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Symbiodiniaceae diversity of *Palythoa tuberculosa* in the central and southern Red Sea influenced by environmental factors

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KEYWORDS

Saudi Arabia, zooxanthellae, zoantharian, sea surface temperature, chlorophyll a

Abstract

Sea surface temperatures (SST) and chlorophyll a concentrations (Chl a) in the southern Red Sea have wide variations based on distance from the coast. To understand how these variations can affect the diversity of symbionts hosted by reef-associated organisms, we conducted a study in the central and southern Red Sea to examine the diversity of Symbiodiniaceae hosted by the zooxanthellate zoantharian *Palythoa tuberculosa* at different distances from the coast: offshore (FBO), midshelf (FBM) and inshore (FBI) of Farasan Banks, and inshore at Thuwal (TI). Genomic DNA was extracted from 198 specimens, followed by amplification of the ribosomal DNA internal transcribed spacer 2 (ITS-2) and non-coding region of the chloroplast plastid minicircle (psbA^{ncr}). *Durusdinium* and six lineages of *Cladocopium* (Pt-1-a, Pt-1-b, Pt-1-c, Pt-1-d, Pt-3-a, Pt-3-b) were identified based on sequences of the two marker regions. Changes in composition of

Symbiodiniaceae lineages were observed from FBI (high SST, high Chl a) to FBO (low SST, low Chl a). Molecular variance analyses (AMOVA) showed that distance from coast was the most likely predictor of differences in *Cladocopium* lineages. Multinomial Logistic Regression (MLR) analysis showed a transition among different *Cladocopium* lineages as SST increased. One *Cladocopium* lineage, Pt-1-b, demonstrated higher prevalences at high SSTs, and increased in prevalences at the same rate as thermotolerant *Durusdinium*. Additionally, *Cladocopium* lineage Pt-3-a had a high affinity to low Chl a concentrations. This study demonstrates that environmental variations in SSTs and Chl a concentrations based apparently are significant predictors for the diversity of dominant Symbiodiniaceae within individual host *P. tuberculosa* colonies. We theorize that flexibility with different lineages of Symbiodiniaceae allows generalist *P. tuberculosa* to live across a wide range of environments in the southern Red Sea.

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Introduction

The Red Sea is a region of high marine biodiversity and endemism levels, and is one of the hottest marine environments in the world (Berumen et al., 2013; DiBattista et al., 2016a; DiBattista et al., 2016b; Ludt et al., 2017). The Red Sea basin was formed approximately 5 million years ago (Bailey et al., 2007; Berumen et al., 2013; Ludt et al., 2017) and is connected at the south with the adjacent Indian Ocean through the shallow opening of Bab al Mandab Strait. The partial oceanographic isolation and geographic location of the Red Sea has resulted in its southern region and inshore waters becoming comparably very warm, with sea surface temperatures (SSTs) regularly exceeding 32 °C during the summer. The shallow Bab al Mandab Strait brings nutrient rich water from the Indian Ocean into the Red Sea, making this region high in primary productivity (Brewin et al., 2015; Krueger et al., 2017; Ngugi et al., 2012; Osman et al., 2017; Raitsoos et al., 2013; Sofianos & Johns, 2003; Sonnewald & El-Sherbiny, 2017). In the southern region of the Red Sea, zooxanthellate scleractinian corals begin to bleach at 32.5 °C (Eladawy et al., 2017; Monroe et al., 2018). Hence, the shallow southern inshore reefs of the Red Sea exist at their upper thermal tolerance limits and are more susceptible to bleaching compared to offshore and deeper reefs that experience slightly lower SSTs (Fine et al., 2013; Monroe et al., 2018; Osman et al., 2017). This makes inshore Red Sea reefs marginal, and such marginal reefs are susceptible to global climate change as SSTs increase rapidly due to anthropogenic causes (Furby et al., 2013; Hughes et al., 2003; Lough et al., 2018; Monroe et al., 2018).

Until now, frequent contact with naturally high SSTs has driven adaptation within Red Sea coral reefs and has favored resilient variants of associated organisms (Coles et al., 2018; Hume et al., 2013; Maynard et al., 2008). One taxon shown to be driven by such environmental factors are the symbiotic dinoflagellates of the family Symbiodiniaceae (previously genus *Symbiodinium*;

LaJeunesse et al., 2018) that live in symbioses with a variety of marine invertebrates, including many anthozoans (Baker, 2003; LaJeunesse et al., 2010; Mies et al., 2017). The host-symbiont ('holobiont') relationship has been at the center of coral reef research as holobionts experience increasing frequencies of thermal-stress-induced mass bleaching events (Baker et al., 2018; Burke et al., 2011; Chakravarti et al., 2017; Hughes et al., 2017; Lough et al., 2018; Núñez-Pons et al., 2017; Smith et al., 2017a). Among Symbiodiniaceae, *Cladocopium* (formerly 'clade C') and *Durusdinium* (formerly 'clade D') are the most common genera found in the Indo-Pacific scleractinians and zoantharians (Burnett, 2002; Mostafavi et al., 2007; LaJeunesse, 2004; LaJeunesse et al., 2018). By using molecular phylogenetic analyses, most genera of Symbiodiniaceae have been shown to contain many species (Hume et al., 2015; Lajeunesse et al., 2018; LaJeunesse et al., 2014; Lewis et al., 2019; Reimer et al., 2017a; Wong et al., 2016).

Various factors have been shown to drive the evolution of Symbiodiniaceae; coevolution with specific hosts, geographical distributions, and the environment (SST, PAR light levels, pH) in which the holobionts are found (Finney et al., 2010; LaJeunesse et al., 2004; LaJeunesse et al., 2010; Tonk et al., 2013). Hence, there are diverse Symbiodiniaceae with specific and optimized niches, and associations with these can improve the survivability and resilience of hosts (Kemp et al., 2006; Sawall et al., 2014). Additionally, in the aftermath of recent bleaching events, some reports have seen a shift or 'reshuffling' of Symbiodiniaceae, especially from *Cladocopium* to more stress-tolerant *Durusdinium trenchii* (D1a) within some host species (Bay et al., 2016; Cunning et al., 2015; Silverstein et al., 2015; Silverstein et al., 2017). However, Tonk et al. (2013) noted that is hard to make robust inferences on the extent to which environment and geographical distributions drive the diversity of Symbiodiniaceae. This is due to the fact that the majority of Symbiodiniaceae are host-specific, and that host species' distributions are often limited, restricting

the potential for wider environmental and geographical comparisons within one widely distributed host (Finney et al., 2010).

Several studies have examined Symbiodiniaceae hosted by the widely distributed zoantharian *Palythoa tuberculosa* (Anthozoa: Hexacorallia: Zoantharia) (Burnett, 2002; Noda et al., 2017; Reimer & Todd, 2009; Reimer et al., 2017a). The host *Palythoa* is common across the Indo-Pacific Ocean (Hibino et al. 2013) and is an ecological generalist, found in a wide variety of environments (acidified reefs, river mouths, and intertidal zones to 50 m depth). Previous research has shown that *P. tuberculosa* hosts *Cladocopium* C1 and *Durusdinium* (Burnett, 2002; Reimer et al., 2017a; Reimer & Todd, 2009), while more recent examinations of the non-coding region of chloroplast plastid minicircle (psbA^{ncr}) have demonstrated the presence of four *Cladocopium* lineages in the Red Sea (Reimer et al., 2017a). The composition of these *Cladocopium* lineages in *P. tuberculosa* changes from areas with lower SSTs in the north (~25 °C) to areas in the warmer central (~29 °C) Red Sea. However, there is still a lack of information on the Symbiodiniaceae in *P. tuberculosa* in the southern Red Sea.

Studying Symbiodiniaceae composition in the southern Red Sea allows us to examine the influence of SSTs and turbidity on symbionts. As these reefs are among the hottest in the world, observed trends may also serve as harbingers for other areas under future climate changes (Roik et al., 2016). Furthermore, targeting a single target host species removes the influence of host identity so as to examine the effect of environmental gradients on symbiont distribution and diversity (LaJeunesse et al., 2004; Tonk et al., 2013). *Palythoa tuberculosa* is among the best candidates for this kind of study as it is widely distributed in the Red Sea and is easily identified in the field (Reimer et al., 2017b). In this study, two molecular markers (ITS-2 of ribosomal DNA and psbA^{ncr}) were used to compare the composition of Symbiodiniaceae hosted by *P. tuberculosa*

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109 colonies at different depths and distances from the coast in two southern and central Red Sea
110 locations, Farasan Banks (southern) and Thuwal (central). Models of expected Symbiodiniaceae
111 probability based on Multinomial Logistic Regression Analysis were constructed with SST and
112 chlorophyll a concentration data in order to observe patterns of Symbiodiniaceae composition
113 change across different distances from coast and depths.

Materials and Methods

Field collection

The study was conducted in reefs in two regions in the central eastern Red Sea; the Farasan Banks and Thuwal, both in Saudi Arabia (Figures

Fig. 1). Specimen collection at Farasan Banks was conducted from April 30 to May 7, 2017 at 19 different sites. Sites within the Farasan Banks were split into three categories based on their distance from the Saudi Arabian coast: offshore (FBO, >55 km), midshelf (FBM, 20-55 km), and inshore (FBI, <20 km). Specimen collection within the Thuwal inshore region (TI) was conducted May 9-10, 2017 at four sites, all of which were <15 km from the coast (Fig. 1, Supplementary 1). The offshore reefs (FBI) are located at the continental shelf edge of the Farasan Banks and are surrounded by deep waters (typically >500 m deep) with steep drop-offs, while inshore reefs (FBI and TI) are usually near to the coast and at shallower depth of less than 80 m (Yao et al., 2014). The midshelf reefs (FBM) are typically found on or nearby to the relatively shallow waters at the continental shelf and are intermediary between inshore and offshore reefs.

Palythoa tuberculosa specimens were collected via SCUBA diving and the roving search method (Raymundo et al., 2008). Each collection dive started from 30 m and slowly ascended the reef slope while searching for colonies. Once located, the depth of each host colony was recorded and 2 cm² subsamples of each colony were taken. Immediately after dives, collected specimens were preserved in 99.5% ethanol. A total of 203 specimens were collected, consisting of 161 from

the Farasan Banks and 42 from Thuwal (Supplementary 1a). Specimens were brought back to the University of the Ryukyus, Japan, for further analyses.

DNA extraction and amplification

Genomic DNA was extracted from specimens using a DNeasy Blood and Tissue extraction kit following the manufacturer's instructions (Qiagen, Tokyo). Two DNA marker regions were amplified via polymerase chain reaction (PCR) targeting specific regions: the internal transcribed spacer 2 of ribosomal DNA (ITS-2) of nuclear ribosomal DNA for all specimens, as well as the non-coding region of the plastid minicircle (*psbA^{ncr}*) for specimens containing *Cladocopium*. The ITS-2 sequences were utilized to place symbiont types within the well-established ITS-2 phylogenetic framework (LaJeunesse & Thornhill, 2011), while the *psbA^{ncr}* sequences were utilized to examine differences at a finer phylogenetic resolution (LaJeunesse & Thornhill, 2011; Reimer et al., 2017a; Noda et al., 2017).

The ITS-2 region was amplified using the primers zITSf (5'-CCG GTG AAT TAT TCG GAC TGACGC AGT-3') and ITS4 (5'-TCC TCC GCT TAT TGATAT GC-3') (White et al., 1990; Rowan & Powers, 1992; Hunter et al., 1997), and *psbA^{ncr}* was amplified using the primers 7.4-Forw (5'-GCA TGA AAG AAA TGC ACA CAA CTT CCC-3') and 7.8-Rev (5'-GGT TCT CTT ATT CCA TCA ATA TCT ACT G-3') (LaJeunesse & Thornhill, 2011; Moore et al., 2003). The PCR mixes (20 µl) were composed of 1.0 µl of genomic DNA, 10.0 µl of HotStarTaq Plus Master Mix, 1.0 µl of each primer (10 pmol), 1.0 µl MgCl₂ (25 mmol), 0.5 Bovine Serum Albumin (20 mg/ml), and 1.5 µl Coral Load. Thermocycle conditions were modified slightly from Noda et al. (2017); ITS-2: 95.0 °C for 5 min; 35 cycles of 94.0 °C for 30 s, 51.0 °C for 45 s, and 72.0 °C for 2 min; with a final extension at 72.0 °C for 10 min; and for *psbA^{ncr}*: 95.0 °C for 5 min; 40 cycles of

94.0 °C for 10 s, 55.0 °C for 30 s, and 72.0 °C for 2 min; with a final extension at 72.0 °C for 10 min. The products were sent to Fasmac (Kanagawa, Japan) for sequencing in both directions.

