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REPORT

Low symbiont diversity as a potential adaptive strategy in a marginal non-reefal environment: a case study of corals in Hong Kong

Tsz Yan Ng[1] · Put Ang Jr.[1]

Abstract Symbiosis with genetically diverse *Symbiodinium* has been shown to affect host coral physiological responses to environmental stresses. Hong Kong, located in a subtropical region, is a marginal environment for coral growth largely due to its wide annual temperature fluctuation with low mean winter sea water temperature (~ 16 °C) and variable salinity conditions. The symbiont diversity in Hong Kong corals is therefore worth investigating to enrich our understanding on symbioses in marginal and fluctuating environments. Examination of 56 scleractinian coral species and five soft coral species using denaturing gel gradient electrophoresis of the internal transcribed spacer region 2 found only five distinct subclades of *Symbiodinium* with C1 the dominant type occurring in all but one scleractinian coral and all soft coral species investigated. C15 and C21 *Symbiodinium* were found in *Porites* spp. and *Montipora peltiformis*, respectively, both of which are vertical transmitters. D8-12 was found in *Oulastrea crispata*, a stress-tolerant species, and D1 in a single sample of *Goniastrea aspera*. No spatial differences in *Symbiodinium* composition were found among different regions of Hong Kong. Seasonal monitoring of tagged *Platygyra acuta* and *Porites* spp. colonies also revealed no changes in their symbiont types despite wide ranges of in situ temperature fluctuation. Hong Kong scleractinian corals hosted a remarkably low symbiont diversity compared with corals in the surrounding regions. The predominance of a single subclade, C1 *Symbiodinium*, suggests that this subclade is best acclimatized to local fluctuating conditions and/or low winter temperature. Forming symbiosis with the best acclimatized symbiont, instead of with a diverse group of symbionts with different physiological performances, either sequentially or simultaneously, may be a strategy used by Hong Kong corals to cope with stressful conditions.

Keywords Corals · *Symbiodinium* · Symbiosis · Diversity · Adaptive strategy

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Introduction

The symbiosis between reef-building scleractinian corals and single-celled dinoflagellates is key to the success of coral reefs. These symbionts, *Symbiodinium* spp., are genetically diverse. They are currently classified into nine subgeneric clades, clades A to I, which can each be further divided into multiple genetically diverge lineages called subclades (Baker 2003; LaJeunesse et al. 2008; Pochon and Gates 2010). This internal transcribed spacer 2-denaturing gradient gel electrophoresis (ITS2-DGGE) nomenclature of the symbiont 'type' is widely used and probably represents distinct species based on genetic, biogeographical, ecological and physiological data (LaJeunesse 2001; Sampayo et al. 2009). Several of the subclades and types of *Symbiodinium* have also been described as binomial species (LaJeunesse et al. 2012, 2014; Jeong et al.

2014), though these designations are yet to be widely accepted.

Spatial differentiation in the global distribution of different clades and/or subclades of *Symbiodinium* spp. has been reported. For example, clades A, B and/or F are more common in high-latitude areas (Baker 2003; Thornhill et al. 2008; Silverstein et al. 2011), while clade C is distributed more widely, but mainly dominates in low-latitude tropical corals (Rodriguez-Lanetty et al. 2001; LaJeunesse et al. 2008). In the Indo-Pacific region, clades C and D have been reported as the dominant types of symbionts (Baker 2003; LaJeunesse 2005).

Differences in physiological performance of various clades and/or subclades are also well documented and may be part of the reason for their spatial distribution patterns (LaJeunesse et al. 2009; Mieog et al. 2009). For example, clade D *Symbiodinium* is suggested to be stress tolerant and has been commonly found in stressful environments with high temperature and high salinity (Fabricius et al. 2004), low light intensity (Ulstrup and van Oppen 2003) and significant terrestrial impact (van Oppen et al. 2001). In contrast, numerous members within clade C *Symbiodinium* are reported to be more thermally sensitive (Rowan 2004), but can contribute to higher host growth rate than within clade D *Symbiodinium* (Mieog et al. 2009; Jones and Berkelmans 2010). Experimental research has also shown that certain types of *Symbiodinium* spp. are more tolerant to one or a few stressors than others. For example, B2 *Symbiodinium* demonstrated quick and full recovery of PSII photochemical efficiency after cold stress treatment, while A3, B1 and C2 *Symbiodinium* failed to recover (Thornhill et al. 2008).

Earlier studies suggested that most scleractinian corals were specific in forming symbiosis, hosting only one type of symbiont (Baker 2003; Goulet 2006). However, with the development of advanced molecular techniques, an increasing number of studies have reported that some corals are able to host multiple clades or subclades of *Symbiodinium* (polymorphic symbiosis) either sequentially or simultaneously (Fay and Weber 2012). The capacity to host multiple symbionts may be advantageous to corals as it provides an opportunity for symbiont displacement, through either switching or shuffling (Jones et al. 2008). By changing symbiont types from one that is stress sensitive to one that is more stress tolerant, coral hosts may be able to survive the changing climate (Buddemeier and Fautin 1993). Yet the flexibility of corals is still likely to be limited to a very specific pool of symbiont types among all those that are available. On the other hand, harbouring a low diversity of symbionts could also be beneficial to coral hosts, as a mixture of different types of symbionts that are not compatible may lead to competition and thus decreased translocation of photosynthates to the hosts. This might eventually lead to lowered resistance of the host to environmental stresses (Frank 1996; Putnam et al. 2012).

Hong Kong (HK), located in the subtropics, is a marginal and stressful environment for coral growth (Ang et al. 2005). The sea water temperature shows high seasonal variability from as low as 13 °C in winter to close to 30 °C in summer. In the western region of HK, summer salinity can be as low as 15 psu because of increased discharge of freshwater from the Pearl River as well as from heavy rainfall. Turbidity is generally high, especially in the southern and western regions. Despite these stresses, HK waters still support a high diversity of scleractinian corals with at least 84 zooxanthellate scleractinian coral species forming non-reefal coral communities (Ang et al. 2003). Marginal non-reefal coral communities are potential refugia for future coral growth and survival in response to climate change (Precht and Aronson 2004; Greenstein and Pandolfi 2008). Unique adaptive genetic and phenotypic traits have also often been reported in organisms in marginal areas (Kawecki 2008; Budd and Pandolfi 2010). Therefore, this study aimed at investigating the symbiotic patterns of HK scleractinian corals and to compare them with those of other regions. Supplementary information on another zooxanthellate cnidarian taxon, octocorals, was also obtained. Given the heterogeneity of environmental conditions, with the presence of significant spatial salinity and turbidity gradients within a short distance and annual temporal variation of temperature (Yeung et al. 2014), it was hypothesized that there should also be spatial and temporal diversity of symbiont types with a prevalence of polymorphic symbioses in corals within HK, comparable to that reported in other areas albeit across a larger spatial scale (Rodriguez-Lanetty et al. 2001; Huang et al. 2011; Silverstein et al. 2011). This study also provides the baseline information that could be used for future assessment of changes in the pattern of symbiosis in a marginal environment under the threat of global climate change.

