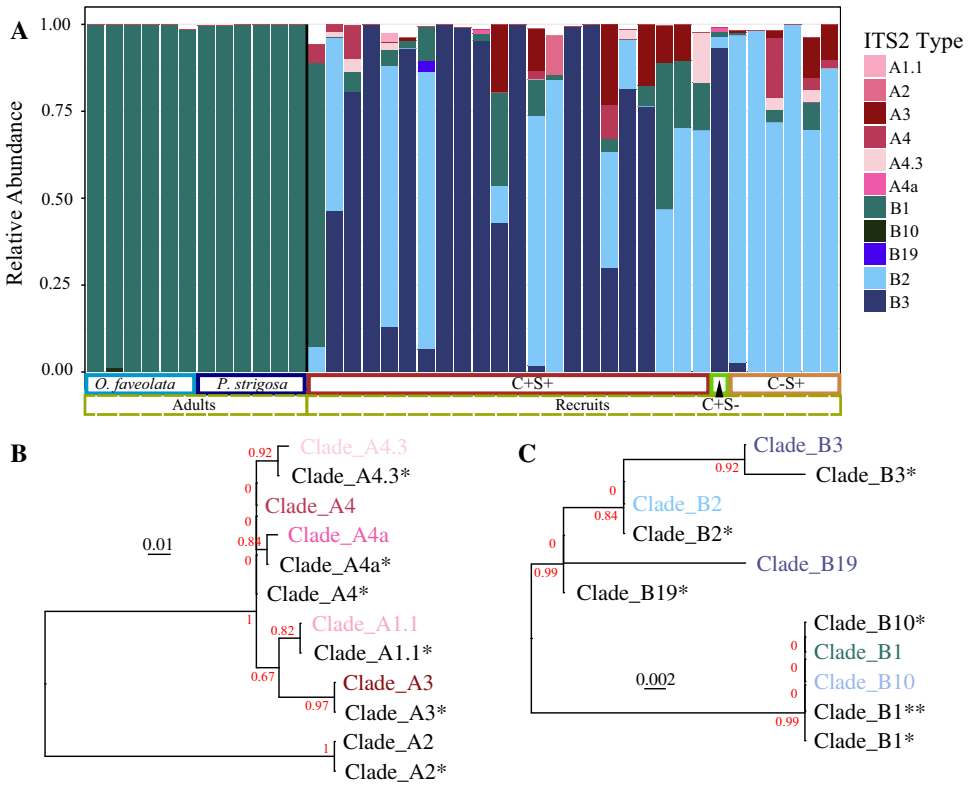

Fig. 2 Symbiodiniaceae communities across adult *P. strigosa* in natal reef sites, adult *O. faveolata* used in uptake experiment (collected from natal reef site) and *P. strigosa* recruits in uptake experiment. **a** Relative abundance of total reads mapping to reference sequences in GeoSymBio ITS2 database where each vertical bar denotes one coral's Symbiodiniaceae community. The experimental uptake treatment of coral recruits and the two species of adult corals are indicated below the barplot. The black line demarcates adult corals from recruits, contrasting the differences in Symbiodiniaceae communities across the two life stages. C−S+ = FGB natal reef sediment only, C+S− = *O. faveolata* coral host fragment only, and C+S+ = FGB natal reef sediment and *O. faveolata* coral host fragment. Phylogenetic analysis of the most abundant unique *Symbiodinium* (**b**) and *Breviolum* (**c**) sequences within coral samples in the present study in addition to reference ITS2 sequences that were successfully mapped to. Branch support values are shown on the branches at divisions between distinct clades in red. The scale bar represents replacements per nucleotide site. *Indicates reference sequences from the GeoSymBio ITS2 database while **indicates B1 reference sequence from Green et al. (2014)

Table 1) and Tukey's post hoc tests confirmed that C+S+ treatments exhibited significantly higher mean Simpson and Shannon alpha diversities when compared to alpha diversities of other treatments and adult host fragments of both species ($p < 0.05$; Table 2). C+S− treatment was not included in pairwise comparisons since only a single recruit achieved symbiosis.

