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REPORT

Species and spatio-environmental effects on coral endosymbiont communities in Southeast Asia

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Abstract Understanding the drivers of algal endosymbiont communities hosted by reef corals is a requisite for predicting coral resilience. For the biodiverse reefs of Southeast Asia, few studies have characterised the spatial variability of Symbiodiniaceae communities amongst reefs and investigated species and environmental effects on community structure and diversity. To profile the endosymbionts associated with reef corals inhabiting Southeast Asia, three common species, *Pachyseris speciosa, Pocillopora acuta* and *Diploastrea heliopora*, were sampled from 10 reef sites along the coasts of the Malay Peninsula. The nuclear internal transcribed spacer 2 region of Symbiodiniaceae was targeted for high-throughput sequencing, and the SymPortal framework was used to establish the identities of endosymbiont genera and types. Effects of environmental variables on endosymbiont community structure and diversity were then tested. Analyses revealed that Symbiodiniaceae diversity in this region is higher than previously known. Endosymbiont communities are structured significantly by host species and are relatively invariant in *D. heliopora*, with *P. speciosa* associating strongly with both *Cladocopium* and *Durusdinium* while *P. acuta* and *D. heliopora* are dominated by *Durusdinium*. Environmental parameters influence Symbiodiniaceae communities and diversity distinctly between host species. In particular, higher sea surface temperature (SST) affects endosymbiont diversity positively for *P. acuta* while higher SST range affects diversity negatively for *P. speciosa* and *D. heliopora*. Overall, this study has uncovered the hidden diversity of Symbiodiniaceae types previously unrecorded in the region and established a baseline for future comparative studies on how Southeast Asian reef corals acclimatise and adapt to changing environments through the natural variation of endosymbiont communities.

Keywords Central Indo-Pacific · Bio-ORACLE · High-throughput sequencing · Scleractinia · Tropical biodiversity · Zooxanthellae

Introduction

Coral reefs are most diverse in the Central Indo-Pacific region, with species diversity of corals and reef fishes reaching a global maximum in the Coral Triangle (Roberts et al. 2002; Hoeksema 2007; Veron et al. 2015)—an area extending from Indonesia and Philippines to the Solomon Islands (Veron et al. 2011). Southeast Asia accounts for a large part of this biodiversity, harbouring over 600 of the

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world's 800 reef-building coral species (Burke et al. 2002; Huang et al. 2015). For example, 480 and 255 species have been recorded on the reefs of Peninsular Malaysia and Singapore, respectively (Huang et al. 2009; Affendi and Rosman 2011). As is the case in many other tropical and subtropical regions, coral reefs form a major marine ecosystem in Southeast Asia and its persistence is highly reliant on the endosymbiotic relationship between reef corals and Symbiodiniaceae dinoflagellates. In terms of carbon sequestration alone, the combined carbon storage value of reefs on the west coast of Peninsular Malaysia and Singapore has been estimated at U$288,700 annually (Chou 2000), though this value would be considerably higher today (Wagner 2021).

Despite their high ecological, cultural and economic values, corals are under threat from various anthropogenic activities and climate change. Indeed, one-third of reef-building corals are deemed to be at an elevated risk of extinction (Carpenter et al. 2008). Due to rapid ocean warming, the greatest threat to corals is coral bleaching, in which the relationship between reef corals and the Symbiodiniaceae dinoflagellates breaks down (Brown 1997). Mass mortality events due to bleaching are occurring at increasing frequency, scale and severity on a global level and can be exacerbated by other anthropogenic stressors (Glynn 1993; Hughes et al. 2017, 2018a, b; Oliver et al. 2018). For instance, high levels of maritime traffic, land reclamation and coastal development can increase sediment smothering of corals, resulting in bleaching (Weber et al. 2006; Bessell-Browne et al. 2017). The ability to recover from coral bleaching can also be impeded by previous or ongoing anthropogenic disturbances such as contaminant retention and land-sea biological exchange from coastal development and urban land use (Carlson et al. 2019).

Studies have observed that dominant Symbiodiniaceae taxa present in corals are associated with bleaching tolerance (Hume et al. 2015; Silverstein et al. 2015) and contribute to the stability and resilience of reef corals during chronic and acute disturbances (Sampayo et al. 2008). Broadly, each genus can be distinct in ecological breadth and function (LaJeunesse et al. 2018): *Symbiodinium* (formally clade A) is adapted to living in high irradiance or variable light conditions (Shoguchi et al. 2018); *Breviolum* (formerly clade B) contains species that can be found in coral hosts with a wide depth range and other species in shallow waters in the Caribbean (e.g. Diekmann et al. 2003; Finney et al. 2010); *Cladocopium* (formally clade C) comprises many host specialists which are physiologically diverse, with many members adapted to various light and temperature conditions (Fabina et al. 2013); and *Durusdinium* (formally clade D) includes species that are tolerant of large fluctuations in temperature (Baker et al. 2013; LaJeunesse et al. 2018). The biogeographic distributions of Symbiodiniaceae genera also differ: *Symbiodinium* and *Breviolum* are more frequently found in higher latitudes while *Cladocopium* and *Durusdinium* are more common in tropical latitudes (see Baker, 2003). Furthermore, different host species can be associated with multiple Symbiodiniaceae types (e.g. Chankong, et al. 2020). The identification of Symbiodiniaceae taxa hosted by corals is now relatively routine in studies involving coral endosymbiosis and commonly relies on high-throughput sequencing (HTS) of high-resolution amplicon markers, of which the most widely used is the internal transcribed spacer 2 (ITS2) rRNA region (e.g. Hume, et al. 2018a, b; Terraneo et al. 2019). Recent development of the analytical SymPortal framework specifically for processing ITS2 data has further enhanced the speed and resolution of Symbiodiniaceae community and diversity analyses (Hume et al. 2019; Howells et al. 2020).

The Malay Peninsula is amongst many areas in Southeast Asia currently facing declining coral diversity and abundance (Chou 2000, 2006; Guest et al. 2016a). Singapore supports one of the world's busiest harbours which yield high levels of maritime traffic, land reclamation and seabed dredging (Chou 2006; Chou et al. 2019). These activities lead to increased sediment load and turbidity in the water column (Dikou and van Woesik 2005; Chow et al. 2019), changes in hydrodynamic patterns and tidal speeds, as well as threats of grounding and accidental spillage of hazardous materials (Chan et al. 2006; Sin et al. 2016). These degraded marine conditions severely threaten coral survival (Wong et al. 2018; Poquita-Du et al. 2019; Bollati et al. 2022; Ng et al. 2021). Along the coasts of Peninsular Malaysia, live coral cover has been variable over the years but is generally declining at many sites (Reef Check Malaysia, 2018) as a result of destructive fishing (e.g. blast fishing and use of cyanide), sedimentation and pollution (Chou 2000; Praveena et al. 2012). Throughout the region, global-scale thermal stress has led to extensive coral bleaching events, such as in 2010 (Guest et al. 2012, 2016b; Chan and Sukarno 2016) and 2016 (Kimura et al. 2018; Toh et al. 2018; Ng et al. 2020).

