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SYMBIODINIUM (DINOPHYCEAE) COMMUNITY PATTERNS IN INVERTEBRATE HOSTS FROM INSHORE MARGINAL REEFS OF THE SOUTHERN GREAT BARRIER REEF, AUSTRALIA[1]

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The broad range in physiological variation displayed by *Symbiodinium* spp. has proven imperative during periods of environmental change and contribute to the survival of their coral host. Characterizing how host and *Symbiodinium* community assemblages differ across environmentally distinct habitats provides useful information to predict how corals will respond to major environmental change. Despite the extensive characterizations of *Symbiodinium* diversity found amongst reef cnidarians on the Great Barrier Reef (GBR) substantial biogeographic gaps exist, especially across inshore habitats. Here, we investigate *Symbiodinium* community patterns in invertebrates from inshore and mid-shelf reefs on the southern GBR, Australia. Dominant *Symbiodinium* types were characterized using denaturing gradient gel electrophoresis fingerprinting and sequencing of the ITS2 region of the ribosomal DNA. Twenty one genetically distinct *Symbiodinium* types including four novel types were identified from 321 reef-invertebrate samples comprising three sub-generic clades (A, C, and D). A range of host genera harbored C22a, which is normally rare or absent from inshore or low latitude reefs in the GBR. Multivariate analysis showed that host identity and sea surface temperature best explained the variation in symbiont communities across sites. Patterns of changes in *Symbiodinium* community assemblage over small geographic distances (100s of kilometers or less) indicate the likelihood that shifts in *Symbiodinium* distributions and associated host populations, may occur in response to future climate change impacting the GBR.

Key index words: **coral reefs; Great Barrier Reef; ITS2; *Symbiodinium*; symbiosis**

***Abbreviations*: DGGE, denaturing gradient gel electrophoresis; GBR, Great Barrier Reef; ITS2, internal transcribed spacer region 2; SST, sea surface temperature**

The community diversity of symbiotic dinoflagellates (genus *Symbiodinium*) that live in association with reef building corals is imperative to the growth and survival of the holobiont (coral host and symbionts). This is especially relevant during periods of environmental stress such as increased sea surface temperature (SST) when the symbiosis is at risk of disruption. This disruption, also known as coral bleaching, has resulted in significant coral mortality over the past two decades (Hoegh-Guldberg 1999, Hughes et al. 2003, Pandolfi et al. 2003) and more recently, in April 2016, a mass coral bleaching event which has affected 93% of surveyed reefs on the (GBR; ARCCoE 2016). Besides reef building corals, bleaching can affect a wide range of reef-dwelling invertebrates and protists that live in association with *Symbiodinium*: soft octocorals, anemones, sponges, zoanthids, corallimorphs, giant clams, hydroids, and foraminifera (van Oppen et al. 2001, LaJeunesse et al. 2003, Pochon et al. 2004). The genus *Symbiodinium* is a large dinoflagellate group that contains nine genetic clades, A to I (Rowan and Powers 1991, Carlos et al. 1999, LaJeunesse and Trench 2000, Pawlowski et al. 2001, Pochon et al. 2004, Pochon and Gates 2010). Recent comparison of two *Symbiodinium* genomes from different clades indicates that divergence is higher than usually assumed (Shoguchi et al. 2013, Lin et al. 2015) with each clade comprising genetically and ecologically distinct lineages referred to as "types" (van Oppen et al. 2001, LaJeunesse et al. 2004b, 2014, Sampayo

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et al. 2007) or "species" although many remain undescribed (LaJeunesse et al. 2014, Parkinson et al. 2015, 2016). *Symbiodinium* species display differences in physiology which in turn can influence the holobionts' photosynthetic efficiency (Iglesias-Prieto et al. 2004), growth (Little et al. 2004) and stress response such as disease susceptibility (Littman et al. 2010) or thermal tolerance (Rowan and Knowlton 1995, Berkelmans and van Oppen 2006, Warner et al. 2006, Jones et al. 2008, Sampayo et al. 2008, Finney et al. 2010). Factors that are known to shape host–symbiont distributions include host identity and specificity (e.g., link between specific *Symbiodinium* types and host species or genera; van Oppen et al. 2001, LaJeunesse et al. 2004a, Frade et al. 2008, Stat et al. 2008, LaJeunesse et al. 2010), longstanding biogeographic partitioning (LaJeunesse et al. 2003, 2004a, 2010) as well as regional and local environmental conditions (Oliver and Palumbi 2009, LaJeunesse et al. 2010, Cooper et al. 2011). Characterizing how host and *Symbiodinium* community assemblages differ across environmentally distinct habitats is needed to provide useful information to assess the vulnerability of reef corals and how they will cope with changing environmental conditions in the fate of current climate change (LaJeunesse 2001, Oliver and Palumbi 2009, LaJeunesse et al. 2010).

A recent compilation of currently available data on dominant *Symbiodinium* communities on the GBR, which is amongst one of the most abundantly studied areas, revealed a surprising lack of information on reef invertebrate-*Symbiodinium* symbioses particularly from inshore areas in the southern region (Tonk et al. 2013b). To address this biogeographic gap, we focus on the dominant *Symbiodinium* types of inshore communities of the Mackay/Capricorn area situated at the southern end of the GBR. These areas are often so-called marginal reefs, characterized by lower species diversity and abundance. Inshore reefs of the southern GBR are exposed to reduced water quality due to terrestrial nutrient and sediment runoff (exacerbated by punctuated flood events), and higher seasonal fluctuations in SST's. Inshore reefs can vary considerably in community structure depending on habitat type and are typically characterized by particle feeders, well-suited to turbid environments (DeVantier et al. 2006).

The relatively lower SST's in the southern GBR may provide potential refugia for some coral species to escape increasing SST, and these areas could therefore play a key role in the persistence of corals under the increasing pressures of climate change. Climate change related migration to cooler areas is in fact observed in a range of terrestrial and marine organisms (Parmesan and Yohe 2003) including corals (Yamano et al. 2011, Baird et al. 2012). Inshore reef communities may already include symbiont types that are more resistant to fluctuations in temperature due to naturally occurring broader seasonal variation.

Here, we aim to expand the investigation of dominant *Symbiodinium* communities in invertebrates of the GBR by including inshore locations of the Mackay/Capricorn area situated at the southern end of the GBR. Adding these locations will contribute to understanding *Symbiodinium* biogeography while the multivariate analysis of *Symbiodinium* community data from inshore reefs combined with data from mid-shelf reefs provides insights into local environmental factors that influence *Symbiodinium* community diversity in the absence of large biogeographical barriers.