Discussion

The reservoirs of free-living Symbiodiniaceae available for uptake by horizontally transmitting corals remain unresolved (Quigley et al. 2017). Here we assessed the relative roles that availability of reef sediment and coral adults play in the establishment of symbiosis in the horizontally transmitting reef-building coral *Pseudodiploria strigosa*. We found that reef sediment appears necessary for the successful establishment of symbiosis in *P. strigosa* coral recruits since recruits in treatments with sediment (C+S+ and C−S+) consistently exhibited significantly higher uptake rates when compared to treatments without sediment (C+S− and C−S−) (Fig. 1C) and C+S+ treatments exhibited the highest genetic diversities (Fig. 3b,c). This outcome is consistent with previous studies investigating symbiont uptake, which have similarly found that sediment serves as an important reservoir of Symbiodiniaceae for horizontally transmitting coral larvae and recruits (Adams et al. 2009; Cumbo et al. 2013; Nitschke et al. 2016) and this sediment has high diversity of available algal symbionts (Quigley et al. 2017). Free-living Symbiodiniaceae are ubiquitous in the reef environment (Coffroth et al. 2006; Pochon et al. 2010; Takabayashi et al. 2012; Quigley et al. 2017; Porto et al. 2008) and their densities in the sediment have been estimated to be up to 15 times higher when compared to densities in the water column (Littman et al. 2008) due to the symbiont being largely immobile and negatively buoyant (Coffroth et al. 2006; Yacobovitch

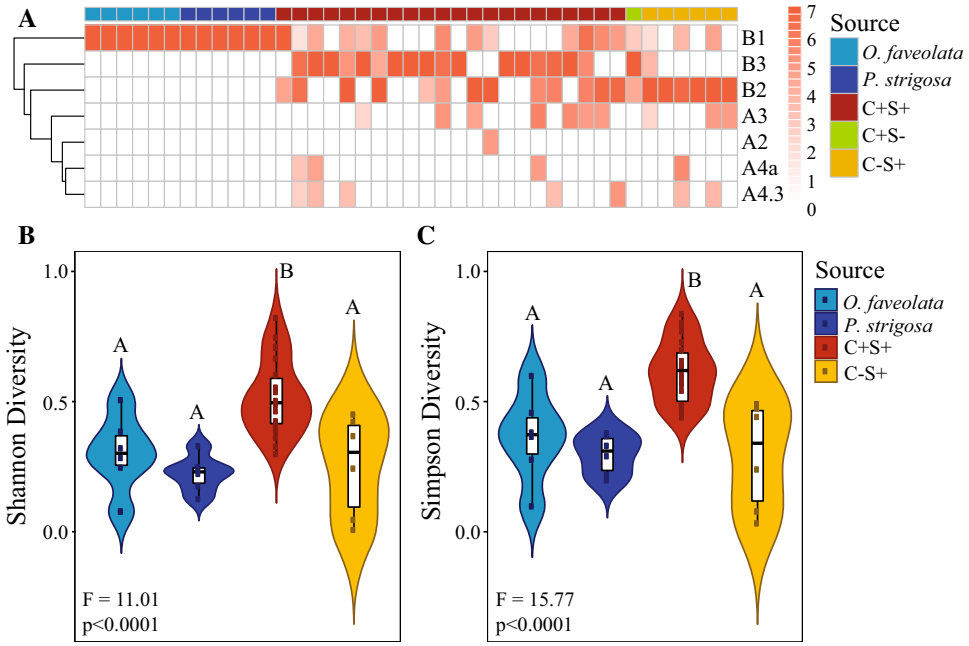

Fig. 3 Symbiodiniaceae community diversity across adult *P. strigosa* in natal reef sites, adult *O. faveolata* used in uptake experiment (collected from natal reef site) and *P. strigosa* recruits in uptake experiment. **a** Heatmap of *log*-normalized counts within an ITS2 type for all sequenced samples. **b** Mean Shannon and Simpson alpha diversities for Symbiodiniaceae communities across adult corals and recruits in experimental uptake treatments. Widths of colored bands in violin plots correspond to the probability distribution of diversity indices. The boxplot and whiskers correspond to the interquartile range, median, and 95% confidence interval of alpha diversity measures. C−S+ = FGB natal reef sediment only, C+S− = *Orbicella faveolata* coral host fragment only, and C+S+ = FGB natal reef sediment and *O. faveolata* coral host fragment. *Orbicella faveolata* coral host fragment only treatments (C+S−) were excluded from analyses due to low sample size ($N = 1$)