Materials and methods

Sample collection

Scleractinian coral and octocoral samples were collected haphazardly by SCUBA diving. Host coral species were identified in the field, and photographic records were taken for later confirmation. Scleractinian coral samples were collected at five sites in the north-eastern region, two sites in the eastern and one site each in the southern and western regions of HK, while octocoral samples were collected from one site (Lan Kwo Shui) in the north-eastern region (Fig. 1). Samples collected were preserved in 95% ethanol at 4 °C until DNA extraction.
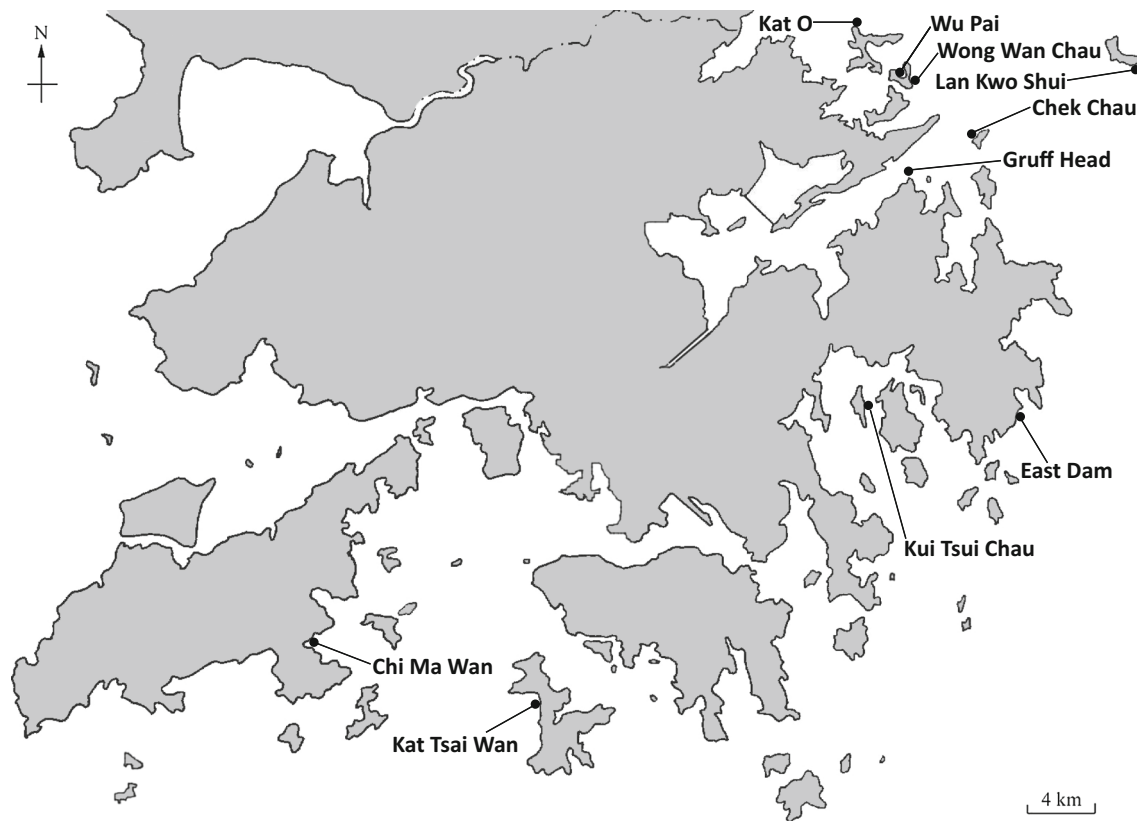
Fig. 1 Map of Hong Kong showing the locations of sampling sites. North-eastern sites: Kat O, Wu Pai, Wong Wan Chau, Chek Chau, Gruff Head, Lan Kwo Shui; eastern sites: East Dam, Kui Tsui Chau; southern site: Kat Tsai Wan; western site: Chi Ma Wan

Seasonal monitoring

To investigate the seasonal stability of symbiosis, 20 *Platygyra acuta* colonies (in Chek Chau) and 20 *Porites* spp. colonies, mainly *Porites lutea*, (in Wu Pai) were tagged in north-eastern HK (Fig. 1). *Platygyra acuta* and *Porites* spp. are the most dominant local coral species; *Porites*, in particular, is widely distributed in different water regions. Samples were collected every 3 months in January, April, July and October 2013. In situ temperature data were recorded using temperature probes located at each sampling site. Other environmental parameters of each site, e.g. salinity and turbidity, were obtained from the HK Environmental Protection Department (HKEPD).

DNA extraction and amplification

The salting-out method (Ferrara et al. 2006) was used to extract total genomic DNA of coral host and *Symbiodinium*. Small fragments of coral tissue were immersed overnight in 200 μL lysis buffer with 5 μL proteinase K (20 mg mL^{-1}) at 60 °C. An equal amount of NaCl (7 M) was then added. The solution was subsequently transferred to a column tube inserted in a collection tube. After centrifugation (8000 rpm,

1 min), the filtrate was discarded and 500 μL of 70% EtOH was then added, followed by another centrifugation (8000 rpm, 5 min). The column tube was later transferred to a new 1.5-mL tube and dried at 37 °C for 15 min. Finally, 50 μL TE buffer was added, followed by centrifugation (13,000 rpm, 3 min) to elute the DNA.

Symbiodinium spp. internal transcribed spacer 2 (ITS2) region was obtained by PCR amplification using a host-excluding primer pair ITS2clamp and ITSintfor2 (LaJeunesse and Trench 2000) with a touchdown thermal cycle: initial denaturation period at 94 °C (3 min); 20 cycles at 94 °C (30 s), annealing from 62 °C (20 s) to final annealing temperature of 50 °C by decrements of 0.5 °C per cycle and 72 °C (45 s); 20 cycles at 94 °C (30 s), 52 °C (45 s), 72 °C (45 s); one cycle of extension at 72 °C (10 min). PCR was run on a C1000 thermal cycler (Bio-Rad, CA, USA). Dilution was performed on some DNA samples to obtain PCR products with high yield.

Denaturing gel gradient electrophoresis of ITS2 region and sequencing

PCR products with satisfactory yield were loaded on acrylamide denaturing gradient gel (45–80%) at 115 V for

15 h using a CBS Scientific System (Del Mar, CA, USA). Marker DNAs with known subclade identity were loaded together with DNA samples for comparison. Gel was stained with SYBE Gold nucleic acid stain (Molecular Probes, Eugene, OR, USA) for 20 min and photographed for further analysis. Prominent bands characteristic of each ITS2-DGGE fingerprint were excised, eluted overnight in ddH$_2$O, re-amplified using primer pair ITS2 (without GC-rich clamp) and ITSintfor2 (LaJeunesse 2002) and then directly sequenced. PCR artifacts or the formation of heteroduplexes were discriminated from occurrence of co-dominant symbionts by intensive re-sequencing of co-occurring bands. DNA sequences were then inspected and assembled using Geneious Pro 6.0.6 and identified using BLAST in Gen-Bank. Sequences have been deposited in GenBank (accession numbers KU841773–KU842043; Table 1).

Data analyses, comparative study and statistical analyses

To observe any spatial difference within the symbiont community of HK, symbiont diversity in corals from the four regions of HK waters (north-eastern, eastern, southern and western; Fig. 1), which have distinct water qualities (Yeung et al. 2014), was compared in terms of the proportion of species hosting C1 symbionts versus those hosting non-C1 symbionts using Pearson's Chi-square test (SPSS 22) (see Electronic Supplementary Material, ESM, Table S1 for details of samples).

