Environmental latitudinal gradients and host-specificity shape Symbiodiniaceae distribution in Red Sea Porites corals

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Abstract
Aim: The aim of the study was to assess the diversity of algal symbionts of the family Symbiodiniaceae associated with the coral genus Porites in the Red Sea, and to test for host-specificity and environmental variables driving biogeographical patterns of algal symbiont distribution.

Location: Saudi Arabian Red Sea.

Taxon: Endosymbiotic dinoflagellates of the family Symbiodiniaceae in association with the reef-building coral genus Porites.

Methods: Eighty Porites coral specimens were collected along the Saudi Arabian Red Sea coast. Species boundaries were assessed morphologically and genetically (putative Control Region – mtCR; ITS region – ITS). Community composition of symbiotic dinoflagellates of the family Symbiodiniaceae was also assessed. Using the ITS2 marker with the SymPortal framework, Symbiodiniaceae data at the genus, majority ITS2 sequence and ITS2 type profile were used to assess symbiont diversity and distribution patterns. These were analysed in relation to coral host diversity, geographic location and environmental variables.

Results: Among the 80 Porites samples, 10 morphologies were identified. These corals were clustered into five lineages (clades I–V) by each of the markers independently. Clades I, II and III each comprised of a single Porites morphology, while clades IV and V contained up to five distinct morphologies. The diversity of Symbiodiniaceae associated with Porites was high and latitudinal differentiation was observed. In particular, a shift from a Cladocopium-dominated to a Durusdinium-dominated community was found along the north–south gradient. Symbiont diversity showed the patterns of geographic-specific association at Symbiodiniaceae genus, majority ITS2 sequence and ITS2 type profile level. Specific associations with host genotypes (but not morphological species) were also recovered when considering Symbiodiniaceae majority ITS2 sequence and ITS2 type profiles.

Main conclusions: This study provides the first large-scale molecular characterization of Symbiodiniaceae communities associated with Porites corals from the Saudi Arabian Red Sea. The use of intragenomic diversity data enabled the resolution of host-symbiont specificity and biogeographical patterns of distribution, previously
unachievable with the ITS2 marker alone. Finally, correlation among symbiont diversity and Red Sea environmental gradients was documented.

**KEYWORDS**
ITS2, Latitudinal gradient, next-generation sequencing, Scleractinia, symbiosis, SymPortal

# 1 INTRODUCTION

Shallow water tropical and subtropical corals rely on their association with microscopic endosymbiotic dinoflagellates of the family Symbiodiniaceae. Providing the corals with up to 95% of their nutritional needs (Falkowski, Dubinsky, Muscatine, & Porter, 1984), these photosynthetic symbionts are crucial for the growth and functioning of coral reefs (Hughes et al., 2017, 2018; Sampayo et al., 2016).

Symbiodiniaceae diversity in reef ecosystems is high and the specificity and variability of the associations that scleractinian hosts form with these symbionts have proven to confer ecological advantages to corals under different ecological conditions (Berkelmans & van Oppen, 2006; Hume et al., 2016; LaJeunesse et al., 2010; Rosic et al., 2015). These associations influence the geographical zonation patterns of corals at large and small scales and provide the corals with different tolerance to light intensity (Baker, 2001) and temperature (Berkelmans & van Oppen, 2006; Glynn, Mate, Baker, & Calderón, 2001; Rowan & Knowlton, 1995). Indeed, different Symbiodiniaceae-host interactions impact the corals’ susceptibility to bleaching events (for a definition of bleaching, see van Oppen & Lough, 2018). The potential of corals to ‘shuffle’ (i.e. replacement of dominant population by a background resident population) or ‘switch’ (i.e. the exogenous uptake of a different population from the environment) their symbiont communities towards more tolerant ones could also play a major role towards ecological resilience of coral reefs (Baker 2004; Sampayo, Ridgway, Bongaerts, & Hoegh-Guldberg, 2008; Kemp, Hernandez-Pech, Iglesias-Prieto, Fitt, & Schmidt, 2014), yet this potential remains questionable, and the diversity of the symbiont communities associated with different hosts seems to be non-random (Rowan, 1991; Trench, 1988, 1992). Different patterns of host–symbiont associations have been documented in response to latitudinal, longitudinal and environmental gradients, for various geographic locations and host taxa (Huang et al., 2011; Hume et al., 2015, 2016; Keshavmurthy et al., 2014; Oliver & Palumbi, 2009; Tonk, Sampayo, Chai, Schrameyer, & Hoegh-Guldberg, 2017; Ziegler, Roder, Büchel, & Voolstra, 2015). Nevertheless, our knowledge of the specificity and diversity of these associations is still poor, limiting our understanding of the ecological benefits that different associations provide (LaJeunesse et al., 2018).

