Genetic Diversity of Symbiodiniaceae Associated with *Porites lutea* and *Pocillopora damicornis* in the Gulf of Thailand Inferred from Nucleotide Sequences of Internal Transcribed Spacer-2

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ABSTRACT

Understanding the variation in symbiotic algae-coral associations can provide insights about how corals respond to environmental changes. We examined community composition of family Symbiodiniaceae in two scleractinian coral hosts, *Porites lutea* and *Pocillopora damicornis* at nine sites (n = 4-5 colonies/species/site) in the Gulf of Thailand (GoT). Species in the family Symbiodiniaceae were resolved from denaturing gradient gel electrophoresis (DGGE) and identified based on Internal Transcribed Spacer-2 (ITS2) DNA sequences. We observed five ITS2 haplotypes including those contained within *Cladocopium goreaui* (C1), *Cladocopium* spp. (C15, C15.7), *Durusdinium glynii* (D1) and *D. glynnii* (D6). *Cladocopium* sp. (C15) was abundant and prevalent in *Por. lutea*. *D. glynnii* (D1) was prevalent in *Poc. damicornis*. PERMANOVA revealed the effects of host species and site interactions on Symbiodiniaceae communities. The sampling sites contributed to the variation in Symbiodiniaceae species composition in *Poc. damicornis* ($P<0.01$), but not in *Por. lutea*. Cluster analysis suggested three groups: (1) *Por. lutea* (dominated by *Cladocopium* sp. (C15), (2) *Poc. damicornis* (dominated by *D. glynnii* (D1)) and (3) a minor group consisting of *Poc. damicornis* from SM with *D. glynnii* (D6). *D. glynnii* (D1) is likely to be widely distributed in the GoT while other is restricted in particular habitats. Despite multiple bleaching events in the GoT, our results suggest the persistence of spatial variation of Symbiodiniaceae communities, and thus stress the importance of local adaptation of coral-symbiont partnerships.

Keywords: Coral, DGGE, Gulf of Thailand, ITS2, Symbiodiniaceae, Zooxanthellae

INTRODUCTION

The Gulf of Thailand (GoT), locates between 5º 00' and 13º 30' N and 99º 00' and 106º 00' E, is a semi-enclosed, shallow coastal basin situated on the northwestern part of the Sundra Shelf, and measuring approximately 400 km by 800 km. The gulf can be considered as the inner and lower GoT, divided by a line drawn from the most southern tip of Chonburi Province (Sattahip District) to Hua Hin District, Prachuap Khiri Khan Province. Due to the enclosed nature of the inner GoT (surrounded by land in the north, east and west), strong influence from major rivers and unique sea surface circulation (Buranapratheprat *et al*., 2008), several islands in the inner GoT harbor a high diversity of scleractinian corals, and the most inner island, Sichang Island, hosts a unique coral community. The eastern part of the inner gulf contains reefs developed along the coastline and near islands in Pattaya and Sattahip,

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Chonburi Province and further along the east coast from Rayong to Trat. Along the west coast of the lower Gulf, a few islands are surrounded by fringing reefs in Prachuap Khiri Khan and Chumphon provinces, and with Losin Island (Pattani Province) being the furthest south (Sudara and Yeemin, 1997; Phongsuwan *et al*., 2013).

Coral reefs in the GoT have been threatened historically by natural and human disturbances, and the pressure is alarming in the present. In the past two decades, coral reefs in the GoT, especially in inshore areas, have experienced at least five mass bleaching events, with mild bleaching in 2003, 2005 and 2008, and severe events in 1998 and 2010 (Sutthacheep *et al*., 2012). Prior to the 1998 bleaching event, reef communities were dominated by *Porites* and *Acropora*, and *Pocillopora* was common, but afterwards, mature colonies of *Acropora* were almost wiped out from the GoT (Boonprakob and Chankong, 2000). *Acropora* and *Pocillopora* were most vulnerable, and 70-90% died, while massive corals such as *Porites* and faviids were less impacted. During the 2010 coral bleaching event, 30-90% of live corals died and previously healthy reefs became deteriorated. However, branching colonies of *Acropora* appeared to resist the 2010 bleaching. In this case the more vulnerable coral taxa were *Porites lutea* and faviids; additionally, *Pocillopora* disappeared from many reefs in the GoT (Sutthacheep *et al*., 2012). Several factors may have contributed to this changing resilience among coral communities in the GoT, including coral-symbiont associations, adaptation to high turbidity and the heterotrophic feeding ability of corals.

The composition of symbiotic zooxanthellae in coral colonies depends on geographical and environmental conditions, as well as host species. In their recent systematic revision of the formerly known genus *Symbiodinium*, LaJeunesse *et al*. (2018) described *Symbiodinium* "clades" as being equivalent to genera in the family Symbiodiniaceae, and some new species were described (previously recognized as subclades). For example, a common C1 type has been revised to '*Cladocopium goreaui*', and D1 and D1-4-6 to '*Durusdinium glynnii*' (LaJeunesse *et al*. 2010, LaJeunesse and Thornhill, 2011, Wham *et al*., 2017). Our preliminary results indicated six ITS2 haplotypes (i.e. *C. goreaui* (C1), *Cladocopium* spp. (C3u, C15, C15.7), *D. glynnii* (D1) and *D. glynnii* (D6)) from four scleractinian corals, including *Porites lutea*, *Platygyra daedalea*, *Pavona decussata* and *Pocillopora damicornis* in the eastern GoT. The diversity of *Durusdinium* spp. (two haplotypes) was less than that of *Cladocopium* spp. (four haplotypes) (Chankong, 2018). Due to changing vulnerability of coral hosts to coral bleaching, in this study we are interested in the association of Symbiodiniaceae in brooder species with branching colony growth form (*Poc. damicornis*) and broadcast spawners with massive growth form (*Por. lutea*) along the reefs in the GoT. We tested the effects of location (inshore-offshore), site and host species on the Symbiodiniaceae community composition within *Poc. damicornis* and *Por. lutea*. Insights from the study will benefit coral rehabilitation programs after future coral bleaching events.