Symbiont diversity of corals in HK was also compared with other sites worldwide. Studies that investigated symbiont diversity over a wider range of host coral species, rather than on one or a few species, were examined from published literature. For direct comparison, only studies using ITS markers and mainly DGGE analysis, which thus detect symbiont types with similar resolution, were chosen (see details in ESM Table S2). Other studies applying alternative symbiont identification techniques such as restriction length fragment polymorphism (RFLP) analysis, cloning and pyrosequencing were not included. The rationale for this is that thus far, studies employing ITS-DGGE analysis provide the most extensive set of data from the greatest number of species in a large number of study sites around the world.

The number of coral host species examined and symbiont types detected from them were extracted from the chosen studies to calculate the average number of symbionts detected per host species and genus. This gives a normalized estimate of symbiont diversity, addressing differences in sampling intensity among studies. Regression analysis (SPSS 22) was also used to test for any relationships between the number of host species examined and the number of symbiont types detected. Both linear and nonlinear relationships were evaluated to find the best-fit regression line, which was set to pass through the origin (0,0) to fulfil the assumption that no symbiont types should be detected when no host was examined.

As different coral communities can have very different coral species composition, a further comparison was made using only sites that shared at least five common coral species with HK. Only those shared coral species and their respective symbionts were included in a direct comparison. Relative *Symbiodinium* diversity was calculated for each site by dividing the number of symbionts detected among those shared coral species. The relative *Symbiodinium* diversity of each site was then compared individually with that of HK, with HK diversity set as 1. Further examination of the number of coral species that showed polymorphic symbiosis was also carried out among these sites. Relative occurrence of polymorphic symbiosis in each site was calculated as the difference in percentage of shared coral species that hosted multiple symbionts compared with corals in HK.

Results

Symbiodinium diversity of HK scleractinian corals

Symbiodinium diversity was determined in 306 samples collected from 56 host scleractinian coral species representing 23 genera (Table 1). Detailed information on the specimens collected is listed in ESM Table S1. Five distinct *Symbiodinium* ITS2 types—C1 (=*Symbiodinium goreaui*), C15, C21, D1 (=*S. glynni*) and D8-12 (=*S. eurythalpos*)—were characterized by PCR-DGGE ITS2 fingerprinting and subsequent sequencing (Fig. 2). C1 *Symbiodinium* was found in all but one sampled coral species. C15 was only harboured by three *Porites* spp.: *Porites aranetai*, *P. lobata* and *P. lutea*. C21 was hosted by *Montipora peltiformis*. D1 was found only in one of the samples of *Goniastrea aspera*. D8-12 was found only in *Oulastrea crispata*. There was no significant difference in *Symbiodinium* diversity among scleractinian corals from the four sampling regions within HK (Pearson's $\chi^2 = 1.988$, $df = 3$, $p = 0.653$).

Symbiodinium diversity in HK octocorals

All zooxanthellate octocoral species recorded in HK are soft corals (eight species) (Ang et al. 2011). *Symbiodinium* diversity was determined in three samples of each of five host soft coral species: *Cladiella* sp., *Lobophytum* sp., *Sarcophyton tumulosum*, *Sansibia* sp. and *Sinularia brassica*. *Symbiodinium* ITS2 type C1 was the only symbiont type detected (GenBank accession numbers KU842024–KU842043).

Table 1 List of host coral species examined in the present study with their reproductive and symbiont information

Host family/species	Reproductive/ symbiont transmission mode[a]	Region of HK[b]	ITS2 subclade[c]	Hong Kong (overall)	GenBank accession no.	Pacific Ocean[d]	Indian Ocean[d]
Acroporidae							
Acropora digitifera	S, H	E	C1(2)	C1	KU841773-76	C3[2,3,6], C3k[5], C3i[5]	C3u[12], D1-4[12]
A. glauca	S	E	C1(1)	C1	KU841777	C3[7]	C3z[13]
A. pruinosa		E	C1(1)	C1	KU841778		C3z[13]
A. solitaryensis	S	E	C1(5)	C1	KU841779-82		
A. valida (=A. tumida)	S, H	NE	C1(6)	C1	KU841783-87	C3[2,3,5], C3i[3]	C3u[12], C3z[12,13], C101[12], C115a[13], D1-4[12]
Montipora peltiformis	S, V	E	C21(4)	C21	KU841788-95		C110[13]
Agariciidae							
Pavona decussata	H	NE	C1(7)	C1	KU841796-800	C1[2]	D1[12]
Dendrophylliidae							
Turbinaria peltata		NE	C1(8)	C1	KU841801-06	C22a[6]	
		S	C1(1)				
Euphylliidae							
Galaxea astreata	S	NE	C1(4)	C1	KU841807-09	C1[1,5,6]	C1b[12], C3u[13], C3u/D1-4[12]
Galaxea fascicularis	S, H	NE	C1(4)	C1	KU841810-13	C1[1,5,6], C21a[3], D1a[2,5], C1/D1a[2]	C1[11], C3u[12,13], C40[11], D1a[11]
Fungiidae							
Lithophyllon undulatum		NE	C1(5)	C1	KU841814-18	C3h[5]	
Lobophylliidae							
Acanthastrea echinata	S, H	NE	C1(8)	C1	KU841819-24	C3[6], D1a[5]	C3u[13]
		E	C1(1)				
A. hemprichii		E	C1(1)	C1	KU841825-27		C3u[13]
		S	C1(1)				
A. lordhowensis		NE	C1(4)	C1	KU841828-30		
A. subechinata	S. H	NE	C1(1)	C1	KU841831		
Echinophyllia aspera		NE	C1(5)	C1	KU841832-36	C3h[5], C3ha[5], C21[6]	C3[13], C3u[12,13]
		E	C1(1)				
Merulinidae							
Cyphastrea chalcidicum	S, H	NE	C1(7)	C1	KU841837-40		C40[11]
C. japonica		NE	C1(1)	C1	KU841841	C1[3]	