MATERIALS AND METHODS

Specimen collection. A total of 321 reef invertebrates were sampled by SCUBA from inshore sites along a latitudinal gradient within the Great Sandy Marine Park (Hervey Bay, 25°16.9337′ S, 152°51.3218′ E; Elliot Heads, 24°55.1393′ S, 152°29.6272′ E; Burkitts Reef, 24°48.7355′ S, 152°28.154′ E) and from inshore sites and a mid-shelf reef within the Mackay/Capricorn section of the Great Barrier Reef Marine Park (respectively; Hummock Hill, 24°00.2214′ S, 151°29.9192′ E, Pancake Creek, 24°01.3736′ S, 151°44.3993′ E and Fitzroy Reef, 23°37.1515′ S, 152°09.3932′ E; Fig. 1, Table 1). Samples were collected in the months May, June, and September of 2008. Inshore sites were sampled at 3–6 m except for Hervey Bay, which was sampled at 0.5–3 m due to the shallow setting of the invertebrate community at that site. The mid-shelf reef at Fitzroy was additionally sampled at 16–18 m due to its more extensive depth range. The sampling design was targeted to encompass a wide range of invertebrate hosts harboring *Symbiodinium* spp. (van Oppen et al. 2001, LaJeunesse et al. 2003, 2004a), and collections spanned various classes of organisms (Anthozoa, Hydrozoa and Mollusca) representing five orders, 16 families, and 33 genera (Table S1 in the Supporting Information). Up to four individuals were sampled per species at each site, and a photographic record and fragment were taken for taxonomic reference. Soft coral identification was based on morphological characteristics and microscopic examination (Fabricius and Alderslade 2001) while scleractinian corals were identified based on whole colony and skeletal characteristics (Veron 2000, 2002). Tissue samples were preserved in 20% DMSO buffered saline (Seutin et al. 1991) and stored at −20°C until further processing.

Genotyping. DNA was extracted using the Wizard DNA extraction protocol (LaJeunesse et al. 2003) and the internal transcribed spacer region 2 (ITS2) of the ribosomal genes was amplified with *Symbiodinium* specific primers "ITSintfor 2" and "ITS2 clamp" (LaJeunesse et al. 2003). PCR amplification was performed according to conditions specified by LaJeunesse et al. (2003) and followed by screening for polymorphisms using denaturing gradient gel electrophoresis (DGGE) on a CBScientific system (LaJeunesse 2001, LaJeunesse et al. 2003). The DGGE was run for 14 h on 8% polyacrylamide gels (37.5:1 acrylamide:bis) with a gradient of 30%–60% denaturants (formamide and urea). Up to five representative samples of each ITS2-DGGE fingerprint were used to identify the prevailing *Symbiodinium* types. Note that DGGE picks up the dominant *Symbiodinium* types that make up >5%–10% of the total population (Thornhill et al. 2006, Mieog et al. 2007). This was done by stabbing the dominant bands from the denaturing gel using a 10 μL pipette tip and placing the tip in 30 μL H_2O for 2 h. The diluted DNA was then re-amplified using the ITSintfor-2 primer and reverse primer ITS2rev (without the GC-clamp) using PCR conditions

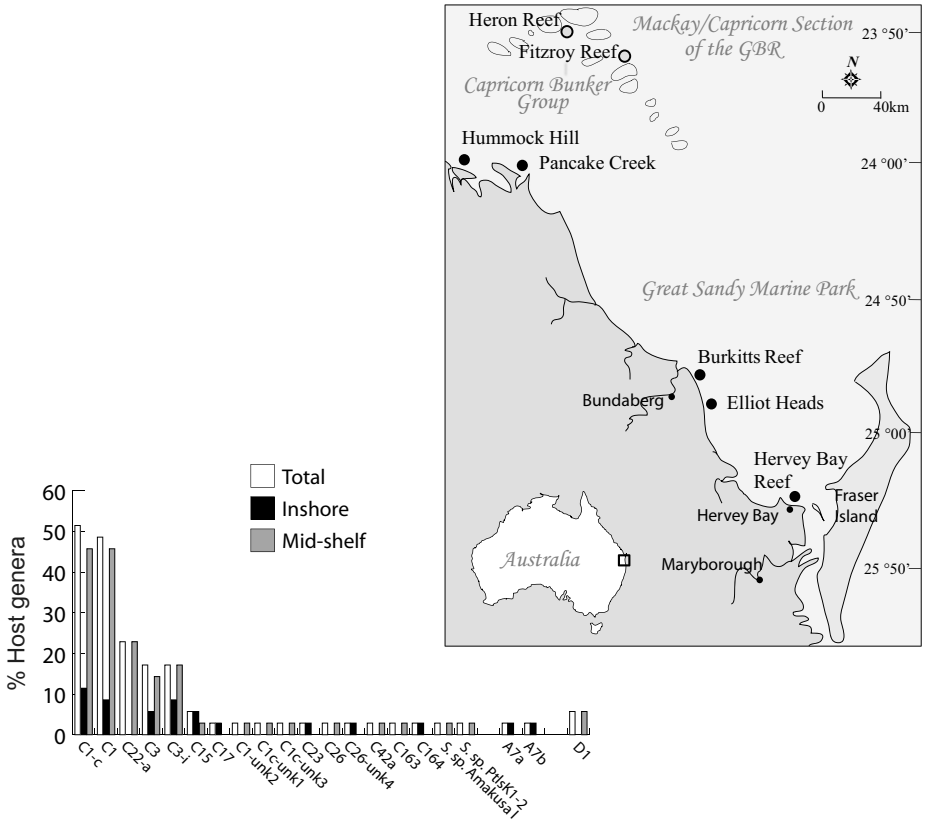

FIG. 1. Map showing the sample locations in the Mackay/Capricorn section of the Great Barrier Reef and Great Sandy Marine Park. A comparison of the dominant *Symbiodinium* types and community structure identified in this study is shown across shelf positions. The *Symbiodinium* types are indicated on the *x*-axis and the percentage of host genera that were associated with each symbiont type is presented on the *y*-axis.

TABLE 1. Site information and environmental parameter data of the sampled sites.