Despite the lack of a single commonly accepted molecular marker for Symbiodiniaceae diversity typing, the Internal Transcribed Spacer II (ITS2) region is currently the most widely used barcode locus within the family (Hume et al., 2016, 2018; Smith, Ketchum, & Burt, 2017). The multicycopy nature of this marker means that both intragenomic and intergenomic sequence variants may be present within any single coral Symbiodiniaceae sample. Distinguishing between these sequence variant sources is difficult, and therefore, the majority of commonly used analytical approaches aim to collapse the confounding intragenomic diversity (Arif et al., 2014; Cunning, Gates, & Edmunds, 2017). However, the intragenomic diversity harboured within every Symbiodiniaceae genome may be taxonomically informative. Gel-based techniques have made use of this diversity to improve taxonomic resolution for more than 15 years (LaJeunesse, 2002). Most recently, the SymPortal analytical framework (symportal.org; github.com/SymPortal/SymPortal_framework; Hume et al., 2019) has been developed to make use of this intragenomic diversity for resolving genetic delineations using next-generation sequencing (hereafter ‘NGS’) ITS2 data. By leveraging the informative nature of Symbiodiniaceae intragenomic diversity, finer scale resolutions of genetic delineations are now possible; these delineations far surpass what were previously achievable with the ITS2 marker (Hume et al., 2019; Smith et al., 2017; Thornhill, Howells, Wham, Steury, & Santos, 2017).

As one of the hottest and most saline regions of the ocean, the Red Sea represents an ideal setting to explore Symbiodiniaceae diversity in a system where natural conditions are already exceeding the thresholds typical for Scleractinia persistence elsewhere in the world. Moreover, due to limited freshwater inflow, low circulatory exchange with the Indian Ocean and high evaporation rates (~2 m/year), the Red Sea displays extreme latitudinal environmental gradients (Trommer et al., 2009; Raitos et al., 2013). In particular, the sea surface temperature (SST) maxima ranges from 26°C (~±1.1°C) in the north to 31.3°C (~±1.1°C) in the south (Osman et al., 2018), the primary productivity increases from northern oligotrophic waters to southern nutrient rich waters (Raitos et al., 2013) and the salinity drops from 41 in the north to 36 in the south (Ngugi, Antunes, Brune, & Stingl, 2012). Symbiodiniaceae diversity in the Red Sea has been recently evaluated from the widespread anthozoan host *Palythoa tuberculosa*, and clear biogeographical patterns of association were recovered along the Red Sea latitudinal gradients (Reimer et al., 2017).

The Red Sea is also recognized as a marine biodiversity hotspot, harbouring more than 2000 species of fish and 50 genera of corals (Berumen et al., 2013; Briggs & Bowen, 2012; DiBattista, Choat, et al., 2016; DiBattista, Roberts et al., 2016). Hermatypic corals of the cosmopolitan genus *Porites* are among the most abundant, widespread and diverse zooxanthellate scleractinians in the Red Sea (Sheppard & Heppard, 1991). Up to 15 species of *Porites* have been reported from the region following traditional morphology-based classifications (Sheer & Pillai, 1983; Sheppard & Sheppard, 1991; Veron,
However, species boundaries within the genus remain unresolved (Dimond, Gamblewood, & Roberts, 2017; Forsman, Barshis, Hunter, & Toonen, 2009; Forsman et al., 2017; Forsman, Wellington, Fox, & Toonen, 2015; Prada et al., 2014). Besides their role as fundamental reef builders, *Porites* corals are also among the most resistant corals to increasing water temperatures (LaJeunesse et al., 2003). During both the 2010 and the 2016 bleaching events, *Porites* was among the least-affected coral genera in the Red Sea, with less than 40% of the resident population showing signs of bleaching (Furby, Bouwmeester, & Berumen, 2013; Monroe et al., 2018).

In this work, we applied NGS to explore the diversity of the Symbiodiniaceae community associated with *Porites* in the Saudi Arabian Red Sea along a 12° latitudinal gradient. We tested for host-specificity as well as geographic and environmental variables driving biogeographical patterns of algal symbiont distribution, with the aim of providing a better understanding of the ecological resilience of Red Sea coral reefs.

2 MATERIALS AND METHODS

2.1 Sampling and identification

A total of 80 *Porites* coral colonies were collected at seven coastal localities along the Saudi Arabian Red Sea between 2013 and 2016 (Figure 1, Appendix S1). Logistical reasons prevented us from sampling all regions at the same time. Each coral colony was imaged in the field using a Canon G15 camera while SCUBA diving. A fragment of approximately 10 cm³ was sampled from each scleractinian colony using hammer and chisel. Once in the laboratory, a subsample of 1 cm³ was taken from each specimen and preserved in 99% ethanol for further molecular analyses. The rest of the coral was bleached in sodium hypochlorite for 48 hr to remove fresh tissue and was air-dried for further morphological observations. Dried skeletons were imaged using a Canon G15 and were used for traditional morphology-based species identification.

