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Endosymbiont Communities in *Pachyseris speciosa* Highlight Geographical and Methodological Variations

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Reef-building corals live in symbiosis with the phototrophic dinoflagellate family Symbiodiniaceae, which comprises diverse genera such as *Cladocopium* and *Durusdinium*. *Pachyseris speciosa*, a widely distributed Indo-Pacific coral found in a variety of reef habitats, is known to be associated with these two Symbiodiniaceae genera, but little is known about the biogeographic variability of the endosymbiont communities across the region. In this study, the diversity and dominance patterns of Symbiodiniaceae at the western and eastern areas of the Central Indo-Pacific region were examined. We sampled *Pachyseris speciosa* colonies at seven and six sites in Singapore and Papua New Guinea, respectively, and genotyped their endosymbionts based on the internal transcribed spacer (ITS) markers using two distinct methods, quantitative polymerase chain reaction (qPCR) and high-throughput sequencing (HTS). Results showed 92% of all colonies in Singapore exhibiting *Cladocopium* dominance. There was a higher abundance of *Durusdinium* compared to *Cladocopium* in certain colonies from one site, Pulau Hantu (mean *Durusdinium* abundance of 90%, compared to 0–14% among all other sites). In contrast, variation in the endosymbiont communities was generally higher among sites in Papua New Guinea. *Cladocopium* expectedly dominated most colonies (75%), although colonies from Kimbe Bay (85%) and Kavieng (65%) showed *Durusdinium* dominance. Between localities, relative genus abundances based on qPCR were not significantly different, but HTS showed that the ratio of *Durusdinium* over *Cladocopium* was significantly higher in Papua New Guinea corals. Notably, 6% of colonies from Singapore and 15% from Papua New Guinea showed endosymbiont dominance patterns that were inconsistent between the two methods, underscoring the need for further validation of symbiotic algal quantification based on HTS. The richness of ITS2 type profiles was clearly lower among colonies from the impacted and turbid reefs of Singapore compared to the less urbanized

reefs of Papua New Guinea. These coral intraspecific variations of Symbiodiniaceae communities within and among localities suggest that local conditions are important drivers of endosymbiosis and may ultimately influence corals' resilience against global stressors such as ocean warming.

Keywords: Central Indo-Pacific, high-throughput sequencing, internal transcribed spacer, qPCR, reef corals, Scleractinia, Symbiodiniaceae

INTRODUCTION

Reef-building stony corals (Cnidaria: Anthozoa: Scleractinia) are associated with the dinoflagellate alga Symbiodiniaceae, forming an ecologically critical symbiotic relationship (Falkowski et al., 1984; Stanley and Fautin, 2001; Baker, 2003). The partnership yields multiple ecological benefits for both the coral host and algal endosymbiont, providing the basis of the tropical coral reef ecosystem and its survival. Symbiotic corals depend on the photosynthetic activities of their mutualistic dinoflagellates (Boilard et al., 2020; LaJeunesse, 2020), or Symbiodiniaceae, to produce the energy required for calcium carbonate skeletal growth of the coral hosts, ultimately driving coral reef development (Al-Horani et al., 2003). In return, *in hospite* Symbiodiniaceae cells are provided with a light-enriched environment and the by-products of host metabolism (Banin et al., 2003).

These extremely diverse microalgae are currently recognized as several phylogenetically distinct genera (LaJeunesse et al., 2018; Boilard et al., 2020). Reef-building stony corals typically host multiple Symbiodiniaceae species and are most commonly associated with four genera (*Symbiodinium, Breviolum, Cladocopium*, and *Durusdinium*) (Rowan and Knowlton, 1995; Baker, 2003; Little et al., 2004; Stat et al., 2008b). The endosymbiont community may grant varying degrees of fitness to the coral host depending on the holobiont's capability to tolerate environmental stressors and bleaching (Baker, 2001; Stat et al., 2011).

Symbiodiniaceae endosymbiont community composition has been shown to vary between biogeographic regions, host species, reef depth, light accessibility and shifting environmental dynamics, among other factors (Rowan and Knowlton, 1995; Rowan et al., 1997; Stat et al., 2008a; Rouzé et al., 2016; Eckert et al., 2020; Hume et al., 2020). In particular, the diversity of Symbiodiniaceae in various geographic locations and host taxa has been studied extensively (LaJeunesse, 2002; LaJeunesse et al., 2004; Huang et al., 2011; Keshavmurthy et al., 2014; Hume et al., 2015; Leveque et al., 2019; Terraneo et al., 2019; Teschima et al., 2019; Tan et al., 2020). For example, latitudinal variation in Symbiodiniaceae genus dominance has been observed in the Saudi Arabian Red Sea, with shifts from *Cladocopium* dominance to *Durusdinium* dominance in *Porites* colonies along a north-south gradient (Terraneo et al., 2019; see also Chen et al., 2019). Fundamentally, with *Cladocopium* and *Durusdinium* known to be the most abundant endosymbionts in tropical reefs, corals hosting *Durusdinium* were initially thought to be more tolerant against thermal stress and turbidity fluctuations (Stat and Gates, 2011; Silverstein et al., 2015), whereas *Cladocopium* was generally considered the more stress-sensitive genus (Chen et al., 2003; Stat and Gates, 2011). However, it is now well understood that *Cladocopium* is found in corals throughout a large depth range and among multiple host species, and even when present in marginal conditions, there may be taxon-specific associations and local adaptations that result in it being the dominant genus (Hume et al., 2015; Rouzé et al., 2016; Innis et al., 2018). For example, corals in the Red Sea exhibit strong species-specific associations and display *Cladocopium* dominance despite a strong latitudinal gradient and facing high temperatures (Ziegler et al., 2017; Osman et al., 2020). Similarly, corals withstanding extreme temperatures in the Persian Gulf maintain predominant associations with *Cladocopium* (Howells et al., 2020).