MATERIALS AND METHODS

Study area and sample collection

During 2016-2017, we collected coral tissue samples by SCUBA diving on reefs from a depth range between 3-19 m at nine locations in GoT (Figure 1). These locations represented inshore (1.5-8 km from shore) and offshore (15-80 km from shore) areas (Table 1). Distance from shore was associated with water turbidity levels, with inshore areas being more turbid than those offshore. Five inshore sites included Sichang Island, Chonburi Province (SC), Samaesan Island, Chonburi (SS), Samet Island, Rayong (SM), Kudee Island, Rayong (KD) and Khai Island, Chumphon (KH) and four offshore sites were Hin Ploeng Pinnacle, Rayong (HP), Rankai Island, Chumphon (RK), Kra Island, Nakhon Sri Thammarat (KR) and Losin Island, Pattani (LS). Characteristics of coral communities and their dominant species varied among sites, except for *Porites* being prevalent across all sites. Corals at these sites have experienced various degrees of coral bleaching in the past 20 years. Coral tissues were sampled from a total of 89 coral

colonies, belonging to a broadcast spawner species (*Por. lutea*, n = 5 colonies/location) and a brooder species (*Poc. damicornis*, n = 4-5 colonies/site). Tissue biopsies were preserved in 95% ethanol for further analysis.

Genomic DNA extraction of Symbiodiniaceae

Coral tissue and calcium skeletons were ground and processed for DNA extraction following a modified salting-out method (Ferrara *et al*., 2006). Approximately 500 mg of each sample was incubated in 1 mL of lysis buffer solution (SDS 20%, EDTA 5 mM, Tris-HCl 10 mM) with 0.1 µL of proteinase K (10 µL/mL) for 3 h at 56ºC to lyse coral and symbiont cells. Proteins were precipitated in saturated CH_3COONa (6.1 M), with 1 mL added for each 4 mL of lysate. Samples were then centrifuged at 10,000 g for 20 min, and the supernatant was transferred to a new tube. Two volumes of 99%

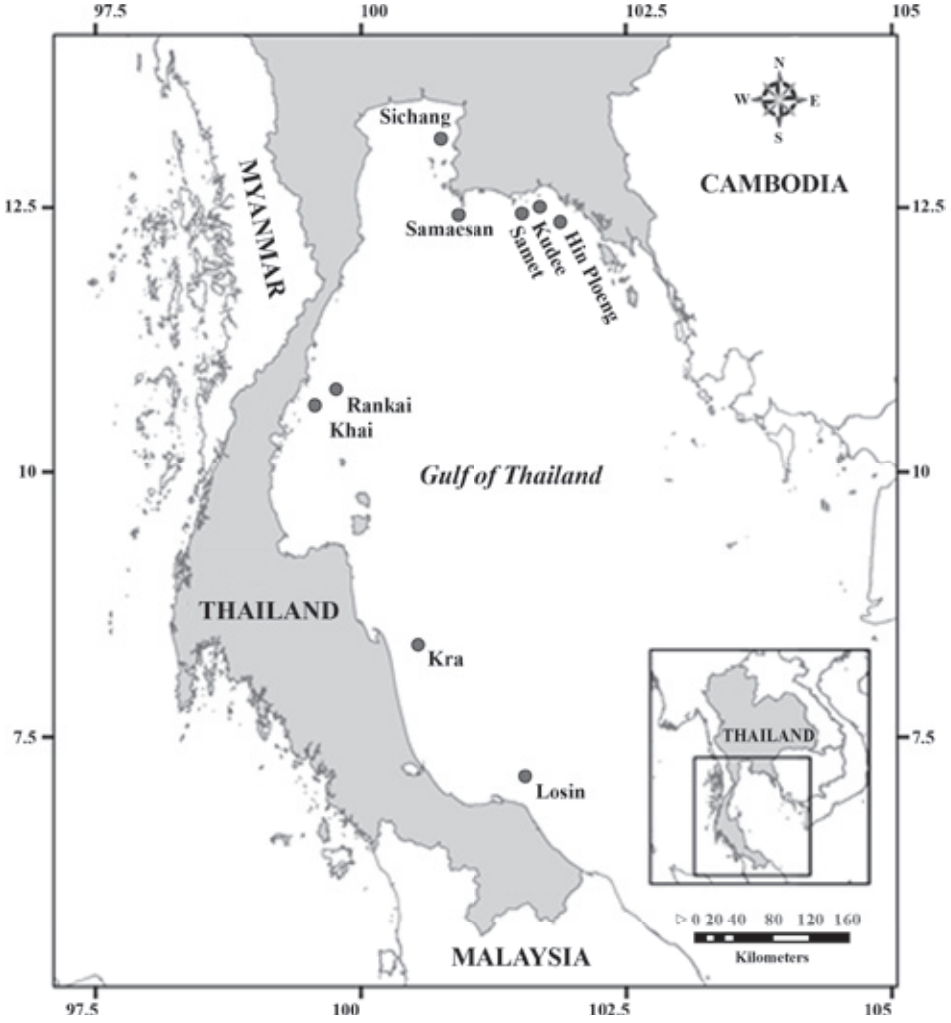

Figure 1. Locations of coral and Symbiodiniaceae collections in the inner Gulf of Thailand (Sichang, Samaesan), eastern Gulf of Thailand (Samet, Kudee, Hin Ploeng) and western Gulf of Thailand (Rankai, Khai, Kra and Losin).