**FIGURE 1** Sampling location and specimen collection overview (a) Red Sea map with sampling localities. b–j, *Porites* morphologies encountered among our 80 collected samples. (b) *Porites fontanesii*, (c) *Porites columnaris*, (d) *Porites sp1*, (e) *Porites rus*, (f) *Porites monticulosa*, (g) *Porites lutea*, (h) *Porites lobata*, (i) *Porites annae*, (j) *Porites echinulata*, (k) *Porites solida*
Specimens were identified based on skeletal morphology features of the corallum and coralites following Sheer & Pillai (1983), Sheppard and Sheppard (1991) and Veron (2000) and are now deposited at King Abdullah University of Science and Technology (KAUST - Saudi Arabia).

2.2 Coral host DNA extraction and PCR amplification

Genomic DNA was extracted from the coral samples using the DNeasy® Blood & Tissue Kit (Qiagen Inc.). Extracted DNA was quantified with AccuBlue High Sensitivity dsDNA quantitation kit (Biotium, Inc.) using a Qubit® fluorometer (ThermoFisher Scientific, Inc.). Details on PCR amplifications and primers are provided in Appendix S2.

Forward and reverse sequences were assembled and edited using Sequencer 5.3 (Gene Codes Corp.). Nuclear sequences that showed intra-individual polymorphisms were phased using Phase (Stephens, Smith, & Donnelly, 2001; available online at http://stephenslab.uchicago.edu/software.html) and SeqPHASE (Flot, 2010; available online at http://seqphase.mpg.de/seqphase/) when the alleles showed the same length, and using Champuru (Flot, 2007; available online at http://seqphase.mpg.de/champuru/) when the two predominant alleles were of different lengths. Alignments were performed using the E-INS-i option in MAFFT 7.130b (Katoh & Standley, 2013) and manually checked using BioEdit 7.2.5 (Hall, 1999). All the produced sequences are deposited at GenBank (see Appendix S1), and alignments are available upon request to the corresponding author.

2.3 Coral host phylogeny reconstructions

For each marker, the best fit substitution model was calculated using PartitionFinder 1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) with unlinked branch lengths, the greedy search algorithm for nucleotide sequence and a partitioning scheme comparison was performed using the corrected Akaike information criterion (AIC) and the Bayesian information criterion (BIC). For the mtCR, PartitionFinder selected the evolutionary model GTR+I, while for the ITS region, the GTR+I+G was the most suitable model. Phylogenetic relationships were reconstructed under two different criteria: Bayesian inference (BI) using MrBayes 3.2.6 (Ronquist et al., 2012) and Maximum Likelihood (ML) using RAxML 2 (Stamatakis, 2014). The CIPRES server (Miller, Pfeiffer, & Schwartz, 2012) was used to run both the BI and ML analyses. BI runs were performed using four Markov Chain Monte Carlo (MCMC) chains for 10 million generations, saving one tree every 100 generations. The tree searches were stopped when all parameters reached stationarity for effective sampling size and unimodal posterior distribution using Tracer 1.6 (Rambaut et al., 2014). The first 25% trees sampled were discarded as burn-in following indications by Tracer. ML topologies were obtained under the default parameters shown on the CIPRES server with a multiparametric bootstrap analysis of 1,000 bootstrap replicates.

2.4 Symbiodiniaceae MiSeq sequencing library preparation

Symbiodiniaceae types were characterized using PCR amplification of the ITS2 region (ITS2) for the Illumina MiSeq platform in the KAUST Bioscience Core Laboratory. Details on PCR amplifications, library preparation and sequencing are provided in Appendix S2.

2.5 Symbiodiniaceae MiSeq data processing

Symbiodiniaceae NGS ITS2 data were analysed using the SymPortal framework (Hume et al., 2019) by submitting paired fastq.gz files directly to the framework. A standardized quality control (QC) of sequences was conducted as part of the submission. Briefly, the standard SymPortal QC is conducted using mothur 1.39.5 (Schloss et al., 2009), the BLAST+ suite of executables (Camacho et al., 2009) and minimum entropy decomposition (MED; Eren et al., 2015). The MED incorporated into the standard SymPortal QC pipeline uses an ‘M’ value cut-off meaning that MED nodes (a proxy for representative sequences) are identified down to a relative within sample, genus-partitioned abundance of 0.4% (i.e. 0.4% of the sequences for a given genus in a given sample; or four sequences if the sequencing is very shallow). As such, the ITS2 type profile predictions in the standard outputs of SymPortal should be viewed as being representative of the more abundant genotypes present in any given sample's genus portioned collection genotypes (sequences are analysed on a genus partitioned basis within the SymPortal framework). While the SymPortal approach of searching for genotypic representative sets of sequences can be applied to identify low abundance genotypes within samples (by searching for identified ITS2 type profiles in the pre-MED decomposition sequences that are also output by the SymPortal QC; providing that sequencing depth is adequate), this study concerned itself with only the standard output and thus the more abundant genotypes in the genus partition. This standard output contains two count tables: one provides sequence abundances listed by sample (see Appendix S3) and the second provides ITS2 type profile abundances listed by sample (see Appendix S3). In the second count table, alongside the ITS2 type profile abundances, different hierarchical levels are given for each of the identified Symbiodiniaceae genotypes. Specifically, for this analysis, the clade (genus), majority ITS2 sequence (most abundant ITS2 sequence) and ITS2 type profile (representative of putative taxa) were used. The source code and detailed documentation of the logic underlying the SymPortal analysis are available from its GitHub repository (Hume, 2019).