Shelf position Site	Depth range (m)	Zsd (m)[a]	Yearly avg SST (SD) (°C)[b]	Yearly avg Chl *a* (SD) (mg · m^3)[b]	Distance to coast (km)	PARz (Einstein · m^{-2} · d)[c]	Host sample size (*n*)
Inshore							
Hummock Hill	3–6	6.04–7.8	23.92 (2.96)	2.32 (0.36)	0.1	19.28	87
Pancake Creek	3–6	6.04–7.8	23.94 (2.70)	0.85 (0.38)	0.05	29.25	28
Burkitts Reef	3–6		23.63 (3.03)	0.79 (0.21)	0.1	28.31	81
Elliot Heads	3–6		23.58 (3.04)	0.82 (0.20)	0.1	28.44	68
Hervey Bay	0.5–2		23.60 (3.11)	2.72 (0.35)	0.03	32.54	26
Mid-shelf							
Heron Island	3–5	13.0–16.7	24.29 (2.08)	0.40 (0.07)	80	31.51	90
	10				80	14.63	37
Fitzroy Reef	3–6	13.0–16.7	24.39 (1.99)	0.37 (0.06)	63.5	33.48	16
	16–18				63.5	16.26	15

[a]Data acquired from e Atlas (e-atlas.org.au).
[b]Data acquired from Giovanni data system (gdata1.sci.gsfc.nasa.gov).
[c]Based on light attenuation coefficient (Kd).

specified in LaJeunesse et al. (2003). PCR products were cleaned using ExoSAP-it (GE Healthcare Limited, Buckinghamshire, UK) according to the manufacturers' protocol and sequenced at the Australian Genome Research Facility (University of Queensland, Australia) and the Pennsylvania State University Nucleic Acid Facility (State College, USA). Both facilities use an ABI 3730*xl* sequencer in combination with BigDye Terminator sequencing reaction kits.

Phylogenetic analysis. Sequence chromatograms were visually checked using Codoncode Aligner version 3.5.3. (Codoncode Corporation, Centerville, Massachusetts, USA) and aligned using ClustalW (Thompson et al. 1994). The resulting sequences were blasted against previously recorded *Symbiodinium* types on GenBank (http://www.ncbi.nih.gov). Phylogenies were estimated by means of Maximum Parsimony and Maximum Likelihood analysis using Paup (Swofford 2001), treating gaps as a 5th character state, counting indels as a single state change and using a delayed transitions model. Bootstrap values were acquired using 1,000 replicates.

Environmental variables. Time series were derived for SST, chlorophyll *a* (Chl *a*) and the diffuse attenuation coefficient (Kd) over the period 2002–2008 (at 4 km spatial resolution) from the MODIS/aqua interface of the Giovanni online data system, developed and maintained by the NASA GES DISC (Fig. 2, A and B). The SST metrics included the monthly mean climatology over the 6 y period as well as the long-term

SST climatology. Similarly, the standard deviation of the long-term climatology over the period of 2002–2008 (SSTstdev) was calculated from the monthly climatology and used as a proxy for the SST range the holobionts (hosts and symbionts) are exposed to. The same metrics were obtained for Chl *a* as a measure for turbidity (Table 1). The Kd was used to calculate the proportion of photosynthetically available radiation (PAR) at each sampling depth (Fig. 2C) using the following formula:

$$\mathrm{PARz} = \mathrm{PAR} * e[-\mathrm{Kd} * z] \quad (1)$$

where Kd is the light attenuation coefficient and z is the depth in meters (Table 1). Due to the proximity to shore and spatial resolution of the time series an alternative pixel was chosen for inshore sites to exclude land data. Additional biophysical parameters used were distance to the mainland and depth (Table 1).

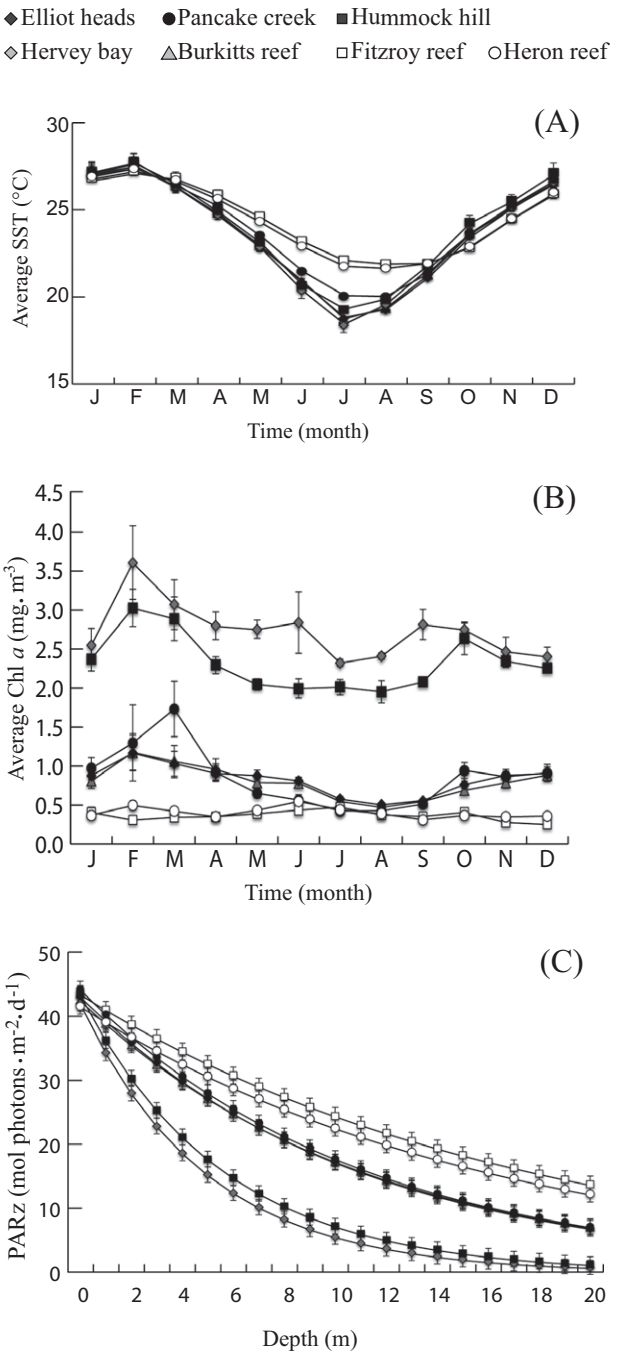

FIG. 2. Monthly averages of (A) SST and (B) chlorophyll *a* (Chl *a*) at sites in the Mackay/Capricorn section of the GBR. Error bars indicate standard error. Average monthly values over a period from July 2002 to June 2008 were acquired from the Giovanni online data system, developed and maintained by the NASA GES DISC. (C) Proportion of incident PAR over depth was calculated from the light attenuation coefficient (Kd) at the different shelf locations.

