Endosymbiont diversity and community structure in *Porites lutea* from Southeast Asia are driven by a suite of environmental variables

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Abstract

Many corals depend upon the highly specialised and intricate relationship they form with Symbiodiniaceae algal symbionts. *Porites lutea* is a massive reef-building coral found throughout Southeast Asia that hosts these endosymbionts obligately. Yet despite the prevalence and importance of *P. lutea* as one of the most dominant corals here, its associated Symbiodiniaceae communities have not been precisely characterised. In this study, we used high-throughput DNA amplicon sequencing of the nuclear internal transcribed spacer 2 (ITS2) to characterise the diversity, community structure and biogeographic distribution of Symbiodiniaceae in *P. lutea* throughout Singapore and Peninsular Malaysia. Consistent with previous studies, we found that *Cladocopium* was the most dominant genus among all samples, and *Cladocopium* C15 was the most dominant type (or subclade) with 100% occurrence in all samples from every study site. Results also revealed numerous Symbiodiniaceae types associated with *P. lutea* that were previously undetected in Southeast Asia. Endosymbiont diversity and community variation are driven by a combination of site-specific mean monthly cloud cover and variance in monthly sea surface temperature. This study contributes baseline data toward understanding differences in Symbiodiniaceae assemblages hosted by *P. lutea*, shedding light on how they might be indicative of particular environmental conditions and coral responses.

Keywords Algal-coral symbiosis · Biodiversity · Coral reef · ITS2 · Peninsular Malaysia · Scleractinia · Singapore · Zooxanthellae

1 Introduction

Southeast Asian marine ecosystems are some of the most biologically diverse on the planet (Burke et al. 2002), with 255 coral species recorded in Singapore and over 400 species known from Peninsular Malaysia (Huang et al. 2009; Affendi and

Rosman 2011; Huang et al. 2015). The coral reefs of Singapore and Malaysia yield tangible benefits through tourism, fisheries and research estimated at US\$563 million per year (Chou 2000). They provide critical ecological services, buffering against storm impact and mitigating the risk of flooding by reducing wave energy (Heery et al. 2018). However, these reefs are increasingly subjected to the negative impacts of coastal urbanisation and climate change (Fabricius 2005; Hoegh-Guldberg et al. 2007; Carpenter et al. 2008; Lough et al. 2018; Toh et al. 2018). Singapore's reefs, in particular, are threatened by decades of land reclamation and coastal development, resulting in high sedimentation rates and reduced light levels (Chou 1992; Dikou and Woesik 2006; Lai et al. 2015; Chow et al. 2019). In Malaysia, reefs are threatened by destructive fishing practices, increasing tourism, as well as sedimentation from logging activities, coastal urbanisation and agricultural runoff (Lau et al. 2019).

The persistence of coral reefs and associated communities depends critically on the mutualistic relationship formed between dinoflagellate endosymbionts and their coral hosts (Arif et al. 2014). These photosynthetic endosymbionts belong to

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the highly diverse family Symbiodiniaceae, more commonly known as zooxanthellae (LaJeunesse et al. 2018). The endosymbionts provide up to 90% of the nutritional requirements of their coral host through photoautotrophy (Muscattine and Porter 1977; Berkelmans and Oppen 2006), increase their host's ability to calcify, and promote the uptake of inorganic nutrients. In return, corals shelter their endosymbionts from grazers, the environment (Arif et al. 2014), and provide them with inorganic nutrients (Stat and Gates 2011). Consequently, Symbiodiniaceae play a critical role in coral reef ecosystems, and the expulsion of these symbionts during extended periods of stress-induced coral "bleaching" can cause widespread mortality of corals and the degradation of coral reef ecosystems (Baker 2003; Stat and Gates 2011; Hughes et al. 2017, 2018).

