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Low Symbiodiniaceae diversity in a turbid marginal reef environment

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Abstract The coastal waters of Singapore support coral reefs that are biodiverse but characterized by high turbidity and sedimentation. Here, we used internal transcribed spacer two (ITS2) amplicon sequencing to investigate the Symbiodiniaceae communities associated with this marginal reef system, as turbid reefs may serve as potential refugia from future thermal stress. Using the analytical framework SymPortal, we identified a predominance of *Cladocopium* among the five coral species studied across six reef sites. *Durusdinium* was present in comparatively lower abundances and was composed of multiple *Durusdinium trenchii* strains. In contrast to other marginal environments, the *Cladocopium* communities exhibited low diversity and lacked the host-specificity of strains reported elsewhere. Nevertheless, we identified a site-specific strain across three species, which was supported by sequencing of the non-coding region of the psbA minicircle (psbA^{ncr}). The overall low diversity of the symbiont communities suggests that, although Singapore's reefs may provide habitat for a diverse coral assemblage, the strong selective pressure exerted by the prevalent turbidity likely limits the diversity of the associated symbiont community.

Keywords Symbiodiniaceae · Marginal reefs · ITS2 · Singapore

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Introduction

Reef-building corals have experienced widespread declines in recent decades due to a variety of natural and anthropogenic stressors (Bruno and Selig 2007; Hoegh-Guldberg et al. 2007; Wilkinson 2008; De'ath et al. 2012). Understanding the effects of extreme environmental conditions on reef-building corals is therefore paramount for predicting how future ocean conditions may impact coral populations (Kleypas et al. 1999; Camp et al. 2018). Studies of corals in marginal habitats provide a unique opportunity to elucidate the mechanisms that underpin the ecological resilience of reef communities at the extremes of their environmental tolerances.

One critical aspect of coral biology that is impacted by environmental conditions is the coral-algal symbiosis (Hennige et al. 2010; Lesser et al. 2010; Hume et al. 2013, 2015, 2016; Howells et al. 2016; Smith et al. 2017c). The symbiotic dinoflagellates of the family Symbiodiniaceae (LaJeunesse et al. 2018) help support the coral host's metabolic demands through the translocation of photosynthetically fixed carbon (Muscatine and Porter 1977).

This relationship is highly sensitive to changes in environmental variables, as evidenced by the phenomenon of coral bleaching, whereby symbiosis breaks down under stressful conditions (Glynn 1983; Brown 1997). Symbiont communities in coral hosts from marginal environments have shown increased presence of stress-tolerant *Durusdinium* genotypes, novel *Cladocopium* types, and/or high symbiont diversity (Fabricius et al. 2004; Hennige et al. 2010; Wicks et al. 2010; Oliver and Palumbi 2011; Hume et al. 2015; Smith et al. 2017b, c; Ziegler et al. 2017). In marginal environments dominated by *Durusdinium*, the stress-tolerant symbiont *Durusdinium trenchii* is commonly encountered (LaJeunesse et al. 2010; Keshavmurthy et al. 2014). Alternatively, in other extreme environments, *D. trenchii* appears to be outcompeted by different lineages, for example, the *Cladocopium thermophilum* lineage in the thermally extreme southern Persian/Arabian Gulf (D'Angelo et al. 2015; Hume et al. 2015, 2016; Smith et al. 2017a, c; Howells et al. 2020). Recent advances have shown that this lineage has diversified into host-specific strains (Smith et al. 2017b). This juxtaposition of high symbiont diversity on reefs with low coral species diversity has also been documented in other marginal environments (LaJeunesse et al. 2008; Wicks et al. 2010).

While low coral species diversity is a common feature of many marginal reef environments, it is not always the case. Singapore's reefs are an excellent example of a system that is characterized by high coral species diversity, with > 250 species recorded despite environmentally stressful conditions (Huang et al. 2009). Singapore's reefs have been subject to decades of extensive anthropogenic impacts from large-scale land reclamation, coastal development, and shipping activities (Guest et al. 2016b). As a consequence, Singapore's reefs are heavily impacted by sedimentation and high turbidity levels, restricting the euphotic zone to the upper 8 m, even at offshore sites (Todd et al. 2004). While coral hosts can exhibit adaptation/acclimatisation strategies to low light (e.g., Todd et al. 2004; Stambler and Dubinsky 2005; Smith et al. 2017d), the low light environment is also likely to affect the associated symbiont communities (Toller et al. 2001; Iglesias-Prieto et al. 2004; Bongaerts et al. 2010). However, as temperature and light act synergistically in thermal bleaching, the low irradiance on turbid reefs can help reduce the local incidence of bleaching (Cacciapaglia and van Woesik 2016; Morgan et al. 2017). This has led to the hypothesis that turbid reefs may serve as important refugia from thermal stress events (Cacciapaglia and van Woesik 2016; Morgan et al. 2017; Camp et al. 2019) and, therefore, it is essential to characterize the symbiont diversity associated with these marginal environments. Previous work has found both *Cladocopium*- and *Durusdinium*-containing assemblages in Singapore (Reimer and Todd 2009; Guest et al. 2016a; Tanzil et al. 2016; Poquita-Du et al. 2020). However, these studies have been limited in their ability to resolve the full diversity of Symbiodiniaceae present due to limited sample sizes and the use of less powerful methods of genotypic delineation (e.g., direct sequencing, DGGE, OTU clustering). As such, the Symbiodiniaceae communities in Singapore, and the region in general, remain largely understudied.

Here, we use high-resolution analyses of next generation sequencing-based internal transcribed spacer two (ITS2) amplicons supported by sequencing of the chloroplastic psbA non-coding region (psbA^{ncr}) to investigate the diversity and structure of the Symbiodiniaceae communities associated with these marginal, yet highly diverse, reefs.