Table 1. Environmental and coral community characteristics of nine sampling locations in the Gulf of Thailand surveyed in 2016-2017.

Inshore/ Offshore	Location	Distance from shore (km)	Depth (m)	Turbidity level (m-FTU)	Coral reef area (km^2) (Number of coral species)	Dominant corals	% coral colonies bleached in 2010
Inshore	1. Sichang Is. (SC) Chonburi Province	8	4-6	1.68	0.0912 (10-15)	*Porites, Pavona,* faviids	5 - 10
	2. Samaesan Is. (SS) Chonburi Province	2	3-6	1.04	0.5424 (15-20)	*Porites, Pavona,* faviids, *Pocillopora*	30 - 50
	3. Samet Is. (SM) Rayong Province	3	3-5	1.60	1.1904 (25-30)	*Porites, Pavona,* faviids, *Acropora*	50 - 70
	4. Kudee Is. (KD) Rayong Province	4	4-6	0.78	0.224 (25-30)	*Porites, Pavona,* faviids, *Acropora*	50 - 70
	5. Khai Is. (KH) Chumphon Province	3	4-6	0.47	0.1824 (20-25)	*Porites, Pavona,* faviids, *Acropora*	10 - 30
Offshore	6. Hin Ploeng (HP) Rayong Province	25	10-13	0.92	0.016 (20-25)	*Porites, Pavona,* faviids, *Pocillopora*	5 - 10
	7. Rankai Is. (RK) Chumphon Province	15	3-5	0.37	0.0096 (20-25)	*Porites, Pavona,* faviids, *Pocillopora*	10 - 30
	8. Kra Is. (KR) Nakhon Sri Thammarat Province	53	5-10	0.35	0.6592 (15-20)	*Acropora,* faviids, *Porites*	30 - 50
	9. Losin Is. (LS) Pattani Province	80	16-19	0.34	0.072 (15-20)	*Acropora,* faviids, *Porites*	10 - 30

cold ethanol were added to each supernatant sample to precipitate the total genomic DNA. The samples were then incubated at -20ºC for 30 min and then centrifuged at 12,000 g for 30 min. The solution was discarded and DNA pellets were allowed to dry for 15 min, then resuspended in 50-100 µL of sterile distilled water. DNA quantity and quality was checked by electrophoresis on a 1.5% agarose gel in 1xTBE buffer.

PCR amplification and DGGE analysis

We inferred the Symbiodinium OTUs from the ITS2 nucleotide sequences, obtained from community DNA profiles on polyacrylamide denaturing gradient gels (LaJeunesse and Trench 2000). The ITS2 region was amplified using the primers ITSintfor2 (5'GAATTGCAGAACTCCG TG-3') and ITS2CLAMP (5'CGCCCGCCGCGC CCCGCGCCCGTCCCGCCGCCCCCGCCCGG GATCCATATGCTTAAGTTCAGCGGGT-3') in polymerase chain reactions (PCR) (LaJeunesse and Trench, 2000). Each reaction (20 µl) contained 1 µL of template DNA, 1 x PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 and 0.4 µM of forward and reverse primers, respectively, and 1 unit of *Taq* polymerase (5 $U \bullet \mu L^{-1}$, Vivantis, Malaysia). To maximize the amplification of Symbiodiniaceae within coral colonies, we employed the touchdown temperature profile where annealing temperature was decreased or increased at a consistent rate (0.5°C per cycle in our case, LaJeunesse and Trench 2000). The temperature profile consisted of a DNA denaturing step at 92°C for 3 min, followed by 20 cycles of 92°C for 30 s, then 62°C-52°C for 40 s (i.e., a touchdown step), and finally 72°C for 30 s and 15 cycles of 92°C for 30 s, 52°C for 40 s, 72°C for 30 s and ending with an extension step at 72°C for 5 min.

The ITS2 fingerprint of the Symbiodiniaceae community in each coral colony was resolved using denaturing gradient gel electrophoresis (DGGE, 8% polyacrylamide gels with a 45-80% gradient of urea and formamide - 3.2 to 5.6 M urea and 18% to 32% formamide) (VS20WAVE-DGGE, Cleaver Scientific, Ltd, Warwickshire, United Kingdom) for 14 - 15 h at 110 V at a constant temperature of 65°C. For visualization, gels were stained with 1 x SYBR Gold (Life Technologies Corporation, Eugene, OR, USA) for 10 min.

Prominent bands of representative ITS2-DGGE fingerprints were excised, eluted overnight in distilled water, and re-amplified using the ITS for 2 and the reverse primer without the GC-rich clamp (ITSrev), using PCR ingredients and a temperature profile similar to the original PCR conditions (annealing temperature of 52°C), excluding the touchdown step. The PCR products were then purified using a commercial column-based purification kit (GF1-AmbiClean, Vivantis, Malaysia) and submitted for sequencing (ABI 3130xl, 1st Base Inc., Malaysia). The sequences of diagnostic bands and the accompanying ITS2-DGGE fingerprints were aligned; the remaining fingerprints were inferred from the diagnostic bands. All nucleotide sequences were checked and edited manually using Sequence Scanner v.1 (Applied Biosystems, USA) and contiged with CAP3, a sequence assembly program (Huang and Madan, 1999). To identify species of family Symbiodiniaceae, the sequences were aligned with existing sequences available in the National Center for Biotechnology Information (NCBI, https://blast.ncbi.nlm.nih.gov/Blast.cgi) database, a custom ITS2 BLAST database (Arif *et al*., 2014) and the SymbioGBR database (http://www.symbioGBR.org) (Tonk *et al*., 2013), using the nucleotide basic local alignment search tool (BLASTn).