Phylogenetic analyses

ITS-2 sequences in both directions were aligned manually to produce consensus sequences using Bioedit (Hall, 1999). Consensus sequences of ITS-2 and psbA^{ncr} forward sequences were edited individually using the same software. Only one direction of psbA^{ncr} sequences were used as in previous research (Hawkins et al., 2016; Kunihiro & Reimer, 2018; Noda et al., 2017; Reimer et al., 2006). Edited sequences for each marker were compiled and aligned in MEGA v.7 (Kumar et al., 2016). Previously reported ITS-2 sequences of genera *Cladocopium* (AB207184, DQ480631, DQ480639, DQ889741, DQ889743), *Durusdinium* (AB704033, EU333712, KU535564, MH243738), *Gerakladium* (AB546599) and *Effrenium* (AY160123) were obtained from GenBank and included in the ITS-2 alignment as references. psbA^{ncr} reference sequences were obtained from Reimer et al. (2017a), including the four lineages previously reported from the Red Sea associated with *P. tuberculosa*; lineages Pt-1-a (KU920804, KU920812, KU920813, KU920816, KU920826), Pt-1-b (KU920803, KU920805, KU920809, KU920810, KU920811), Pt-3-a (KU920703, KU920706, KU920707, KU920714, KU920718, KU920719), and Pt-3-b (KU920720, KU920733, KU920734, KU920737, KU920743, KU920744). The final ITS-2 and psbA^{ncr} alignments were comprised of 154 (451 bp length) and 126 (239 bp) sequences, respectively. Novel sequences of psbA^{ncr} from this study were deposited in GenBank (MT250281-MT250506), and the alignment file is available as supplementary data (Supplementary_psbAF_126_239bp.fas).

The ITS-2 and psbA^{ncr} alignments were used to construct phylogenetic trees with maximum likelihood (ML), neighbor-joining (NJ), maximum parsimony (MP), and Bayesian

inference (BI) methods. The best substitutional methods were chosen for ML and NJ phylogenetic constructions for both alignments based on NJ tree reference under automatic model selection of MEGA. With this, Kimura-2 and Jukes-Cantor model with 1000 bootstraps phylogeny test at uniform rates were selected to construct the ML, NJ, and MP trees of the ITS-2 and psbA^{ncr} alignments (Hasegawa et al., 1985). Bayesian inference trees for ITS-2 (chain length=5,000,000; burn-in=1,250,000) and psbA^{ncr} (chain length=15,000,000; burn-in=3,750,000) using the same substitution model as mentioned above were constructed via MrBayes v.3.2.6 x64 (Huelsenbeck & Ronquist, 2001). The genetic differences between and within psbA^{ncr} forward sequences with references from Reimer et al. (2017a) were calculated in MEGA utilizing the Maximum Composite Likelihood Model. The values of within group mean distances of each reference sequence were used to determine the grouping of sequences based on the pairwise genetic distances (GD).

The psbA^{ncr} alignment was exported into fasta file format via MEGA. The alignment was imported into Microsoft Excel via the add-on GenAlEx (Genetic Analysis in Excel) version 6.5 and converted into numbers corresponding to each nucleotide. The polymorphic nucleotide (PN) positions of the alignment were extracted and ambiguous bases were removed. Each sequence was assigned to a distance from coast category (FBO, FBM, FBI, TI), and to a depth (0-10 m, 10.1-20 m, 20.1-30 m, and >30 m). Analysis of Molecular Variance (AMOVA) was conducted on the PN data sets to observe genetic differences between populations.

Symbiodiniaceae composition

The genera/subclade (ITS-2) and lineages (psbA^{ncr}: *Cladocopium* only) of the specimens were sorted based on distance from coast and depth. Shannon-Wiener Diversity Indices (SWDI,

H') and Pielou's evenness (Evenness, J') of *Cladocopium* lineages were calculated for each distance from coast and depth category. The prevalences of *Cladocopium* lineages were analyzed via ecological statistical methods using R studio with “vegan”, “Mass”, and “dplyr” packages (Oksanen, 2015; R Core Team, 2017). The dataset was transformed with Bray-Curtis dissimilarity matrices and represented with the dissimilarity composition in dendrogram cluster tree (hclust) (Oksanen, 2015).

Environmental data

Sea surface temperature (SST, °C) and sea surface chlorophyll-a (Chl a, mg m⁻³) data for the period between December 31, 2014 to May 31, 2017 were obtained from the National Aeronautical and Space Administration (NASA) Giovanni website (<https://giovanni.gsfc.nasa.gov/giovanni>) (Acker & Leptoukh, 2007). The time-averaged SST and Chl a data with 4 km resolution derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua database were superimposed on a map of the region investigated in this study (from 37.6172 °E, 17.4023 °N to 42.3633 °E, 23.9063 °N), and generated maps were examined using QGIS 3.6.0 (QGIS Development Team, 2019) to estimate the values of SST and Chl a for each sampling location in this study. Shapiro-Wilk normality tests showed both SST and Chl a datasets were not normal. Thus, we conducted a non-parametric Kruskal-Wallis tests on the SST and Chl a datasets with the locations (FBI, FBM, FBO, TI) as factors, followed by Dunn post-hoc test with Bonferroni correction. Spearman's rho correlation test was conducted to check the correlation among locations, depths, SST, Chl a, and Symbiodiniaceae (four *Cladocopium* psbA^{ncr} lineages and *Durusdinium*). Multinomial Logistics Regression analysis (MLR) was conducted using modified script from Reimer et al. (2017a) to identify good predictors of Symbiodiniaceae composition in the Red Sea environment using available parameters (location, depth, SST, Chl a)

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223 via R console with package “nnet” and “VGAM” (R Core Team, 2017; Ripley & Venables, 2016;
224 Yee, 2015). All statistical tests were conducted with statistical significance detection at $p=0.05$.

Results

Phylogeny of Symbiodiniaceae

In total, 198 ITS-2 region sequences from specimens were successfully amplified (Supplementary 1b). Preliminary analyses showed 164 specimens hosted Symbiodiniaceae identified as belonging to genus *Cladocopium*. Within these *Cladocopium*, 43 sequences either had double peaks within the sequences and/or shorter sequences. We interpreted these results as the possible presence of intragenomic variation in these 43 specimens, and this subset was removed from the subsequent ITS2 phylogenetic analyses. Our ITS-2 phylogenetic tree (Supplementary 2) showed distinct two clusters of Symbiodiniaceae. The remaining *Cladocopium* specimens (n=121) were grouped in the cluster of *Cladocopium* subclade C1/C1-related sequences (DQ889741, DQ889743, DQ480639, DQ480631; Reimer et al., 2017a), similar to those previously reported from *P. tuberculosa* in southern Japan and Saudi Arabia. A second, smaller cluster (n=34) was within the genus *Durusdinium* (EU333712) as previously reported from *P. tuberculosa* in Singapore (Reimer & Todd, 2009) and other parts of the Indian Ocean (Burnett, 2002). Based on obtained sequences, this cluster is likely *D. trenchii* or a closely related lineage.

Out of the 164 *P. tuberculosa* specimens hosting *Cladocopium*, 126 *psbA^{ncr}* region sequences were obtained (Supplementary 1c). The phylogenetic tree of *psbA^{ncr}* (Fig. 2) showed two strongly supported monophyletic large groupings corresponding to previously reported Red Sea lineages: Pt-1 and Pt-3 sensu Reimer et al. (2017a) (ML=99%, NJ=99%, MP=100%, BI=1.00). Within the large Pt-3 clade there were two subclades as previously seen in Reimer et al. (2017a): lineage Pt-3-a (n=33; ML=64%, NJ=60%, MP=100%, BI=1.00), and lineage Pt-3-b (n=57; ML=64%, NJ=64%, MP=100%, BI=0.65). In the Pt-1 cluster, Pt-1-a (n=14; ML=78%, NJ=78%,

MP=100%, BI=1.00) and Pt-1-b (n=15) each formed well-supported clusters, with Pt-1-b as the basal cluster. Between Pt-1-a and Pt-1-b, there were two additional strongly supported lineages; Pt-1-c (n=4; ML=99%, NJ=97%, MP=100%, BI=1.00), and Pt-1-d (n=3; ML=99%, NJ=99%, MP=100%, BI=1.00). Pt-1 lineages had a very large mean genetic difference (GD) within (GD: 0.000-0.040) and among (GD: 0.070-0.180) the lineages compared to those of Pt-3 lineages (Supplementary 3, GD: within<0.008; among=0.027).

The AMOVA test based on *psbA^{ncr}* sequences showed that genotype diversity of the *Cladocopium* at different locations (FBI, FBM, FBO, TI) was primarily within each location (=60%) more than among the locations (=40%). A similar pattern was also observed for depths, but with higher diversity within a single depth category (=96%), as compared to among different depths (=4%). The total genetic variations for *Cladocopium* among locations (PhiPT=0.401, P<0.001) and depths (PhiPT=0.043, P=0.038) were found to be statistically significant (Supplementary 4).

Symbiodiniaceae distribution

Each *P. tuberculosa* colony had a dominant Symbiodiniaceae genus (*Cladocopium* or *Durusdinium*) and lineage (for *Cladocopium* only) based on the results from ITS-2 and *psbA^{ncr}* sequences, respectively. The composition of identified ITS-2 and *psbA^{ncr}* specimens were grouped based on the distance from coast (=location) and depth in percentage abundance (Fig. 3). ITS-2 analyses grouped the specimens into two genera (*Cladocopium* and *Durusdinium*); while *psbA^{ncr}* showed six lineages of *Cladocopium* (Pt-1-a, Pt-1-b, Pt-1-c, Pt-1-d and Pt-3-a, Pt-3-b). *Cladocopium* was the dominant genus, found in 82.8% of the *P. tuberculosa* examined, and made up >50% of colonies at every site (Fig. 3a & b, Supplementary 1b & c). In *psbA^{ncr}* sequences

(Supplementary 1c), the most common *Cladocopium* lineage was Pt-3-b (n=57 colonies, 45.2%), followed by Pt-3-a (n=33, 26.2%), Pt-1-b (n=15, 11.9%), Pt-1-a (n=14, 11.1%), Pt-1-c (n=4, 3.2%), and lastly Pt-1-d (n=3, 2.4%).

By location, *Durusdinium* had highest prevalence at FBI (n=16/30, 34.8%), followed by FBM (n=14/54, 20.6%), with FBO (n=2/41, 4.7%) and TI (n=2/39, 4.9%) having similar and low prevalences (Fig. 3a). FBO, FBM and FBI had the same number of *Cladocopium* lineages present (n=5), while at TI only two *Cladocopium* lineages were detected. Lineage Pt-3-b (Fig. 3c) was the most common lineage in FBM (n=20/37, 54.1%) and TI (n=32/35, 91.4%). Pt-3-a (n=25/34, 73.5%) was the most abundant lineage at FBO, while FBI was dominated by Pt-1-b (n=11/20, 55.0%). The diversity of *Cladocopium* lineages increased from FBO ($H'=0.900$), across FBM ($H'=1.235$), to FBI ($H'=1.304$), while the lowest diversity was at TI ($H'=0.293$). Similar patterns were observed for evenness, with highest at FBI ($J'=0.810$), FBM ($J'=0.768$), FBO ($J'=0.559$), to lowest at TI ($J'=0.422$). A dissimilarity matrix of the *Cladocopium* lineages showed that TI and FBM shared 63.9% similarity (Fig. 4a). These two sites were 47.9% similar to composition of FBO, and FBI composition was only 28.1% similar to composition of the rest of the sites.

Durusdinium (Fig. 3b) was absent at depths greater than 30 m and increased in prevalence between 20-30 m (n=2/53; 3.8%) and 10-20 m (n=21/78; 26.9%). However, the shallowest 0-10 m showed a reduction of *Durusdinium* prevalence to 18.0% (n=11/61). On the other hand, the shallowest depths (0-10 m) had the highest number of *Cladocopium* lineages recorded (n=6), followed by 10.1-20 m (n=5), 20.1-30 m (n=4), and lastly >30 m (n=3). *Cladocopium* lineage Pt-3-b was the most common lineage at 0-10 m (n=20/39, 51.3%), 10.1-20 m (n=19/43, 44.2%), and >30 m (n=3/6, 50.0%), while Pt-3-a was the most common lineage at depths between 20.1-30 m (n=18/38, 47.4%; Fig. 3d). Shannon-Weiner indices showed a gradual increase in diversity

towards the shallows, from >30 m ($H'=1.011$), 20.1-30 m ($H'=1.076$), 10.1-20 m ($H'=1.343$), to 0-10 m ($H'=1.468$). However, evenness showed a different pattern, from 20.1-30 m ($J'=0.776$), 0-10 m ($J'=0.819$), 10.1-20 m ($J'=0.834$), >30 m ($J'=0.921$). The composition of *Cladocopium* lineages (Fig. 4b) between 0-10 m and 10.1-20 m was 80.5% similar. This was followed by 76.5% similarity between 20.1-30 m range and the shallower groups. Finally, the >30 m *Cladocopium* lineage composition was 27.3% similar to the compositions of the rest of the depths.