Materials and methods

Five locally abundant coral species [*Pectinia alcicornis* (Saville Kent 1871), *Merulina ampliata* (Ellis and Solander 1786), *Platygyra daedalea* (Ellis and Solander 1786), *Podabacia crustacea* (Pallas 1766) and *Pachyseris speciosa* (Dana 1846)] were sampled across six fringing reefs in the Southern Islands of Singapore (Fig. 1). Samples were identified according to Veron et al. (2015) and Huang et al. (2014). Within each site, a small coral fragment (1–2 cm^2) was collected from 15 individual colonies for each species at 3–4 m depth. Samples were placed in individual zip-lock bags and transferred to salt-saturated DMSO immediately following each dive. The samples were stored at 4 °C prior to DNA extractions. DNA extractions and ITS2 amplicon library preparation [using the Sym-Var primer set (Hume et al. 2018b)] were performed according to an established protocol as previously described (ESM Supplementary Methods; Smith 2017b). Sequencing was performed on an Illumina MiSeq at the New York University Abu Dhabi Sequencing Core facility using the 600-cycle kit.

Raw sequencing reads were demultiplexed using the *process_radtags* module of Stacks (Catchen et al. 2013). Demultiplexed reads were then loaded into SymPortal (Hume et al. 2019). SymPortal uses the co-occurrence of ITS2 intragenomic variants within samples to identify Symbiodiniaceae profiles, analogous to DGGE 'types' but exploiting the greater sequencing depth and sensitivity of amplicon approaches (Hume et al. 2019). Sequencing data are analysed by SymPortal using a standardized approach across all analytical steps to ensure reproducibility and transferability across studies (Hume et al. 2019). Paired FASTA files are quality filtered in mothur (Schloss et al. 2009), screened for Symbiodiniaceae sequences within the range 184–310 bp, and then collapsed to associate the

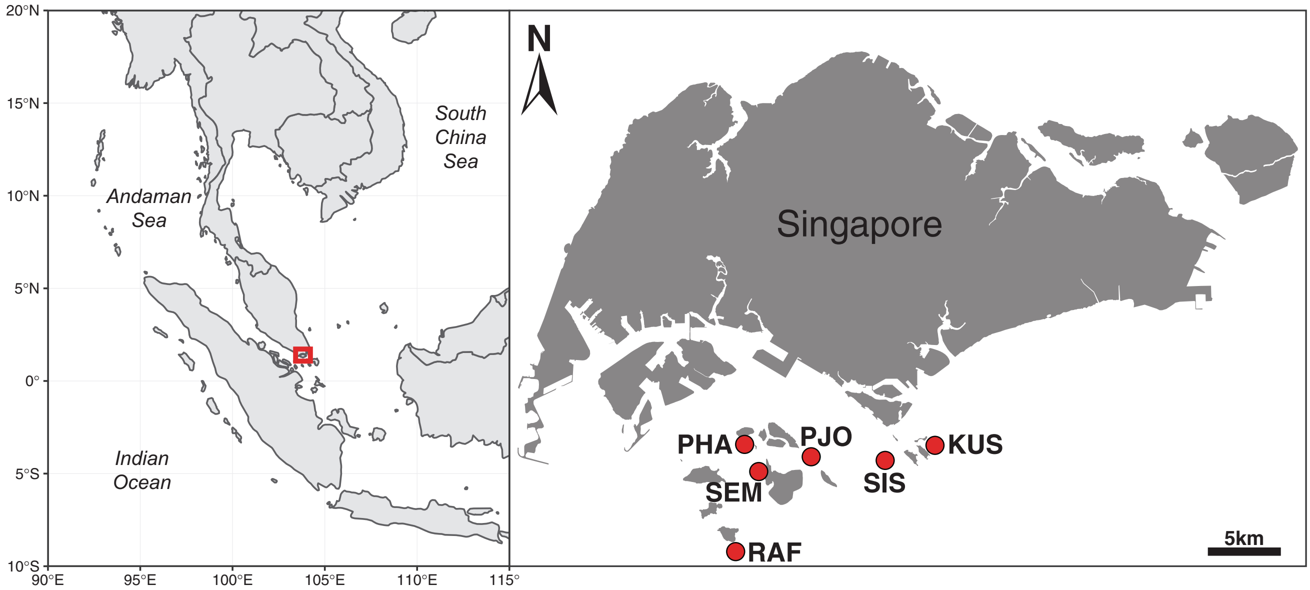


Fig. 1 Location of study site. Left: The geographic situation of Singapore. Right: Locations of the six sampling sites (RAF, Raffles; PHA, Pulau Hantu; SEM, Pulau Semakau; PJO, Pulau Jong; SIS, Sisters' Island; KUS, Kusu)

rarest variants to more common variants on a sample by sample basis using minimum entropy decomposition (Eren et al. 2015). After quality control, SymPortal calculates the presence and relative abundance of ITS2 profiles within each Symbiodiniaceae genus through the identification of co-occurring defining intragenomic variants (DIVs) within samples, and those in the existing database. As different combinations of DIVs form distinct ITS2 profiles, the number of ITS2 profiles identified can exceed the number of DIVs reported in a set of samples. The naming of the profiles reflects the DIVs present in the profile, in order of their relative abundance. For example, *C27-C21-C27a* describes a symbiont genotype that contains the ITS2 sequences C27, C21, and C27a, where C27 is the most abundant ITS2 sequence in that symbiont. Where two or more DIVs are separated by the '/' character, this signifies that they are co-dominant in that profile.