To generate a phylogenetic matrix, alignments were performed using MEGA7 software. The length of the sequences used in the analysis was 282 bp. Based on the maximum likelihood approach implemented in MEGA 7, the best-fit nucleotide substitution model was Kimura 2-parameter (lowest Bayesian Information Criterion score). All positions containing gaps and missing data were eliminated. Maximum likelihood phylogenetic trees of the ITS2-DGGE sequences were constructed using the maximum likelihood (mL) approach based on Kimura 2-parameter model (Kimura, 1980) with 1,000 replicates to generate a supporting value for each node (MEGA7 software, Kumar *et al*., 2016). Clade A was used as an outgroup (accession no. MH211593.1).

Statistical analysis

All statistical analyses of Symbiodiniaceae community composition were performed using the software package PRIMER 6 (Clarke and Gorley, 2006) with PERMANOVA+ (Anderson *et al*., 2008) (Multivariate statistics were executed with the software PRIMER v6 and the add-on software). All analyses used a Bray-Curtis similarity matrix, based on presence/absence data for Symbiodiniaceae assemblages. We evaluated global effects of location (inshore-offshore), site, and host species on Symbiodiniaceae assemblage using a permutational multivariate analysis of variance (PERMANOVA) (Anderson *et al*., 2008). Post-hoc pairwise tests were also performed and *P* values were generated by 1,000 permutations. To test for host specificity, we performed a one-way analysis of similarity (ANOSIM) of Symbiodiniaceae assemblages between the two host species and performed similarity percentage analysis (SIMPER) to identify the species contributing to the composition differences. An unweighted pair group method with arithmetic mean (UPGMA) dendrogram was also generated to reveal the clustering pattern of *Symbiodinium* assemblages by host coral species, and the SIMPROF test was conducted to determine statistical significance of the dendrogram nodes.

RESULTS

Phylogenetic relationships

We recovered 52 Symbiodiniaceae ITS2 sequences obtained from 45 *Por. lutea* colonies and 44 *Poc. damicornis* colonies. All Symbiodiniaceae sequences were assigned to genus *Cladocopium* (3 DGGE profiles: 1 profile of C1, 1 profile of

C15, 1 profile of C15.7) and genus *Durusdinium* (2 DGGE profiles: 1 profile of D1 and 1 profile of D6) (Table 2). Phylogenetic analysis of family Symbiodiniaceae, inferred from ITS2 sequences (271-283 bp, variation 57.45%), revealed that prominent genera *Cladocopium* and *Durusdinium* were genetically distinct (bootstrap values = 99, Figure 2). Genus *Cladocopium* formed a monophyletic group consisting of three haplotypes namely *C. goreaui* (C1), *Cladocopium* sp. (C15) and *Cladocopium* sp. (C15.7). All haplotypes are associated with *Por. lutea*, *Cladocopium* spp. (C15 and C15.7) haplotypes formed closely related group (bootstrap value = 98) while *C. goreaui* (C1) is separated. Genus *Durusdinium* consisted of four haplotypes: *D. glynnii* (D1, D1v1 (a novel *D. glynnii* (D1) variant 1, 1 bp different form ITS2 type of *D. glynnii* (D1)), D1v2 (a novel *D. glynnii* (D1) variant 2, 1 bp different form ITS2 type of *D. glynnii* (D1)) and *D. glynnii* (D6). All haplotypes associated with *Poc. damicornis*, which *D. glynnii* (D1) and D1v1 formed a monophyletic group (bootstrap value = 100). *D. glynnii* (D1v2) and *D. glynnii* (D6) were more genetically similar to each other than to *D. glynnii* (D1) (bootstrap value = 53).

Table 2. GenBank accession number for Symbiodiniaceae ITS2 haplotypes.

Symbiodiniaceae ITS2 haplotype	GenBank accession number	Custom database (Arif *et al*., 2014)
Cladocopium goreaui (C1)	AF333515	lcl|Query_46063
Cladocopium sp. (C15)	AY236639	lcl|Query_239713
Cladocopium sp. (C15.7)	HE579015.1	lcl|Query_10097
Durusdinium glynii (D1)	EU449061	lcl|Query_239913
Durusdinium glynii (D6)	EU812742	lcl|Query_40547

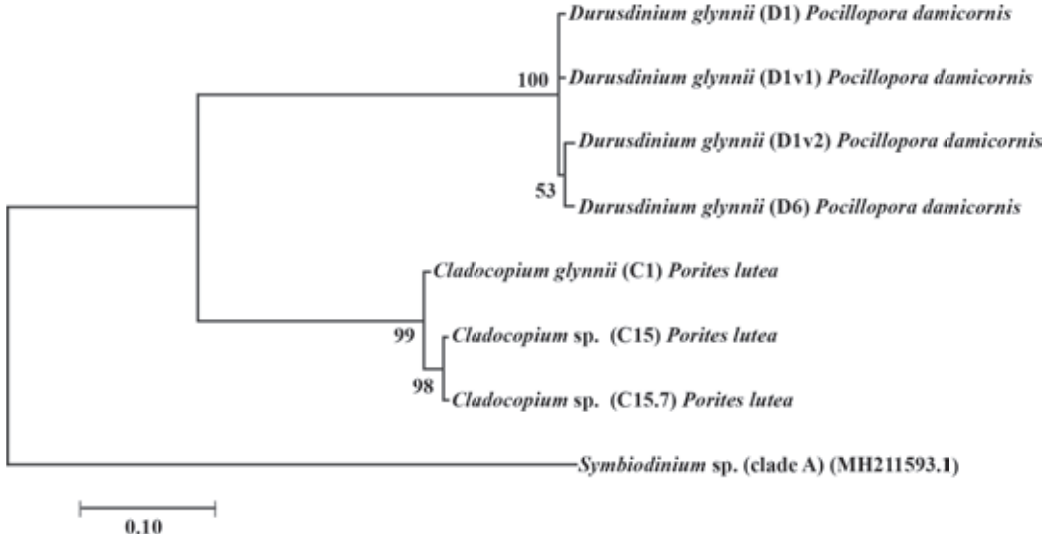

Figure 2. Maximum likelihood phylogenetic tree of unique Symbiodiniaceae ITS2 sequences (Kimura 2-parameter model) in *Por. lutea* and *Poc. damicornis* in the Gulf of Thailand. Branch labels indicate bootstrap values (1,000 replicates).