2.6 Environmental data

A total of four environmental variables were considered in this study, namely chlorophyll-a [Chl-a], sea surface temperature (SST), particulate organic carbon (POC) and salinity. The first three variables were gathered for each of the seven sampling localities of the Red Sea from 2010 to 2017 from the National Aeronautic and Space
Administration (NASA) Giovanni website (Acker & Leptoukh, 2007), developed and maintained by the NASA Goddard Earth Sciences Data and Information Services Center (https://giovanni.gsfc.nasa.gov/giovanni/). In particular, monthly average Chl-a, SST and POC data were derived from 4 km resolution data from the Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua database. For each sampling location, annual averages were then calculated. Following Reimer et al. (2017), salinity for each sampling site was gathered from March 2010 (winter) data in Ngugi et al. (2012), as representative salinity occurring at each site. All environmental data are listed in Appendix S3.

2.7 Statistical analyses

Statistical analyses were performed using PRIMER 6.1.15 (Primer-E) with the add-on PERMANOVA+package (Anderson, Gorley, & Clarke, 2008). Permutational multivariate analysis of the variance (PERMANOVA) was performed on Bray–Curtis distance matrices to test for significant differences in Symbiodiniaceae community composition along the Red Sea latitudinal gradient, and to test for morphological and molecular host-specificity with Porites corals. In particular, the three Symbiodiniaceae input datasets (genus level, IT52 majority sequence, IT52 type profile) were tested for compositional differences for three biodiversity metrics: Red Sea locality (seven levels: Gulf of Aqaba, Duba, Al Wajh, Yanbu, Thuwal, Farasan Banks, Farasan Islands), Porites molecular clade (five levels: clades I, II, III, IV, V) and Porites morphological species (10 levels: P. annae, P. echinulata, P. fontanesii, P. columnaris, P. lobata, P. lutea, P. monticulosa, P. rus and P. solida). The factors were fixed and orthogonal. Moreover, given that some Symbiodiniaceae communities may change over time the three Symbiodiniaceae input datasets (genus level, IT52 majority sequence, IT52 type profile) were tested for sampling time (March 2013, September 2013, October 2014, November 2015, December 2015, January 2016, February 2016). The factors were fixed and orthogonal. Prior to running the PERMANOVA, we verified the homogeneity of the dispersions of the categorical variables using PERMDISP. We chose PERMANOVA to test for differences among the levels of our explanatory variables, as it can cope with the uneven sample size of Porites at each studied location (Anderson et al., 2008).

Canonical Analysis of Principal coordinates (CAP) for each single factor was performed as a validation, effectively testing how well CAP can correctly reallocate the samples to their respective groups (Anderson & Willis, 2003).

A marginal test in distance-based linear modelling (DistLM) was used to explore the correlation between Symbiodiniaceae diversity (genus level, majority ITS2 sequence, ITS2 type profile) and the four environmental variables [salinity, Chl-a, DOM and SST] (Anderson et al., 2008). A Bray–Curtis dissimilarity matrix was built with the inclusion of a dummy variable (+1) to accommodate for zeros in the biological data. Bi-plots of the CAP ordination with Bray–Curtis distance were computed using R and the package ‘vegan’ (Oksanen et al., 2013) to visualize the relationship between environmental variables (i.e. Chl-a, SST, POC and salinity) and each biodiversity metrics (i.e. locality, Porites molecular clade, Porites morphological species) and Symbiodiniaceae at the genus level, ITS2 majority sequence and ITS2 type profiles.

3 RESULTS

3.1 Traditional and molecular identification of Porites

Among the 80 Porites colonies collected, a total of nine nominal species were identified based on morphological characters: Porites annae, P. echinulata, P. fontanesii, P. columnaris, P. lobata, P. lutea, P. monticulosa, P. rus and P. solida. One sample did not match any of the existing original descriptions of Porites species in the literature and, therefore, was referred to as Porites sp1.

Sequence data for the mtCR and ITS region were obtained from all 80 analysed samples (see Appendix S1). One sequence of Goniopora sp. was added to the two datasets and used as outgroup in both the reconstructions following Kitano et al. (2014). The mtCR sequence alignment consisted of 1,292 bp, with 68 polymorphic and 31 parsimony informative sites. The ITS alignment encompassed 795 bp with 103 variable sites, 88 of which were parsimony informative. BI and ML tree topologies obtained from the two regions were largely congruent, recovering five highly supported molecular clades in our samples (Clades I–V) (Figure 2). Three clades were comprised of a single morphospecies, and their monophyly was highly supported: clade I included all P. fontanesii material, clade II grouped P. columnaris and clade III was comprised solely of a sample from P. sp1. Conversely, clades IV and V grouped more than one morphospecies of Porites. Namely, clade IV included P. rus and P. monticulosa, while clade V comprised five different morphologies of Porites, that is, P. annae, P. echinulata, P. lobata, P. lutea and P. solida.