Reef-building stony corals (Cnidaria: Anthozoa: Scleractinia) associate with a wide range of Symbiodiniaceae taxa (Berkelmans and Oppen 2006). Nine different genus-level clades are currently recognised, with each clade consisting of a number of subclades or types (LaJeunesse et al. 2004a, 2018; Tanzil et al. 2016). Different endosymbionts are associated with varying tolerances to different environmental conditions (Rouzé et al. 2016). For example, the genera *Symbiodinium* (formerly clade A) and *Breviolum* (formerly clade B) are more prevalent in shallow-water corals which typically experience higher irradiance (Chen et al. 2003), while *Cladocopium* (formerly clade C), though widespread, has been found in corals living at greater depth and light-attenuated environments (Innis et al. 2018). *Durisdinium* (formerly clade D) has been linked to an increased thermal tolerance among host colonies and is thought to be indicative of marginal reef environments (Chen et al. 2003; Stat and Gates 2011; Rouzé et al. 2016). Particular associations between the coral host and endosymbionts can provide ecological advantages in different environments (Berkelmans and Oppen 2006; Fisher et al. 2011), and thus the specific composition of Symbiodiniaceae types living in a coral colony can impact the capacity of the coral host to thrive in its habitat (Muller-Parker et al., 2015).

The factors driving heterogeneity of endosymbiont associations among coral populations are complex (Innis et al. 2018). Previous studies have shown that Symbiodiniaceae diversity in corals is influenced by a variety of biotic and abiotic environmental factors, including (but not limited to) thermal stress, irradiance, freshwater input, salinity, sedimentation, types of symbionts present in the environment, as well as host identity and physiology (Baker 2003; Finney et al. 2010; Kennedy et al. 2016; Ziegler et al. 2017). These effects are in turn influenced by larger scale processes such as ocean circulation in addition to regional and global climate cycles (Stenseth 2002). Consequently, the spatial and geographic distribution of host corals can structure endosymbiont communities. For example, *Cladocopium* is dominant throughout tropical locations while *Symbiodinium*, *Breviolum* and

Fugacium (formerly clade F) are more prevalent at higher latitudes (Baker 2003), while *Breviolum* is dominant across the Caribbean but is rare or absent in the Indo-Pacific (Lewis et al. 2018). Similarly, thermal history can strongly influence Symbiodiniaceae clade composition (Chen et al. 2003; Fitt et al. 2009; Fisher et al. 2011; Stat and Gates 2011; Hume et al. 2013; Hauff et al. 2016).

Porites lutea is an ecologically important and widespread coral species that contributes substantially to the formation and maintenance of coral reefs throughout the Indian and Pacific Oceans (Stat and Gates 2011; Tanzil et al. 2013). The composition of Symbiodiniaceae communities hosted by this species is known from Japan, Persian Gulf, Red Sea, northern South China Sea, Gulf of Thailand and the Great Barrier Reef (LaJeunesse et al. 2004a; Hume et al. 2013; Gong et al. 2018; Chankong et al. 2019; Terraneo et al. 2019). However, despite the high levels of biodiversity in Southeast Asian marine ecosystems, only limited work using denaturing gel gradient electrophoresis (DGGE) – which is limited in its ability to detect symbiont types of low abundance – has been performed in Singapore (Tanzil et al. 2016), and currently no data exist for corals from Peninsular Malaysia.

In this study, we identified Symbiodiniaceae taxa within *Porites lutea* collected from Singapore and around Peninsular Malaysia, and sought to verify whether *Cladocopium* is the dominant genus of endosymbionts as previously identified by Tanzil et al. (2016). We also assembled environmental data from throughout the region (sea surface temperature and mean cloud cover) to establish any correlation with Symbiodiniaceae diversity and community structure.

2 Methods

2.1 Sample collection and DNA extraction

Between 14 and 21 tissue samples measuring approximately 3 cm² were collected from visibly healthy *Porites lutea* colonies at each of 15 reef sites across Singapore, eastern and western Peninsular Malaysia (Fig. 1). As per Wainwright et al. (2019), tissues were placed in individual sealed containers that remained in the shade. All samples were immersed in water to minimise thermal stress and preserved in 100% molecular-grade ethanol within six hours of collection. DNA was extracted with a QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's recommended protocol.