The diversity and functioning of specific coral-Symbiodiniaceae relationships rely on the sensitivity of symbionts to thermal stress, irradiance and/or host-specificity (LaJeunesse et al., 2004). The composition of the symbionts within the host can be influenced by changing environments and driven by various host or symbiont processes (Kemp et al., 2014). Community alterations are achieved via "switching" (changes in the endosymbiont community composition via exchange of symbionts from the environment) or "shuffling" (shifts in the relative abundances of present symbionts) (Baker, 2003). The holobiont's capacity to undergo these "switching" or "shuffling" processes is vital for its resilience against environmental stress (Baker, 2004; Sampayo et al., 2008; Kemp et al., 2014). More generally, the persistence of coral reefs and the survival of its associated organisms depend greatly on the responses of the coral-Symbiodiniaceae relationship to environmental change (Arif et al., 2014). Seeing as temperature anomalies due to global warming are predicted to occur more frequently (Hughes et al., 2017a,b), studying the diversity of symbionts in different regions is critical for understanding geographic variations in coral reef persistence.

The zooxanthellate coral, *Pachyseris speciosa*, is one of the most common and abundant corals found in the Central Indo-Pacific region with a wide distribution through various depths (DeVantier and Turak, 2017; Bongaerts et al., 2021). Symbiodiniaceae communities hosted by *P. speciosa* have been examined in the Great Barrier Reef, Red Sea, French Polynesia, and Singapore (LaJeunesse et al., 2004; Bongaerts et al., 2011; Ziegler et al., 2015b; Tanzil et al., 2016; Goulet et al., 2019; Meistertzheim et al., 2019; Jain et al., 2020; Smith et al., 2020). However, little is known about the biogeographic variabilities of its endosymbionts across the Central Indo-Pacific as comparisons among localities are limited.

Despite chronically high sediment deposition, turbidity and low light levels, the hyper-urbanized shores of Singapore have

been home to 255 stony coral species (Huang et al., 2009). *Pachyseris speciosa* is one of the most common corals dominating the shallow reefs here (Wong et al., 2018; Chow et al., 2019) and is associated with *Cladocopium* and *Durusdinium* (Tanzil et al., 2016; Jain et al., 2020; Smith et al., 2020). By contrast, Papua New Guinea, which is generally much less affected by urbanization, has a greater coral diversity and is part of the Coral Triangle (Allen et al., 2003; Veron et al., 2011, 2015). Its reefs hold more than 500 stony species that are of vital importance to the local communities and their self-sustainable lifestyles (Allen et al., 2003; Nicholls, 2004; The Nature Conservancy, 2004). Apart from a study investigating the response of endosymbiont communities to elevated carbon dioxide, little is known about the Symbiodiniaceae communities associated with *P. speciosa* here (Noonan et al., 2013).

In this study, we characterize the relative abundances of *Cladocopium* and *Durusdinium* across various sites in Singapore and Papua New Guinea using two distinct quantification methods, quantitative polymerase chain reaction (qPCR) and high-throughput sequencing (HTS). The universal presence and known association of *Pachyseris speciosa* with *Cladocopium* and *Durusdinium* allow their relative abundances to be examined among coral colonies from the two regions. Here, we quantify genus abundances and dominance by the relative concentrations of the nuclear ribosomal internal transcribed spacer 1 (ITS1) region for *Durusdinium* and *Cladocopium* as evaluated by qPCR, and by the relative HTS read abundances of the internal transcribed spacer 2 (ITS2) region for the two genera. In accordance with the SymPortal framework (Hume et al., 2019), we also report on the ITS2 type profiles of *P. speciosa* that account for intragenomic variation and are representative of putative Symbiodiniaceae species. Our data on the relative abundances of the endosymbiont taxa between the western and eastern Central Indo-Pacific highlight the regional biogeographic drivers of similarities and differences in Symbiodiniaceae diversity. Furthermore, a sympatric species complex representing three distinct lineages of *P. speciosa* has been discovered recently (Bongaerts et al., 2021; Feldman et al., 2021). Using a cleaved amplified polymorphic sequence (CAPS) assay, we uncover the different *P. speciosa* lineages from our collections and determine whether they affect Symbiodiniaceae genus dominance. Additionally, we test the interchangeability of using qPCR and HTS to detect relative abundances of *Cladocopium* and *Durusdinium* based on a larger and geographically wider set of samples relative to our previous study (Jain et al., 2020).

MATERIALS AND METHODS

Field Collections

Reefs at Singapore and Papua New Guinea were accessed via SCUBA and *Pachyseris speciosa* colonies were haphazardly sampled using a hammer and chisel. A total of 266 colonies were sampled, including 146 from seven different sites in Singapore between 25th and 31st May 2018. Between 18 and 25 samples were collected each from Terumbu Pempang Tengah (1°13′45.1″N 103°43′50.5″E), St. John's Island (01°13′09.0″N 103°50′44.0″E), Pulau Semakau (01°12′11.5″N 103°45′15.0″E), Sisters' Island (or Pulau Subar Laut; 01°12′49.0″N 103° 49′59.0″E), Pulau Hantu (01°13′36.5″N 103°44′47.0″E), Raffles Lighthouse (or Pulau Satumu; 01°09′36.5″N 103°44′24.0″E) and Kusu Island (or Pulau Tembakul; 01°13′33.0″N 103°51′34.0″E). From Papua New Guinea, 120 samples were taken, comprising 20 colonies from each of six sites – Kimbe Bay (5°09′55.2″S 150°30′00.2″E), Rabaul (4°12′13.9″S 152°09′54.6″E), Kavieng (2°33′02.1″S 150°47′42.2″E), Madang (5°13′54.0″S 145°48′05.3″E), Milne Bay (10°25′50.5″S 150°43′13.4″E) and Port Moresby (9°27′54.0″S 147°08′34.5″E) – between 1st and 28th September 2018 (**Figure 1**). All sampled fragments were transported to the laboratory in seawater and fixed in 100% molecular grade ethanol, or stored in 20% salt-saturated DMSO immediately following collection.

DNA Extraction

Total genomic DNA was extracted from all the 146 Singapore samples and 120 Papua New Guinea samples using the DNeasy Blood and Tissue Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's protocol. A 2–4 cm^2 fragment of coral tissue was used for each sample extraction. Fragments were cut into smaller pieces using sterile forceps and scissors prior to addition of buffers. DNA was eluted in 150 μL nuclease-free water. Extracted DNA samples were stored at −80°C until further processing.