Diversity of Symbiodiniaceae in corals in the Gulf of Thailand

Cladocopium and *Durusdinium* were found in similar proportions in the assemblages, 48.4% and 51.6%, respectively (Table 3). We detected five ITS2 haplotypes of dominant Symbiodiniaceae species, including *C. goreaui*, *Cladocopium* (C15, C15.7), *D. glynnii* (D1) and *D. glynnii* (D6) (Figure 3). Colonies typically contained a single species, with a few rare cases having two species (i.e., three colonies of *Por. lutea* and one colony of *Poc. damicornis* at SC and SS, Chonburi Province and SM, Rayong Province). The number of Symbiodiniaceae species observed in *Por. lutea* (at least 3 species) was higher than in *Poc. damicornis* (1 species). *Cladocopium* sp. (C15) was prevalent in *Por. lutea* populations, while *D. glynnii* (D1) was prevalent in *Poc. damicornis* populations.

Overall, every Symbiodiniaceae species observed in this study was found at least one of the inshore sites, while at the offshore sites only *Cladocopium* sp. (C15) (in *Por. lutea*) and *D. glynnii* (D1) (in *Poc. damicornis*) were recorded (Table 3, Figure 4). However, the spatial pattern of Symbiodiniaceae species distribution differed between the two coral host species.

Table 3. Occurrence of Symbiodiniaceae ITS2 haplotypes and DGGE profiles in coral hosts (expressed as the number of colonies) from five inshore sites and four offshore sites in the Gulf of Thailand.

Location (Inshore/ Offshore)	Sites	Coral species	C1	C15	C15.7	D1	D6	Total DGGE profiles
Inshore	Sichang Is. (SC)	*Porites lutea*	1	5	-	-	1	3
		Pocillopora damicornis	-		-	4	-	1
	Samaesan Is. (SS)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	4	1	2
	Samet Is. (SM)	*Porites lutea*	-	3	1	2	-	3
		Pocillopora damicornis	-	-	-	-	5	1
	Kudee Is. (KD)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	5	-	1
	Khai Is. (KH)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	5	-	1
Offshore	Hin Ploeng (HP)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	5	-	1
	Rankai Is. (RK)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	5	-	1
	Kra Is. (KR)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	5	-	1
	Losin Is. (LS)	*Porites lutea*	-	5	-	-	-	1
		Pocillopora damicornis	-	-	-	5	-	1

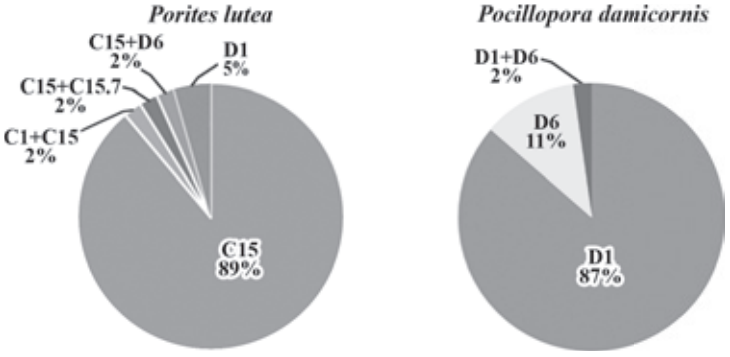

Figure 3. Percentage of occurrence of Symbiodiniaceae communities in *Porites lutea* (n=45 colonies) and *Pocillopora damicornis* (n=44 colonies) from five inshore sites and four offshore sites in the Gulf of Thailand (Refer to the text for Symbiodiniaceae species names).

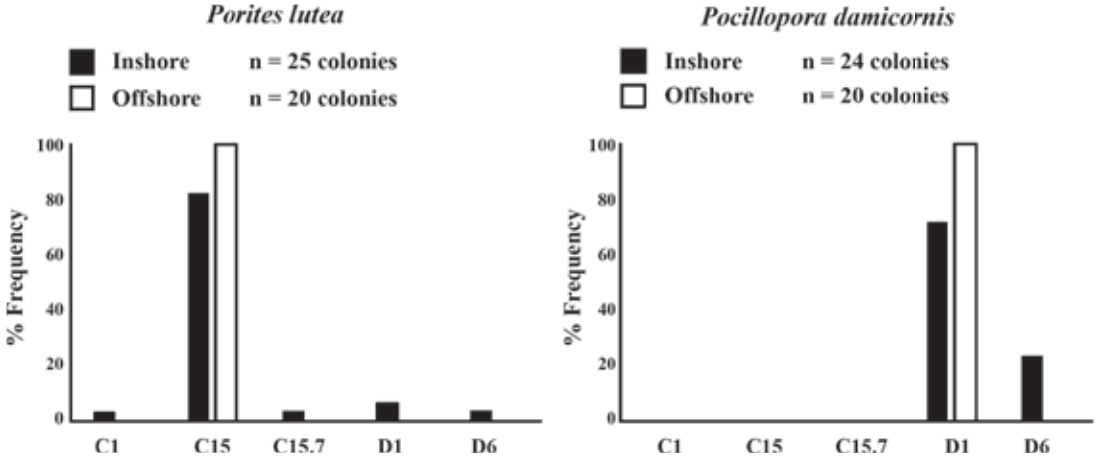

Figure 4. Frequency of occurrence of Symbiodiniaceae ITS2 haplotypes observed in *Por. lutea* and *Poc. damicornis* at inshore and offshore sites in the Gulf of Thailand.

