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REPORT

Species richness and generalists–specialists mosaicism of symbiodiniacean symbionts in corals from Hong Kong revealed by high-throughput ITS sequencing

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Abstract Hong Kong is considered to be a marginal area for coral growth due to its subtropical geography with relatively low winter sea temperatures. Corals can only form non-reefal communities and are believed to host only a low diversity of symbionts with limited flexibility in their symbiont changes. Whether these previously predicted low symbiont diversity is true or is simply a technical artifact and whether these symbionts are generalists or specialists have not fully resolved. To address these issues, we investigated symbiodiniacean diversity and community structure of 30 species of Hong Kong corals using high-throughput sequencing of the nuclear ribosomal RNA gene ITS2. We found high Symbiodiniaceae species richness, with each coral hosting multiple distinct ITS2 symbiont types. Using SymPortal pipeline to alleviate effects of intraspecific ITS polymorphism, the 4662 Symbiodiniaceae ITS2 sequence variants (DIVs) found in our samples were collapsed into 13 distinct ITS2-type profiles, covering *Symbiodinium*, *Breviolum*, *Cladocopium*, and *Durusdinium* genera, seven of which were coral specific. *Cladocopium goreaui* was the most diversified (six profiles) and prevalent lineage, dominating the symbiont communities in 29 of the 30 corals species examined. The stress-tolerant *Oulastrea crispata* was exceptional as its symbionts were dominated by *Durusdinium eurythalpos* (D13-D13b-D12-D13c profile). Interestingly, *Cladocopium* C15 was diversified into two ITS2-type profiles, one being *Porites lobata* specific, while the other was associated with both *P. aranetai* and *P. lutea*. Overall, most corals harbor a single dominant generalist symbiont, while some corals host both generalist and specialist symbionts. This work lays the foundation for future research to understand how the generalist and specialist as well as dominant and rare symbionts contribute to the responses and resilience of their host corals against environmental fluctuations in a marginal coral ecosystem, like that in Hong Kong.

Keywords Symbiodiniaceae · Diversity · Generalist/specialist symbionts · rRNA gene, ITS2 · Marginal coral

Introduction

The success of a coral community is driven by the mutualism between coral polyps and their endosymbionts from the Symbiodiniaceae family of dinoflagellate (Baker 2003). Knowledge on symbiodiniacean symbiont composition and dynamics is thus a key to understanding coral-symbiosis

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interaction, evolution, development, and resilience (Baker et al. 2004; Lin et al. 2015). Symbiodiniaceae is comprised of at least 15 genera, with hundreds of genetically, physiological, and ecologically distinct genotypes (Pochon and Gates 2010; LaJeunesse et al. 2018; Nitschke et al. 2020; Pochon and LaJeunesse 2021).

Over the past decades, our knowledge about the diversity of mixed symbiont communities within corals (Rowan and Powers 1991) has been greatly enhanced with updated notions about generalist symbiont vs. generalist coral, i.e., one symbiont multiple corals versus one coral multiple symbionts, respectively (Thornhill et al. 2014; LaJeunesse et al. 2018). In the current framework, a symbiodiniacean species or type can be recognized as a “generalist” or “specialist” depending on its range of host, geography or habitat (LaJeunesse et al. 2018; Hume et al. 2020). While generalist symbionts are widely distributed across different hosts and habitats (e.g., *Cladocopium goreaui* [C1 type]), specialist symbionts are uniquely associated with certain hosts or habitats (e.g., *Cladocopium* [C17 type] specific to *Montipora* spp.) (LaJeunesse et al. 2003). The rapid growth of research data has also provided evidence that different dominant symbiodiniacean lineages/genotypes or different combinations of multiple lineages/genotypes may confer coral holobionts with different performance and adaptability against environmental stress and diseases (Mieog et al. 2009; Starzak et al. 2014; Burt et al. 2020).

The increasing accessibility of high-throughput sequencing (HTS) (Arif et al. 2014; Quigley et al. 2014; Cunning et al. 2017) and qPCR (Correa et al. 2009; Yamashita et al. 2011; Saad et al. 2020) has allowed detection of low-abundance symbiont types in different corals. These symbiont types might have been missed previously in many coral samples, resulting in their being incorrectly recognized as specialists. On the contrary, the increasing resolving power of analytical methods has also provided refined symbiont type distinction leading to increasing recognition of more specialists. This is largely a result of utilizing highly variable molecular markers such as ribosomal RNA internal transcribed spacer genes (ITS1 and ITS2) (Sampayo et al. 2009; Pochon and Gates 2010), the chloroplast large subunit (cp23S) ribosomal RNA gene (Santos et al. 2002; Pochon et al. 2006), chloroplast psbA minicircle noncoding region (psbA^{ncr}) (LaJeunesse and Thornhill 2011; Reimer et al. 2017), and microsatellites (Coffroth and Santos 2005).