Few studies have characterised the Symbiodiniaceae communities present in reef corals of the Malay Peninsula. Using denaturing gel gradient electrophoresis, Tanzil et al. (2016) documented the dominance of *Cladocopium* and *Durusdinium* in common corals in Singapore, the high prevalence of *Durusdinium* in three of seven coral species examined, as well as variable symbiosis in *Pachyseris speciosa* which associated with both *Cladocopium* and *Durusdinium*. In another study, however, high-throughput amplicon sequencing of endosymbionts in five coral species established *Cladocopium* as the dominant clade, with low community diversity which was attributed to the high turbidity of Singapore's coastal environment (Smith et al. 2020). Across the Malay Peninsula, Tan et al. (2020) found *Cladocopium* to be the most dominant genus hosted by *Porites lutea* as expected (see Gong et al. 2018, 2019; Pootakham et al. 2018; Terraneo et al. 2019), with community diversity driven by a combination of mean monthly cloud cover, variance in monthly sea surface temperature and the interaction between both factors. These findings and studies in other regions also suggest that endosymbiont communities in various host species differ in how they are structured spatially and environmentally (e.g. Chen et al. 2019; Leveque et al. 2019; Qin et al. 2019; Hume et al. 2020; Tong et al. 2020).

The present study expands upon the sampling of Symbiodiniaceae communities in Southeast Asia, focusing on three common host species—*Pachyseris speciosa* (Agariciidae; plating; Jain et al. 2020, 2021), *Pocillopora acuta* (Pocilloporidae; branching; Poquita-Du et al. 2017) and *Diploastrea heliopora* (Diploastraeidae; mounding; Huang et al. 2014) (Fig. 1). While samples were collected with those of Tan et al. (2020), this study goes beyond that work in two ways. First, it examines and compares three different host species of distinct lineages and colony forms. Colony form is a very important factor to consider because it has been observed to be associated with bleaching vulnerability; branching *Pocillopora* is highly vulnerable to thermal bleaching but massive and encrusting corals tend to be more resistant (Loya et al. 2001; Darling et al. 2012; Ng et al. 2020; but see Swain et al. 2018a). There is also evidence showing that skeletal structures of different host species scatter light distinctly and modulate light availability to the algal endosymbionts (Swain et al. 2018b). Second, this study analyses a larger suite of environmental variables with greater model complexity. These parameters include those related to thermal conditions (e.g. temperature and cloud cover), productivity and nutrient levels (e.g. photosynthetically available radiation, phosphate and nitrate concentrations), which are known to affect Symbiodiniaceae communities (Baker et al. 2013; Chow et al. 2019). Our primary objectives are to profile the diversity and distribution patterns of Symbiodiniaceae in the three coral species along the Malay Peninsula (Fig. 1) and to assess the influence of relevant satellite-derived environmental parameters on endosymbiont community structure and diversity. We show that corals here are dominated by *Cladocopium* and *Durusdinium*, whose communities are structured by host species and site. Various spatio-environmental factors influence Symbiodiniaceae community structure and diversity, but the effects differ between host species. By identifying the coral species and populations hosting particular Symbiodiniaceae taxa and endosymbiont communities that are sensitive or resistant to stress, our findings contribute to the growing literature on coral resilience and establish a baseline for reef rehabilitation studies.