Quantitative Polymerase Chain Reaction

The ITS1 region, located between the 18S and 5.8S rRNA genes, was targeted for the quantification of relative *Cladocopium* and *Durusdinium* rDNA copy numbers. Two samples from a previous study were used to generate concentration standards for the ITS1 amplicons of *Cladocopium* and *Durusdinium* (Jain et al., 2020). The amplicons were quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, MA, United States) with the Life Technologies Qubit 3.0 Fluorometer (Thermo Fisher Scientific, MA, United States) and then diluted to 50 ng/μL. For amplification of *Cladocopium*, the universal forward primer [5′–AAG GAG AAG TCG TAA CAA GGT TTC C–3′ (Ulstrup and Van Oppen, 2003)] and *Cladocopium*-specific reverse primer [5′–AAG CAT CCC TCA CAG CCA AA–3′ (Ulstrup and Van Oppen, 2003)] were used. For *Durusdinium*, the universal forward primer and the *Durusdinium*-specific reverse primer [5′–CAC CGT AGT GGT TCA CGT GTA ATA G–3′ (Ulstrup and Van Oppen, 2003)] were used. The amplification profile consisted of an initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 30 s, 53°C for 45 s, 72°C for 45 s and a 5 min extension step at 72°C. The size of the PCR products (∼100 bp) was confirmed with gel electrophoresis. The products were then purified using SureClean Plus (Bioline Inc., London, United Kingdom) and quantified with the Qubit assay as mentioned above. Amplicon concentration standards were prepared by diluting the purified PCR product to 1 ng/μL, and then serially diluting to get 10 standards (1 to 10^{-9} ng/μL) each for *Cladocopium* and *Durusdinium*. All standards were run alongside each qPCR experiment.

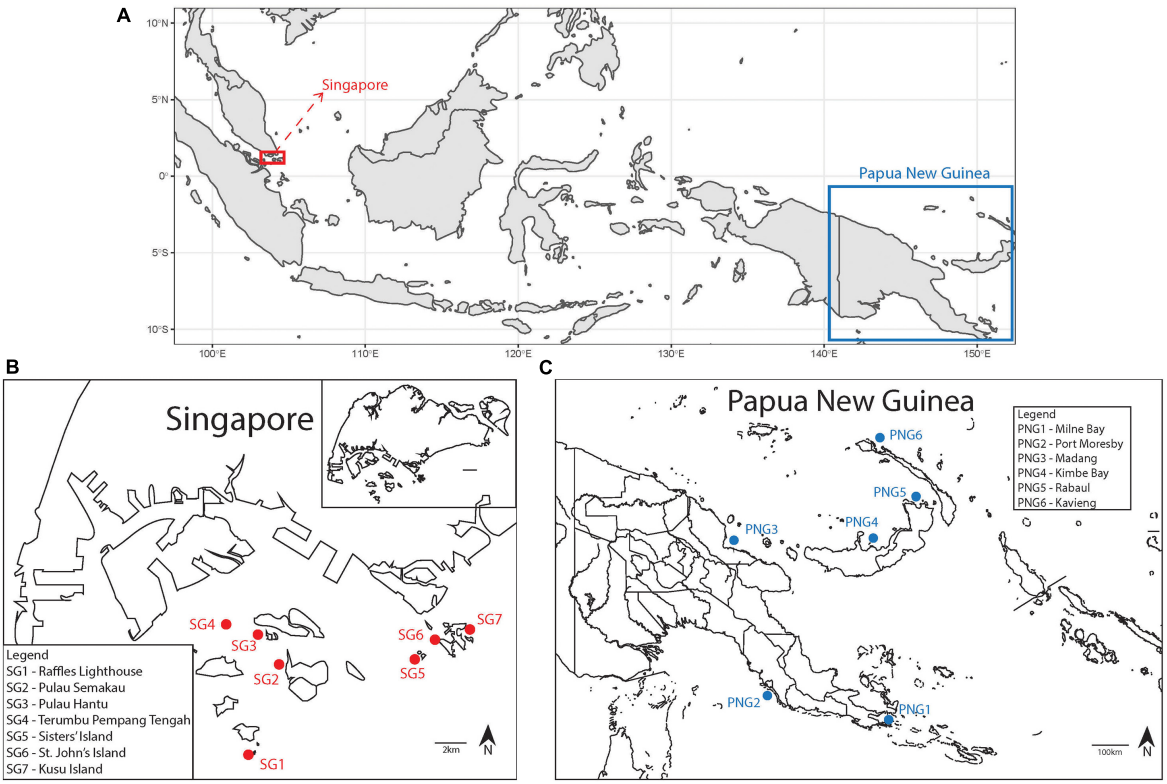

FIGURE 1 | (A) Map showing Singapore and Papua New Guinea. **(B)** Map of Singapore with study sites (SG1 to SG7) indicated in red (inset: Map of the entire Singapore island for reference). **(C)** Map of Papua New Guinea with study sites (PNG1 to PNG6) indicated in blue.

All DNA extracts were quantified and diluted to 1 ng/μL. SsoAdvanced SYBR Green SuperMix (Bio-Rad, CA, United States), the above-mentioned primers (Ulstrup and Van Oppen, 2003) and diluted standardized DNA template were used for qPCR using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, CA, United States). All standards and samples were run in triplicates. The thermocycling profile used consists of an initial denaturation at 98°C for 3 min followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. A melt curve analysis from 65 to 95°C (0.5°C increase per 5 s) was subsequently performed to verify the specificity of the amplification.

CFX Manager Software (Bio-Rad, CA, United States) was used to analyze the efficiency of standards and to enumerate the relative quantities of *Cladocopium* and *Durusdinium* (expressed as log-scaled ratio of *Durusdinium* concentration to *Cladocopium* concentration) in each sample based on the standard curve, following Jain et al. (2020).