Table 1 continued

Host family/species	Reproductive/symbiont transmission mode[a]	Region of HK[b]	ITS2 subclade[c]	Hong Kong (overall)	GenBank accession no.	Pacific Ocean[d]	Indian Ocean[d]
C. microphthalma		NE; E	C1(4); C1(1)	C1	KU841842-44	C1[5]	
C. serailia	S, H	NE; S; W	C1(9); C1(3); C1(1)	C1	KU841845-52	C1[1,2,5,6], C1i[6], C3[5], C102[7], C104[7]	C3u[12], C3u/D1-4[12]
Dipsastraea favus (=Favia favus)	S, H	E	C1(2)	C1	KU841853-54	C21[6]	C3u[12]
D. lizardensis (=F. lizardensis)	S, H	NE	C1(6)	C1	KU841855-58		C3u[12]
D. rotumana (=F. rotumana)	S	NE; E; S; W	C1(4); C1(1); C1(2); C1(3)	C1	KU841859-66		
D. speciosa (=F. speciosa)	S, H	E	C1(1)	C1	KU841867	C1[5], C3[2], C15[1]	C3u[12]
D. veroni (=F. veroni)	S, H	E	C1(1)	C1	KU841868-69	C3[2]	
Favites abdita	S, H	NE; S; W	C1(7); C1(1); C1(2)	C1	KU841870-76	C1[7], C3[5,6], C3h[5], C1/C15[1]	C1[11], C3, C3u[12], C3z[12], C101[12], C129[11], D1a[11], D1-4[12], D1-4-10[12], C3u/C101[12], C3u/C3z[12], C3u/D1-4[12]
F. acuticollis	S	NE	C1(3)	C1	KU841877-79		
F. chinensis	S	NE; E; S; W	C1(1); C1(3); C1(1); C1(3)	C1	KU841880-85		C3u[13]
F. complanata	S	NE	C1(2)	C1	KU841886-87	C3[6]	C3u[12], D1-4[12]
F. pentagona	S, H	NE	C1(6)	C1	KU841888-91	C1[1], C21a[2]	C1c[11], C1gg-hh[11], C3u[13], D1a[11], D1-4[12], C119/D1-4[12]
Goniastrea aspera	S/B, H/V	NE; S	C1(7), C1/D1(1); C1(3)	C1, C1/D1	KU841892-99	C3[2,6]	
G. favulus	S, H	E	C1(1)	C1	KU841900	C1c[7], C3w[7], C108[7], D1a[6]	C3z[13], D1-4-10/C3u[12]
Hydnophora exesa	S, H	NE	C1(5)	C1	KU841901-04	C3[5], C3h[5], C21a[2,3], C27[3]	C3[13], C3u[12,13], C3u/C15[12]
Phymastrea curta	S, H	NE	C1(6)	C1	KU841905-08	C1[7,9], C3[6], C3/D1a[5]	
Platygyra acuta	S, H	NE; W	C1(7); C1(3)	C1	KU841909-14		

Table 1 continued

Host family/species	Reproductive/symbiont transmission mode[a]	Region of HK[b]	ITS2 subclade[c]	Hong Kong (overall)	GenBank accession no.	Pacific Ocean[d]	Indian Ocean[d]
P. carnosus		NE	C1(4)	C1	KU841915-19		
		E	C1(3)				
		W	C1(2)				
P. ryukyuensis		E	C1(4)			C3h[5]	
P. verweyi	S, H	E	C1(1)	C1	KU841920-22		C3u[12]
P. yaeyamaensis		E	C1(1)	C1	KU841923-24		
Pocilloporidae					KU841925-28		
Stylocoeniella guentheri		NE	C1(5)	C1	KU841929-32		
Poritidae							
Goniopora columna	S	NE	C1(8)	C1	KU841933-36	C15[1]	C1b-s[13], D1-4[12]
G. djiboutiensis	S	NE	C1(6)	C1	KU841937-39	C1[5]	D1-4[12]
G. lobata	S	NE	C1(6)	C1	KU841940-42		
G. planulata	H	E	C1(4)	C1	KU841943-44	C3[2]	
G. stutchburyi	S	NE	C1(7)	C1	KU841945-49	C15[1]	
Porites aranetai		NE	C15(5)	C1, C15	KU841950-59		
		E	C1(1), C15(3)				
P. lobata	S, V	E	C15/C1(1)	C15/C1	KU841960-62	C15[4,10]	C3[14], C15[12,13], C15i[13]
P. lutea	S, V	NE	C15(5)	C1, C15, C15/C1	KU841963-78	C15[2,3]	C3[14], C15[12,13], C15i[13]
		E	C1(1), C15(2), C15/C1(3)				
		S	C15(5)				
Psammocoridae							
Psammocora haimeana		NE	C1(2)	C1	KU841979-84		C1[13]
		E	C1(6)				
P. nierstraszi		E	C1(2)	C1	KU841985-86		
P. superficialis	S	NE	C1(2)	C1	KU841987-88	C1f[8]	
		E	C1(2)				
		W	C1(1)				
Siderastreidae							
Coscinaraea sp.		S	C1(1)	C1	KU841989		

Table 1 continued

Host family/species	Reproductive/symbiont transmission mode[a]	Region of HK[b]	ITS2 subclade[c]	GenBank accession no.	Hong Kong (overall)	Pacific Ocean[d]	Indian Ocean[d]
Incertae sedis							
Leptastrea pruinosa		NE	C1(4)	KU841990-92	C1	C1[5], C3h[5]	D1-4[12], C3u/D1-4[12]
L. purpurea	S/B, H	NE	C1(7)	KU841993-2000	C1	C1[5], C1b[5], C1f[4], C3[2]	C1b-s[13]
		W	C1(4)				
Oulastrea crispata	S/B, H/V	NE	C1(3), C1/D8-12(8)	KU842001-18	C1, C1/D8-12	C3[16], D8[22], D8-12[22], D12-13[22], D15[16,22]	
Plesiastrea versipora	S	NE	C1(8)	KU842019-24	C1	C1[1]	B18[11], C1b-s[13], C1b-y[13]
		W	C1(1)				

Symbiodinium ITS2 type detected in each species is compared to that detected in the same species reported from other sites in the Pacific and Indian Oceans. Details of samples collected (collection site, depth, season) can be found in ESM Table S1

[a] Reproductive means and/or symbiont transmission methods recorded: S: spawning; B: brooding; H: horizontal transmission; V: vertical transmission (Baird et al. 2009; Fabina et al. 2012; Huang et al. 2013)

[b] Regions in Hong Kong waters where the coral samples were collected from (see Fig. 1)

[c] Number after subclade indicates number of host coral colonies examined

[d] Superscript number indicates sites for comparison: [1]Xuwen of Guangdong Province (Liu et al. 2012), [2]Sanya, Hainan Island (Zhou and Huang 2011), [3]Okinawa (LaJeunesse et al. 2004b), [4]Hawaii (LaJeunesse et al. 2004a), [5]Central Great Barrier Reef (LaJeunesse et al. 2004b), [6]Southern Great Barrier Reef (LaJeunesse et al. 2003), [7]Eastern Australia (Wicks et al. 2010), [8]Gulf of California (LaJeunesse et al. 2008), [9]New Zealand (Wicks et al. 2010), [10]Johnston Atoll (Stat et al. 2009), [11]Western Australia (Silverstein et al. 2011), [12]Thailand (LaJeunesse et al. 2010), [13]Tanzania (LaJeunesse et al. 2010), [14]Abu Dhabi, Persian Gulf (Hume et al. 2013), [22]various locations from tropical Japan to temperate Japan (Lien et al. 2013b), [16]Jeju Island, Korea (De Palmas et al. 2015)