Statistical analyses were performed using the vegan package (v.2.5-6; Oksanen et al. 2019) in R (v.3.6.2; R Core Team 2019). The proportion of different Symbiodiniaceae genera and ITS2 profiles within samples were arcsine square-root transformed before the calculation of a Bray–Curtis distance matrix using the vegdist command. We tested for significant differences among host species and sites using permutational multivariate analysis of variance (PERMANOVA) via the *adonis* function. As differences in dispersion among groups can impact PERMANOVA analyses, we also performed an analysis of multivariate homogeneity of dispersion (variance) using *betadisp*. By evaluating the degree of imbalance in group sample sizes and variance ratios in relation to the simulations of Anderson and Walsh (2013), we concluded that our PERMANOVA results were robust to Type I errors caused by heterogeneous dispersions (see ESM: Supplementary Methods and Results; Hume et al. 2020).

To validate the identification of a novel symbiont type found at Sisters' Island, the psbA^{ncr} marker was analysed. The psbA^{ncr} was amplified from a subset of *Platygyra daedalea*, *M. ampliata* and *Podabacia crustacea* samples (as these species hosted the novel type) using the PSBA_NC_F and PSBA_NC_R primer pair (Smith et al. 2017c). The amplification was performed using the high-fidelity enzyme Primerstar GXL according to the establish protocol for these primers (Smith et al. 2017c). Sequencing of the PCR products was performed at Bioneer (South Korea).

PsbA^{ncr} chromatograms were visually inspected and were cropped according to the extent of unambiguous base calls as sequence quality rapidly deteriorates after poly-A/T regions. Low quality sequences resulting from the presence of psbA^{ncr} intragenomic or intergenomic variants were discarded. The resulting sequences (GenBank accession numbers: MT513104-MT513119) were manually aligned in MEGA X (Kumar et al. 2018) due to the presence of multiple large indels (Moore et al. 2003; LaJeunesse and Thornhill 2011). The sequence alignment was analysed with MrBayes (Ronquist et al. 2012) v3.2.6 using the Jukes-Cantor model. The MCMC was run for 2×10^6 generations, and sampled every 1000 generations with a burn-in of 0.25. The majority 50% consensus tree was imported into *ggtree* (Yu et al. 2017) v2.0.2 for visualization.

Results and discussion

Sequencing of the ITS2 amplicons yielded 25,869,576 raw demultiplexed reads. Merging of the overlapping reads resulted in 12,934,788 raw contigs, yielding 8,000,718 contigs after quality control from 438 individuals (mean: 18,266 contigs/individual). From these quality-controlled contigs, SymPortal identified 27 profiles (7 *Cladocopium*; 20 *Durusdinium*) within our dataset based on 28 DIVs (10 *Cladocopium*; 18 *Durusdinium*) (Figs. 2, 3).

Among the five coral species sampled across reefs in Singapore, only two symbiont genera were identified: *Cladocopium* and *Durusdinium* (Fig. 2), consistent with previous reports of symbiont communities in Singapore (Reimer and Todd 2009; Guest et al. 2016a; Tanzil et al. 2016) and other Indo-Pacific reef systems (Lien et al. 2007; Hennige et al. 2010; LaJeunesse et al. 2010; Ng and Ang 2016; Wong et al. 2016). However, the absence of genera such as *Symbiodinium* differs from other marginal environments, most notably in cases where *Symbiodinium* has been reported in conspecifics (Hume et al. 2015; Smith et al. 2017a, c; Ziegler et al. 2017). It has been suggested that stress-tolerant *Durusdinium* strains may play a crucial role in Singapore's reefs due to their association with warm, turbid reefs, and the apparent resilience of these reefs to multiple bleaching events (Guest et al. 2016a; Tanzil et al. 2016). However, of the two Symbiodiniaceae genera observed, *Cladocopium* was the most common, accounting for 89% of the symbiont community, and the most dominant (i.e., accounting for > 50% of reads in the sample) genus observed in 91% of colonies. In contrast, *Durusdinium* was considerably less abundant, accounting for only 11% of the symbiont community (Fig. 2). The relative abundance of *Durusdinium* on Singapore reefs is lower than previous records for neighbouring reef provinces, including the Andaman Sea (relative *Durusdinium* abundance ~ 35%), where corals also experience high turbidity (LaJeunesse et al. 2010). This difference in *Durusdinium* abundance could be related to temporal changes in the symbiont community after bleaching events (e.g., Jones et al. 2008, but see McGinley et al. 2012, Smith et al. 2017a, Howells et al. 2020) or due to differences in sampled host taxa between studies (different host taxa may show a higher affinity for *Durusdinium* associations). Among our samples, we identified variation in *Durusdinium* abundance among coral species with the mean abundance ranging from 21 ± 3% (mean ± SE) in *Pectinia alcicornis* to 3 ± 1% (mean ± SE) in *Platygyra daedalea*. PERMANOVA analyses identified a significant species effect on the symbiont community (ESM Supplementary Table 1); therefore, caution should be taken when comparing across studies with different host taxa. Indeed,