Illumina Library Preparation, Sequencing and Bioinformatics

The ITS2 region, located between the 5.8S and 28S rRNA genes, was targeted for conventional PCR and HTS. All DNA extracts were quantified and diluted to 1 ng/μL. Reagent volumes, PCR cycling conditions and PCR clean-up protocols followed the 16S Metagenomic Sequencing Library Preparation guide for the Illumina MiSeq System (Illumina, 2013). This two-step protocol included an amplicon PCR (1st step) with the ITS2-specific SYM_VAR primer pair [SYM_VAR_5.8S2: 5′–GAATTGCAGAACTCCGTGAACC–3′ (Hume et al., 2015), SYM_VAR_REV: 5′–CGGGTTCWCTTGTYTGACTTCATGC–3′ (Hume et al., 2013)]. The index PCR (2nd step) added unique dual indexes (see **Supplementary Table 1**) and adapters complementary to the MiSeq flow cell for binding. Indexed PCR products were normalized using the SequalPrep™ Normalization Plate Kit (Thermo Fisher Scientific, MA, United States), pooled and sequenced on the Illumina MiSeq sequencing platform (V3 chemistry; 30% PhiX spike-in) for 300-bp paired-end reads.

Demultiplexed paired fastq.gz files containing Symbiodiniaceae ITS2 reads were analyzed using the SymPortal framework (Hume et al., 2019), run locally. Briefly, sequence filtering and a standardized quality control pipeline were conducted using mothur 1.39.5 (Schloss et al., 2009), the BLAST + suite (Camacho et al., 2009) and minimum entropy decomposition (MED; Eren et al., 2015).

Identifying Distinct Lineages of *Pachyseris speciosa*

Bongaerts et al. (2021) discovered distinct lineages within *Pachyseris speciosa* that represented a sympatric species complex and developed a CAPS assay to identify each of the lineages. The three lineages were referred to as "green," "blue" and "red"

(see also Feldman et al., 2021). To determine whether the different lineages influenced Symbiodiniaceae genus dominance in the corals studied here, we performed the CAPS assay on our *P. speciosa* samples.

DNA extracted from all the colonies from Singapore (n = 146) was assigned to one of the three lineages using the CAPS assay following Bongaerts et al. (2021). As we found no link between host lineage and Symbiodiniaceae genus dominance in the Singapore samples (see below), up to five randomly chosen samples from each site in Papua New Guinea (n = 21) were tested. Briefly, total DNA extracted from each sample was amplified using lineage-specific primers (PsPe-Green, PsPe-Blue and PsPe-Red) and purified using SureClean Plus. Purified products were then digested with the associated restriction enzyme (Green-*Hha*I, Blue-*Hae*III, and Red-Taqα1, respectively) and digestion verified on a 3% gel. Following enzymatic digestion, the "green" or "blue" lineage was determined with positive digestion observed using the associated *Hha*I and *Hae*III restriction enzyme. The "red" lineage was assigned to samples showing no digestion using the Taqα1 restriction enzyme. To verify digestion of PCR amplicons that could not be visualized through gel imaging, the purified PCR products were sequenced using Sanger sequencing. The sequences were analyzed in Geneious Prime 2020.2.4 (Kearse et al., 2012) and a virtual digest was performed to determine the lineages.

Statistical Analyses

Data were analyzed using R 3.6.1 (R Core Team, 2017). Two separate runs of the nested analysis of variance (ANOVA) were performed to assess the differences in ITS1 and ITS2 *Durusdinium* to *Cladocopium* ratios among sites in each locality and between the two localities. Specifically, the ratio of *Durusdinium* to *Cladocopium* ITS1 concentrations for each sample obtained from the qPCR experiments were compared between localities (Singapore vs. Papua New Guinea) with sites nested within localities. Separately, the ratios of *Durusdinium* to *Cladocopium* ITS2 HTS read abundances were also compared between the two localities with sites nested within localities. The Tukey test was further performed to resolve statistical differences between sites in each locality.

To determine if and how the qPCR tests and HTS reads were comparable in the relative *Durusdinium* vs. *Cladocopium* levels obtained, we ran a linear model to quantify the relationship between relative (*Durusdinium* divided by *Cladocopium*) ITS1 concentrations and ITS2 read abundances at the sample level. Data were log-transformed for normality and homoscedasticity.

To characterize the geographical variation of Symbiodiniaceae communities at different localities and sites, we report the ITS2 type profiles obtained from SymPortal.

RESULTS

Quantitative Polymerase Chain Reaction

The qPCR quantification of the relative abundances of *Durusdinium* vs. *Cladocopium* ITS1 in the 146 colonies sampled across Singapore showed limited variation among the seven sites examined (Terumbu Pempang Tengah, St. John's Island, Pulau Semakau, Sisters' Island, Pulau Hantu, Raffles Lighthouse and Kusu Island; p = 0.392, df = 6, F = 1.056; **Figure 2**). *Post hoc* Tukey tests further revealed insignificant pairwise site differences in the ratios of the relative abundances of *Durusdinium* vs. *Cladocopium* (p values ranging from 0.4 to 1).

Among the 120 Papua New Guinea colonies, the ITS1 ratios were significantly different between sites (Port Moresby, Madang, Milne Bay, Rabaul, Kavieng, and Kimbe Bay; $p < 0.001$, df = 5, F = 7.136) with *post hoc* Tukey tests revealing significantly greater proportions of *Durusdinium* in colonies sampled from Kavieng compared to the other four sites (Madang, Milne Bay, Rabaul and Port Moresby; $p < 0.001$). There were no significant differences in the relative abundances of *Cladocopium* and *Durusdinium* between samples from Madang, Milne Bay, Rabaul and Port Moresby. Overall, ITS1 *Durusdinium* to *Cladocopium* ratios between Singapore and Papua New Guinea were not significantly different (p = 0.494, df = 1, F = 0.470).

High-Throughput Sequencing

Illumina sequencing reads of the 146 *Pachyseris speciosa* colonies sampled across the seven sites in Singapore yielded a total of 19,721,036 raw reads, resulting in 9,860,518 contigs. Following quality control and sequence filtering, a total of 8,113,743 Symbiodiniaceae ITS2 sequences comprising 77,067 unique sequences were retained. The relative abundances of *Durusdinium* vs. *Cladocopium* ITS2 reads within the 146 coral colonies were not significantly different between the seven sites in Singapore (p = 0.118, df = 6, F = 1.732; **Figure 2**). *Post hoc* Tukey tests indicated higher proportions of *Durusdinium* in colonies from Pulau Hantu compared to Raffles Lighthouse (p = 0.131), although no statistically significant differences were detected among all sites. These statistical similarities and differences between the sites in Singapore were generally consistent with patterns obtained with ITS1 ratios.