Statistical analyses. Multivariate analyses were performed in PRIMER-e (v6.1.13) with the PERMANOVA add-on (v1.03; Anderson et al. 2008) to test for the effect of environmental parameters and host identity on the *Symbiodinium* community composition across sites. Additional invertebrate-*Symbiodinium* data from Heron Island (LaJeunesse et al. 2003) situated north of Fitzroy Reef (Fig. 1) was added to improve comparative analyses across shelf position. The factors shelf and depth were used to define the groups of samples in five inshore shallow groups and four mid-shelf groups (two deep and two shallow groups). The environmental dataset was analyzed using the Pearson coefficient to calculate co-linearity amongst variables. Correlation analyses were rerun and redundant variables were removed prior to subsequent analyses (Pearson's $r > 0.8$ or $r < -0.8$; excluding monthly mean SST and Chl *a* climatologies, standard deviation of the long-term climatology and distance to shore), maintaining the long-term mean SST climatology, the long-term mean Chl *a* climatology, and relative irradiance (% PAR). A similarity matrix of the environmental data (log transformed and normalized, Euclidean distance) was used to perform a permutational multivariate ANOVA (PERMANOVA) between sites based on shelf position and depth (Table 1). PERMANOVA makes the assumption that dispersions are roughly constant across groups. Therefore, PERMDISP (permutational analysis of multivariate dispersions) was used to test for heterogeneity in groups defined by the factors shelf and depth. A principal component analysis (PCA) was performed to visualize spatial patterns between sites as a function of environment.

A similarity matrix of the host and *Symbiodinium* data (presence/absence, S7 Jaccard) was used to perform PERMANOVA and PERMDISP between sites based on depth (Table 1). Similarity (S7 Jaccard) in the host and symbiont data (presence/absence) was analyzed using Principle Coordinate Analyses (PCO). RELATE was used to test for a significant relation between host and the environment and the host and symbiont data matrices (Spearman rho, rho ≈ 0 indicates no relation is found, rho = 1 indicates a perfect relation). Distance-based analysis on a linear model (distLM) was used to model the relationship between the symbiont dissimilarity data and the environmental variables chosen. Host–symbiont specificity can obscure multivariate analysis assessing the relative contribution of environmental drivers in the distribution of *Symbiodinium* due to the well-established connection between specific *Symbiodinium* types and host species or genera. To include the influence of the host on the symbiont matrix, PCO1 and PCO2 of the host presence/absence data (HPCO1 and HPCO2 for continued reference) were added as covariates to the environmental data matrix in subsequent linear regression data analyses (cf. Tonk et al. 2013b). No further PCO's were added since this reduced the % explained variance in the fitted model as well as to prevent over-fitting of the covariates in the multiple linear regression analyses (distLM). Another way to reduce the host effect is by focusing on particular species or life history traits, for example reproductive or symbiont transmission mode. Therefore, a separate analysis was done on host genera that have an

open symbiont transmission and acquire symbionts from the environment (horizontal transmission) as opposed to species with a direct transmission that obtain their symbionts from the maternal colony (vertical transmission). Subsequently symbiont data from a total of twenty host genera that are known to broadcast spawn (Kerr et al. 2011) were used in a separate distLM analysis.

In the distLM analysis, marginal tests assessed the importance of each variable separately while in the sequential tests a forward search of the optimal fit of a combination of environmental variables was used by sequentially adding variables. The data were visualized with distance based redundancy analyses (dbRDA) ordination plots. Vector overlays using the environmental data and symbiont data independently as predictor variables (drawn as multiple partial correlations) were applied to visualize the effect, strength and direction of the different variables in the ordination plots.

RESULTS

Descriptive data of Symbiodinium *communities.* A total of 21 genetically distinct *Symbiodinium* types were identified from clades A (2 types), C (18 types), and D (1 type) in 34 host genera across six sites (Fig. 1). While most *Symbiodinium* types found here have previously been recorded in hosts from the GBR (LaJeunesse et al. 2003, 2004b, Stat et al. 2008), *Symbiodinium* sp. Amakusa I (a C1 derivative) and *Symbiodinium* sp. Ptlsk1-2 (a C3 derivative) have thus far only been documented in the northwestern Pacific (Rodriguez-Lanetty and Hoegh-Guldberg 2003, Reimer et al. 2006; Table S1). In addition, four novel *Symbiodinium* types were characterized from two inshore and a mid-shelf reef (C163, C164, A7a, and A7b, GenBank accession nos': KJ801946–KJ801949). C163 is a C15 derivative detected in six anemones of the species *Heteractis crispa* whereas C164 is a C21 derivative found in a *Montipora flabellata* colony (Fig. 3). A7a and A7b were found in *Millepora* colonies (fire coral). The octocorals *Guaiagorgia* sp. ($n = 4$) and *Dendronephthya* sp. ($n = 7$) that are believed to be azooxanthellate appeared to harbor C1c and C1c, C3i and C22a respectively (Table S1). However, histological examination could not confirm *Symbiodinium* presence in the tissue of these specimens, which was degraded by the use of 20% DMSO for DNA preservation.

Both host-generalist and host-specific *Symbiodinium* types were found. The prevailing host generalists included types C1c (found associated with 70 samples across 17 genera), C1 a.k.a. *Symbiodinium* goreaui (Thornhill et al. 2014, Wham and LaJeunesse 2016, in 85 samples across 17 genera), and C22a (in 36 samples across six genera; Fig. 1; Table S2 in the Supporting Information). Most soft corals harbored C3i (54%) or C1c (37%). The majority of the hard corals harbored C1 (48%) or C22a (20%; Table S2). Other types were detected in a single host genus or species such as C17, C23, C26, *Symbiodinium* sp. Ptlsk1-2, D1, and the four novel types (Table S2). Such types were generally consistent with previously identified host-specific

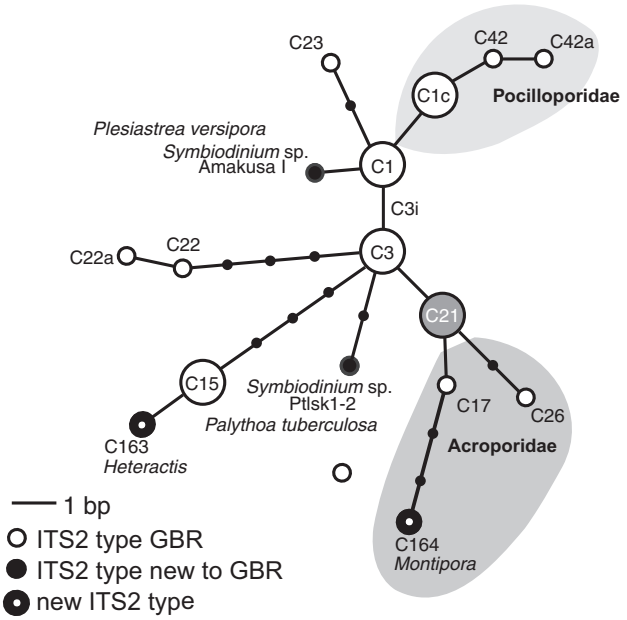

FIG. 3. Maximum Parsimony unrooted tree of clade C *Symbiodinium* ITS2 diversity. Open circles indicate *Symbiodinium* spp. found in this study that are similar to previously detected combinations. C21 (in gray) was not identified in this study. Black dots indicate symbiont types new to the GBR but previously detected in hosts elsewhere. Novel types are indicated with thick open circles and include the host genus. Small black dots indicate 1 base change.