Fig. 2 ITS2-DGGE fingerprint of *Symbiodinium* showing representative bands of subclades C1, C15, C21, D1 and D8-12 (*open triangles*). Bands indicated in *arrows* are due to PCR artifacts or heteroduplex formation verified by subsequent sequencing
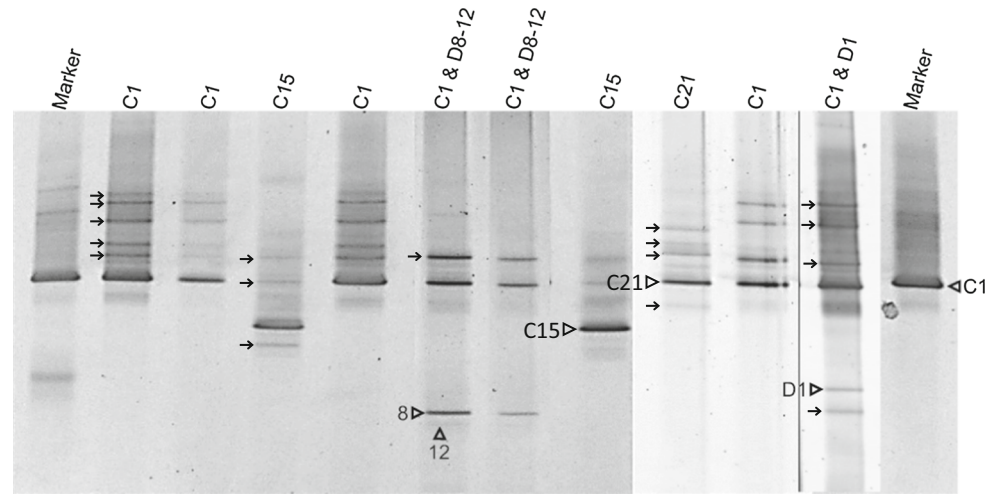
Flexibility of HK scleractinian corals-*Symbiodinium* symbioses

The vast majority of scleractinian coral species examined had only one clade (96.4%, $n = 54$ of 56) and subclade (91.1%, $n = 51$ of 56; either C1 ($n = 50$) or C21 ($n = 1$)) (Table 1). Only five coral species—*P. aranetai*, *P. lobata*, *P. lutea*, *G. aspera* and *O. crispata*—hosted multiple subclades. The three *Porites* spp. hosted mainly C15, while C1 was occasionally found in trace amounts simultaneously with C15 or as the only symbiont type. All *G. aspera* colonies hosted C1, but D1 was also found in a single colony together with C1. Eight of 11 *O. crispata* samples hosted both C1 and D8-12 simultaneously, and the remaining three samples hosted only C1.

Seasonal monitoring of *Symbiodinium* types of *Platygyra acuta* and *Porites* spp.

Tagged colonies of *P. acuta* hosted C1 *Symbiodinium*, while *Porites* spp. hosted C15 *Symbiodinium* throughout the sampling period without temporal change in symbiont types, despite a large temperature fluctuation during this period, from 16.7 to 30.1 °C in Chek Chau and 16.4 to 30.4 °C in Wu Pai. Salinity in the monitoring stations nearest to Chek Chau and Wu Pai varied from 30.1 to 32.8 psu and 29.5 to 32.7 psu, respectively, while turbidity ranged from 0.3 to 17.4 NTU and 0.8 to 6.8 NTU, respectively (EPD-HKSAR 2013).

Comparative studies of dominant *Symbiodinium* spp. in scleractinian corals from other sites

The predominance of clade C *Symbiodinium* in HK scleractinian corals is consistent with the pattern reported in the Indo-Pacific region, in which subclades C1 and C3 are most commonly found (Table 2). C1, dominating 98.2% of coral species examined in HK (both exclusively or with other subclades), also dominates the coral communities in Xuwen of Guangdong Province in northern South China Sea (76.0%) (Liu et al. 2012), New Zealand (66.7%) (Wicks et al. 2010) and Johnston Atoll of the eastern Pacific Ocean (66.7%) (Stat et al. 2009). It shares dominance with C3 in Okinawa and C3h in the central Great Barrier Reef (LaJeunesse et al. 2004b). Apart from Okinawa, C3 is also dominant in other Pacific Ocean regions including Sanya of Hainan Island in the South China Sea (50.0%) (Zhou and Huang 2011), southern Great Barrier Reef (57.5%) (LaJeunesse et al. 2003) and eastern Australia (41.7%) (Wicks et al. 2010), but is absent in HK corals. In contrast, *Symbiodinium* dominance is more complex in the Indian Ocean, with Al, C1, C3, C3u, C40, D1 and D1a dominating or being found in more than half of the coral species in various regions (Table 2). In the Atlantic Ocean, clades A and B are more commonly detected and B1 is reported as the dominant subclade, while C1 and C3 also contribute to a high proportion of symbiosis in the Bahamas, Mexico (LaJeunesse 2002), Barbados and Belize (Finney et al. 2010) in the Caribbean Sea.

Clade D *Symbiodinium* was also found in HK scleractinian corals but only in two species, *G. aspera* and *O. crispata*. *Oulastrea crispata* has been reported to form symbiosis with clade D *Symbiodinium* in various regions of the Pacific Ocean (Lien et al. 2013b). Yet clade C could also be detected in all the *O. crispata* samples collected in HK and three samples examined even hosted only clade C but no clade D symbionts. *Goniastrea aspera* has also been reported to host clade D in Nansha, South China Sea (Huang et al. 2006), Thailand (LaJeunesse et al. 2010) and

Table 2 Dominant types of *Symbiodinium* spp. found in Hong Kong (this study) and other sites around the world

Site[a]	Most dominant	%[b]	Second most dominant	%[b]	Third most dominant	%[b]
Pacific Ocean						
Hong Kong (56)	C1	98.2	C15	5.4	C21, D1, D8-12	1.8
Xuwen[1] (25)	C1	76.0	C15	36.0		
Johnston Atoll[10] (12)	C1	66.7	C3	50.0	C1ca, C21, C3.2, C45	33.3
New Zealand[9] (3)	C1	66.7	C1z, C22a, C3w	33.3		
Jeju Island, Korea[16] (5)	C1	28.6	C17, C3, D15, F-Ajap	14.3		
Okinawa[3] (66)	C1, C3	30.3	C21a	16.7	C27	9.1
Sanya[2] (44)	C3	50.0	C1	15.9	C21a	11.4
Eastern Australia[7] (12)	C3	41.7	C1	25.0	C100, C102, C1c, C3w	16.7
Southern Great Barrier Reef[6] (73)	C3	57.5	C1	12.3	C21	11.0
Central Great Barrier Reef[5] (124)	C1, C3h	30.6	C3	29.0	C3k	8.9
Hawaii[4] (23)	C1f	21.7	C15	17.4	C1	13.0
Gulf of California[8] (11)	C1b-c, C1f, D1	36.4	C1c	18.2	C1, C66, C66a, C66b, C75	9.1
Indian Ocean						
Chagos Archipelago[17] (12)	C1	100.0	C1c	42.9	A1, C3i, C3z	28.6
Abu Dhabi[14] (7)	C3	100.0	A1	57.1	C15, C21, C36	14.3
Thailand (Andaman Sea)[12] (141)	C3u	60.3	D1-4	31.9	C101	19.1
Tanzania (Zanzibar)[13] (110)	C3u	50.9	C3z	12.7	D1-4	9.1
Western Australia[11] (19)	C40, D1a	31.6	C1	15.8	B18, C7d	10.5
Atlantic Ocean						
Bahamas, Caribbean[21] (16)	B1	25.0	A3	18.8	C3, C12, D1a	12.5
Mexico, Caribbean[18] (34)	B1	35.3	C1	17.7	C3a	14.7
Barbados, Caribbean[19] (31)	B1	48.4	C3	38.7	D1a	22.6
Belize, Caribbean[20] (36)	B1	30.6	C3	16.7	C1, D1a	11.1

References for sites, indicated as number in superscript, are given in Table 1 and Fig. 3

[a] Number in brackets indicates the number of host coral species investigated

[b] Percentage occurrence as calculated by the percentage of coral host species that hosted the listed type of *Symbiodinium*

Western Australia (Silverstein et al. 2011). However, only one of eight colonies examined in HK hosted clade D together with clade C, suggesting that clade D is not the predominant type of symbiont harboured by this coral species locally.