2.2 Polymerase chain reaction and MiSeq library preparation

Polymerase chain reactions (PCRs) were carried out to amplify the nuclear ribosomal internal transcribed spacer 2 (ITS2) region (LaJeunesse 2002; Arif et al. 2014) using the forward

Fig. 1 Sample collection sites throughout Singapore and Peninsular Malaysia

primer ITSintfor2 (5′–GAATTG CAG AAC TCC GTG–3′) (LaJeunesse and Trench 2000), and reverse primer ITS-Reverse (5′–GGG ATC CAT ATG CTT AAG TTC AGC GGG T–3′) (Coleman et al. 1994). Unique 8 base-pair (bp) barcodes were generated using Barcode Generator 2.8 (Comai and Howell 2012) and attached to the 5′-end of each primer pair for multiplexed sequencing. PCR was performed in a 25 µl reaction volume containing 12.5 µl of GoTaq® Green Master Mix (Promega Corporation), 8.5 µl of water, 1 µl each of reverse and forward primers at 10 µM, and 2 µl of DNA (diluted 1:10). Thermocycling followed the profile of Lim et al. (2019), with 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 45 s at 72 °C, and a final extension at 72 °C for 3 min. PCR products were purified with Sera-Mag beads (GE Healthcare). PCR products were normalised and pooled into four separate libraries, with unique 8-bp barcodes in each library. Negative PCR controls without template DNA were included to check for potential contamination. Libraries were sequenced at the Genome Institute of Singapore on the Illumina MiSeq platform using the MiSeq Reagent Kit v2 to generate 2x250bp reads.

2.3 Bioinformatics

Raw data were processed following the pipeline of Sze et al. (2018). Briefly, the Paired-End reAd mergeR (PEAR) program was used to align and merge paired-end reads with overlapping regions of at least 100-bp (Zhang et al. 2013). The following steps were performed in OBITools version 1.2.0 (Boyer et al. 2016): (1) *ngsfilter* was used to assign reads to individual samples and remove primer sequences; (2)

obiannotate was used to label sequences according to sample number and sequence length; (3) *obisplit* then split each sequence file into a set of subfiles according to their sample numbers; (4) de-replication was carried out using *obiuniq*, and lastly (5) *obigrep* was used to filter out sequences represented by single reads as these are indicative of possible amplification or sequencing errors.

Sequences were then searched against the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST) to positively identify Symbiodiniaceae subclades present in each *P. lutea* sample, based on sequence similarity of $\geq 97\%$ and E-value $\leq 10^{-5}$. Sequences that were not matched to a GenBank record with type-level identity following the above criteria were discarded.

2.4 Environmental parameters

Monthly sea surface temperature (SST) and monthly SST variance in °C were obtained for each site for the months of January 2017 to December 2018 using the NOAA Coral Reef Watch database (<https://coralreefwatch.noaa.gov/vs/data.php>), which provides global SST measurements via satellite data at a resolution of 5 km. Monthly cloud cover (%) was collected for each site using global cloud cover data measured between 2000 and 2014 from Wilson and Jetz (2016), with a resolution of 1 km (SI Table 1).

2.5 Statistical analyses

For each coral sample, endosymbiont type (subclade) richness, Shannon diversity index and proportional abundance

were characterised. Proportional abundance data were consolidated in a community matrix. The effects of different environmental factors—mean monthly SST, monthly SST variance and mean monthly cloud cover, as well as their interactions—on Shannon diversity were estimated using a generalised linear model (GLM). The Akaike information criterion (AIC) was used for model selection following stepwise simplification of the predictors.