Materials and methods

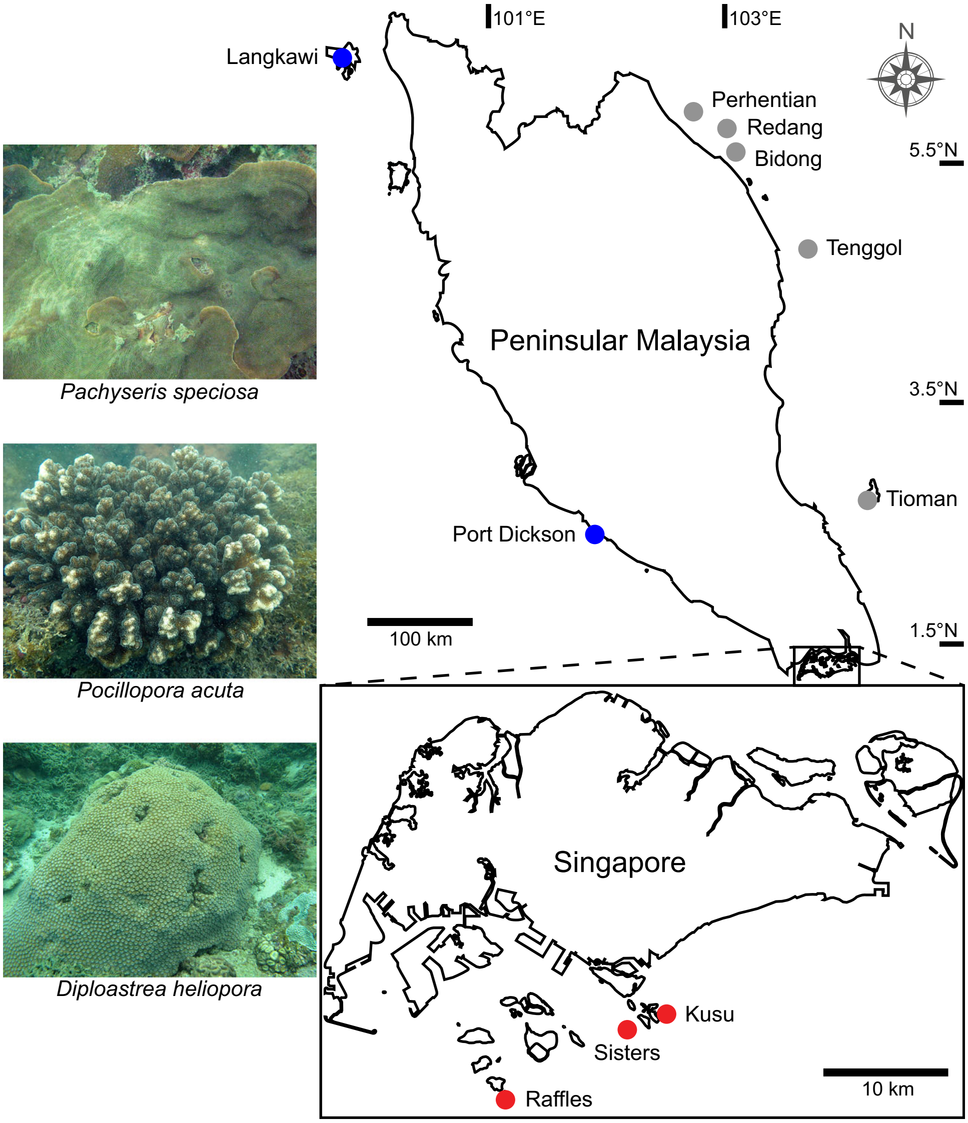
Coral sampling

Pachyseris speciosa, *Pocillopora acuta* and *D. heliopora* were sampled between November 2017 and July 2018 from seven sites around Peninsular Malaysia (PM) and three sites in Singapore (Raffles Lighthouse, Sisters' Islands and Kusu Island) (Fig. 1), alongside the sampling for Tan et al. (2020). Of the seven sites in PM, two are from the western coast (West PM; Langkawi with two collection points, Pulau Payar and Datai Beach, as well as Port Dickson) and five are from the eastern coast (East PM; Pulau Perhentian, Pulau Redang, Pulau Bidong, Pulau Tenggol and Pulau Tioman). At least 20 samples of *Pachyseris speciosa* and *Pocillopora acuta* were collected from each site, except for *Pachyseris speciosa* at Port Dickson and Tenggol (six and 17 samples, respectively). Collection of *D. heliopora* was more variable—Langkawi, Pulau Tioman and Pulau Tenggol yielded 20 samples each while 9–16 samples were collected from Port Dickson, Raffles Lighthouse, Kusu Island, Pulau Perhentian and Pulau Redang (Electronic Supplementary Material, ESM Table S1). Sampling protocol followed Tan et al. (2020). Briefly, tissue samples were collected from coral colonies visually determined to be healthy at depths of 2–6 m in Singapore and 4–25 m in PM, spanning the full depth ranges of reefs at the respective sites. The vast majority of samples from PM were collected between 4 and 11 m depth. Samples were immersed in water in separate sealed containers and kept in the shade until processing. Samples were preserved in 100% molecular-grade ethanol within 6 h and thereafter stored at −80 °C upon return to the laboratory.

DNA library preparation and sequencing

Symbiodiniaceae DNA was extracted from coral samples using the DNeasy Blood and Tissue Kit (Qiagen, Singapore) according to the manufacturer's recommended protocol. We followed the 16S Metagenomic Sequencing Library Preparation guide for the Illumina MiSeq System (Illumina 2013) using the SYM_VAR primer pair for Symbiodiniaceae ITS2 amplification (ESM Table S2) (Hume et al. 2018b). Polymerase chain reaction (PCR) was performed using 2.5 µL of sample DNA, 5 µL of forward and reverse primers each and 12.5 µL of 2 × KAPA HiFi HotStart ReadyMix to make a total volume of 25 µL per reaction mix. The PCR conditions were initial denaturation at 95 °C for 3 min, followed by 25 cycles of 95 °C for

Fig. 1 Sampling sites in Peninsular Malaysia and Singapore for *Pachyseris speciosa*, *Pocillopora acuta* and *Diploastrea heliopora*, with representative colonies shown in the images



30 s, 55 °C for 30 s and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. Amplicon purification was carried out using AMPure XP magnetic beads (Agencourt).

Adaptor-index PCR was then performed using 2.5 µL of sample DNA, 2.5 µL each of Index 1 (i5F) and Index 2 (i7R) primers (Integrated DNA Technologies, Coralville, IA, USA), 12.5 µL of 2 × KAPA HiFi HotStart ReadyMix and 5 µL of PCR-grade water to make a total volume of 25 µL per reaction mix. The PCR conditions were initial denaturation at 95 °C for 3 min, followed by 8 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and a final extension step at 72 °C for 5 min. PCR purification was then carried out again using the AMPure XP magnetic beads (Agencourt).

Successful adaptor-index PCR was checked by running 1 µL of each PCR product on a 1% agarose gel. Samples were also quantified on a Qubit 3 Fluorometer using the dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) and 1 µL of PCR product. Sample normalisation was performed using the SequelPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA, USA) to achieve a concentration of 1–2 ng/µL. Samples for each library were then pooled for a minimum concentration of 5 nM. Libraries were sequenced on the Illumina MiSeq platform (V3 chemistry, 300-bp paired-end reads) at Macrogen (Seoul, South Korea).

Bioinformatics

Sequence data were processed locally using the SymPortal framework (Hume et al. 2019). Demultiplexed and paired forward and reverse FASTQ files were submitted to SymPortal, where sequence filtering and quality control were carried out using Mothur 1.43.0 (Schloss et al. 2009), BLAST + suite of executables (Camacho et al. 2009) and minimum entropy decomposition (MED) (Eren et al. 2015).

Mothur-processing generated contiguous sequences and sequences were screened with maximum ambiguities allowed set to 0 and maximum homopolymer set to 5 in order to discard sequences putatively generated from sequencing errors. Distinct sequences were identified, and singletons and doubletons were removed. Non-Symbiodiniaceae sequences were then filtered out using BLASTn searches against the SymPortal reference database (accessed on 3 October 2019) on a sample-by-sample basis. Sequences that had an identity $> 80\%$ and coverage $> 95\%$ to any sequence in the reference database were assigned to be of Symbiodiniaceae origin. Size screening was performed with minimum and maximum cut-offs of 184 bp and 310 bp, respectively ($\sim$ 50 bp ± average lengths for the shortest and longest fragments across Symbiodiniaceae genera). Finally, MED nodes within a given set of sequence were identified by the nucleotide positions most informative in differentiating between these sequences to yield relative abundance data for Symbiodiniaceae types. Samples with fewer than 10,000 sequencing reads were removed, and rarefaction curves were plotted for the retained samples to verify sufficient sequencing depth had been achieved (ESM Fig. S1).