Environmental data

The average SST dataset showed FBI (29.9 ± 0.2 °C) was the warmest between 2015-2017 (Fig. 5a). This was followed by FBM (29.6 ± 0.2 °C) and FBO (29.3 ± 0.3 °C), with TI (28.5 ± 0.0 °C) as the coldest location in this study. There were significant differences in SST among the locations (Kruskal-Wallis: $X^2=17.642$, $p<0.001$), with the SST of TI significantly lower than those of FBI (Dunn post hoc: $Z=4.064$, $p<0.001$) and FBM (Dunn post hoc: $Z=2.729$, $p=0.038$) (Table 1).

Average Chl a between 2015-2017 showed the highest value at FBI (0.8 ± 0.2 mg m⁻³) (Fig. 5b), followed by TI (0.7 ± 0.2 mg m⁻³), FBM (0.5 ± 0.1 mg m⁻³) and FBO (0.4 ± 0.1 mg m⁻³). There were significant differences in Chl a levels among the locations (Kruskal-Wallis: $X^2=16.61$, $p<0.001$), with Chl a at FBI significantly lower than FBM (Dunn post hoc: $Z=2.977$, $p=0.017$) and FBO (Dunn post hoc: $Z=3.536$, $p=0.002$) (Table 1).

Comparisons of Symbiodiniaceae (*Cladocopium* psbA^{ncr} lineages and *Durusdinium*) with depth, SST, Chl a, and locations were conducted with Spearman's rho correlation (Fig. 6). There was a significantly strong correlation between SSTs of different locations (Spearman: $r_s=-0.92$, $p<0.001$), and a significantly negative correlation between Chl a levels and depths (Spearman: $r_p=-$

0.48, $p < 0.001$). Furthermore, there were significant correlations between Symbiodiniaceae with locations (Spearman: $r_s = 0.49$, $p < 0.001$) and SSTs (Spearman: $r_s = -0.52$, $p < 0.001$).

Multinomial logistic regression (MLR) tests were conducted to examine the misclassification rate of highest probability observing the given nominal outcome (*Cladocopium* psbA^{ncr} lineages and *Durusdinium*) based on a set of independent variables (distance from coast, depth, SST, Chl a). Only four lineages of *Cladocopium* psbA^{ncr} lineages (Pt-1-a, Pt-1-b, Pt-3-a, Pt-3-b) were used in this model, as Pt-1-c and Pt-1-d were removed due to low numbers ($n < 5$). All predictors were required for best predicting the distribution of Symbiodiniaceae (correct rate = 70.39%). The global model (intercept) showed that *Durusdinium* distribution was different to those of all *Cladocopium* lineages to the exception of Pt-1-b (Table 2). SST was the main predictor for the distribution of Symbiodiniaceae (6/10 comparisons), while Chl a was the main predictor of Pt-3-a distribution.

As SST and Chl a both showed significant influences in predicting the distribution of Symbiodiniaceae in *P. tuberculosa*, the probability of observing different symbionts were reconstructed based on SST (Fig. 7a) and Chl a (Fig. 7b) gradients. The SST (Fig. 7a) model showed that from 29–30 °C, which corresponds to the SST range of the Farasan Banks, there was a transition in *Cladocopium* psbA^{ncr} lineages from Pt-3-a to Pt-3-b to Pt-1-b. Furthermore, *Durusdinium* was observed to increase in probability at almost the same rate as *Cladocopium* lineage Pt-1-b. At <29 °C, Pt-3-b had the highest probability of occurrence. Pt-1-a showed low probability of prevalence throughout the examined temperature range. For the Chl a gradient (Fig. 7b), lineage Pt-3-a was only found at low Chl a concentrations (<0.5 mg m⁻³), while Pt-3-b had a peak occurrence at 0.6 mg m⁻³, and *Durusdinium* had a higher occurrence probability at >0.5 mg

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335 m⁻³. Both Pt-1 *Cladocopium* lineages did not show any clear pattern across the Chl a concentration
336 gradient.

Discussion

This study revealed a transition of Symbiodiniaceae composition in *Palythoa tuberculosa* at different distances from the coastline in the southern Red Sea. Reimer et al. (2017a), focusing on the northern to the central Red Sea (spanning ≈ 900 km), suggested that SST was a strong predictor of the *Cladocopium* lineages across latitude. However, results from the current study showed that within the Farasan Banks, across a much smaller geographical scale (<60 km), not only SST but also other environmental factors influenced the diversity of Symbiodiniaceae within *P. tuberculosa*. These results confirm previous studies that have shown shifts in prevalences among closely related Symbiodiniaceae may occur in host populations over small geographic distances of tens of kilometers (LaJeunesse et al., 2004; Noda et al., 2017; Wee et al., 2019). Based on our present analyses, sea surface temperatures (SST) and chlorophyll a concentrations (Chl a) were strong predictors of dominant Symbiodiniaceae genera and of *Cladocopium* lineages in *P. tuberculosa* in the southern Red Sea (Acker & Leptoukh, 2007; Eladawy et al., 2017; Raitsos et al., 2013; Sofianos & Johns, 2003).

There are two Symbiodiniaceae genera hosted by *P. tuberculosa* in the Red Sea and across the Indo-Pacific, *Cladocopium* and *Durusdinium*. These two Symbiodiniaceae genera have also been reported in the sister species *P. caribaeorum* in the Caribbean region (Kemp et al., 2006). Lineages of *Cladocopium* have been shown to be the dominant Symbiodiniaceae in *P. tuberculosa* across the Indo-Pacific Ocean, with reports of *Durusdinium* lineages from warm and turbid areas (Burnett, 2002; LaJeunesse, 2004; Noda et al., 2017; Reimer & Todd, 2009; Reimer et al., 2017a). Spatially, one *Durusdinium* lineage had higher abundances in the southern Red Sea, particularly at inshore (FBI) and shallow depths (<20 m), compared to offshore (FBO) and deeper depths (>20 m). At the Thuwal inshore (TI) sites of the central Red Sea, the prevalence of *Durusdinium* in *P.*

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4 360 *tuberculosa* was within the range of previously reported rates of occurrence for coral reef
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6 361 holobionts in the eastern coast of the Red Sea (<10%) (Reimer et al., 2017a; Ziegler et al., 2017).
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9 362 On the other hand, *P. tuberculosa* hosted only *Cladocopium* lineages at depths >30 m, with the
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11 363 *Durusdinium* lineage slowly increasing in prevalence towards shallower water. *Durusdinium*
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14 364 lineages are commonly found near the equator, at shallower depths, and at more turbid and/or
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16 365 inshore reefs (Bongaerts et al., 2013; Burnett, 2002; LaJeunesse et al., 2010; Reimer & Todd, 2009;
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22 367 Within *Cladocopium*, six lineages were identified based on *psbA*^{ncr} sequences; four
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25 368 previously reported lineages (Pt-1-a, Pt-1-b, Pt-3-a, and Pt-3-b; Reimer et al., 2017a) and two
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27 369 newly observed lineages (Pt-1-c and Pt-1-d) that were phylogenetically located within the Pt-1
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30 370 radiation. Pt-3-b was the most abundant *Cladocopium* lineage at Thuwal (TI), and this agrees with
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32 371 the results of Reimer et al. (2017a) in the central Red Sea. Compared to TI, the Farasan Banks had
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35 372 higher diversity of *Cladocopium* lineages at each location (FBO, FBM, FBI: n=5). Nevertheless,
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37 373 there were significant differences in both the genetic diversity and lineage composition among the
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40 374 locations of the Farasan Banks. These results agree with those of LaJeunesse et al. (2004), who
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42 375 noted that geographic gradients determined the composition and selection of Symbiodiniaceae by
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44 376 host. On the other hand, there was little differentiation of *Cladocopium* lineages among depths
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47 377 (AMOVA and composition similarity). This in agreement with Finney et al. (2010) and Reimer et
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49 378 al. (2017a), who both observed that geographical separation influenced Symbiodiniaceae diversity
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52 379 (both at genera level and *Cladocopium* lineages) more than depth.

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55 380 SSTs and Chl a were the two main environmental parameters that changed with distance
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58 381 from the coast in the southern Red Sea (Eladawy et al., 2017; Raitsos et al., 2013; Sofianos &
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60 382 Johns, 2003). Although the SST range in this study was much narrower when compared that
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observed in Reimer et al. (2017a), SSTs showed significant differences, especially between the warmer inshore Farasan Banks and colder Thuwal (Sofianos & Johns, 2003). In general, SSTs were observed to increase towards the south and nearer to the coast (Eladawy et al., 2017; Monroe et al., 2018).

The Chl a values followed a similar trend as SST data, from lower values in the north ($<0.01 \text{ mg m}^{-3}$) to higher values in the south ($>1 \text{ mg m}^{-3}$) (Raitsos et al., 2013). Chl a results in this study matched well with the values previously observed for the region (1 mg m^{-3} ; Raitsos et al., 2013). Furthermore, inshore waters had generally higher Chl a concentrations compared to offshore waters at the same latitude (Raitsos et al., 2013; Sofianos & Johns, 2003). The higher productivity and more variable Chl a concentration is thought to be attributable to the intrusion of nutrient rich water from the Gulf of Aden and Indian Ocean (Raitsos et al., 2013; Sofianos & Johns, 2003). Thuwal had higher Chl a concentrations compared to offshore and midshelf Farasan Banks, similar to previous studies that reported the coastal waters along the eastern Red Sea with higher concentrations of Chl a than concentrations of areas away from the coast (Eladawy et al., 2017; Sofianos & Johns, 2003).

Based on MLR analyses, the order of parameters with the most significance to the least significance in predicting the Symbiodiniaceae distribution was $\text{SST} > \text{Chl a (turbidity/irradiance)} > \text{depth}$. Among the predictors, SST was the strongest predictor of the distribution of Symbiodiniaceae (*Cladocopium* psbA^{ncr} and *Durusdinium*), agreeing with Reimer et al. (2017a) in which SST predicted *Cladocopium* lineage distributions well in the north to central Red Sea. Furthermore, with the higher SST ranges in this study compared to those in Reimer et al. (2017a) ($24\text{--}28^\circ\text{C}$), we finally can explain Pt-1-b distribution, as this lineage was previously found only in low numbers in the north and central Red Sea and thus no hypothesis on its distribution was

previously made. From our results, Pt-1-b appears to be one of the most thermotolerant *Cladocopium* lineages. This lineage was absent in Thuwal and increased in number as SSTs increased from offshore to inshore in the Farasan Banks. Notably, Pt-1-b prevalence increased with SST gradients at the same rate as thermotolerant *Durusdinium*. Hence, based on Reimer et al. (2017a) and the current study, *Cladocopium* psbA^{ncr} lineage transition with SST increases was observed as Pt-1-a<Pt-3-a<Pt-3-b<Pt-1-b.

The other strong predictor of Symbiodiniaceae prevalence we observed was Chl a concentration. Chl a was used in this study as a proxy for turbidity, which can affect the distribution of Symbiodiniaceae (Caballero et al., 2019; Otero & Carberry, 2005). The MLR model results showed changes in the prevalence of different Symbiodiniaceae across the Chl a concentration gradient (0.2-1.0 mg m⁻³), especially for *Cladocopium* lineage Pt-3-a, which had higher prevalence at reefs further away from the coast with lower Chl a (Brewin et al., 2015; Eladawy et al., 2017; Raitos et al., 2013; Zhu et al., 2007). Furthermore, Pt-3-a was absent in Thuwal, which had lower SSTs than the offshore Farasan Banks (FBO) but higher Chl a. These results suggest that *Cladocopium* lineage Pt-3-a has higher prevalences in environments with lower irradiance as compared to environments with lower SSTs. The MLR model also showed *Cladocopium* lineage Pt-3-b and *Durusdinium* had higher occurrences at locations with higher Chl a than Pt-3-a. These patterns could be observed in this study as we investigated sites across a wider and higher Chl a range (0.2-1.0 mg m⁻³) than compared to Reimer et al. (2017a) (0.1-0.4 mg m⁻³). It appears from our results that Chl a levels (= turbidity, or in other words available light) is a good predictor for the distribution of Symbiodiniaceae. However, the influence of Chl a was slightly less than that of SST on Symbiodiniaceae communities, which agreed with Leydet and Hellberg (2016).