3.2 Symbiodiniaceae community structure

A total of 8,159,993 sequences were generated using Illumina MiSeq. After filtering, 5,506,746 sequences were analysed with the SymPortal framework, and 156 Symbiodiniaceae-defining ITS2 intragenomic variants were recorded within our 80 Porites samples. A total of 77 ITS2 type profiles were recovered that were represented by 38 distinct ITS2 sequences found to be the most abundant in any one of the ITS2 type profiles (see Appendix S3). Symbiodiniaceae community composition at the genus level, the majority ITS2 sequence level (most abundant ITS2 sequence for a given ITS2 type profile) and the ITS2 type profile level was visualized using stacked bar charts to compare the relative abundance for three factors, namely, locality, Porites molecular clade and Porites morphological species (Figure 3).

Overall, the most abundant genus of Symbiodiniaceae associated with Porites in the Red Sea was Durusdinium (51%), followed by Cladocopium (46%); only 3% of the sequences belonged to the
genus *Symbiodinium* (Figure 3). PERMANOVA analysis identified a significant geographical structure in the Symbiodiniaceae genera distribution along the latitudinal gradient of the Saudi Arabian Red Sea ($F_{4,73} = 6.42, p = .001$, Table 1), with the cross-validation analysis reassigning 42.5% of the sequences to the correct geographic location (Table 1). In particular, in the northern Red Sea (i.e. Gulf of Aqaba and Duba), *Porites* colonies exclusively harboured *Cladocopium*. From north to south, the Symbiodiniaceae community shifted gradually from *Cladocopium* dominated towards *Durusdinium* dominated. Indeed, *Durusdinium* represented 80% of the community in the southern Red Sea (i.e. Farasan Islands). Finally, the genus *Symbiodinium* appeared below 1% in the northern (Duba, Al Wajh, Yanbu) and central (Thuwal, Farasan Banks) Red Sea, while in the Farasan Islands, represented 8% of the symbiont community (Figure 3). No significant correlations between Symbiodiniaceae genera and *Porites* morphological species ($F_{11,68} = 0.73, p = .74$) or *Porites* molecular lineages ($F_{11,75} = 1.14, p = .30$, Table 1) were recorded.

The majority of ITS2 sequences for a given ITS2 type profile was C15 (accounting for 28% of the entire major ITS2 sequences), followed by D1 (18% of the total diversity) (Figure 3). A significant latitudinal structure of Symbiodiniaceae community emerged by analysing the distribution of the majority ITS2 sequence for the ITS2 type profiles predicted ($F_{4,73} = 2.27, p = .001$; cross-validation analysis reassigned 38.75% of the majority ITS2 sequences to the correct location – Table 1). In the north, from the Gulf of Aqaba to Al Wajh, the C15 sequence was the most abundant majority ITS2 sequence, contributing to the entire diversity in the Gulf of Aqaba (100%) and to more than 40% of the diversity in Duba (58%) as well as in Al Wajh (42%). In Yanbu, D1 (34%) and C15 (38%) were the most abundant majority ITS2 sequences, while in Thuwal, C15 was the most abundant majority ITS2 sequence (39%) together with D1 or D4 (23%) and C15 or C60a (23%). Similarly, in the Farasan Banks, C15, D1 and D4 sequences were the most abundant majority ITS2 sequences (31%, 21% and 19% respectively). Finally, in the Farasan Islands, D1 or D6 and D1 or D4 sequences mostly contributed to the majority ITS2 sequences of the community (36% and 21% respectively; Figure 3).

Symbiodiniaceae host-specificity was recovered when considering the majority of ITS2 sequences in relation to *Porites* molecular clade ($F_{11, 68} = 1.71, p = .01$; cross-validation reassigned 48.75% of the majority ITS2 sequences to correct *Porites* molecular clade – Table 1). In *Porites* clades I, II, III, and IV, the majority ITS2 sequences of the ITS2 type profiles was represented by one or two sequences. In particular, in clade I: C15 sequence (42%) and D1 or D4 or D1n (32%); clade II: C15 sequence (45%) and D1 sequence (41%); clade III: A1 sequence (100%); clade IV: D1 or D4 (37%) and D1 (16%). Finally, in *Porites* clade V, three sequences of Symbiodiniaceae made up more than 50% of the diversity: C15 (25%), D1 or D6, and D1 (Figure 3). No correlation between *Porites* morphological species and Symbiodiniaceae ITS2 majority was identified ($F_{11, 68} = 1.19, p = .1$ – Table 2).