To visualise variation among coral hosts in terms of their endosymbiont community structure, non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity was performed using the *vegan* package in R (Oksanen et al. 2018; R Core Team, 2013). Vectors indicating the effects of mean monthly SST, monthly SST variance and mean monthly cloud cover were fitted onto the ordination. Analysis of Similarities (ANOSIM; 999 permutations) was performed using the Bray-Curtis dissimilarity measure to determine if there were differences in Symbiodiniaceae community structure among regions (i.e. Singapore, eastern and western Peninsular Malaysia).

In addition to the primary dataset which excluded sequences with single reads, a secondary dataset with a more stringent minimum three-read count criterion was assembled to examine the potential effects of false positives, a possible but unlikely consequence of DNA sequencing errors that could be retained in our primary dataset.

3 Results

Quality-filtered reads from all four libraries were combined after Illumina MiSeq sequencing, and redundant reads were collapsed (via *obiuniq*). A total of 42,132,578 assembled paired-end reads were retained, representing a total of 270 samples in the primary dataset (SI Table 2). There were 234 samples in the minimum-three-read secondary dataset. Unless otherwise stated, results and discussions are based on the primary dataset, with any differences highlighted.

A total of 23 distinct Symbiodiniaceae types (subclades) were identified across all samples ($n = 270$) (Table 1). For Singapore samples ($n = 153$), a total of 21 distinct types were identified, with 3 *Symbiodinium*, 13 *Cladocopium* and 5 *Durisdinium* types. For samples from eastern Peninsular Malaysia ($n = 84$), a total of 14 distinct types were identified, with 2 *Symbiodinium*, 9 *Cladocopium* and 3 *Durisdinium* types. For western Peninsular Malaysia samples ($n = 33$), a total of 17 distinct types were identified, with 3 *Symbiodinium*, 11 *Cladocopium* and 3 *Durisdinium* types.

Consistent with previous work in Southeast Asia (LaJeunesse et al. 2010; Leveque et al. 2019; Qin et al. 2019), the top three most common types identified in host colonies from all three regions belonged to the genus *Cladocopium* (Table 1), though the specific composition

differed between regions (Fig. 2 & SI Fig. 1). *Cladocopium* C15 was the most common type identified in all regions and was present in all host colonies sampled (Fig. 2 and SI Fig. 1).

Subclade richness was highest in Singapore, followed by western and then eastern Peninsular Malaysia (Table 1). NMDS and ANOSIM performed on both datasets did not show community structuring or significant differences in endosymbiont diversity between regions (Fig. 3 & SI Fig. 2, ANOSIM minimum-two-read dataset $R = 0.042$, $p = 0.098$; ANOSIM minimum-three-read dataset $R = -0.035$, $p = 0.838$).

For both datasets, the optimum GLM as determined with AIC was ‘Shannon index ~ Monthly SST variance * Mean monthly cloud cover’. For the minimum-two-read dataset all factors were significant, while none were significant at the arbitrarily-defined significance threshold of 0.05 in the more stringent minimum-three-read dataset. However, monthly SST variance and the interaction between monthly SST variance and mean monthly cloud cover were marginally significant ($p = 0.071$ and $p = 0.062$ respectively) (Table 2, SI Table 3). The difference between model results was likely due to the reduction in sample size from 270 in the minimum-two-read dataset to 234 in the minimum-three-read dataset, weakening statistical power. Despite this difference, ANOSIM and NMDS were in agreement for both datasets (Fig. 3 & SI Fig. 2).

4 Discussion

In this first study to comprehensively characterise Symbiodiniaceae communities in *Porites lutea* from Peninsular Malaysia and Singapore, we document numerous endosymbiont types that hitherto have not been detected in Southeast Asia. *Cladocopium* C15 is the most dominant type and present in all corals from all sampled sites. This dominance pattern in *P. lutea* corals from Singapore is consistent with the findings of Tanzil et al. (2016), which identified subclade C15 as the only type present in *P. lutea* corals based on DGGE. However, our study demonstrates that a greater diversity of subclades within *Symbiodinium*, *Cladocopium* and *Durisdinium* are hosted by corals from Singapore than previously reported. More broadly in Southeast Asia and in agreement with our work, *Cladocopium* C15 has recently been identified as the dominant endosymbiont associated with *P. lutea* in the Gulf of Thailand (Chankong et al. 2019).