Despite the wide use of ITS in high-throughput molecular analysis for documenting Symbiodiniaceae diversity, the inter- and intragenomic sequence polymorphism poses challenges in assigning sequences to species (LaJeunesse and Thornhill 2011; Arif et al. 2014; Hume et al. 2019). The same issue occurs in other dinoflagellates (Miranda et al. 2012). This inter- and intragenomic complexity hinders the accurate transformation of numerous distinct HTS sequence variants into their true diversity and hence the abundances of the designated taxa (Ebenezer et al. 2012; Gong and Marchetti 2019). Alternative genetic markers such as chloroplast cp23S, psbA^{ncr}, and the mitochondrial cytochrome oxidase c subunit I (COI) and cytochrome oxidase b (COB) are available, but ITS remains a preferred choice of marker because it has conserved regions for primer design and enough sequence variations for taxon resolution. Recently, SymPortal analysis framework approach was developed to tackle the polymorphism problem (Hume et al. 2019) and has been recognized as a powerful analytical framework for characterizing fine-scale Symbiodiniaceae diversity (Hume et al. 2020; Smith et al. et al. 2020). SymPortal recognizes the co-occurrence of intragenomic sequence variants (DIVs) within the samples in question by comparing sample sequences with those hosted in SymPortal Symbiodiniaceae ITS2 database where DIVs are defined. Different DIV combinations are then used to confirm distinct ITS2 type profiles, proxies of authentic Symbiodiniaceae taxa (Hume et al. 2019), which are placed in subgenera, subclades, species, types, subtypes, ITS2 types, or genotypes. To avoid nomenclatural confusion, in this paper we define “ITS2 type” (shortened as “type” when repeated) using the most abundant sequence in a ITS2 profile (shortened as “profile” when repeated) that contains a pool of sequence variants, following the methodology of Hume et al. (2019). For example, *C. goreaui* is referred to as C1 type even though its ITS2-type profile contains C1/C1c/C3-C1b-C72k, because C1 is most dominant within this profile.

Hong Kong is located in the northern South China Sea (SCS) where the subtropical environmental settings (annual seawater temperature 14–31 °C, salinity 14-32 PSU) impede complete reef development (Perry and Larcombe 2003; Ang et al. 2005; Ng and Ang 2016). However, these marginal coral communities can play a vital role as alternative and refuge habitats in future warming climate for the tropical coral species (Thomson et al. 2011; Camp et al. 2018). These subtropical corals have long been subjected to seasonal fluctuations in environmental conditions (Ng and Ang 2016; Xie et al. 2020). Sea surface temperature is about 31 °C in summer (May–October) and drops to 14 °C in the winter season (November–April). Salinity is about 32 PSU in winter and can drop to 15 PSU in the summer season due to heavy rains and local runoff (Ang et al. 2005; Yeung et al. 2014). These profound temperature and salinity swings inevitably impose selection pressure on plasticity in corals, including their endosymbiotic assemblages. Like tropical corals, these marginal corals also undergo bleaching and degradation at stress climate or environmental episodes (Ng and Ang 2016; Xie et al.

2020). Previous studies suggested the presence of low symbiont diversity in Hong Kong corals, with *C. goreaui* (C1) being the most prevalent symbiont among 56 of the coral species examined (Ng and Ang 2016). It was hypothesized that this low symbiont diversity may be an adaptive strategy against the highly fluctuating conditions of the marginal environment. With the advent of more advanced molecular tools, it is worth re-examining the symbiont community composition and structure of Hong Kong corals. Discovery of cryptic symbiont species diversity may provide additional insights into the possible dynamics of symbiont–coral host interactions which is hitherto poorly understood in a marginal coral ecosystem.

In this study, we uncovered the Symbiodiniaceae species diversity in Hong Kong corals using ITS2-Illumina sequencing platform. We examined 30 common scleractinian coral species which were also previously examined using ITS2-denaturing gel gradient electrophoresis (ITS2-DGGE) method in Ng and Ang (2016). Results shed light on Symbiodiniaceae diversity and cryptic symbiont diversity across different environmental settings in Hong Kong coral ecosystem, providing a baseline for future research on Symbiodiniaceae diversity, coral resilience, and responses to climate change.

Materials and methods

Sample collection

Zooxanthellate scleractinian coral samples were collected during 2010–2011 from seven sites in east to northeastern Hong Kong waters where dominant coral communities are found: Kat O, Wu Pai, Chek Chau, Gruff Head, Kui Tsui Chau, Wong Wan Chau, and East Dam (Fig. 1). These collection sites are all far from the main development areas in Hong Kong (Kowloon and northern Hong Kong Island), although some of them were close to smaller towns and cities in western Hong Kong and eastern Hong Kong. The collection was in compliance with coral control regulations of the government of the Hong Kong Special Administration Region. These selected sites were relatively sheltered and away from the influence of freshwater outflow of the Pearl River in the west. In total, 84 scleractinian coral fragments (about 2 cm each) representing 42 scleractinian coral species ($n = 2$ per species) were collected using SCUBA from 1 to 4 m water depth (Tables 1, S1). Each fragment was separately kept in a Ziploc bag. Coral specimens were identified in situ and confirmed later from recorded photographs (Ang et al. 2003). Species names were further updated to conform with taxonomic revisions made in recent years (Ang and Ng 2018). Once out of water, coral specimens were immediately preserved in 90% ethanol and kept in an insulated cooler and later stored at − 20 °C upon arrival at the laboratory. For prolonged preservation, the coral fragments were cleaned and dried off from the ethanol solution and then crushed using a mortar and pestle and finally preserved in DNA lysis buffer at − 20 °C until analyzed.