associations such as C17 and C26 with *Montipora* (LaJeunesse et al. 2003, 2004a). Mixtures of two *Symbiodinium* types were detected in seven host colonies of which two harbored *Symbiodinium* types from different clades (clade C and D; Table S1). The average *Symbiodinium* to host diversity ratio found in this study was 0.62 ITS-types/host genera and varied from 0.37 to 1.14 for inshore sites and was 0.79 for mid-shelf Fitzroy reef (Table S1).

The inshore communities structurally varied per site in the amount of host diversity and the ratio of soft to hard corals. For example, the host community at Hervey Bay was low in diversity (eight host genera sampled) and mostly consisted of soft corals (*Sinularia*, *Klyxum*, *Lobophytum*, and *Cladiella*) with only two sampled hard coral species (*Turbinaria spp*). Whereas the host community at most inshore sites consisted of a variety of both soft and hard coral species, Pancake Creek represents an *Acropora* dominated inshore reef situated in an estuary along the east bank near the broad entrance of the creek. At least 13 different *Acropora* species were found at this site Veron 2000, resulting in a relatively high symbiont type/host genera ratio of 1.14 (8 symbiont types/7 host genera).

Environmental and multivariate data analyses. The average monthly SST showed largest differences between the austral summer (January/February) and winter months (July/August) within each location (Fig. 2A). The mid-shelf reef Fitzroy had the

highest yearly SST (24.39°C; Table 1) but no significant differences were found between monthly SST averages at different locations (Tukey's HSD). The average monthly Chl *a* concentrations of the inshore sites varied throughout the year with highest values in February/March (highest for Hervey Bay; Table 1) and lowest in July/August. In contrast, mid-shelf reefs of Fitzroy and Heron showed little variation throughout the year (Fig. 2B).

The environmental parameters long-term mean SST climatology, long-term mean Chl *a* climatology and PAR at sample depth (*z*) were used in a permutational multivariate analysis to assess for differences between sites based on shelf position and depth (Table 1). Inshore sites significantly differed from mid-shelf sites (PERMANOVA: Pseudo-$F_{1,6}$ = 7.056, P = 0.013) and shallow versus deep locations also differed significantly (PERMANOVA: Pseudo-$F_{1,6}$ = 5.088, P = 0.003). PERMDISP showed no significant differences in dispersions across groups. The PCA of the environmental data clearly shows that the ordination of inshore sites is separated from mid-shelf sites based on decreasing Chl *a* and increasing SST over an inshore to mid-shelf gradient whereas most shallow sites are distinct from deeper sites due to the percentage of incident PAR at those depths (Fig. S1 in the Supporting Information).

The sampled host community showed no significant differences between shelf positions or between shallow versus deep sites (PERMANOVA). The *Symbiodinium* community showed no significant differences between shallow versus deep sites but a significant difference was found between *Symbiodinium* communities from inshore sites versus mid-shelf sites (PERMANOVA; Pseudo-$F_{1,5}$ = 2.063, P = 0.017). However, the dispersions of symbiont groups significantly differed based on shelf position (PERMDISP; $F_{1,7}$ = 6.362, P = 0.042) and it is therefore not clear whether the difference in *Symbiodinium* community between shelf positions is compounded by the unequal dispersion of groups. PERMDISP showed no significant differences in dispersions across host groups or symbiont groups based on depth. A significant relation between the host and symbiont data matrix (RELATE; rho = 0.575, P = 0.035) indicated that host identity played an imperative role in the symbiont assemblages found across sites. The first two principal coordinate axes (PCO1 and PCO2) of the PCO on the host presence/absence data explained 50.4% of the variation in the host data matrix (Fig. S2 in the Supporting Information) and these two coordinate axes were added as covariates to the environmental data matrix in subsequent regression analyses to include the host effect (denoted as HPCO1 and HPCO2; Fig. 4, A and B). The environmental parameters significantly influenced both host and symbiont distributions across sites (RELATE; rho = 0.716, P = 0.004 and rho = 0.561 and P = 0.002 respectively). A dbRDA on the symbiont data (transformed to presence/absence) indicated that 60.4% of the variation in the original data was explained by the fitted model. Within the fitted model 47.1% of the total variation was explained by the parameters chosen (Fig. 4A). Of these parameters, long-term mean SST climatology, long-term mean Chl *a* climatology and HPCO1 were independent significant drivers of the sampled *Symbiodinium* community (distLM, marginal tests; Table 2). A forward search for the optimal fit of a combination of variables by sequential addition of these variables showed that long-term mean SST climatology was the main significant variable, but the addition of HPCO1, Chl *a*, PARz, and HPCO2 contributed to the selection of the model that best described *Symbiodinium* diversity assemblages across sites (distLM, sequential tests; Table 2; Fig. 4A). The ordination of mid-shelf sites was separated from inshore sites by the influence of HPCO1, lower SST and higher PARz and Chl *a* (Fig. 4A).

A dbRDA on a subset of the data consisting of symbiont information from host genera with an open transmission mode showed that the reduced fitted model itself was more apt at explaining the variation in the original data (85.8%) and the total variation in the fitted model explained by the parameters chosen was slightly higher (49%; Fig. 4B). SST, Chl *a*, and HPCO1 were significant drivers of the *Symbiodinium* community of this data subset when assessed separately (Table 2). In a forward search for the optimal fit of a combination of variables, SST and HPCO2 were found significant and HPCO1 contributed to the selection of the best model (Table 2; Fig. 4B). Mid-shelf sites were separated from inshore sites under the influence of HPCO1 and higher SST when using data of host genera with an open symbiont transmission mode (Fig. 4B).