PERMANOVA suggested that the Symbiodiniaceae assemblages were highly structured by host species and site (*P=0.005*). Symbiodiniaceae species compositions in *Por. lutea* and *Poc. damicornis* were significantly different in four pairs of sites (SS-SM, SC-SM at $P \leq 0.05$; SM-KD, SM-KH at $P \leq 0.01$). We also observed highly significant interactions between site and host species (*P=0.005*). The Symbiodiniaceae species compositions in Poc. damicornis differed among sites (SS-SM, SC-SM, SM-KD and SM-KH at $P \leq 0.05$). However, we did not observe the same pattern in the Symbiodiniaceae communities in *Por. lutea*. The pairwise tests suggested no significant differences in Symbiodiniaceae species composition in *Por. lutea* among sites.

Global ANOSIM tests showed that the Symbiodiniaceae species association with coral species was significant (R = 0.789, P = 0.001), indicating host specificity. SIMPER analysis revealed that the proportions of *Cladopopium* sp.

(C15) and *D. glynnii* (D1) or their combinations contributed to a distinctive composition in each coral species. *Por. lutea* was mainly associated with *Cladopopium* sp. (C15) and *Poc. damicornis* was associated with *D. glynnii* (D1). The UPGMA dendrogram revealed a consistent clustering pattern with each coral species harboring a distinctive Symbiodiniaceae species composition (Figure 5). The dendrogram provided additional insights into host species and site interactions. At 65% similarity, the dendrogram suggested three main clusters, with one major cluster containing mostly *Por. lutea* and another containing *Poc. damicornis*, and a small but quite unique cluster of *Poc. damicornis* with D6 from SM. In addition, we found some coral specimens (from SS, SC and SM) that contained more than one species of Symbiodinaceae.

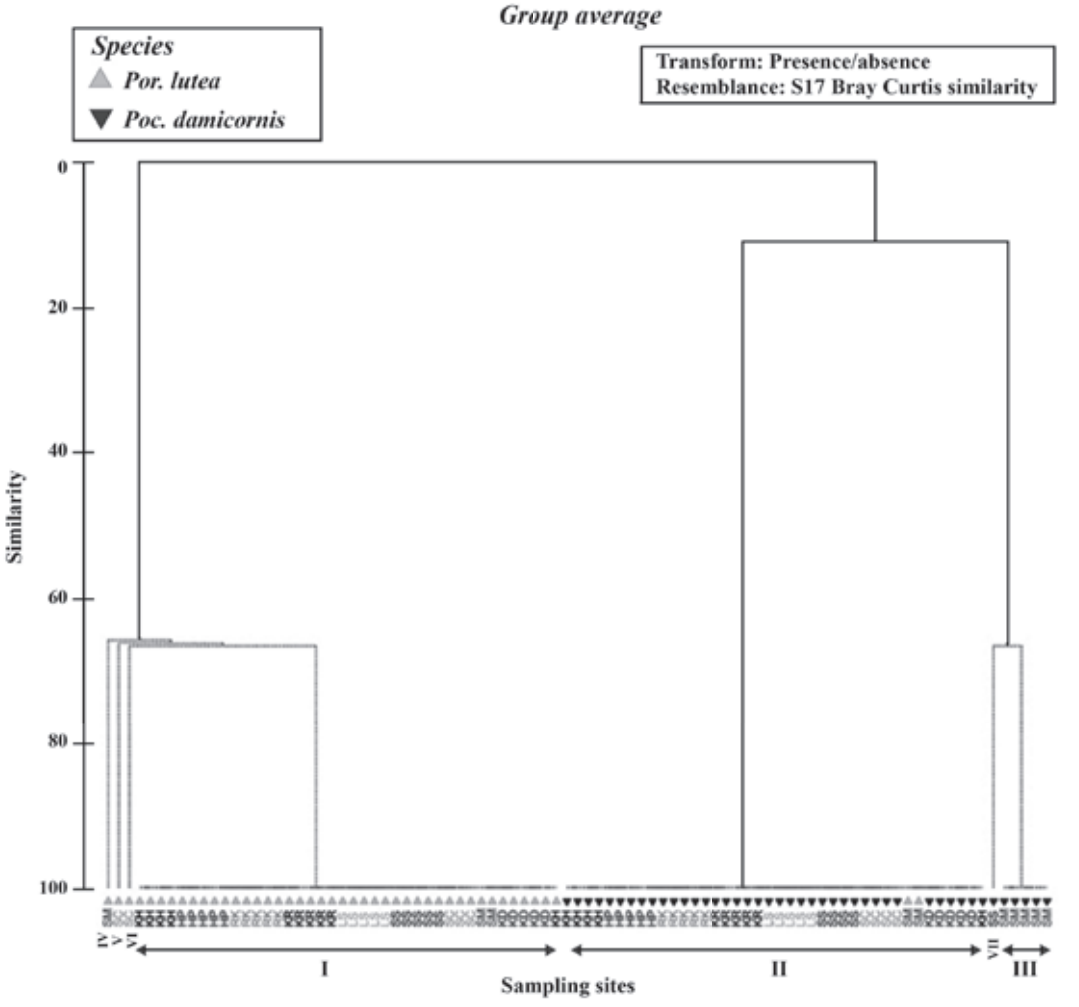

Figure 4. UPGMA dendrogram based on Bray-Curtis similarity of Symbiodiniaceae species composition in *Por. lutea* and *Poc. damicornis* from the Gulf of Thailand. *Red line* indicates significant groupings ($P < 0.05$) based on the SIMPROF test. Labels for nine sampling sites are SC, Sichang Is.; SS, Samaesan Is.; SM, Samet Is.; KD, Kudee Is.; HP, Hin Ploeng Pinnacle; KH, Khai Is.; RK, Rankai Is.; KR, Kra Is. and LS, Losin Is. The host-symbiotic algae associations are superimposed on the significant clusters (I to VII).