The four main *Cladocopium* psbA^{ncr} lineages examined in this study (Pt-1-a, Pt-1-b, Pt-3-a, and Pt-3-b) were shown to have distinct environmental niches based on the MLR analyses of SST and Chl a. In general, species delineation is mostly through genetic variations that define the ecological, physiological, or morphological traits as distinct ecotypes or strains (Cohen, 2002; Sampayo et al, 2009, 2007; Thornhill et al., 2017). This is the cohesion species concept, which states that the closer the genetic distance between two species is, the closer their ecological niche is (Cohen, 2002). Based on the cohesion species concept, in this study one could infer that the lineages within each of the Pt-1 and Pt-3 clades should have almost the same ecological niche as they are very closely phylogenetically related. However, while this is true for Pt-3-a and Pt-3-b, closely related Pt-1-a and Pt-1-b showed two distinct ecological niches at two different temperature ranges (Reimer et al., 2017a). In this study, Pt-1-b had higher prevalences in warmer waters (>29°C) and increased at almost the same rate as the thermotolerant *Durusdinium*, while Pt-1-a preferred colder temperatures (<26°C) at higher latitudes in the Red Sea (Reimer et al., 2017a). These results demonstrate that genetically closely related Symbiodiniaceae do not necessarily share the similar physiological traits and niches (Sampayo et al., 2007), further evidence of the high amounts of species diversity within the family (LaJeunesse, 2004; Pochon et al., 2014; Smith et al., 2017b; Thornhill et al., 2014). Results from other genetic markers and examinations of allele exchange could help clarify the relationships between the *Cladocopium* lineages observed in this study.

In this study, we showed that environmental changes over relatively small geographical scales can influence the kinds of symbiont associated with a host (<60 km LaJeunesse et al., 2004). Agreeing with previous studies (LaJeunesse et al., 2004; Finney et al., 2010), variations in SST and Chl a with distance from coast were shown to be good predictors for the distribution of *Cladocopium* psbA^{ncr} lineages and *Durusdinium* hosted in *P. tuberculosa*. Nevertheless, more

studies in the future are required to examine other environmental factors, such as nutrient (nitrogen families, phosphate) levels, which can also be driven by distance from coast, and thereby influencing Chl a concentrations and Symbiodiniaceae diversity (Fahmy, 2003; Grupstra et al., 2017). Furthermore, two closely related *Cladocopium* lineages in this study (Pt-1-a, Pt-1-b) had two distinct environmental niches. These findings indicate that the psbA^{ncr} marker resolves lineages of *Cladocopium* that possess distinct physiological and ecological attributes (Noda et al., 2017; Reimer et al., 2017a). Hence, future research utilizing hypervariable DNA regions (such as psbA^{ncr}) combined with fine-scale environmental data to examine diversity of Symbiodiniaceae is strongly recommended (LaJeunesse & Thornhill, 2011; Smith et al., 2017a). Such higher-resolution patterns of diversity will become more important in the future as more robust ecological analyses will be utilized to study Symbiodiniaceae diversity in the face of global climate change.

Acknowledgements

This work was partially funded by the King Abdullah University of Science and Technology (KAUST) (award URF/1/1389-01-01, Red Sea Research Center funds, and baseline research funds to MLB and TR), JSPS Kakenhi-Kiban B grant entitled ‘Global evolution of Brachycnemina and their *Symbiodinium*’ to JDR, and Sasagawa Research Foundation funding to HBW (29-751). We wish to thank the captain and crew of the MV Dream Master, the KAUST Coastal and Marine Resources Core Laboratory, A. Gusti (KAUST), Roberto Arrigoni, Darren Coker, Tane Sinclair-Taylor and other members of the KAUST Reef Ecology Lab for fieldwork assistance in the Red Sea. Comments from two reviewers greatly improved an earlier version of this manuscript.

Conflict of interest statement

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

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Figures

Fig. 1 **Sampling sites of (a) Thuwal and (b) Farasan Banks.** Different colours represent FBO (red), FBM (orange), FBI (yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM=Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 2 **Phylogeny of Symbiodiniaceae *Cladocopium* lineages from *Palythoa tuberculosa* at Farasan Banks and Thuwal.** Maximum Likelihood (ML) tree of forward sequences of *Cladocopium* chloroplast psbA non-coding region (psbA^{ncr}) with reference sequences from Reimer et al. (2017a). Coloured dots on the tree represent each specimen found at FBO (red), FBM (orange) and FBI (yellow), and TI (green). Values at the branches represent ML, neighbor joining (NJ) and maximum parsimony (MP) bootstrap supports, and Bayesian inference (Bayes) posterior probability, respectively. Dotted boxes in the figure represent lineages of *Cladocopium*. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 3 **Symbiodiniaceae composition based on molecular identity (Sampling sites of (a) Thuwal and (b) Farasan Banks.** Different colours represent FBO (red), FBM (orange), FBI (yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 2). Based on ITS-2, genera *Cladocopium* and *Durusdinium* composition at different (a) locations and (b) depths. psbA^{ncr} marker identified six lineages of *Cladocopium* and composition is shown based on (c) locations and (d) depth (meters). FBO=Farasan Banks Offshore; FBM=Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 4 **Similarity dendrogram cluster of *Cladocopium* lineages based on psbA^{ncr} composition.**

Percentage similarity between sites based at different (a) locations and (b) depths (meter).

FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore;

TI=Thuwal.

Fig. 5 **Map depicting (a) sea surface temperature (SST, °C) and (b) chlorophyll a**

concentration (Chl a, mg m⁻³). The colour bars on each map represent the values of SST and Chl

a, respectively. The sites are represented as filled circles for FBO (red), FBM (orange), FBI

(yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf;

FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 6 **Spearman's rho correlations among variables Symbiodiniaceae (*Cladocopium* psbA^{ncr}**

lineages and *Durusdinium*) and predictors; depth, sea surface temperature (SST),

chlorophyll a (Chl a) and locations. Diagonal histograms represent the comparisons within each

variable. The scatter plots below the histograms represent pairwise linear regression among

variables, the numbers above the histograms represent Spearman's rho correlation coefficient

values among variables with significance markings (0.1., 0.01*, 0.05**, <0.001***). The x and y

axis of the graph represent either the gradients (SST and Chl a) or arbitrary values representing the

categories in order (Locations, Depth and Symbiodiniaceae) of each variable.

Fig. 7 **Probability of observing Symbiodiniaceae (*Cladocopium* psbA^{ncr} lineages and**

***Durusdinium*) in *Palythoa tuberculosa* of the central and southern Red Sea along (a) sea**

surface temperatures and (b) chlorophyll a levels. Probabilities were calculated from

multinomial logistic regression of SST and Chl a, respectively.

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Tables

Table 1 **Test of normality distribution (Shapiro-Wilk test) and significance (Kruskal-Wallis with Dunn post-hoc test) for sea surface temperature (SST) and chlorophyll a (Chl a) among locations.** * represent values with significant difference ($p < 0.05$). FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Table 2 ***p*-values of multinomial logistic regression (MLR) coefficients among the Symbiodiniaceae for locations (FBI vs. FBM, FBO and TI), depth, sea surface temperature (SST) and chlorophyll a (Chl a).** * represent values with significant differences ($p < 0.05$). FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Supplementary

Supplementary 1 **Number of specimens based on locations and depths represented in tables**

of: (a) total number of specimens collected in sampling, (b) numbers of generated ITS-2 sequences, and (c) of numbers of generated *psbA^{ncr}* sequences obtained. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Supplementary 2 **Phylogeny of Symbiodiniaceae from *Palythoa tuberculosa* at Farasan Banks**

and Thuwal. Maximum Likelihood (ML) tree of the internal transcribed spacer 2 of ribosomal DNA (ITS-2) of nuclear ribosomal DNA of Symbiodiniaceae with reference sequences from Reimer et al. (2017a). Coloured dots on the tree represent each specimen found at FBO (red), FBM (orange) and FBI (yellow), and TI (green). Values at the branches represent ML, neighbor joining (NJ) and maximum parsimony (MP) bootstrap supports, and Bayesian inference (Bayes) posterior probability, respectively. Dotted boxes in the figure represent genera of Symbiodiniaceae. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Supplementary 3 **Genetic difference among lineages in this study (Pt-1-a, Pt-1-b, Pt-1-c, Pt-1-**

d, Pt-3-a, Pt-3-b). Two tables represent the (a) within-group genetic difference with mean and standard deviation (StDev), and (b) pairwise genetic differences between groups with mean (bottom black) and standard deviation (top, grey).

Supplementary 4 **Analysis of the Molecular Analysis (AMOVA) for *Cladocopium psbA^{ncr}*.** Two

statistical comparisons were conducted based on populations sorted by (a) locations and (b) depths (meter).

1
2 Fig. 1 **Sampling sites of (a) Thuwal and (b) Farasan Banks.** Different colours represent FBO (red), FBM (orange), FBI (yellow) and TI (green) sites.
3 FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 2 **Phylogeny of Symbiodiniaceae *Cladocopium* lineages from *Palythoa tuberculosa* at Farasan Banks and Thuwal.** Maximum Likelihood (ML) tree of forward sequences of *Cladocopium* chloroplast psbA non-coding region (psbA^{ncr}) with reference sequences from Reimer et al. (2017a). Coloured dots on the tree represent each specimen found at FBO (red), FBM (orange) and FBI (yellow), and TI (green). Values at the branches represent ML, neighbor joining (NJ) and maximum parsimony (MP) bootstrap supports, and Bayesian inference (Bayes) posterior probability, respectively. Dotted boxes in the figure represent lineages of *Cladocopium*. FBO=Farasan Banks Offshore; FBM=Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 3 **Symbiodiniaceae composition based on molecular identity** (Fig. 2). Based on ITS-2, genera *Cladocopium* and *Durusdinium* composition at different (a) locations and (b) depths. psbA^{ncr} marker identified six lineages of *Cladocopium* and composition is shown based on (c) locations and (d) depth (meters). FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 3 **Similarity dendrogram cluster of *Cladocopium* lineages based on *psbA^{ncr}* composition.** Percentage similarity between sites based at different (a) locations and (b) depths (meter). The percentage values represent the similarity percentage between the dichotomous branches at each node. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 4 Map depicting (a) sea surface temperature (SST, °C) and (b) chlorophyll a concentration (Chl a, mg m^{-3}). The colour bars on each map represent the values of SST and Chl a, respectively. The sites are represented as filled circles for FBO (red), FBM (orange), FBI (yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 5 Spearman's rho correlations among variables Symbiodiniaceae (*Cladocopium psbA^{ncr}* lineages and *Durusdinium*) and predictors; depth, sea surface temperature (SST), chlorophyll a (Chl a) and locations. Diagonal histograms represent the comparisons within each variable. The scatter plots below the histograms represent pairwise linear regression among variables, the numbers above the histograms represent Spearman's rho correlation coefficient values among variables with significance markings (0.1., 0.01*, 0.05**, <0.001***). The x and y axis of the graph represent either the gradients (SST and Chl a) or arbitrary values representing the categories in order (Locations, Depth, and Symbiodiniaceae) of each variable.

Fig. 6 Probability of observing Symbiodiniaceae (*Cladocopium* psbA^{ncr} lineages and *Durusdinium*) in *Palythoa tuberculosa* of the central and southern Red Sea along (a) sea surface temperatures and (b) chlorophyll a levels. Probabilities were calculated from multinomial logistic regression of SST and Chl a, respectively.