DISCUSSION

Closing knowledge gaps of reef invertebrate-dinoflagellate genetic diversity and identifying the environmental factors influencing community patterns is needed to improve predictions on how the symbiotic reef community will respond to a changing environment. This study complements *Symbiodinium* information from the GBR and shows that the dominant *Symbiodinium* community on inshore sites in the southern GBR is less diverse compared to more offshore positioned reefs, but comparable to reefs situated inshore at lower latitudes. The predominant *Symbiodinium* types (C1, C1c, and C22a) were clearly more abundant in inshore hosts. In addition, this study showed that host identity and the environmental factors, long-term SST climatology and long-term Chl *a*, best explained the variation in symbiont communities across sites. Similar results explaining even more of the variation found in the *Symbiodinium* community data were obtained when only

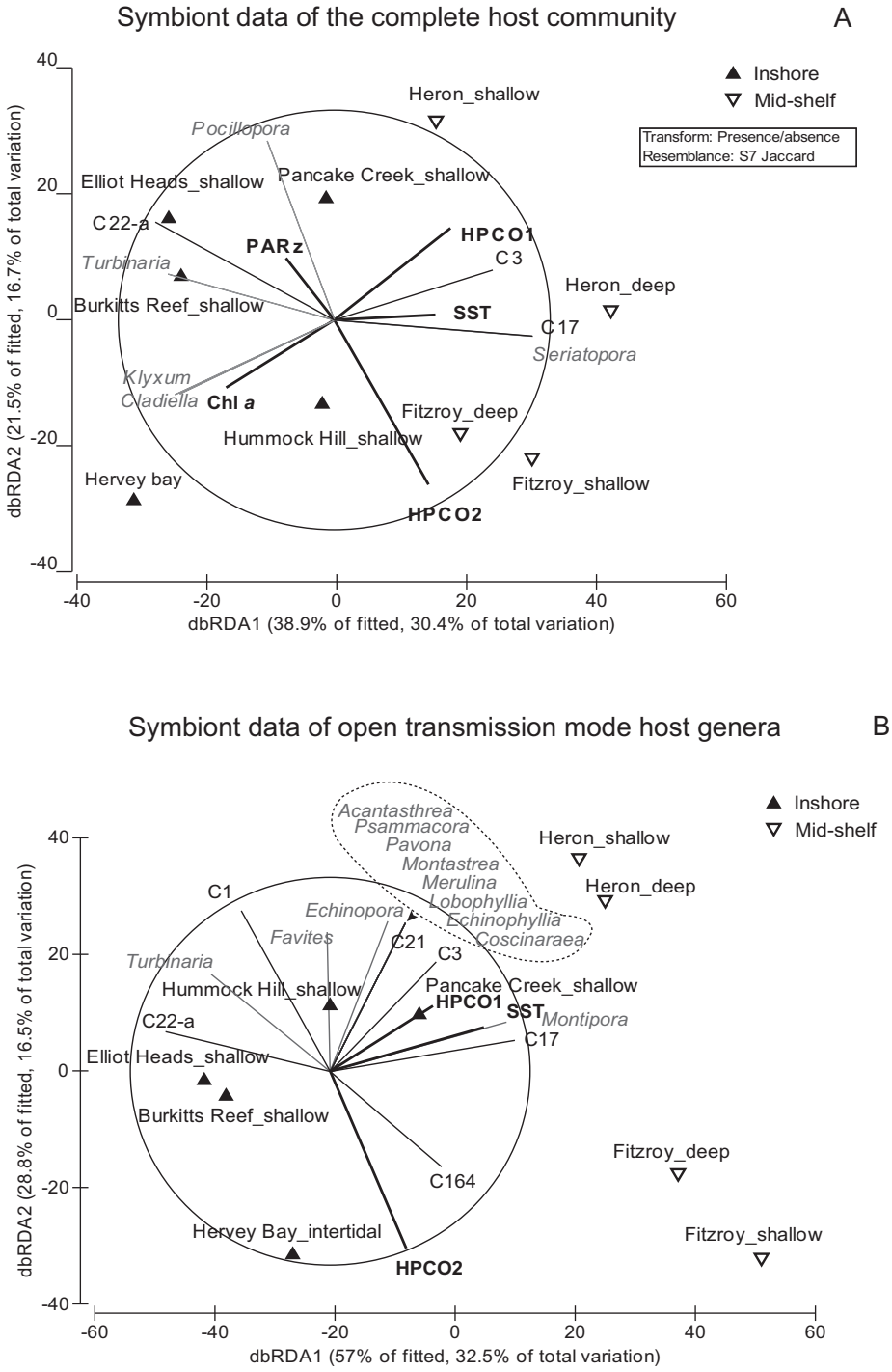

FIG. 4. Distance-based RDA plots relating environmental variables and host genus information to *Symbiodinium* data of the complete host community (A) and of 20 host genera with open symbiont transmission mode (B). Biplot projections are shown for the effect of environmental factors (in bold), symbiont ITS-type data (in black) and host data (in gray). "the % of fitted" explains the percentage of the variability in the original data explained by the axis, and "the % of total variation" indicates the percentage of variation in the fitted matrix explained by the axis.

using symbiont data from host genera with an open symbiont transmission mode.

Symbiodinium *community assemblage & host specificity. Symbiodinium* type/host genera ratio of the inshore sites (except the site Pancake Creek) appeared low (Table S2) when compared to the ratio found on Fitzroy reef (0.79 ITS- types/host genera; this study) and sites around Heron Island (0.85, 34 ITS-types/40 host genera; LaJeunesse et al. 2003, Sampayo et al. 2007, Stat et al. 2008, Ulstrup et al. 2008). This was highly dependent on the local habitat generating variation in host communities across sites. The high ITS-type/host genera ratio (1.14) at Pancake Creek was due to the high amount of *Acropora* species sampled at that site resulting in relatively few sampled host genera (7). However, most inshore sites showed a much lower symbiont type/host genera ratio, comparable to the

TABLE 2. Summary of the distLM analyses. Table shows output for model selection of the relationship between *Symbiodinium* communities, host and/or environmental variables for A: the total host community and B: open transmission host genera. For the sequential test results only those variables are shown that are selected in the best model. The percentage of variance explained (% var. expl.) by each of the environmental variables selected.