A total of 77 Symbiodiniaceae ITS2 type profiles were recovered by the SymPortal analytical framework, distributed as follows: 38 type profiles belonged to the genus *Cladocopium*, 26 to *Durusdinium* and 13 to *Symbiodinium* (see Appendix S3). Locality, *Porites* molecular clade and *Porites* morphological species correlated with the Symbiodiniaceae type profiles recovered ($F_{11, 75} = 1.49, p = .0004; F_{11, 75} = 1.79, p = .001; F_{11, 68} = 1.31, p = .006$, respectively – Figure 3).

**FIGURE 2** RAxML phylogeny reconstructions of Red Sea *Porites* at two molecular loci. (a) Mitochondrial Control Region, (b) nuclear Internal Transcribed Spaces region. Number on the branches represents support values corresponding to Bayesian posterior probabilities (>90%), ML bootstrap values (>70%). *Goniopora* was selected as outgroup in both the analyses. In (b), black curved lines connect the two sequences of heterozygous individuals.
but the cross-validation analyses only reassigned correctly ITS2 type profiles to the geographic location (42.5%), and the Porites molecular clade (55%), while only 10% of them were regrouped into correct morphological species (Table 1).

Finally, the PERMANOVA analysis identified a significant effect of the sampling time on the Symbiodiniaceae genera ($F_{6,73} = 5.18$, $p = .001$), majority of ITS2 sequence ($F_{6,73} = 2.35$, $p = .001$) and ITS2 type profiles ($F_{6,73} = 1.63$, $p = .001$) in the Saudi Arabian Red Sea (see Appendix S5).

3.3 Symbiodiniaceae diversity in relation to environmental variables

DistLM showed that the four environmental variables included in the analyses (i.e., salinity, Chl-$a$, POC and SST) were statistically significantly explaining the variation of Symbiodiniaceae diversity at the Symbiodiniaceae genus level, majority ITS2 sequences and the ITS2 type profile ($p < .002$, Table 2; Appendix S4). Interestingly, in all the datasets analysed, salinity was the most influential variable, which alone explained 31.3%, 10.9% and 4.9% of the entire variability (Symbiodiniaceae genus, majority ITS2 sequence and ITS2 type profile respectively), followed by Chl-$a$, POC and SST at all the levels (Table 2; Appendix S4).

4 DISCUSSION

4.1 Porites morpho-molecular diversity

Porites is one of the most speciose zooxanthellate coral genera in the world, accounting for over 160 described species, 62 of which are currently recognized as valid (WoRMS, 2018). Almost a quarter of these have been previously recorded in sympathy in the Red Sea (Sheer & Pillai, 1983; Sheppard & Sheppard, 1991; Veron, 2000), rendering the region a biodiversity hotspot for Porites corals. In our study, we characterized 10 Red Sea Porites morphospecies at two molecular loci. Only three of the 10 morphospecies proved to be monophyletic (i.e., P. fontanisi, P. columnaris and P. sp1) and showed concordant morpho-molecular species boundaries. The remaining seven species (P. annae, P. echninulata, P. lobata, P. lutecia, P. solidia, P. monticulosa and P. rus) clustered into two evolutionary lineages (clades IV and V). Our molecular data demonstrate that the identification of Porites species based exclusively on morphological features does not match with the molecular lineages occurring in the Red Sea, at least based on the markers used herein (Figure 2). As such, a deep gap in our understanding of species boundaries and evolutionary relationships in the genus is confirmed (Forman et al., 2009, 2017, 2015). The use of a multidisciplinary taxonomic approach has proven useful to fill
this gap for several coral genera (Benzoni, Stefani, Pichon, & Galli, 2010; Kitahara, Fukami, Benzoni, & Huang, 2016; Schmidt-Roach, Miller, Lundgren, & Andreakis, 2014). Indeed, recent studies showed that coupling multilocus molecular evidence with new morphological evidence (e.g. micromorphology and microstructure), reproductive biology data and symbiosis insights could provide us with a new understanding of Scleractinia systematics and evolution.

### 4.2 Symbiodiniaceae biogeography across Red Sea gradients

The Red Sea environmental gradients correlated with the Symbiodiniaceae biogeographical patterns observed. Sequence data showed a shift from the genus *Cladocopium* to *Durusdinium* going from the north (Gulf of Aqaba and Duba) to the south (Farasan Islands) of the Red Sea, with corals in the central part of the Red Sea (Al Wajh, Yanbu, Thuwal, Farasan Banks) harbouring both *Cladocopium* and *Durusdinium* (Figures 1 and 3). *Symbiodinium* appears mainly in the Farasan Islands at relative low abundance, and at very low abundance (never above 1%) in the rest of the localities. Similar biogeographical patterns highlighting a community break south of the Gulf of Aqaba have been previously found in different anthozoans symbiont communities. For example, similar patterns with Symbiodiniaceae community break at the entrance of the Gulf of Aqaba were also recorded by Reimer et al., (2017) for the Zoantharia *P. tuberculosa*. Nevertheless, although a different *Cladocopium* community was found outside the Gulf of Aqaba, this former work found *Cladocopium* from the Gulf of Aqaba to the Thuwal area, where *Durusdinium* also appeared. Sawall, Al-Sofyani, Banguera-Hinestroza, and Voolstra (2014) found discontinuity in the

### TABLE 1 PERMANOVA results calculated for three biodiversity metrics (Red Sea locality, *Porites* molecular clade and *Porites* morphological species) for the datasets Symbiodiniaceae genus, Symbiodiniaceae majority ITS2 sequences and Symbiodiniaceae ITS2 type profile.