Table 1 Test of normality distribution (Shapiro-Wilk test) and significance (Kruskal-Wallis with Dunn post-hoc test) for sea surface temperature (SST) and chlorophyll a (Chl a) among locations. * represent values with significant difference ($p < 0.05$). FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

		SST		Chl a	
		W	p	W	p
Shapiro -Wilk		0.834	<0.001*	0.825	<0.001*
		X ²	p	X ²	p
Kruskal-Wallis		17.642	0.001*	16.61	0.001*
		Z	p	Z	p
Dunn post-hoc test	FBI - FBM	1.764	0.467	2.977	0.018*
	FBI - FBO	2.53	0.069	3.536	0.003*
	FBM - FBO	1.017	1.000	0.936	1.000
	FBI - TI	4.065	0.001*	0.645	1.000
	FBM - TI	2.729	0.039*	-1.946	0.311
	FBO - TI	1.628	0.623	-2.572	0.061

Table 2 p-values of multinomial logistic regression (MLR) coefficients among the Symbiodiniaceae for locations (FBI vs. FBM, FBO and TI), depth, sea surface temperature (SST) and chlorophyll a (Chl a). * represent values with significant differences ($p < 0.05$). FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

	(Intercept)	FBM	FBO	TI	depth	SST	Chl a
<i>Durusdinium</i> vs Pt-1-a	0.006*	0.267	0.099	0.640	0.099	0.003*	0.070
<i>Durusdinium</i> vs Pt-1-b	0.239	0.849	0.413	<0.001*	0.184	0.604	0.051
<i>Durusdinium</i> vs Pt-3-a	0.002*	0.001*	0.001*	<0.001*	0.446	0.001*	0.006*
<i>Durusdinium</i> vs Pt-3-b	0.002*	0.436	0.766	0.785	0.001*	0.001*	0.460
Pt-1-a vs Pt-1-b	0.001*	0.429	0.417	<0.001*	0.845	0.001*	0.672
Pt-1-a vs Pt-3-a	0.259	0.423	0.376	<0.001*	0.435	0.443	0.001*
Pt-1-a vs Pt-3-b	0.195	0.664	0.153	0.498	0.147	0.229	0.144
Pt-1-b vs Pt-3-a	0.006*	0.008*	0.006*	-	0.600	0.013*	0.001*
Pt-1-b vs Pt-3-b	0.002*	0.564	0.798	<0.001*	0.152	0.002*	0.505
Pt-3-a vs Pt-3-b	0.322	<0.001*	<0.001*	0.548	0.024*	0.539	0.002*

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Supplementary Material

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Supplementary Material

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1 **Symbiodiniaceae diversity of *Palythoa tuberculosa* in the central**
2 **and southern Red Sea influenced by environmental factors**

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15 KEYWORDS
16 Saudi Arabia, zooxanthellae, zoantharian, sea surface temperature, chlorophyll a

17 **Abstract**

18 Sea surface temperatures (SST) and chlorophyll a concentrations (Chl a) in the southern Red Sea
19 have wide variations based on distance from the coast. To understand how these variations can
20 affect the diversity of symbionts hosted by reef-associated organisms, we conducted a study in the
21 central and southern Red Sea to examine the diversity of Symbiodiniaceae hosted by the
22 zooxanthellate zoantharian *Palythoa tuberculosa* at different distances from the coast: offshore
23 (FBO), midshelf (FBM) and inshore (FBI) of Farasan Banks, and inshore at Thuwal (TI). Genomic
24 DNA was extracted from 198 specimens, followed by amplification of the ribosomal DNA internal
25 transcribed spacer 2 (ITS-2) and non-coding region of the chloroplast plastid minicircle (psbA^{ncr}).
26 *Durudinium* and six lineages of *Cladocopium* (Pt-1-a, Pt-1-b, Pt-1-c, Pt-1-d, Pt-3-a, Pt-3-b) were
27 identified based on sequences of the two marker regions. Changes in composition of

28 Symbiodiniaceae lineages were observed from FBI (high SST, high Chl a) to FBO (low SST, low
29 Chl a). Molecular variance analyses (AMOVA) showed that distance from coast was the most
30 likely predictor of differences in *Cladocopium* lineages. Multinomial Logistic Regression (MLR)
31 analysis showed a transition among different *Cladocopium* lineages as SST increased. One
32 *Cladocopium* lineage, Pt-1-b, demonstrated higher prevalences at high SSTs, and increased in
33 prevalences at the same rate as thermotolerant *Durisdinium*. Additionally, *Cladocopium* lineage
34 Pt-3-a had a high affinity to low Chl a concentrations. This study demonstrates that environmental
35 variations in SSTs and Chl a concentrations based apparently are significant predictors for the
36 diversity of dominant Symbiodiniaceae within individual host *P. tuberculosa* colonies. We
37 theorize that flexibility with different lineages of Symbiodiniaceae allows generalist *P.*
38 *tuberculosa* to live across a wide range of environments in the southern Red Sea.

39

Introduction

The Red Sea is a region of high marine biodiversity and endemism levels, and is one of the hottest marine environments in the world (Berumen et al., 2013; DiBattista et al., 2016a; DiBattista et al., 2016b; Ludt et al., 2017). The Red Sea basin was formed approximately 5 million years ago (Bailey et al., 2007; Berumen et al., 2013; Ludt et al., 2017) and is connected at the south with the adjacent Indian Ocean through the shallow opening of Bab al Mandab Strait. The partial oceanographic isolation and geographic location of the Red Sea has resulted in its southern region and inshore waters becoming comparably very warm, with sea surface temperatures (SSTs) regularly exceeding 32 °C during the summer. The shallow Bab al Mandab Strait brings nutrient rich water from the Indian Ocean into the Red Sea, making this region high in primary productivity (Brewin et al., 2015; Krueger et al., 2017; Ngugi et al., 2012; Osman et al., 2017; Raitso et al., 2013; Sofianos & Johns, 2003; Sonnewald & El-Sherbiny, 2017). In the southern region of the Red Sea, zooxanthellate scleractinian corals begin to bleach at 32.5 °C (Eladawy et al., 2017; Monroe et al., 2018). Hence, the shallow southern inshore reefs of the Red Sea exist at their upper thermal tolerance limits and are more susceptible to bleaching compared to offshore and deeper reefs that experience slightly lower SSTs (Fine et al., 2013; Monroe et al., 2018; Osman et al., 2017). This makes inshore Red Sea reefs marginal, and such marginal reefs are susceptible to global climate change as SSTs increase rapidly due to anthropogenic causes (Furby et al., 2013; Hughes et al., 2003; Lough et al., 2018; Monroe et al., 2018).

Until now, frequent contact with naturally high SSTs has driven adaptation within Red Sea coral reefs and has favored resilient variants of associated organisms (Coles et al., 2018; Hume et al., 2013; Maynard et al., 2008). One taxon shown to be driven by such environmental factors are the symbiotic dinoflagellates of the family Symbiodiniaceae (previously genus *Symbiodinium*;

LaJeunesse et al., 2018) that live in symbioses with a variety of marine invertebrates, including many anthozoans (Baker, 2003; LaJeunesse et al., 2010; Mies et al., 2017). The host-symbiont ('holobiont') relationship has been at the center of coral reef research as holobionts experience increasing frequencies of thermal-stress-induced mass bleaching events (Baker et al., 2018; Burke et al., 2011; Chakravarti et al., 2017; Hughes et al., 2017; Lough et al., 2018; Núñez-Pons et al., 2017; Smith et al., 2017a). Among Symbiodiniaceae, *Cladocopium* (formerly 'clade C') and *Durisdinium* (formerly 'clade D') are the most common genera found in the Indo-Pacific scleractinians and zoantharians (Burnett, 2002; Mostafavi et al., 2007; LaJeunesse, 2004; LaJeunesse et al., 2018). By using molecular phylogenetic analyses, most genera of Symbiodiniaceae have been shown to contain many species (Hume et al., 2015; LaJeunesse et al., 2018; LaJeunesse et al., 2014; Lewis et al., 2019; Reimer et al., 2017a; Wong et al., 2016).

Various factors have been shown to drive the evolution of Symbiodiniaceae; coevolution with specific hosts, geographical distributions, and the environment (SST, PAR light levels, pH) in which the holobionts are found (Finney et al., 2010; LaJeunesse et al., 2004; LaJeunesse et al., 2010; Tonk et al., 2013). Hence, there are diverse Symbiodiniaceae with specific and optimized niches, and associations with these can improve the survivability and resilience of hosts (Kemp et al., 2006; Sawall et al., 2014). Additionally, in the aftermath of recent bleaching events, some reports have seen a shift or 'reshuffling' of Symbiodiniaceae, especially from *Cladocopium* to more stress-tolerant *Durisdinium trenchii* (D1a) within some host species ~~in the Indo-Pacific region~~ (Bay et al., 2016; Cuning et al., 2015; Silverstein et al., 2015; Silverstein et al., 2017). However, Tonk et al. (2013) noted that is hard to make robust inferences on the extent to which environment and geographical distributions drive the diversity of Symbiodiniaceae. This is due to the fact that the majority of Symbiodiniaceae are host-specific, and that host species' distributions

are often limited, restricting the potential for wider environmental and geographical comparisons within one widely distributed host (Finney et al., 2010).

Several studies have examined Symbiodiniaceae hosted by the widely distributed zoantharian *Palythoa tuberculosa* (Anthozoa: Hexacorallia: Zoantharia) (Burnett, 2002; Noda et al., 2017; Reimer & Todd, 2009; Reimer et al., 2017a). The host *Palythoa* is common across the Indo-Pacific Ocean (Hibino et al. 2013) and is an ecological generalist, found in a wide variety of environments (acidified reefs, river mouths, and intertidal zones to 50 m depth). Previous research has shown that *P. tuberculosa* hosts *Cladocopium* C1 and *Durusdinium* (Burnett, 2002; Reimer et al., 2017a; Reimer & Todd, 2009), while more recent examinations of the non-coding region of chloroplast plastid minicircle (psbA^{ncr}) have demonstrated the presence of four *Cladocopium* lineages (~~OTU at least species~~) in the Red Sea (Reimer et al., 2017a). The composition of these *Cladocopium* lineages in *P. tuberculosa* changes from areas with lower SSTs in the north (~25 °C) to areas in the warmer central (~29 °C) Red Sea. However, there is still a lack of information on the Symbiodiniaceae in *P. tuberculosa* in the southern Red Sea.

Studying Symbiodiniaceae composition in the southern Red Sea allows us to examine the influence of SSTs and turbidity on symbionts. As these reefs are among the hottest in the world, observed trends may also serve as harbingers for other areas under future climate changes (Roik et al., 2016). Furthermore, ~~examining-targeting~~ a single target host species ~~reduces-removes the influence of~~ host ~~factors-identity so as to examine that potentially influence Symbiodiniaceae diversity~~ the effect of environmental gradients on symbiont distribution and diversity (LaJeunesse et al., 2004; Tonk et al., 2013). *Palythoa tuberculosa* is among the best candidates for this kind of study as it is widely distributed in the Red Sea and is easily identified in the field (Reimer et al., 2017b). In this study, two molecular markers (ITS-2 of ribosomal DNA and psbA^{ncr}) were used to

109 compare the composition of Symbiodiniaceae hosted by *P. tuberculosa* colonies at different depths
110 and distances from the coast in two southern and central Red Sea locations, Farasan Banks
111 (southern) and Thuwal (central). Models of expected Symbiodiniaceae probability based on
112 Multinomial Logistic Regression Analysis were constructed with SST and chlorophyll a
113 concentration data in order to observe patterns of Symbiodiniaceae composition change across
114 different distances from coast and depths.

Materials and Methods

Field collection

The study was conducted in reefs in two regions in the central eastern and Thuwal, both in Saudi Arabia (Figures

Fig. 1). Specimen collection at Farasan Banks was conducted from April 30 to May 7, 2017 at 19 different sites. Sites within the Farasan Banks were split into three categories based on their distance from the Saudi Arabian coast: offshore (FBO, >55 km), midshelf (FBM, 20-55 km), and inshore (FBI, <20 km). Specimen collection within the Thuwal inshore region (TI) was conducted May 9-10, 2017 at four sites, all of which were <15 km from the coast (Fig. 1, Supplementary 1). The offshore reefs (FBI) are located at the continental shelf edge of the Farasan Banks and are surrounded by deep waters (typically >500 m deep) with steep drop-offs, while inshore reefs (FBI and TI) are usually near to the coast and at shallower depth of less than 80 m (Yao et al., 2014). The midshelf reefs (FBM) are typically found on or nearby to the relatively shallow waters at the continental shelf and are intermediary between inshore and offshore reefs.

Palythoa tuberculosa specimens were collected via SCUBA diving and the roving search method (Raymundo et al., 2008). Each collection dive started from 30 m and slowly ascended the reef slope while searching for colonies. Once located, the depth of each host colony was recorded and 2 cm² subsamples of each colony were taken. Immediately after dives, collected specimens were preserved in 99.5% ethanol. A total of 203 specimens were collected, consisting of 161 from the Farasan Banks and 42 from Thuwal (Supplementary 1a). Specimens were brought back to the University of the Ryukyus, Japan, for further analyses.

136 *DNA extraction and amplification*

137 Genomic DNA was extracted from specimens using a DNeasy Blood and Tissue extraction
138 kit following the manufacturer's instructions (Qiagen, Tokyo). Two DNA marker regions were
139 amplified via polymerase chain reaction (PCR) targeting specific regions: the internal transcribed
140 spacer 2 of ribosomal DNA (ITS-2) of nuclear ribosomal DNA for all specimens, as well as the
141 non-coding region of the plastid minicircle (*psbA^{ncr}*) for specimens containing *Cladocopium*. The
142 ITS-2 sequences were utilized to place symbiont types within the well-established ITS-2
143 phylogenetic framework (LaJeunesse & Thornhill, 2011), while the *psbA^{ncr}* sequences were
144 utilized to examine differences at a finer phylogenetic resolution (LaJeunesse & Thornhill, 2011;
145 Reimer et al., 2017a; Noda et al., 2017).