Environmental data

Environmental data associated with the coral reef environment were extracted from Bio-ORACLE v2.1 (Tyberghein et al. 2012; Assis et al. 2018) (ESM Table S3). All data layers were at a resolution of 5 arcmin, or approximately 9.2 km at the equator, and the long-term averages for the selected variables used in this study at each site were extracted from their respective layers. All 14 parameters obtained were analysed as mean values to characterise baseline environmental conditions at each study site (ESM Table S4). Environmental conditions within each region (West PM, Singapore and East PM) were more similar to one another than between regions (ESM Fig. S2). Within each region, however, these parameters also varied considerably between sites (ESM Table S4), so data analyses were performed at the site level to take this variation into account.

Data analyses

All analyses were performed in R version 3.6.3 (R Core Team 2013). Symbiodiniaceae richness was calculated for each host species based on absolute abundance count tables of post-MED ITS2 sequences, and the majority (top 20 most abundant) ITS2 sequences for each species were identified.

A Bray–Curtis dissimilarity matrix was computed for the entire dataset using the vegan package (Oksanen et al. 2018). Permutational multivariate analysis of variance (PERMANOVA) was performed to test the significance of host species and collection site in structuring Symbiodiniaceae communities. Following results showing significant differences between coral species, data for each host species were analysed separately. Analysis of similarities (ANOSIM) was performed for each host species using the Bray–Curtis dissimilarity measure to test for differences in community structure between sites, and distance decay of similarities was performed with Mantel test on the shortest over-water distance between each pair of collection sites.

Variation of Symbiodiniaeceae communities was visualised via non-metric multidimensional scaling (NMDS; Bray–Curtis dissimilarity; vegan package; Oksanen et al. 2018) of the count tables of the entire dataset as well as separately for each host species. Using the *envfit* function from the vegan package, environmental data modelled below were fitted onto the NMDS for each host species to assess the correlation between these parameters and Symbiodiniaceae community structure. The *betadisper* function from the same package was used to test for differences in group dispersions.

Shannon diversity index was calculated for each host species. Generalised linear mixed models (GLMMs) were constructed using the lme4 package (Bates et al. 2015) to analyse the fixed effects of environmental variables on Symbiodiniaceae diversity, with monsoon period (October to March, and April to September) and site indicated as random factors. Multicollinearity tests were performed using the GGally package (Schloerke et al. 2021) and parameters with pairwise collinearity values greater that 0.7 were removed. The parameters retained were scaled using the *scale* function in R due to being of different orders of magnitude. The maximal GLMM was constructed and variables that remained collinear (variable inflation factor > 2.5) were identified using the *vif* function from the car package (Fox and Weisberg 2011) and sequentially removed. Models were assessed by the Akaike information criterion corrected for small sample sizes (AICc). Model averaging was carried out using the MuMIn package for models with $\Delta\text{AICc} \leq 2$. Coefficient estimates reported are from the "full" average which assumes that a variable is included in every model; where the model does not originally include the variable, the coefficient of that variable is set to zero.

Results

Sequencing resulted in average sample read counts of 143,079 for *Pachyseris speciosa*, 140,547 for *Pocillopora acuta* (excluding samples that returned with 0 reads) and 184,664 for *D. heliopora*. Samples with read counts less than 10,000 were removed for poor quality; of the 430 samples processed and sequenced, 100 were removed from the final analyses. Thereafter, average sample read counts increased to 145,488 for *Pachyseris speciosa*, 211,365 for *Pocillopora acuta* and 221,211 for *D. heliopora.* All sequencing data have been deposited at the National Center for Biotechnology Information (BioProject ID PRJNA785764).

Comparing Symbiodiniaceae communities between species, *Pocillopora acuta* ($n = 89$) hosted the highest Symbiodiniaceae richness with a total of 315 unique Symbiodiniaceae types and *Pachyseris speciosa* ($n = 142$) had an intermediate richness of 269 types, while *D. heliopora* ($n = 99$) hosted the lowest richness of 165 types (Table 1). Samples of *D. heliopora* and *Pachyseris speciosa* had mean richness of 13.4 (SD = 5.54 and 5.18, respectively) while *Pocillopora acuta* samples had a lower mean richness of 10.8 (SD = 5.84).

The top 20 most abundant ITS2 sequences comprised 97.7% of the total read abundance present in *Pachyseris speciosa*, while the proportion was 89.2% for *Pocillopora acuta* and 96.4% for *D. heliopora*, revealing that *Pocillopora acuta* had a greater representation of background types than the other two host species (ESM Fig. S3). These differences were statistically significant (Kruskal–Wallis test: Chi-squared = 33.5, df = 2, $p < 0.05$; pairwise Wilcoxon rank sum tests: $p < 0.05$). *Pachyseris speciosa* was found to be strongly associated with both *Cladocopium* and *Durusdinium* while *Pocillopora acuta* and *D. heliopora* were dominated by *Durusdinium* (Fig. 2a; ESM Fig. S3 and Table S5). *Gerakladium* was detected in the background in one *Pocillopora acuta* sample from Perhentian and one *Pachyseris speciosa* sample from Redang, both being the northernmost sites in East PM. PERMANOVA results revealed host species identity was a significant driver of Symbiodiniaceae community (df = 2, $F = 172$, $p < 0.05$) and the interaction between species and site was significant (df = 11, $F = 17.6$, $p < 0.05$).

Symbiodiniaceae community composition was variable amongst sites for every host species (Fig. 2). ANOSIM showed that communities were significantly different between sites, with communities in *Pachyseris speciosa* being the most dissimilar amongst sites while those in *D. heliopora* were least dissimilar (Table 2). Results of the combined NMDS supported this pattern as *D. heliopora* had the smallest spread of data points (Fig. 3a), with significant differences in the community dispersion of Symbiodiniaceae between host species (betadisper: df = 2, $F = 19.9$, $p < 0.05$).

NMDS performed separately for each host species showed evidence of Symbiodiniaceae communities clustering by region (Fig. 3b), except in *D. heliopora* for which the different regions overlapped substantially. Community dispersion of Symbiodiniaceae amongst the three regions according to betadisper was significantly heterogeneous in *Pachyseris speciosa* (df = 2, $F = 5.20$, $p < 0.05$) and *Pocillopora acuta* (df = 2, $F = 11.5$. $p < 0.05$) but not *D. heliopora* (df = 2, $F = 0.0372$, $p = 0.964$). These results are in line with the outcomes of the distance decay analysis based on Mantel tests, which showed isolation by distance of Symbiodiniaceae communities in *Pachyseris speciosa* and *Pocillopora acuta*, but no correlation between community dissimilarity and spatial separation in *D. heliopora* (Table 2). In other words, endosymbiont communities in *Pachyseris speciosa* and *Pocillopora acuta* were more similar between corals in closer proximity than those farther apart, but this was not evident for *D. heliopora*.

