Baseline

Stable Symbiodiniaceae composition in three coral species during the 2017 natural bleaching event in subtropical Hong Kong

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ABSTRACT

Adaptive changes in endosymbiotic Symbiodiniaceae communities have been reported during and after bleaching events in tropical coral species, but little is known about such shifts in subtropical species. Here we examined the Symbiodiniaceae communities in three coral species (Montipora peltiformis, Pavona decussata, and Platygyra carnosa) based on samples collected during and after the 2017 bleaching event in subtropical Hong Kong waters. In all of the collected samples, ITS2 meta-sequencing revealed that P. decussata and P. carnosa were predominantly associated with Cladocopium C1 and Cladocopium C1c, whereas M. peltiformis was mainly associated with two Cladocopium C21 types and Clade C. For each species, the predominant endosymbionts exhibited high fidelity, and the relatively low abundance ITS2-types showed minor changes between the bleached and recovered corals. Our study provided the first details of coral-algal association in Hong Kong waters, suggesting the selection of certain genotypes as a potential adaptive mechanism to the marginal environmental conditions.

The symbiotic relationship with the dinoflagellate family Symbiodiniaceae allows many scleractinian corals to have access to the photosynthesates produced by the algae, and thus thrive in shallow tropical and subtropical waters (Hoegh-Guldberg, 1999). However, this symbiosis is sensitive to seawater temperature changes – a prolonged exposure to 1–2 °C above the average summer temperature maximum can result in bleaching – a breakdown of the symbiosis that reduces the algal density and produces the whitish bleaching phenotype (Goreau et al., 2000). During the last half-century, large-scale abnormal seawater warming has triggered several regional to global scale coral bleaching events, including those that occurred during 1982–1983 (Glynn, 1991), 1997–1998 (Wilkinson, 1998), 2010 (Alemu and Clement, 2014), and 2014–2017 (Eakin et al., 2019). As the seawater temperature is predicted to continually increase and there will be more heatwaves in this century (IPCC, 2014), understanding the coral-algal symbiosis and how such symbiosis responds to extreme environmental conditions are fundamental to predicting the future of coral reef ecosystems.

The family Symbiodiniaceae comprises the genera Symbiodinium, Brevisiolum, Cladocinium, Durusdinium, Effrenium, Fugaciun, Freudenthalidium, Gerakladium, Halluxium, Clade I, and Clade J (LaJeunesse et al., 2018; Yorifuji et al., 2021). Among these genera, the former four are common symbionts of scleractinian corals. The genetic diversity of a coral’s endosymbiont reflects its stress tolerance due to the differential photosynthetic efficiencies and environmental tolerance of Symbiodiniaceae genotypes (Baker, 2003; Pochon and Gates, 2010; Russnak et al., 2021). In general, coral colonies that host the stress-tolerant Durusdinium have a 1–2 °C higher thermal tolerance threshold than those that are associated with other genotypes such as Cladocopium (Mostafavi et al., 2007; Silverstein et al., 2015; Fuller et al., 2020). Some corals exhibit flexibility in their endosymbiont communities (e.g., shifting the algal partner from Cladocopium to Durusdinium during bleaching event (Ziegler et al., 2017; Gong et al., 2018; Gardner et al., 2019; Claar et al., 2020). However, even within Cladocopium, the sensitivity to thermal stress varies with genotypes (Hume et al., 2016), and certain coral species or populations still maintain highly stable symbiotic communities over broad temperature gradients and through bleaching events (LaJeunesse et al., 2004; Smith et al., 2017; Osman et al., 2020; Voolstra et al., 2021). Therefore, for many coral species, especially those in subtropical regions that are less well-studied than tropical corals (Beger et al., 2014; Sully et al., 2019; Eddy et al., 2021), there is an urgent need to analyze the composition of Symbiodiniaceae community and its changes in response to extreme climate conditions.