The *Symbiodinium* diversity of corals in HK, as represented by the number of symbionts detected per host coral species/genus, is one of the lowest in the world (Fig. 3). A significant logarithmic relationship ($R^2 = 0.883$, $F_{(1,19)} = 143.561$, $p < 0.001$) was found between the number of symbiont types detected and the number of hosts examined (Fig. 4). Based on this model ($y = 4.881 \ln x$), with 56 host species examined, HK is predicted to have 19.65 types of symbionts detected. However, only five symbiont types were actually detected, suggesting that HK corals have a relatively low symbiont diversity compared with the global pattern.

Moreover, *Symbiodinium* diversity at all sites was greater than 1 relative to HK (Table 3), indicating that there are more diverse symbiont communities at all other sites relative to HK. All sites with shared coral species with HK, except Sanya and the southern Great Barrier Reef, had positive values of relative occurrence of polymorphic symbiosis (Table 3), indicating that these sites have a higher proportion of coral species hosting multiple symbionts than HK.

Discussion

Many of the 84 species of zooxanthellate scleractinian corals recorded in HK are locally rare or uncommon (Ang et al. 2003). Hence, only 56 of these species (67%) were collected, and these represent the most commonly found scleractinian coral species in HK waters. Of the 67 octocorals recorded in HK, only soft corals (eight out of 29 species) have been recorded to host *Symbiodinium* (Ang et al. 2011). The five soft coral species in five genera collected represent the most commonly found zooxanthellate soft coral species in HK waters.
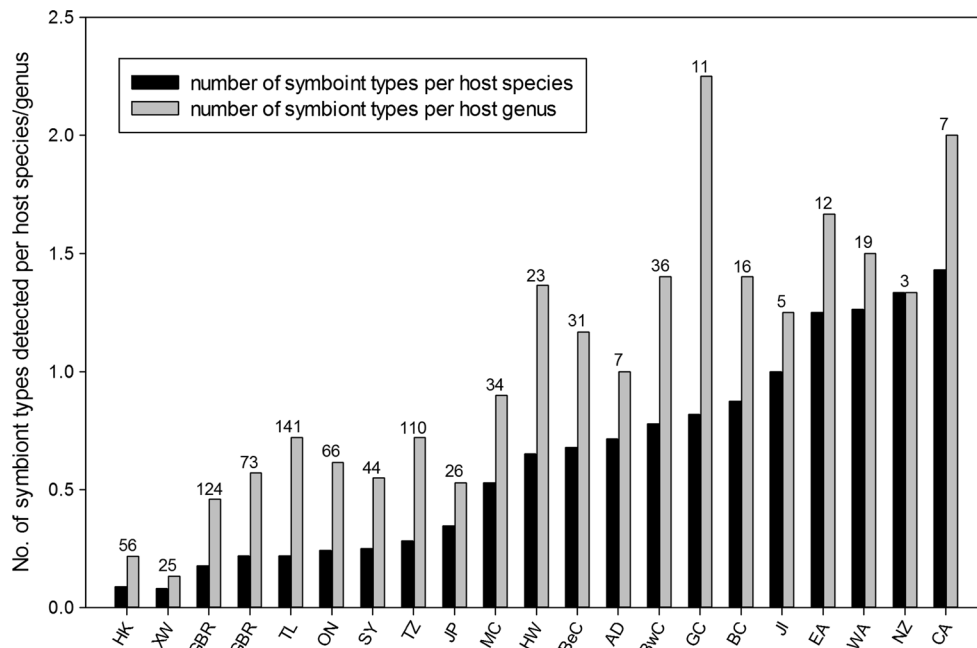
Fig. 3 Symbiont diversity of scleractinian corals in HK (this study) and in other regions as represented by number of *Symbiodinium* types detected per host coral species/genus. *Numbers* on *top* of *each bar* indicate the number of coral species investigated at each site. Note that different coral species may have been examined in different sites. HK: Hong Kong; XW: Xuwen of Guangdong Province[1]; cGBR: central Great Barrier Reef[5]; sGBR: southern Great Barrier Reef[6]; TL: Thailand[12]; ON: Okinawa[3]; SY: Sanya of Hainan Island[2]; TZ: Tanzania[13]; JP: temperate Japan[15] (Lien et al. 2013a); MC: Mexico,

Caribbean[18] (LaJeunesse 2002); HW: Hawaii[4]; BeC: Barbados, eastern Caribbean[19] (Finney et al. 2010); AD: Abu Dhabi, Persian Gulf[14]; BwC: Belize, western Caribbean[20] (Finney et al. 2010); GC: Gulf of California[8]; BC: Bahamas, Caribbean[21] (LaJeunesse 2002); JI: Jeju Island[16]; EA: eastern Australia[7]; WA: western Australia[11]; NZ: New Zealand[9:]; CA: Chagos Archipelago[17] (Yang et al. 2012). References for sites indicated only by numbers in superscript are given in Table 1

Spatial homogeneity and temporal stability of scleractinian coral symbioses in HK

Different regions of HK have been recognized as distinct water quality zones based on differences in their water
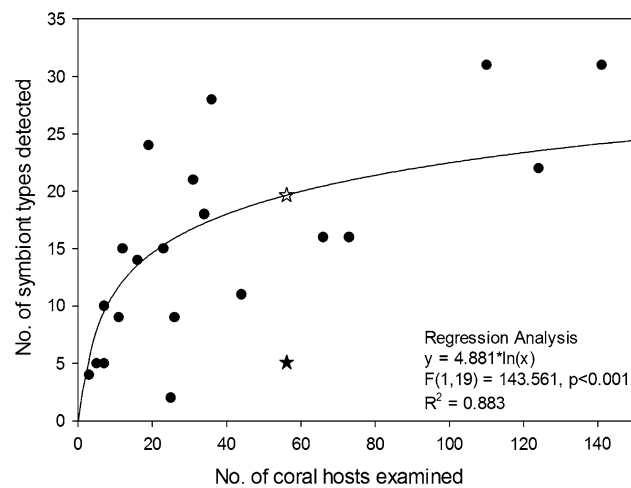

Fig. 4 Logarithmic relationship between the number of symbiont types detected and the number of coral host species examined in different sites globally. The low diversity of symbiont types actually detected in HK corals (*solid star*) is well below that predicted (*open star*) from this global pattern

qualities (Yeung et al. 2014). Southern and western regions are characterized by seasonal low salinity and high turbidity due to high levels of sedimentation, in contrast to northeastern and eastern regions. Despite these differences, distinct spatial *Symbiodinium* subclade variability was not detected among corals collected from these regions, including those from the same coral species (Table 1).