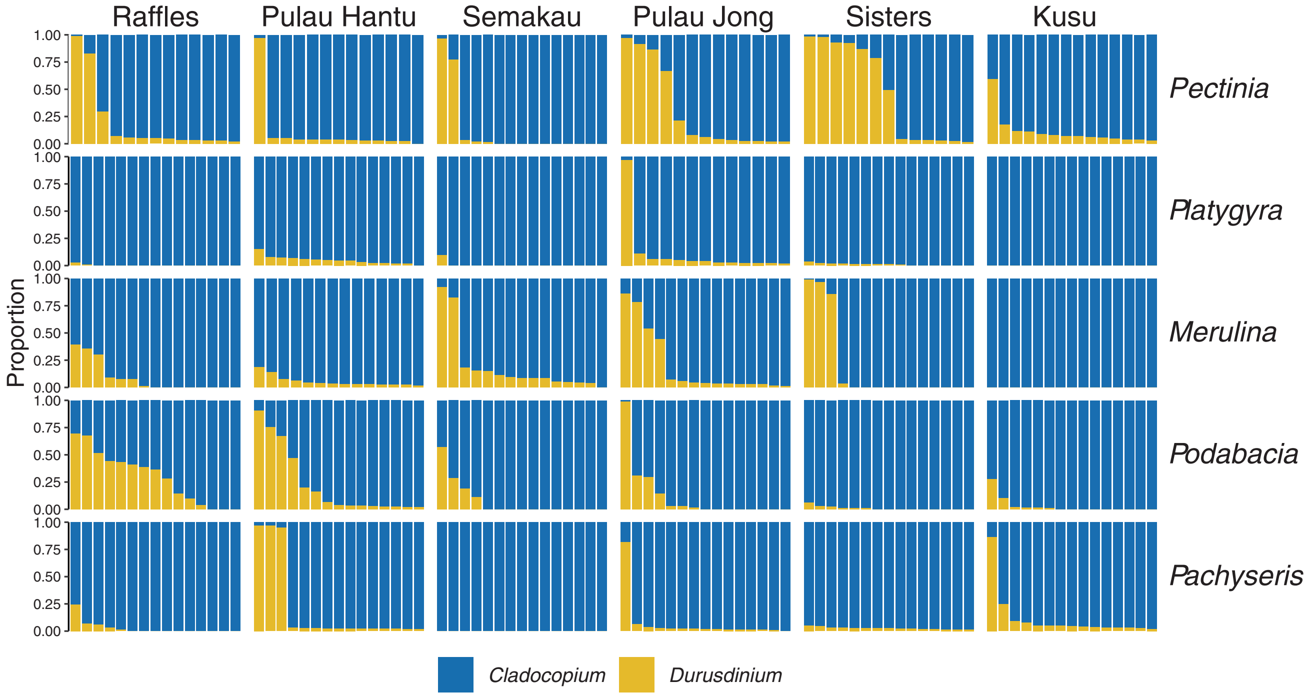


Fig. 2 Symbiodiniaceae genera composition of Singaporean corals. Each species-by-site section of the matrix plot is comprised of a series of stacked bar plots showing the relative composition of *Cladocopium* (blue) and *Durusdinium* (yellow) ITS2 sequences within a single sample. Each stacked bar represents a single individual