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Located in a subtropical environment with strong seasonal fluctuations in seawater temperature from 14 to 31 °C and a strong gradient of water quality due to the presence of the Pearl River Estuary to the west, Hong Kong’s marine waters represent a marginal environment for coral reef development. Even in the eastern oceanic waters where coral communities are better developed, they typically grow on volcanic rocks as non-reefal colonies and are restricted to shallow waters <10 m deep (Morton and Morton, 1983; Yeung et al., 2021). A total of 84 scleractinian corals have been recorded in Hong Kong (Chan et al., 2005). Previous studies have found that these corals harbor a low diversity of Symbiodiniaceae, with the majority being Cladocopium genotypes (Ng and Ang, 2016; Wong et al., 2016; Saad et al., 2021). Despite being located in a subtropical environment, Hong Kong’s coral communities have been affected by three bleaching events that occurred in the summers of 1997–1998 (McCorry, 2002), 2014 (Xie et al., 2017), and 2017 (Xie et al., 2020). However, no investigation has been conducted to understand the endosymbiotic compositional changes during natural bleaching events in Hong Kong, or any of the fringing coral communities widely distributed along the coasts of the neighboring Guangdong Province of mainland China (Zhao et al., 2022).

In this study, we examined the endosymbiotic dinoflagellates communities associated with three common coral species in Hong Kong (Yeung et al., 2021), including Montipora peltiformis, Pavona decussata, and Platygyra carnosa (Fig. 1), based on samples collected during the 2017 heatwave. These selected species exhibited contrasting patterns of loss and recovery of algal symbiont/pigmentation indicating their differential bleaching susceptibility (Xie et al., 2020; Ip et al., 2022; Zhang et al., 2022). We compared the endosymbiotic communities using high throughput metabarcoding in coral samples collected from colonies with differential bleaching phenotypes (non-bleached and bleached) during the bleaching event (sampled in Jun-Aug 2017), as well as the same colonies (bleached) that have recovered from the bleaching event (sampled in Sep 2017; details in Supplementary Fig. S1). The results will improve our understanding of Symbiodiniaceae diversity in subtropical coral species, and form a baseline for monitoring Symbiodiniaceae community structure in the future.

We collected samples of M. peltiformis, P. decussata, and P. carnosa by SCUBA at 2–4 m water depth from Bluff Island (22°19′27.6″N 114°21′14.0″E), Sharp Island (22°21′54.3″N, 114°17′20.3″E), and Lai Chi Wo (22°32′01.5″N 114°16′04.1″E), respectively, during the summer and fall of 2017 (Fig. 1). We collected the coral samples during June to September 2017 when the three species were found to undergo bleaching, tagged bleached colonies and revisited them roughly three months later (Ip et al., 2022; Zhang et al., 2022) The seawater surface temperature recorded from sites near these coral communities ranged from 26.2 °C to 31.4 °C when bleaching was found (details in Supplementary Fig. S1). We used the Coral Health Chart of CoralWatch (Siebeck et al., 2006) to help determine the bleached and non-stressed colonies during the field sampling. There were four health conditions: (1) ‘unbleached’: a fragment from a colony without bleaching; (2) ‘stressed-unbleached’: an unbleached fragment from a partially bleached colony; (3) ‘stressed-bleached’: a bleached fragment from the same colony as in the ‘stressed-unbleached’ condition; (4) ‘recovered’: a fragment from a colony recovered from partial bleaching. For each health condition, we collected four biological replicates (colonies) at least two meters apart to avoid sampling of clones. Samples were kept in

![Fig. 1. Photographs of Montipora peltiformis (sampling site: Bluff Island), Pavona decussata (Sharp Island), and Platygyra carnosa (Lai Chi Wo) show the paled/bleached (left) and recovered (right) colonies during and after the 2017 bleaching event.](image-url)
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Coral samples were grounded in liquid nitrogen with a mortar and pestle, and genomic DNA was extracted using the CTAB method (Porbiski et al., 1997). We qualified and quantified DNA using agarose gel electrophoresis and a Qubit fluorometer (Thermo Fisher Scientific, USA), respectively. We submitted DNA samples to Novogene (Tianjin, China) for high-throughput sequencing of the internal transcribed spacer 2 (ITS2) region using the dinoflagellate-specific primers ITSInt-For2 (5′-GAAGTCAGAAGTCAGTGG-3′ (LaJeunesse and Trench, 2000)) and ITS-Reverse (5′-GGATCCATATGCTTAAGTTCAGGGGTT -3′ (Coleman et al., 2010)). The sequencing libraries were prepared using the NEBNext Ultra DNA Library Prep Kit (Illumina, USA) and sequenced on an Illumina NovaSeq sequencer to obtain paired-end reads with a read length of 250 bp. There were four amplicon libraries for *M. peltiformis*, but only three amplicon libraries for *P. decussata* and *P. carnosa* due to the small amount of genomic DNA available for these two species.