The two continuously monitored coral taxa, *Platygyra acuta* (in Chek Chau) and *Porites* spp. (in Wu Pai), are locally dominant and exhibit different symbiont transmission modes, with *Platygyra acuta* a horizontal transmitter and *Porites* spp. a vertical transmitter. Moreover, *Platygyra acuta* colonies seldom bleach while *Porites* spp. bleach during winter and normally recover in late spring and summer in HK (Choi 2002; Choi 2003). Change in symbiont types may occur after bleaching but not always (Chen et al. 2005; Hsu et al. 2012), and thus monitoring of both bleaching and non-bleaching colonies is equally important. Yet throughout the sampling period, despite a ~14 °C change in in situ sea water temperature, no change in symbiont types was observed in either coral taxon (one bleached and one did not bleach). Furthermore, for other local coral species that were repeatedly sampled in different seasons (ESM Table S1), e.g. *Pavona decussata*, *Plesiastrea versipora* and *Platygyra*

Table 3 *Symbiodinium* diversity and occurrence of polymorphic symbiosis of scleractinian corals at different sites relative to those in Hong Kong

	Hong Kong	Xuwen[1]	Sanya[2]	Okinawa[3]	Temperate Japan[15]	Central Great Barrier Reef[5]	Southern Great Barrier Reef[6]	Thailand[12]	Tanzania[13]	Western Australia[11]	Eastern Australia[7]
No. of coral spp. shared with Hong Kong	–	9	13	6	16	17	13	21	21	5	5
No. of *Symbiodinium* types among shared coral spp. in each respective site	–	2	5	6	6	8	6	9	11	7	7
No. of *Symbiodinium* types among shared coral spp. in Hong Kong	–	1	3	2	2	1	2	3	4	2	1
Relative *Symbiodinium* diversity (compared to Hong Kong)[a]	1	2.00	1.67	3.00	3.00	8.00	3.00	3.00	2.75	3.50	7.00
No. of shared coral spp. with multiple symbionts in each respective site	–	1	1	2	6	9	1	9	7	3	2
No. of shared coral spp. with multiple symbionts in Hong Kong	–	0	2	0	0	0	1	3	3	1	0
Relative occurrence of polymorphic symbiosis (%)[b]	–	11.11	−7.69	33.33	37.50	52.94	0.00	28.57	19.05	40.00	40.00

References for sites, indicated as number in superscript, are given in Table 1 and Fig. 3

[a] Relative *Symbiodinium* diversity is expressed as ratio of number of *Symbiodinium* types in coral species shared with Hong Kong at each site relative to the number in Hong Kong, with the *Symbiodinium* diversity in Hong Kong set as 1. Higher values indicate greater symbiont diversity elsewhere compared to Hong Kong

[b] Relative occurrence of polymorphic symbiosis is expressed as difference in percentage of shared coral species that host multiple symbionts. Higher values indicate a greater number of shared coral species that host multiple symbionts elsewhere than in Hong Kong

carnosus, temporal stability was also observed. It is thus likely that HK corals have very stable relationships with their symbionts both spatially and temporally. Nevertheless, more extensive investigation on more local coral taxa, especially those hosting different symbiont types, such as *O. crispata* (C1 and D8-12), *M. peltiformis* (C21) and *G. aspera* (C1 and D1), is still needed, as there may be species-specific differences in temporal flexibility of their relationships with the symbionts (Putnam et al. 2012). Furthermore, a change in dominant symbiont types could occur over a longer period of time (Edmunds et al. 2014). Continuous monitoring over a longer period should be put in place to capture any longer-term variability, especially with respect to extreme events that do not occur regularly.

Flexibility of HK scleractinian corals-*Symbiodinium* symbioses

Flexibility/specificity of coral symbiosis is a continuous variable (Baker 2003). Flexibility is suggested to be advantageous as it may provide an opportunity for hosts to choose among physiologically different symbionts in response to a changing environment, such as shifting to thermal-tolerant symbionts during a thermal-related bleaching event (Baker 2003; Jones et al. 2008; LaJeunesse et al. 2009). Yet the flexibility/specificity of many host species is still unclear and may vary spatially and temporally. Based on the ITS2-DGGE results, most HK corals engage in symbiosis with only one or a few symbiont subclades. However, symbiont types representing <5–10% of the total symbiont community are likely to be undetectable by the conventional PCR-DGGE method, and the actual detection limit varies for different symbiont types (Thornhill et al. 2006b; LaJeunesse et al. 2008). Therefore, clades/subclades with density lower than the detection limit of ITS2-DGGE analyses might be overlooked. With the increased sensitivity provided by advanced techniques such as *Symbiodinium* amplicon pyrosequencing, a higher diversity of symbionts may be discovered (Arif et al. 2014; Thomas et al. 2014).

Despite the possibility that DGGE analyses may miss some background symbiont types, comparison of the number of host species showing polymorphic symbiosis between HK and other studies (Table 3) was made from data taken from studies that also applied the ITS2-DGGE technique with a similar detection limit (ESM Table S2) for comparable results. HK stands out as having a comparatively low proportion of coral species hosting multiple symbionts. Furthermore, some coral species found in HK hosted different *Symbiodinium* types in other regions (Table 1), suggesting that they have potential to host multiple symbionts.

Diversity of *Symbiodinium* in HK soft corals

Symbiodinium ITS2 type C1 was the only symbiont type detected in the five soft corals investigated. This predominance of clade C *Symbiodinium* is consistent with that reported globally; 23 soft coral species from six geographical locations all hosted clade C *Symbiodinium*, and two species also hosted clade D *Symbiodinium* (Goulet et al. 2008). Many C-type *Symbiodinium* have been reported in Indo-west Pacific soft corals, with some types (C3j, C71a) found exclusively in soft coral species but not in other octocorals. These novel types of *Symbiodinium* were not detected in the present study. Nevertheless, greater symbiont diversity may be revealed in soft corals by using more sensitive detection methods.

Low symbiont diversity in HK scleractinian corals

Only five distinct ITS2 *Symbiodinium* types were detected from the 306 samples of 56 scleractinian coral species in 23 genera examined in the present study. This is far below what would be expected based on the established global relationship between the number of symbiont types detected and the number of host coral species examined (Fig. 4). This low diversity of symbionts was further skewed by the dominance of a single C1 subclade which was the only symbiont type harboured by 89.3% of HK scleractinian coral species examined. Many of these coral species hosted symbiont types other than C1 elsewhere in the world (Table 1). For example, *Hydnophora exesa* hosted C21a and C27 in Okinawa, C3 and C3h on the Great Barrier Reef (LaJeunesse et al. 2004b), C3u and C15 in Thailand and C3 and C3u in Tanzania (LaJeunesse et al. 2010). In contrast, such flexibility was not observed in HK. Given the known detection limit of DGGE, it is possible that more symbiont types could be found if more sensitive detection methods were applied. However, most studies mentioned above also employed a similar DGGE technique and were able to detect different *Symbiodinium* subclades. Therefore, failure to find other subclades of symbiont in HK corals is likely to reflect comparatively low symbiont variability in these corals, rather than limitations in the technique employed.