146 The ITS-2 region was amplified using the primers zITSf (5'-CCG GTG AAT TAT TCG
147 GAC TGACGC AGT-3') and ITS4 (5'-TCC TCC GCT TAT TGATAT GC-3') (White et al., 1990;
148 Rowan & Powers, 1992; Hunter et al., 1997), and *psbA^{ncr}* was amplified using the primers 7.4-
149 Forw (5'-GCA TGA AAG AAA TGC ACA CAA CTT CCC-3') and 7.8-Rev (5'-GGT TCT CTT
150 ATT CCA TCA ATA TCT ACT G-3') (LaJeunesse & Thornhill, 2011; Moore et al., 2003). The
151 PCR mixes (20 µl) were composed of 1.0 µl of genomic DNA, 10.0 µl of HotStarTaq Plus Master
152 Mix, 1.0 µl of each primer (10 pmol), 1.0 µl MgCl₂ (25 mmol), 0.5 Bovine Serum Albumin (20
153 mg/ml), and 1.5 µl Coral Load. Thermocycle conditions were modified slightly from Noda et al.
154 (2017); ITS-2: 95.0 °C for 5 min; 35 cycles of 94.0 °C for 30 s, 51.0 °C for 45 s, and 72.0 °C for 2
155 min; with a final extension at 72.0 °C for 10 min; and for *psbA^{ncr}*: 95.0 °C for 5 min; 40 cycles of
156 94.0 °C for 10 s, 55.0 °C for 30 s, and 72.0 °C for 2 min; with a final extension at 72.0 °C for 10
157 min. The products were sent to Fasmac (Kanagawa, Japan) for sequencing in both directions.

158 *Phylogenetic analyses*

159 ITS-2 sequences in both directions were aligned manually to produce consensus sequences
160 using Bioedit (Hall, 1999). Consensus sequences of ITS-2 and psbA^{ncr} forward sequences were
161 edited individually using the same software. Only one direction of psbA^{ncr} sequences were used as
162 in previous research (Hawkins et al., 2016; Kunihiro & Reimer, 2018; Noda et al., 2017; Reimer
163 et al., 2006). Edited sequences for each marker were compiled and aligned in MEGA v.7 (Kumar
164 et al., 2016). Previously reported ITS-2 sequences of genera *Cladocopium* (AB207184, DQ480631,
165 DQ480639, DQ889741, DQ889743), *Durusdinium* (AB704033, EU333712, KU535564,
166 MH243738), *Gerakladium* (AB546599) and *Effrenium* (AY160123) were obtained from GenBank
167 and included in the ITS-2 alignment as references. psbA^{ncr} reference sequences were obtained from
168 Reimer et al. (2017a), including the four lineages previously reported from the Red Sea associated
169 with *P. tuberculosa*; lineages Pt-1-a (KU920804, KU920812, KU920813, KU920816, KU920826),
170 Pt-1-b (KU920803, KU920805, KU920809, KU920810, KU920811), Pt-3-a (KU920703,
171 KU920706, KU920707, KU920714, KU920718, KU920719), and Pt-3-b (KU920720, KU920733,
172 KU920734, KU920737, KU920743, KU920744). The final ITS-2 and psbA^{ncr} alignments were
173 comprised of 154 (451 bp length) and 126 (239 bp) sequences, respectively. Novel sequences of
174 psbA^{ncr} from this study were deposited in GenBank (MT250281-MT250506), and the alignment
175 file is available as supplementary data (Supplementary_psbAF_126_239bp.fas).

176 The ITS-2 and psbA^{ncr} alignments were used to construct phylogenetic trees with
177 maximum likelihood (ML), neighbor-joining (NJ), maximum parsimony (MP), and Bayesian
178 inference (BI) methods. The best substitutional methods were chosen for ML and NJ phylogenetic
179 constructions for both alignments based on NJ tree reference under automatic model selection of
180 MEGA. With this, Kimura-2 and Jukes-Cantor model with 1000 bootstraps phylogeny test at

181 uniform rates were selected to construct the ML, NJ, and MP trees of the ITS-2 and psbA^{ncr}
182 alignments (Hasegawa et al., 1985). Bayesian inference trees for ITS-2 (chain length=5,000,000;
183 burn-in=1,250,000) and psbA^{ncr} (chain length=15,000,000; burn-in=3,750,000) using the same
184 substitution model as mentioned above were constructed via MrBayes v.3.2.6 x64 (Huelsenbeck
185 & Ronquist, 2001). The genetic differences between and within psbA^{ncr} forward sequences with
186 references from Reimer et al. (2017a) were calculated in MEGA utilizing the Maximum
187 Composite Likelihood Model. The values of within group mean distances of each reference
188 sequence were used to determine the grouping of sequences based on the pairwise genetic distances
189 (GD).

190 The psbA^{ncr} alignment was exported into fasta file format via MEGA. The alignment was
191 imported into Microsoft Excel via the add-on GenAlEx (Genetic Analysis in Excel) version 6.5
192 and converted into numbers corresponding to each nucleotide. The polymorphic nucleotide (PN)
193 positions of the alignment were extracted and ambiguous bases were removed. Each sequence was
194 assigned to a distance from coast category (FBO, FBM, FBI, TI), and to a depth (0-10 m, 10.1-20
195 m, 20.1-30 m, and >30 m). Analysis of Molecular Variance (AMOVA) was conducted on the PN
196 data sets to observe genetic differences between populations.

197 *Symbiodiniaceae* composition

198 The genera/subclade (ITS-2) and lineages (psbA^{ncr}: *Cladocopium* only) of the specimens
199 were sorted based on distance from coast and depth. Shannon-Wiener Diversity Indices (SWDI,
200 H') and Pielou's evenness (Evenness, J') of *Cladocopium* lineages were calculated for each
201 distance from coast and depth category. The prevalences of *Cladocopium* lineages were analyzed
202 via ecological statistical methods using R studio with "vegan", "Mass", and "dplyr" packages

203 (Oksanen, 2015; R Core Team, 2017). The dataset was transformed with Bray-Curtis dissimilarity
204 matrices and represented with the dissimilarity composition in dendrogram cluster tree (hclust)
205 (Oksanen, 2015).

206 *Environmental data*

207 Sea surface temperature (SST, °C) and sea surface chlorophyll-a (Chl a, mg m⁻³) data for
208 the period between December 31, 2014 to May 31, 2017 were obtained from the National
209 Aeronautical and Space Administration (NASA) Giovanni website
210 (<https://giovanni.gsfc.nasa.gov/giovanni>) (Acker & Leptoukh, 2007). The time-averaged SST and
211 Chl a data with 4 km resolution derived from the Moderate Resolution Imaging Spectroradiometer
212 (MODIS) Aqua database were superimposed on a map of the region investigated in this study
213 (from 37.6172 °E, 17.4023 °N to 42.3633 °E, 23.9063 °N), and generated maps were examined
214 using QGIS 3.6.0 (QGIS Development Team, 2019) to estimate the values of SST and Chl a for
215 each sampling location in this study. Shapiro-Wilk normality tests showed both SST and Chl a
216 datasets were not normal. Thus, we conducted a non-parametric Kruskal-Wallis tests on the SST
217 and Chl a datasets with the locations (FBI, FBM, FBO, TI) as factors, followed by Dunn post-hoc
218 test with Bonferroni correction. Spearman's rho correlation test was conducted to check the
219 correlation among locations, depths, SST, Chl a, and Symbiodiniaceae (four *Cladocopium* psbA^{ncr}
220 lineages and *Durusdinium*). Multinomial Logistics Regression analysis (MLR) was conducted
221 using modified script from Reimer et al. (2017a) to identify good predictors of Symbiodiniaceae
222 composition in the Red Sea environment using available parameters (location, depth, SST, Chl a)
223 via R console with package “nnet” and “VGAM” (R Core Team, 2017; Ripley & Venables, 2016;
224 Yee, 2015). All statistical tests were conducted with statistical significance detection at p=0.05.

Results

Phylogeny of Symbiodiniaceae

In total, 198 ITS-2 region sequences from specimens were successfully amplified (Supplementary 1b). Preliminary analyses showed 164 specimens hosted Symbiodiniaceae identified as belonging to genus *Cladocopium*. Within these *Cladocopium*, 43 sequences either had double peaks within the sequences and/or shorter sequences. We interpreted these results as the possible presence of intragenomic variation in these 43 specimens, and this subset was removed from the subsequent ITS2 phylogenetic analyses. Our ITS-2 phylogenetic tree (Supplementary 2) showed distinct two clusters of Symbiodiniaceae. The remaining *Cladocopium* specimens (n=121) were grouped in the cluster of *Cladocopium* subclade C1/C1-related sequences (DQ889741, DQ889743, DQ480639, DQ480631; Reimer et al., 2017a), similar to those previously reported from *P. tuberculosa* in southern Japan and Saudi Arabia. A second, smaller cluster (n=34) was within the genus *Durusdinium* (EU333712) as previously reported from *P. tuberculosa* in Singapore (Reimer & Todd, 2009) and other parts of the Indian Ocean (Burnett, 2002). Based on obtained sequences, this cluster is likely *D. trenchii* or a closely related lineage.

Out of the 164 *P. tuberculosa* specimens hosting *Cladocopium*, 126 psbA^{ncr} region sequences were obtained (Supplementary 1c). The phylogenetic tree of psbA^{ncr} (Fig. 2) showed two strongly supported monophyletic large groupings corresponding to previously reported Red Sea lineages: Pt-1 and Pt-3 sensu Reimer et al. (2017a) (ML=99%, NJ=99%, MP=100%, BI=1.00). Within the large Pt-3 clade there were two subclades as previously seen in Reimer et al. (2017a): lineage Pt-3-a (n=33; ML=64%, NJ=60%, MP=100%, BI=1.00), and lineage Pt-3-b (n=57; ML=64%, NJ=64%, MP=100%, BI=0.65). In the Pt-1 cluster, Pt-1-a (n=14; ML=78%, NJ=78%,

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MP=100%, BI=1.00) and Pt-1-b (n=15) each formed well-supported clusters, with Pt-1-b as the basal cluster. Between Pt-1-a and Pt-1-b, there were two additional strongly supported lineages; Pt-1-c (n=4; ML=99%, NJ=97%, MP=100%, BI=1.00), and Pt-1-d (n=3; ML=99%, NJ=99%, MP=100%, BI=1.00). Pt-1 lineages had a very large mean genetic difference (GD) within (GD: 0.000-0.040) and among (GD: 0.070-0.180) the lineages compared to those of Pt-3 lineages (Supplementary 3, GD: within<0.008; among=0.027).

The AMOVA test based on *psbA^{ncr}* sequences showed that genotype diversity of the *Cladocopium* at different locations (FBI, FBM, FBO, TI) was primarily within each location (=60%) more than among the locations (=40%). A similar pattern was also observed for depths, but with higher diversity within a single depth category (=96%), as compared to among different depths (=4%). The total genetic variations for *Cladocopium* among locations (PhiPT=0.401, P<0.001) and depths (PhiPT=0.043, P=0.038) were found to be statistically significant (Supplementary 4).

Symbiodiniaceae distribution

Each *P. tuberculosa* colony had a dominant Symbiodiniaceae genus (*Cladocopium* or *Durusdinium*) and lineage (for *Cladocopium* only) based on the results from ITS-2 and *psbA^{ncr}* sequences, respectively. The composition of identified ITS-2 and *psbA^{ncr}* specimens were grouped based on the distance from coast (=location) and depth in percentage abundance (Fig. 3). ITS-2 analyses grouped the specimens into two genera (*Cladocopium* and *Durusdinium*); while *psbA^{ncr}* showed six lineages of *Cladocopium* (Pt-1-a, Pt-1-b, Pt-1-c, Pt-1-d and Pt-3-a, Pt-3-b). *Cladocopium* was the dominant genus, found in 82.8% of the *P. tuberculosa* examined, and made up >50% of colonies at every site (Fig. 3a & b, Supplementary 1b & c). In *psbA^{ncr}* sequences

269 (Supplementary 1c), the most common *Cladocopium* lineage was Pt-3-b (n=57 colonies, 45.2%),
270 followed by Pt-3-a (n=33, 26.2%), Pt-1-b (n=15, 11.9%), Pt-1-a (n=14, 11.1%), Pt-1-c (n=4, 3.2%),
271 and lastly Pt-1-d (n=3, 2.4%).