Illumina sequencing reads of the 120 *Pachyseris speciosa* colonies sampled across the six sites in Papua New Guinea yielded a total of 20,018,361 raw reads, resulting in 10,365,381 raw contigs. Following quality control and sequence filtering, a total of 7,688,010 Symbiodiniaceae ITS2 sequences comprising 93,003 unique sequences were retained. The relative abundances of *Durusdinium* vs. *Cladocopium* ITS2 reads were significantly different among the six Papua New Guinea sites (p = 0.0032, df = 5, F = 3.801). *Post hoc* Tukey test showed larger proportions of *Durusdinium* detected in colonies from Kavieng compared to those from Madang, Milne Bay, Rabaul and Port Moresby ($p < 0.05$). There were no significant differences in the relative abundances of *Cladocopium* and *Durusdinium* between samples from Madang, Milne Bay, Rabaul and Port Moresby. Colonies from Kimbe Bay and Kavieng had similar proportions of *Durusdinium.* These results were consistent with the relative abundances derived from qPCR. However, the ITS2 *Durusdinium* vs. *Cladocopium* ratios based on HTS showed significant differences

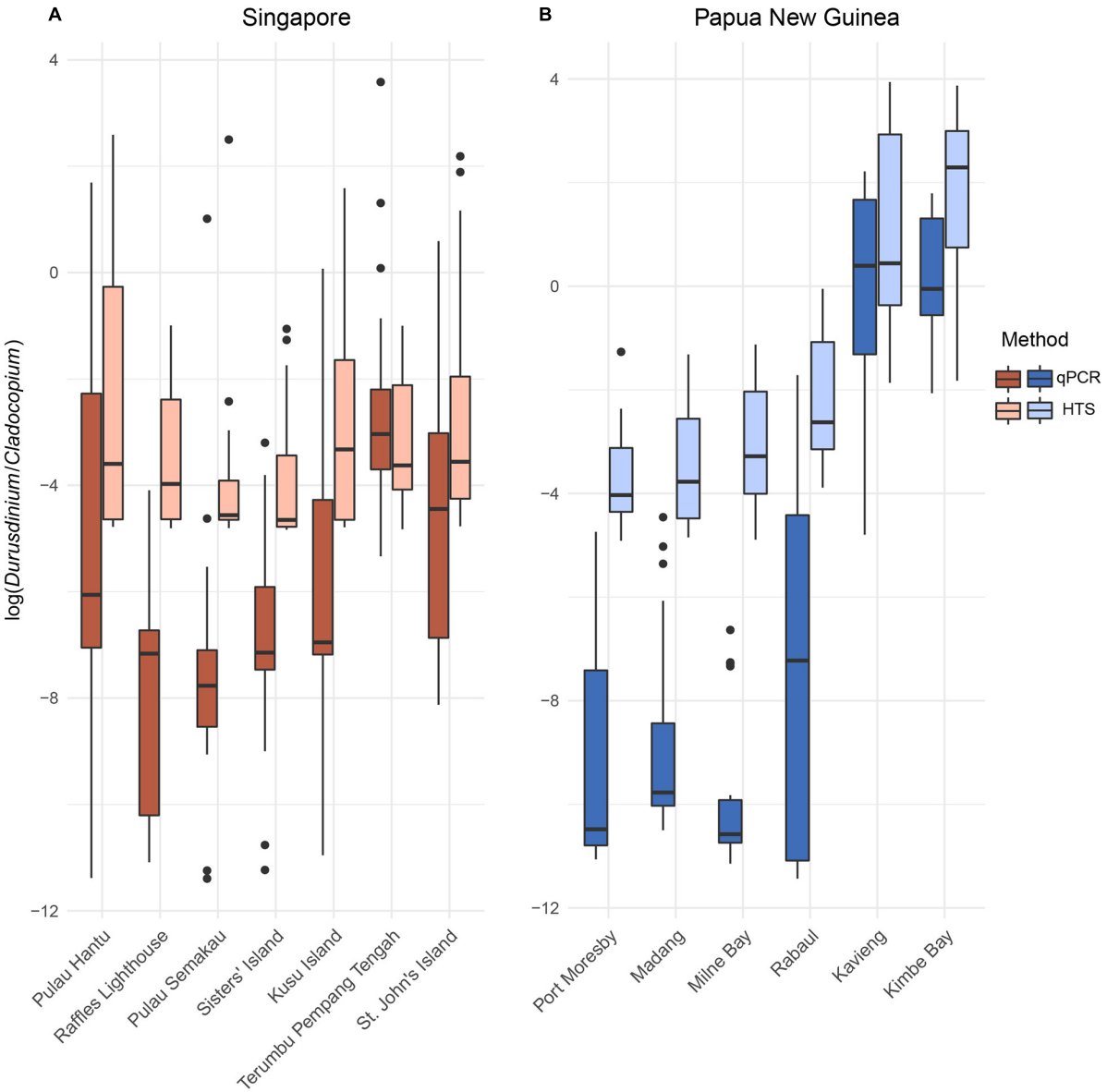

FIGURE 2 | Ratios of *Durusdinium* vs. *Cladocopium* relative abundances (log-transformed ratios) in the **(A)** seven sites across Singapore and **(B)** six sites across Papua New Guinea obtained via quantitative polymerase chain reaction (qPCR) and high-throughput sequencing (HTS).

between Singapore and Papua New Guinea (p = 0.0015, df = 1, F = 10.352).

Methodological and Lineage Variations

Overall, the patterns of relative *Durusdinium* vs. *Cladocopium* abundances were consistent between the qPCR tests and HTS reads (**Figure 2**). At the sample level, relative ITS1 concentrations determined via qPCR and ITS2 HTS read abundances could be explained by a significant linear relationship at each locality (Singapore: R^2 = 0.435, $p << 0.001$; Papua New Guinea: R^2 = 0.778, $p << 0.001$; **Figure 3**). However, six and 15 colonies from Singapore and Papua New Guinea, respectively, recorded as having *Durusdinium*-dominated communities with HTS were actually *Cladocopium*-dominated according to qPCR. Furthermore, three colonies each from Singapore and Papua New Guinea were recorded as *Durusdinium*-dominated with qPCR but actually showed *Cladocopium* dominance using HTS.