Some suggestions have been put forward to explain the higher symbiont diversity found in certain regions. For example, the Caribbean regions (MC-Mexico and BC-Bahamas in Fig. 3) harbour a diverse pool of *Symbiodinium*, including clades A, B, C and D, and hence provide more subclades from these lineages (LaJeunesse 2002). Remote, isolated Western Australia (WA in Fig. 3) harboured diverse and novel *Symbiodinium* communities (Silverstein et al. 2011). Silverstein et al. (2011) suggested several possible reasons for this, including variable environmental

histories, relative isolation of the sites and frequent transport of symbionts from Indo-Malay reefs nearby. Wicks et al. (2010) also attributed the high diversity of symbionts in high-latitude Australia and New Zealand to the locally challenging and highly fluctuating environmental conditions. In contrast, while it is common that one or a few generalist symbionts dominate coral communities in Indo-Pacific regions, the symbiont diversity in HK revealed in the present study is still relatively low even compared with sites in the nearby Indo-Pacific region. Thus far, no hypothesis has been put forward to explain such low diversity.

Prevalent stability of coral–*Symbiodinium* symbiosis has been shown in some DGGE-based seasonal monitoring studies (Thornhill et al. 2006a, b). Goulet (2006) analysed 43 studies of 442 coral species and suggested that most corals do not change their symbionts. Yet several cases of seasonal change in symbiont type, probably in response to temperature and/or irradiance variation, were reported by Chen et al. (2005) and Hsu et al. (2012), suggesting that one-time sampling may fail to detect some symbiont types. In the present study, monitoring of locally dominant species over a year as well as comparison between host samples collected in different seasons (ESM Table S1) showed no seasonal change in symbiont types, suggesting temporally stable symbiosis. Moreover, comparison of symbiont diversity in different regions within HK waters revealed no significant spatial differentiation. Furthermore, an earlier study of *Platygyra acuta* showed a consistent association of this locally dominant coral species in HK with *Symbiodinium* C1, irrespective of the locations within individual colonies (top vs. basal regions) and despite the presence of a significant gradient of photosynthetic activity along the surface of the colonies (Tsang 2010). Hence, neither temporal nor spatial factors are likely to be masking HK symbiont diversity.

Symbiont diversity may be affected by mode of symbiont transmission, either horizontal or vertical. LaJeunesse et al. (2004a) indicated that the high symbiont diversity in Hawaii was due to the dominance of vertically transmitting host taxa. Meta-analysis of a global dataset of coral–*Symbiodinium* interaction records in tropical Indo-Pacific and Atlantic Oceans from 1991 to 2010 (Fabina et al. 2012) showed a strong correlation between symbiont specificity and symbiont transmission mode, with more symbiont types in vertically transmitting corals. In contrast, van Oppen (2004) found that mode of symbiont transmission did not affect symbiont diversity in two acroporid genera. Among 41 of the HK coral species examined with known spawning behaviours (Table 1), 90.2% are broadcast spawning corals, 2.4% are brooding corals and 7.3% exhibit both spawning behaviours. For those ($n = 28$) whose mode of symbiont transmission has been recorded

elsewhere (Table 1), 82.1% are horizontal transmitters, 14.3% are vertical transmitters and 3.6% exhibit both transmission modes (Table 1). Corals that are horizontal transmitters share the same environmental source of symbionts. They therefore often have a lower number of symbiont types than the vertical transmitters (Fabina et al. 2012). The dominance of HK coral communities by broadcast spawning corals and/or horizontal transmitters of symbionts may contribute to the low diversity of symbiont types. All local horizontal transmitters hosted only a generalist symbiont, C1 *Symbiodinium* ($n = 23$). For the few species that exhibit vertical or mixed modes of transmission, more specific symbionts were found (e.g. C21 in *M. peltiformis* and C15 in *Porites* spp.). Coexistence of clades C and D *Symbiodinium* was only found in two species, *G. aspera* and *O. crispata*, which transmit symbionts both horizontally and vertically. However, the pattern is not always consistent, as *Stylocoeniella guentheri*, a vertical transmitter, also hosted only C1 symbionts. This suggests that the mode of symbiont transmission may only partly explain the patterns of local symbiont diversity observed.

High symbiont specificity as a strategy for symbiont adaptation and interaction in HK corals

Unique adaptive traits are often reported in marginal areas (Kawecki 2008; Budd and Pandolfi 2010). The fluctuating and stressful environment for corals suggests that HK corals should host more stress-tolerant symbiont types such as members of clade D *Symbiodinium*, unique and novel types of symbionts, and/or a diverse pool of different symbiont types. However, the diversity of symbiotic *Symbiodinium* spp. forming symbiosis with both scleractinian and soft corals in HK is apparently low with only five ITS2 types detected and dominated by symbiont type C1. This symbiosis is highly specific and stable both spatially and temporally, in contrast to those found in other neighbouring sites. The low diversity and high specificity of HK symbiotic *Symbiodinium* may provide a different perspective on, and insight into, coral–symbiont interactions.

C1 *Symbiodinium* is an ancestral type of symbiont that is commonly found in both Indo-Pacific and Atlantic-Caribbean corals (LaJeunesse 2005). It is more thermally sensitive than other symbiont types found in HK (i.e. C15, C21, D1 and D8-12; LaJeunesse et al. 2003; Stat and Gates 2011). Yet the summer sea surface temperature in HK may not have been sufficiently high (≤ 31 °C) to reach the upper thermal limit of C1 *Symbiodinium* or to provide a competitive advantage to other more thermally tolerant symbiont types. On the other hand, the continuous dominance of C1 *Symbiodinium* even in winter at temperatures as low

as 13 °C suggests that this type of symbiont is acclimatized to a wide local temperature range and possibly to other local stressors such as varying salinity and high turbidity. Reimer et al. (2006) also suggested that C1 may be a 'generalist' since it lives in a variety of environments over a wide latitudinal range. The presence of C1 in high latitudes such as Southern Japan (Reimer et al. 2006) and Jeju Island in South Korea (De Palmas et al. 2015) provides further support that C1 is highly tolerant to low temperature. Howells et al. (2012) demonstrated divergent thermal tolerance in C1 *Symbiodinium* types occurring in two different thermal environments, suggesting that *Symbiodinium* types can adapt to local differences in physical environment. Pettay and LaJeunesse (2013) also showed a strong differentiation between subtropical and tropical populations of D1 *Symbiodinium* corresponding with fluctuating environmental conditions, suggesting the possibility for the same *Symbiodinium* type to adapt to different local environments. The 'environmental generalist' nature of C1 may make it the best candidate for coral symbiosis under the stressful and fluctuating environmental conditions in HK. Thus, forming symbiosis with the best acclimatized symbiont, instead of with a diverse group of symbionts with different physiological performance, may be a strategy exhibited by HK corals to cope with stressful conditions. This strategy may prove to be most cost-effective without the risk of failure to re-establish a new interaction with a new symbiont type in each change in environmental conditions nor to bear the cost arising from competition between different types of symbionts occurring in polymorphic symbiosis (Frank 1996; Putnam et al. 2012). To what extent HK corals retain their ability to change their symbiotic partners in the event of further climate changes remains to be investigated. Nonetheless, keeping a low *Symbiodinium* diversity with the best acclimatized *Symbiodinium* type, an environmental generalist C1 symbiont type in the present case, may turn out to be an effective strategy for corals coping with an uncertain world.

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