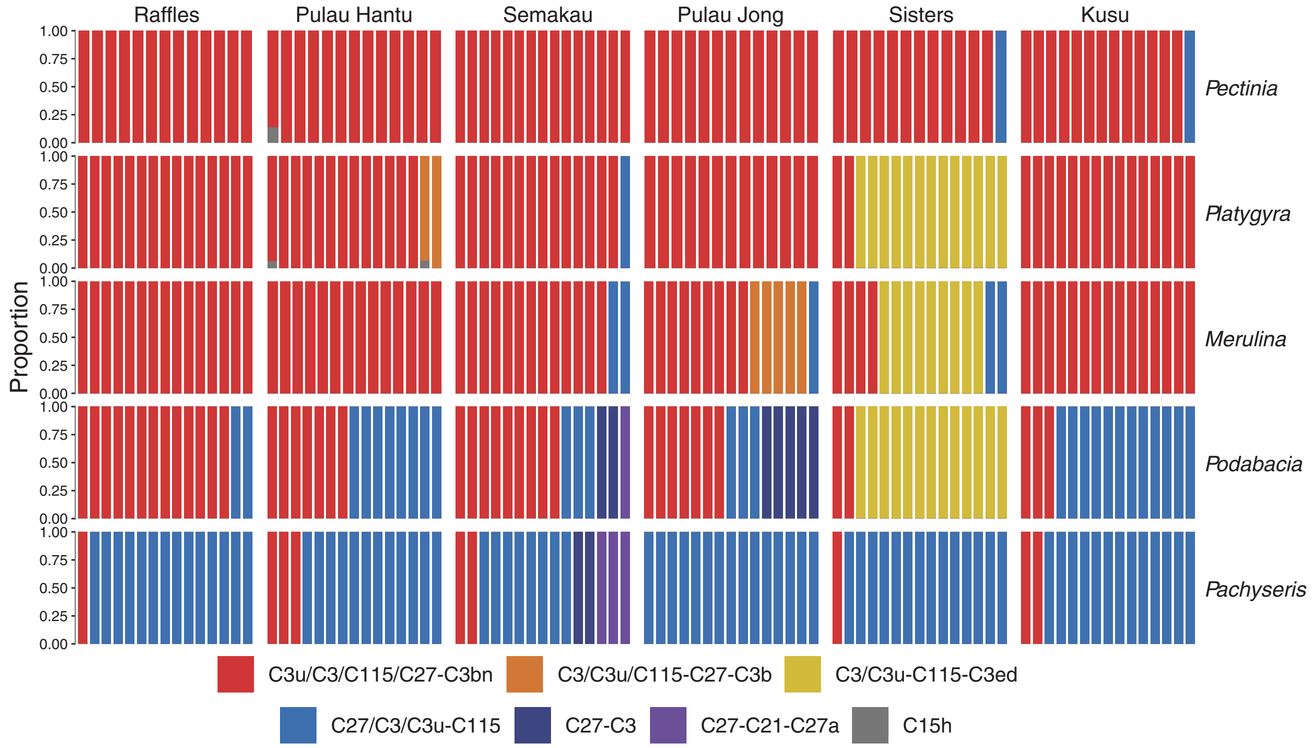


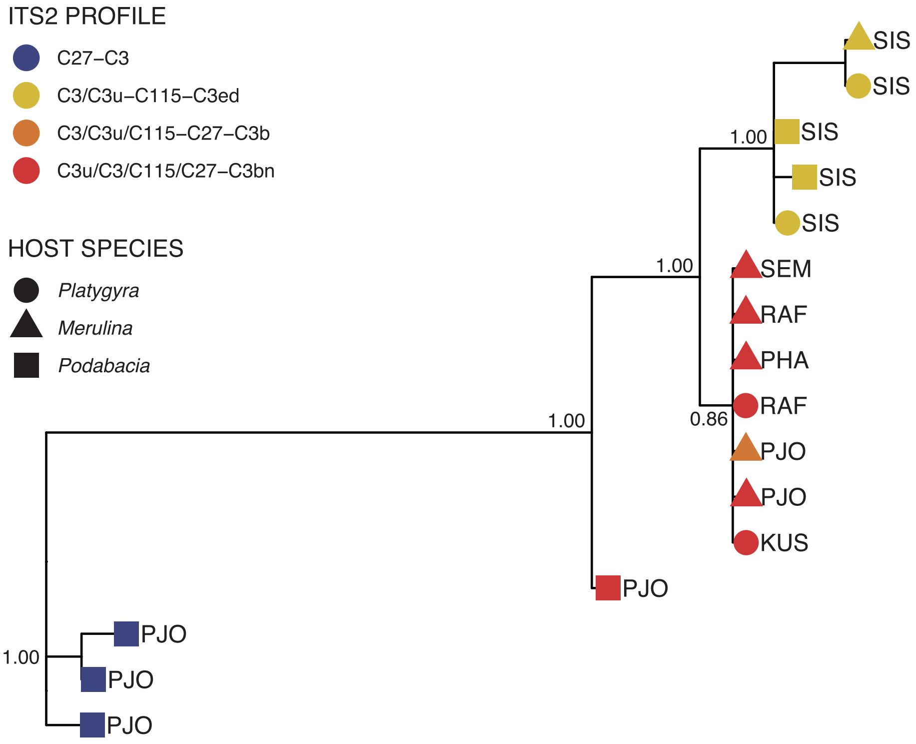
Fig. 3 *Cladocopium* profile composition of Singaporean corals. Each species-by-site section of the matrix plot is comprised of a series of stacked bar plots showing the relative proportion of the seven *Cladocopium* profiles within a sample. Each stacked bar represents a single individual

although we did not observe *Durusdinium*-dominated communities among our sampled taxa, there is evidence among a limited number of samples that *Pocillopora acuta* and *Diploastrea heliopora* colonies in Singaporean reefs may have high *Durusdinium* fidelity (Tanzil et al. 2016; Poquita-Du et al. 2020).

Using SymPortal (SymPortal.org), we found that the diversity of *Durusdinium* profiles were most likely from the stress-tolerant species, *D. trenchii*, due to the dominant co-occurrence of the D1 and D4 ITS2 sequences and presence across multiple hosts (ESM Supplementary Figure 1) (LaJeunesse et al. 2014). Of the 20 *Durusdinium* profiles identified, 17 were *D1-4* derivatives while for two other profiles it was not possible to determine whether they belonged to the *D1-4* or *D1-4-6* lineage due to the differences in sensitivity between the DGGE method used for the original symbiont descriptions, and the amplicon approach used in the current study. Nevertheless, the occurrence of *D. trenchii* on Singapore's highly impacted, turbid reefs is consistent with previous studies showing elevated abundances of this species on marginal reefs (LaJeunesse et al. 2010; Keshavmurthy et al. 2014). Importantly, our observation of multiple *D. trenchii* profiles suggests further diversity within this important symbiont species. Additional sampling where *D. trenchii* is more abundant and the application of additional genetic markers (e.g., LaJeunesse et al. 2014) is warranted to better understand the ITS2 profile diversity and the distribution of these putative strains across different sites and species.

There were seven ITS2 profiles identified by SymPortal in the most abundant genus, *Cladocopium* (Fig. 3). Based on the UniFrac similarity between profiles (ESM Supplementary Figure 2), these profiles can be broadly split into three main groups, group A (*C3/C3u/C115-C27-C3b*, *C3/C3u-C115-C3ed*, *C3u/C3/C115/C27-C3bn*), group B (*C27-C21-C27a*, *C27-C3*, *C27/C3/C3u-C115*) and group C (*C15*). PERMANOVA analyses of ITS2 profile composition indicated that host species had the greatest impact on the ITS2 profile community structure ($R^2 = 0.377$; $p < 0.001$), with site ($R^2 = 0.113$; $p < 0.001$) and species-by-site ($R^2 = 0.128$; $p < 0.001$) interactions also playing a significant role (ESM Supplementary Table 2). Rather than reflecting host-specific Symbiodiniaceae strains, the large proportion of variance attributed to the coral host describes the association of different host-generalist strains with different coral taxa. The group A profiles were widespread across samples from all sites for *Pectinia alcicornis*, *M. ampliata* and *Platygyra daedalea*, whereas group B profiles were the dominant profiles in *Pachyseris speciosa* samples. *Podabacia crustacea* hosted a combination of group A and

Fig. 4 Chloroplast psbA^{ncr} phylogeny of *Cladocopium* samples classified to different ITS2 profiles by SymPortal. The tree was constructed through Bayesian inference using a Jukes-Cantor model. Samples are labelled according to site (RAF, Raffles; PHA, Pulau Hantu; SEM, Pulau Semakau; PJO, Pulau Jong; SIS, Sisters' Island; KUS, Kusu). Support for nodes is shown where the posterior probabilities exceed 0.8



group B profiles potentially reflecting lower fidelity for specific strains in this coral species.