Based on the CAPS assay following Bongaerts et al. (2021), two lineages ("green" and "blue") occur sympatrically within Singapore and Papua New Guinea. No "red" lineage was identified. To associate the host lineages with the Symbiodiniaceae communities, we compared *Cladocopium* and *Durusdinium* dominance in each colony with the assigned lineage (**Supplementary Table 2**). Colonies that showed a dominance of *Durusdinium* were assigned to either a "green" or "blue"

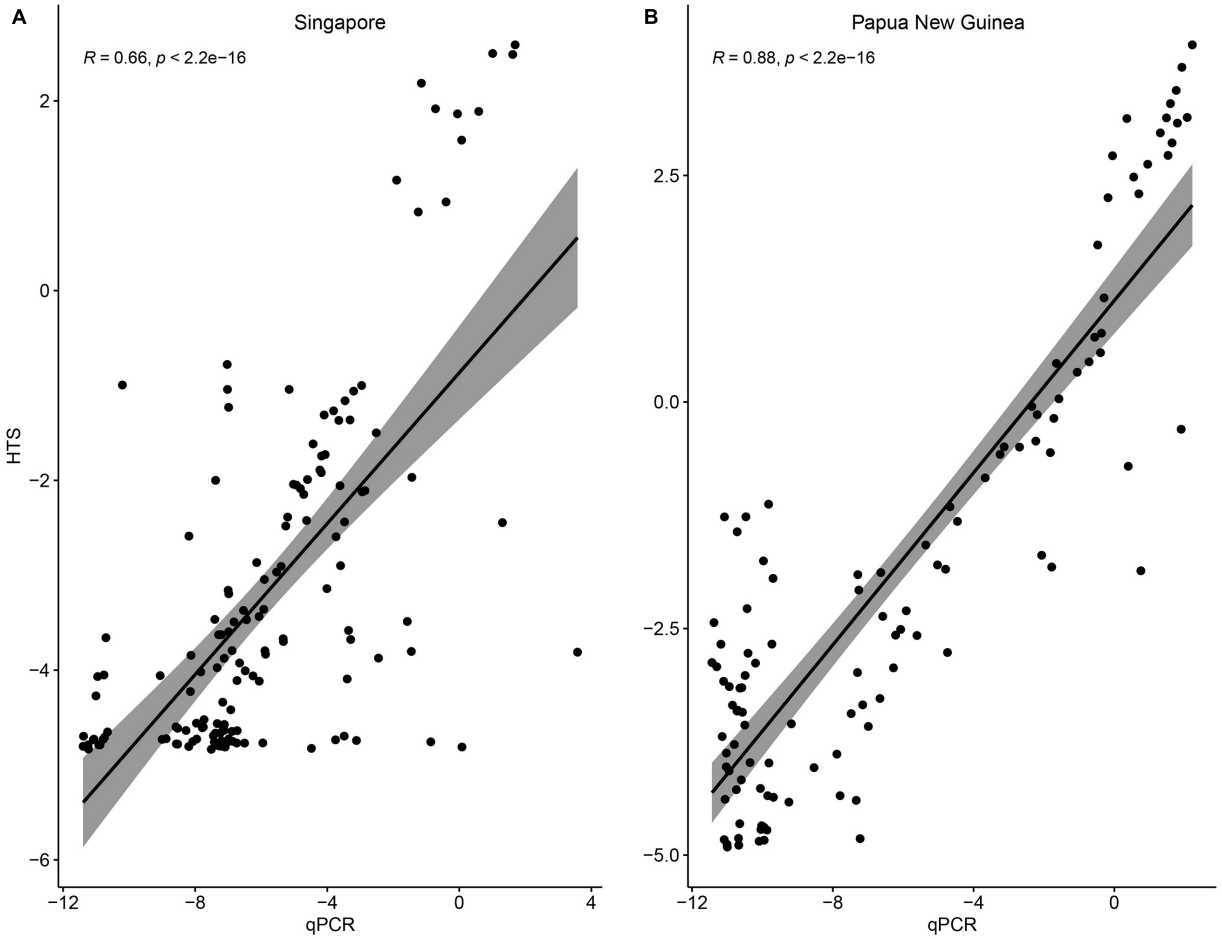

FIGURE 3 | Relationship between the log-transformed relative abundances of *Durusdinium* vs. *Cladocopium* ITS1 concentrations (quantitative polymerase chain reaction) and ITS2 read abundances (high-throughput sequencing) on a sample level among colonies from **(A)** Singapore and **(B)** Papua New Guinea.

lineage. There was no clear association of the "green" lineage with dominance of *Durusdinium*, so we surmised that there was no discernible link between host lineage and endosymbiont community composition.

ITS2 Type Profiles

Based on the SymPortal framework, nine ITS2 type profiles were found in Singapore *P. speciosa*, including five *Cladocopium* and four *Durusdinium* profiles (**Figure 4**). The five profiles of *Cladocopium* were associated with eight unique ITS2 sequences and the four profiles of *Durusdinium* were also associated with eight unique ITS2 sequences. The profile C27-C3-1319-C27a was detected in colonies across all sites except for St. John's Island where only C27-C3-C27a was detected, suggesting the absence of the *Cladocopium* sequence "1319" at this particular site. Colonies from Raffles Lighthouse, Terumbu Pempang Tengah and Sisters' Island showed none of the *Durusdinium* profiles. Colonies from the other sites contained *Durusdinium* type profiles, with D1/D4-D1c detected predominantly in colonies from Pulau Hantu and St. John's Island, and D1-D4-D4c-D4f-D1l detected in colonies from Pulau Semakau and Kusu Island.

Colonies from Papua New Guinea showed a higher number of ITS2 type profiles (18) as compared to colonies from Singapore. There were 12 ITS2 type profiles assigned to *Cladocopium* and six type profiles assigned to *Durusdinium* across all the six sites sampled (**Figure 4**). Each site showed dominance of a distinct ITS2 type profile. The dominant type profile detected in colonies from Port Moresby, Madang, Milne Bay and Rabaul belonged to *Cladocopium* whereas colonies from Kavieng and Kimbe Bay showed dominance by *Durusdinium* type profiles (Port Moresby: C21-C21u-C3-C21v-34025; Madang: C21; Milne Bay: C3/C21/6451-C3av-C3b-C3at-C3dp; Rabaul: C40-C3-C115; Kavieng: D1-D4-D4c-D1c and Kimbe Bay: D1-D4-D4c-D3b-D1l-3732-D4f).