The SymPortal framework v0.3.22 was used locally to analyze the Symbiodiniaceae ITS2 data (Hume et al., 2019). Briefly, we quality filtered all demultiplexed and paired forward and reverse fastq.gz files using mothur v1.43.0 (Schloss et al., 2009), conducted BLASTn search against the SymPortal reference ITS2 database, which revealed a total of 62 endosymbiotic ITS2-types belonging to the genus *Cladocopium* (formerly Clade C; Fig. 2; details in Supplementary Table S2). Of the 62 endosymbiont ITS2-types, 45 (72 %) were not characterized by SymPortal, therefore were considered as novel types. To characterize these novel genotypes, we conducted BLASTn search against the SymPortal reference ITS2 database, which revealed a total of 20 C1 variants, 11 C3 variants, 22 C21 variants, and 9 other genotypes of *Cladocopium* according to Symbiodiniaceae phylogenetic relationships (Supplementary Table S2, LaJeunesse, 2005; LaJeunesse et al., 2018). In addition, in all collection times, SymPortal revealed *Cladocopium* 1466-C21-C1-4118-C3 as the single ITS2-type profile in all *M. peltiformis* samples, and *Cladocopium* C1-C1c-1466 as the only profile for all *P. decussata* and *P. carnosa* samples (Fig. 1).

To understand the general endosymbiont composition within each species, we examined the ITS2-type and -abundance at species-level, combining all coral samples collected during and after the bleaching event. Samples of *M. peltiformis* (Supplementary Table S2) harbored 20 to 25 ITS2-types; among them three C21 variants (average 42.9 % of 1466_C, 18.4 % of C21, and 4.9 % of 4118_C), C1 (7.0 %), and C3 (3.9 %) were predominant, accounting for 80 % of the total abundance. All other *M. peltiformis* genotypes were of low abundances (1.0–2.4 %), including two C3 variants (C3.14 and 5864_C) and six C21 variants (C21ac, C21k, 5865_C, 5866_C, 5867_C, and 5869_C). Samples of *P. decussata* were associated with 16 to 24 ITS2-types, with C1 (59.9 %), C1c (17.4 %), and C21 (14.6 %) predominant, accounting for 80.9 % of the total abundance. Other ITS2-types are shown, other sequences are represented by grey bars on the top.

Analysis of Variance (ANOVA) followed by the Tukey test to compare each ITS2-type with relative abundance >1 % among the four health conditions using SPSS Statistics 19 (SPSS Inc., Illinois, USA).

ITS2 amplicon sequencing (n = 40) generated a total of 3.59 million pair-end reads (average = 9.0 thousand reads per sample) from three coral species (Supplementary Table S1). Among these reads, SymPortal revealed 7,753 Symbiodiniaceae ITS2 sequence variants (DIVs) and further clustered them into 62 endosymbiotic ITS2-types belonging to the genus *Cladocopium*. Principal coordinate analyses (PCoA) were conducted based on the Bray–Curtis distance matrices obtained from the SymPortal as part of the output files. We tested significant differences among host species and health conditions with Permutational Multivariate Analysis of Variance (PERMANOVA) using the “adonis2” function in the R package “vegan”. We used a one-way Analysis of Variance (ANOVA) followed by the Tukey test to compare each ITS2-type with relative abundance >1 % among the four health conditions using SPSS Statistics 19 (SPSS Inc., Illinois, USA).