DNA extraction

The crushed corals preserved in DNA lysis buffer were thawed at room temperature and vortexed for about 30 s for homogenization, and then subsequently 1 mL was pipetted into a 2-mL tube. DNA was extracted following

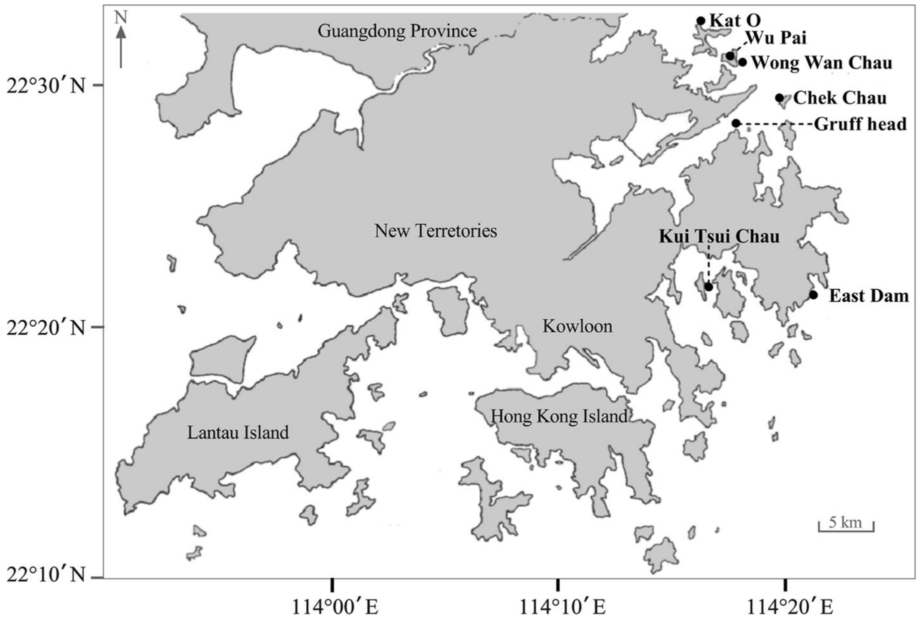

Fig. 1 Location of the seven sampling sites for Hong Kong corals

Table 1 The 60 coral samples used in the present study, with their species name, reference code, and collection location given

Coral species	Coral reference code	Collection site	Coral species	Coral reference code	Collection site
Acanthastrea echinata	Aca_e1	Gruff Head	*Goniopora planulata*	Gon_p1	Kui Tsui Chau
A. echinata	Aca_e2	Gruff Head	*G. planulata*	Gon_p2	Kui Tsui Chau
Acropora digitifera	Acr_d1	Kui Tsui Chau	*Hydnophora exesa*	Hyd_e1	Kat O
A. digitifera	Acr_d2	Kui Tsui Chau	*H. exesa*	Hyd_e2	Wu Pai
A. solitaryensis	Acr_s1	Kui Tsui Chau	*Lithophyllon undulatum*	Lit_u1	Kat O
A. solitaryensis	Acr_s2	East Dam	*L. undulatum*	Lit_u2	Wu Pai
A. valida	Acr_v1	Kat O	*Oulastrea crispata*	Oul_c1	Chek Chau
A. valida	Acr_v2	Kat O	*O. crispata*	Oul_c2	Wu Pai
Cyphastrea serailia	Cyp_s1	Kat O	*Pavona decussata*	Pav_d1	Wu Pai
C. serailia	Cyp_s2	Gruff Head	*P. decussata*	Pav_d2	Wu Pai
Dipsastraea favus	Dip_f1	Kui Tsui Chau	*Astrea curta*	Phy_c1	Wu Pai
D. favus	Dip_f2	Kui Tsui Chau	*A. curta*	Phy_c2	Wu Pai
D. lizardensis	Dip_l1	Gruff Head	*Platygyra acuta*	Pla_a1	Kat O
D. lizardensis	Dip_l2	Gruff Head	*P. acuta*	Pla_a2	Chek Chau
D. rotumana	Dip_r1	Gruff Head	*P. carnosa*	Pla_c1	Chek Chau
D. rotumana	Dip_r2	Gruff Head	*P. carnosa*	Pla_c2	Chek Chau
Favites acuticollis	Fav_a1	Gruff Head	*Porites aranetai*	Por_a1	Kui Tsui Chau
F. acuticollis	Fav_a2	Wu Pai	*P. aranetai*	Por_a2	Kui Tsui Chau
F. chinensis	Fav_ch1	Kui Tsui Chau	*P. lobata*	Por_l1	Kui Tsui Chau
F. chinensis	Fav_ch2	Kui Tsui Chau	*P. lobata*	Por_l2	Kui Tsui Chau
F. complanata	Fav_c1	Chek Chau	*P. lutea*	Por_u1	Kui Tsui Chau
F. complanata	Fav_c2	Kat O	*P. lutea*	Por_u2	Kui Tsui Chau
Galaxea astreata	Gal_a1	Wu Pai	*Psammocora haimiana*	Psa_h1	Kui Tsui Chau
G. astreata	Gal_a2	Wu Pai	*P. haimiana*	Psa_h2	Kui Tsui Chau
G. fascicularis	Gal_f1	Wu Pai	*P. nierstraszi*	Psa_n1	Kui Tsui Chau
G. fascicularis	Gal_f2	Wu Pai	*P. nierstraszi*	Psa_n2	Kui Tsui Chau
Coelastrea aspera	Gon_a1	Gruff Head	*P. profundacella*	Psa_s1	Kui Tsui Chau
C. aspera	Gon_a2	Gruff Head	*P. profundacella*	Psa_s2	Kui Tsui Chau
Goniopora lobata	Gon_l1	Chek Chau	*Turbinaria peltata*	Tur_p1	Wu Pai
G. lobata	Gon_l2	Chek Chau	*T. peltata*	Tur_p2	Wu Pai

Refer to Fig. 1 for the physical location of the collection site within Hong Kong. Refer to Table S1 for other details of these samples

our optimized bead beating cetyltrimethylammonium bromide (CTAB)-Zymo column method (Saad et al. 2020). Briefly, each sample was injected with 10 µL Proteinase K (200 µg/mL) and then incubated at 56 °C for three nights for cell lysis. The remaining intact cells at the end of the incubation period were subjected to bead beating homogenization on FastPrep-24 bead mill (MP Biomedicals, USA) for repeated cycles until all cells were broken as verified microscopically. After removing organic contaminants using CTAB, DNA purification was carried out using the DNA Clean and Concentrator kit (Zymo Research Kit, Orange, CA, USA). DNA was finally eluted in 50 µL of 10 mM Tris–HCl solution (pH 8.0), and the quantity and quality were measured using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Franklin, MA, USA).