DISCUSSION

Geographical Variation

In this study, the relative abundances of Symbiodiniaceae genera and ITS2 type profiles hosted by the common reef-building stony coral, *Pachyseris speciosa*, were compared among colonies

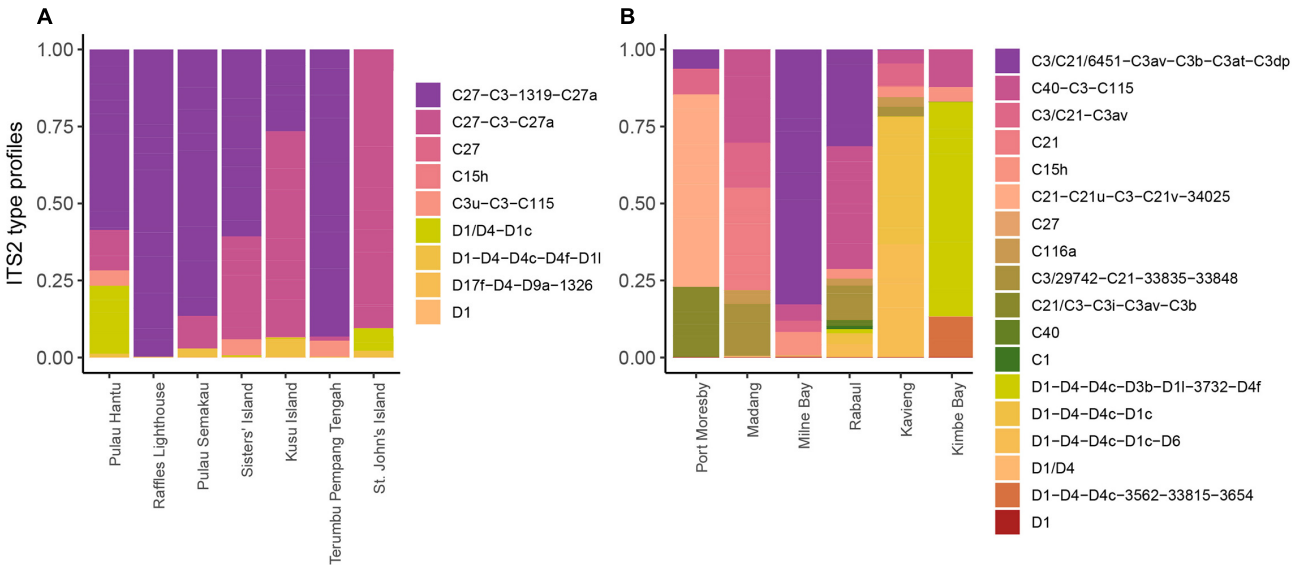

FIGURE 4 | Diversity and relative abundances of Symbiodiniaceae ITS2 type profiles among the sites studied in **(A)** Singapore and **(B)** Papua New Guinea.

from Singapore and Papua New Guinea. *Cladocopium* C27-C3-1319-C27a was the most abundant ITS2 type profile found in all colonies across sites in Singapore with the exception of St. John's Island where C27-C3-C27a was detected (**Figure 4**). This is expected as previous studies have recorded similar results across multiple sites in Singapore (Jain et al., 2020; Smith et al., 2020) with C27 dominating most *P. speciosa* colonies. However, the type profile C27/C3/C3u-C115 was the most dominant across all six sites examined by Smith et al. (2020). This marginal difference may be the result of 3 years separating the sampling by Smith et al. (2020) in 2015 and the present study in 2018. In particular, a global-scale coral bleaching event (GCBE) occurred here in 2016 (Toh et al., 2018), leading to a shift in the Symbiodiniaceae communities in at least one of the sites, Pulau Hantu (Jain et al., 2020), even as the corals had recovered by 2018 (Ng et al., 2020).

Generally, ITS2 type profiles of *Cladocopium* were more abundant among colonies in Singapore compared to type profiles of *Durusdinium*, corroborating the findings at Pulau Hantu by Jain et al. (2020). While there were five *Cladocopium* and four *Durusdinium* type profiles, the dominant *Cladocopium* type profiles included the sequence of C27 and all the *Durusdinium* type profiles included the sequence of D1. These were also the dominant sequences detected for each of the genera in Jain et al. (2020). High *Durusdinium* abundance was detected in 26% of colonies from Pulau Hantu (Jain et al., 2020), with fewer colonies from Pulau Semakau (5%), Kusu Island (6%), and St. John's Island (14%). There were no *Durusdinium*-dominated colonies at Raffles Lighthouse, Sisters' Island and Terumbu Pempang Tengah.

The increased relative abundances of *Durusdinium* in colonies from Pulau Hantu compared to others could be a result of thermal or urbanization-related stress (Jain et al., 2020), given that certain *Durusdinium* species are commonly associated with resistance to high temperatures and turbidity fluctuations (Baker, 2003; Ulstrup and Van Oppen, 2003; Jones et al., 2008). *Durusdinium* has been suggested to play an important role in the survival of Singapore's corals (Poquita-Du et al., 2020). The warm, turbid environment subject corals growing here with challenges not usually ideal for coral health (e.g., close proximity to a major urban center and high sedimentation rates), and furthermore, these reefs are susceptible to major bleaching events (Guest et al., 2016; Ng et al., 2020). Indeed, *Durusdinium* has mostly been associated with other marginal reefs comparable to those of Singapore (LaJeunesse et al., 2010; Keshavmurthy et al., 2014). Slight changes in the endosymbiont communities are known to be influenced by various environmental stressors (Buddemeier and Fautin, 1993; Baker, 2001). The limited differences in endosymbiont communities between various sites in Singapore suggest the stresses (e.g., thermal, sediment) that *P. speciosa* is exposed to may be similar among sites.