The environmental parameters that were significant in structuring Symbiodiniaceae communities varied amongst host species (Table 3). Of the main effects, calcite and phosphate concentrations and sea surface temperature (SST) were significant in structuring endosymbiont communities in *Pachyseris speciosa*. Cloud fraction, salinity and SST range were significant for *Pocillopora acuta*, while only primary productivity was significant for *D. heliopora*. Symbiodiniaceae communities were not structured by monsoon period of collection (ESM Fig. S4).

Shannon diversity of Symbiodiniaceae types was found to be significantly different amongst the three host species (df = 2, $F = 29.7$, $p < 0.05$) and between sites (df = 9, $F = 11.7$, $p < 0.05$) based on a two-way ANOVA. Overall, Shannon diversity was least variable in *D. heliopora* and

Table 1 Symbiodiniaceae richness in terms of the number of ITS2 types hosted by each coral species

Species	*Cladocopium*	*Durusdinium*	*Gerakladium*	Total	Within sample (mean ± SD)
Pachyseris speciosa	111	145	13	269	13.4 ± 5.18
Pocillopora acuta	58	256	1	315	10.8 ± 5.84
Diploastrea heliopora	89	76	0	165	13.4 ± 5.54

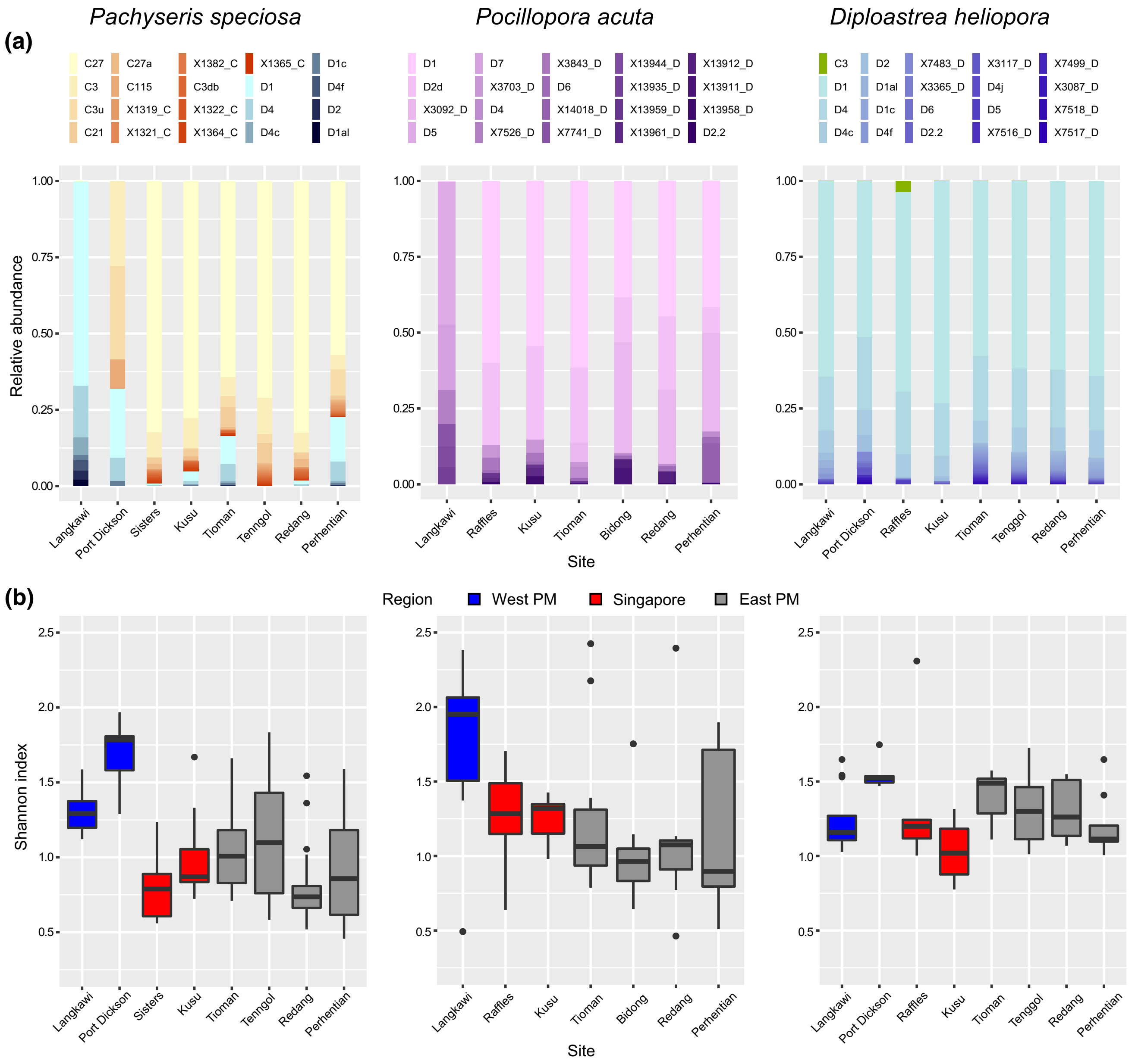


Fig. 2 Diversity of Symbiodiniaceae communities hosted by *Pachyseris speciosa*, *Pocillopora acuta* and *Diploastrea heliopora* at each sampling site. The top 20 most abundant ITS2 sequences composed 97.7% of the total read abundance present in *Pachyseris speciosa*, 89.2% in *Pocillopora acuta* and 96.4% in *D. heliopora.* **a** Stacked bar charts showing the relative abundances of the 20 most abundant ITS2 types. **b** Boxplots showing Shannon diversity index of Symbiodiniaceae communities. Colours represent regions in Peninsular Malaysia (PM) and Singapore

Table 2 Analysis of similarities (ANOSIM) amongst sites and distance decay of similarity (Mantel tests) in Symbiodiniaceae communities hosted by each coral species

Species	ANOSIM statistic *R*	Mantel statistic *r*
Pachyseris speciosa	0.456*	0.501*
Pocillopora acuta	0.367*	0.496*
Diploastrea heliopora	0.183*	− 0.0610

Significant statistic values ($p < 0.05$) are indicated with an asterisk

the greatest variation occurred in *Pocillopora acuta* (Table 4, Fig. 2b). Across sites and regions, Symbiodiniaceae diversity was higher in *Pocillopora acuta* and *D. heliopora* compared to *Pachyseris speciosa* (Table 4). While sites in West PM had the highest Symbiodiniaceae diversity across all host species, sites in Singapore had the lowest diversity for *Pachyseris speciosa* and *D. heliopora* but not for *Pocillopora acuta*, indicating the presence of site by species interaction (df = 11, $F = 3.79$, $p < 0.05$).