272 By location, *Durusdinium* had highest prevalence at FBI (n=16/30, 34.8%), followed by
273 FBM (n=14/54, 20.6%), with FBO (n=2/41, 4.7%) and TI (n=2/39, 4.9%) having similar and low
274 prevalences (Fig. 3a). FBO, FBM and FBI had the same number of *Cladocopium* lineages present
275 (n=5), while at TI only two *Cladocopium* lineages were detected. Lineage Pt-3-b (Fig. 3c) was the
276 most common lineage in FBM (n=20/37, 54.1%) and TI (n=32/35, 91.4%). Pt-3-a (n=25/34, 73.5%)
277 was the most abundant lineage at FBO, while FBI was dominated by Pt-1-b (n=11/20, 55.0%).
278 The diversity of *Cladocopium* lineages increased from FBO ($H' = 0.900$), across FBM ($H' = 1.235$),
279 to FBI ($H' = 1.304$), while the lowest diversity was at TI ($H' = 0.293$). Similar patterns were observed
280 for evenness, with highest at FBI ($J' = 0.810$), FBM ($J' = 0.768$), FBO ($J' = 0.559$), to lowest at TI
281 ($J' = 0.422$). A dissimilarity matrix of the *Cladocopium* lineages showed that TI and FBM shared
282 63.9% similarity (Fig. 4a). These two sites were 47.9% similar to composition of FBO, and FBI
283 composition was only 28.1% similar to composition of the rest of the sites.

284 *Durusdinium* (Fig. 3b) was absent at depths greater than 30 m and increased in prevalence
285 between 20-30 m (n=2/53; 3.8%) and 10-20 m (n=21/78; 26.9%). However, the shallowest 0-10
286 m showed a reduction of *Durusdinium* prevalence to 18.0% (n=11/61). On the other hand, the
287 shallowest depths (0-10 m) had the highest number of *Cladocopium* lineages recorded (n=6),
288 followed by 10.1-20 m (n=5), 20.1-30 m (n=4), and lastly >30 m (n=3). *Cladocopium* lineage Pt-
289 3-b was the most common lineage at 0-10 m (n=20/39, 51.3%), 10.1-20 m (n=19/43, 44.2%),
290 and >30 m (n=3/6, 50.0%), while Pt-3-a was the most common lineage at depths between 20.1-30
291 m (n=18/38, 47.4%; Fig. 3d). Shannon-Weiner indices showed a gradual increase in diversity

292 towards the shallows, from >30 m ($H'=1.011$), 20.1-30 m ($H'=1.076$), 10.1-20 m ($H'=1.343$), to
293 0-10 m ($H'=1.468$). However, evenness showed a different pattern, from 20.1-30 m ($J'=0.776$), 0-
294 10 m ($J'=0.819$), 10.1-20 m ($J'=0.834$), >30 m ($J'=0.921$). The composition of *Cladocopium*
295 lineages (Fig. 4b) between 0-10 m and 10.1-20 m was 80.5% similar. This was followed by 76.5%
296 similarity between 20.1-30 m range and the shallower groups. Finally, the >30 m *Cladocopium*
297 lineage composition was 27.3% similar to the compositions of the rest of the depths.

298 *Environmental data*

299 The average SST dataset showed FBI (29.9 ± 0.2 °C) was the warmest between 2015-2017
300 (Fig. 5a). This was followed by FBM (29.6 ± 0.2 °C) and FBO (29.3 ± 0.3 °C), with TI (28.5 ± 0.0 °C)
301 as the coldest location in this study. There were significant differences in SST among the locations
302 (Kruskal-Wallis: $X^2=17.642$, $p<0.001$), with the SST of TI significantly lower than those of FBI
303 (Dunn post hoc: $Z=4.064$, $p<0.001$) and FBM (Dunn post hoc: $Z=2.729$, $p=0.038$) (Table 1).

304 Average Chl a between 2015-2017 showed the highest value at FBI (0.8 ± 0.2 mg m⁻³) (Fig.
305 5b), followed by TI (0.7 ± 0.2 mg m⁻³), FBM (0.5 ± 0.1 mg m⁻³) and FBO (0.4 ± 0.1 mg m⁻³). There
306 were significant differences in Chl a levels among the locations (Kruskal-Wallis: $X^2=16.61$,
307 $p<0.001$), with Chl a at FBI significantly lower than FBM (Dunn post hoc: $Z=2.977$, $p=0.017$) and
308 FBO (Dunn post hoc: $Z=3.536$, $p=0.002$) (Table 1).

309 Comparisons of Symbiodiniaceae (*Cladocopium* psbA^{ncf} lineages and *Durisdinium*) with
310 depth, SST, Chl a, and locations were conducted with Spearman's rho correlation (Fig. 6). There
311 was a significantly strong correlation between SSTs of different locations (Spearman: $r_s=-0.92$,
312 $p<0.001$), and a significantly negative correlation between Chl a levels and depths (Spearman: $r_p=-$

0.48, $p < 0.001$). Furthermore, there were significant correlations between Symbiodiniaceae with locations (Spearman: $r_s = 0.49$, $p < 0.001$) and SSTs (Spearman: $r_s = -0.52$, $p < 0.001$).

Multinomial logistic regression (MLR) tests were conducted to examine the misclassification rate of highest probability observing the given nominal outcome (*Cladocopium* psbA^{ncr} lineages and *Durusdinium*) based on a set of independent variables (distance from coast, depth, SST, Chl a). Only four lineages of *Cladocopium* psbA^{ncr} lineages (Pt-1-a, Pt-1-b, Pt-3-a, Pt-3-b) were used in this model, as Pt-1-c and Pt-1-d were removed due to low numbers ($n < 5$). All predictors were required for best predicting the distribution of Symbiodiniaceae (correct rate = 70.39%). The global model (intercept) showed that *Durusdinium* distribution was different to those of all *Cladocopium* lineages to the exception of Pt-1-b (Table 2). SST was the main predictor for the distribution of Symbiodiniaceae (6/10 comparisons), while Chl a was the main predictor of Pt-3-a distribution.

As SST and Chl a both showed significant influences in predicting the distribution of Symbiodiniaceae in *P. tuberculosa*, the probability of observing different symbionts were reconstructed based on SST (Fig. 7a) and Chl a (Fig. 7b) gradients. The SST (Fig. 7a) model showed that from 29-30 °C, which corresponds to the SST range of the Farasan Banks, there was a transition in *Cladocopium* psbA^{ncr} lineages from Pt-3-a to Pt-3-b to Pt-1-b. Furthermore, *Durusdinium* was observed to increase in probability at almost the same rate as *Cladocopium* lineage Pt-1-b. At <29 °C, Pt-3-b had the highest probability of occurrence. Pt-1-a showed low probability of prevalence throughout the examined temperature range. For the Chl a gradient (Fig. 7b), lineage Pt-3-a was only found at low Chl a concentrations (<0.5 mg m⁻³), while Pt-3-b had a peak occurrence at 0.6 mg m⁻³, and *Durusdinium* had a higher occurrence probability at >0.5 mg

335 m⁻³. Both Pt-1 *Cladocopium* lineages did not show any clear pattern across the Chl a concentration
336 gradient.

Discussion

This study revealed a transition of Symbiodiniaceae composition in *Palythoa tuberculosa* at different distances from the coastline in the southern Red Sea. Reimer et al. (2017a), focusing on the northern to the central Red Sea (spanning ≈ 900 km), suggested that SST was a strong predictor of the *Cladocopium* lineages across latitude. However, results from the current study showed that within the Farasan Banks, across a much smaller geographical scale (<60 km), not only SST but also other environmental factors influenced the diversity of Symbiodiniaceae within *P. tuberculosa*. These results confirm previous studies that have shown shifts in prevalences among closely related Symbiodiniaceae may occur in host populations over small geographic distances of tens of kilometers (LaJeunesse et al., 2004; Noda et al., 2017; Wee et al., 2019). Based on our present analyses, sea surface temperatures (SST) and chlorophyll a concentrations (Chl a) were strong predictors of dominant Symbiodiniaceae genera and of *Cladocopium* lineages in *P. tuberculosa* in the southern Red Sea (Acker & Leptoukh, 2007; Eladawy et al., 2017; Raitsos et al., 2013; Sofianos & Johns, 2003).

There are two Symbiodiniaceae genera hosted by *P. tuberculosa* in the Red Sea and across the Indo-Pacific, *Cladocopium* and *Durusdinium*. These two Symbiodiniaceae genera have also been reported in the sister species *P. caribaeorum* in the Caribbean region (Kemp et al., 2006). Lineages of *Cladocopium* has-have been shown to be the dominant Symbiodiniaceae in *P. tuberculosa* across the Indo-Pacific Ocean, with reports of *Durusdinium* lineages from warm and turbid areas (Burnett, 2002; LaJeunesse, 2004; Noda et al., 2017; Reimer & Todd, 2009; Reimer et al., 2017a). Spatially, one *Durusdinium* lineage had higher abundances in the southern Red Sea, particularly at inshore (FBI) and shallow depths (<20 m), compared to offshore (FBO) and deeper depths (>20 m). At the Thuwal inshore (TI) sites of the central Red Sea, the prevalence of

360 *Durusdinium* in *P. tuberculosa* was within the range of previously reported rates of occurrence for
361 coral reef holobionts in the eastern coast of the Red Sea (<10%) (Reimer et al., 2017a; Ziegler et
362 al., 2017). On the other hand, *P. tuberculosa* hosted only *Cladocopium* lineages at depths >30 m,
363 with the *Durusdinium* lineage slowly increasing in prevalence towards shallower water.
364 *Durusdinium* lineages are commonly found near the equator, at shallower depths, and at more
365 turbid and/or inshore reefs (Bongaerts et al., 2013; Burnett, 2002; LaJeunesse et al., 2010; Reimer
366 & Todd, 2009; Tonk et al., 2013).

367 Within *Cladocopium*, six lineages were identified based on *psbA^{ncr}* sequences; four
368 previously reported lineages (Pt-1-a, Pt-1-b, Pt-3-a, and Pt-3-b; Reimer et al., 2017a) and two
369 newly observed lineages (Pt-1-c and Pt-1-d) that were phylogenetically located within the Pt-1
370 radiation. Pt-3-b was the most abundant *Cladocopium* lineage at Thuwal (TI), and this agrees with
371 the results of Reimer et al. (2017a) in the central Red Sea. Compared to TI, the Farasan Banks had
372 higher diversity of *Cladocopium* lineages at each location (FBO, FBM, FBI: n=5). Nevertheless,
373 there were significant differences in both the genetic diversity and lineage composition among the
374 locations of the Farasan Banks. These results agree with those of LaJeunesse et al. (2004), who
375 noted that geographic gradients determined the composition and selection of Symbiodiniaceae by
376 host. On the other hand, there was little differentiation of *Cladocopium* lineages among depths
377 (AMOVA and composition similarity). This in agreement with Finney et al. (2010) and Reimer et
378 al. (2017a), who both observed that geographical separation influenced Symbiodiniaceae diversity
379 (both at genera level and *Cladocopium* lineages) more than depth.

380 SSTs and Chl a were the two main environmental parameters that changed with distance
381 from the coast in the southern Red Sea (Eladawy et al., 2017; Raitos et al., 2013; Sofianos &
382 Johns, 2003). Although the SST range in this study was much narrower when compared that

383 observed in Reimer et al. (2017a), SSTs showed significant differences, especially between the
384 warmer inshore Farasan Banks and colder Thuwal (Sofianos & Johns, 2003). In general, SSTs
385 were observed to increase towards the south and nearer to the coast (Eladawy et al., 2017; Monroe
386 et al., 2018).

387 The Chl a values followed a similar trend as SST data, from lower values in the north
388 ($<0.01 \text{ mg m}^{-3}$) to higher values in the south ($>1 \text{ mg m}^{-3}$) (Raitsos et al., 2013). Chl a results in this
389 study matched well with the values previously observed for the region (1 mg m^{-3} ; Raitsos et al.,
390 2013). Furthermore, inshore waters had generally higher Chl a concentrations compared to
391 offshore waters at the same latitude (Raitsos et al., 2013; Sofianos & Johns, 2003). The higher
392 productivity and more variable Chl a concentration is thought to be attributable to the intrusion of
393 nutrient rich water from the Gulf of Aden and Indian Ocean (Raitsos et al., 2013; Sofianos & Johns,
394 2003). Thuwal had higher Chl a concentrations compared to offshore and midshelf Farasan Banks,
395 similar to previous studies that reported the coastal waters along the eastern Red Sea with higher
396 concentrations of Chl a than concentrations of areas away from the coast (Eladawy et al., 2017;
397 Sofianos & Johns, 2003).