Table 1 Tukey post hoc pairwise statistics for Simpson and Shannon alpha genetic diversities with respect to recruits in different uptake treatments and adult coral host Symbiodiniaceae communities

Treatment	Simpson *p* value	Shannon *p* value
Adult *O. faveolata*—*P. strigosa*	0.8404	0.7645
Adult *P. strigos*a—C+S+	0.0017**	0.0124*
Adult *P. strigos*a—C−S+	0.8119	0.9402
Adult *O. faveolata*—C+S+	0.0001***	0.0004***
Adult *O. faveolata*—C−S+	0.9999	0.9773
C−S+–C+S+	0.0001***	0.0018**

p values *< 0.05; **< 0.01; ***< 0.001 Note: Adult host fragment only treatments (C+S−) were not included given that too few recruits were observed to uptake algal symbionts. C−S+ = FGB natal reef sediment only and C+S+ = FGB natal reef sediment and *O. faveolata* coral host fragment

et al. 2004), and because reef sediment is likely a growth niche for certain symbiodiniaceans (e.g., Nitschke et al. 2015). In light of these Symbiodiniaceae distributions, it is perhaps not surprising that we observed significantly higher uptake rates in treatments with sediment available (C−S+, C+S+) (Fig. 1a) and higher genetic diversities in C+S+ treatments (Fig. 3b, c).

We observed that only a single coral recruit established symbiosis in the presence of adult coral but in the absence of sediment (C+S−); notably, most of these symbiont types were not detected in adult tissue (Fig. 2). Therefore, similarly to the algal communities established from sediment, these symbiont types likely represent free-living Symbiodiniaceans populating the coral's exposed skeletal surface rather than algal symbionts establishing symbiosis directly from the adult coral's endosymbiont community. This result is in contrast to Nitschke et al. (2016), which found similar uptake rates between their C+S− and C−S+ treatments. However, it is important to note that in the Nitschke et al. (2016) study, recruits were settled on pre-conditioned terracotta tiles, and symbionts could have potentially transitioned from adult corals to microhabitats on these tiles, which may have then served as a source of algal symbionts for recruits. The glass

slides used in our study may not have provided the microhabitat refugium to allow for this secondary transmission. In addition, the adult coral symbiont sources provided in this study were not conspecifics, whereas the Nitschke et al. (2016) study provided conspecific symbiont sources. Although these two coral species hosted the same ITS2-type, there could be genetic differences between these B1 ITS2-types that were not detected here that might explain the lack of algal symbiont uptake in the C+S− treatment.

The most surprising of our results is the finding that the combined C+S+ treatment exhibited significantly higher ($p = 0.0218$) uptake rates than can be expected from just the sum of individual C−S+ and C+S− effects (Fig. 1). Perhaps the presence of an adult coral alone increases uptake rates through the use of chemical cues. Previous work has linked chemical cues between corals and algal symbionts (Fitt and Trench 1981; Hagedorn et al. 2015; Fitt 1984; Takeuchi et al. 2017); however, facilitation of the onset of symbiosis via adult specific cues is a novel hypothesis. In turn, the presence of sediment might facilitate uptake of algal symbionts from other sources. If the B3 symbiont type, which was never detected in adult coral colonies and was detected nearly exclusively in C+ treatments, is derived from the surface of the adult coral, then the sediment appears to have strongly promoted its uptake (Fig. 2a). It is possible that sediment is required for the alga to complete a certain life cycle transition before it can infect recruits or a threshold of coral-specific cue mediated by the presence of adult host tissue must be met in order to induce behavioral changes in algal symbionts that promote symbiosis establishment.