ITS2 sequencing, quality control, and data processing

The ITS2 gene of Symbiodiniaceae was amplified using ITS-DINO: 5′-GTGAATTGCAGAACTCCGTG-3′ (Pochon et al. 2001) and ITS2Rev2 reverse primer: 5′-CCTCCGCTTACTTATATGCTT-3′ (Stat et al. 2009) primers barcoded with extra eight bases unique to each sample. The PCRs products were purified, pooled, and sequenced on Illumina HiSeq2500 platform using 2 × 250 bp paired-end protocol at Guangzhou Genedenovo Biotechnology Co., Ltd (China). The resulting raw sequences were submitted to NCBI (SRA) (Accession number PRJNA666313).

The Illumina raw sequence data (Demultiplexed forward and reverse. Fastq.gz file) were submitted to SymPortal analytical framework at symportal.org for quality control and analyses. The portal contains a quality control pipeline for removal of non-Symbiodiniaceae, low quality, and ambiguous reads, taxa delamination algorithm, and defining intraspecific ITS polymorphism (Hume et al. 2019). SymPortal pipeline aligns the query sequences with the predefined Symbiodiniaceae ITS2 sequences database to identify the intraspecific ITS2 sequence variants (DIVs), which were then used to identify the ITS2-type profile that represents a taxon. For example, D1 and D4 are DIVs sequences of a D-type taxon, which if appearing in sufficient numbers across different samples would define the D1-D4 ITS2-type profile (putatively *Durusdinium trenchii*). Moreover, to resolve the relationship between coral samples and between ITS2-type profiles, distance matrices were generated based on weighted UniFrac method using square-root-transformed abundance data and visualized in plots of the principal coordinate analyses (PCoA) as part of SymPortal output files.

Results

Overall symbiodiniaceae profiling among Hong Kong corals

Initially, we collected and processed 84 coral clones. However, based on NanoDrop measurements, only 60 high-quality DNA samples (A260/A280 ratio ranged from 1.8 to 2.0 and A260/A230 ratio ranged from 2.0 to 2.2) were selected for further analyses, representing 30 different coral species ($n = 2$) (Table S1). From Illumina HiSeq sequencing, a total of 3,918,273 (on average 65,304 per sample) high-quality sequences were obtained after quality control and filtering process. Subsequent to this, SymPortal results reveal 4662 Symbiodiniaceae ITS2 sequence variants (DIVs). Although seven symbiodiniacean genera (formerly clades A-G), namely *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Effrenium*, *Fugacium*, and *Gerakladium*, were detected in the ITS2 sequences dataset (Fig. 2), only the first four genera were recognized by the 13 Symbiodiniaceae ITS2-type profiles (Figs. 3, S1, S2, S3). This is because SymPortal pipeline only integrates those DIVs that occur in a sufficient number of samples within both the sequences dataset being examined and the SymPortal ITS2 sequence database to define ITS2-type profiles (the taxonomic unit of SymPortal framework). The genetic distances among these profiles (one distance matrix per Symbiodiniaceae genus) can be seen in plots of the principal coordinate analyses (PCoA) as part of the SymPortal output (Figs. S1, S2, S3). The majority of these profiles (7 out of 13) belong to the *Cladocopium* genus, six of which were assigned to *C. goreaui* (C1 type). Of the remaining profiles, three belong to *Durusdinium*, two to *Breviolum minutum*, and one to *Symbiodinium microadriaticum.*

Generalist/specialist symbiodiniaceae among Hong Kong corals

Consistent with the growing evidence for one coral-multiple Symbiodiniaceae types notion, all Hong Kong corals were associated with multiple ITS2 type lineages (Fig. 2, Table S2). However, only one symbiodiniacean ITS2 type (*C. goreaui* [C1]) was shared by all our corals (Fig. 2, Table S2). Furthermore, about half of Symbiodiniaceae types we detected were hosted by more than one coral species (e.g., *Breviolum* B1, *Cladocopium* C1c, C15, C3, and *Durusdinium* D13). The other half number of symbiodiniacean types appeared to be “specialists,” as they were each exclusively associated with one coral species (Table S2). The majority of these specialist (specific) symbiont ITS2 types were in relatively low abundance ($< 1\%$) (named rare biosphere hereafter), such as *Symbiodinium* A1ey, *Breviolum* B1s, *Cladocopium* C21, C15ch, 1272035_C, *Durusdinium* D1c, and 1272045_D (Table S2). Notably, the corals *Acropora valida*, *Oulastrea crispata*, and *Turbinaria peltata* hosted 38, 36, 35 unique symbiont ITS2 types, respectively, the highest number of specialized types among all corals we examined (Table S2). These specialist symbiodiniacean types were closely related to lineages such as B1, C1, C15, and D13 in general. However, to limit the diversity overestimation, we mainly focus on ITS2-type profile level for diversity analyses.