398 Based on MLR analyses, the order of parameters with the most significance to the least
399 significance in predicting the Symbiodiniaceae distribution was $\text{SST} > \text{Chl a (turbidity/irradiance)} >$
400 depth. Among the predictors, SST was the strongest predictor of the distribution of
401 Symbiodiniaceae (*Cladocopium* psbA^{ncr} and *Durusdinium*), agreeing with Reimer et al. (2017a)
402 in which SST predicted *Cladocopium* lineage distributions well in the north to central Red Sea.
403 Furthermore, with the higher SST ranges in this study compared to those in Reimer et al. (2017a)
404 ($24\text{--}28^\circ\text{C}$), we finally can explain Pt-1-b distribution, as this lineage was previously found only in
405 low numbers in the north and central Red Sea and thus no hypothesis on its distribution was

previously made. From our results, Pt-1-b appears to be one of the most thermotolerant *Cladocopium* lineages. This lineage was absent in Thuwal and increased in number as SSTs increased from offshore to inshore in the Farasan Banks. Notably, Pt-1-b prevalence increased with SST gradients at the same rate as thermotolerant *Durusdinium*. Hence, based on Reimer et al. (2017a) and the current study, *Cladocopium* psbA^{ncr} lineage transition with SST increases was observed as Pt-1-a<Pt-3-a<Pt-3-b<Pt-1-b.

The other strong predictor of Symbiodiniaceae prevalence we observed was Chl a concentration. Chl a was used in this study as a proxy for turbidity, which can affect the distribution of Symbiodiniaceae (Caballero et al., 2019; Otero & Carberry, 2005). The MLR model results showed ~~patterns-changes in the prevalence of different of-Symbiodiniaceae prevalencee~~ across the Chl a concentration gradient (0.2-1.0 mg m⁻³), especially for *Cladocopium* lineage Pt-3-a, which had higher prevalence at reefs further away from the coast with lower Chl a (Brewin et al., 2015; Eladawy et al., 2017; Raitos et al., 2013; Zhu et al., 2007). Furthermore, Pt-3-a was absent in Thuwal, which had lower SSTs than the offshore Farasan Banks (FBO) but higher Chl a. These results suggest that *Cladocopium* lineage Pt-3-a has higher prevalences in environments with lower irradiance as compared to environments with lower SSTs. The MLR model also showed *Cladocopium* lineage Pt-3-b and *Durusdinium* had higher occurrences at locations with higher Chl a than Pt-3-a. These patterns could be observed in this study as we investigated sites across a wider and higher Chl a range (0.2-1.0 mg m⁻³) than compared to Reimer et al. (2017a) (0.1-0.4 mg m⁻³). It appears from our results that Chl a levels (= turbidity, or in other words available light) is a good predictor for the distribution of Symbiodiniaceae. However, the influence of Chl a was slightly less than that of SST on Symbiodiniaceae communities, which agreed with Leydet and Hellberg (2016).

The four main *Cladocopium* psbA^{ncr} lineages examined in this study (Pt-1-a, Pt-1-b, Pt-3-a, and Pt-3-b) were shown to have distinct environmental niches based on the MLR analyses of SST and Chl a. In general, species delineation is mostly through genetic variations that define the ecological, physiological, or morphological traits as distinct ecotypes or strains (Cohen, 2002; Sampayo et al., 2009, 2007; Thornhill et al., 2017). This is the cohesion species concept, which states that the closer the genetic distance between two species is, the closer their ecological niche is (Cohen, 2002). Based on the cohesion species concept, in this study one could infer that the lineages within each of the Pt-1 and Pt-3 clades should have almost the same ecological niche as they are very closely phylogenetically related. However, while this is true for Pt-3-a and Pt-3-b, closely related Pt-1-a and Pt-1-b showed two distinct ecological niches at two different temperature ranges (Reimer et al., 2017a). In this study, Pt-1-b had higher prevalences in warmer waters (>29°C) and increased at almost the same rate as the thermotolerant *Durusdinium*, while Pt-1-a preferred colder temperatures (<26°C) at higher latitudes in the Red Sea (Reimer et al., 2017a). These results demonstrate that genetically closely related Symbiodiniaceae do not necessarily share the similar physiological traits and niches (Sampayo et al., 2007), further evidence of the high amounts of species diversity within the family (LaJeunesse, 2004; Pochon et al., 2014; Smith et al., 2017b; Thornhill et al., 2014). Results from other genetic markers and examinations of allele exchange could help clarify the relationships between the *Cladocopium* lineages observed in this study.

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In this study, we showed that environmental changes over relatively small geographical scales can influence the kinds of symbiont associated with a host (<60 km LaJeunesse et al., 2004).~~In this study, we showed that Symbiodiniaceae composition in a single host species (*P. tuberculosa*) changes with environmental variation driven by distances from coast at smaller geographical scales compared to Reimer et al. (2017a) (<60 km vs. ~900 km; LaJeunesse et al.,~~

2004). Agreeing with previous studies (Finney et al., 2004; LaJeunesse et al., 2004; Finney et al., 2010), variations in SST and Chl a with distance from coast were shown to be good predictors for the distribution of *Cladocopium* psbA^{ncr} lineages and *Durusdinium* hosted in *P. tuberculosa*. Nevertheless, more studies in the future are required to examine other environmental factors, such as nutrient (nitrogen families, phosphate) levels, which can also be driven by distance from coast, and thereby influencing Chl a concentrations and Symbiodiniaceae diversity (Fahmy, 2003; Grupstra et al., 2017). Furthermore, two closely related *Cladocopium* lineages in this study (Pt-1-a, Pt-1-b) had two distinct environmental niches. These findings indicate that the psbA^{ncr} marker resolves lineages of *Cladocopium* that possess distinct physiological and ecological attributes (Noda et al., 2017; Reimer et al., 2017a). These results demonstrate the psbA^{ncr} region marker can provide a detailed resolution of Symbiodiniaceae *Cladocopium* lineage diversity that can be reconciled with ecological and functional data (Noda et al., 2017; Reimer et al., 2017a). Hence, future research utilizing hypervariable DNA regions (such as psbA^{ncr}) combined with fine-scale environmental data to examine diversity of Symbiodiniaceae is strongly recommended (LaJeunesse & Thornhill, 2011; Smith et al., 2017a). Such higher-resolution patterns of diversity will become more important in the future as more robust ecological analyses will be utilized to study Symbiodiniaceae diversity in the face of global climate change.

Acknowledgements

This work was partially funded by the King Abdullah University of Science and Technology (KAUST) (award URF/1/1389-01-01, Red Sea Research Center funds, and baseline research funds to MLB and TR), JSPS Kakenhi-Kiban B grant entitled ‘Global evolution of Brachynemina and their *Symbiodinium*’ to JDR, and Sasagawa Research Foundation funding to HBW (29-751). We wish to thank the captain and crew of the MV Dream Master, the KAUST Coastal and Marine

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475 Resources Core Laboratory, A. Gusti (KAUST), Roberto Arrigoni, Darren Coker, Tane Sinclair-
476 Taylor and other members of the KAUST Reef Ecology Lab for fieldwork assistance in the Red
477 Sea. [Comments from two reviewers greatly improved an earlier version of this manuscript.](#)

478 **Conflict of interest statement**

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

480

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Figures

Fig. 1 **Sampling sites of (a) Thuwal and (b) Farasan Banks.** Different colours represent FBO (red), FBM (orange), FBI (yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM=Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 2 **Phylogeny of Symbiodiniaceae *Cladocopium* lineages from *Palythoa tuberculosa* at Farasan Banks and Thuwal.** Maximum Likelihood (ML) tree of forward sequences of *Cladocopium* chloroplast psbA non-coding region (psbA^{ncf}) with reference sequences from Reimer et al. (2017a). Coloured dots on the tree represent each specimen found at FBO (red), FBM (orange) and FBI (yellow), and TI (green). Values at the branches represent ML, neighbor joining (NJ) and maximum parsimony (MP) bootstrap supports, and Bayesian inference (Bayes) posterior probability, respectively. Dotted boxes in the figure represent lineages of *Cladocopium*. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 3 **Symbiodiniaceae composition based on molecular identity (Sampling sites of (a) Thuwal and (b) Farasan Banks.** Different colours represent FBO (red), FBM (orange), FBI (yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

Fig. 2). Based on ITS-2, genera *Cladocopium* and *Durusdinium* composition at different (a) locations and (b) depths. psbA^{ncf} marker identified six lineages of *Cladocopium* and composition is shown based on (c) locations and (d) depth (meters). FBO=Farasan Banks Offshore; FBM=Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

765 Fig. 4 **Similarity dendrogram cluster of *Cladocopium* lineages based on psbA^{ncr} composition.**
766 Percentage similarity between sites based at different (a) locations and (b) depths (meter).
767 FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore;
768 TI=Thuwal.

769 Fig. 5 **Map depicting (a) sea surface temperature (SST, °C) and (b) chlorophyll a**
770 **concentration (Chl a, mg m⁻³).** The colour bars on each map represent the values of SST and Chl
771 a, respectively. The sites are represented as filled circles for FBO (red), FBM (orange), FBI
772 (yellow) and TI (green) sites. FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf;
773 FBI= Farasan Banks Inshore; TI=Thuwal.

774 Fig. 6 **Spearman's rho correlations among variables Symbiodiniaceae (*Cladocopium* psbA^{ncr}**
775 **lineages and *Durusdinium*) and predictors; depth, sea surface temperature (SST),**
776 **chlorophyll a (Chl a) and locations.** Diagonal histograms represent the comparisons within each
777 variable. The scatter plots below the histograms represent pairwise linear regression among
778 variables, the numbers above the histograms represent Spearman's rho correlation coefficient
779 values among variables with significance markings (0.1., 0.01*, 0.05**, <0.001***). The x and y
780 axis of the graph represent either the gradients (SST and Chl a) or arbitrary values representing the
781 categories in order (Locations, Depth and Symbiodiniaceae) of each variable.

782 Fig. 7 **Probability of observing Symbiodiniaceae (*Cladocopium* psbA^{ncr} lineages and**
783 ***Durusdinium*) in *Palythoa tuberculosa* of the central and southern Red Sea along (a) sea**
784 **surface temperatures and (b) chlorophyll a levels.** Probabilities were calculated from
785 multinomial logistic regression of SST and Chl a, respectively.

786

788 **Tables**

789 Table 1 **Test of normality distribution (Shapiro-Wilk test) and significance (Kruskal-Wallis**
790 **with Dunn post-hoc test) for sea surface temperature (SST) and chlorophyll a (Chl a) among**
791 **locations.** * represent values with significant difference (p< 0.05). FBO=Farasan Banks Offshore;
792 FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

793 Table 2 **p-values of multinomial logistic regression (MLR) coefficients among the**
794 **Symbiodiniaceae for locations (FBI vs. FBM, FBO and TI), depth, sea surface temperature**
795 **(SST) and chlorophyll a (Chl a).** * represent values with significant differences (p< 0.05).
796 FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore;
797 TI=Thuwal.

798

799 **Supplementary**

800 Supplementary 1 **Number of specimens based on locations and depths represented in tables**
801 **of:** (a) total number of specimens collected in sampling, (b) numbers of generated ITS-2 sequences,
802 and (c) of numbers of generated psbA^{ncr} sequences obtained. FBO=Farasan Banks Offshore;
803 FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore; TI=Thuwal.

804 Supplementary 2 **Phylogeny of Symbiodiniaceae from *Palythoa tuberculosa* at Farasan Banks**
805 **and Thuwal.** Maximum Likelihood (ML) tree of the internal transcribed spacer 2 of ribosomal
806 DNA (ITS-2) of nuclear ribosomal DNA of Symbiodiniaceae with reference sequences from
807 Reimer et al. (2017a). Coloured dots on the tree represent each specimen found at FBO (red), FBM
808 (orange) and FBI (yellow), and TI (green). Values at the branches represent ML, neighbor joining
809 (NJ) and maximum parsimony (MP) bootstrap supports, and Bayesian inference (Bayes) posterior
810 probability, respectively. Dotted boxes in the figure represent genera of Symbiodiniaceae.
811 FBO=Farasan Banks Offshore; FBM= Farasan Banks Midshelf; FBI= Farasan Banks Inshore;
812 TI=Thuwal.

813 Supplementary 3 **Genetic difference among lineages in this study (Pt-1-a, Pt-1-b, Pt-1-c, Pt-1-**
814 **d, Pt-3-a, Pt-3-b).** Two tables represent the (a) within-group genetic difference with mean and
815 standard deviation (StDev), and (b) pairwise genetic differences between groups with mean
816 (bottom black) and standard deviation (top, grey).

817 Supplementary 4 **Analysis of the Molecular Analysis (AMOVA) for *Cladocopium* psbA^{ncr}.** Two
818 statistical comparisons were conducted based on populations sorted by (a) locations and (b) depths
819 (meter).

820