Both adult coral species (*O. faveolata* and *P.* strigosa) were found to associate with the same ITS2-type B1 (Fig. 2a); however, it is important to note that we cannot be certain that these coral species host the same algal symbiont type without additional sequencing of markers with increased resolution (i.e., $psbA^{ncr}$, microsatellites; LaJeunesse et al. 2018). Regardless of what the adult corals hosted, *P. strigosa* recruits established symbiosis with many other Symbiodiniaceae ITS2 types that were undetectable in adult *O. faveolata* and hosted a small proportion of these "adult-like" B1 symbionts (Figs. 2a, 3a). Stark differences in Symbiodiniaceae communities between early life stages and adults have been observed in multiple horizontal-transmitting corals, including Pacific acroporids (Abrego et al. 2009a, b; Little et al. 2004; Gómez-Cabrera et al. 2007), Caribbean *Orbicella faveolata* (McIlroy and Coffroth 2017), and Caribbean *Briareum asbestinum* (Poland et al. 2013). We demonstrate similar results for a divergent Caribbean coral species (*P. strigosa*), suggesting that this phenomenon is a common feature of horizontally transmitting corals.

We did not assess the Symbiodiniaceae diversity present in the sediment, so we cannot determine if uptake of symbionts from the sediment was random or if certain algal symbionts were more infectious. Future studies should sequence sediment Symbiodiniaceae communities to address this shortcoming, especially given that Quigley et al. (2017) found that Symbiodiniaceae communities in the sediment had four times as many Operational Taxonomic Units (OTUs) (equivalent to ASV used in our study) when compared with Symbiodiniaceae communities hosted by juvenile *Acropora* recruits. In addition to finding more OTUs, Quigley et al. (2017) determined that very few OTUs were shared among juveniles and sediment, indicating that Symbiodiniaceans differ in their infection capabilities or their benefits they offer to the coral host.

While high diversity symbiont communities in juvenile horizontally transmitting corals are well-established, the reason for this remains unclear. Increased diversity in recruits could be due to the lack of robust symbiont recognition mechanisms (Cumbo et al. 2013). Alternatively, harboring a more diverse Symbiodiniaceae community as recruits could confer varied functional and physiological advantages, perhaps even allowing them to cope with a variable local environments (Thornhill et al. 2017). Interestingly, uptake of B1 was not significantly higher in the presence of the coral fragment (C+S+) relative to the sediment only treatment (C−S+), suggesting that endosymbiotic B1 cells that do successfully infect recruits did not transition directly from coral hosts in treatment tanks. That said, it is notable that several C+S+ recruits did uptake higher proportions of B1 with one C+S+ recruit being dominated by the B1 ITS2-type (Figs. 2a, 3a). Still, on average, these differences between treatments were not significant and overall these data suggest that infective Symbiodiniaceae cells are free-living, and that transition to this state from the state of endosymbiosis is either indirect or takes considerable time.

Our results corroborate prior work showing that both sediment and host corals enhance the establishment of symbiosis in horizontally transmitting corals. Most notably, we found that the presence of adult corals interacted synergistically with the presence of sediment. Clearly, more work on the life history of Symbiodiniaceae is required to explain these observations and to understand all the steps leading to transmission of resident endosymbionts to the next generation of coral hosts.

Acknowledgements Funding was provided by the National Science Foundation Grant DEB-1054766 to M.V.M., a departmental graduate student start-up award from the Section of Integrative Biology at the University of Texas at Austin to S.W.D., and the PADI Foundation Award to S.W.D. In addition, the Flower Garden Banks National Marine Sanctuary is acknowledged for boat time aboard the R/V Manta. We acknowledge Marie Strader for her confocal microscopy work and Galina Aglyamova for help in the molecular lab. Lastly, we would like to thank two anonymous reviewers for their critical feedback that greatly improved the manuscript.

Compliance with ethical standards

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

References

Abrego D, Van Oppen MJH, Willis BL (2009a) Highly infectious symbiont dominates initial uptake in coral juveniles. Mol Ecol 18:3518–3531

Abrego D, Van Oppen MJH, Willis BL (2009b) Onset of algal endosymbiont specificity varies among closely related species of *Acropora corals* during early ontogeny. Mol Ecol 18:3532–3543

Abrego D, Ulstrup KE, Willis BL, Van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. Proc R Soc B Biol Sci 275:2273–2282

Adams LM, Cumbo VR, Takabayashi M (2009) Exposure to sediment enhances primary acquisition of *Symbiodinium* by asymbiotic coral larvae. Mar Ecol Prog Ser 377:149–156