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<td>p .307</td>
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<tr>
<td><em>Porites</em> morphological species</td>
<td>df 11</td>
<td>F 0.7351</td>
<td>p .74</td>
</tr>
<tr>
<td>Res</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: df, degrees of freedom; Res, Residual degree of freedom; CAP, Cross validation of the PERMANOVA results testing how well CAP routine can correctly reallocate the samples to their respective groups (Anderson & Willis, 2008).

### TABLE 2 Marginal test results of the DistLM analyses for each of three datasets (Symbiodiniaceae genus, Symbiodiniaceae majority ITS2 sequence and Symbiodiniaceae ITS2 type profile) against the environmental factors SST, Chl-a, POC and Salinity.

<table>
<thead>
<tr>
<th>Environmental factor</th>
<th>F</th>
<th>p</th>
<th>% var</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbiodiniaceae genus</td>
<td>SST</td>
<td>20.289</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Chl-a</td>
<td>34.536</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>POC</td>
<td>35.656</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>35.656</td>
<td>.001</td>
</tr>
<tr>
<td>Symbiodiniaceae majority ITS2 sequence</td>
<td>SST</td>
<td>5.3512</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Chl-a</td>
<td>9.3287</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>POC</td>
<td>8.829</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>9.639</td>
<td>.001</td>
</tr>
<tr>
<td>Symbiodiniaceae ITS2 type profile</td>
<td>SST</td>
<td>2.5913</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>Chl-a</td>
<td>3.8551</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>POC</td>
<td>3.6152</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Salinity</td>
<td>4.0011</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: SS, Sum of squares; %var, percent of variance explained by each predictor variable.
symbiont communities associated with the coral *Porites verrucosa* between the Gulf of Aqaba and the rest of the Red Sea. Similar to our findings, *P. verrucosa* colonies from the Gulf of Aqaba were characterized by the unique association with the genus *Cladocopium*, while only *Symbiodinium* was found in the remaining sites of the central and southern Red Sea. Similarly, Arigoni, Benzioni, Terraneo, Caragnano, and Berumen (2016) investigated the symbiont community of Red Sea *Stylophora*. All samples from the Gulf of Aqaba harboured *Cladocopium*, while outside coral host colonies also associated with *Symbiodinium*. This break has been proposed to be mainly driven by cooler temperature at the entrance of the Gulf of Aqaba and, in particular, has been proposed that *Cladocopium* might have colder water preference (Sawall et al., 2014; Ulstrup, Berkelmans, Ralph, & Oppen, 2006). However, while this cooler water preference hypothesis may be an effective explanation for individual *Symbiodiniaceae* taxa, it is important to note that this trend should not be extrapolated to include the whole of the genus *Cladocopium*, especially when exceptionally thermally tolerant members exist, that is, *C. thermophilum* (D’Angelo et al., 2015).

Genetic breaks have also been documented among populations between the central Red Sea and the Farasan Islands in the south. For example, genetic breaks have been reported in different fish populations, (Frouk & Kochzius, 2007; Nanninga, Saenz-Agudelo, Manica, & Berumen, 2014; Saenz-Agudelo et al., 2015), sponges (Giles, Saenz-Agudelo, Hussey, Ravasi, & Berumen, 2015), and mussels (Shefer, Abelsson, Mokady, & Geffen, 2004). From these studies, the genetic brakes matched with environmental transitions occurring around 16°–20° N in the Red Sea. In contrast to the rest of the Red Sea, the Farasan Islands region is a shallow reef system, characterized by less saline but warm, eutrophic and turbid waters (Sheppard & Sheppard, 1991). Our data show a predominance of *Durusdinium* sequences in this environment, with high proportions of *D. trenchii* (D1-D4) expected. This distribution fits into theories that *D. trenchii* is a stress-resilient taxon within the *Symbiodiniaceae*, and thus found in association with warm and turbid environments or in response to stressful events (Baker, 2001; LaJeunesse et al., 2008). Recent studies showed association of *P. lutea*, *P. lobata* and *P. harrisoni* with *C. thermophilum* symbionts in the southern Arabian Gulf where SST (36°C) and salinity (42) levels exceed the ones from the Red Sea (D’Angelo et al., 2015; Hume et al., 2016, 2018). D’Angelo et al. (2015) showed a community transition along the temperature and salinity gradient occurring between the southern Arabian Gulf and the Gulf of Oman: *C. thermophilum* was associated with 100% of *Porites* in the southern Arabian Gulf; in the transition zone between the Arabian Gulf and the Gulf of Oman, *Porites* associated with *C. thermophilum* as well as C15 lineages and *D. trenchii*; finally, in the Gulf of Oman, where environmental conditions more resemble those present elsewhere in the tropical Indo-Pacific belt, *Porites* associated mainly with C15 lineages, as elsewhere in *Porites* hosts. In the Red Sea, we find a similar pattern. In the north and central Red Sea, *Porites* is found mainly in association with C15 radiation, the most common symbiont association within *Porites*. In the south, where the thermal stressors are higher (although not reaching levels as extreme as in the Arabian Gulf), *Porites* associates with the more resilient *D. trenchii*.