The number of distinct *Cladocopium* ITS2 profiles in this study of Singaporean corals is comparable to other studies employing SymPortal. For example, previous studies have identified five distinct *Cladocopium* ITS2 profiles from the Great Barrier Reef (GBR) and eight from the Red Sea (Camp et al. 2019; Terraneo et al. 2019). However, the reduced taxonomic scope (GBR: 2 coral genera; Red Sea; 1 genus) and fewer samples (GBR: $n = 14$; Red Sea: $n = 80$) in these studies suggest *Cladocopium* diversity in Singapore is lower than other regions (ESM Supplementary Figure 3). Indeed, a recent study from the Red Sea with a more comparable sampling design (seven host species, six sites, $n = 603$) identified 74 *Cladocopium* ITS2 profiles (Hume et al. 2020). Low *Cladocopium* diversity on reefs with high coral species diversity is notable for two reasons. First, it suggests that while the refuge effect of a low light environment may help maintain coral species diversity, it may not support diverse symbiont communities. Secondly, these patterns of diversity are in stark contrast to other marginal reef systems (LaJeunesse et al. 2008; Wicks et al. 2010), such as the Persian/Arabian Gulf where few coral species associate with a diverse *Cladocopium* lineage, *C. thermophilum* (Hume et al. 2016, 2018a; Smith et al. 2017b). The host specificity of *Cladocopium* strains found in the Persian/Arabian Gulf (Smith et al. 2017b; Howells et al. 2020), and other marginal reefs (LaJeunesse et al. 2008), were not observed in Singapore, where single strains (represented by an ITS2 profile) are found across multiple host species (e.g., *C3u/C3/C115/C27-C3bn*). The distinct differences in the symbiont diversity among these marginal environments may be reflective of the duration under which these reefs have been exposed to stressful conditions. The high turbidity on Singapore's reefs is a relatively recent phenomenon, occurring as a consequence of increasing anthropogenic activities over the past 60 yrs (Chia et al. 1988; Dikou and Van Woesik 2006). Conversely, the Persian/Arabian Gulf has experienced thermally extreme conditions for ~ 4000 yrs, providing greater opportunity for adaptive radiation in the symbiont community (Hume et al. 2016).

Despite low diversity of the *Cladocopium* communities in Singapore, we found a distinct *Cladocopium* ITS2 profile at Sisters' Island. To verify that the Sisters' Island associated ITS2 profile was distinct from other closely related profiles, we sequenced the psbA^{ncr} for a subset of samples. Coral samples from Sisters' Island formed a monophyletic clade in our psbA^{ncr} tree (Fig. 4), in agreement with the distinct SymPortal ITS2 profile. Across an alignment of 427 nucleotides, the Sisters' Island samples shared two nucleotide substitutions that were distinct from all other samples. A similar degree of psbA^{ncr} sequence differentiation was observed between strains inferred using ITS2 metahaplotype approach (Smith et al. 2017b), highlighting the utility of the psbA^{ncr} to provide additional support for closely related strains identified through ITS2 intragenomic variant-based analyses. The cause of the apparent site specificity of this profile is unclear as Sisters' Island is not located at the geographic extremes of our sampling regime and because all sites are within 16 km of each other. Fine-scale differences in *Cladocopium* genotypes over short distances (tens of kilometres) have been observed in other marginal environments and were linked to local environmental conditions (Noda et al. 2017; Hume et al. 2018a). Deployment of in situ loggers to assess key environmental parameters (temperature, light, salinity) will help elucidate microhabitat differences and the potential environmental drivers for fine-scale differences observed in this study.

This study provides the most comprehensive survey of Symbiodiniaceae in Singaporean corals to date, covering five of the most locally dominant coral species at six reef sites. We show that a few host-generalist *Cladocopium* symbionts dominate the symbiont community and that the less abundant *Durusdinium* community is diverse, consisting of multiple *D. trenchii* strains. The higher taxonomic resolution afforded by SymPortal identified fine-scale differences in the Singaporean Symbiodiniaceae communities that would have been undetected by previous techniques. Our analyses highlight that as our resolution improves, fine-scale environmental data will be needed to help understand drivers of diversity on local and regional scales. As the high-resolution ITS2 analyses become more widespread and the usage of SymPortal framework increases, it will be possible to identify emerging trends with regard to important aspects of symbioses, such as the prevalence of local adaptive radiations in marginal environments and the timescales over which they arise.

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Compliance with ethical standards

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

References

Anderson MJ, Walsh DC (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol Monogr 83:557–574

Bongaerts P, Riginos C, Ridgway T, Sampayo EM, van Oppen MJ, Englebert N, Vermeulen F, Hoegh-Guldberg O (2010) Genetic divergence across habitats in the widespread coral *Seriatopora hystrix* and its associated Symbiodinium. PLoS ONE 5:e10871

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:S129–S138

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS ONE 2:e711

Cacciapaglia C, van Woesik R (2016) Climate-change refugia: shading reef corals by turbidity. Glob Chang Biol 22:1145–1154

Camp EF, Schoepf V, Mumby PJ, Hardtke LA, Rodolfo-Metalpa R, Smith DJ, Suggett DJ (2018) The future of coral reefs subject to rapid climate change: lessons from natural extreme environments. Front Mar Sci 5:4

Camp EF, Edmondson J, Doheny A, Rumney J, Grima AJ, Huete A, Suggett DJ (2019) Mangrove lagoons of the Great Barrier Reef support coral populations persisting under extreme environmental conditions. Mar Ecol Prog Ser 625:1–14