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. Trends Ecol Evol 24:16–20

Baker AC (2003) Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of *Symbiodinium*. Annu Rev Ecol Evol Syst 34:661–689

Barfield SJ, Aglyamova GV, Bay LK, Matz MV (2018) Contrasting effects of *Symbiodinium* identity on coral host transcriptional profiles across latitudes. Mol Ecol 27:3103–3115

Baums IB, Devlin-Durante MK, Lajeunesse TC (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. Mol Ecol 23:4203–4215

Berkelmans R, van Oppen MJ (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. Proc R Soc B Biol Sci 273:2305–2312

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, Van Oppen MJH (2016) Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. ISME J 10:2693–2701

Byler KA, Carmi-Veal M, Fine M, Goulet TL (2013) Multiple symbiont acquisition strategies as an adaptive mechanism in the coral *Stylophora pistillata*. PLoS ONE 8:e59596

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583

Coffroth MA, Lewis CF, Santos SR, Weaver JL (2006) Environmental populations of symbiotic dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians. Curr Biol 16:PR985–R987

Coffroth MA, Poland DM, Petrou EL, Brazeau DA, Holmberg JC (2010) Environmental Symbiont Acquisition May Not Be the Solution to Warming Seas for Reef-Building Corals. PLoS ONE 5:e13258

Coffroth MA, Santos SR, Goulet TL (2001) Early ontogenetic expression of specificity in a cnidarian-algal symbiosis. Mar Ecol Prog Ser 222:85–96

Cumbo VR, Baird AH, van Oppen MJH (2013) The promiscuous larvae: Flexibility in the establishment of symbiosis in corals. Coral Reefs 32:111–120

Cunning R, Vaughan N, Gillette P, Capo TR, Mate JL, Baker AC, Morgan SG (2015) Dynamic regulation of partner abundance mediates response of reef coral symbioses to environmental change. Ecology 96:1411–1420

Davies SW, Meyer E, Guermond SM, Matz MV (2014) A cross-ocean comparison of responses to settlement cues in reef-building corals. PeerJ 2:e333

Davies SW, Rahman M, Meyer E, Green EA, Buschiazzo E, Medina M, Matz MV (2013) Novel polymorphic microsatellite markers for population genetics of the endangered Caribbean star coral, *Montastraea faveolata*. Mar Biodivers 43:167–172

Davies SW, Scarpino SV, Pongwarin T, Scott J, Matz MV (2015a) Estimating Trait Heritability in Highly Fecund Species. G3 (Bethesda) 5:2639–2645

Davies SW, Treml EA, Kenkel CD, Matz MV (2015b) Exploring the role of Micronesian islands in the maintenance of coral genetic diversity in the Pacific Ocean. Mol Ecol 24:70–82

Dereeper A, Audic S, Claverie JM, Blanc G (2010) BLAST-EXPLORER helps you building datasets for phylogenetic analysis. BMC Evol Biol 10:8

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res 36:W465–W469

Douglas AE (1998) Host benefit and the evolution of specialization in symbiosis. Heredity (Edinb) 81:599–603

Fitt WK (1984) The role of chemosensory behavior of *Symbiodinium microadriaticum*, intermediate hosts, and host behavior in the infection of coelenterates and molluscs with zooxanthellae. Mar Biol 81:9–17

Fitt WK, Trench RK (1981) Spawning, development, and acquisition of zooxanthellae by *Tridacna squamosa* (Mollusca, bivalvia). Biol Bull 161:213–235

Fournier A (2013) The story of symbiosis with zooxanthellae, or how they enable their host to thrive in a nutrient poor environment. Master Biosci Rev - Ec Norm Supérieure Lyon, p 8

Franklin EC, Stat M, Pochon X, Putnam HM, Gates RD (2012) GeoSymbio: a hybrid, cloud-based web application of global geospatial bioinformatics and ecoinformatics for *Symbiodinium*-host symbioses. Mol Ecol Resour 12:369–373

Gómez-Cabrera MC, Ortiz JC, Loh WKW, Ward S, Hoegh-Guldberg O (2007) Acquisition of symbiotic dinoflagellates (*Symbiodinium*) by juveniles of the coral *Acropora longicyathus*. Coral Reefs 27:219–226