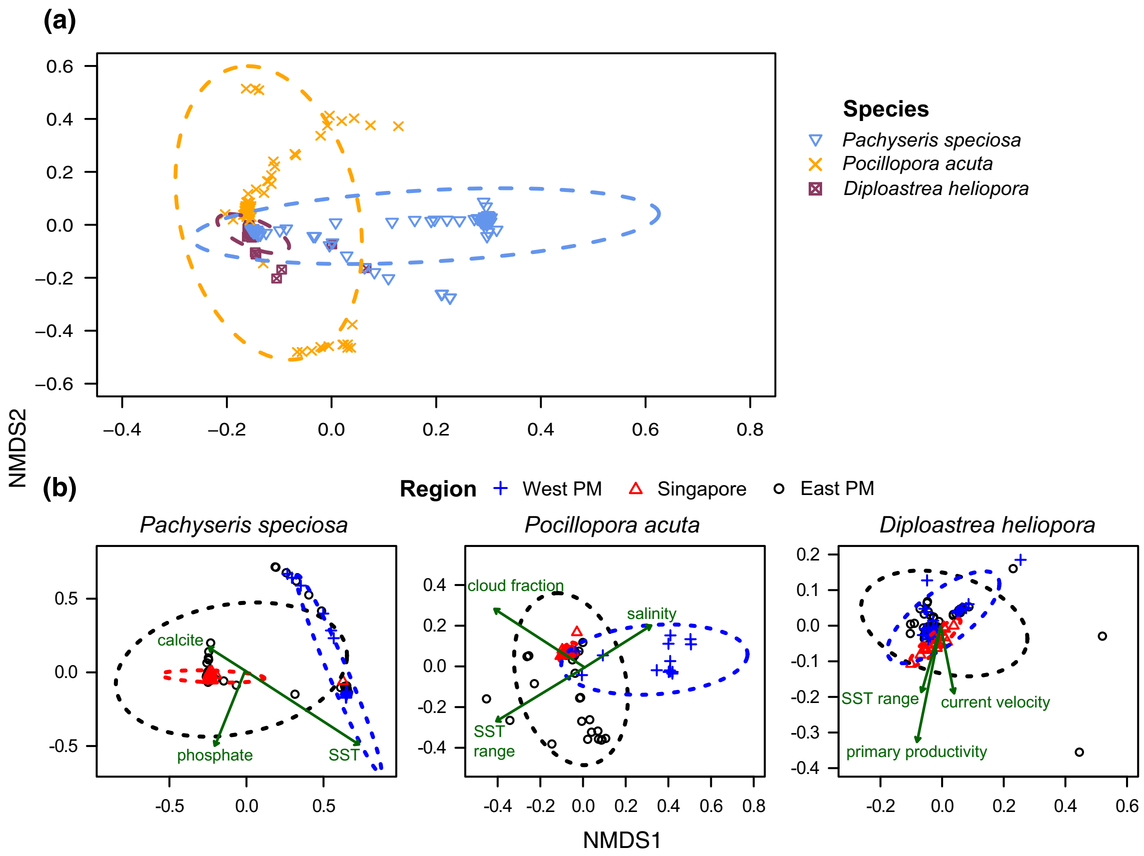


Fig. 3 Non-metric multidimensional scaling (NMDS) of Symbiodiniaceae communities based on Bray–Curtis dissimilarity. Each point represents a coral sample; ellipses denote 95% confidence level. **a** NMDS plot of all three host species (stress = 0.0776). **b** NMDS plot for each species (stress < 0.2). In each plot, symbols represent regions in Peninsular Malaysia (PM) and Singapore, with fitted vectors of environmental variables denoted by arrows

Table 3 Permutational multivariate analysis of variance (PERMANOVA) pseudo-*F* statistic for the main environmental effects structuring Symbiodiniaceae communities hosted by each coral species

Species	Pseudo-*F* statistic		
Pachyseris speciosa	Calcite concentration	Phosphate concentration	SST
	10.2*	14.7*	86.4*
Pocillopora acuta	Cloud fraction	Salinity	SST range
	28.5*	6.72*	9.13*
Diploastrea heliopora	Current velocity	Primary productivity	SST range
	1.08	3.18*	1.67

Significant statistic values ($p < 0.05$) are indicated with an asterisk. Parameters that are highly collinear have been removed from the analysis

Table 4 Shannon diversity index (mean ± SD) of Symbiodiniaceae ITS2 types hosted by each coral species

Species	West PM	Singapore	East PM	Overall
Pachyseris speciosa	1.39 ± 0.227	0.877 ± 0.231	0.959 ± 0.351	1.02 ± 0.350
Pocillopora acuta	1.78 ± 0.488	1.25 ± 0.260	1.13 ± 0.465	1.28 ± 0.462
Diploastrea heliopora	1.34 ± 0.221	1.12 ± 0.329	1.31 ± 0.198	1.28 ± 0.246

For Shannon diversity data pooled across all three host species, the full GLMM model had high levels of multicollinearity. Since the ANOVA above showed that Shannon diversity differed significantly between host species, GLMMs for each coral species were used to analyse the environmental parameters. Assessment of all candidate models with strong support (ΔAICc ≤ 2) resulted in two models for *Pachyseris speciosa* and six models each for *Pocillopora acuta* and *D. heliopora* (ESM Table S6). Results showed that different sets of environmental variables affected Symbiodiniaceae diversity distinctly amongst species (Table 5). After model averaging, the only significant parameters were SST ($p < 0.05$) and calcite concentration × phosphate concentration ($p < 0.01$) for *Pachyseris speciosa*, although calcite and phosphate concentrations were also present in the best model. Cloud fraction, salinity and their interaction were associated with Symbiodiniaceae diversity in *Pocillopora acuta*. For *D. heliopora,* current velocity, primary productivity, SST range and current velocity × SST range were the most important predictors. Overall, parameters related to temperature (SST and SST range) influenced Symbiodiniaceae diversity in all three host species, though with varying effects—SST had a positive effect in *P. speciosa* while SST range had negative effects in *Pocillopora acuta* and *D. heliopora*. Primary productivity and nutrient availability also had different effects, shown by the positive relationship with phosphate concentration in *Pachyseris speciosa* but negative relationship with primary productivity in *D. heliopora* (Table 5).