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. Mol Ecol 22:3124–3140

Chia LS, Khan H, Chou LM (1988) The coastal environmental profile of Singapore. WorldFish

D'Angelo C, Hume BC, Burt J, Smith EG, Achterberg EP, Wiedenmann J (2015) Local adaptation constrains the distribution potential of heat-tolerant *Symbiodinium* from the Persian/Arabian Gulf. ISME J 9:2551

Dana JD (1846) Zoophytes. In: Wilkes C (ed) United States Exploring Expedition During the years 1838, 1839, 1840, 1841, 1842. Lea and Blanchard, Philadelphia, pp 1–740

De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27–year decline of coral cover on the Great Barrier Reef and its causes. Proc Natl Acad Sci USA 109:17995–17999

Dikou A, Van Woesik R (2006) Survival under chronic stress from sediment load: spatial patterns of hard coral communities in the southern islands of Singapore. Mar Pollut Bull 52:1340–1354

Ellis J, Solander DC (1786) The natural history of many curious and uncommon zoophytes: collected from various parts of the globe. Benjamin White and Son, and Peter Elmsly, London

Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH, Sogin ML (2015) Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME J 9:968

Fabricius K, Mieog J, Colin P, Idip D, Van Oppen M (2004) Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. Mol Ecol 13:2445–2458

Glynn PW (1983) Extensive 'bleaching'and death of reef corals on the Pacific coast of Panama. Environ Conserv 10:149–154

Guest J, Low J, Tun K, Wilson B, Ng C, Raingeard D, Ulstrup K, Tanzil JTI, Todd P, Toh T (2016a) Coral community response to bleaching on a highly disturbed reef. Sci Rep 6:20717

Guest J, Tun K, Low J, Vergés A, Marzinelli E, Campbell AH, Bauman A, Feary D, Chou L, Steinberg P (2016b) 27 years of benthic and coral community dynamics on turbid, highly urbanised reefs off Singapore. Sci Rep 6:36260

Hennige SJ, Smith DJ, Walsh S-J, McGinley MP, Warner ME, Suggett DJ (2010) Acclimation and adaptation of scleractinian coral communities along environmental gradients within an Indonesian reef system. J Exp Mar Bio Ecol 391:143–152

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Howells EJ, Abrego D, Meyer E, Kirk NL, Burt JA (2016) Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. Glob Chang Biol 22:2702–2714

Howells EJ, Bauman AG, Vaughan GO, Hume BC, Voolstra CR, Burt JA (2020) Corals in the hottest reefs in the world exhibit symbiont fidelity not flexibility. Mol Ecol 29:899–911

Huang D, Tun KP, Chou LM, Todd PA (2009) An inventory of zooxanthellate scleractinian corals in Singapore, including 33 new records. Raffles Bulletin of Zoology 22:69–80

Huang D, Benzoni F, Fukami H, Knowlton N, Smith ND, Budd AF (2014) Taxonomic classification of the reef coral families Merulinidae, Montastraeidae, and Diploastraeidae (Cnidaria: Anthozoa: Scleractinia). Zool J Linn Soc 171:277–355

Hume B, D'angelo C, Burt J, Baker A, Riegl B, Wiedenmann J (2013) Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: prevalence of clade C3 *Symbiodinium*, host fluorescence and ex situ temperature tolerance. Mar Pollut Bull 72:313–322

Hume BC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. Sci Rep 5:8562

Hume BC, Voolstra CR, Arif C, D'Angelo C, Burt JA, Eyal G, Loya Y, Wiedenmann J (2016) Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to Holocene climate change. Proc Natl Acad Sci U S A 113:4416–4421

Hume BC, D'Angelo C, Burt JA, Wiedenmann J (2018a) Fine-scale biogeographical boundary delineation and sub-population resolution in the *Symbiodinium thermophilum* coral symbiont group from the Persian/Arabian Gulf and Gulf of Oman. Front Mar Sci 5:138

Hume BC, Ziegler M, Poulain J, Pochon X, Romac S, Boissin E, De Vargas C, Planes S, Wincker P, Voolstra CR (2018b) An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. PeerJ 6:e4816

Hume BC, Smith EG, Ziegler M, Warrington HJ, Burt JA, LaJeunesse TC, Wiedenmann J, Voolstra CR (2019) SymPortal: a novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. Mol Ecol Resour 19:1063–1080

Hume BC, Mejia-Restrepo A, Voolstra CR, Berumen ML (2020) Fine-scale delineation of Symbiodiniaceae genotypes on a previously bleached central Red Sea reef system demonstrates a prevalence of coral host-specific associations. Coral Reefs 1–19

Iglesias-Prieto R, Beltran V, Lajeunesse TC, Reyes-Bonilla H, Thome P (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc Biol Sci 271:1757–1763

Jones AM, Berkelmans R, van Oppen MJ, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc Biol Sci 275:1359–1365

Keshavmurthy S, Meng P-J, Wang J-T, Kuo C-Y, Yang S-Y, Hsu C-M, Gan C-H, Dai C-F, Chen CA (2014) Can resistant coral-*Symbiodinium* associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. PeerJ 2:e327

Kleypas JA, McManus JW, Menez LA (1999) Environmental limits to coral reef development: where do we draw the line? Am Zool 39:146–159

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through psbA non-coding region genotyping. PLoS ONE 6:e29013

LaJeunesse TC, Bonilla HR, Warner M, Wills M, Schmidt G, Fitt W (2008) Specificity and stability in high latitude eastern Pacific coral-algal symbioses. Limnol Oceanogr 53:719–727

LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA (2014) Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) Clade D are different species. Phycologia 53:305–319