Green EA, Davies SW, Matz MV, Medina M (2014) Quantifying cryptic *Symbiodinium* diversity within *Orbicella faveolata* and *Orbicella franksi* at the Flower Garden Banks, Gulf of Mexico. PeerJ 2:e386

Hagedorn M, Carter V, Zuchowicz N, Phillips M, Penfield C, Shamenek B, Vallen EA, Kleinhans FW, Peterson K, White M, Yancey PH (2015) Trehalose is a chemical attractant in the establishment of coral symbiosis. PLoS ONE 10:e0117087

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. 25: Coral reefs, ecosystems of the world. Elsevier Science Publishers, Amsterdam

Hill R, Ralph PJ (2007) Post-bleaching viability of expelled zooxanthellae from the scleractinian coral *Pocillopora damicornis*. Mar Ecol Prog Ser 352:137–144

Howells EJ, Berkelmans R, Van Oppen MJH, Willis BL, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. Ecology 94:1078–1088

Kenkel CD, Bay LK (2018) Exploring mechanisms that affect coral cooperation: symbiont transmission mode, cell density and community composition. PeerJ 6:e6047

Kenkel CD, Goodbody-Gringley G, Caillaud D, Davies SW, Bartels E, Matz MV (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. Mol Ecol 22:4335–4348

Klein HU, Bartenhagen C, Kohlmann A, Grossmann V, Ruckert C, Haferlach T, Dugas M (2011) R453Plus1Toolbox: An R/Bioconductor package for analyzing Roche 454 sequencing data. Bioinformatics 27:1162–1163

Kolde R (2015) Pheatmap: Pretty Heatmaps. R Package Version 1.0.8 http://cran.rproject.org/web/packages/pheatmap/index.html

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol 28:2570.e6–2580.e6

LaJeunesse TC, Smith R, Walther M, Pinzón J, Pettay DT, McGinley M, Aschaffenburg M, Medina-Rosas P, Cupul-Magaña AL, Pérez AL, Reyes-Bonilla H, Warner ME (2010) Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. Proceedings Biol Sci 277:2925–2934

Little AF, Van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science (80) 304:1492–1494

Littman RA, van Oppen MJH, Willis BL (2008) Methods for sampling free-living *Symbiodinium* (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef). J Exp Mar Bio Ecol 364:48–53

McIlroy SE, Coffroth MA (2017) Coral ontogeny affects early symbiont acquisition in laboratory-reared recruits. Coral Reefs 36:927–932

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8:e61217

Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of Zooxanthallae by a reef coral. Bio Bull 137:506–523

Muscatine L, Porter JW (1977) Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. Bioscience 27:454–460

Nitschke MR, Davy SK, Cribb TH, Ward S (2015) The effect of elevated temperature and substrate on free-living *Symbiodinium* cultures. Coral Reefs 34:161–171

Nitschke MR, Davy SK, Ward S (2016) Horizontal transmission of *Symbiodinium* cells between adult and juvenile corals is aided by benthic sediment. Coral Reefs 35:335–344

Parkinson JE, Banaszak AT, Altman NS, LaJeunesse TC, Baums IB (2015) Intraspecific diversity among partners drives functional variation in coral symbioses. Sci Rep 5:15667

Pochon X, Pawlowski J, Zaninetti L, Rowan R (2001) High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. Mar Biol 139:1069–1078

Pochon X, Stat M, Takabayashi M, Chasqui L, Chauka LJ, Logan DDK, Gates RD (2010) Comparison of endosymbiotic and free-living *Symbiodinium* (dinophyceae) diversity in a hawaiian reef environment. J Phycol 46:53–65

Poland DM, Coffroth MA (2017) Trans-generational specificity within a cnidarian–algal symbiosis. Coral Reefs 36:119–129

Poland DM, Mansfield JM, Hannes AR, Fairbank Lewis CL, Shearer TL, Connelly SJ, Kirk NL, Coffroth MA (2013) Variation in *Symbiodinium* communities in juvenile *Briareum asbestinum* (Cnidaria: Octocorallia) over temporal and spatial scales. Mar Ecol Prog Ser 476:23–37