At ITS2-type profile and genus level, majority of the coral species were occupied by the *Cladocopium* genus, primarily *C. goreaui* (C1/C1c/C3-C1b-C72k profile). This genus dominated the symbiont communities in 38 of the 60 coral samples examined in the present study (Fig. 3). Interestingly, *Porites* corals were separated into two groups; one of them harbored *C. goreaui* C15/C1 profile (*P. aranetai and P. lutea*), and the other was associated with *Cladocopium* C15-C15bn-C15by-C15ai profile (*P. lobata*). While *Cladocopium* was predominant in all our coral samples, *O. crispata* was exceptional as its symbionts were dominated by *Durusdinium*, with *D. eurythalpos* (D13-D13b-D12-D13c) being the most dominant ITS2-type profile (Fig. 3). Symbiont genera other than *Cladocopium* and *Durusdinium* were found in low abundances. *Symbiodinium microadriaticum* (A1 ITS2-type profile) was found in only one coral species (*A. valida*) and its presence

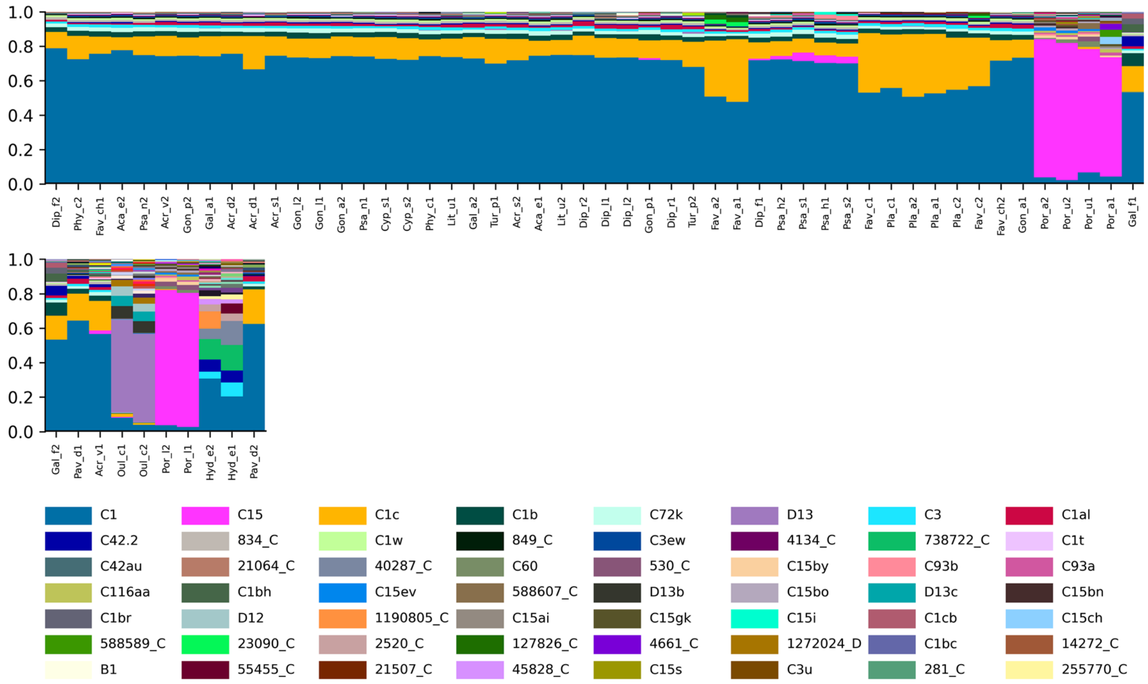

Fig. 2 Normalized relative abundance of recovered Symbiodiniaceae ITS2-type sequences from Hong Kong corals. Only the most abundant ITS2 sequences are displayed in the order of overall abundance. ITS2-type that have been labelled with names, e.g., C1, C15 or D13 refer to sequences that are commonly found in the literature or have been used to characterize ITS2-type profiles that have been run through the SymPortal analytical framework. Less common sequences, and those that have not been used to characterize ITS2-type profiles, are named according to a unique database ID and their genera, e.g., 843_C refers to a sequence with ID 834 derived from the *Cladocopium* genera. Each stacked bar represents a single individual coral sample. The full names of the coral species and the reference codes are given in Table S1

in low abundance raised the question of whether it is specific to this coral species, as the case in the jellyfish *Cassiopea* (Santos et al 2002). In contrast, the other low-abundance taxon, *B. minutum* (B1-B1g profile) displayed a debatable generalist behavior by being hosted by four different corals (*A. valida*, *Dipsastraea favus*, *D. lizardensis*, and *D. rotumana*). Strikingly, our results indicated that *D. trenchii* D1/D4/D4c profile was restricted to only one coral (*Coelastrea aspera*), while *D. eurthalpos* D13-D13b-D12-D13c and D13 ITS2-type profiles were relatively more common among Hong Kong corals, associated with *Lithophyllon undulatum*, *O. crispata*, *Platygyra acuta*, and *T. peltata* (Fig. 3).

Among the 30 corals collected from Hong Kong, *A. valida* appeared to be highly flexible (generalist) in its symbiont associations, hosting four different ITS2-type profiles namely *S. microadriaticum* A1, *B. minutum B1-B1g*, *C. goreaui* C1/C1c/C3-C1b-C72k, and *C. goreaui* C1-C1b-C1c-C42.2-C1bh-C1br-C1cb-C72k (Fig. 3, Table S3). Other common "generalists" corals included but were not limited to *C. aspera* and *O. crispata*, each of which was associated with three different ITS2-type profiles.

Relative abundances of different symbiodiniaceae species and ITS2 types

Overall, the majority of corals in Hong Kong (29 out of 30) were dominated by members of the *Cladocopium* genus, which accounted for over 97% relative abundance of the total ITS2 sequences dataset (Figs. 2 and 3). Ranked second for its contribution toward the total ITS2 sequences dataset was *Durusdinium*, which accounted for ∼ 2.5% of overall ITS2 sequences dataset, with *O. crispata* being the only coral in Hong Kong that was dominated by *Durusdinium* member (*D. eurythalpos* D13-D13b-D12-D13c ITS2-types profile). Finally, both *S. microadriaticum* (A1 ITS2-types profile) and *B. minutum* (B1-B1g and B1 ITS2-types profiles) were present in the lowest abundances and accounted for about 0.05% and 0.11% of the total ITS2 sequences (Fig. 3, Table S2).