Fig. 2. Symbiodiniaceae community composition of three coral species during and after the 2017 bleaching event. Relative abundance (%) of ITS2-types from four health conditions is plotted above the horizontal black line (in X-axis). The predicted ITS2-type profile is plotted below. Only the top 15 relative abundance ITS2-types are shown, other sequences are represented by grey bars on the top. Designated names (e.g., C1) represent ITS2 sequences characterized in the SymPortal (Hume et al., 2019), while the uncharacterized/novel sequences are designated to a unique ID with the associated clade/genera (e.g., 1466_C). Sampling sites: Bluff Island (*Montipora peltiformis*), Sharp Island (*Pavona decussata*), and Lai Chi Wo (*Platygryra carnosa*).
1466_C (4.0 %) being most dominant; all samples contained five low abundance Cladocopium, including C1b, C1al, C3, C72k, and 1365_C. Samples of P. carnosa exhibited similar endosymbiont composition with a. decussata, consisting of C1 (54.9 %), C1c (25.8 %), and 1466_C (4.9 %) and five low abundance ITS2-types (C1b, C3, C21, C72k, and 1373_C) in all samples. Notably, we also detected 20, 9, and 7 unique ITS2-types in M. peltiformis, P. decussata, and P. carnosa, respectively (Supplementary Fig. S2). These genotypes were closely related to lineages of C21 in M. peltiformis and C1 in P. decussata and P. carnosa, respectively.

To reveal the potential changes in symbiont composition during the bleaching event, we compared the endosymbiont community structure among coral species and health conditions. The endosymbiont community structure exhibited a high level of host-specificity (PERMANOVA, \( F = 520.38, R^2 = 0.965, p < 0.0001 \); Supplementary Table S3), while the PCoA plot revealed clustering of samples by species (Fig. 3), further confirming host identity as the major contributor for the differences in endosymbiont community structure among the samples. Each coral host was associated with three to five predominant endosymbionts that were present in the three coral species in different proportions. Notably, four ITS2-types (C1, C1c, C21, and 1466_C) were among the most abundant endosymbiotic dinoflagellates (Supplementary Fig. S2) in the three coral species under all four health conditions.

Within each species, the endosymbiont types generally did not vary significantly among the four health conditions (PERMANOVA, \( F = 1.52, R^2 = 0.004, p = 0.226 \)). Nevertheless, when we compared each ITS2-type (relative abundance > 1 %) among the four health conditions within each coral host, we found that the ‘recovered’ M. peltiformis samples contained lower proportions of C1 (3.4 %) and C1c (1.1 %) than the other health conditions (6.9 %–10.0 % and 2.3 %–3.5 %, respectively; Tukey test, \( p > 0.05 \); Supplementary Table S4). Although there was a reducing tendency of C21 types in ‘stressed-bleached’ M. peltiformis (71.5 %), compared with the other health conditions (75.3 %–80.4 %), this difference was not statistically significant. In addition, there was a slight increase in C1c in the stressed P. decussata (17.6 %–18.8 %), compared with the ‘unbleached’ corals (15.2 %), although the differences were insignificant. In P. carnosa, there was no significant difference among the four health conditions, except for a slightly lower abundance of C1c in the ‘unbleached’ condition (22.8 %) than in other conditions (26.2 %–27.2 %). Thus, our results showed that the coral host was the primary determining factor of endosymbiont variability. Among the three coral species, ‘stressed-bleached’ M. peltiformis (32) was associated with more ITS2-types than the other two species (26 in P. decussata and 19 in P. carnosa; Supplementary Fig. S2), and there were slight changes in C1 and C1c, which are two ITS2 genotypes common to the subtropical corals of Hong Kong, Guangdong Province, and Guangxi Province in the South China Sea (Chen et al., 2019; Saad et al., 2021).