Porto I, Granados C, Restrepo JC, Sánchez JA (2008) Macroalgal-associated dinoflagellates belonging to the genus *Symbiodinium* in caribbean reefs. PLoS ONE 3:e2160

Quigley KM, Bay LK, Willis BL (2017) Temperature and Water Quality-Related Patterns in Sediment-Associated *Symbiodinium* Communities Impact Symbiont Uptake and Fitness of Juveniles in the Genus *Acropora*. Front Mar Sci 4:401

Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz MV, Bay LK (2014) Deep-sequencing method for quantifying background abundances of *Symbiodinium* types: Exploring the rare *Symbiodinium* biosphere in reef-building corals. PLoS ONE 9:e94297

R Core Team (2017) R: a language and environment for statistical computing. R Found Stat Comput Vienna, Austria

Ralph PJ, Gademann R, Larkum AWD (2001) Zooxanthellae expelled from bleached corals at 33 °C are photosynthetically competent. Mar Ecol Prog Ser 220:163–168

Rippe JP, Matz MV, Green EA, Medina M, Khawaja NZ, Pongwarin T, Pinzón CJH, Castillo KD, Davies SW (2017) Population structure and connectivity of the mountainous star coral, *Orbicella faveolata*, throughout the wider Caribbean region. Ecol Evol 7:9234–9246

Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proc Natl Acad Sci 105:10444–10449

Sharp KH, Ritchie KB, Schupp PJ, Ritson-Williams R, Paul VJ (2010) Bacterial acquisition in juveniles of several broadcast spawning coral species. PLoS ONE 5:e10898

Stat M, Baker AC, Bourne DG, Correa AMS, Forsman Z, Huggett MJ, Pochon X, Skillings D, Toonen RJ, van Oppen MJH, Gates RD (2012) Molecular Delineation of Species in the Coral Holobiont. Adv Mar Biol 63:1–65

Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of *Symbiodinium* and scleractinian hosts-symbiosis, diversity, and the effect of climate change. Perspect Plant Ecol Evol Syst 8:23–43

Swain TD, Chandler J, Backman V, Marcelino L (2016) Consensus thermotolerance ranking for 110 *Symbiodinium phylotypes*: an exemplar utilization of a novel iterative partial-rank aggregation tool with broad application potential. Funct Ecol 31:172–183

Takabayashi M, Adams LM, Pochon X, Gates RD (2012) Genetic diversity of free-living *Symbiodinium* in surface water and sediment of Hawai'i and Florida. Coral Reefs 31:157–167

Takeuchi R, Jimbo M, Tanimoto F, Tanaka C, Harii S, Nakano Y, Yasumoto K, Watabe S (2017) Establishment of a model for chemoattraction of *Symbiodinium* and characterization of chemotactic compounds in *Acropora tenuis*. Fish Sci 83:479–487

Therneau TM, T. Lumley (2015) Package 'survival.' R Top Doc

Thornhill DJ, Howells EJ, Wham DC, Steury TD, Santos SR (2017) Population genetics of reef coral endosymbionts (*Symbiodinium*, Dinophyceae). Mol Ecol 26:2640–2659

Trench RK, Blank RJ (1987) *Symbiodinium microadriaticum* Freudenthal, *S. goreauii* sp. nov., *S. kawagutii* sp. nov. and *S. pilosum* sp. nov.: gymnodinioid dinoflagellate symbionts of marine invertebrates. J Phycol 23:469–481

van Oppen MJH (2004) Mode of zooxanthella transmission does not affect zooxanthella diversity in acroporid corals. Mar Biol 144:1–7

Weis VM, Reynolds WS, DeBoer MD, Krupp DA (2001) Host-symbiont specificity during onset of symbiosis between the dinoflagellates *Symbiodinium* spp. and planula larvae of the scleractinian coral *Fungia scutaria*. Coral Reefs 20:301–308

Yacobovitch T, Benayahu Y, Weis VM (2004) Motility of zooxanthellae isolated from the Red Sea soft coral *Heteroxenia fuscescens* (Cnidaria). J Exp Mar Bio Ecol 298:35–48

Yu G, Smith DK, Zhu H, Guan Y, Lam TTY (2017) ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol 8:28–36