Mean cloud cover, a proxy for irradiance levels, monthly SST variance, and the interaction between these two factors are significant predictors of endosymbiont diversity in *P. lutea*. Model estimates indicate that higher SST variance leads to lower diversity (Table 2), and correspondingly, eastern Peninsular Malaysia has the lowest richness of endosymbionts (Table 1). This pattern is consistent with previous

Table 1 List of subclades identified in *Porites lutea* colonies from Singapore, eastern Peninsular Malaysia (PM) and western PM

Genus	List of subclades identified			
	All regions	Singapore	eastern PM	western PM
<i>Symbiodinium</i>	A3	✓	✓	✓
	A3c	✓	-	✓
	A6	✓	✓	✓
<i>Cladocopium</i>	C1	✓**	✓*	✓*
	C114	✓	✓	✓
	C116	✓	✓**	✓**
	C15	✓***	✓***	✓***
	C161	✓	✓	✓
	C162	✓	✓	✓**
	C19	✓	✓	✓
	C21	✓	-	-
	C3	✓*	✓	✓
	C35a	✓	-	-
	C4	✓	-	-
	C60	✓	✓	✓
	C69a	✓	-	-
	C80	-	-	✓
	C97	-	-	✓
<i>Durusedinium</i>	D1	✓	✓	✓
	D1a	✓	✓	✓
	D1b	✓	-	-
	D2	✓	-	-
	D5	✓	✓	✓
Total	23	21	14	17

Asterisks indicate the most commonly found subclades in that region, with (***) denoting most common, (**) denoting second-most common, and (*) denoting third-most common

studies showing that SST variance plays a significant role in influencing the diversity of coral endosymbionts (Baker and Romanski 2007; Keshavmurthy et al. 2017). It is conceivable that higher SST variance exerts a selective pressure against endosymbiont types that have lower tolerance for temperature

fluctuations or those that are less heat-resistant (Ziegler et al. 2017). Consequently, endosymbiont richness is reduced throughout eastern Peninsular Malaysia where SST variance is highest. Similar ideas have been used to explain the absence of endosymbiont subclades in environments where they may

Fig. 2 Symbiodiniaceae subclade prevalence in *Porites lutea* colonies based on proportional read abundance from Singapore, eastern and western Peninsular Malaysia (PM). Error bars indicate standard deviation

Fig. 3 NMDS based on proportional read count of individual subclades of the primary dataset (minimum read count = 2); each point represents a coral colony sample (stress = 0.0717). Ellipses show how samples from each of the three regions (Singapore, eastern and western PM) are clustered based on a 95% confidence interval. Length of each vector indicates the amount of influence the environmental factor has in driving variation in subclade communities, with longer vectors indicating greater influence

perform sub-optimally relative to others (Chen et al. 2003; Toller et al. 2001; Stat and Gates 2011).

The influence of mean cloud cover on Symbiodiniaceae diversity is likely due to the effects of irradiance on the photosynthetic abilities of endosymbionts, and consequently the types of endosymbionts taken up by the coral. Studies show that symbiotic associations are, at least to some extent, influenced by the abilities of specific symbionts to exploit different photic environments (Iglesias-Prieto & Trench, 1994). For example, *Cladocopium* is more prevalent on shaded sides of *Montastraea* coral colonies indicating its potential role as a “shade specialist” (Rowan 1998; Savage et al. 2002; Innis et al. 2018). Although the Shannon diversity pattern is not reflective of this effect (Table 2, SI Table 3), our richness data appear to lend support since *P. lutea* found in the extremely turbid and light-attenuated waters of Singapore (Chou 1992;

Dikou and Woesik 2006; Chow et al. 2019) harbours the highest number of *Cladocopium* types overall (Table 1).