Discussion

Building extensively upon the works of Tanzil et al. (2016), Smith et al. (2020) and Tan et al. (2020), we provide the most comprehensive profiling thus far of the community structure of Symbiodiniaceae along the Malay Peninsula and furthermore assess the influence of environmental parameters on endosymbiont community diversity. We focus on host species which are representative of reef corals in the region and of different lineages, colony forms and life histories. Importantly, the use of SymPortal for the identification of Symbiodiniaceae types reveals a high diversity of Symbiodiniaceae, higher than what might have been predicted from previous studies in this region (Leveque et al. 2019; Tan et al. 2020). Our analyses further highlight the dominance of *Cladocopium* and *Durusdinium*, with the presence of *Gerakladium* in the background. *Cladocopium* has been reported to be the most common genus in several Indo-Pacific host genera (e.g. Leveque et al. 2019; Tan et al. 2020). While *Cladocopium* dominate assemblages in the western Indian Ocean (LaJeunesse et al. 2010) and western Pacific Ocean (LaJeunesse et al. 2004), *Durusdinium* had been found to occur frequently in the Andaman Sea and the Gulf of

Table 5 Model-averaged coefficient estimates for all variables present in generalised linear mixed models with ΔAICc ≤ 2

Species	Explanatory variable	Coefficient estimate	Adjusted SE	p	Relative importance
Pachyseris speciosa (2 models)	Intercept	0.104	0.0739	0.162	
	Calcite concentration	− 0.0283	0.109	0.796	1.00
	Phosphate concentration	0.162	0.0853	0.0580	1.00
	SST	0.0764*	0.0338	0.0241	1.00
	Calcite concentration × **phosphate concentration**	− 0.219*	0.0534	0.0000422	1.00
	Phosphate concentration × SST	− 0.118	0.217	0.585	0.39
Pocillopora acuta (6 models)	Intercept	0.203*	0.0645	0.0018	
	Cloud fraction	− 0.000535	0.0255	0.983	0.44
	Salinity	0.0213	0.0606	0.752	0.59
	SST range	− 0.0605	0.0627	0.335	0.61
	Cloud fraction × **salinity**	− 0.0662	0.111	0.552	0.28
	Cloud fraction × SST range	0.0162	0.0406	0.690	0.16
	Salinity × SST range	− 0.00967	0.0266	0.716	0.16
Diploastrea heliopora (6 models)	Intercept	0.302*	0.0841	0.000322	
	Current velocity	0.0467	0.0973	0.631	0.41
	Primary productivity	− 0.0567	0.104	0.585	0.65
	SST range	− 0.0819	0.0877	0.351	0.64
	Current velocity × primary productivity	− 0.0582	0.135	0.667	0.18
	Current velocity × **SST range**	− 0.108	0.139	0.437	0.41
	Primary productivity × SST range	0.0267	0.0626	0.670	0.18

Significant parameters ($p < 0.05$) are indicated with an asterisk. Bold text indicates that the variable is present in the best model

Thailand (LaJeunesse et al. 2010; Chankong et al. 2020). This pattern has been attributed to higher water temperatures and turbidity (LaJeunesse et al. 2010), factors that can also be extended to the tropical coastal waters off Peninsular Malaysia and Singapore. Indeed, Tanzil et al. (2016) found high prevalence of *Durusdinium* in three of seven coral species examined, highlighting the importance of this Symbiodiniaceae genus for the persistence of urban coral reefs in Singapore (Poquita-Du et al. 2020). The 20 most abundant Symbiodiniaceae types make up more than 89% of total abundance of Symbiodiniaceae for each host species, with hundreds making up the low-abundance background types that may be transient or permanent. While Lee et al. (2016) has suggested that background Symbiodiniaceae are transient and have minimal ecological significance in temperate reef corals, emerging studies indicate that background Symbiodiniaceae may have a functional role in host coral stress response and resilience to perturbations (Ziegler et al. 2018).

Pachyseris speciosa is associated with two Symbiodiniaceae genera (*Cladocopium* and *Durusdinium*) while both *Pocillopora acuta* and *D. heliopora* are dominated by *Durusdinium*. *Pocillopora acuta* has the highest Symbiodiniaceae richness, consistent with findings of large genetic variation in the *Durusdinium* types present in this species (Tanzil et al. 2016). A recent study of samples from the same localities found that *Porites lutea* was dominated by *Cladocopium*, but it was also associated with *Symbiodinium* and *Durusdinium* (Tan et al. 2020). Comparisons of Symbiodiniaceae richness between host species and their life histories can be informative for characterising the relationship between the endosymbionts and the resilience of the holobiont against environmental stressors. *Pocillopora acuta* is considered a weedy, opportunistic coloniser of recently disturbed habitats, *D. heliopora* a stress-tolerant species, while *Pachyseris speciosa* is a generalist with traits overlapping between the weedy and stress-tolerant life histories (Darling et al. 2012, 2013). These life history classifications appear consistent with their Symbiodiniaceae richness, with *Pocillopora acuta* having the highest total richness, in line with its opportunistic life history, and *D. heliopora* having the lowest total richness (Fig. 2a), possibly as fewer endosymbiont types are required for a stress-tolerant coral species residing in stressful urban marine environments, especially Singapore (Smith et al. 2020). *Pachyseris speciosa*, with a life history that partially overlaps with *Pocillopora acuta* and *D. heliopora*, has an intermediate Symbiodiniaceae total richness. Another stress-tolerant coral, *Porites lutea*, also registered relatively low community richness in the Malay Peninsula (Tan et al. 2020), and this is consistent with the recent finding that urbanised environments tend to depress Symbiodiniaceae richness (Smith et al. 2020; Jain et al. 2021).

Symbiodiniaceae communities in *Pachyseris speciosa* are most dissimilar amongst sites while those in *D. heliopora* are least dissimilar (Table 2). The site-wise distinctiveness exhibited by *Pachyseris speciosa* is contributed by the different combinations of *Cladocopium* and *Durusdinium*. At the regional level, this is most evident where the West PM confidence ellipse in the NMDS is largely distinct from the East PM and Singapore ellipses (Fig. 3b), as supported by the ANOSIM results showing significantly distinct communities between sites. Analysis of the most abundant Symbiodiniaceae types demonstrates that *Pachyseris speciosa* in West PM has a greater proportion of *Durusdinium* (mean 0.838 ± SD 0.325; ESM Fig. S3), with *Cladocopium* present in the background in very low abundances in Langkawi (Fig. 2a). For *Pocillopora acuta*, 12 of the majority Symbiodiniaceae types are unique to one region only, with eight types unique to West PM, three to Singapore and one to East PM. These biogeographic patterns contribute to dissimilarities in Symbiodiniaceae communities.