In each coral species, we observed a high host fidelity in their predominant endosymbiotic dinoflagellates: Cladocopium C21 in M. peltiformis, and C1/C1c in P. decussata and P. carnosa. These ITS2 types have been reported in non-bleached corals in Hong Kong previously (Tong et al., 2017; Saad et al., 2021). The dominance of Cladocopium has also been reported in nearby subtropical coral communities in Taiwan (Chen et al., 2005) and the South China Sea (Gong et al., 2018; Chen et al., 2019). Such host specificity within a region has been reported in tropical and subtropical coral reefs (e.g., Australia’s Great Barrier Reef (Tonk et al., 2013), the Red Sea (Hume et al., 2020; Osman et al., 2020), the Andaman Sea and Zanzibar (LaJeunesse et al., 2010)). The host-symbiont fidelity in Hong Kong likely indicates strong local selection pressure to the subtropical climate (Saad et al., 2021). A similar high fidelity of host-symbiont association after bleaching was reported for corals inhabiting the northern Red Sea (Hume et al., 2020; Voolstra et al., 2021), and this has been suggested as a specific adaptation to the relatively warm water that has selected the heat-resistant Symbiodiniaceae (Silverstein et al., 2015; Boulou et al., 2016). In corals of the Persian/Arabian Gulf, Smith et al. (2017) reported stability of endosymbiont composition, with Cladocopium C3 being the dominant genotype in the lagoonal reefs, and no increase in the proportion of Durusdinium during and after a bleaching event. They proposed that this was due to the absence of a more heat tolerant genotype than the dominant C3 in the study region. Notably, our SymPortal analysis revealed 45 out of 64 ITS2-types as novel Cladocopium types. Our BLASTn search revealed that 59 of them were highly homologous with the Symbiodiniaceae types that have previously been reported in the Symbiodiniaceae (Chen et al., 2019; Saad et al., 2021). Our previous field and laboratory studies revealed differential sensitivity to heatwave in the three coral species, with P. decussata and P. carnosa being most resilience than M. peltiformis (Xie et al., 2020; Ip et al., 2022; Zhang et al., 2022). Specifically, the bleaching susceptible M. peltiformis was associated with host-specialist symbiont Cladocopium C21 with relative higher symbiont diversity, which is restricted to only a few exposed sites facing the open ocean. The resilient P. decussata and P. carnosa were associated with host-generalist C1 with lower symbiont diversity and they are widely distributed in the protected bays of eastern Hong Kong waters (Yeung et al., 2021). These results appeared to lend support to the speculation that the low symbiont diversity is an adaptive mechanism in marginal coral communities, where the strong selective pressure may favor the selection of certain well-adapted genotypes (Ng and Ang, 2016; Smith et al., 2020; Saad et al., 2021).

Although Hong Kong’s coral communities have suffered from three
bleaching events, i.e., 1997–1998, 2014, and 2017, little is known about the endosymbionts responses in these marginal corals. Here, for the first time, we provided compositional details of the endosymbiotic dinoflagellates in three coral species in the subtropical Hong Kong waters during and after the 2017 bleaching event. We showed that endosymbiont community composition might have contributed to the differential bleaching susceptibility of the three coral species. Specifically, *P. carnosoa* and *P. decussata* with a high proportion of generalist C1/C1c types had better resilience to heatwave as evidenced by lower reductions in algal symbiont and coral pigmentation (Ip et al., 2022; Zhang et al., 2022). The more susceptible *M. peltiformis* having a larger loss of endosymbiont density and stronger photoinhibition in the remaining endosymbiont (Ip et al., 2022) hosted the specialist C21 types. In addition, we identified 45 novel *Cladocium* types, highlighting the genetic diversity of endosymbionts inhabiting the northern South China Sea. Overall, our study has provided new knowledge on the changes in coral Symbiodiniaceae composition during a subtropical bleaching event, and a baseline for future coral research and management in Hong Kong and the surrounding Greater Bay Area. The results highlight the importance of locally adapted genotypes in coral reef resilience.

CRediT authorship contribution statement

JWQ designed the research. YHY and JXY collected the samples. YZ extracted the DNA. JCH analyzed the data and drafted the manuscript. All authors edited the manuscript and approved the submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Sequence data determined in this study are available in the NCBI SRA database under the BioProject PRJNA884662.

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Appendix A. Supplementary Figures S1-S2 and Supplementary Tables S1-S5

Supplementary data to this article can be found at https://doi.org/10.1016/j.marpolbul.2022.114224.

References