The clustering patterns were associated with dominant Symbiodiniaceae species. The first major group consisted of most *Por. lutea* colonies hosting *Cladocopium* sp. (C15) (subgroup I). The second major group consisted of most *Poc. damicornis* colonies hosting *D. glynnii* (D1) and two *Por. lutea* at SM (subgroup II). The third major group consisted of *Poc. damicornis* colonies hosting *D. glynnii* (D6) (subgroup III). The other smaller groups (IV-VII) each of which contained only a coral colony, included *Por. lutea* colonies at SC hosting *Cladocopium* sp. (C15) in a combination with *C. goreaui* (C1) or *D. glynnii* (D6); an SM *Por. lutea* colony containing *Cladocopium* sp. (C15) and *Cladocopium* sp. (C15.7) and an SS *Poc. damicornis* colony hosting *D. glynnii* (D1-6) (subgroup VII).

DISCUSSION

Compared to other Indo-Pacific regions, we found relatively diverse Symbiodiniaceae species in *Por. lutea* (5 ITS2 haplotypes) and in *Poc. damicornis* (2 haplotypes) across nine sites from the GoT. *Cladocopium* sp. (C15) was dominant in *Por. lutea* while *D. glynnii* (D1) was dominant in *Poc. damicornis*. Symbiodiniaceae communities were highly structured by host coral species and by site. We observed significant interactions between the coral host species and two geographic factors on Symbiodiniaceae species compositions.

Symbiodiniaceae communities in relation to local environment and geography

The overall number of Symbiodiniaceae ITS2 haplotypes (or profiles) detected for *Por. lutea* and *Poc. damicornis* in the GoT (this study and Putchim (2017)) is lower (5 haplotypes) than in reports from the Andaman Sea (11 haplotypes) (LaJeunesse *et al*., 2010; Putchim 2017), but higher than for reefs in Singapore, Hong Kong and Taiwan (Tanzil *et al*., 2016; Wong *et al*., 2016; Keshavmurthy *et al*., 2014). In this study, we found three ITS2 haplotypes of *Cladocopium* spp. (*C. goreaui* (C1), *Cladocopium* sp. (C15) and *Cladocopium* sp. (C15.7)) and two *Durusdinium* spp. (*D. glynnii* (D1), *D. glynnii* (D6)), while Putchim (2017) reported one additional haplotype, namely *Cladocopium* sp. (C17). In the Andaman Sea, LaJeunesse *et al*., (2010) and Putchim (2017) reported 11 haplotypes in *Porites* and *Pocillopora*, with *Por. lutea* being associated with five haplotypes (*Cladocopium* spp. 4 haplotypes and *D. trenchii*), and *Poc. damicornis* being associated with seven haplotypes (*Cladocopium* spp. 4 haplotypes and *Durusdinium* spp. 3 haplotypes). In Singapore, Hong Kong, and Taiwan, *Porites* harboured only *Cladocopium* sp. (C15) and *Pocillopora* was associated with only a few haplotypes (Keshavmurthy *et al*., 2014; Tanzil *et al*., 2016; Wong *et al*., 2016).

The Indo-Pacific Symbiodiniaceae species in both coral genera are generally less diverse than those observed in the reefs off the Arabian Peninsula (Persian/Arabian Gulf, PAG, Sea of Oman, SO, and Red Sea, RS) (Ziegler *et al*., 2017). Ziegler *et al*. (2017) reported 10 (PAG) - 28 (RS) haplotypes in *Porites* and 3 (PAG) - 8 (RS) haplotypes in *Pocillopora* across all three regions, with PAG harbouring the lowest number of haplotypes. The lower diversity in other Indo-Pacific regions may be due to fewer species within each genus being sampled, the molecular techniques employed (DGGE vs. next generation sequencing) or the environments being examined having a narrower environmental gradient. In extreme environmental gradients among regions, Ziegler *et al*. (2017) found a shift in Symbiodiniaceae species composition from *Cladocopium* spp. dominant reefs in the Red Sea and the Sea of Oman (cooler water) to *Durusdinium* spp. dominant reefs in the Persian/Arabian Gulf (highly saline, warmer water). These results suggested that an extreme selective pressure (such as in PAG) can drastically alter the Symbiodiniaceae species composition with a tendency to lower Symbiodiniaceae diversity. Also, the physiology of each coral species may play an important role in maintaining its Symbiodiniaceae association within each environment.

In the GoT, site had a significant effect on the distribution of Symbiodiniaceae species for *Poc. damicornis*, but not for *Por. lutea*. In *Por. lutea*, most sites had one common haplotype (*Cladocopium* sp. (C15)), and some colonies in Sichang and Samet

Islands harbored additional, less common haplotypes. This observation of few haplotypes per site in this coral species has been made in other areas in the GoT (Putchim, 2017), the Andaman Sea (LaJeunesse *et al*., 2010; Putchim, 2017) and other Indo-Pacific regions (Putnam *et al*., 2012; Keshavmurthy *et al*., 2014; Wong *et al*., 2016).