Group	Pseudo-*F*	*P*-value	% var. expl.
(A1) Marginal tests: symbiont community sGBR (complete dataset)			
SST	2.63	0.007[a]	27.3
Chl *a*	2.12	0.011[a]	23.3
PARz	1.07	0.346	13.2
HPCO1	2.18	0.012[a]	23.8
HPCO2	1.76	0.054	20.1

Group	Adjusted R^2	Pseudo-*F*	*P*-value	% var. expl.	df
(A2) Sequential tests: symbiont community sGBR (complete dataset)					
SST	0.17	2.63	0.006[a]	27.3	7
HPCO1	0.23	1.50	0.093	14.5	6
Chl *a*	0.28	1.42	0.18	12.8	5
PARz	0.31	1.28	0.30	11.0	4
HPCO2	0.41	1.68	0.18	12.3	3

Group	Pseudo-*F*	*P*-value	% var. expl.
(B1) Marginal tests: symbiont community sGBR (dataset restricted to hosts with open transmission mode)			
SST	3.20	0.002[a]	31.4
Chl *a*	2.11	0.025[a]	23.2
PARz	0.91	0.528	11.4
HPCO1	2.34	0.025[a]	25.0
HPCO2	1.77	0.081	20.2

Group	Adjusted R^2	Pseudo-*F*	*P*-value	% var. expl.	df
(B2) Sequential tests: symbiont community sGBR (dataset restricted to hosts with open transmission mode)					
SST	0.22	3.20	0.001[a]	31.4	7
HPCO2	0.31	2.00	0.009[a]	17.2	6
HPCO1	0.31	1.00	0.545	8.6	5

[a]Significant values.

northern situated inshore site of Curaçao Island (0.44 ITS- types/host genera; LaJeunesse et al. 2004a,b). Host species diversity is generally lower in high nutrient inshore environments (Fabricius et al. 2005), reducing opportunities for symbiont diversity. In addition the smaller depth range of shallower inshore reefs as opposed to mid-shelf or outer reefs can, at least partly, explain the difference in *Symbiodinium* communities over an inshore to outer reef gradient. Sampling over a wider depth range, corresponding to a larger irradiance range, increases the chance of including host colonies that live in association with depth specialist symbionts (Rowan et al. 1997, Sampayo et al. 2007, Frade et al. 2008). The smaller irradiance range of inshore regions is exacerbated by the higher turbidity at these locations, providing a more uniform light habitat.

Concurrent with previous findings on the GBR, clade C dominance was found throughout the investigated sites as well as the commonly found pattern of few host generalists and various host specific *Symbiodinium* types (LaJeunesse et al. 2003, 2004a, LaJeunesse 2005). The most prevalent host generalist types were C1c, C1, C22a, C3, and C3i. While C22a has been found associated with *Lobophyllia* and two *Turbinaria* species on the GBR in the past (LaJeunesse et al. 2003), this study found C22a associated with eight host genera although most frequently detected in *Turbinaria*. While C1, C3, and C1c are tightly linked to the ancestral core, C22a does not display the typical central phylogenetic position of these generalist types (LaJeunesse 2005) making it an interesting host generalist candidate that may be more abundant in marginal or high latitude reef communities.

While most *Symbiodinium* types matched with previously detected types in the southern GBR (LaJeunesse et al. 2003), two types were only detected at sites south of Japan (Rodriguez-Lanetty and Hoegh-Guldberg 2003, Reimer et al. 2006). These northwestern Pacific *Symbiodinium* types occurred at similar latitudes. Mackay sites are situated between 23°37′ S and 25°16′ S and Amakusa and Ishigaki Island at 24°20′ N and 32°00′ N respectively. The sporadically detected types were, however, found in different host species (*Symbiodinium* sp. Amakusa I in *Sarcophyton* sp., GBR, instead of *Plesiastrea versipora*, Japan; and *Symbiodinium* sp. Ptlsk1-2 in *Turbinaria* sp., GBR, instead of *Palythoa tuberculosa*, Japan). In general, rare types are often host specialists or endemic species. Our finding of *Symbiodinium* sp. Amakusa I and *Symbiodinium* sp. Ptlsk1-2 on the GBR suggests that these types have either evolved in parallel despite geographic separation and different host environments or perhaps are less specific than previously thought. However, fine-scale genetic markers may reveal different lineages within ITS types, indicating genetic isolation between these regions (LaJeunesse and Thornhill 2011, Thornhill et al. 2014). Of the four novel types, two (A7a and A7b) were identified from *Millepora* spp. (fire coral) colonies at Fitzroy reef and showed most similarity with A7, previously found in *Millepora platyphylla* on the southern GBR near Heron Island (LaJeunesse et al. 2003). It should be noted that these types are likely genetic variants of the same species lineage. The novel type C163, a close relative of C15, was detected in six anemones of the species *H. crispa* at Burkitts Reef and Elliot Heads. Other GBR members of the *Stichodactylidae* family are mainly known to harbor C1 derivatives like C25, C67, C68, and C69a (LaJeunesse et al. 2003, 2004a) instead of members of the C15 radiation (commonly found in Poritidae, *Montipora*; LaJeunesse 2005). It is noteworthy that C15 has previously been linked to environments high in SST and turbidity (LaJeunesse et al. 2010) and displays an increased temperature tolerance (Fisher et al. 2012). The application of different phylogenetic markers would improve

species delineation as in certain instances distinct ITS2 types might represent population variants of the same species (such as in the case of A7a, A7b) while in other instances the same type might actually represent various species (such as C1c, C1, or C3 – highlighted by LaJeunesse and Thornhill 2011, Thornhill et al. 2014).

Some host species are capable of harboring multiple *Symbiodinium* types from either the same or from different genetic clades. In our study, 2% of the sampled hosts contained a mixture of two dominant *Symbiodinium* types (Table S1). Studies in the northern part of the GBR using a similar sampling approach and detection technique have shown that mixtures of dominant *Symbiodinium* in a single host are in fact more common. Around Lizard Island and the far northern region a mixture of *Symbiodinium* was detected in, respectively, 4% (Tonk et al. 2014) and 33% (Tonk, unpublished data) of the sampled host colonies. This relative increase is potentially linked to the higher SST's and the occurrence of "bleaching hotspots" in this low latitude region. Bleaching hotspots are defined by SST's exceeding the climatological maximum by 1°C or more, conditions under which corals are more likely to bleach (Rayner et al. 2003). Our chosen identification technique, DGGE, which is mostly used because of its ability to detect a range of *Symbiodinium* types has detection levels of 5%–10% and is likely to underestimate the total *Symbiodinium* diversity of some host colonies (Thornhill et al. 2006, Mieog et al. 2007). On the other hand the higher sequence diversity that is detected with more sensitive sequencing based approaches overestimates diversity by the inclusion of low abundant intragenomic variants, transient opportunistic *Symbiodinium* or even non-symbiotic types (e.g., ingested types, contamination from seawater or symbionts living on the host mucus; Arif et al. 2014, Lee et al. 2016). These ecologically cryptic or "background" types do not contribute to the coral's physiology in the same way that high abundant and stable *Symbiodinium* types do and may confound the *Symbiodinium* distribution data thereby preventing the detection of environmental factors that explain distribution patterns (Arif et al. 2014, Lee et al. 2016).