In colonies from Papua New Guinea, *Cladocopium* type profiles were the most abundant at all sites except Kimbe Bay and Kavieng, where *Durusdinium* dominated most colonies. Rather than the C27-C3-1319-C27a dominance exhibited by corals in Singapore, colonies from Papua New Guinea hosted mostly distinct *Cladocopium* type profiles at different sites. Colonies from Kimbe Bay and Rabaul had the most abundant C40-C3-C115, whereas colonies from Kavieng, Milne Bay, Madang and Port Moresby hosted C3/C21-C3av, C3/C21/6451-C3av-C3b-C3at-C3dp, C21 and C21-C21u-C3-C21v-34025, respectively. These *Cladocopium* type profiles included sequences of either C3 or C21, or both. Notably, *Cladocopium* C3, C21, and C40 are regarded as generalists due to their associations with a wide range of reef coral taxa in the tropics at various depths (LaJeunesse et al., 2003, 2004). In fact, the C3 group comprises many divergent lineages that are symbiotic with distinct host taxa

(LaJeunesse, 2005; Thornhill et al., 2014). The relatively high variability in the dominant type profile between sites observed at Papua New Guinea suggests a certain level of local adaptation even as *Cladocopium* C3, C21 and C40 share very similar ITS2 sequences (LaJeunesse et al., 2003).

Pachyseris speciosa colonies from Kimbe Bay and Kavieng were dominated by *Durusdinium*. The distinction of these two sites based on *Durusdinium* dominance does not appear to be spatially driven, although Kimbe Bay, Rabaul and Kavieng are on the islands of New Britain and New Ireland off the New Guinea mainland and samples from these sites contained higher *Durusdinium* levels than the others. It is known that the abundance of *Durusdinium* may be elevated in corals undergoing bleaching, and may not revert to the pre-bleaching community post-recovery (Tye Pettaya et al., 2015; Boulotte et al., 2016; LaJeunesse et al., 2016). However, these sites do not appear to have bleached in recent years (Mana, R. R., unpublished data), suggesting that local environmental factors such as light intensity, sea surface temperature and currents may promote *Durusdinium* dominance in Kimbe Bay and Kavieng. In particular, Kavieng faces the Pacific Ocean and is directly exposed to the South Equatorial Current, likely experiencing distinct local conditions compared to the other more sheltered sites to the south. Further tests and environmental measurements are needed to explain the Symbiodiniaceae dominance patterns at Papua New Guinea.

Comparison of the type profiles between Singapore and Papua New Guinea showed highly diverse endosymbiont communities within and between localities (**Figure 4**). In particular, the SymPortal framework revealed 18 type profiles throughout Papua New Guinea, which is twice the number detected in Singapore. It remains unclear why the endosymbiont compositions between Singapore and Papua New Guinea differ (LaJeunesse et al., 2004; Bongaerts et al., 2011; Cooper et al., 2011; Ziegler et al., 2015b), but these biogeographical patterns could be a consequence of different environmental factors and thermal regimes as discussed above (Tonk et al., 2013; Ziegler et al., 2015a, 2017). We note that the spatial distances between sampling sites in Papua New Guinea (∼100 km) are considerably greater than those in Singapore (∼10 km). This could mean greater connectivity and/or more similar environmental conditions among sites in Singapore, and may explain the relatively homogenized Symbiodiniaceae communities within Singapore compared to Papua New Guinea.

Methodological Variation

Clearly there is a need to extend our comparisons of the endosymbiont communities to more localities in the Central Indo-Pacific using complementary approaches. As we have demonstrated, several colonies recorded as having *Durusdinium*-dominated communities with HTS were actually *Cladocopium*-dominated according to qPCR (**Figure 3**). Therefore, it may even be necessary to revisit samples that have been used in the past with HTS to infer that symbiosis with *Durusdinium* is associated with thermal tolerance (Baker, 2001; Baker et al., 2004; Stat et al., 2013). Similarly, in our previous study which explored the Symbiodiniaceae community patterns in *P. speciosa* during and after the 2016 GCBE (Jain et al., 2020), three colonies were found to be *Durusdinium*-dominant based on HTS and *Cladocopium*-dominant based on qPCR. Nevertheless, overall patterns in the relative abundances of *Durusdinium* vs. *Cladocopium* remained consistent.

In this study, differences between localities in the levels of *Durusdinium* vs. *Cladocopium* appeared to be magnified with HTS. Our results showed significant differences in the relative abundances of *Durusdinium* and *Cladocopium* between Singapore and Papua New Guinea for the HTS results but not for qPCR, likely driven by the higher richness of ITS2 type profiles detected in corals from Papua New Guinea. qPCR is a sensitive method for detecting endosymbionts at a much higher resolution compared to more traditional methods like DGGE. While the technology of HTS has allowed us to detect sequence variants much more easily, it needs to be validated further for the purpose of understanding coral-algal symbiosis as the PCR step may be prone to amplification bias (Neilson et al., 2013). PCR may also pick up free-living Symbiodiniaceae in the corals' immediate external environment (Santos, 2004; Coffroth et al., 2006; Sze et al., 2018; Fujise et al., 2021). Hence, further exploration of the Symbiodiniaceae community around the coral is important to quantify the coral-algal symbiosis more precisely by accounting for environmental DNA signals. We suggest that water and sediment surrounding the coral colony of interest should be sampled at the same time for Symbiodiniaceae characterization. Overall, since the inferences of Symbiodiniaceae dominance differ at a sample level, we urge caution when examining endosymbiont abundances, and suggest verifying HTS results with qPCR for more precise estimates.

Indeed, precise characterization of the roles of different Symbiodiniaceae taxa would provide insights on the adaptation of coral reefs as global stressors exacerbate. The large colony-level variations of the coral-Symbiodiniaceae relationship even within a small reef area suggest that local adaptation is ongoing that may allow certain colonies to persist despite the accelerating environmental change (McClanahan et al., 2004; Hoogenboom et al., 2017). Such mosaicism of coral fitness at the reef level would be important for forecasting the performance of local reefs amid global climate change.

DATA AVAILABILITY STATEMENT

All sequencing reads associated with this work have been deposited at the National Center for Biotechnology Information under the BioProject Accession PRJNA754701.

AUTHOR CONTRIBUTIONS

SJ, BW, and DH contributed to conception and design of the study. SJ performed the molecular analyses and analyzed the data. SJ and DH wrote the first draft of the manuscript. All authors contributed to the collection of samples, contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.759744/full#supplementary-material

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