Among the environmental variables tested, salinity resulted as partitioning the most variation in the *Symbiodiniaceae* community distribution along the Red Sea latitudinal gradient. This result was expected since, in the Red Sea, salinity changes linearly along the north–south latitudinal gradient. Published datasets show that salinity in the Red Sea is relatively stable among years (Roik et al., 2016); nevertheless, oscillation in salinity can occur seasonally during the year as result of evaporation rates, precipitations and mixing of low saline surface inflow from Gulf of Aden (Eshel & Heavens, 2007; Sofianos, Johns, & Murray, 2002). Monitoring physio-chemical variables for three reefs in the central Red Sea across 2 years, Roik et al. (2016) show that although salinity oscillations occur seasonally (up to 1.43), they are smaller in comparison to other reef systems affected by riverine and precipitation inputs, such as on inshore reefs in the Great Barrier Reef, where salinity can fluctuate from five to ten (Roik et al., 2016). Comparing Ngugi et al., (2012) salinity dataset, with annual mean salinity data from the World Ocean Atlas 2018 available at https://www.nodc.noaa.gov/OC5/woa18/ (Conkright et al., 2002; Zeng et al., in prep), the salinity trend along the north–south latitudinal gradient in the Red Sea appeared stable, so we decided to include salinity in our analyses.

The residual variance not partitioned to the environmental factors tested in this study would suggest that other sources of variation not considered in the present study might be involved in explaining the dataset’s variability across the gradient. For example, bathymetric distribution has been shown to influence symbiotic association (Bongaerts et al., 2013, 2015; Frade, Jongh, Vermeulen, Bleijwijk, & Bak, 2008). We thus suggest the incorporation of additional variables in future work.

### 4.3 Host-symbiont specificity

By identifying previously overlooked *Symbiodiniaceae* ITS2 sequences, we were able to identify host-specific association patterns. Indeed, our data showed coupling between *Porites* genotypes (clade I–V) and *Symbiodiniaceae* ITS2 type profiles and the majority sequences that represent them. C15 radiation sequences were shared among four of the five genetic lineages of *Porites*, and made up the most of the ITS2 sequences in Clade I, II and V, confirming that the C15 radiation is commonly associated with *Porites* (Franklin, Stat, Pochon, Putnam, & Gates, 2012; Keshavmurthy et al., 2014; LaJeunesse, 2004). Nevertheless, association is not exclusive in our data and might vary depending on the host and the environment. Clade III was mainly associated with A1 sequences, although small sample size prevents us from drawing any firm conclusion about this association.

No specific pattern of association among morphologically described nominal species of *Porites* and *Symbiodiniaceae* was recovered. This is an informative result in an era of coral taxonomic revolution, supporting the evidence that coral skeletal morphology
alone can lead to misleading classifications. Evolutionary relationships in corals are being revised at every taxonomic level by combining genomic evidence with other lines of evidence. Our results highlighted that such evidence can come from detailed analyses of the associated symbiont community, a trait that has been so far only considered in a limited number of studies.

5 CONCLUSIONS

This study provides an overview of Symbiodiniaceae diversity associated with *Porites* corals in the Saudi Arabian Red Sea, one of the hottest and most saline environments in the world. This study was able to define zooxanthellae diversity at a new level through the use of an analytical approach leveraging the taxonomically informative intragenomic sequence diversity harboured in every Symbiodiniaceae genome. Even if *Porites* clades inherently harboured a certain degree of variability at each location due to sample size and clade distribution, biogeographical patterns of symbiont distribution could be distinguished along 2000 km of Red Sea coast, and a correlation among symbiont diversity and Red Sea environmental gradients was found.

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**BIOSKETCH**

Using a suite of different approaches, from modern molecular techniques to fine-scale morphology and patterns of reproduction, T.I.T aims to provide a better understanding of corals taxonomy and evolution.

Author contributions: T.I.T, R.A, M.L.B designed the study; T.I.T and F.B collected specimens; T.I.T performed molecular work and data analyses; B.C.C.H ran the SymPortal analysis; M.F performed statistical analyses; T.I.T wrote the paper with comments by B.C.C.H, C.R.V, R.A, M.F, Z.H.F, F.B, M.L.B.

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**Cell to cell signals in plant, animal and microbial symbiosis**