Symbiodinium *distribution.* The percentage of host genera harboring C1 and C3 at the investigated inshore sites was comparable to those found at Heron Island (LaJeunesse et al. 2004a). C1 is the predominant type in the central and northern GBR followed by C3 (northern GBR) and C3h (central GBR; LaJeunesse et al. 2010, Tonk et al. 2014). Our results show that, despite being common amongst host genera throughout the GBR, C1c was considerably more abundant in the southern inshore GBR. C3i was prevalent in the southern GBR but is also common throughout the GBR whereas C22a, which was prevalent amongst inshore sites, appeared largely restricted to host genera in the southern GBR (LaJeunesse et al. 2003, 2010, Tonk et al. 2013a). C22a was also found in *Turbinaria* at the Kermadec Islands, New Zealand (Wicks et al. 2010) and its occurrence might be linked to marginal reef environments.

Members of the enigmatic group of clade D *Symbiodinium* were only sporadically found as dominant symbionts (less than 1% of the sampled colonies). Particularly, *Symbiodinium* D1 and *Symbiodinium trenchii* (D1–4 or D1a; LaJeunesse et al. 2014) are often found in stressful conditions such as high SST and high turbidity (Ulstrup and Van Oppen 2003, Fabricius et al. 2004, LaJeunesse et al. 2009, 2010, Oliver and Palumbi 2009), however, they are also found in relation to low to medium SST and high turbidity, conditions similar to the inshore southern GBR (Chen et al. 2003, Lien et al. 2007). Our results are in line with a relatively low occurrence of clade D as part of the dominant *Symbiodinium* assemblage in hosts throughout GBR sites (van Oppen et al. 2005, Tonk et al. 2013b).

Factors influencing Symbiodinium *assemblages.* Multiple studies have provided evidence that host identity best explains *Symbiodinium* distribution (Frade et al. 2008, Stat et al. 2008, Finney et al. 2010, LaJeunesse et al. 2010, Wicks et al. 2010, Tonk et al. 2013b, 2014). Combined the HPCO1 and HPCO2, which together represent host identity, accounted for most of the variance explained in the marginal tests of both multivariate approaches. In the sequential tests host identity explained a sizeable 26.8% and 25.8% of the variance in, respectively, the complete dataset and the dataset restricted to hosts with an open transmission mode. The significant relationship found between host and symbiont matrices underlines the importance of host identity in community-scale analyses of *Symbiodinium.* In such analyses the mix of host-specific and host-generalist symbionts can prevent environmental driven patterns from being revealed due to the link between specific *Symbiodinium* types and host species (host-specificity). We highlight this by reanalyzing the data restricted to host species with an open symbiont transmission mode. These acquire their symbionts from the environment and are less likely to display high levels of symbiont-specificity. The increase in explained variation in the *Symbiodinium* distribution indicates that at least some part of the patterns seen are obscured by consistent relationships between certain hosts and symbionts. Besides host-specificity, host identity influences the direct environment of *Symbiodinium* cells (Tonk et al. 2014). Only when the analysis is performed on a single host species can this effect truly be ruled out. However, environmental factors shaping *Symbiodinium* distribution patterns vary between host species (Tonk et al. 2013b), emphasizing the need for a community based approach when investigating factors influencing the spatial occurrence of symbiont assemblages at a reef-wide scale.

At a regional scale SST derived metrics are amongst the most important environmental drivers of *Symbiodinium* distributions. This is consistent at the level of community-based approaches as well as in single host species across the GBR (Cooper et al. 2011, Tonk et al. 2013b). On a more local scale different variables, i.e., water quality (Cooper et al. 2011), turbidity, light regime or % PAR (Ulstrup and van Oppen 2003, LaJeunesse et al. 2010) can be of importance alongside SST (Fabricius et al. 2004, Oliver and Palumbi 2009). In this study, SST was a significant variable in both multivariate approaches. Moreover, in the sequential tests SST accounted for most of the variance explained. Beside SST, host identity was found to significantly contribute to the optimal fit of a combination of variables in the sequential test on the *Symbiodinium* distribution of open transmission host genera. In addition, Chl *a* and host identity were found significant as individual variables in the marginal tests. It should be noted that the variables SSTstdev, summer SST and winter SST that were omitted from the multivariate analysis due to co-linearity with long-term SST climatology are effectively concurrent factors of importance. A similar cross-shelf analysis in the northern section of the GBR revealed winter SST most heavily affecting *Symbiodinium* distribution while Chl *a* and PAR explained additional variation (Tonk et al. 2014). Although most of the variation in the fitted model on *Symbiodinium* distribution data was explained by host identity, SST, Chl *a,* and PARz, other parameters not included in this study may likely explain additional variation.

Collectively these results support the view of host identity as a key influence on *Symbiodinium* distribution. Whereas SST has previously been established as a major factor influencing *Symbiodinium* distribution on a regional scale, our results indicate that SST is also important at local scales regardless of latitude (Tonk et al. 2014). In addition, water quality related variables such as Chl *a* and available PAR have repeatedly emerged as essential parameters. With rising SSTs, these results point toward potential future shifts in *Symbiodinium* distribution, which likely will have downstream effects on the associated host population in the southern GBR. These data underline the need to understand the implications on a community level. Southern GBR inshore coral communities are vulnerable due to their proximity to shore especially in areas with coastal development. Notwithstanding they exist in a variety of habitats and portray substantial invertebrate-*Symbiodinium* diversity that seeds adjacent (marginal) reefs. In a time of changing environmental conditions these communities are ever more so important since they may provide suitable conditions for lower latitude symbiotic reef invertebrates as they are driven southwards by increasing SST.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Principal component analysis (PCA) on the environmental data across sites. The distribution and designation of inshore and mid-shelf sites and environmental factor projections are shown.

Figure S2. Principal coordinate analysis (PCO) on the host distribution data (presence/absence) across sites. The "% of total variation" indicates the percentage of total variation in the resemblance matrix explained by the axis.

Table S1. Sample list of host genera (*n* indicates amount of sampled host colonies) from Mackay/Capricorn sites, Australia and associated *Symbiodinium* ITS2 types. Note the difference between e.g., C22-a (C22 band plus the co-dominant intragenomic variant C22a) and for example C42a (only C42a band is detected).

Table S2. Sample list of identified *Symbiodinium* ITS2 types from sites in the Mackay/Capricorn area of the GBR, Australia and associated host genera. *N* indicates the amount of sampled host colonies.