Durussinium richness is also highest in Singapore. It is well-established that *Durussinium* species are tolerant against thermal stress (Chen et al. 2003; Gong et al. 2018; Brian et al. 2019). Studies also suggest that the genus is a possible indicator of marginal reef environments and that its members tend to be more prevalent on reefs with higher levels of environmental disturbance (Chen et al. 2003; Stat and Gates 2011). The close proximity of coral reefs in Singapore to a large urban population and Singapore’s role as a major maritime shipping and petrochemical processing hub (Chou 2006; Heery et al. 2018) likely create stresses and disturbances that are not present in other regions (Poquita-Du et al. 2019). On the one hand, subclades D1b and D2 are not associated with *P. lutea* outside of Singapore possibly because they have been excluded by other endosymbionts better adapted to less stressful environments. In more stressful environments, on the other hand, D1b and D2 may be at a competitive advantage (Chen et al. 2003; Toller et al. 2001), thus accounting for the higher richness of *Durussinium* in the more impacted waters of Singapore.

The processes structuring coral symbiont communities can be complex and wide-ranging (Archer et al. 2019), in addition to temperature and cloud cover, Symbiodiniaceae assemblages can be influenced by variables including water chemistry, oceanic processes, sedimentation and pollution (Stat and Gates 2011; Yang et al. 2012; Keshavmurthy et al. 2017; Gong et al. 2018). Seasonally, the varying effects of the northeast monsoon (December to March) and southwest monsoon (June to September) can have different biophysical influences on the three studied regions. The east coast of Peninsular Malaysia has direct exposure to the South China Sea and the northeast monsoon, making it highly susceptible to flooding and freshwater runoff during this period (Varikoden et al. 2011). In comparison, the west coast of Malaysia is reasonably sheltered from southwest monsoonal storms by Sumatra. Furthermore, annual rainfall in eastern Peninsular Malaysia is becoming increasingly variable and torrential (Varikoden et al. 2011; Mayowa et al. 2015). The environmental differences on either side of Peninsular Malaysia are likely responsible, or at least contributing factors that are responsible, for generating the observed disparity in endosymbiont richness between the two regions.

Table 2 Summary of optimal GLM model: Shannon index ~ Monthly SST variance

	Estimate	Std. Error	p-value
(intercept)	55.2193	16.4637	0.0009
Monthly SST variance	-101.9227	29.7158	0.0007
Mean monthly cloud cover	-0.7746	0.2265	0.0007
Monthly SST variance:Mean monthly cloud cover	1.4021	0.4126	0.0008

*Mean monthly cloud cover

5 Conclusion

Congruent with other work performed in the Indo-Pacific, our research shows that *Cladocopium* is the dominant endosymbiont genus hosted by *Porites lutea*, with 100% of sampled colonies containing *Cladocopium* C15 (LaJeunesse et al. 2004b). However, through the use of high-throughput DNA sequencing techniques we show that many more, previously undetected Symbiodiniaceae types are present in *P. lutea* from Singapore and Peninsular Malaysia.

Considering that *Porites lutea* is a major reef-building coral in Singapore, Peninsular Malaysia and throughout Southeast Asia, this study contributes important baseline information with which we can begin to understand how corals acclimatise and adapt to changing environmental pressures through adjustments in their endosymbiont communities. Long-term replicated studies conducted at regular intervals on Southeast Asian reefs will provide greater insights into the ways distinct endosymbiont assemblages reflect different environmental conditions. Ultimately, research of this nature allows us to understand, predict, respond to, and manage potential coral bleaching events in this highly biodiverse marine region.

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Compliance with ethical standards

Ethical approval All applicable permits, international, national, and/or institutional guidelines required to perform the work were followed. Collections from Malaysia were made under permit JTLM 630-7Jld.9(9) and from Singapore under permit numbers NP/RP16–156 and NP/RP18–035.

Conflict of interest The authors declare no conflicts of interests.

Data availability statement The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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