Discussion

This work provides the first broad yet in-depth analyses of Symbiodiniaceae diversity and community structure from a marginal coral system using ITS2 high-throughput metabarcoding technology. Out of 30 of the 84 hermatypic

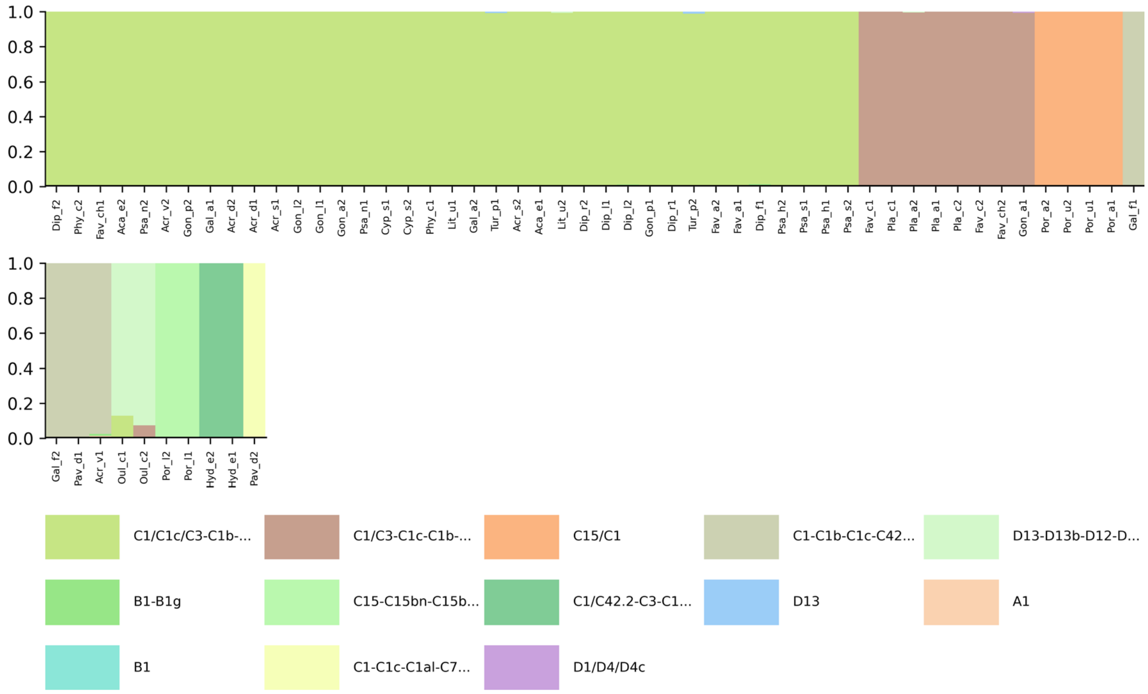

Fig. 3 Normalized relative abundance of predicted Symbiodiniaceae ITS2-type profiles from Hong Kong corals. ITS2-type profiles are listed in the order of overall abundance. The name of each ITS2-type profile is made up of the defining intragenomic variants (DIVs) used to characterize them, in the order of average abundance. DIVs names preceded by a '/' were found as the most abundant sequence in at least one of the samples, and DIVs names preceded by a '–'were not. Each stacked bar represents a single individual coral sample. The full names of the coral species and the reference codes are given in Table S1

coral species recorded in Hong Kong, our results show that Hong Kong corals have markedly higher Symbiodiniaceae ITS2 types (genotypic) richness than previously reported (Ng and Ang 2016), thanks to the power of high throughput sequencing in uncovering hidden symbiont diversity. A higher number of replicates for each coral species would be desirable for surveys like this, but given the small size of Hong Kong coral communities and the governmental regulation, this was not possible. Nonetheless, employing the SymPortal analysis pipeline removed potential overestimation of diversity caused by intragenomic sequence variants (DIVs) (Fig. 2) and provided more robust results of taxon presence in multiple samples. From the SymPortal analysis, 13 distinct Symbiodiniaceae ITS2-type profiles were finally obtained (Figs. 3, S1, S2 and S3), compared to our original 95 OTUs found without using the SymPortal pipeline (Table S4). However, this is likely a conservative estimate because the SymPortal pipeline only integrates those ITS2 sequences (DIVs) that occur in a sufficient number of samples within both the sequences dataset being examined and the SymPortal ITS2 sequence database into the ITS2 type profiles. These 13 profiles represent four Symbiodiniaceae genera: *Symbiodinium* (one profile), *Breviolum* (two profiles), *Cladocopium* (seven profiles), and *Durusdinium* (three profiles).

Our results reveal that symbiodiniacean endosymbionts are more diverse than previously thought in Hong Kong marginal corals, presenting a new perspective for future study on the Symbiodiniacean–coral interaction and resilience to environmental stress. As earlier investigation only applied classical molecular methods to describe Hong Kong symbiodiniacean communities (Ng and Ang 2016), higher Symbiodiniaceae diversity would be expected from HTS analyses, as has been demonstrated by different authors (e.g., Arif et al. 2014; Hume et al. 2020; Smith et al. 2020). Consistent with previous report, our results also indicated lower Symbiodiniaceae diversity indices than those reported from other areas in general (Ng and Ang 2016; Hume et al. 2020; Smith et al. 2020).