In *Poc. damicornis*, on the other hand, we consistently observed more Symbiodiniaceae species at the inshore sites (2 haplotypes) than at the offshore sites (1 haplotype). An opposite pattern was observed for the Andaman Sea, where LaJeunesse *et al*. (2010) reported three *Durusdinium* spp. at the inshore sites and six haplotypes (3 *Cladocopium* spp. and 3 *Durusdinium* spp. haplotypes) at offshore sites. Our observation in the GoT may be explained by local adaptation of corals and Symbiodiniaceae to highly disturbed environments nearshore. Constant exposure to selective agents (high turbidity, freshwater discharge from major rivers, high sedimentation) might have allowed for diversification of Symbiodiniaceae species (Anthony and Fabricius, 2000; LaJeunesse *et al*., 2010; Sawall *et al*., 2014). Also, coral colonies in the nearshore areas, where the water flow was high, were not as badly bleached as some of the offshore areas in the 2010 bleaching event (Phuket Marine Biological Center, 2010). In addition to potential local adaptation to persistent environmental disturbances, light penetration through the water column is decreased at these sites, thus reducing the combined temperature/light stresses that induce bleaching (Phuket Marine Biological Center, 2010)

Host specificity

In our study, we observed associations between the 'host specific' *Cladocopium* sp. (C15) and *Por. lutea* and between 'host generalist' types, *D. glynnii* (D1), and *Poc. damicornis*. We detected a very high prevalence of *Cladocopium* sp. (C15) (90% of total coral colonies) and *D. glynnii* (D1) (4%) associated with *Por. lutea*. This pattern is consistent with the observations of Putchim (2017) in other sites in the GoT, where 60-100% of *Por. lutea* colonies hosted *Cladocopium* sp. (C15) and 20-40% hosted *C. goreaui*. Similarly, LaJeunesse *et al*. (2010) and Putchim (2017) found *Cladocopium* sp. (C15) to be associated with 75-90% of *Por. lutea* colonies in the Andaman Sea. In addition, Tanzil *et al*. (2016) found only *Cladocopium* sp. (C15) associated with *Por. lutea* at three shallow reefs around Singapore's southern islands (i.e. Pulau Tekukor, Kusu Island and Pulau Satuma).

In this study, *Poc. damicornis* with *D. glynnii* (D1) was found in both locations (inshore sites and offshore sites), whereas *D. glynii* (D1) was regularly found associated with *Pocillopora* inhabiting turbid and shallow habitats in the far tropical eastern Pacific (Pinzón and LaJeunesse, 2011). In addition, we observed a difference in species dominance at an inshore site in the eastern GoT (*D. glynnii* (D6), Samet Island) (Figures 3, 4). These dominant Symbiodiniaceae species in *Poc. damicornis* in the GoT differ from several locations in other Indo-Pacific areas (e.g., LaJeunesse *et al*., 2010; Stat *et al*.,2015; Tanzil *et al*., 2016). In particular, the prevalence of *D. glynnii* (D6) that was found alone at SM and found in combination with *Cladocopium* sp. (C15) at SS showed a difference from other sites in the GoT. Conversely, in the Andaman Sea *Poc. damicornis* was found associated with *Durusdinium* sp. (D5) (LaJeunesse *et al*., 2010). Interestingly, at Cape Panwa, an inshore reef in Phuket which has similar reef type to SM and SS, LaJeunesse *et al*. (2010) reported *D. glynnii* (D6) in combination with D1-4 (or D1-4-6) in *Poc. damicornis*. For comparison, in central GBR where there was high temperature and turbidity, C3h was the dominant symbiont (LaJeunesse *et al*., 2004).

The dominance of *D. glynnii* (D1) at the offshore sites in the GoT was unexpected as these sites are less turbid, less disturbed by human activities and possibly cooler than the inshore sites. The recent recolonization of *Poc. damicornis* in the GoT after the 2010 severe mass bleaching event (Sutthacheep *et al*., 2012; Kuanui *et al*., 2015) may explain this association. This species was severely bleached in 2010 and absent from many sites, but juvenile colonies reappeared in 2014 (Kuanui *et al*., 2015) and they tended to have increased bleaching resistance. A similar phenomenon occurred in the Andaman Sea (Putchim, 2017). The increased prevalence of *D. glynnii* (D1) in our

samples is probably due to the adaptation of new *Poc. damicornis* recruits after the 2010 bleaching, similar to *Poc. acuta* in Singapore (Tanzil *et al*., 2016).

CONCLUSION

Symbiodiniaceae fingerprints, inferred from ITS2 haplotypes, in two scleractinian coral hosts, *Por. lutea* and *Poc. damicornis*, at nine sites in the GoT consisted of one or a combination of five dominant Symbiodiniaceae haplotypes. *Cladocopium* sp. (C15) was highly abundant in *Por. lutea*. while *D. glynnii* (D1) were prevalent in *Poc. damicornis*. Symbiodiniaceae species composition varied significantly by site and host species, and by the interactions between site and host species. Based on coral-Symbiodiniaceae association, the UPGMA dendrogram suggested three major groups, (1) *Por. lutea* colonies associated with *Cladocopium* sp. (C15) and its combination with less common haplotypes; (2) *Poc. damicornis* colonies associated with *D. glynnii* (D1); and (3) *Poc. damicornis* colonies associated with *D. glynnii* (D6). Despite multiple bleaching events and other selective pressures due to coastal influence on coral reefs, nearshore coral-Symbiodiniaceae associations can still maintain some diversity and resilience. However, recent drastic environmental changes may have altered coral-Symbiodiniaceae associations in 'less tolerant' reefs offshore, leading to lower overall diversity. For the long-term resilience of coral reefs in the region, the protection of these Symbiodiniaceae refugia nearshore as well as those 'less tolerant' offshore may provide such a safeguard.

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