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol 28(2570–2580):e2576

LaJeunesse TC, Pettay DT, Sampayo EM, Phongsuwan N, Brown B, Obura DO, Hoegh-Guldberg O, Fitt WK (2010) Long-standing environmental conditions, geographic isolation and host–symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus Symbiodinium. J Biogeogr 37:785–800

Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A (2010) Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. Ecology 91:990–1003

Lien Y-T, Nakano Y, Plathong S, Fukami H, Wang J-T, Chen C (2007) Occurrence of the putatively heat-tolerant *Symbiodinium* phylotype D in high-latitudinal outlying coral communities. Coral Reefs 26:35–44

McGinley MP, Aschaffenburg MD, Pettay DT, Smith RT, LaJeunesse TC, Warner ME (2012) *Symbiodinium* spp. in colonies of eastern Pacific *Pocillopora* spp. are highly stable despite the prevalence of low-abundance background populations. Mar Ecol Prog Ser 462:1–7

Moore RB, Ferguson KM, Loh WK, Hoegh-Guldberg O, Carter DA (2003) Highly organized structure in the non-coding region of the psbA minicircle from clade C *Symbiodinium*. Int J Syst Evol Microbiol 53:1725–1734

Morgan KM, Perry CT, Johnson JA, Smithers SG (2017) Nearshore turbid-zone corals exhibit high bleaching tolerance on the Great Barrier Reef following the 2016 ocean warming event. Front Mar Sci 4:224

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27:454–460

Ng TY, Ang P (2016) Low symbiont diversity as a potential adaptive strategy in a marginal non-reefal environment: a case study of corals in Hong Kong. Coral Reefs 35:941–957

Noda H, Parkinson JE, Yang S-Y, Reimer JD (2017) A preliminary survey of zoantharian endosymbionts shows high genetic variation over small geographic scales on Okinawa-jima Island, Japan. PeerJ 5:e3740

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) vegan: Community Ecology Package. R package version 2.5-6

Oliver T, Palumbi S (2011) Many corals host thermally resistant symbionts in high-temperature habitat. Coral Reefs 30:241–250

Pallas PS (1766) Elenchus zoophytorum sistens generum adumbrationes generaliores et specierum cognitarum… descriptiones etc. Varrentrapp, Den Haag

Poquita-Du RC, Huang D, Chou LM, Todd PA (2020) The contribution of stress-tolerant endosymbiotic dinoflagellate *Durusdinium* to *Pocillopora acuta* survival in a highly urbanized reef system. Coral Reefs 1–11

R Core Team (2019) R: A language and environment for statistical computing. Austria, Vienna

Reimer JD, Todd PA (2009) Preliminary molecular examination of zooxanthellate zoanthid (Hexacorallia, Zoantharia) and associated zooxanthellae (*Symbiodinium* spp.) diversity in Singapore. Raffles Bulletin of Zoology 22:103–120

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542

Saville Kent W (1871) On some new and little known species of Madreporaria, or stony corals, in the British Museum collection. Proceedings of the Zoological Society of London 2:275–286

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Smith EG, Vaughan GO, Ketchum RN, McParland D, Burt JA (2017a) Symbiont community stability through severe coral bleaching in a thermally extreme lagoon. Sci Rep 7:2428

Smith EG, Ketchum RN, Burt JA (2017b) Host specificity of *Symbiodinium* variants revealed by an ITS2 metahaplotype approach. ISME J 11:1500–1503

Smith EG, Hume BC, Delaney P, Wiedenmann J, Burt JA (2017c) Genetic structure of coral-*Symbiodinium* symbioses on the world's warmest reefs. PLoS ONE 12:e0180169

Smith EG, D'angelo C, Sharon Y, Tchernov D, Wiedenmann J (2017d) Acclimatization of symbiotic corals to mesophotic light environments through wavelength transformation by fluorescent protein pigments. Proc Biol Sci 284:20170320

Stambler N, Dubinsky Z (2005) Corals as light collectors: an integrating sphere approach. Coral Reefs 24:1–9

Tanzil JTI, Ng APK, Tey YQ, Tan BHY, Yun EY, Huang D (2016) A preliminary characterisation of *Symbiodinium* diversity in some common corals from Singapore. Cosmos 12:15–27

Terraneo TI, Fusi M, Hume BC, Arrigoni R, Voolstra CR, Benzoni F, Forsman ZH, Berumen ML (2019) Environmental latitudinal gradients and host-specificity shape Symbiodiniaceae distribution in Red Sea Porites corals. J Biogeogr 46:2323–2335

Todd PA, Ladle RJ, Lewin-Koh N, Chou LM (2004) Genotype × environment interactions in transplanted clones of the massive corals *Favia speciosa* and *Diploastrea heliopora*. Mar Ecol Prog Ser 271:167–182

Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. Biol Bull 201:348–359

Veron JE, Stafford-Smith M, Turak E, DeVantier L (2015) Corals of the World (http://www.coralsoftheworld.org/)

Wicks L, Sampayo E, Gardner J, Davy S (2010) Local endemicity and high diversity characterise high-latitude coral–*Symbiodinium* partnerships. Coral Reefs 29:989–1003

Wilkinson C (2008) Status of coral reefs of the world: 2008. Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville

Wong JC, Thompson P, Xie JY, Qiu J-W, Baker DM (2016) *Symbiodinium* clade C generality among common scleractinian corals in subtropical Hong Kong. Reg Stud Mar Sci 8:439–444

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

Ziegler M, Arif C, Burt JA, Dobretsov S, Roder C, LaJeunesse TC, Voolstra CR (2017) Biogeography and molecular diversity of coral symbionts in the genus *Symbiodinium* around the Arabian Peninsula. J Biogeogr 44:674–686