While *Cladocopium* and *Durusdinium* genera are well documented in the symbiont communities of Hong Kong corals, the detection of *S. microadriaticum* and *B. minutum* is particularly interesting because these species are well known only from other regions, e.g., Red Sea (Sawall et al. 2014; Hume et al. 2020), Western Atlantic (LaJeunesse et al. 2012), Belize Barrier Reef (Eckert et al. 2020), and Caribbean (Cunning et al. 2017) regions. A recent work using similar framework analyses as in our study has shown that *Symbiodinium* and *Breviolum* were absent from Singapore corals, a nearby turbid marginal coral region (Smith et al. 2020). Nevertheless, these novel findings support the recent observation of *Breviolum* being associated with *A. valida* coral from Hong Kong using qPCR assays (Saad et al. 2020). Despite the importance of the present findings in extending the geographical range of these two symbiont species, it is still unclear whether they are involved in a long-term and stable symbiosis with Hong Kong marginal corals. It is surprising that even *Effrenium*, known as an exclusively free-living symbiodiniacean lineage, was found in some of the Hong Kong coral samples. Whether this could have been cells attached on the surface of the corals remains to be investigated further in the future.

Despite the appreciable taxon richness shown above, the majority of Symbiodiniaceae ITS2 types detected in this study were in relatively low or rare abundance ($< 1\%$, rare ITS2 types). These rare ITS2 types seem to be host specific in general (Table S2). Their low abundance can explain why the majority of them escaped detection in previous studies (Ng and Ang 2016). Failure to detect these rare ITS2 types could also be exacerbated in part by the very high ITS copy number of the predominant species *C. goreaui* (C1) among all the symbiodiniacean taxa examined so far (Saad et al. 2020). When *C. goreaui* (C1) is dominant, the combination of its high ITS copy number and high cell abundance could mask the rare ITS2 types. In this context, it is noteworthy that previous studies have reported much higher symbiont diversity in coral communities when *C. goreaui* is less prevalent, e.g., Hawaii (LaJeunesse et al. 2004), St. John, US Virgin Islands, USA (Cunning et al. 2017), Red Sea, Sea of Oman, and Persian/Arabian Gulf (Ziegler et al. 2017; Hume et al. 2020). While *P. lutea* symbiont diversity in our study was clearly lower than those from reef corals in tropical (Sanya Bay and Leizhou Peninsula, China) and subtropical areas (Daya Bay, China, located east of Hong Kong) (Gong et al. 2018), *A. valida* in Hong Kong seemed to have a higher Symbiodiniaceae diversity compared to their counterparts in subtropical and tropical reefs (Gong et al. 2018). We should note that different pipeline framework of analyses may lead to slightly different results. For example, in order to reduce the potential sequencing and bioinformatics noise and the resulting diversity overestimation, Gong et al. (2018) applied a higher filter cutoff value ($\leq 3\%$ relative abundance) to remove rare ITS2 types (OTUs), which might explain the lower Symbiodiniaceae diversity they detected in their corals.

Previously, *C. goreaui* (C1) has been recognized as a common and generalist symbiont in Hong Kong corals (Ng and Ang 2016). This is further confirmed in our present study. However, in our study, *C. goreaui* is distinguished into six distinct ITS2-types profiles (Figs. 3, S1), with *C. goreaui* C1/C1c/C3-C1b-C72k being the most common, prevalent, and abundant generalist symbiodiniacean lineage dominating about 60% of Hong Kong coral samples. Genetic radiation among generalist symbiodiniacean species has been suggested as one mechanism that could lead to host-specific lineage proliferation and niche diversification. This has been illustrated in *B. minutum* (B1 type) from the Caribbean reef using combined markers such as ribosomal ITS2, chloroplast 23S, and mitochondria COB (Parkinson et al. 2015; Cunning et al. 2017). Among the *C. goreaui* lineages (profiles) detected in the present study, two were host-specialists: C1/C42.2-C3-C1au were specific to *Hydnophora exesa* and C1-C1c-C1al-C72k specific to *Pavona decussata* corals. Thus, with more sampling effort and application of alternative and higher-resolution markers, more host-symbiont specialists could be expected. Other noticeable common symbiont ITS2-type profiles (generalists) observed in the present study include and not limited to *B. minutum* B1-B1g, *C. goreaui* C1/C3-C1c-C1b-C1w-C42.2, *C. goreaui* C15/C1, and *D. eurythalpos* D13-D13b-D12-D13c (Fig. 3, Table S3).

Cladocopium goreaui and the other generalists identified here might be physiologically plastic and suited for the highly seasonal environmental conditions and the relatively low seawater temperature in winter and have thus been selected for Hong Kong coral system (Ng and Ang 2016; Smith et al. 2020). In addition, *Cladocopium* spp. in general provide their host with higher amounts of carbon, greater fitness, and growth rate compared to other symbiodiniacean genera (Cantin et al. 2009; Mieog et al. 2009). This latter trait makes the species generally favorable for corals, as evidenced by its presence in worldwide environmental setting/geographical regions (LaJeunesse et al. 2018), in diverse hosts including corals and other cnidarians, foraminifera and molluscs (Pochon et al. 2014), and in its capacity to adapt to pelagic and benthic habitats (Pochon et al. 2014; Decelle et al. 2018).

In contrast, *Cladocopium* C3 lineage diversification and distribution were relatively low in Hong Kong compared to other geographical regions, including the nearby Hainan Island also in northern SCS (Zhou and Huang 2011), in Singapore reefs (Smith et al. 2020) and the Great Barrier Reef in Indo-Pacific, in Caribbean reefs (LaJeunesse et al. 2003, 2004), Persian/Arabian Gulf (Hume et al. 2013), and the western and northeastern Indian Ocean (LaJeunesse et al. 2010). This could be due to it being less tolerant to environmental seasonality and colder winter water temperature in Hong Kong. However, experimental evidence to support this has yet to emerge.