Interestingly, even though *D. heliopora* has relatively high overall Symbiodiniaceae diversity (Table 4), its endosymbiont communities are largely similar amongst sites based on the low ANOSIM statistic (Table 2), overlapping NMDS confidence ellipses (Fig. 3b), and insignificant betadisper result. The relative stability of Symbiodiniaceae communities amongst *D. heliopora* colonies is an interesting parallel to findings of limited genetic and morphological variation amongst *D. heliopora* colonies (Todd et al. 2004; Lam et al. 2006; Huang et al. 2014), suggesting that host similarity may constrain Symbiodiniaceae types and community structure. More research on coral species of varying host characteristics would help clarify this relationship. Relatedly, the lack of distance decay of similarity in *D. heliopora* could suggest either high connectivity between sites or an endosymbiont community structure that is relatively unaffected by environmental variation. In view of the genetic invariance of *D. heliopora* and its stress-tolerant life history, the latter may be the more likely explanation. That *Porites lutea*, also a stress-tolerant species, exhibits similar Symbiodiniaceae composition throughout the Malay Peninsula (Tan et al. 2020) further lends support to this hypothesis.

Different environmental variables appear to structure Symbiodiniaceae communities distinctly amongst host species (Table 3), a finding that is in line with work elsewhere (e.g. Great Barrier Reef (Tonk et al. 2013) and Red Sea (Terraneo et al. 2019; Osman et al. 2020)) showing host-specific community responses to various environmental variables. In particular, our GLMM results reveal that across the three host species, different sets of environmental parameters have varying effects on Symbiodiniaceae diversity (Table 5). Common to all is sea surface temperature (mean SST or SST range), which also consistently features across GLMMs with high relative importance across host species. Higher SST seems to be associated with an increase in Symbiodiniaceae diversity in *Pachyseris speciosa*; at warmer sites, Symbiodiniaceae types that are more thermally tolerant may be recruited as *Pachyseris speciosa* "shuffles" its symbionts (Jain et al. 2020), potentially driving diversity higher. Meanwhile, smaller SST range is associated with higher Symbiodiniaceae diversity in *Pocillopora acuta* and *D. heliopora*. Inhabiting shallow reefs, temperature stability may be an important influencing factor for these two host species.

Primary productivity and nutrients also have varying effects on Symbiodiniaceae diversity (Table 5). In particular, primary productivity is negatively associated with endosymbiont diversity for *D. heliopora* while phosphate concentration has a positive influence for *Pachyseris speciosa*, underscoring the fact that different host species tend to have different water quality or nutrient requirements. With nutrients being limiting in reef waters, influx of phosphates can impact corals greatly (Duprey et al. 2016). The increase in *Pachyseris speciosa*'s Symbiodiniaceae diversity on reefs with higher phosphate concentration may be a sign that *Pachyseris speciosa* could adapt to poorer water quality by altering its Symbiodiniaceae community. Finally, surface current velocity has a moderately important positive effect on endosymbiont diversity in *D. heliopora*, possibly resulting in greater water movement surrounding the coral colonies. The enhanced flushing may moderate the effects of extremely high temperature or nutrient concentrations (West and Salm 2003; Fabricius 2005), preventing the dominance of Symbiodiniaceae types that are more well-adapted to stressful conditions (Smith et al. 2020).

While these findings reveal species-specific effects of different environmental parameters on Symbiodiniaceae diversity, the details of interactions amongst modelled predictors remain challenging to explain because the physiologies of most Symbiodiniaceae types are yet to be fully characterised (LaJeunesse et al. 2018). Other factors known to affect Symbiodiniaceae communities but are not tested here add to the complexity of variations in endosymbiont diversity. Such parameters can include turbidity, sedimentation rate, sediment type and nutrients (Cooper et al. 2011; Savage 2019; Zhou et al. 2021), which require concerted in situ measurements. The depth of host corals can also potentially drive Symbiodiniaceae community structure because temperature and light levels tend to be lower at deeper habitats (Chow et al. 2019; Eckert et al. 2020). Bacterial associates in corals are also important in maintaining the dynamic relationship between the coral host and its endosymbionts (Peixoto et al. 2017). For example, Epstein et al. (2019) found that *Pocillopora acuta* in the Great Barrier Reef had a relatively stable microbiome and Symbiodiniaceae community throughout a thermal stress event that likely contributed to the species' thermal resilience. While the dynamics between the trio of coral host, Symbiodiniaceae and bacteria are not fully understood, it is possible that associated bacterial microbes may play a role in determining the composition of Symbiodiniaceae that is taken up by the coral host throughout its lifetime (Quigley et al. 2019; Claar et al. 2020; Matthews et al. 2020). Thus, the environment may not always affect Symbiodiniaceae community structure directly, but it may do so indirectly by influencing the composition of the coral microbiome (see Chen et al. 2021).

Critically, studies monitoring corals over seasonal to interannual timescales are needed to uncover fine-scale temporal variations in Symbiodiniaceae communities and physico-chemical parameters, preferably with comparable sampling intervals between the biotic and abiotic data. Studies suggest that corals under thermal stress may become increasingly reliant on *Durusdinium* (Baker et al. 2004; Kemp et al. 2014; Huang et al. 2020), and this may have long-term implications on coral nutrition and growth. Since *Durusdinium* is known to withhold more nutrition from the coral host under warm-water conditions (Baker et al. 2018), growth rates may be reduced for corals which host *Durusdinium*. This effect may also differ between corals dominated by *Durusdinium*, such as *Pocillopora acuta* and *D. heliopora*, and those which associate with both *Cladocopium* and *Durusdinium*, like *Pachyseris speciosa*. Endosymbiont communities and growth rates of corals at the sites examined here could be quantified over time and environmental gradients to assess if such a prediction—where corals become increasingly reliant on *Durusdinium* as SST rises (Heron et al. 2016; Hughes et al. 2018b)—holds true in this region and to predict future coral performance under climate change.

This study has shed light on the natural variation of Symbiodiniaceae communities hosted by different coral species inhabiting the reefs of Malay Peninsula, showing how spatio-environmental variables affect endosymbiont diversity. The high richness of Symbiodiniaceae present in the region is also uncovered here for the first time, filling a critical biodiversity knowledge gap. Our results have established a baseline and foundation for future studies aiming to better understanding how different Symbiodiniaceae assemblages confer adaptive advantages on corals amidst local impacts and climate change.

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Declarations

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

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