Moreover, compared to other regions, diversification in both *Cladocopium* and *Durusdinium* genera was limited in Hong Kong corals. For example, in Singapore tropical reefs, while still characterized as a low symbiont diversity coral community (Smith et al. 2020), twofold more symbiont ITS2-type profiles were observed in only five coral species than in all profiles combined detected for Hong Kong corals.

The comparison of these results suggests that Hong Kong corals may have reduced their symbiont diversity as a cost-effective adaptation to cope with the local suboptimal-environmental conditions (Ng and Ang 2016; Chen et al. 2019; Tsang and Ang 2019). Similar adaptation has been suggested for corals under stressful environments such as the acidified seawater in southern Japan reef (Wee et al. 2019) and the high turbidity habitats in Singapore reefs (Smith et al. 2020), where strong selection pressures may be imposed on the corals to select for only certain well-adapted symbiont lineages, instead of multiple lineages (Ng and Ang 2016).

Moreover, detection of *D. eurythalpos* (D13-D13b-D12-D13c and D13 ITS2-type profiles) in many different coral species (e.g., *T. peltata, L. undulatum, and P. acuta*) in Hong Kong is unexpected because this symbiont species is typically known to be *O. crispata* specific (LaJeunesse et al. 2014). Another somewhat unexpected result is that *D. trenchii* (D1 and D4) was only observed in a single coral sample (*C. aspera*). This again might be due to the lower winter temperature in Hong Kong, given that *D. trenchii* is heat tolerant. Further evidence of this may be gained from *Galaxea fascicularis* corals in the nearby tropical region of SCS areas (i.e., Hainan Island, Yongxing Island of Xisha Islands, and Zhubi Reef of Nansha Islands) which were all found to be associated with multiple symbiont types of *Cladocopium* and *Durusdinium* genera (Huang et al. 2011; Zhou et al. 2017), while the same coral species in Hong Kong only hosts a single ITS2-type profile (*Cladocopium* C1-C1b-C1c-C42.2-C1bh-C1br-C1cb-C72k). Hong Kong corals might have been less frequently exposed to extreme heat stress that imposes strong selection pressure for the opportunistic *D. trenchii* species (but see Xie et al. 2020). These findings suggest that the restricted *D. trenchii* occurrence in the marginal coral reef environment like that in Hong Kong might reflect a tradeoff between heat tolerance and growth in colder environments.

In general, our results showed that corals in Hong Kong appear to host lower Symbiodiniaceae diversity compared to those in many other reef systems, including St. John, US Virgin Islands, USA (Cunning et al. 2017), Red Sea, Sea of Oman, Persian/Arabian Gulf (Ziegler et al. 2017), central equatorial Pacific (Claar et al. 2020), and even regions nearby our study area in tropical and subtropical South China Sea (Gong et al. 2018; Chen et al. 2019; Smith et al. 2020). In contrast, however, *A. valida* in Hong Kong appears to be highly flexible (generalist) in its symbiotic associations, hosting four different ITS2-type profiles including *S. microadriaticum* A1, *B. minutum* B1-B1g, *C. goreaui* C1/C1c/C3-C1b-C72k, and *C. goreaui* C1-C1b-C1c-C42.2-C1bh-C1br-C1cb-C72k. Other common "generalists" corals in Hong Kong include *C. aspera*, *Dipsastraea favus*, *D. lizardensis*, *D. rotumana*, *L. undulatum*, *O. crispata*, *P. acuta*, and *T. peltata*. While *C. aspera* and *O. crispata* have been documented to host *C. goreaui* and *D. trenchii* (Ng and Ang 2016; Saad et al. 2020), other coral-symbiont associations reported are novel findings in the present study.

While similarly reported in other coral reefs to be a generalist (Putnam et al. 2012), *A. valida* in Hong Kong supports a higher Symbiodiniaceae diversity compared to its counterparts in other subtropical and tropical reefs (Gong et al. 2018). The relatively high flexibility in *A. valida*, and in other generalist corals found in the present study, however, should not be a surprise as these corals are suggested to exercise horizontal transmission to acquire short-term unusual and opportunistic symbionts in addition to vertical transmission of long-term symbionts (Ng and Ang 2016). Meanwhile, the specialist corals tended to secure long-term fidelity in symbiosis with the inheritable partners, as seen between *Porites* spp. and the *Cladocopium* C15 lineage.

These unique coral–symbiont associations could be physiologically and ecologically different and could have the potential to support their hosts with different levels of fitness and flexibility to cope with environmental stress (Ziegler et al. 2018). Given that the latent specialist symbionts are highly similar to the common and dominant lineages, they might simply be variants that recently emerged as a result of environmental stress selection and/or generally rapid genetic drift of Symbiodiniaceae (LaJeunesse 2005; Thompson 2009; Thornhill et al. 2014). More research is needed to explore the potential adaptive roles of these rare ITS2 types in coral holobiont physiology and performance (Lee et al. 2016). The roles played by the dominant and the rare symbiont types in their hosts in responding to different environmental stresses in both tropical coral reefs and marginal coral communities need to be further investigated. Our study provides a baseline to facilitate such future endeavor.

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Author contributions SL conceived and supervised the project. SL, OS, LL, and XL designed the study. TN and PA collected and processed the environmental coral samples. OS and XL conducted data analysis. OS carried out the experiments and wrote the first draft of the manuscript. All authors edited and approved the manuscript.

Declarations

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

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